BIOTECHNOLOGY AND GENETIC ENGINEERING FOR DEVELOPMENT IN THE ISLAMIC WORLD

Biotechnology and Genetic Engineering for Development in the Islamic World

Proceedings of the Conference on Biotechnology and Genetic Engineering for Development in the Islamic World held in Rabat, Morocco
22-26 October 2001

Edited by

Abdel Salam Majali
Mehmet Ergin
and
Moneef R. Zou'bi

Published by

Islamic Academy of Sciences
Amman, Jordan

Publication sponsored by

Islamic Development Bank
Jeddah, Saudi Arabia

OPEC Fund for
International Development
Vienna, Austria

Copyright © Islamic Academy of Sciences, Amman, Jordan 2004.

All rights reserved. No part of this publication may be reproduced or transmitted in any form or by any means, without permission.

First published in 2004 by
The Islamic Academy of Sciences
PO Box 830036 Zahran
Amman 11183 – Jordan.

Telephones: + 9626 55 22 104
+ 9626 552 33 85
Fax: + 9626 55 11 803

Email: ias@go.com.jo
http://www.iasworld.org

Type-setting at the Secretariat of the Islamic Academy of Sciences, Amman, Jordan.

Printed in Jordan by the National Printing Press, Amman.

Jordan National Library Classification Deposit Number: 126/1/2003.
*Descriptors: \ Genetic Engineering \\ Sustainable Development \\ Islamic World \\ Heredity *

ISBN 9957-412-07-8

Price: US$ 30-00.

*** The views expressed in this Proceedings are those of the authors, and not necessarily of the editors, the Council or the General Assembly of the Islamic Academy of Sciences. The Islamic Academy of Sciences, providing a free forum for the exchange of scientific opinions and being a non-political institution of the whole of the Islamic Ummah, does not hold itself responsible for the statements made or ideas expressed by the contributors.*

** Cover photos courtesy of 'Al-Oloum' Magazine.*

Contents

Preface 1

Acknowledgements 3

Organisations sponsoring the Eleventh Conference – 2001 5

IAS Rabat Declaration 7

Conference Report 11

PART ONE: STATEMENTS AT THE INAUGURAL SESSION

Message of His Excellency the President of the Islamic Republic of Pakistan, and Patron of the Islamic Academy of Sciences 17

Message of His Royal Highness Prince Al Hassan Ibn Talal of Jordan, and Patron of the Islamic Academy of Sciences 19

Address of His Excellency Prof. Abdul Salam Majali, President of the Islamic Academy of Sciences 23

Address of His Excellency Prof. Abdulaziz Twaijri, Director General, ISESCO 29

Address of Prof. Abdelhafid Lahlaidi FIAS, Chairman of the Local Organising Committee 33

PART TWO: BIOTECHNOLOGY: WHERE DO WE STAND?

Biotechnology in the Developing World 39

Edgar J. Dasilva, Elias Baydoun and Adnan Badran

Biotechnology in the Islamic World: Challenges and Opportunities 87

Anwar Nasim and Zabta K. Shinwari

Biotechnology for Developing Countries: Challenges and Opportunities 103

Salleh Mohd Nor and P.A. Avadhani

PART THREE: MEDICAL BIOTECHNOLOGY FOR THE FUTURE

Biotechnology: The Discovery of Disease Causing Genes and the Treatment of Inherited Diseases in Man 119

S. Q. Mehdi, Abdul Hameed, Muhammed Ismail, Aiysha Abid and Shagufta Khaliq

Technical Aspects of Genetic Engineering: Current and Future Trends 141

Naima Alami-Ouahabi, M. Lahrichi and A. Belmahi

New Opportunities for Novel Drugs and Vaccines against HIV/AIDS based on the Structure and Functions of the HIV Accessory Proteins Nef and Vpr 175

Ahmed A. Azad

Gene Therapy for the Treatment of Tumours: A Perspective 187

Naima Alami-Ouahabi

The Candidate Genes of Cardiovascular Diseases: Study of the Cholesterol Ester Transfer Protein and of the Apolipoprotein E Polymorphism 223

O. Kabbaj, N. Lahlali-Kacemi, A. Touzani, Y. Bamou, N. Alami, M. Hassani and M. Lahrichi

Advances in Cancer Gene Therapy 239

Saima N. Waqar and Muhammad Anwar Waqar

PART FOUR: SOME ASPECTS OF BIOTECHNOLOGY AND FOOD SECURITY

Biotechnology in the Palm Oil Industry: The Experience of the Malaysian Palm Oil Board (MPOB) 253

Ahmad Parveez Ghulam Kadir, Siti Nor Akmar Abdullah and S. Ravigadevi

Use of Biotechnology and Molecular Tools for Crop Improvement and Sustainable Agriculture 277

Ahmad S. Islam and Zeba I. Seraj

Application of RT-RCR Technology for Detection of Bluetongue and Epizootic Hemorrhagic Disease Viruses 293

Imadeldin E. Aradaib and Bennie I Osborn

PART FIVE: NATIONAL BT SCENARIOS

Biotechnology Research and Development in Tunisia 307

Radhouane Ellouz

Development of the Biotechnology Sector: The Iraq Perspective 315

Sami Al-Mudhaffar

Present Status and Future Prospects of Biotechnology in Sudan with Special Reference to Veterinary Medicine 329

Hashim M. El-Hadi and Imadeldin E. Aradaib

Arsenic Contamination of Drinking Water in Bangladesh 345

S. Z. Haider

PART SIX: BIOTECHNOLOGY EDUCATION

Common Characteristics of the Required Strategy in Biotechnology in Islamic Countries: Educational, Scientific and Economic Aspects 357

Faiq Billal

Genetic Engineering and the Ecobalance 371

Munir Ozturk and Iffet Irem Uzonur

Conservation and Utilization of Genetic Resources: An Issue for the Developing World 389

Mohamed B. E. Fayez

Some Educational Activites in Biotechnology in Iraq 397

M. A-H. Kadhim

PART SEVEN: ETHICS, SOCIAL VALUES AND BIOTECHNOLOGY

Islam and Biology 425

Muhammad Al-Habib Ibn Al-Khoja

The Twenty First Century is a Century of Biology: Prospects and Challenges 437

Mohamed Al-Mokhtar Essalami

Ethical Issues of Genetic Engineering: An Islamic Perspective 451

Anwar Nasim

Convergence and Divergence between Religious Thought and Rational/Scientific Thought in Islam: A Preliminary Survey 461

Mazhar M. Qurashi

Biotechnology and Health 471

Mostefa Khiati

PART EIGHT: APPENDIXES

Conference Committees 481

Chairpersons of Conference Sessions 483

List of Conference Participants 485

IAS Council Members and Executive Staff 493

IAS Patrons and Honorary Fellows 495

IAS Fellows 497

IAS Deceased Fellows and Ibrahim Award Laureate 501

IAS Supporters..... 503

IAS Waqf 505

Preface

The world is living through a dizzyingly rapid expansion in biotechnology, and a widening biotechnological gap is emerging between developed and developing including Islamic countries.

This "genetic divide" is likely to have far-reaching consequences for the developing countries because of the radical nature of biotechnology and its implications for agriculture, human health and environmental management. Unlike the information revolution, which was the subject of the IAS's 2000 conference, advances in biotechnology have the potential to alter the patterns of food production and distribution in fundamental ways, as well as influence the pharmaceutical industry.

Today's biotechnology is largely identified with applications in medicine and agriculture based on our knowledge of the genetic code of life. Various terms have been used to describe this form of biotechnology including genetic engineering and genetic modification technology, or GM technology.

The complete sequencing of the Human Genome, which has been described as a historic milestone in the history of science, raises unlimited possibilities in, among many things, the human quest to combat centuries old diseases. It moreover poses a number of ethical, legal and social questions that need to be examined by our academicians, religious scholars and scientists.

The many crucial decisions to be made in the area of biotechnology by the private and the government sectors as well as individuals in our countries will affect the future of humanity and the planet's natural resources. These decisions must be based on sound scientific information in order to allow effective choices of policy options.

The IAS has developed a tradition of addressing issues that influence Islamic and developing countries. Biotechnology is no exception. This is done in order to provide recommendations to the decision-makers, developers and overseers of such issues in our countries, and to offer scientific perspectives to the ongoing debate on the potential role of biotechnology and genetic engineering in 'human' development.

Since its launch in 1986, Islamic Academy of Sciences (IAS) has followed advances in science and technology and has focused especially on developmental issues. This focus is evident in the choice of themes for each of the ten conferences it has organised, the proceedings books of which have been distributed worldwide.

IAS activities have attracted the attention of several national institutions and international organisations leading to their sponsorship. The World Bank, UNESCO, Islamic Development Bank, COMSTECH, the OPEC Fund for International Development, and ISESCO are some such collaborators.

This publication, which carries the same title as the conference, has been divided up into six parts with respect to the conceptual aim of the presented material.

Part One consists of the statements and messages presented at the opening ceremony of the conference. **Part Two** which is entitled, "Biotechnology: Where do we stand?" is made up of three general papers that look at the international biotechnology and genetic engineering scene. Some selected aspects of medical biotechnology research are presented in **Part Three**. **Part Four** includes papers that address some facets of the relationship between biotechnology and food security.

Part Five looks at the biotechnology research scene in a number of OIC-countries; Tunisia; Iraq; Sudan; and also includes a paper from Bangladesh that looks at a real-life problem in that country; namely "Arsenic contamination."

Some broad educational aspects related to biotechnology are looked at in **Part Six**.

Part Seven is the "food for thought" part with presentations that look at the Islamic viewpoint of some contemporary biotechnological questions.

Part Eight of the book is the appendix that includes the list of participants in the conference, the conference scientific and organising committees, the names of IAS Fellows and Council members as well as various other details about the Islamic Academy of Sciences.

Overall, this book will help the reader better understand how the current Biotechnology scene will evolve. He/she will be able to get a picture of the potential of BT to help resolve the major problems that developing countries face. Moreover, one will be exposed to a number of varied and interesting facets of BT applications in areas that affect our lives.

Abdel Salam Majali
Mehmet Ergin
Moneef R. Zou'bi

Acknowledgements

The Islamic Academy of Sciences is grateful to His Majesty King Mohammed VI, for his patronage of the Eleventh IAS Conference. The continuous support of the two Academy patrons; His Excellency the President of the Islamic Republic of Pakistan, and His Royal Highness Prince Al-Hassan Ibn Talal of Jordan is gratefully acknowledged.

The IAS extends its appreciation to all the organisations that sponsored the convening of the conference foremost among which are the Academy of the Kingdom of Morocco and its Secretary General Prof. Abdelatif Berbeich, the Islamic Development Bank (IDB), COMSTECH, UNESCO, ISESCO as well as the OPEC Fund for International Development.

We also wish to thank all the Moroccan organisations that sponsored and supported the convening of the IAS conference in Morocco.

We are very grateful to all the eminent speakers who participated in the conference and to all the specialists and the various participants who made the effort to take part in the conference.

The preliminary work done by the IAS Council and the efforts volunteered by Dr Abdelhafid Lahlaidi, Dr Hamza Kettani, Dr M Ergin Mr Moneef R Zou'bi during the meetings of the scientific committee and indeed during the conference itself are gratefully acknowledged.

The IAS Council headed by Dr A S Majali has done a lot too to help realise this activity.

The dedicated staff at IAS Secretariat including Ms Taghreed Saqr, Ms Lina Jalal, Ms Hind Bilbeissi; as well as members of staff at the RSS printing unit; Mr George Anz and Ms Amal Mizher have all worked hard to produce this book. To them all we are grateful.

Abdel Salam Majali
Mehmet Ergin
Moneef R. Zou'bi

Organisations sponsoring the Eleventh IAS Conference - 2001

(1) Islamic Academy of Sciences (IAS), Amman, Jordan;
(2) Academy of the Kingdom of Morocco, Rabat, Morroco;
(3) Islamic Development Bank (IDB), Jeddah, Saudi Arabia;
(4) OIC Ministerial Committee on Scientific and Technological Co-operation (COMSTECH), Islamabad, Pakistan;
(5) OPEC Fund for International Development, Vienna, Austria;
(6) Islamic Educational, Scientific and Cultural Organisation (ISESCO), Rabat, Morocco;
(7) United Nations Educational Scientific and Cultural Organisation (UNESCO), Paris, France;
(8) Faculty of Medicine and Pharmacy, University of Mohammed V, Rabat, Morocco; and
(9) International Institute of Higher Education (IIHEM), Rabat, Morocco.

6

IAS Rabat Declaration
on
Biotechnology and Genetic Engineering for Development in the Islamic World

Adopted in Rabat (Morocco) on
8 Sha'aban 1422
24 October 2001

PREAMBLE

WHEREAS Allah (God) Subhanahu-Wa-Ta'ala has created Man in the best of forms, provided him with the abilities and resources to improve his well-being, endowed him with reason, dignified and honoured him, and granted mankind the inheritance of life and resources of nature;

WHEREAS Allah has made the pursuit of knowledge an absolute obligation and its acquisition a source of pride and dignity, and has urged human beings to seek, utilize and disseminate it for the benefit of humanity;

WHEREAS the teachings of Islam emphasize the importance of the well-being of man, and underline the fact that Man's relationship to the universe and to his fellow-man must be one of stewardship and complementarity respectively, and never one of mastery;

WHEREAS the Holy Qur'an is replete with numerous references to biological sciences;

WHEREAS Muslim contribution to the establishment and advancement of biological sciences throughout the period of Islamic renaissance has been significant;

WHEREAS Islam promotes a needed ecological balance between all living beings and their life-sustaining environment;

WHEREAS the Islamic Value-System provides a unique Code of Ethics, which should govern our outlook on contemporary Genetic Engineering issues, such as Cloning and Organ Transplants;

WHEREAS Biotechnology and Genetic Engineering have now become areas at the forefront of basic and applied research and are continuously reaching new levels in advancement and complexity;

AND WHEREAS the majority of OIC and developing countries have not mapped out a stand on the science or ethical features of Biotechnology and Genetic Engineering and are lagging behind in these areas;

NOW, THEREFORE, the Islamic Academy of Sciences:

(i) **REALIZING** that the applications of biotechnology could have far-reaching effect and favourable impact in developing countries, many of which suffer from large and rapidly increasing populations, chronic food-shortages and malnutrition, poor health, and profound environmental problems;

(ii) **ACKNOWLEDGING** that Biotechnology and Genetic Engineering are areas where rapid and meaningful advancement can readily be made by OIC countries, especially in attaining food security, promoting the pharmaceutical industry;

(iii) **APPRECIATING** the activities being carried out by many governments, academic institutions, and non-governmental organizations in the fields of Biotechnology and Genetic Engineering, especially in Agriculture;

(iv) **ACKNOWLEDGING** that advancement in Biotechnology and Genetic Engineering underlines the importance of investment in basic sciences, which are the backbone of sustainable S&T advancement, especially as there is very little biotechnological R & D in the developing countries;

(v) **ACKNOWLEDGING** the significance of the sequencing of the human genome, an event compared to man's landing on the moon and described as an historic milestone in the history of science that will enhance research in human biology focused on diseases such as Cancer, Alzheimer, Diabetes and Cardiovascular disorders; and

(vi) **TAKING INTO CONSIDERATION** the Universal Declaration on the Genome and Human Rights, adopted by the General Conference of UNESCO in 1997, which is the first worldwide instrument in the field of biology, medicine, and genetics;

MOREOVER

(a) **OBSERVING WITH CONCERN** the lack of a long-term Biotechnology policy at the national level in most OIC countries;

(b) **NOTING WITH CONCERN** the limited number of enrolled students and quality-graduates in Biotechnology-related disciplines, as well as the poor level of instruction at most institutions;

(c) **BEING CONCERNED** at the lack of adequate infrastructure for BT - research available in most OIC countries, to sustain this growing sector;

(d) **NOTING WITH CONCERN** the absence of co-ordination between the various agencies involved in Biotechnological research and application;

(e) **NOTING** the lack of up-to-date curricula in Biotechnology for all stages of the educational process, as well as the shortage of suitably qualified and motivated teaching staff;

(f) **NOTING WITH CONCERN** that the major breakthroughs in molecular biology and genetic engineering have raised many serious legal, ethical and social questions;

(g) **COGNIZANT** of the fact that genetic engineering has been defined as an unnatural insertion of a foreign sequence of genetic codes in the midst of the orderly sequence of genetic codes developed through millions of years, which is a profound intervention, with unpredictable consequences;

THE ISLAMIC ACADEMY OF SCIENCES MEETING, AT RABAT (MOROCCO) DURING OCTOBER 2001, CALLS UPON the international community to:

(i) **EXTEND**, in the spirit of co-operation, all possible help to developing countries in the area of technology-transfer, as well as debt relief to enable them to divert more resources to research in critical areas of Biotechnology;
(ii) **CONTINUE** to support research projects of importance in the developing countries;
(iii) **SET-UP** an appropriate mechanism for the protection of national genetic resources;

AND CALLS UPON the leaders and decision-makers of Islamic countries to:

(a) **RE-DEFINE** national developmental objectives in the area of science and technology, especially Biotechnology and Information Technology, in view of globalization and free-trade arrangements that are being adopted by countries;
(b) **DEFINE** their BT strategies and, where possible, incorporate them into national S&T policies;
(c) **INTRODUCE** BT awareness programmes at various stages of the educational process;
(d) **INTRODUCE** appropriate legislation, including tax and customs-exemptions, to promote the various aspects of the pharmaceutical industry;
(e) **ESTABLISH** linkages and partnerships between OIC member countries, in Biotechnology and Genetic Engineering, to facilitate inter-country co-operation by governments, industry and academia;
(f) **ALLAY** fears that stringent patents and intellectual property rights, legislation and countries' accession to the World Trade Organisation (WTO) could cripple drug manufactures in OIC countries;

AND WOULD MOREOVER

(i) **ASSERT** that exploiting a technology, which may give rise to unexpected substances that could be damaging to health, before this risk has been carefully investigated, should be avoided;
(ii) **ASSERT** that exploiting a technology that may have irreparable environmental effects, before it has been proven that the products do not cause significant harm to the environment, should be avoided;

(iii) **ASSERT** that it is not appropriate to expose people and the environment to even the smallest hazard, considering that present genetically engineered products are of little value;

(iv) **ASSERT** that it is not right to justify the exploitation of a potentially hazardous technology today because of a scientifically unfounded principle that it might generate useful products in the future;

(v) **ASSERT** that, if genetically engineered organisms are to gain greater acceptance, decisions that address concerns associated with the application of biotechnology to agriculture must be science-based;

AND FURTHER CALLS UPON the relevant OIC, and other, organisations to:

(a) **ENCOURAGE** and support OIC-based BT and pharmaceutical industrial ventures;

(b) **DEVELOP** databases of BT and GE human resources in OIC countries, to facilitate appraisal of national strengths and weaknesses;

(c) **SUPPORT** inter-disciplinary research and development in various fields related to BT and ensure the requisite development of human resources;

(d) **PROVIDE** funds as well as governmental support for diagnostic as well as curative medical applications of BT, Gene Therapy, etc.;

(e) **CONTINUE** to address developments in basic sciences and not to marginalize this backbone of S&T development;

(f) **ENCOURAGE** and promote the publication of quality research-material of OIC scientists especially in the area of Biotechnology in internationally renowned journals and on the Internet;

AND FURTHER PROPOSES

(i) **THE CREATION OF AN ISLAMIC BT FUND** to help poorer OIC countries to transfer Biotechnology know-how from other countries and develop and utilise it to achieve national food-security;

(ii) **THE ENFORCEMENT OF A MORATORIUM** on the release of genetically engineered organisms and on the use of Genetically Engineered (GE) foods, until sufficient knowledge has been acquired to make it possible to judge how far it is safe for human health and the environment to exploit this technology; and

(iii) **THE DEVELOPMENT OF A MULTI-DISCIPLINARY GROUP** of scientists, technologists and Islamic scholars to study the various facets of social and ethical issues emanating from the recommendations of this conference.

Eleventh Conference of the Islamic Academy of Sciences *Biotechnology and Genetic Engineering for Development in the Islamic World*

22-26 October 2001
Rabat, Morocco

Conference Report

Under the patronage of His Majesty King Mohammed VI, the king of Morocco, the Islamic Academy of Sciences convened its eleventh international conference under the title *Biotechnology and Genetic Engineering for Development in the Islamic World*, during 22-26 October 2001.

The conference, which was held at the Academy of Morocco Building, was an open activity in which over 120 participants representing over 25 countries participated. It was organised and sponsored by the following organisations:

(1) Islamic Academy of Sciences (IAS), Amman, Jordan;
(2) Academy of the Kingdom of Morocco;
(3) OIC Ministerial Committee on Scientific and Technological Co-operation (COMSTECH), Islamabad, Pakistan;
(4) Islamic Development Bank, Jeddah, Saudi Arabia;
(5) OPEC Fund for International Development, Vienna, Austria;
(6) Islamic Educational, Scientific and Cultural Organisation (ISESCO), Rabat, Morocco;
(7) United Nations Educational Scientific and Cultural Organisation (UNESCO);
(8) Faculty of Medicine and Pharmacy, University of Mohammed V, Rabat, Morocco; and
(9) International Institute of Higher Education in Morocco (IIHEM), Rabat, Morocco.

The main objectives of the conference were:

(a) To appraise some contemporary concepts in Biotechnology with the aim of disseminating them in OIC and developing countries;
(b) To define some R&D areas of importance in BT, and develop innovative proposals for future activities in BT;

(c) To assess the impact of recent advances in GE on medical research dedicated to combating disease;
(d) To assess the status of GE research in the Islamic World, with particular reference to countries with extensive experience in this field;
(e) To formulate an Islamic scientific stand where possible on BT and GE off-shoot issues;
(f) To define a role for governments in BT and GE research in terms of priorities, regulation, funding, private-public sector collaboration;
(g) To establish paradigms that define the impact of developments in BT on the environment, agriculture; and
(h) To facilitate the free exchange of views among experts on BT and GE;

In addition to an international keynote that overviewed the state of Biotechnology in developing countries, 2 other keynotes were presented. The first overviewed the changing role of science and technology as a key for development in the Islamic world. The second keynote addressed ethical issues related to Biotechnology.

In order to generate academic interaction among the participants, the conference was mostly divided up into roundtable discussions (four in all), in which short communications were followed by discussions on a number of important issues. The roundtables addressed the themes of Biotechnology and Food Security; Biotechnology and Education; National Biotechnology Scenarios and Ethics, Social Values and Biotechnology. The latter was a specialised session jointly organised by the IAS, COMSTECH and the Islamic Development Bank.

In the "Scenarios" session, speakers from Tunisia, Iraq, Morocco, Sudan and Malaysia were invited to present highlights of their national experiences in the BT sector. Emphasis was especially placed on the future courses of action that these countries will take in the field of BT and its applications.

At the conclusion of the conference, the Islamic Academy of Sciences adopted the IAS Rabat Declaration on Biotechnology and Genetic Engineering for Development in the Islamic World.

The declaration noted that the majority of OIC and developing countries have not mapped out a stand on the scientific or ethical features of Biotechnology and Genetic Engineering nor fully realized the favourable impact of Biotechnology and Genetic Engineering as means of achieving national food and pharmaceutical security.

It emphasized that BT can represent an area where rapid and meaningful advancement can readily be made by developing countries.

The declaration acknowledged the significance of sequencing the human genome and described it as a historical milestone which will lead to advanced research in combating diseases such as Alzheimer, Diabetes and Cardiovascular disorders.

The declaration emphasized the importance of addressing the link between ethics, social values and Biotechnology through specialized multi-disciplinary groups of scientists, technologists and Islamic scholars.

As part of the follow-up action to the conference, the Academy will circulate the IAS Rabat Declaration to concerned individuals and relevant agencies throughout the world, so that measures can be taken to implement the recommendations decided upon at the conference.

The Academy will also publish the complete proceedings of the conference in a quality volume that will be distributed internationally. Such a book, like all other published IAS proceedings, will become a valuable reference for experts that draw up and implement Biotechnology policies and/or are involved in Genetic Engineering applications.

Through IAS Fellows, personal contact and correspondence, the IAS will promote the ideas that were developed at the conference among the decision making circles of the Islamic world, and will provide whatever help it can to get the various recommendations implemented.

14

PART ONE
STATEMENTS AT THE INAUGURAL SESSION

16

Message* of
His Excellency General Pervez Musharraf
President of the Islamic Republic of Pakistan, and
Patron of the Islamic Academy of Sciences

As one of the Patrons of the Islamic Academy of Sciences, I am pleased to greet you on the Eleventh Annual Conference of the Islamic Academy of Sciences. I thank His Majesty King Mohammed VI and his government for hosting this important event in Rabat and generously supporting the activities of the Islamic Academy of Sciences.

The theme chosen for the Academy's Eleventh Conference "Biotechnology and Genetic Engineering," represents one of the most important areas of science in the new millennium.

About half a century ago, when Watson and Crick discovered the structure of DNA, no one imagined how rapidly genetic technology would develop. Today, biotechnology and genetic engineering present challenges unprecedented in history. These new technologies enable researchers to redesign living organisms by moving genes across species. The results essentially are genetically modified organisms, capable of perpetuating in nature.

These revolutionary opportunities and breakthroughs have the potential to produce new microorganisms, plants and animals and thus have far reaching economic benefits in agriculture, environment, industry, and human health. Biotechnology now has the potential to cure diseases, prolong life and alleviate human suffering. However, these opportunities have given rise to a number of ethical, legal and social questions that need to be addressed and critically examined. For the Muslim World the real challenge is the need to undertake an in-depth analysis of the risks and benefits and to develop regulatory procedures and guidelines that will make these practices environmentally safe. It is indeed important to appreciate that biotechnology is already a multibillion-dollar industry and in agriculture alone, more than 75 million acres of genetically modified crops are now being sown worldwide. It is estimated that 30,000 products in US grocery stores are also genetically modified in some fashion. Its impact on society is therefore here to stay, and it is expected to be so enormous that further delay in realizing its potentials and comprehending its pitfalls would be unwise and detrimental.

---

* Delivered by Dr M A Mahesar, Executive Secretary, COMSTECH.

At the dawn of the Third Millennium, another serious question that confronts the *Ummah* is the tendency to view biotechnological developments as an issue threatening our ethical and religious notions. It is now up to our religious scholars and researchers to examine the ethical issues in the light of the teachings of Islam. Such efforts obviously need to be carried out in close co-operation with scientists. I am indeed pleased to learn that this august assembly is going to debate these important aspects and suggest ways that can be used as future strategies.

The role of Islamic Academy of Sciences in shaping future policies in scientific development of *Ummah* is indeed vital. As a think-tank and a brain trust of *Ummah*, the Islamic people look towards you for initiating debates on fundamental scientific issues confronting our age. I am confident that with the rich knowledge base that is available in the Islamic Academy of Sciences, it would be possible for you to critically analyse all aspects of biotechnology and propose an effective set of guidelines at the end of the conference.

Keeping in view the nature and significance of Biotechnology and Genetic Engineering. COMSTECH has always attached special importance to this essential field of scientific development. And, in spite of serious resource difficulties, COMSTECH has managed to find means to support several programmes in biotechnology capacity building and organized several international symposia. As Chairman of COMSTECH and a Patron of the Islamic Academy of Sciences, it is obviously a matter of great personal pleasure for me to see that COMSTECH and IAS are working closely together, and pursuing a common goal of strengthening the science and technology infrastructure in the OIC member states. I am certain that these two organizations will continue to work together in this important area.

I sincerely hope that the Eleventh Conference of Islamic Academy of Sciences in Rabat will mark a new beginning and a new era in the scientific and technological development of the OIC region.

May *Allah* give us the courage to boldly cope with all the challenges in accordance with the teachings of Islam and bless us with a true and genuine commitment to promote the cause of education, scientific research, technological advancement, and welfare of all the citizens of the OIC region.

Message* of His Royal Highness Prince El Hassan Ibn Talal of Jordan, Founding Patron of the Islamic Academy of Sciences

بسم الله الرحمن الرحيم

والصلاة و السلام على نبيه الأمين و اله وصحبه أجمعين

Your Excellency, Dr Najib Zerwali, Minister of Higher Education and Scientific Research, Kingdom of Morocco
Dear Colleagues, Fellows of the Academy
Ladies and Gentlemen

السلام عليكم ورحمة الله وبركاته

It is a distinct pleasure for me to be able to take this opportunity to pay tribute to His Majesty King Mohammed VI for his generous patronage of this, the eleventh IAS Conference in Rabat. I would like to express my gratitude to the Academy of the Kingdom of Morocco, whose gracious patron is His Majesty the king himself, for hosting the conference. Let me, at the same time, extend my greetings to all participants of this distinguished scientific forum.

The Islamic Academy of Sciences is fifteen years old this very month. This might still be a little short of full maturity; but it is definitely the blissful beginning of puberty. It is the age when attempts are tirelessly made to reach out to new realms of imagination, creativity and constructive change. A great achievement is a labour of love triggered by a great imagination and pursued with much toil, persistence and devotion. How else can we understand the staggering achievements of – to mention one prominent example – our own civilisation in its heyday?

Over the past decade many conferences – local, pan-Arab, pan-Muslim, and Third-World – have been held on the topic of biotechnology and genetic engineering. Although these have been very useful in stimulating discussions and in familiarising workers with each other, very few practical results have emerged.

---

* Delivered by Prof. Adnan Badran FIAS, President of Philadelphia University, Jordan.

Surely, the deficit will be addressed during the sessions of this conference. As its mission statement avers, the purpose of the Islamic Academy of Science is "to provide an institutional set up for the utilisation of Science and Technology for the development of Islamic countries and humanity at large." I would like to underline the word *utilisation*. Since its inception in 1986, the IAS programme has addressed many contemporary issues facing the Islamic world, with a view of not only benefiting the *Ummah*, but also of benefiting all mankind by means of a well-informed, co-operative, pragmatic and humanitarian approach in scientific and technological development.

Many of us have already benefited over the years from the Academy's publications of previous conference proceedings. The IAS has so far discussed – among other topics – information technology; education; water in the Islamic world; health and nutrition; the environment; manpower; technology transfer; and S & T policy. In each case, the Academy has been concerned above all with the question of *development* – that is, with the practical measures that can be taken by individuals and organisations within the scientific community to maintain values alongside innovative changes, and to establish a culture of scientific education and creativity. In this respect, it was a great pleasure for me to attend the IAS launch of the Culture and Science Initiative earlier this year in March.

Biotechnology and genetic engineering carry great potential for economic and social development in the Islamic world. Of all emerging technologies, biotechnology has three obvious advantages:

- Low cost compared with IT, micro-technologies, and so forth;
- Availability of trainable manpower;
- Adaptability to division of labour even in a narrow field – not just between workers in different laboratories, but between workers in different countries – resulting in a more economically viable system.

We are likely to identify a commercially viable field for which no more than a handful of individuals (researchers and technicians) would need to be trained in fewer than a dozen specialised techniques.

Biotechnology with genetic engineering is a diverse field. Not all of it needs to be introduced at one go. Nor is it required that the techniques used in the manufacture of one product be all practised in one place, so long as the final goal is clearly decided. Not the least advantage of the field is the co–operative approach between institutions and nations, which will be required for effective sharing of information and resources. For example, producing marketable polypeptides requires the following steps:

- Isolation of the gene;
- Introduction of the gene into a suitable organism;
- Multiplication of the organism on a large scale;
- Isolation of the protein;

- Quality control and testing of the protein product; and
- Packaging and marketing of the product.

Each of these six steps could take place in a different institution.

Information-sharing and resource-sharing will be dominant factors. Recently, it seems, scientists worldwide have begun to adopt a synthesising approach. For life-scientists especially, a brave new world is dawning – the world of structures and dynamics that lie between the cell and the atom. In this broadly-defined field, many disciplines have come to overlap and share information and techniques. Single polypeptides command the attention of – for example – protein crystallographers studying folding structure, geneticists investigating the finer function of the human genome, pharmaceutical research and development teams developing 'intelligent medicines,' cytologists getting to grips with the complex and delicate ecosystem of the cell, medical researchers seeking the origins of degenerative disease, and plant scientists hoping to increase crop yields.

We are constantly astounded by the richness of Creation and by the fact that in biotechnology and genetic engineering we are now granted the privilege of taking up the conductor's baton to direct – if not the *symphony* of life – then at least, perhaps, a brief violin solo or two.

Here, I would like to quote from the preamble of the IAS Tunis Declaration on Information Technology for Development in the Islamic World. As we all know, this document originated in last year's dynamic conference on Information Technology. Quote:

"Allah has made the pursuit of knowledge an absolute obligation and its acquisition a source of pride and dignity, and has urged human beings to seek, utilise and disseminate it for the benefit of humanity;

The teachings of Islam emphasise the importance of the well-being of man, and underline the fact that Man's relationship to the universe and to his fellow-man must be one of stewardship and complementarity, respectively, and never one of mastery." (Unquote.)

As scientists it is our duty to pursue new knowledge and new technology. But as we are living organisms ourselves – and, I should add, non-governmental organisms at that – I would like to suggest that it is also our duty to think about how we may pursue *wisdom* in scientific endeavour. I have mentioned the issue of co-operation and sharing across institutional and national boundaries. We must also consider the issue of appropriateness – appropriateness of technology, appropriateness of application. For new techniques in themselves are no substitutes for the pragmatic and humanitarian application of information in a clearly-defined ethical context.

Bioethics must form a cornerstone of any involvement in biotechnology and genetic engineering. Let me say here too that in today's high-speed, information-rich age, to be credible in the world market one must take into account not only local imperatives but global factors. Multinational and transnational corporations are at the forefront of the biotech revolution. We seek

investment and profit so that we may further research and development schemes which are highly necessary in our parts of the world. Yet economic imperatives, subject as they are to political whim, cannot on their own establish the consistent good practice that must prevail.

Scientific and technological developments have given human beings powers that threaten to far outstrip their collective good judgement. We need to identify those universal values to which we should all adhere and which will guide the biotech revolution into biotech evolution, and not devolution. It is time to consider the guidelines that will allow technology to progress according to a universally-recognised code of conduct which has the welfare of the human individual at its centre. Consequently, and bearing in mind the importance of pragmatism in the context of this pan-Islamic conference, I would suggest that *policies* rather than *politics* should dominate our discussions of any global ethic regarding investment and research in our field.

Perhaps we may take advantage of the opportunity that this very important and inclusive conference provides for us to consider the establishment of a *virtual* institution or 'invisible college' of biotechnology. The IAS Secretariat could possibly arrange for some peripheral meetings to take place that might select one or more narrow areas of biotechnology and identify the laboratories and companies, which could establish such an invisible college. Such an organisation would require no new labs, personnel or buildings and relatively little effort and money. It could well become a model for Islamic co-operation in addition to (potentially) generating income.

Finally, I would like to say that I regret not being with you in person today to listen to you. But I do look forward to studying carefully your contributions, with the view of not only promoting new developments and collaborations in the field of biotechnology and genetic engineering, but also of pursuing a course that will lead to greater happiness and security – that is to say, the 'soft security' of human dignity – for the generations that are to come.

Thank you, Ladies and Gentlemen, and God bless you.

أحيّيكم، وأسلم عليكم.

Address of
His Excellency Dr Abdel Salam Majali FIAS
President of the
Islamic Academy of Sciences

Your Excellency Prof. Najib Zerwali, Minister of Higher Education and Scientific Research, Representative of the Kingdom of Morocco
Excellencies
Eminent Fellows of the Islamic Academy of Sciences
Ladies and Gentlemen

السلام عليكم ورحمة الله وبركاته

May I at the outset request that we recite Al-Fatiha for our late friend, Prof. Ali Kettani, IAS Vice-President, and Founding Fellow of the Academy.

Upon the gracious invitation of His Majesty King Mohammed VI, we convene again this year in a spirit of brotherly interaction to define our scientific outlook concerning important scientific themes that affect our lives, namely Biotechnology and Genetic Engineering. We are honoured to meet in this House of Knowledge, the Academy of Morocco, in this historical city, with the same open-minded spirit that the towering figures of our Islamic history had, to address issues important to our present and to our future.

The number and calibre of the persons gathered here reflect the admiration that the Islamic scientific community has for the Kingdom of Morocco, and its enlightened leader who represents an honourable ruling dynasty famous for its patronage and support of science and scientists.

The twentieth century has been called the century of matter. The twenty-first century will in all probability be that of life. Our knowledge of living beings and of the world around us has, in the space of a few decades, been shaken to its foundations by discoveries with far-reaching ramifications. For the first time, thanks to findings in genetics, neurobiology and embryology, humans have gained access to some knowledge of their own vital mechanisms.

Humans have also gained the power to transform the development processes of every species of living being, including their own. The impact of this new-found power is immense and we have not yet gone into its depths.

Advances in medicine and biology and the cognitive and practical breakthroughs made possible by their association with other disciplines – informatics, to name but one – now offer a hope of a better life. The

development of gene therapies will enable us to treat or prevent hitherto incurable genetic diseases. Pharmacological progress and greater knowledge of immunity mechanisms have led to a series of qualitative leaps in the area of grafts and transplants. The development of the neurosciences, robotics and medical imaging has opened up a new era of surgery. Progress is being made all the time in prenatal medicine, embryology and medically assisted procreation.

We can only wonder at the advances made since 1953, when the double helical structure of DNA was discovered in all its beauty. All this leads us to believe that humanity is on the way to ending the diseases and other scourges that have plagued it for thousands of years.

The progress of the biomedical sciences is raising unprecedented expectations. It is also a source of great complexity whenever the question of its moral legitimacy is raised.

Excellencies
Ladies and Gentlemen

Islam contains plentiful constructive implications and lofty goals all of which emanate from the general denotation of the first Qur'anic word to be sent down as a heavenly revelation, "Read: in the name of thy Lord who Created, Created man from a clot. Read: And thy Lord is the Most Bounteous, Who taught by (the use of) the Pen, Taught man that which he knew not."

Science, knowledge and civilisation are the best aspirations a Muslim can have in search for which he beseeches help through the kind care of God. God whose help is sought for the achievement of the knowledge and learning pursued through reading. Learning and teaching are natural and even necessary for any human civilisation and progress. Thus Islam has accorded a high status to learning by making the latter a sort of worship and an article of faith.

Learning is a blessing bestowed by God on man through which he can discover the Creator's originality by means of the miraculous creation and wonderful dexterity. In this, there is an intimation to this living being, his nature, structure, distinctive qualities, qualifications, gifts and capacities. God has stressed these blessings through the talent of teachableness and learning. The Almighty has also emphasised them through the secrets of man's creation. He also singled man out by honouring him through His imperative injunction to man to read, alluding by that to benefits accruing from the pen without which no religion can be established and no life can smoothly go on.

The Qur'an is replete with references to biological sciences. Some of them are related to the creation of man, to the species of animals or to the realm of plants. All of these give hints to scientific facts inherent in man's creation, which lead to explanations that reveal indications noted by science, and confirmed by experimentation and tests.

Muslim contributions in a number of fields in biology are also significant, and indeed the contributions of early Muslim celebrities in this field are

distinctive including those of Al-Razi, Al-Zahrawi, Ibn Sina, Ibn Al-Haytham, Ibn Zuhr and others.

They translated the basic original works of this science from Greek and other languages and added to that their experimentations and their own original contributions and pioneering discoveries that made them paragons and outstanding examples to be followed. Most of the works of these Muslim worthies were translated into Latin and other tongues.

If we go back to the theoretical side in medical science we notice that during the early Abbasid period, Muslims directed their attention to books of pharmacology and botany, which they translated into Arabic.

Excellencies
Ladies and Gentlemen

In the aftermath of our countries gaining their independence and facing up to the realities of the modern world, the IAS, as well as a number of other OIC off-shoot organisations, were conceived during the late sixties onwards as a result of fundamental heart-searching on the part of some enlightened leaders. The realities of ignorance, disease, hunger and indeed of facing political crises emanating from our geo-strategic location, and what God has endowed us in terms of wealth in natural resources – became very evident.

This interest in science as a means for us as an *Ummah* to achieve progress has led to a number of interesting questions. For example, "Is everything that is technically feasible ethically acceptable?" As the potential of biology, medicine and genetics diversifies and new ways are found of putting this potential into practice, we are becoming increasingly aware of the inadequacy of our moral and philosophical reference points and the gaps in our institutional and legal frameworks. At the dawn of the twenty first century, the debate on cloning and, more recently, on the eventuality of research on embryonic stem cells showed that many societies feel the need to reflect on the ethical implications and to adopt a position. I wonder here whether we as the *Ummah* of *Aqeedah* have fully realised our potential?

Given its intellectual and ethical capability, the science community in the Islamic world has both the ability and the will to promote this dialogue, which is more necessary than ever, and to see to its coordination. We can and must work out guidelines that enshrine universally shared ethical principles.

Moreover, we can prepare an international charter on the human genome through adopting the pragmatic approach that is based on the latest advances in genetics and its applications. In this mission, we should examine the ethical and legal implications of genetic screening and testing. We should address the issues of the confidentiality of genetic data, population genetics, the development of vegetable and animal biotechnologies. We should opt for the greatest possible transparency, and widely disseminate the findings of our work.

The Universal Declaration on the Genome and Human Rights, adopted by the General Conference of UNESCO in 1997, is the first worldwide instrument

in the field of biology, medicine and genetics. Its chief virtue is that it strikes a balance between guaranteeing the observance of rights and fundamental freedoms and ensuring the freedom of research. By endorsing it in 1998, the UN General Assembly recognised the importance of setting out ethical principles for the application of science to human beings.

The statement in Article 1 of the Universal Declaration on the Human Genome and Human Rights that "in a symbolic sense [the human genome] is the heritage of humanity" set the touchstone for all subsequent applications of genomics – and the validity of this touchstone has not been diminished by our growing mastery of the vocabulary and subsequently the syntax of the genetic language.

This said, the rapid development of genetic engineering, especially since the 1970s, has led to a much wider range of patents. The extension of patentability to new sectors immediately provoked misgivings and tensions, to which the reactions varied widely, from country to country and from culture to culture, depending on the protagonists involved and the interests at stake.

Whatever protection is to be given to a discovery – and protection must, of course, be given – another sacrosanct principle must apply to the use made of the meteoric progress in the sequencing of the human genome: free access to the raw data in question, a principle that is crucial to the dissemination of knowledge and knowledge-sharing.

On 14 March 2000, when human genome sequencing was nearing completion, the then President of the United States of America, Mr Bill Clinton, and the British Prime Minister, Mr Tony Blair, issued a joint statement calling for free access to human genome data. Calls followed from a number of circles stressing that it was both important and urgent that the international community as a whole should ensure free access to raw fundamental data on the human genome on the basis of the ethical principles set forth in the Universal Declaration on the Human Genome and Human Rights.

Moreover, the United Nations Millennium Declaration adopted on 8 September 2000 reasserted the need to ensure free access to information on the human genome sequence. I earnestly hope that this would eventually lead to solving some of the problems posed by the protection of gene-linked intellectual property.

Progress made in genome sequencing (and not only that of the human genome) opens up mind-boggling prospects in farming, medicine and industry. There is however a belief that the technology is not yet quite ready.

Public opinion, often ill-informed about such complex issues, is equally changeable. In short, the seeds of confusion are there and passions will be inflamed by the clash of convictions and interests.

I firmly believe that all the conditions are there for a radical review, stocktaking, a dispassionate attempt to compare the competing opinions, policies and practices, especially amongst our OIC-member countries.

We have to present our clear scientific stand on these issues to world leaders. A stand that is based on our beliefs and interests. Only through this would we ensure that no other scenario is forced upon us in the future.

Excellencies
Ladies and Gentlemen

The world is living through unprecedented turmoil. The events of the last few weeks have produced a flood of reactions that are directed against Islam, most of which are unjust, unrealistic and unjustified. Our mission now as the science leaders of the *Ummah* is to firstly address the world and project our pride in our Islamic civilisation and what it has achieved and how it contributed to Western civilisations, for we belong to a civilisation that believes in dialogue. We are the sons of a civilisation the core of which is a divine message that promulgates respect for human life and human rights and most of all justice.

Our mission now is not to close our doors but open up to the world and present our case and point out to despair generating conflicts in our world. We too need to activate our bilateral interaction, and jump-start our transnational thinking, in our quest to achieve food and water security, science security and ultimately national security.

We, in this world, cannot obtain good unless we have a wakeful conscience and unless we follow in the crystal clear paths of right that are free from fluctuation and confusion. We, the science leaders of the *Ummah*, have to promulgate a science policy for the *Ummah* and request our leaders to put it into action before it is too late.

I have every confidence that our meeting here will give our stand on this subject a considerable boost. I thank all of you wholeheartedly for agreeing to take part in this meeting, whether in the chair or from the floor, in a spirit of curiosity and open-mindedness and with the firm resolve to advance our exchanges.

I would like to thank His Majesty for cultivating science and scientists in this oasis of knowledge. He is afterall the descendent of an honourable lineage that founded Islam in these blessed lands.

Thank you.

28

Address of His Excellency Dr Abdulaziz Othman Altwaijri, Director General of the Islamic Educational, Scientific and Cultural Organization (ISESCO)

In the name of Allah, Most Gracious, Most Merciful

Praise be to God, Prayer and Peace on Mohammed the Messenger of God, and on his kith and kin

Your Excellency, Dr Najib Zerouali, Minister of Higher Education and Scientific Research, Government of the Kingdom of Morocco
Your Excellency, Dr Abdel Salam Majali, President of the Islamic Academy of Sciences
Your Excellency, Dr Moneef R. Zou'bi, Director General of the Islamic Academy of Sciences
Distinguished Guests
Ladies and Gentlemen

Assalamu Alaikum Warahmatu Allah Wabarakatuh

It is indeed a matter of immense pleasure to be here at the opening ceremony of the Islamic Academy of Sciences' Eleventh General Conference on "Biotechnology and Genetic Engineering for Development in the Islamic World," which is being held under the patronage of His Majesty King Mohammed VI, may God preserve him. I am grateful to the Islamic Academy of Sciences (IAS) for taking the initiative to hold this conference on a topic which is of prime importance in the development of the Islamic countries.

Excellencies, Ladies and Gentlemen

This conference will help us to asses the present status of biotechnology in the Islamic world, facilitate the free exchange of views among the renowned experts on biotechnology and genetic engineering and will help to define important research and development areas in biotechnology. Applications of Biotechnology have given boost in fields like healthcare, agriculture, energy production, population control and environmental protection. It has enabled development of useful products and processes, which have contributed directly

towards socio-economic development. In Agriculture, genetically modified crops have been developed that have resistance to pests, viruses and drought. In the marine field, Biotechnology has provided a boost to fish and seaweed farming. In Environmental protection, we are able to produce pollution fighting enzymes and microbes. Biotechnologies have also helped production of energy from biomass.

It is extremely important for developing countries to assign this field a very high priority and implement realistic, ambitious and calculated programmes for the benefit of their economies and well-being of their populations. The economy of most of the Muslim countries is dependent on Agriculture and Agro-forestry. Biotechnology presents solutions to overcome their major problems like poor crop yields, while reducing crop losses due to pests and drought.

Muslim countries are called upon to focus on this important area to utilize and propagate biotechnologies for at least meeting growing food requirements of our fast growing population. I am pleased to know that the present conference is also focusing to assess the impact of recent research in genetic engineering on medical research, which will help guide us in drafting suitable planning.

Excellencies, Ladies and Gentlemen

The Islamic Educational, Scientific and Cultural Organization is fully aware of the importance of science and technology in the socio-economic development of the Islamic Countries. It has already announced a number of programmes in various scientific and technological fields and is undertaking appropriate measures to tackle various challenges being faced by the Member States to strengthen their capacities to enable them to move towards sustainable development. Under its scientific programmes, ISESCO has given one of the top priorities to the development of the biotechnology base of the Islamic countries and announced a number of programmes to promote the applications of biotechnology. The implementation mechanisms of the Strategy for the Development of Science and Technology in Islamic countries, which was prepared in collaboration with OIC Standing Committee for Scientific and Technological Co-operation (COMSTECH), and adopted by the 8th Islamic Summit Conference, also contained special emphasis on boosting biotechnological development in the Islamic countries.

The first meeting of the consultative council for the implementation of the Strategy for Development of Science and Technology in Islamic Countries, held last month, at Fes, Kingdom of Morocco, has also recommended to the ISESCO to set up expert panels to prepare a strategy for promotion of biotechnology in the Islamic countries. I would like to take this opportunity to request the renowned scientists and biotechnology experts who are amongst us to kindly contribute in this ISESCO-led process to enable us to achieve a tangible result in strengthening the biotechnological base of Islamic countries.

Excellencies, Ladies and Gentlemen

It is my pleasure to reiterate my thanks and consideration to the Director General of the Islamic Academy of Science for the honour given to me to address this august assembly. I would like to congratulate the Moroccan Academy for organizing this important conference and for making the excellent arrangements for the participants. I am confident that this conference will serve as a milestone in the development of the biotechnological base of the Islamic *Ummah*.

May Allah guide us and bless our efforts towards the progress and prosperity of our Muslim *Ummah*.

Wassalamu Alaikum Warahmatu Allah Wabarakatuh

32

Address of
Prof. Abdelhafid Lahlaidi FIAS
Chairman, Moroccan Organising Committee

Allow me dear scientists to first and foremost welcome you to the land of Morocco. The land that has shepherded science and scientists, ever since its various states, kingdoms and peoples were engulfed by the flag of Islam, in testimony of the Almighty's words,

﴿هل يستوي الذين يعلمون والذين لا يعلمون﴾، وقوله ﴿إنما يخشى الله من عباده العلماء﴾، وقوله ﴿ يا معشر الجن والإنس إن استطعتم أن تنفذوا من أقطار السموات والأرض فأنفذوا، لا تنفذون إلا بسلطان﴾.

I am honoured that this meeting is being held at this unique academic edifice, the pillar of which was constructed by His Late Majesty King Hassan II may Allah bless his soul, and the cornerstones of which were raised by the inheritor of the late king's secret, his son and successor His Majesty King Mohammed VI who is patronising this conference with the heart of a true believer who believes that genuine science is the gate to faith, and the mind of an alert open-minded person, who knows that the sublimity of nations is only measured by their contribution to the building of the edifice of human civilisation, and the actions of their sons in it and their influence in the direction it takes.

Ever since your Academy was founded in Amman during 1986, under the patronage of HRH Prince Hassan Ibn Talal, you have been striving and struggling. You have concerned yourselves with "Food Security in the Islamic World," at your Amman conference in 1987. You have contemplated on "Science and Technology Policies for Self Reliance in the Islamic World," at Islamabad in 1988.

You have sought to arrive at the "New Technologies and the Development of the Muslim World," in Kuwait in 1989. You were interested in "Technology Transfer for Development in the Islamic World," in Antalya (Turkey) in 1990. You have realised that strength does not come except through "Science and Technology Manpower Development," in Amman in 1991, and through attention to the "Environment and Development in the Islamic World," in Kuala Lumpur in 1992. You have been careful to address "Health, Nutrition and Development in the Islamic World," at Dakar (Senegal) in 1993. You have concerned yourselves with "Water in the Islamic World: An Imminent Crises,"

in Khartoum in 1994. You have realised the importance of "Science and Technology Education for Development in the Islamic World," in Tehran in 1999, and "Information Technology," in Tunis in 2000.

Dear Eminent Scientists

Whomsoever looks at these activities finds that you have addressed the most prominent of contemporary issues that affect Man today. Your objective from such specialised activities has been to preserve and protect the human being, his existence, his needs of food, water, health, clean environment, and indeed information. You have in this quest utilised active forces at the centre of which is Man himself both in mind and spirit. The force of the transfer of knowledge and technology and its control and the mastery of its tools. Your objective has been to serve the Islamic nation in a world over burdened with conflict for the sake of profit and not for spreading bliss. A world over burdened with haughtiness of invention for the sake of boasting and hegemony not for mercy or lenity.

Dear Scientists

This Academy made from the planet a one homeland, and from science it made tenderness, and made from serving Man an objective. You have provided it with the material means to grow. It has developed relations with the effective agencies of nations in terms of policy, management and science. The Academy has provided the whole of humanity a compilation of scientific publications and academic periodicals, and modern communication tools that help attain science and help with it.

Dear Eminent Scientists

We meet today to discuss an important, dangerous and distinct science. To the extent that it holds such a promise for humanity, it also calls for contemplation and care. It is a science that looks into the most dangerous of subjects, into the secret of secrets, into the greatest manifestations of creation "Genes." Delving into its secrets can represent the peak of science that God Almighty has endowed in Man's brain today. The ability of Man to decipher all the unknowns of this science will surely change the face of the world.

We hope that this change will benefit Man and be to his good. "Biotechnology and Genetic Engineering" is a science that cannot be given a peculiar quality. It does not carry the colour of ideology. This is a quality of all true sciences. Inspite of this, the track of many sciences, for many reasons, might be affected negatively or positively by emotions. While we are shaded by the spirit of Islam, and oblige the objectives of our Academy namely enhancing the interaction between our scientists and the exchange of scientific concerns, as well as the preservation of the Islamic brain reservoir, we are too driven by this to action while paying our respect to the creation and realising that we cannot

introduce changes in it, “No alteration to Allah’s creation "لا تبديل لخلق الله" ”. These bases lead us to strive at a science that is beneficial to mankind in terms of mind and spirit. A science that promises extreme happiness, a science that has always been in front of our scientist forefathers’ eyes. It is maybe for this reason that they were physicians, philosophers as well as religious scholars at the same time, for their objective was to heal the spirit as well as the body and the pleasing of God Almighty and the happiness of creatures.

Dear Eminent Scientists

You are among friends and you are in your country. You have from this country of yours, and your host, immense gratitude for the trouble you have taken, our love and our great welcome, from the top to the bottom. I, at the conclusion of my statement, would like to thank the Fellows (members) of the Academy who have exerted some effort in travelling numerous times and stayed up late on many occasions to organise this meeting and set out its programme and arrange its proceedings and planning every minute within it.

The tongue is speechless and cannot present its gratitude and eloquent praise to the high determination of the patron of this honourable kingdom, and the energiser of its scientific capabilities, the youthful King Mohammed VI, for what he has furnished to us in terms of strong determination and comprehensive and generous patronage, that added status to the status of these eminent scientists. This is not strange for a young monarch who has inherited these virtues from his forefathers some of whom were religious scholars and historians. This is not strange as he is the son of a monarch who divided up his interest between politics and science until he was called the scholar of kings. This was no strange matter too for afterall he is the graduate of the most wellborn universities who has practised in the midst of international organisations. Indeed he is an experienced monarch. He followed the detailed arrangements of our meeting with diligence and granted us his complete attention and accorded our group his best favours. He made sure that you all are included in his affection, that you are in the hearts of this honourable kingdom.

It is the duty of scholars to show gratitude to their fellow scholars and that is why we have to thank Prof. Abbas Jirrari, advisor of His Majesty and member of the Academy of Morocco, for all the help he extended to us in our endeavour.

I would also like to thank the permanent secretary of the Academy of the Kingdom of Morocco, Prof. Abdellatif Berbich for the generosity and patience with which he provided us with this everlasting house of science so that it shades us with its affection and science, so that we may all work for the same noble objectives namely serving our Islamic *Ummah* in its past and its present.

Gentlemen

He was a modest man proud to belong to this Academy. He was supposed to be here today to embrace and welcome people and be happy. The will of God Almighty did not allow us but to remember him and ask for mercy for his soul. Professor Ali Kettani attends with us today in spirit. May Allah bless his soul.

والسلام عليكم ورحمة الله ومرحبا ووفق الله لصالح الأعمال.

"وقل اعملوا فسيرى الله عملكم ورسوله والمؤمنون". صدق الله العظيم.

PART TWO
BIOTECHNOLOGY:
WHERE DO WE STAND?

38

Biotechnology in the Developing World

EDGAR J. DASILVA*
ELIAS BAYDOUN**
and
ADNAN BADRAN***

1 ABSTRACT

Scientific advances in the life sciences, and especially in cell and molecular biology, microbiology, and the neurosciences, offer opportunities for revolutionizing human welfare activities. Enriched by inputs from genomic research, biotechnology is becoming a major force for development in all countries. Closely interrelated with culture and ethical values, biotechnology can help contribute to the solving of global problems such as food availability and water insecurity which hamper national development in developing countries and threaten peace. Several developing countries and especially the least developed countries (LDCs) face R & D problems that result from a lack of facilities and available professional skills in the biosciences and the biotechnologies. Furthermore, these inadequacies limit their full participation in take-off activities in national self-sufficiency, and self-reliant regional ventures in sustainable development. Nevertheless all is not bleak. Progress in biotechnology in several developing countries is impressive and sustainable.

2 INTRODUCTION

Scientific advances in the life sciences, and especially in cell and molecular biology, microbiology, and the neurosciences, offer opportunities for revolutionizing human welfare activities and improvements in the quality and quantity of healthcare. Notwithstanding the availability of these knowledge-rich instruments, global problems such as food and water security and the advent of new and re-emergent diseases hamper national development especially in developing countries and constitute a threat to peace. Furthermore, least

* International Scientific Council for Islam and Development (INSULA), UNESCO House, Paris, France.
** Professor of Biology, Faculty of Arts and Science, American University of Beirut, Beirut, Lebanon.
*** Professor of Biology and Fellow of the IAS, President of Philadelphia University, Jordan, and former Deputy Director General of UNESCO, Paris.

developed countries (LDCs) are further disadvantaged by the lack of facilities and available professional skills in biosciences and biotechnologies. These inadequacies limit their full participation in international activities and restrict their full take-off in self-sufficiency, national and self-reliant regional ventures in sustainable development. Nevertheless, a number of biotech initiatives concerning GMOs, food security, desert agriculture, intellectual property rights, etc., have been made by several developing countries. Indeed, progress and achievements in several of these countries, to date, are impressive.

3 BIOTECHNOLOGY FOR DEVELOPMENT

The UN Human Development Report (HDR) "*Making New Technologies Work for Development,*" (*UN*, *2001a*) identified biotechnology as a key avenue for the socio-economic advancement of developing countries. A veritable goldmine of opportunities in the corporate world, biotechnology is enriching the way we do and teach science that has become a global player on the international scene. Moreover, it is full of entrepreneurial opportunities for networking the technological transformation of the developing world to design solutions to devise bioclean industrial processes; to enhance soil fertility, to increase crop yield, and to engage in molecular farming for the production of new bio-products, efficient drugs, novel crops and therapies.

During the early decades of the last century, petroleum-based industrial and utility products gradually replaced similar products in use between 1910 and 1940, and made from biological materials. Today, biobased industrial products e.g. biofuels are beginning to compete in the contexts of energy and the environment with highly fashionable petroleum-based products, which in the mid-1970s were curtailed by the rising costs of fossil-derived fuels. Reliance on biobased processes is growing since it contributes to long-term sustainability of natural resources, industrial development, environmental quality, and national security. Use of the enzymatic machinery of the invisible microbe and genetic tailoring will increase in the future to obtain a variety of bioproducts ranging from biofuels, bioplastics, biodetergents, biolubricants, and biopharmaceuticals to bio-ornamentals with new plant and floral architecture (Box 1).

Box 1: Biobased industries

- *Established technologies:*
  - Recycled packaging materials from agro-industrial residues;
  - Bioabsorbents for use in odour control, pet litter, animal bedding;
  - Biocement in building, insulation, and road materials;
  - Biocontrol products - soil amendments, fertilizers and pesticides; and
  - Biobased products - cellulose-based paints, cosmetics, textiles, and landscape products e.g. decorative parks, floral ornamentals.

- *Emerging technologies:*
  - Plant-based plastics, starch-based biodegradable polymers and films;
  - Biodegradable soybean oil-derived lubricants, and functional greased-refined fluids;
  - Soybean derived inks and paints;
  - Enzymes - Cellulases for fruit juice clarification and stone-washed jeans; amylases for nutrition enhancement;
  - Renewable carton packaging materials from bagasse, kenaf;
  - Biochips involving mating of bacterial proteins with silicon chips; and
  - Edible vaccines, rub-on vaccines.

Source: *DaSilva* (*2001c*).

4 GMOS AND DEVELOPMENT

GMOs (also known as *Living Modified Organisms -LMOs*) are obtained from parent animals, plants and microorganisms. Concerns, fears, hopes and promises have been expressed with GM crops and foods. Such sentiments are not associated with a wide range of fermented foods that contain whole or parts of natural organisms, and foods prepared in near-to-safe hygienic conditions, that are ingested world-wide with pride, relish and satisfaction.

Debate concerning GM crops and foods is fierce and emotional (Box 2).

Box 2. GM crops and developing countries

- Benefits poor-resource farmers in use of number/amount of herbicides/pesticides;
- High yields and more productive crops are part of the solution to the global food crisis;
- No evidence that commercial transgenic crops contain new allergens nor have negative impact on human health;
- Genetic engineering techniques, generally are applied to crops important to the industrialised world, and not to crops on which the world's hungry depend;
- Fears concerning GM foods and crop result from:
  - the occurrence and spread of *Mad Cow Disease* in Europe;
  - Reported occurrence of chemical dioxin in foods and soft drinks; and of sludge in animal food;
  - Inclusion of GM food in animal in food for humans (*tortillas*);
  - Concentration of seed companies in a few hands;
  - Lack of, and/or inadequate testing of GM crops in tropical environments;
  - Improper identification and labelling of GM foods in food markets; and
  - Absence of public awareness and consumer education programmes.
- In summary, "*the use of the newer biotechnological techniques does not result in food which is less safe than that which is produced by conventional ones.*"

Source: *WHO* (*1991*) - extract from a joint FAO/WHO Consultation Report: *Strategies for assessing the safety of foods produced by biotechnology.*

Arguments focus on the economic loss of crop genetic diversity and biodiversity; threat to use of generic medicinal products, inadequate compensation costs; the indiscriminate appropriation of native intellectual property resources without adequate compensatory measures; non-conformity with religious, cultural, and ethical issues; and monopolistic trends (10 top life science industries have ownership of 15 major food and non-food crops). To counteract hazards to health, that may arise from the release of GMOs into the environment (*WHO*, *2000*), there is a continuing need of safety assessment of GM foods that requires a comparative approach between such foods and their conventional counterparts in the identification of potential safety and nutritional issues (*FAO/WHO*, *2000*).

Notwithstanding randomized controlled trials, an integrated stepwise approach, and on a case to case approach would go a long way in the assuring the safety of GM foods as well as for allowing an update in safety assessment

issues. Brazil, has introduced restrictive conditions on the imports of GM products starting this year in relation to environmental and food safety pending further review. And Sri Lanka has banned the imports of GMOs (*Anderson and Yao, 2000*). Public protest in the industrialized societies has fuelled demands for risk-assessment research in the cause-effect phenomena associated with GM crops and stricter regulation has given rise to differing transatlantic viewpoints (*Levidov and Carr, 2001*). Emphasis is on the biosafety component in GM agriculture. Whereas fifteen developing countries have biosafety guidelines in force, several others were in the process of drafting such regulatory review.

GM agriculture is not new. It has been in practice over several thousands of years, encouraged and practised by Mother Nature. Furthermore, it has its origin in Gregor Mendel's principles of inheritance, and in the subsequent application of the science of genetic engineering to crop improvement. In the absence of evidence of new allergenicity, GM agriculture has many benefits for developing countries. These range from an improvement in quantity and quality of meat, milk and livestock production; enhanced market possibilities with higher crop yields, and low dependence of poor-resource farmers on expensive chemical-based fertilizers. Ancillary benefits are an environmental motivation for development of bio-based clean technologies, and the absence of a deterioration of human health following consumption of GM foods (*FAO/WHO*; *Schlundt, 2001*).

Developing countries are beginning to embrace GM agriculture (*Krattiger, 1994*). Approximately 160 GMO releases have been conducted in about 25 developing countries. Field trials with transgenic *cotton, maize, potato, soybean, tomato, banana and sugarcane* crops are reported in *Argentina, Belize, Bolivia*, *Brazil, Colombia*, *Costa Rica, Chile, Cuba, Dominican Republic, Guatemala, Mexico, Peru* and *Venezuela.* In Africa, the Arab States and Asia, *China, Egypt, India, Indonesia, Malaysia, South Africa, Thailand* and *Zimbabwe* are engaged with 5 transgenic crops: *cotton, corn, potato, soybean and tomato*; and in *Ghana, Kenya, Nigeria, Senegal, Uganda, Uruguay* as well as in countries in Eastern Europe - Bulgaria, Romania and the Ukraine, field trials, in 2000, had just begun or were scheduled to get underway. Today, almost 45 million hectares of GMO crops are grown worldwide involving especially Argentina, Canada, China and the USA. In China, 13 gene-altered crops (i.e. rice, wheat, beet, potato, tomato, corn, peanut, rapeseed, sweet pepper and cotton) have been released in the agricultural sector, over a 10-year period, since 1986. Currently, some 50 per cent of all crops are engineered genetically; and of the 45 million hectares cultivated with biotech crops, the share of the developing countries' amounts to 24%.

In China, over 50 per cent of all crops, today, are expected to have been engineered genetically. Gene-altered crops rice, wheat, beet, potato, tomato, corn, peanut, rapeseed, sweet pepper and cotton crops have been grown since 1986. Research in India with GM crops - rice, rapeseed, potato, aubergine, cauliflower, chilly, and tobacco is being conducted with built-in biosafety and

monitoring protocols through several academic, governmental and private institutions. Indonesia, became the first Southeast Asian country to produce a GM crop-cotton, commercially following successful trials conducted in the districts of Bantaseng, Bone, Bulakumba, Gowa, Soppeng, Talakar and Wajo by the Hasnuddin University and Universitas Gadjah Mada

GM fruits-avocadoes, pineapples and mangos already exist. GM plants are being constructed with bioremediating functions that helps protect the environment. One example is the development of plant-based biodegradable plastics to replace plastics derived from fossil fuels GM plants planned for release within this decade are expected to improve food yields in developing countries by up to 25%. These include caffeine-free coffee plants, tobacco plants containing a diabetes vaccine, and soybeans with a "heart-healthier "oil profile, and an improved digestible protein content.

The bioengineering of plants with economic advantage can help many developing countries transform arid zones into food factories. Crop production in arid lands can benefit from genetic improvement of cultivated species and their resistance to environmental stresses. The use of genetically-modified trees in forest ecosystems, landscape and plantation development has been described by *Rautner* (*2001*). The *argan* tree - *Argania spinosa*, naturally occurring in Morocco is considered promising in saline soils. Furthermore, development of greenhouse technologies and microbial treatment of wastewaters contribute to nutrient recycling and rational use of water resources. Indeed, saline aquaculture can be an alternate source of food and feed, e.g. fish, algae, and non-seasonal crops (*Moffat*, *1990*).

5 FOOD SECURITY, HUNGER, AND POVERTY

In several developing countries, and particularly in the Islamic world, biotechnology has become a source of economic development and social progress (*DaSilva*, *1997, 1998*). Biotechnology is, also, a means to provide access to credit, technology and peer markets to especially the rural poor (*Holaday*, *1999*; *Lalljee* and *Facknath*, *1999*), who are confronted with the growing insecurity of dwindling water resources (*Serageldin*, *1999a*). Food shortages and food insecurity, compounded by the-lack of adequate purchasing power, poverty, inequity, low crop yields, and a deteriorating environment, which lead to hunger (Box 3).

Over 80 low-income food-deficit countries developing countries possess neither the ability to produce sufficient food to feed their own populations nor the foreign-exchange reserves to import food supplies to cover the deficits. The region of sub-Sahara, susceptible to political instability and dysfunctional economies, is the most vulnerable as it is host to about 25% of the population in 67 low-income developing countries.

Box 3: Hunger, poverty and population

Hunger

- About 33,000 people starve to death everyday;
- 830 million people affected world-wide;
  - One out of every six persons, in the developing world, has no access to food;
- Of 830 million people suffering from malnutrition and hunger;
  - 790 million live in developing countries, often, home of the poor and hungry;
  - 200 million are children under 5 years of age;
  - ⅓ of all pre-school children are food-insecure;
- The ***undernourished*** subsist on 1,800 calories per day or less instead of a general requirement of 2000 per adult per day;
  - In ***sub-Saharan Africa*****:** 180 million are undernourished; In ***Asia***: 525 million are undernourished; and in ***Latin America***: 53 million are undernourished;
- At the World Food Summit (1996), 186 countries committed themselves to a reduction by half in the number of undernourished people by 2015;
- Only in ***rare*** cases is hunger due to actual shortfalls of food;
- Nearly 18 million people die every year from communicable diseases; and
- As a ***rule*** hunger is a direct consequence of dire poverty.

Poverty

- Poverty is essentially a rural phenomenon, yet creeping into urban areas;
- 2.8 billion people live on less than US$ 2 per day;
- Of the 1.3 billion people in the sub-region of South Asia;
  - 800 million live on less than US$ 1 per day;
  - 400 million are illiterate adults;
  - 260 million lack access to health services; and 230 million lack access to safe water;
  - 80 million children under 4 years of age are malnourished;
  - 100 million children live or work on the streets;
- In 1998, the 48 least developed countries attracted less than US$3 billion in foreign direct investments, a mere 0.4 per cent of the total;
- The combined wealth of the world's 200 richest people hit $ 1 trillion in 1999; and
- The combined incomes of the 582 million people living in the 43 least developed countries is $ 146 billion.

Population

- Of the approximately 90 million newborn, 95% are born in developing countries;
- 40 million births each year are not registered world-wide;
- 70% of people in the developing countries below the poverty line are rural women;
- Of the approximately 1100 million farmers worldwide;
  - 50 million farmers are in the developed world;
  - 1050 million farmers are in the developing countries with little financial resources;
- By 2050, two-third's of the world's population is likely to be urban;
- 90 per cent of the world's population will begin life in a developing country, and in all probability in poverty as a result of the use of genetic engineering; and
- Poverty in urban areas in coming decades will overtake rural numbers.

Sources: Common Fund for Commodities (*1996*), *Novartis* (*2000*), *Union of Concerned Scientist*s (2000), *White Paper* (*1997*); *World Food Programme* (*2001*), *WMO* (*2000*).

Opportunities and constraints in agricultural biotechnology in developing countries are of significance in the response to the challenge of poverty in the 21st century (*Persley and Lantin*, *2000*); and, which influence national strategies that help minimise environmental, health and social risks; and, address the dietary needs of poor-resource farmers and nutrition of rural consumers who are often the last beneficiaries of advancements in science. Indeed, improvements in dietary intakes and nutrition requires consumer education and awareness programmes. As President Jimmy Carter said: "Responsible biotechnology is not the enemy; starvation is. Without adequate food supplies at affordable prices, we cannot expect world health or peace."

Several of these low-income food-deficit countries are poverty-prone or poverty-stricken. Poverty, already emerging in urban in some industrialised societies, will overtake rural numbers in the coming decades. Food production, population and poverty are closely interwined (Table 1). Of the world's population, a fifth lives in poverty. And in the last decade, nations with the richest 20 per cent of the world's population received 82 per cent of the global income. Nations with the poorest 20 per cent received only 1.4 per cent of the income

Table 1. Population growth and food production

Year	Author	Essay/Report	Remark
1798	**Thomas Malthus**	Essay on the Principles of Population.	Proposes that human fertility would outstrip its ability to produce enough food.
1972	**Club of Rome**	The Limits to Growth.	Mass starvation could result from unchecked growth.
1983	**Amaryta Sen**	Essay on Entitlement and Deprivation.	Poverty, not shortage of physical food, is the cause of hunger.
1990	**P.R. and A.H. Ehrlich**	The Population Explosion.	Focus on population problems vis-à-vis food availability and production.
1996	**World Bank**	Food Security for the World.	Solution to hunger is distribution of purchasing power to the poor.
2000	**Jonathan Latham**	More Food Means Less Hunger.	There's enough food for all, but the poor cannot afford to buy it.

The United Nations Decade for the *Eradication of Poverty* Decade (*1997 - 2006*), draws attention to the environment, development, human rights, vulnerable groups, etc. Combating poverty involves actions that increase food security, that improve the availability and quality of basic services, and that generate opportunities for sustainable livelihoods and for empowerment of rural women and men to access productive resources such as land credit, emerging technologies, training, commercial markets, and participation in community decision-making processes. Profit-oriented research in agricultural biotechnology is becoming concerned with poverty alleviation, food security, and conservation of the environment, and sustainable development. Concerns focus on the involvement of resource-poor farmers, in particularly the LDCs, in the design and formulation of field trials; and in funding the dissemination of socio-economic benefits through them as transmitters of good practice knowledge to rural communities.

Such provision of opportunities and participation is helping rural and village farming communities to gain access to best practices and productive services in agricultural development, to improve gender participation in rural farming processes, to benefit from property and proprietary rights, and to overcome diffidence in self-reliance, Active participation in such community development assists in the way out of poverty. *Karanja et al.* (*2000*) have briefly described close collaboration with participating NGOs in co-financed experimental trials in four Senegalese villages. In these village-based science education exercises

conducted by the Dakar MIRCENS[*], a total of 331 farmers, inclusive of 140 women farmers, were exposed to environmental and societal benefits emanating from the use of biological nitrogen-fixation technology. This demonstration/training/information-cum-service facility is catalytic and rewarding for rural folk where different kinds of technology, new crop varieties, floriculture, aquaculture and micro-enterprises such as mushroom production, etc., are tested, all with the active involvement of the villagers. Talents and skills, individual and collective, are crucial in the constructive evolution of an important bridge between the rural poor, the government and other institutions.

In India, the United Nations Development Programme pioneered the biovillage approach with provision of support in 1999 to the Pillayarkuppam village in the Pondichgerry district. Eighteen other villages, with a population of 25.000 people are committed to this concept which provides technological empowerment, transforms village communities into ecological entrepreneurs, augments individual rural resources with additional incomes, improves gender involvement and contributes to food security which involves availability, either through production or importation of safe and nutritive food in adequate quantities. The easy economic and uninterrupted access to nutritious and wholesome foods, that are easy to prepare for use in daily food intakes by all communal and societal segments - men and women, the aged and the young, the poor and the rich, and those in rural and urban areas .is best exemplified in *the 2020 Vision* of the International Food Policy Research Institute (IFPRI) that foresees:

"*A world where every person has access to sufficient food to sustain a healthy and productive life where malnutrition is absent, and when food originates from efficient, effective and low-cost food systems that are compatible with sustainable use of natural resources.*" In the context of "*food necessary for well-being,*" the value of GMO foods and of fermented foods for human welfare; and for counteracting world hunger, cannot be ignored nor underestimated in the diets of millions of poor people in especially the developing world. Furthermore, fermented foods contribute in no negligible way to counteracting food insecurity amongst hungry populations (*Steinkraus*, *1995*).

Food scarcity results from the inaccessibility to food as a consequence of inadequate sources of income and buying power**.** Also armed conflicts, which destabilize rural and urban development, have been a major factor in the causation of food insecurity, scarcity and hunger. Hunger in Rwanda, Angola, Sierra Leone, Burundi, Liberia and the former Yugoslavia results from social instability and armed conflict rather than from a shortage of natural resources. In the Horn of Africa - Djibouti, Eritrea, Ethiopia, Kenya and Somalia, about 70 million people suffer from malnutrition, food scarcity and famine. Harsh and inhospitable climates hamper efficient agricultural productivity (*FAO*, *2000a*). Resilient communities live under harsh drought conditions e.g. little rainfall,

[*] Part of UNESCO's Global Network of Microbial Resources Centres (Editors).

soil erosion, and lack of access to opportunity in farming. Moreover, due to scant savings, populations live on less that US$1 per day. Poor rural economies result from overgrazing, pollution, little access to agricultural facilities and equipment, weak or authoritarian governance, and inadequate donor aid for the poorest communities.

Food scarcity is a subtle form of surrogate destabilization and bioeconomic warfare. Conflicts over shared water resources in some regions of the world are commonplace; human health, food security and the management of the environment are threatened (*Serageldin*, *1999b*); by emerging diseases (*DaSilva and Iaccarino*, *1999*) and the intentional release of harmful and pathogenic organisms in bioteroristic actions that destroy cash crops (*Rogers et al., 1999*). Research in plant biotechnology contributes to food security, and enables small farmers, be they in Argentina, India, Morocco, and Uganda, or in Australia, France, Canada and the USA to obtain, equally and universally, increased and sustainable crop yields. Success stories in food security in some developing countries e.g. Burkina Faso, China, Ecuador, India, Thailand, Tunisia, and Zimbabwe, amongst other, have been monitored by the *FAO* (*1996*). Similar activities are encountered in Brazil, Chile, Costa Rica, Egypt, Iran, Jordan, Kenya, Mexico, Philippines, Republic of Korea, and South Africa.

6 ARID LAND BIOTECHNOLOGY

The Middle East, with its varied characteristics in culture, economies, the environment, governance and religion, is home to varied semi-urban and urban agriculture that seems to have originated in the *Fertile Crescent of the Middle East*, homeland of the first farmers some 10,000 to 12,000 years ago (*Wilford*, *1997*). This crescent area, rich in the *eight founder crops*: wheat, barley, legumes, grapes, melons, dates, pistachios and almonds, nurtured through individual farming initiatives and routine practices, evolved, between 9,000 and 6,000 years ago, into a successful agricultural movement of domesticated animals and plants that spread to other regions, near and far (*Boguchi*, *1996*). The practice of agriculture ranged from walled-in-home and community gardens in poor-income households and communal gatherings, through nomadic and settlement farming around oases and terraced terrains, to production of market crops in controlled greenhouse environments in saline and desert soils. The commonalities and contrasts of agriculture in Middle Eastern cities and, the contributory growth and sustainability of urban agriculture in greening the brown tints of arid and semi-arid terrains have been reviewed in the context of the region's changing food patterns, needs and opportunities (*Nasr and Kaldjian*, *1997*).

Seventy per cent of Africa is constituted by deserts and arid lands with half the continent's population being found in these areas. Some of the poorest countries in the world, with heavy population growth, meagre national

resources, a weak or negligible technological base, and with primary level educational and technical infrastructures, are found in Africa (Box 4).

Box 4. Arid lands

- 70% of the earth's dryland is affected by desertification;
- 1 billion of the world's 6 billion, in more than 100 countries, is affected by desertification;
- Loss in agricultural goods, due to desertification, is more than US$40 billion p.a.;
- Of the 42 high in-debt countries, 39 are located in tropical desert regions; and
- Farmers are faced with the problems of soil erosion, plant viruses, high-cost chemical-based fertilizers and pesticides.

Source: *UN* (*2000*).

In several developing countries in Africa, agriculture is linked to water availability and irrigation. Vulnerability of agricultural and water resources, ecosystems, food production, utility goods, shelter, and human health is high in regions threatened by weakened infrastructures. Of the 19 water-stressed countries, more are in Africa than in other regions. Several of these African water-stressed countries are dependent on a singular economic base-agriculture, which in the Middle Eastern OPEC countries, is complemented by another naturally-occurring economic export-value resource-oil. These dry land African countries, oft recognised as LDCs, do not have the economic means to respond effectively, well ahead in time, to the onset of malnutrition and poverty, and to the frequent occurrence of vector-borne diseases in implementing well-defined strategies and responses designed for sustainable development. Agriculture in these countries, which have a low livelihood base and inadequate socio-cultural services, is further disadvantaged by fragile ecosystems and the phenomenon of globalisation. Against this background, the use of GM technology could make an impact by focusing on the use of improved seeds and disease-free high-quality plantlets to grow high-value commercial crops in low-rainfall. In addition, rural education could help promote the benefits of such technology in diversifying complementary agricultural practices by rearing fisheries and floriculture.

Often written off as a no-win situation, several indicators encourage the continued utility of supporting biotechnological research ventures in Africa (*Brink et al.*, *1998*). Life-spans of research capacities in Cameroon, Ethiopia, Ghana, Kenya, Malawi, Nigeria, South Africa, Uganda, and Zimbabwe in plant biotechnology are severely limited in scope and are donor-dependent. Future donor support in these countries was recommended to institutes endowed with the capability and capacity to engage in contemporary agricultural research, institute biosafety mechanisms, and new-look

management aspects of biotechnology *Komen et al.* (*2000*). The periodic internal and external peer-review of programme plans and performance would help strengthen the emergence of sustainable competence in agricultural biotech research in these countries. Such measures would also help attract private and public sectors e.g. in Indonesia, Kenya, Mexico and Zimbabwe to engage in contemporary biotechnology with a pronounced user focus (*Falconi*, *1999*), and attract back African expatriate biotechnological expertise and humanpower that has been the mainstay of progress in several newly-industrialized Asian countries.

In Latin America and the Caribbean, desertification and vulnerability is due to repeated farming on marginalized lands, and destruction of the rain forests and to fragile ecosystems in several island countries. Initiatives to address these problems focus on building partnerships and programmes. Several Caribbean and Pacific island countries in their quest for sustainable and economic development have adopted a biotechnological approach (*DaSilva and Taylor*, *1998*).

Sixty to eighty per cent of employment in developing countries with agrarian-based economies can be traced to agriculture. Issues of agricultural biodiversity; bioenergy; drylands and rural development; and water have been dealt with in the framework of the Multifunctional Character of Agriculture and Land (*FAO*, *1999*). The containment of desertification occurs in the ability to bioconvert the ecological disadvantages of arid lands to economic benefits through the cultivation of desert crops; development of saline agriculture and pisciculture, and rational use of water and wastewater resources.

The evolution of biotech policies in the areas of environmental protection, sustainable agriculture, rural development, and international trade needs to be encouraged. And, the roles of CGIAR, the Global Forum for Research, COMSTECH, and UNESCO's Global Networks of Microbial Resources Centres (MIRCENS) and of biosphere reserves in the Man the Biosphere programme (MAB), etc, need to be further used in capacity-building (*ATAS*, *1992*). A range of biological activities in arid and semi-arid regions is provided in Table 2. The introduction of high-yielding, drought tolerant and early ripening varieties into Central and West Africa have led to impressive gains in maize production which have led to the development and availability of supplementary food markets in Burkina Faso, Congo, Ghana, Guinea, Mali, and Nigeria. The Punjab Agricultural University (Ludhiana) in India; the Agricultural University (Peshawar), the Pakistan Agricultural Research Council (Islamabad), and the University of Agriculture (Faisalabad) in Pakistan; the Agronomic Research Service (Aragon) in Spain and the Centre for Arid Zone Studies (Bangor), and the John Innes Centre (Norwich) in the UK has carried out laboratory studies and field trials within the European Union programme to evaluate environmental risks of biocontrol measures introduced into Europe.

Table 2. Range of biological activities in different arid and semi-arid regions

Algeria	• Micropropagation of resistant *Deglat Nour* date-palm varieties to *Fusarium* wilt disease; • Recyclage of agro-industrial wastes for production of biofuels (ethanol, methanol, methane);
Chile	• Test planting of the leguminous tamarugo tree in the Atacama desert for resource development with the Aymara communities; • Fundacion Chile pioneers, in 1999, use of biotech gene tools in improving forestry species in new joint venture;
Pakistan	• Production of commercial biofertilizers for agriculture in OIC States; • *Biotechnology for Development*, Seminar, January 2000, Islamabad, with Islamic Educational, Scientific and Cultural Organization (ISESCO), and Inter-Islamic Network on Genetic Engineering and Biotechnology (INOGE);
Qatar	• Conservation of marine environment and marine resources; • Establishment of gene bank to conserve genetic resources; • Use of non-conventional waters-saline and recycled, to irrigate crops and combat drought and desertification;
Senegal	• Development of BNF technologies for application in desert agriculture in the Arab world and OIC States;
Tunisia	• Enzyme technology in production of glucose and fructose syrups, Centre de Biotechnologie de Sfax.

Several national experiences to combat desertification and mitigate drought in developing countries - Algeria, Burkina Faso, India, Iran, Iraq, Jordan, Saudi Arabia, Tunisia and Yemen as well as regional initiatives by the Arab Maghreb Union (UMA) and the Southern African Development Community (SADC) are available (*UN, 2000*). In brief, arid and semi-arid countries can benefit in multiple ways from appropriate biotech applications. These involve the production of high-quality tissue culture reared plantlets; seeding of rural biotech industries e.g. ornamentals and floriculture which provide complementary sources of income for women; production of GMOs through use of DNA-based protocols for food production; and production of biobased products such as biodiesel, biofertlizers, biopesticides, etc. Examples of regional and international activities in dryland agriculture are provided in Table 3.

Table 3. Arid land agriculture - examples of regional activities

Organization	Biotechnology Activities	Countries
Association of Agricultural Research Institutions in the Near East and North Africa (AARINENA)	• Maghreb/Mashreq Project - Development of Integrated Crop/Livestock Systems in the low rainfall areas (Maghreb- Mashreq Region);	Algeria, Libya, Malta, Mauritania, Morocco, Tunisia, Cyprus, Jordan, Iraq, Lebanon, Palestine, Syria;
	Development of Wheat cultivars resistant to Leaf and Stem rusts in the Nile Valley and Red Sea Regions;	Djibouti, Egypt, Sudan, Somalia, Yemen;
	• Arab Peninsula Region; and	Bahrain, Kuwait, Oman, Qatar, Saudi Arabia, UAE;
	• West and Central Asia Region;	Afghanistan, Iran, Kazakhstan, Kyrghyzstan, Pakistan, Tajikstan, Turkey, Turkmenistan, Uzbekistan;
Asia Pacific Association of Forestry Research Institutes (APAARI)	• **Strengthening of Regional Networks;** - Cereals and Legumes Asia Network (CLAN) - Tropical Asian Maize Network (TRAMNET) - Underutilized Tropical Fruits Asia Network (UTAFNET) - Fisheries and Aquatic Research Network (FARN)	China, India, Indonesia, Iran, Malaysia, Philippines, Thailand;
Central Asia and Caucasus Region (CACR)	• Maximise dual function of agriculture in economic development: Production of food on a continuous and sustainable basis; and provision of gainful employment;	Armenia, Azerbaijan, Georgia, (Caucasus), Kazakhstan, Kyrghyzstan, Tajikistan, Turkmenistan, Uzbekistan (Central Asia);
Regional Forum on Agricultural Research and Development (FORAGRO)	• Reassessment of agriculture in LAC as a key component of economic development;	Member countries of the Latin American and Caribbean regions;

7 COASTAL AGRICULTURE

Saline or seawater agriculture is full of promise (*NAS*, *1990*). Seawater agriculture is the growth of salt-tolerant crops on land irrigated with ocean waters. It also involves a variety of halophytic crops ranging from grasses and shrubs to trees, and which are encountered in coastline marshes or in saline desert terrains. Such grasses and plants are: *Distichlis palmeri* (salt grass), *Salicornia* (glasswort), *Atriplex* (saltbush), *Suaeda* (sea blithe), and the succulent *Batis*, which can be used as feed supplements to bolster meagre sources and intakes of normal palatable plants for livestock feeds. Halophyte farms of *Salicornia* and *Atriplex* species have been established in India, Mexico, the United Arab Emirates, Pakistan, Egypt and Saudi Arabia (*Glenn et al., 1998*). China has embarked upon sea-water irrigated agriculture involving almost 300,000 hectares of coastal land along the Shandong, Hebei, Guandong and Hainan provinces.

Processes and strategies for managing saline soils and increasing soil fertility, involve mulching and biological nitrogen-fixation in degraded soils in Egypt, Ethiopia, Ghana, Kenya, Malawi, South Africa, Tanzania, Uganda, Zambia, and Zimbabwe (*FAO*, *2000b*). In the Islamic world, Egypt, Iran, Libya, Morocco, Oman, Pakistan, Saudi Arabia, Sudan, Tunisia, and UAE are involved in a regional network on the date-palm - *Phoenix dactylifera*, with emphasis on the date-palm as a fruit and as a food crop. These countries along with Namibia are the world's major date producing countries. Opportunities for tissue culture of date palm production in the Republic of Djibouti; and for rehabilitation of date palm production and marketing in the Bari region in Somalia have been identified. AVT Biotechnology established, in 1984 in Kerala, India, the first commercial Plant Tissue Culture Laboratory which maintains high quality date palm germplasm like Medjool, Khanezi, Khalas, Khasab, Barhee, Fard, Nagal etc., obtained from India, Oman, and the USA. Laboratory services and tissue cultured date palm are marketed in Kuwait, Lebanon, Oman, the UAE and Gujarat State in India.

Current research activities in saline agriculture in some arid and semi-arid countries deal with domestication of ornamentals and halophytes in recycled water systems, and with the evaluation of crop responses to protocols for enhancing salt tolerance (*Chourk-Allah et al.*, *1996*) Halophytes are cultured for landscaping and fodder use in Egypt, development of nurseries and ornamentals in Morocco, and for greening and landscaping of arid soils in Tunisia, Saudi Arabia and the UAE. *Salicornia bigelovi* is cultivated in Australia for its potential as an oilseed crop. And, *Atriplex canescens* (USA) and *A. hortensis* (India), *Salicornia* sp. (Mexico) and *Tacticornia verrucosa* (Australia) are halophytes grown for fodder use. Halophytes of economic advantage in coastal agriculture are provided in Table 4.

Table 4. Some examples of halophytes in current use*

Halophyte species	Purpose of use	Region of growth and use
Atriplex angualata;	Fodder plant;	Australia;
Atriplex nummularia;	Fodder plant;	North Africa;
Atriplex semibaccata;	Fodder plant;	Argentine, Bolivia, Chile;
Atriplex volutans;	Fodder plant;	South America;
Halogeton sativus;	Vegetable;	Mediterranean countries;
Juncus acutus;	Paper production;	Egypt;
Noronhia emarginata;	Ornamental;	Madagascar, and other African countries;
Pithcolobium dulce;	Landscape tree, fodder for animals, curry, dye, glue;	India;
Salicornia fruticosa;	Vegetable oil production;	North European coastline and France**;
Tamarix aphylla;	Furniture wood, wind shelter in plantations, firewood;	Arab countries;

* *Source: Cash Crop Halophytes for Future Halophyte Growers –Leith, H, Lohmann, Güth, M, and Menzel, U.,—EC ICI8CT96 –0055 project /UNESCO MAB program (partners:UNESCO/MAB; Institut fur Umweltsystemforschung, and University of Osnabrück).*

** *Introduced into more than 40 countries.*

Desert and arid land biodiversity can be enhanced through reliance on saline agriculture (Table 5). Well-known successes encountered in Australia, Israel, Saudi Arabia, the United Arab Emirates, and the USA (*Chourk-Allah et al.*, *1996*) have stimulated cropping systems with seawater irrigation in Belgium, France, the Netherlands, And Portugal (*Huiskes, 1996*).

Table 5. Some initiatives concerning the greening of deserts

Country	Climatic feature	Species	Remarks
Cape Verde	Group of islands with desert-like low rainfall;	Prosopis trees;	• Joint Project: Cape Verde National Institute with Henry Doubleday Research Association (HDRA), UK; Women main beneficiaries;
China	Saline soils;	Interplantation of amaranthus grain and trees;	• Shandong Teachers University project with HDRA;
India	Thar desert;	Prosopis cineraria;	• Cross trials with between Oman and India using drought-resistant trees;
Mexico, USA	Chihuahua desert;	Berberis trifolia Cernis canadensis var. mexicana;	• Landscape plants, research in desert ecosystems;
Namibia	Kalahari desert;	Scleorocarya birrea;	• Developed with Israel as an orchard crop. Fruit exploited as source of food supplements e.g. vitamins;
Oman	Wahiba Sands desert;	Prosopis cineraria;	• Bark provides edible gum project with Sultan Qaboos University, Durham University (UK);

Source: DaSilva (2001b).

8 MEDICINAL PLANTS

Medicinal plants, since times immemorial, have been used as a source of medicine in nearly all cultures. Herbal remedies and healthcare preparations obtained from commonly used traditional herbs and medicinal plants have been traced to the occurrence of natural products with medicinal properties (Table 6). The use of traditional medicine and medicinal plants in most developing countries preparations have become more popular in the industrialized societies in the treatment of minor ailments ties on account of the increasing costs of personal health maintenance.

Active markets for traditional medicine in developing countries are found in China, India, Pakistan, Sri Lanka and Thailand. About 40% of the total medicinal consumption, in China, is attributed to traditional tribal medicines. In Thailand, herbal medicines are encountered in the legumes *Caesalpiniaceae*, the *Fabaceae*, and the *Mimosaceae*. In the mid-90s, receipts of more than US$2.5 billion have been estimated to result from the sales of herbal medicines.

Africa is a rich source of medicinal plants. Extracts of *Phytolacca dodecandra*, or *endod* are used as a molluscicide to control schistosomiasis. *Catharanthus roseus* which yields the anti-tumour agents *vinblastine* and *vinvristin*e; and *Ricinus communis* which yields the laxative--castor oil, are amongst the best known examples in the pharmaceutical industry. In Botswana, Lesotho, Namibia and South Africa, *Harpagophytum procumbens* is produced as a crude drug for export. Other exports are *Hibiscus sabdariffa* from Sudan and Egypt; *Pausinystalia yohimbe*, which yields *yohimbine*, from Cameroon, Nigeria and Rwanda*;* and *Rauwolfia vomitoria*, from Congo, Madagascar and Mozambique which is exploited to yield reserpine and ajmaline.

The medicinal plants, *Eupatorium perfoliatum* (bonest), *Podophyllum peltatum* (mayapple), and *Panax quinquefolium* (ginseng), also appreciated for their aesthetic and ornamental value, have long been associated with the American Indians e.g. the *Talamancas* (Costa Rica); the *Pipiles* (El Salvador); the *Pech*, *Lencas*, and *Xicaques* (Honduras); the *Miskitos* and *Sumus* (Honduras and Nicaragua); the *Maya* Indians (Mexico) and the *Guaymis* and *Kunas* (Panama).

Table 6. Some examples of national activities concerning medicinal plants

COUNTRY	ACTIVITIES	REMARKS
Australia	• Asian-Australian Centre for the Study of Bioactive Medicinal Plant Constituents, set up in 1992 at La Trobe University, for conduction of research in collaboration with Chulalongkorn and Chieng-Mai Universities in Thailand;	• Inter-university research work focuses on: - The bioactive constituents of turmeric e.g. *curcumin* - Islamic medicinal plants - Antifungal proteins in crop plants, e.g. in cotton and yellow mustard *Sinapis alba* - Marine toxins - Joint collaboration on medicinal plants with Indonesia, Thailand, Bangladesh, Singapore, Kuwait and New Zealand.
Bhutan	• Sustainable protection and use of forest resources and development of plant tissue culture;	• Capacity building in plant biotechnology for rural markets and development of forest seedlings Indigenous Hospital in Thimphu has recorded 180 species of medicinal and aromatic plants.
China	• Root extracts of *Astragalus membranaceus* used as a general tonic food and for boosting immunity;	• Used in Chinese herbal medicine to strengthen vital energy *Qi* in general health. Product widely used in China, and South Asia.
	• Institute of Medicinal Plants established in 1983 with branches in provinces of Yunnan, Hainan and Guangxi;	• Recognised as a *WHO Collaborating Centre on Traditional Medicine* for conservation and use of medicinal plant resources, and discovery of new potent drugs.
Dominican Republic	• Launched in 1982 as a traditional medicine for the islands (TRAMIL) network, activities focus on developing proven plant remedies as alternatives to expensive patent drugs for rural populations;	• Regional node established in Panama in 1994 to cover area from Belize to Panama. Over 150 medicinal plants evaluated and recorded in *Caribbean Pharmacopoeia*. TRAMIL is recognised in Dominican Republic, Honduras, Nicaragua and Panama as an effective mechanism in devising primary healthcare programmes.
Guatemala	• *Farmaya* Laboratory, engaged in organic	• Collaborates with the Central America Centre of Studies on

	cultivation of medicinal plants, pharmacological research, production of plant-derived pharmaceuticals, and development of protocols for safe use and screening of 700 different plants, has developed 15 pharmaceutical products using traditional knowledge of indigenous and rural groups;	Appropriate Technologies (CEMAT) • National Commission for the Use of Medicinal Plants serves as model for Latin American countries in developing guidelines and protocols for plant-based pharmaceuticals; Co-operates in IDRC project on the *Application, Research and Dissemination of the Use of Medicinal Plants in the Caribbean.*
India	• Programme launched in 1993 for implementation by an NGO called "Foundation for Revitalisation of Local Health Traditions;"	• 30 *in situ* "Medicinal Plant Conservation Areas" (MPCA), 15 *ex situ* "Medicinal Plant Conservation Parks (MPCP), and one Model Production Unit (MPU) established in the programme, for large-scale production in Karnataka, Tamil Nadu and Kerala.
	• Herbal Gene Bank at the Tropical Botanic Garden Research Institute at Thiruvananthapurnam;	• All-India ethnobiological project for the development of drugs from medicinal plants and herbs. Promotion of ethnopharmacological research.
	• Central Institute of Medicinal and Aromatic plants in Lucknow;	• Institute deals with plant tissue culture of medicinal plants of commercial significance; monitors All-India Co-ordinated project on Conservation of Endangered Plant species; maintenance of living herbaria or plant gardens that feed traditional systems of medicine such as Ayurveda, Unani and Sidha. Ayurveda recognised by WHO as an alternate system of medicine.
Korea, Republic of	• Natural Products Research Institute;	• Designated in 1977/1978 as *Headquarter Centre for UNESCO Regional Network for the Chemistry of Natural Products in Southeast Asia*, and in 1988 as *WHO Collaborating Centre for Traditional Medicine*. In 1997, the Institute was appointed as the *Korea-China Collaborating*

		Centre for Traditional Oriental Medicines Research.
Nepal	• Plant biotechnology, mushroom cultivation, bioenergy production, environmental microbiology and medicinal plants;	• Medicinal plants widely cultivated in Shivpuri, Doti, Tistung, Urindavan and Tarakava Herbal farms. Herbal products widely marketed as Ayurvedic therapeutics.
Nigeria	• Preservation of Nigerian genetic patrimony comprised of 5000 acquisitions of edible, fodder, forest, industrial and medicinal plants;	• Research supported by National Centre for Genetic Resources and Biotechnology which functions also as affiliate of International Centre for Genetic Engineering and Biotechnology.
Pacific Islands	• *Noni*, a Tahitian herbal tonic derived from *Morinda citrifolia* is used as a general tonic food and energizer;	• Product widely used in China, and South Asia and widely marketed throughout the Pacific islands.
South Africa	• Traditional medicines programme (TRAMED) started in 1994 at University of Cape Town to promote the cultural, health, environmental, scientific and socio-economic benefits obtained from the use of Eastern and Southern African medicinal plants;	• Integral part of *WHO collaborating Centre for Drug Policy* based in Universities of Cape Town and of the Western Cape. • TRAMED activities focus on: development of database for east and southern Africa; evolution of an All-Africa network of rural traditional medicines; inventorization of indigenous tribal knowledge; and articulation of national policy for use of traditional medicines in South Africa.
Turkey	• Medicinal and Aromatic Plant and Drug Research Centre (established in 1982 at Anadolu University, Eskeşehir).	• Centre hosts UNIDO/Turkey project on Production of Pharmaceutical Materials from Medicinal and Aromatic Plants for preparation of pharmaceuticals, perfumes, dyes and surveys concerning assessment of phytopharmaceutical units.

9 GENDER AND BIOTECHNOLOGY

The "LDCs greatest assets are their women, men and children whose potentials as both agents and beneficiaries of development must be fully realized. Efforts at development of human capacities in LDCs have been affected by low school enrolment and low health, nutrition and sanitation status and by the prevalence of the HIV/AIDS pandemic, particularly in Africa, and malaria, tuberculosis and other communicable diseases, as well as by natural and man-made disasters" (*UN, 2001b*).

Women and children in most LDCs occupy a central role. The normal transmitters of traditional civic customs and values, women are crucial in the assimilation and acceptance of new technologies as is evidenced by the number of TV advertisements that are aimed at women.. In rural and village communities, women provide the first example of working together in a co-operative venture as the may ends meet with meager income (Box 5).

Box 5. Women in partnership with Biotechnology

- African proverb: *Without women we all go hungry;*
- In developing countries, women produce 75% of the food;
- In the ACP economies, women produce 80% of staple foods;
- In Asia 90% of the work in rice fields is carried out by women;
- As the male component move to urban areas for better incomes or to serve in war, women constitute the new generation of farmers in several LDCs;
- Women are custodians of traditional knowledge that is of significance in rural medicine;
- Women's health and the quality of a woman's life are being redefined by biotechnology; and
- Biotech medicines and diagnostic aids are helping women to become owners of their own health.

Women in rural and lower middle-income societies make an economic contribution to agricultural and healthcare markets. Women farmers collect, keep, store, conserve and sow seeds for use by peasant societies. Women healers are, in the keepers and providers of traditional knowledge concerning herbal- and plant-based medicine. Again, for example, some of the world's delicacies that are encountered in the domain of fermented foods. Their recipes and preparations are closely guarded secrets that have been handed down orally from generation to generation and which are at the basis of sustaining nutritional inputs, albeit inadequate, of families on a virtually, daily basis. Indeed women, are the primary food producers, food gatherers, and food processors, worldwide, in the developing world. In rural areas, women, not

masters of their own time, plant, weed, help harvest and even see to crop sales. Similarly in the energy sector of indigenous village communities, women trudge miles in search of biomass bush material as a source of fuel for cooking and other domestic purposes. Application of biotechnological principles, on the one hand, releases rural women from the drudgery of tiring manual labor in the energy and food sectors , and on the other hand provides them with more opportunities for cultural, societal, and technical education in improving the quality of family and community life. The impact of modern biotechnologies in agriculture on the position of women and their capability to The role and situation of women, often, the major providers of the livelihoods of their families Africa, Asia and Latin America, may be affected by the modern biotechnologies in agriculture (*Zweifel*, *1995*).

In Indian biotech, women, preferentially, opt for career development and reward in the life sciences. Mathematics, the engineering sciences and space technology attract more the male component of the cream of Indian scientific humanpower rather than potential women scientists. The role of women in biotechnology is best summed up in the *Mission Statement*: "To provide opportunities for professionally qualified women to take to a career of remunerative self-employment through the organisation of environment friendly biotechnological enterprises" of the UNDP-UNIFEM meeting of women scientists at the and M. S. Swaminathan Research Foundation at Chennai, December, 1996.

Subsequently, the Government of Tamil Nadu, and the Department of Biotechnology, Government of India , approved the establishment of the first Women's Biotechnology Park in the country at Kelambakkam, located at 41 km from Chennai amongst other approved programmes commemorating the 50th anniversary of India's independence.

The Park (Figure 1), which came into being in July,1998, and coming up to 20 acres of land at an estimated cost of Rs. 10 crore, aims to develop an integrated approach involving technology identification, incubation, dissemination, training and retraining, development of necessary techno-infrastructure through feasibility studies using the criteria of value addition and market demand.

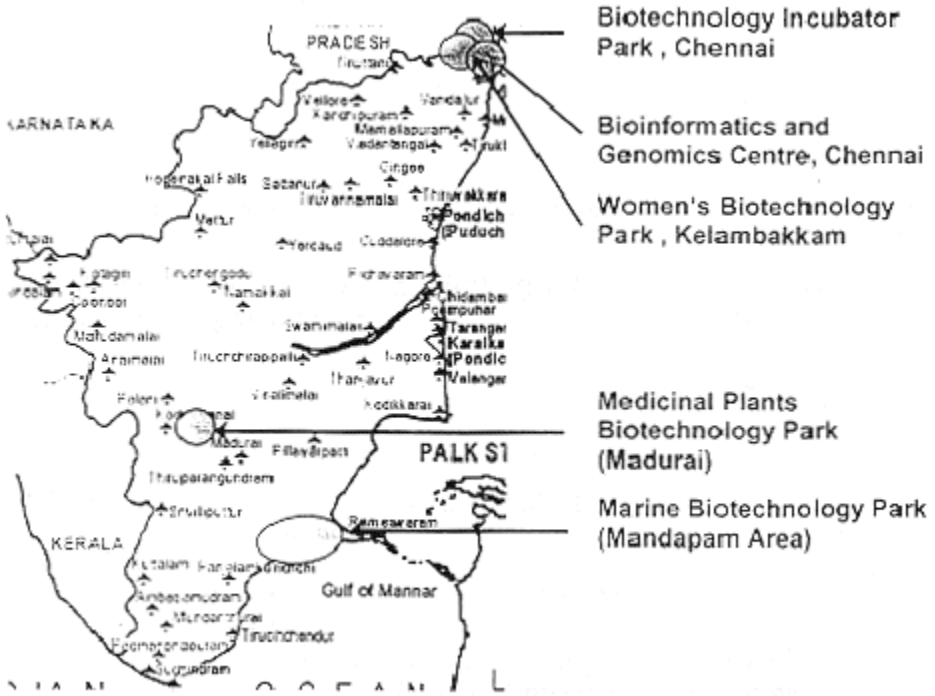


Figure 1. Outlay of women's biotechnology park in Chennai, India.

The Park, designed on the principle of decentralised production supported by appropriate centralised services, promotes a series of high tech biotechnology based enterprises that aim to capture a number of niche markets in the areas of Agri-biotech, Food biotech, Medical biotech etc. When fully developed, this Park will consist of industrial incubation centres, ultra modern multimedia information complex, quality verification reference laboratories etc. The R&D institutions, the corporate sector and the financial institutions will assist the women entrepreneurs to achieve the objectives of the Park that should serve as a model to foster the technological and economic empowerment of women.

10 ENVIRONMENTAL CONSIDERATIONS

Today's technological revolution, powered by advances in biotechnology and communications technology, has altered the global economy and the environment as a consequence of the changing nature of markets, competition and sources of comparative advantages.

Bioremediating technology is a key instrument in environmental management. Monitoring and conservation of air and water quality is dependent upon the bioremediating action of microbial agents in developing clean technologies and in the recycling of wastewaters. Use of bioconversion technologies to transform renewable organic wastes into utility products can

help augment rural incomes and seed entrepreneurial ventures. In agricultural biotechnology, resort is being made to the use of non-polluting biofertilizers to boost crop production; to genetically-engineered micro-organisms to develop disease-resistant crops and plant pesticides; and to improve animal health. Marine biotechnology and new approaches in aquaculture are already contributing to control and eradication of fish diseases and improved quality of edible fish (*Fan and Bao, 2000*). In Asia, the fisheries and aquaculture industries contribute about 45 per cent of world fish production. These industries are indisputable contributors to the food supply, livelihood, foreign-exchange earnings, and the socio-economic fabric and stability of multiple rural areas in several Asian countries. The production of cultured fish is at once a source of food and export products and a means of counteracting the global threat of overharvesting of the world's oceans. In China, the aquaculture and marine biotechnologies occupy a prominent place in national development plans. The success of grass carp in fish cell culture; the development of the antifreeze ability of *Tilapia,* and of transgenic salmon at the Freshwater Fishery Research Institute of the Zhejiang Province is widely recognised. Furthermore, the aquaculture and marine biotechnologies cover the domain of natural products that possess commercial significance. Valuable compounds and products such as pharmaceutical principles, sterols, alginates, oils, etc are being extracted from micro- and macro-algae in several regions of the world. Aquatic invertebrates are being screened as a source of bio-active compounds that may have anticarcinogenic and antiviral properties; and marine bacteria, are being developed for use in treatment of oil spills, and of holding tanks in tanker ships.

Diversity in the physiological machinery of microorganisms influences the widespread impact of the microbial world on human existence. Given the spectacular breakthroughs achieved in the realm of microbial prospecting, current efforts focus on tapping the genetic content and performance of microorganisms resources in the aquatic and marine environments through BETCEN and MIRCENS research and training activities e.g. in China, Costa Rica, and India in the molecular genetics of marine animals, plants and microorganisms, the extraction and characterization of pharmaceuticals and potential drugs from the oceans, and the designing of biobased techniques for environmental clean-up and biofilm prevention.

The goals of environmental considerations are to increase productivity, protect the environment, safeguard against loss of biodiversity, improve environmental management policies, and strengthen national research programmes. Table 7 provides segmental elements relevant to environmental conservation.

Table 7. Environment segments – relevant to development in arid and semi-arid areas

Environment segment	Areas of Interest, Activity and Domain
The Socio-economic and Ecological Enviroment;	– Population growth, poverty, dwindling natural resources (e.g. water, arable land); – Social science research: commonality of intellectual, cultural, and social property.
The Governance Environment;	– Move from "Food Self Sufficiency" to "Food Security;" – Convention on Biological Diversity.
The Scientific Environment;	– Use of quantitative trait loci (QLTs) in enhancing diseases resistance, etc; – GMOs; – Use of GIS in developing an inter - disciplinary approach in building electronic institutions as brokers between electronic database resources and electronic clients and users.
The Educational Environment;	– Need for increased training of rural and urban communities; – Popularizing the use of biotechnologies such as gene engineering, bioinformatics, database development, etc; – Involvement of rural communities in governance and networking management of natural agricultural resources.
Market Environment;	– Development of potential market-oriented bio ventures involving - poultry, livestock, aquaculture, cash crops, landscaping and ornamental materials; – Use of legal practices to protect heritage, traditional knowledge, intellectual property rights.

Source: *DaSilva* (*2001b*).

11 INTELLECTUAL PROPERTY RIGHTS (IPRs)

The spectacular research developments in biotechnology are pushing science into uncharted waters thus emphasizing the need for intellectual property rights, The role of the university as a centre for converting research, educational and innovative knowledge into capital is crucial in the creation and use of intellectual property for development (*Kornhauser, 2001*). Moreover, the growing intertwining pace of information technology, molecular nanotechnology and biotechnology is focusing attention on the need for clarity with relation to IPRs, and the issues of how biotech research knowledge is generated, shared and owned. There is need for debate of IPRS relating to biosafety, ethics, environmental governance and poverty restriction. Such issues are:

- The intense reaction in some public quarters to GMOs;
- Growth of the prosperity gap between the Northern and Southern hemispheres;
- In appropriate and inadequate compensatory measures to safeguard against the loss of indigenous genetic resources and traditional knowledge; and
- Loss of biodiversity of economic significance in rural communities resulting from development of market biosubstitution products.

Recent litigation on biotech issues precipitated by the application of genetic engineering has emphasised the need of safeguarding indigenous traditional knowledge and its use in the absence of adequate compensation costs. As a consequence, many developing countries participate in UN sponsored conventions and treaties dealing with micro-organisms and the protection of new plant varieties. In addition several regional efforts have crystallised into regional entities that cover licensing agreements, patenting issues, policies for transfer of technology and industrial property materials and IPRs (*DaSilva*, *2001a*). The transformation of new biotechnological knowledge into marketable products necessitates the development of partnerships, networking and workshop consultation to clarify IPR issues of relevance to academia and the industry, to developed and developing countries, economists, jurists, ethicists, regulatory bodies and social scientists. Such need derives from the following:

- Biotechnology, in the next two decades will be a major factor in economic development;
- Innovations in biotechnology will aim at poverty reduction;
- Biotechnological research is not blocked by patent law and IPRs;
- Development of biotechnological databases will help reduce the information gap;
- Expanding growth of university-industry co-operation in biotechnology; and
- Questioning and Understanding current IPRs is important for their future evolution.

12 STRATEGIC BIOTECH INITIATIVES BY DEVELOPING COUNTRIES

Developing countries, which account for about 78 per cent of the world's population, are already devising and using strategic biotechnologies to solve problems of local, regional and global significance (Table 8). Within the framework of the Lomé Convention, the European Union (EU) promotes technical co-operation with 70 countries in Africa, the Caribbean and the Pacific. The major occupation, and employer in these ACP countries is agriculture (Figure 2), which is the beneficiary of intense EU scientific and technological collaboration. Research institutes and universities are engaged in competitive breakthrough peer-reviewed research are constantly attracting scientific excellence.

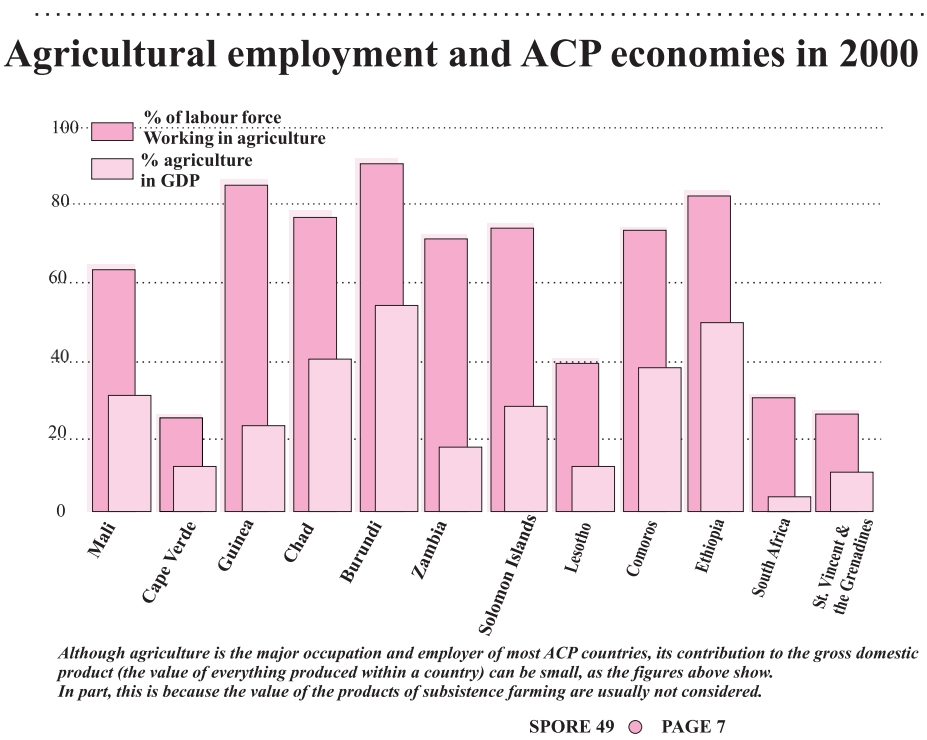


Figure 2. Agriculture and labour indicators in ACP countries.

(*Source: SPORE*, pg. 7; August 2001).

The horizontal flow of research amongst and between developing countries strengthens South-South collaboration and regional and international co-operation. Many developing and least developed countries are confronted by the challenge of a "stand alone" economy, a process that involves diversification of agricultural production, industrial enterprises and a well-developed human resource base.Self-sufficiency and self-reliance, the twin hallmarks of a "stand alone" market-oriented economy are crucial and can be achieved only through networking, sharing of experience, regional co-operation and knowledge-rich resources. South-South and technical development and economic co-operation programmes have proven useful in the transfer of technology (*Rath and Lealess*, *2001*) and as below:

- Use of Senegalese fish-smoking technique to meet local taste preferences of traditional community in Ghana;
- Transfer of biogas technology from China to Brazil and Costa Rica, and from India to Cambodia;
- Development of the "Jaipur foot" prothesis in India and its technology transfer to Malaysia and Thailand;
- Development of the "Ear-lift" unit in Thailand, and subsequently used with success in Lao People's Democratic Republic and Kenya;
- The Africa/Asia Joint Research Project: interspecific hybridization between African and Asian Rice Species which is developing radically new low-management, high-yielding varieties of rice through cross-breeding of African and Asian rice. The new rice varieties have the capacity to suppress weeds and withstand environmental stresses and diseases, and due to their increased height, help women (carrying babies on their backs) to harvest them more easily. In 1997/1988, the project dispatched back-cross progenies to Asia, Latin America and seventeen African nations, including Benin, Burkina Faso, Cameroon, Ivory Coast, Gambia, Ghana, Guinea, Guinea Bissau, Nigeria, Sierra Leone, and Togo;
- Biopharmaceuticals in Latin America in which the Centro de Ingenieria Genetica y Biotecnologia (CIGB) and Finlay Institute, in co-operation with ELEASA, a large Argentine pharmaceutical company find commercial channels for their products;
- The marine biodiversity co-operation among small island developing states in Which twenty-two island States in the South Pacific have participated in co-operative initiatives in marine biodiversity through such regional institutions as the South Pacific Commission, South Pacific Environment Programme, the Forum Fisheries Agency and the University of the South Pacific. Approximately 10000 People per year are trained by the University of the South Pacific Marine Study Programme; and
- Biotica (Argentina) and Sementes Agroceres (Brazil) which reflected the partnership between Biotica and SA developed by the Brazilian-Argentinean Centre for Biotechnology (CABBIO), an association of firms and individuals which is jointly funded by the two governments but is privately run. Biotica is a small research-oriented firm specializing in vegetable micro propagation and new potato seed technology, while SA is a leading manufacturer of agricultural seeds and animal food in Brazil. The objective of the partnership is to develop, produce and market a new variety of potato seed. A new variety of potato was successfully developed and tested in Brazil, where it is accounting for 2% of the Brazilian market and competing successfully with imports from Europe.

Source: Rath and Lealess (*2000*).

Regional and international activities pioneered in the Islamic world are provided in Table 8.

Table 8. Status of Biotechnology in some developing countries

Brazil	• *Rhizobium* Culture Collection at FEPAGRO/UFRGS MIRCEN laboratories functions safety depositary; • Quality control of nationally used legume inoculants ensured by MIRCEN laboratories; • Sugarcane EST Genome Project (SUCEST) schedule to end 2004, identifying around 50,000 genes networking with 38 Brazilian research institutions; • Production of growth hormone in Brazilian corn –joint venture with universities of Campinas and Sao Paulo; • Development of (1) Papaya resistant to local strain of ring-spot virus, and (2) common beans resistant to golden mosaic virus; • Increased yield of sugarcane cultivar *Gueitang 11* obtained with BGA fertilisers in Guangxi province;	• Development of national competence and capacity in IPRs; • Promotion of university-industry joint ventures;	*FAPESP Genome Program (1999);* *Karanja et al (2000);* *Sampaio (1999)* Meidanis and Guimães (2000);* **(see in: Persley, G. J. and Lantin, M. M.).*
Burkina Faso	• Improvement achieved in the household food security situation in the country in 1990 in comparison to that of the mid-1970s following political commitment;	• Development of mix of policy protocols concerning-soil conservation and water use;	
China	• Use of modern molecular technologies for varied biotech uses; • Development and use of the Desert Film Linen Technique (DFLT) with young rice plants in the Badanjlin Desert; technique under test by UNDP as preventive tool in arresting desertification; • National Committee monitors field testing of GMO crops; • Large-scale use of Blue-Green Algal fertilisers to obtain 7% rice yields increase and 30% decrease in use of chemical products; • Production in 2000 if super rice hybrid *Liang You Pei Jin;*	• Increase food production and improve product quality through clean technology use; • Development of national competence and capacity in IPRs; • More extensive use of genomics in agriculture;	*Zhang (1999)*;*

Table 8. (continued)

Chile	• Of the 120 biotech enterprises BiosChile, Biotechnica de Chile, Bioforest and Laboratorio de Chile have national and international profiles that focus on: – micropropagation of fruits (kiwis, peaches, etc) and plant ornamentals; – diagnostic kits and vaccines for human and animal use; – biotech products for use with fruit juice, wine and vegetable production;	• Development of knowledge-rich biotech base; • Development of national competence and capacity in biosafety policies and IPRs;	*(Nef, 1998);*
Costa Rica	• National System of Conservation Areas; • Bioprospecting Program of National Biodiversity Institute (INBio); • Rice Biotechnology Programme aiming at elimination of constraints in crop improvement and production; • Bioprospecting for bacterial genes for use in rice genomics; • Development of transgenic products;	• Expansion of bioprospecting activities covering bioresources and gene pools; • Development of knowledge-rich biotech base; • Development of national competence and capacity in biosafety policies and IPRs;	*Sittenfeld et al (1999)*;*
Egypt	• AGERI established in 1990 to bridge food gap and achieve self-reliance; • Success in development of transgenic potato, maize, faba beans, and tomato crops;	• Development of knowledge-rich biotech base; • Development of national competence and capacity in biosafety policies and IPRs;	*Madkour (1999)*;*

Table 8. (continued)

India	• Establishment of: – National Biotechnology Board in 1982; – Department of Biotechnology (DBT) in 1986 under Ministry of Science and Technology; – Micropropagation Technology Parks in New Delhi and Pune for development of a plant tissue culture industry; – National Bioinformatics Network with 10 nodal centres and 35 sub-nodal centres processing bioinformation; – National Plant Genome Research Centre, Jawarharlal Nehru University; – National Bioscience Career Development Awards with special awards for women scientists; • Successful *White Revolution* with 80% of milk produced in small and rural farms; • World's first IVF buffalo calf—*Pratham,* reared through embryo transfer at National Dairy Research Institute, Karnal; • Research and training programmes involving States of Gujarat, Rajasthan, Madhya Pradesh, Orissa, wesr Bengal, Haryana, Punjab, Jammu & Kashmir, Mizoram, Andhra Pradesh and Uttar Pradesh with more than 5000 research publications, and 4000 postdoctoral students;	• Establishment of a National Brain Research Centre Development of a recombinant vaccine for tuberculosis and hepatitis; • Expansion of university-industry co-operation, established since 1986, in biotechnologies; • Promotion of aquaculture and marine resource development to yield annual target of 10 million metric tons of fish; • 59 MS, postdoctoral and MD training programmes either in progress or approved for immediate start; • Jai Vigyan mission with focus on development of genomic databases, vaccines, and diagnostics; • E-Biotech government web-site with details on technology profiles, services and commercial opportunities;	*Sharma (1999)*;* •*DBT (2001) - http://dbtindia.nic.in*

Table 8. (continued)

Iran	• 340 biotechnologists recognised in Iran; • 46 institutes/centres engaged in biotechnologies; • *Razi* Institute designated as Reference Laboratory in Diagnostics; • National Research Centre for Genetic Engineering and Biotechnology established in 1988: – Covers wide range of research in biosciences, pharmacology, molecular biology, applied microbiology; – Produces growth hormone hepatitis B vaccine; • Iran Research Organization for Science and Technology establishes Biotechnology Centre in 1982, that hosts Persian Type Culture Collection; • Active private sector e.g. – *Rana Agro-Industry Corporation* produces tissue-culture date palm and banana; – *Cina Gene* produces restriction enzymes, etc.	• Mass production of uniform and disease-free date-palm plants, cherry apple, bananas sugarcane, etc through research at the Agricultural Biotechnology research Institute of Iran; • Combating salinity problems; • Development of national competence and capacity in biosafety policies and IPRs; • Expand biotech expertise and excellence through newly established Iranian Society for Biotechnology; • Widen impact of ongoing co-operation of Razi Vaccine and Serum Institute, Pasteur Institute of Iran with WHO; • Expand: – pilot university-industry project (Radha Co. and Tarbiat Modarres University) on bioleaching of copper from sulphide ores to industrial scale by 2002; – Ministry of Mines and Metals project with Pharmacy Faculty, Teheran Medical school on recovery of gold and desulfurization of coal into university-industry joint ventures; – Activities of newly established *Iran Biotechnology Society;* – Biotech activities of *Iran Centre of Industrial Research and Development* (ICIRAD);	*Ghareyazi (1999)** *Shojaosadati (2000)* *http://ibw.nrcgeb.ir*

Table 8. (continued)

Jordan	• Academic sector provides for R&D in wide variety of fields: natural products biotechnology; immunodiagnostics; bioreactor technology for production of bioenergy and biofuels; embryo transfer in animals; applied microbiology, BNF technology; • Active R&D industrial activities in immunobiologicals, tissue culture and developing of appropriate laboratory equipment;	• Develop academic competence and excellence for university-industry ventures in tissue culture ornamentals and products; monoclonal antibodies, single-cell-protein, enzyme production; molecular modelling; molecular genetics; • Establishment of a national biotechnological centre to drive and sustain on-going national efforts in diverse biotechnologies;	*Ajlouni and Malkawi (1999)*;*
Kenya	• MIRCEN pioneers builds on pioneering work concerning use of legume inoculants, and market penetration in East Africa; • Widespread use of tissue culture technology with banana, cassava, potato, pyrethrum and sugarcane crops: – Production of transgenic sweet potato resistant to feathery mottle virus;	• Development of knowledge-rich biotech base; • Development of national competence and capacity in biosafety policies and IPRs;	*Ndiritu (1999)*; Karanja et al (2000);*
Kuwait	• Successful bioremediation of Park in Ahmadi in which park soil is freed from oil pollutants;	• Pioneering research in arid land biotechnology In the Gulf region; • Kuwait Institute for Scientific Research (KISR) expanding profile into international centre of excellence in biotech research;	
Mexico	• GMO crop trials begin in 1988; • Biosafety Committee issues monitoring guidelines for GMOs and risk assessment;	• Development of knowledge-rich biotech base; • Development of national competence and capacity in biosafety policies and IPRs;	*Alvarez-Morales (1999)* ;*

Table 8. (continued)

Philippines	• Establishment of: – National Institute of molecular Biology and Biotechnology (BIOTECH) in 1980 at University of the Philippines (UP) at (Los Banos; – Three centres, in 1995, within framework of the UP system at campuses in: Diliman (industrial biotechnology) Manila (human health) Visayas (marine biotechnology); • National Research, Development and Extension Network system for 13 biobased commodities: (*rice, corn, tubers, coconut, plantation crops, fibre crops, fruits/nuts, vegetables /spices, ornamentals, fisheries, aquaculture, livestock and poultry, and legumes*); and 5 discipline – oriented networks: *fishery post-harvest and marketing; soil and water resources; agricultural and fisheries engineering; postharvest food and nutrition, and social science policy and biotechnology*; • As of 1999, 250 top-level scientists engaged in high-level biotech R& D; • Several national institutions conduct biotechnological activities in plant breeding, animal sciences, food science, etc;	• Deployment of biotechnology to boost agricultural productivity with minimal inputs; • Rural and public science education; • programmes to create public awareness and participation to: – attract private sector sponsorship; – counteract anti-biotech sentiment; – acceptance of GMOs and IPRs; – develop biosafety guidelines; – engage in biotech bioprospecting; – to e-access new technologies; • R&D biotech projects (1980 – 1999)**; ---------- **Extracted from :*De la Cruz* (1999).	*De la Cruz (1999);**

Type of biotechnology R&D	*Number of projects*	*Percent of total*
Biocontrol	55	20.5
Soil amendments	44	16.5
Food/beverage	43	16.3
Tissue culture	52	19.5
Feed component	20	7.5
Enzymes	16	6.0
Diagnostics	7	2.6
Farm waste utilization	4	1.5
Vaccines	3	1.1
Animal reproduction	3	1.1
Molecular markers	12	4.6
GMOs	7	2.7
Total	**266**	**100.0**

Source: Survey conducted by UPLB BIOTECH, 1999.55

Table 8. (continued)

South Africa	• Well advanced activities in the agricultural sector • Five-fold increase in protein content of sweet potatoes: – Production of transgenic tobacco and cotton varieties resistant to herbicides; • GMO Act of 1997 controls import of GM products and protects consumer and the environment; • Over 600 biotechnology research projects in progress covering the medical, agricultural, food, environment and biosafety fields; • 55 companies exist in the plant and medical biotechnologies;	• Development of a national strategy, starting 2001, decreed by the Ministry of Arts, Culture, Science and Technology which foresees establishment of a Bioethics Committee and a Biotechnology Advisory Council covering improvement of crop yields, local production of vaccines, development of regional innovation centres and research laboratories have been targeted for early action; • Development of fungal resistant strawberries, maize and sorghum; insect-resistant sugarcane, and virus-resistant potatoes;	*Njobe-Mbuli (1999);**
Thailand	• In 1991, establishment of National Centre for Genetic Engineering and Biotechnology (BIOTEC) with focus on R&D in transfer of technology and capacity-building: – Establishment of National Biosafety Committee in 1993 to provide guidance and monitor field trial evaluations of GMO crops; – In 1995, Cassava and Starch Technology program established; Production of new variety of tapioca—*Kasetsart 50;* • Expansion of agricultural sector by 2.8% achieved in 1998: – Establishment of Rice Genome Project Thailand; conservation of high-quality fragrant rice cultivar *Khao-Hom Dawk-Mali;* – In 1999, establishment of Shrimp Biotechnology Program to sustain Thai shrimp farming and eradicate shrimp diseases;	• Need to improve crop diversification and yield; and postharvest conservation; • Use of biotech industries: food and animal feed; rubber; and landscape and ornamental wood materials to establish Thailand as key export centre by 2002; • Development of transgenic crops (papaya, pepper,) resistant to viral diseases;	*Tanticharoen, (1999);**

Table 8. (continued)

	– Commercial production of *Trichoderma* to control *Sclerotium rolfsii* Sacc., and Ch*aetomium* to control *Phytophora*		
UAE	• Al Jarf area --transformed desert land with large date plantation; • Al Ajaban area -- orchard plantation: mango, guava, and date palms; • Ras Al-Khaimah poultry enterprise in Digdagga –vast egg production facility; • Mafraq wastewater facility provides irrigation waters for public gardens, parks;	• International Centre on Biosaline Agriculture pioneering research in saline agriculture and electronic communications network in arid land biotechnology; developing *greening* of coastlines; • Conversion of desert terrain into green terrain;	*DaSilva (2001b);*
Zimbabwe	• Department of Agricultural Research and Specialist Services monitors development of hybrid maize; • Kutsaga Research Station oversees tobacco breeding, pathology, etc; • Introduction in 1980 of Master's Degree in the University of Zimbabwe to promote biotech science education; Zimbabwe Biotechnology Training Programme funded by Dutch Government, SAREC (Sweden) and Rockefeller Foundation; • Establishment of Biotechnology Research Institute (BRI) in which research on drought-tolerant maize is predominant;	• Establishment of Zimbabwe Biosafety Board in 1999 and promulgation of Zimbabwe Biosafety Regulations and development of IPR guidelines; • Capacity-building in agricultural biotechnology and biosafety programmes;	*Chetsanga (1999)*;*

Amongst a number of prestigious catalytic Islamic sponsors, the Islamic Academy of Sciences, through a series of pioneering and visionary conferences, has focused policy and research attention in the Muslim world on vital issues such as food security in Amman (1987); new technologies and development in Kuwait (1989); technology transfer for development in Antalya (1990); environment in Kuala Lumpur (1992), health, nutrition and development in Dakar (1993); and, water in the Islamic World in Khartoum (1994). Several Arab and Islamic countries have embraced biotechnology as a vehicle of economical and social progress (Table 9).

Table 9. Biotech activities in the Islamic World

Organization	Biotechnology Activities	Countries
Arab League Educational, Scientific and Cultural Organization (ALECSO);	• Several biotechnologies (plant, food, environmental, industrial) are encountered at different levels of practice. Most countries have biological treatment plants for sanitary waste waters recycled for use in agriculture.	*Algeria, Bahrain, Egypt, Jordan, Libya, Mauritania, Morocco, Saudi Arabia, Syria, UAE, Yemen.*
	• Bioconversion of agro-industry residues into utility products-feed, fertilizers and fuels	*Iraq, Morocco, Syria, Turkey, Yemen.*
	• Traditional fermentation industries - Panary dairy fermentations	*Egypt, Iraq, Kuwait, Lebanon, Oman, Saudi Arabia.*
	• Bioindustries: - Alcohol and organic acid production - Methanol and acetic acid - Acetone, butanol and citric acid	*Egypt, Iraq, Lebanon, Syria.*
Committee on Scientific and Technological Co-operation of the Organization of the Islamic Conference (COMSTECH);	• Production of biofertilizers for sustainable agriculture in OIC countries; • Combating Desertification; • Use of biofertilizers in increasing sustainable crop production and development of desert soils in Egypt;	*Bangladesh, Chad* Egypt.*
	• Production and quality improvement of orchids through plant biotechnology;	*Indonesia.*
	• Biologically safe methods of cotton and rice pest control; • Ecological problems of mammal conservation in the mountains of Djoungar-Ala-Tou; • New Isolates of *Bacillus thuringiensis* in Kazakhstan and Pakistan; • Bacterial leaching of metals from ores; • Isolation of biologically active compounds from Kazakhstani plants; • Biosorption - based process for removal of heavy metals from industrial waste water;	*Kazakhstan.*

Table 9. (continued)

Organization	Biotechnology Activities	Countries
	• Joint Kazakhstan/Pakistan project on the development of leaf curl virus tolerant varieties of cotton; • Wheat Breeding for Rust Resistance;	
	• Use of biofertilizers for increasing crop production in Muslim countries; • Use of *Rhizobium* fertilizer for increasing sustainable production: multilocational bean (*Phaseolus vulgaris*) trial inoculation; • Biofertlilizers for sustainable agriculture in IOC Member States; • Strengthening of biomedical training; • Workshop on *Management of Agricultural Drainage Water*, Islamabad, (23-28 January, 2001); • the fate of organic compounds in environment and technologies • Bioinformatics Program at TUBITAK-MARAMARA Research Center; • Seminar on *Genetics and Molecular Biology Stress Tolerance in Plants,* Kocaeli, (15 – 21 February, 2001).	*Pakistan* *Senegal* *Sierra Leone* *Pakistan*** *Turkey* *Turkey****
Islamic Scientific, Educational and Cultural Organization (ISESCO)	• Seminars, workshops etc. on conservation, biodiversity, medicinal plants, applications of genetic engineering;	*In OIC Member States --Brunei, Burkina Faso, Pakistan with COMSTECH , and Islamic Network on Genetic Engineering.*

* *Co-organizers: UNESCO, UN Convention to Combat Desertification (UNCCD), and the Sahara-Sahel Observatory (OSS).*
* * *Within the framework of Inter-Islamic Network on Water Resources Development and Management.*
** * *In co-operation with Islamic Development Bank, and INOGE.*

12 CONCLUSION

Spectacular breakthrough research with the genetic information of microbial, plant and animal cell systems has enriched the discipline of biology Their translation into numerous applications through innovative bioprocesses and genetic engineering has given birth to the Age of Biotechnology. The sciences of *bioinformatics* an*d genomics*, which closely interact with information technology and nannotechnology, underpin the emergence and establishment of new biotechnologies that are of significance in networking by developing countries in the different biological sciences and biotechnologies. Such efforts contribute to the proactive development of capacity-building in the biotechnologies.

In many developing countries, education in biotechnology is now targeting the attainment and maintenance of economic competitiveness with inputs from the academic, governmental and industrial sectors. Leadership in biotechnology flows from a resource of well-trained and well-qualified scientists, engineers, and managers, within the framework of university-industry co-operation. Furthermore, such partnerships spawn new biotechnological ventures and the emergence of bio-market specialists. Experiences concerning food-producing systems for arid and semi-arid lands (*Manassah and Briskey, 1981*) have helped further the understanding of the complex issues of management and development encountered with dryland areas.

Developing countries, which account for about 78 per cent of the world's population, are already devising and using strategic biotechnologies to solve problems of local, regional and global significance (Table 10). Their participation in several regional and international pedigree programmes contributes to an on-stream worldwide resource that reflects to some extent, the human face of globalization. Flexibility, scientific companionship, and co-shared funding help developing countries respond to the common challenges that demand biotechnological solutions for the benefit of all humankind.

Table 10. Priorities by needs, vision and national goals - situation in Developing Countries

African Lions:	♦ **Gabon** (*immunology*), **Kenya** (*biofertilizers*), **South Africa** (*genetic engineering*, *ornamental plants*), **Senegal** (*biofertilizers*);
Arab Stallions:	♦ **Egypt** (*genetic engineering*)**, Bahrain (***marine biotech***), Kuwait** (pioneer SCP and Bioconversion Technology research in Gulf region), **Morocco** (*germplasm bank*), **Oman** (*marine biotech*), **UAE** (*Biosaline Agriculture Centre*);
Asian Dragons:	♦ **China** (*Shanghai Biotechnology Park*), **Hong Kong** - (Window to Southeast Asia and Pacific-Rim Biotechnology, rural electrification) (*national biogas production*);
Asian Elephants:	♦ **India** - Government Department of Biotechnology, Women's Biotechnology Park: - Bioinformatics programme (University of Poona),Institute of Microbial Resources (Chandigarh); ♦ **Indonesia** - Environmental Biotechnology and Bioindustries;
Asian Tigers:	♦ **Malaysia**, **Republic of Korea**, **Singapore**, **Thailand:** - Well advanced in genetic engineering, different biotechnologies; - Well-known ASEAN database in each country co-ordinated in Singapore;
Latin American Jaguars:	♦ **Argentine, Brazil, Chile, Costa Rica, Cuba, Mexico:** - Well advanced in molecular biology, genetic engineering, production of commodity, chemicals (ethanol), etc., - (Biotech market - immunodiagnostics, etc (*Cuba*).

Source : DaSilva (2001b).

REFERENCES

1. Advanced Technology System –ATAS (1992). *Biotechnology Development, Expanding the Capacity to Produce Food,* Department of Economic and Social Development, U.N., New York, pp. 393-395.
2. Ajlouni, M. M. and Malkawi, H. (1999). *Jordan: Status and Future Prospects of Biotechnology*, (*In:* Persley, G. J. and Lantin, M. M. ---see below).
3. Alvarez-Morales, A. (1999). *Mexico: Ensuring Environmental Safety while Benefiting from Biotechnology,* (*In:* Persley, G. J. and Lantin, M. M. ---see below).
4. Anderson, K. and Yao, S. (2001). GMOs and World Trade: Implication for China as a WTO member, Contribution to International Conference on Greater China and the WTO, Hong Kong, 22- 24 March.
5. Brink, J.A., Woodward, B.R. and DaSilva, E. J. (1998). Plant Biotechnology: A Tool for Development in Africa, *Electronic Journal of Biotechnology*, Vol:1, No.3----*http://ejb.ucv.cl*
6. Boguchi, P. (1996). The Spread of Early Framing in Europe, *American Scientist,* Vol. 84: 242-253.
7. CGIAR. (1997), World's Dryland Farmers Need New Agricultural Technology - "Green Revolution Never Reached Them"--- *http/www.worldbank.org/html/cgiar/press/dyland.html*
8. Chetsanga, C. (1999). *Zimbabwe: Exploitation of Biotechnology in Agricultural Research.In:* Persley, G. J. and Lantin, M. M. (see below).
9. Chourk-Allah, R., Malcolm, C.V. and Hamdy, A. (1996). eds. *Halophytes and Biosaline Agriculture*, Marcel dekker, New York, pg.400.
10. Common Fund for Commodities (1996). Conference on Commodities ----*http://www.inzet.nl/projecten/commodities/com_keynotes.htm*
11. DaSilva, E.J. (1997). Biotechnology in the Islamic World*, Nature Biotechnology*, Vol. 15: 733 – 735.
12. DaSilva, E. J. (1998). Biotechnology, Developing Countries and Globalization*, World Journal of Microbiology and Biotechnology*, Vol.14:463-486.
13. DaSilva, E. J. and Taylor, M. (1998). Island Communities and Biotechnology, *Electronic Journal of Biotechnology*, Vol: 1,No.1, ---- *http://www.ejb.org*
14. DaSilva, E.J. and Iaccarino, M. (1999). Emerging diseases: a global threat, *Biotechnology Advances* Vol: 17: 363-384.
15. Dasilva, E.J. (2001a). Intellectual Property Rights, Biotechnology and Developing Countries, In*: Biotechnological Property at Universities—scientific, technological ,economic, legal and ethical aspects,* Proceedings of Workshop-Expert Meeting, ICCS, Ljubljana, 21 – 24, 2001, ed. Kornahauser, A. and DaSilva, E. J. (Co-ed)., pp. 10 – 16.

16. DaSilva, E. J. (2001b). Biotechnology, Arid Lands and Globalization: Proceedings of the *First International Conference on the Application of Biotechnology for Drylands, (in press).*
17. DaSilva, E. J. (2001c). GMOs and Development, ---- *Electronic Journal of Biotechnology*, Vol:4, No.2, - *http://www.ejb.org*
18. De la Cruz, R. E. (1999). *Philippines : Challenges, Opportunities, and Constraints in Agricultural Biotechnology, In:* Persley, G. J. and Lantin, M. M. (see below)).
19. Falconi, A. (1999). Agricultural Biotechnology Research Capacity in Four Developing Countries*, ISNAR Briefing Paper 42*, December, pg.8.
20. Fan, T-J. and Bao, Z. (2001). Proceedings of *International Symposium on Marine Biotechnology*, October 2000, Ocean University Press, Qingdao, China.
21. FAO (1996). Success Stories in Food Security, Vol. 1., *----http//www.fao.org/wfs/funds/e/volume 1/t2 sum-e.htm*
22. FAO (1999). Cultivating Our Futures, Proceedings of FAO/Netherlands Conference on *The Multifunctional Character of Agriculture and Land,* 12-17 September, Masstricht, The Netherlands, Number 368.
23. FAO (2000*a*) Food Supply Situation and Crops Prospects in Sub-Saharan Africa, *Global Information and Early Warning System on Food and Agriculture,* pg. 66. Rome, Italy.
24. FAO (2000*b*). Management and Rehabilitation of Degraded Soils. MADSEA Network---- *http://www.fao.org/waicent/faoinfo/agricult/agl/madsea/topics5.htm*
25. FAO/WHO (2000). Safety Aspects of Genetically Modified Foods of Plant Origin, Joint FAO/WHO Consultation Report on Foods Derived From Biotechnology, WHO, Geneva, Switzerland, 29 May – 2 June, pg. 35.
26. FAO/WHO (2001). Evaluation of Allergenicity of Genetically Modified Foods, Joint FAO/WHO Consultation Report on Allergenicity of Foods Derived from Biotechnology, Rome, Italy, 22 – 25 January, pg. 29.
27. FAPESP (1999). FAPESP Genome Programme ---- *http://watson.fapesp.br/sucest.htm#bio*
28. Glenn, E.P. Brown, J.J. and O' Leary, J.W. (1998). Irrigating Crops with Seawater, *Scientific American*, August.
29. Ghareyazie, B. (1999). *Iran: Hopes, Achievements, and Constraints in Agricultural Biotechnology,* (*In:* Persley, G. J. and Lantin, M. M. - see below).
30. Holaday, J.W. (1999). Competing in the New Millennium: Challenges Facing Small Biotechnology Firms--- *Congressional Testimony* - Subcomittee on Technology, *----http:www.veterans.house.gov/science/holaday-102799.htm*
31. Holmes, A., Govan, J. and Goldstein, R. (1998). Aquacultural use of *Burkholderia (Pseudomonas) cepacia*: A threat to human health, *Emerging Infectious Diseases*, Vol. 4:221-227.

32. Huiskes, A. H. L. (1966). *Saline Crops*. Report ECAIR3CT92-0198, Wilhelmindorp, Stichting marine culture, Osterelde, pg. 86.
33. Karanja. N., Freire, J. Gueye, M. and DaSilva, E. (2000), MIRCEN Networking: Capacity-building in Africa and Latin America, *Agbiotechne*t, Vol. 2., March, ABN 041, pp. 1-5.
34. Komen, J., Minouna, J. and Webber, H. (2000). Biotechnology in African Agriculture Research: Opportunities for Donor Organizations, *ISNAR Briefing Paper 43*, pg. 8.
35. Kornhauser, A. (2001). Knowledge as Capital ----Technological, Economic and Socio-Cultural Aspects of Intellectual Property, In: *Biotechnological Property at Universities—scientific, technological ,economic, legal and ethical aspects,* Proceedings of Workshop-Expert Meeting, ICCS, Ljubljana, 21 – 24, 2001, ed. Kornahauser, A. and DaSilva, E, J. (Co-ed)., pp. 5 – 9.
36. Krattiger, A. (1994). Field Releases of Genetically Modified Plants: World-wide Data from 1986 to 1993/1994. In: *Biosafety for Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere*, eds. Krattiger, A.F. and Rosemarin, A., ISAAA: Ithaca SEI, Stockholm, pp. 247-266, 1994.
37. Lalljee, R. and Facknatt, S. (1999). Water Harvesting and Alternate Sources of Water for Agriculture, *PROSI Magazine*, September. No. 368
38. Levidov, L. and Carr. S. (2000). Unsound science? Transatlantic regulatory disputes over GM crops, *Int. J. Biotechnology*, Vol. 2: 257 – 253.
39. Madkour, M.A. (1999). *Egypt: Biotechnology from Laboratory to the Marketplace:Challenges and Opportunities, In:* Persley, G. J. and Lantin, M. M. (see below).
40. Manassah, J. T. and Briskey, E. J. (1981). eds. *Advances in Food-Producing Systems for Arid and Semiarid lands*, Parts A and B, Academic Press, New York, USA.
41. Meidanis, J. and Guimães, K. The Bioinformatics of the SUCEST project - *http://sucest.lad.ic.unicamp.br/en/About/LBI/bioinfo_lab.html*
42. Moffat, A. S. (1990). Engineering useful plants, *Mosaic*, Vol.21:34-43.
43. Nasr, J. and Kaldjian, P. (1997). Agriculture in Middle Eastern cities; Commonalities and Contrasts, *Aridlands, Newsletter*, No. 42 ---- *http//ag.arizona/OALS/ALN/aln42/nasr.html*
44. National Academy of Sciences (1990). *Saline Agriculture: Salt-Tolerant Plants for Developing Countries*, publ. National Academy Press, Washington, D.C., pg. 143.
45. Ndiritu, C. G. (1999). *Kenya: Biotechnology in Africa: Why the Controversy, In:* Persley, G. J. and Lantin, M. M. (see below)).
46. Nef, J. (1998). The Interface between "hard" and "soft" technologies: biotechnology, venture capital and management in Chile. Report for IDRC Latin American and Caribbean Office, Centro Internacional de Investigaciones para el Desarrollo, Montevideo, Uruguay, February 26.

47. Njobe-Mbuli, B. (1999). *South Africa: Biotechnology for Innovation and Development, In:* Persley, G. J. and Lantin, M. M. (see below).
48. Novartis (2000). Food Security for a Growing World Population:2000 Years.
49. After Malthus, Still an Unsolved Problem ----*http:www.foundation.novartis.com/food_security_population.htm*
50. Persley, G. J. and Lantin, M. M. (2000). ,eds. *Agricultural Biotechnology and the Poor,* Proceedings of an International Conference, Washington, D.C., 21 – 22 October, 1999 CGIAR, Wasnington, pg. 233.
51. Rath, A. and Lealess, S. (2000). The Forum on South-South Co-operation in Science and Technology, An Overview Document , Policy Researxch Inc., UNDP Seoul Conference, Republic of Korea, 14 – 17 February.
52. Rautner, M. (2001). Designer Trees, *Biotechnology and Development Monitor,* No.44/45, pp. 2 – 7.
53. Rogers, P., Whitby, S. and Dando, M. (1999). Biological warfare against crops, *Scientific American,* Vol: 280:70-75.
54. Sampaio, M.J.A. (1999). Brazil: Biotechnology and Agriculture to Meet the Challenges of Increased Food Production, *In:* Persley, G. J. and Lantin, M. M. (see above).
55. Schlundt, J. (2001). FAO/WHO Expert Consultations – Foods Derived from Biotechnology, In: International Conference on: *New Biotechnology Foods and Crops: Science, Safety and Society*, 10 – 12 July, Bangkok, Thailand (*http://www.oecd.org/bangkok/speeches.htm*)
56. Serageldin, I. (1999a). Biotechnology and Water Security in the 21st Century, ----*http://www.mssrf.orgsg/d99-biotech-water.html*
57. Serageldin, I. (1999b). Biotechnology and Food Security in the 21st Century, *Science*, Vol. 285: 387 – 389.
58. Sharma, M. (1999). *India: Biotechnology Research and Development, In:* Persley, G. J. and Lantin, M. M. (see above).
59. Shojaosadati, S. A. (2000). *Biotechnology in the Republic of Iran:Present Status, Achievements and Prospects*, Author Communication.
60. Sittenfeld, A, Espinoza, A. M., Muñoz, M. and Zamona, A. (1999*). Costa Rica: Challenges and Opportunities in Biotechnology and Biodiversity, In:* Persley, G. J. and Lantin, M. M. (see above).
61. Steinkraus, K. (1995). ed. *Handbook of Indigenous Fermented Foods*, Marcel Dekker, Inc., New York.
62. Tanticharoen, M. (1999). *Thailand: Biotechnology for Farm Products and Agro-Industries, In:* Persley, G. J. and Lantin, M. M. (see above).
63. Union of Concerned Scientists (2000). Biotechnology and the World Food Supply ----*http:www.ucusa.org/agriculture/worldfood.html*
64. UN (2000), Information Sharing Segment ----Intergovernmental Negotiating Committee for a *Convention to Combat Desertification*, First Substantive Session ----*http:www.unced.int/convention/history/INCDinfoSeg/party-1.php*

65. UN (2001). Draft Programme of Action for the Least Developed Countries for the Decade 2001 – 2002, *Third United Nations Conference on the Least Developed Countries*, Brussels, Belgium 14 – 20 May, Document A/CONF.191/L.18, 20 May 2001.
66. UN (2001a). Making New Technologies Work for Human Development, UNDP *Human Development Report 2001*, pg. 274; Oxford University Press, NC27513,USA.
67. White Paper (1997).Eliminating World Poverty: A Challenge for the 21st Century, UK
----*http://www.dfid.gov.uk/public/what/engwptxt.html*
68. Wilford, J.N. (1997). Tracing Origins of Farming - Genetic Studies Fix Locations, *International Herald Tribune*, 20 November, Paris, France
69. World Food Programme (2001). WFP Hunger Map ---- *http://www.wfp.org/map-request.htm*
70. WHO. (2000). Release of Genetically Modified Organisms in the environment: is it a health hazard?, WHO/EURO-ANPA Seminar Report, WHO European Centre for Environment and Health, Rome Division, Rome, Italy 7 – 8 September, pg. 17.
71. World Meterological Organization –WMO. (2000) ----Inter-agency Task Force for the Horn of Africa: Long-term Commitment to Fight Hunger and Poverty----*http://www./wmo.ch/web/press/press651.html*
72. Zhang, Q. (1999). *China: Agricultural Biotechnology Opportunities to Meet the Challenges of Food Production, In:* Persley, G. J. and Lantin, M. M. (see above).
73. Zweifel, H. (1995. Modern biotechnologies in agriculture: Impact on women in the South, *Biotechnology and Development Monitor***,** No. 23, pp. 10 – 13.

Biotechnology in the Islamic World: Challenges and Opportunities

ANWAR NASIM* and ZABTA K SHINWARI
COMSTECH
Islamabad, Pakistan

1 BACKGROUND

Since 1975, genetic engineering has initiated a profound revolution in science with enormous technological and social consequences. This laid the foundation of New or Modern Biotechnology. This last era is neither constrained by nor dependent upon the scientific experience of the past, but is based on a totally novel technology, particularly recombinant DNA technology - the technology that has helped us to embark upon what may lead to yet another industrial revolution!

According to the 2002 report of the National Centre for Food and Agricultural Policy, in 2001, the eight transgenic crop varieties adopted by US growers increased crop yields by 4 billion pounds, saved growers $1.2 billion by lowering production costs, and reduced pesticide use by 46 million pounds. Biotechnology-based animal health products and services are estimated to be worth $2.8 billion and are expected to grow to $5.1 billion by 2005. At the end of 2001, there were 2,494 different biologics available for use against 197 different animal diseases. Modern Biotechnology has already made significant contribution to the health and agriculture sector. Development of several drugs, various pharmaceuticals, and vaccines using recombinant DNA technology has given rise to multi billion dollar industry. In addition, PCR-based diagnostics has also emerged as an important component of health care.

Biotechnology can make an important contribution to national prosperity, particularly in the area of human health (including HIV/AIDS, Malaria, Hepatitis, reproductive health, maternal and child health, typhoid and TB), besides food security and the environment. In pursuit of these priorities, OIC member states are fortunate in that they can be guided by the experiences of several developed and developing countries.

In 2002, 57 OIC members collectively published 4,026 scientific papers (on agriculture, biology, and environmental sciences), which was less than a single developing country - India – which published 4,096. Compared to 111,865 life science publications in the USA in 2002, OIC countries had just 5,512; a paltry

* Fellow of the Islamic Academy of Sciences; Adviser Science, COMSTECH Secretariat, 3-Constitution Avenue G-5/2, Islamabad, Pakistan. Email: anwar_nasim@yahoo.com

5% of what the USA did. Even tiny Switzerland published more, 6063, than our combined efforts.

OIC countries should anticipate and respond to current and future opportunities and challenges of biotechnology. There is however hardly any printed material specifically addressing the status of biotechnology and its economic potential within the Muslim world, except a paper by Dasilva, 1997. The guidelines however for a biotechnology strategy have been discussed by *Shinwari et al.* (2003) in detail.

2 INTRODUCTION

Biotechnology simply is the use of living systems to give us better foods, medicines and other products. It is the DNA, the structure of which was described 50 years ago that is involved in all these innovations. During the last three decades scientists have developed a large number of extremely powerful techniques that provide mechanisms to manipulate DNA in a variety of ways. To illustrate the basic molecular biology, which provides the basis for this science, the central dogma of molecular biology is presented in Figure 1.

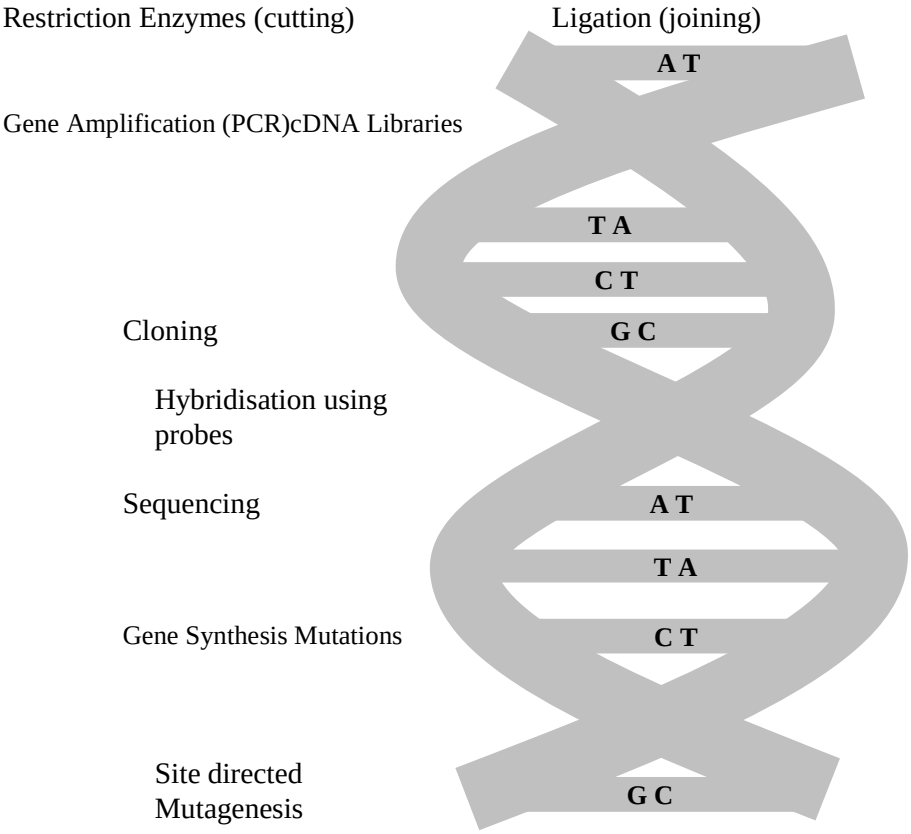


Figure 1. DNA manipulations.

Biotechnology has undergone a spectacular development and is now interacting with many disciplines of scientific research. It branched out into a wide variety of scientific and technological fields. Figure 2 below elaborates it graphically. Two powerful disciplines (Information Technology and Biotechnology) have given rise to hybrid science of Bioinformatics. Bioinformatics is the most recent example of this ongoing interaction between different disciplines.

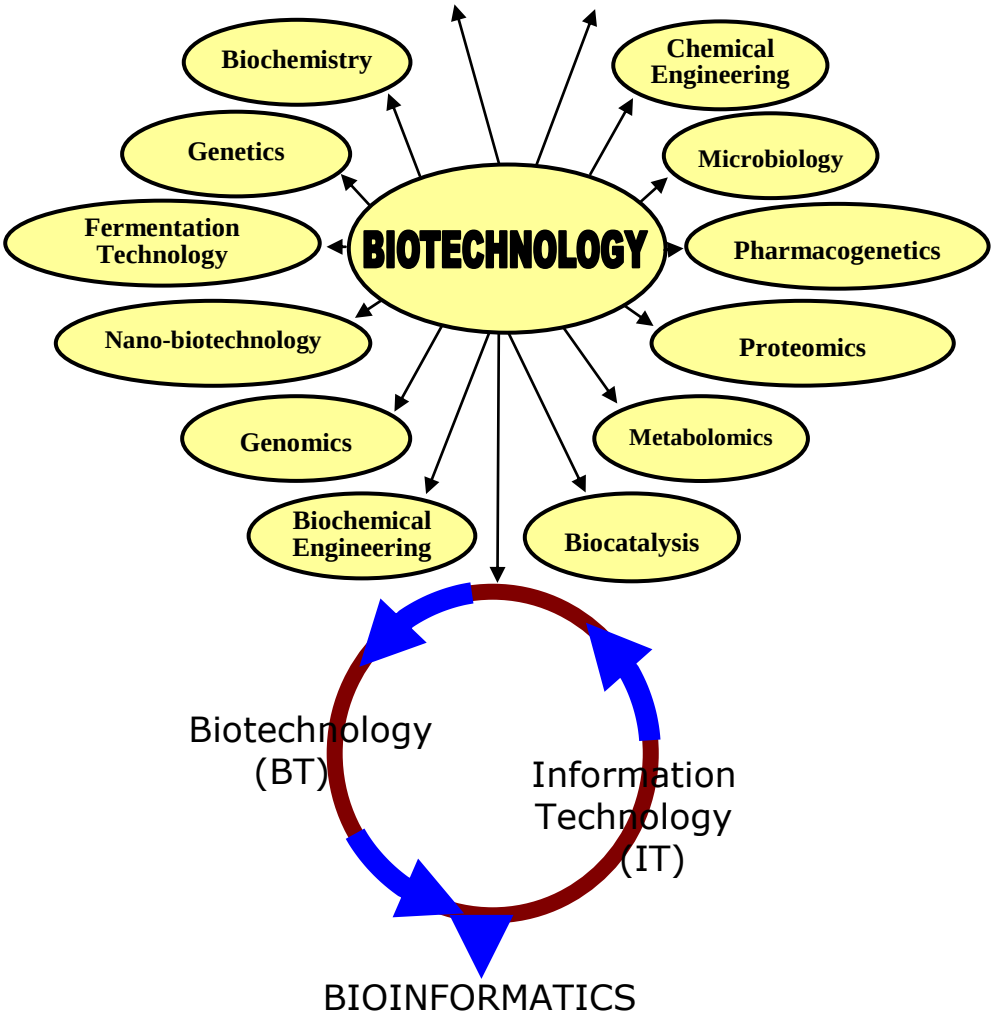


Figure 2. Biotechnology branching out.

Being a multidisciplinary science, biotechnology provides number of novel products including diagnostics, vaccines, pharmaceuticals, transgenic animals, transgenic crops and growth hormones etc (as shown below) Figure 3.

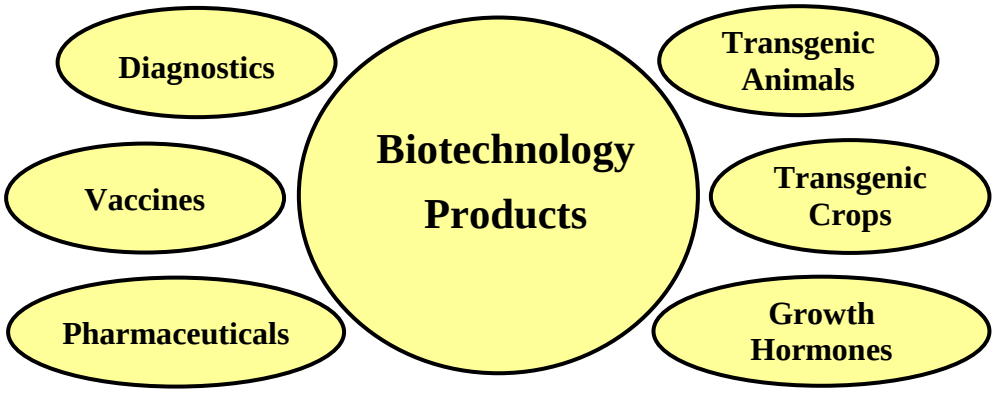


Figure 3. The products of Biotechnology.

As a result of the above two aspects, biotechnology has become a pivotal tool for socio-economic development. In this context, the concept of sustainable development has become a priority for the world's policy-makers. The Islamic world is no exception to this. It is thus crucial that the policy makers in the Muslim world focus continually on the concept of sustainable development to ensure desirable quality of life for future generations. Among the broad range of technologies potentially supportive to reach the goal of sustainability, biotechnological means and methods could take an important place in at least four fields:

- Food Production;
- Renewable energy;
- Pollution prevention; and
- Bioremediation.

Biotechnology, the application of an explosion of biological knowledge, gives humankind the ability to alter the structure of life itself. Whether biotechnology proves to be a miracle or a menace depends on how it is used and controlled? Biotechnology is virtually applied at all levels; even the difficult taxonomic problems are solved through DNA comparisons (*Shinwari, 1995*).

3 BIOTECHNOLOGY IN ACTION: OPPORTUNITIES

3.1 Biotechnology in agriculture

We have always relied on plants and animals for food, shelter, clothing, and fuel, and for thousands of years we have been changing them to better meet our evolving needs. Society's demand for resources provided by plants and animals

will increase as the world's population grows. The global population, which numbered approximately 1.6 billion in 1900, has surged to 6 billion and is expected to reach 10 billion by 2030. The United Nations Food and Agriculture Organization (FAO) estimates world food production will have to double on existing farmland if it is to keep pace with the anticipated population growth.

Biotechnology can help meet the ever increasing need by increasing yields, decreasing crop inputs such as water and fertilizer, and providing pest control methods that are more compatible with the environment. For centuries, farmers have made improvements to crop plants through selective breeding and, more recently, hybridisation – the controlled pollination of plants. Plant biotechnology is an extension of traditional plant breeding with one very important difference: It allows for an easier transfer of selected traits or character in a more precise and controlled manner than conventional cross breeding (Figure 4).

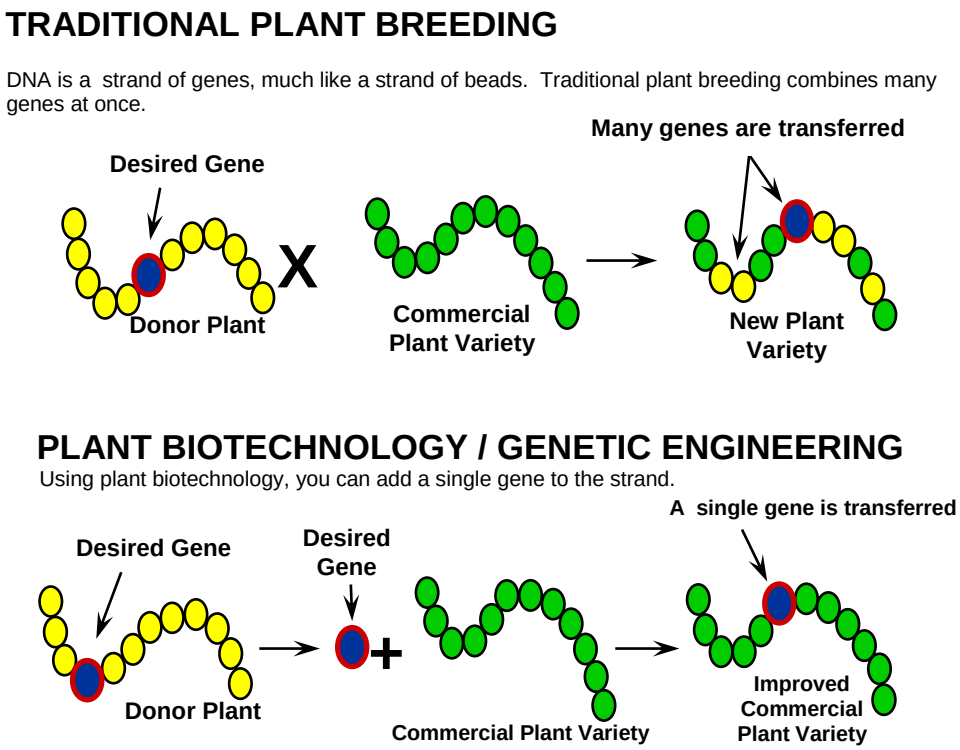


Figure 4. Traditional plant breeding with a difference.

Unlike traditional plant breeding, which involves the crossing and subsequent selection of hundreds of genes in long ten-year cycles, plant biotechnology allows for the transfer of only one or a few desirable genes at a time, leaving all other characteristics on the plant unchanged. Thus modified

crops are referred to as “genetically modified” or “genetically improved” (*Nasim, 2001*).

Agricultural biotechnology has demonstrated the potential to enhance sustainable development and food security by increasing crop yields, reducing the need for chemical and water inputs, and increasing resistance to crop stress such as drought (Nakashima et al. 2000). It can also cut losses from spoilage, pests, and crop diseases, extend product shelf life, and deliver life-saving medicines and vaccines more affordably and accessibly to large sections of population. This new technology will be especially useful for developing countries with growing populations, food shortages, and land, water, and other resource constraints.

3.2 Novel products

3.2.1 Background

It is a combination of these two aspects, i.e. the multidisciplinary nature and the wide range of products produced which now, using genetic modification, make it possible to apply all this know how to achieve novel products. These are only a few examples…
Currently, we are using genetic modification to:

- Produce new and safer vaccines;
- Treat some genetic diseases;
- Provide new and better medicines (Insulin, Interferon, Growth Hormones.);
- Increase crop-yields;
- Improve food nutritional value;
- Increase livestock productivity;
- Develop biodegradable plastics; and
- Decrease water and air pollution.

Many firms and public institutions in the world are offering products and services that are the result of the new technology – BIOTECHNOLOGY. In the USA alone, there are 300 public (biotechnology based) companies with a market capitalization of $353 billion and an annual turnover of $22 billion. Moreover, the growth of biotech-based industry is not restricted to the developed countries. Developing countries such as Cuba, Brazil, India, and China have been quick to identify the potential benefits of the technology.

Development of a large number of diagnostics for major diseases, genetic disorders, cancer, tuberculosis, HIV, malaria, better understanding of the human brain from molecular to systems level to enable control and treatment of a large number of currently incurable neurological disorders and development of new generation vaccines including the DNA vaccine would move fast. It is this ability, which provides the basis for producing transgenic plants, transgenic animals, edible vaccines, and pharmaceutical products like Humulin, interferon and growth hormones.

3.2.2 The Cuban model

The example of Cuba is really worth looking at critically since this can serve as a possible model for the Muslim countries to follow.

With an initial investment of one billion US dollars, Cuba is now a world leader in biotechnology and has many products on the market including human vaccines (Table 1). With 35 national research institutes dedicated to health-related biotechnology and 25 agricultural centres, Cuba has generated a variety of biomedical and agricultural products. It now produces the world's only successful anti-meningococcal vaccine, which is patented worldwide. Other successful vaccines include those for hepatitis B and cholera (Table 2).

Table 1. Biotechnology in Cuba: The Milestones

1959	Triumph of the Cuban Revolution
1961	National campaign against illiteracy
1962	Cuban Academy of Sciences reactivation
1963	Scientific institutes of the Ministry of Health
1965	National Centre for Scientific Research (CENIC)
1981	House No. 149 (Leukocyte Interferon)
1981	Creation of the biological front
1986	Inauguration of the Centre for Genetic Engineering and Biotechnology (CIGB)
1990-96	Investment Program (around US$ 1 billion)
1992	Creation of West Havana Bio-Cluster of 52 facilities and more than 1,500 researchers

Table 2. The Cuban Biotechnology Strategy in brief

Huge Investment by Cuban Government
Biotechnology is part of the health care system programme
Domestic market as first priority
Based on Cuban scientists and professionals
Fully integrated institutions, from research to post-marketing follow-up
National collaboration instead of individual competition; coordination between institutions doing R & D and institutions applying results
"Spin off" companies derived from scientific or production institutions
Gaining international competition ability: quality, production volumes, cost, novelty, joint ventures

3.3 Sustainable Development

Economic prosperity, development and, more so, sustainable development are complex issues, which are the end-result of a whole set of interacting factors.

The concept of sustainable development is based on the conviction that it should be possible to increase the basic standard of living of the world's growing population, without unnecessarily depleting our finite natural resources and further degrading the environment in which we live. Emerging biotechnologies, based on new scientific discoveries, offer novel approaches for striking a balance between developmental needs and environmental conservation. Biotechnology is continuously and rapidly developing in an increasing number of sectors that improve the effectiveness of the way in which products and services are provided. However, the transfer and development of biotechnology, in an environmentally sound manner, requires a variety of conditions, including capital inputs that, in the case of many developing OIC countries, are not readily available.

One of the important aspects of biotechnology is its role in the sustainable development of various sectors. Biotechnology is one technology, which has rapidly developed over the past few decades and has great potential for solving many problems pertaining to agriculture, industry, environment and health which has direct relevance to the sustainable development (*Zechendorf 1999*).

3.4 Animals

In addition to research on plants that can result in treatments for human beings, scientists are also investigating ways to bioengineer animals to produce human medical treatments for disorders or diseases such as cystic fibrosis. There are efforts underway to modify sheep to be able to produce fibrinogen; a major component in blood clotting that may be used for wound treatment. Scientists are also using genetic engineering to try to use animal tissue and organs for transplantation into humans. With biotechnology, however, researchers may be able to genetically modify pigs to suppress a natural protein that causes the animal's tissue or organs to be rejected in humans. "Harvest on the Horizon," however, does not address any of the animal welfare, ethical or other policy issues that have been raised with respect to biotechnology research on animals.

Another area of animal research is the production of spider silk from genetically modified goats. Spider silk, ultra-strong materials that can be used in a variety of industrial products, including bullet-proof vests, are difficult to cultivate commercially. Industry researchers are now able to produce spider silk by harvesting a protein from the milk of goats engineered with a spider gene.

Moreover, it has been estimated that expected percentage increase in the annual demand for meat in the developed and developing countries in 2013 would be 15% and 114% respectively. Similarly, change in the demand for milk for the same period in the developed and developing countries will be 7% and 133% respectively (*Delegado et al., 1999*). Current traditional livestock production methods are unlikely to be able to meet the increased demands for food production of animal origin for human consumptions. "Gene Revolution" on the pattern of "Green Revolution" of the 1970s is essentially required for

massive increase in milk and meat production to address the problems of food security faced by millions of impoverished people.

Biotechnology aids have been applied in many areas including selection of high-yielding animals; mechanisms to increase successful pregnancies (hormones, artificial insemination, estruses / pregnancy detection tests); disease monitoring with vaccination of pregnant animals to protect their offspring from disease; disease monitoring with vaccination of neonatal offspring and prophylactic antibiotic supplements in feed to continue this protection; and growth promoters to maximize feed to continue this protection; and growth promoters to maximize feed conversion ratios.

3.5 Industrial Applications

Table 3. Industrial applications of Biotechnology in brief

The chemical industry Using biocatalysts to produce novel compounds, reduce waste byproducts and improve chemical purity.
The plastics industry To decrease the use of petroleum for plastic production by making "green plastics" from renewable crops such as corn or soybeans.
The paper industry To improve manufacturing processes, including the use of enzymes to lower toxic byproducts from pulp processes.
The textiles industry To lessen toxic byproducts of fabric dying and finishing processes. Fabric detergents are becoming more effective with the addition of enzymes to their active ingredients.
The food industry For improved baking processes, fermentation-derived preservatives and analysis techniques for food safety.
The livestock industry Adding enzymes to increase nutrient uptake and decrease phosphate byproducts.

4 THE OIC BIOTECHNOLOGY SCENARIO: A SNAPSHOT

The Islamic Foundation for Science, Technology and Development (IFSTAD) by mandate from the Fourth Islamic Summit at Casablanca (16-19 January 1984) organized several meetings in different OIC-member states to launch a number of Inter-Islamic networks, one of which was assigned *Biotechnology and Genetic Engineering* as it main area of interest.

The first meeting for the Inter-Islamic Network of Genetic Engineering and Biotechnology (INOGE) was held at Cairo (Egypt) during September 13 -14, 1986. The Network is an inter-state, non-political, and non-profit agency. It is an independent, autonomous, and self-governing institution under the umbrella of COMSTECH. Since its launch, INOGE has organized/ sponsored several related activities (training and thematic workshops), the most recent of which was entitled "Bioethics: How to adapt biotechnology to culture and values (Beirut, March 1-2, 2003)."

Earlier, COMSTECH held a workshop on "Recent Advances in Biotechnology" in Jakarta, Indonesia from 26-28 November 1990 to exchange information about the current state of development of biotechnology in member countries and to devise mechanisms that would promote cooperation among laboratories in the Muslim countries addressing similar goals (Proceedings 1990).

Recognizing the importance of biotechnology in development, COMSTECH organized a seminar in January 2000 in Islamabad on "Biotechnology for Development."

At a different level, some of the OIC member states are focal points of International Centre of Genetic Engineering and Biotechnology (ICGEB) that includes; Afghanistan, Algeria, Bangladesh, Egypt, Iran, Iraq, Jordan, Kuwait, Morocco, Nigeria, Pakistan, Senegal, Sudan, Syria, Tunisia and Turkey.

5 BIOTECHNOLOGY IN ACTION: CHALLENGES

5.1 General

In the recent past, scientific breakthroughs in biology opened unlimited and unimaginable opportunities to mankind. However, no opportunity is an unmitigated blessing and all such opportunities carry their risks and intellectual challenges. Some of the risks and challenges are technological while others are socioeconomic. It is the latter that poses greater threats and bigger challenges to OIC member countries. In the new global scenario, there is a need to set up our centres of excellence for biotechnology; the Biovalley initiative of Malaysia is a very good example.

5.2 The facts

(1) Many OIC member countries do not have the resources for the "state of the art" research. Hence they are dependent on the so-called technology transfer from developed countries. This does not come cheap either;

(2) There is the question of intellectual property rights. Unfortunately, while most of biological resources are located in developing countries, present day technologies as well as the required capital are available mostly in developed countries. Many people in the poor countries feel that they are being exploited. In many cases, they do have the knowledge for intelligent utilization of resources. Moreover, present day practices require production on a scale that is not conducive for conservation;

(3) Many OIC member countries have yet to develop their own guidelines and laws on biosafety while at the same time, the importance and consumption of genetically engineered foods continue to increase. There is an urgent need to institute appropriate legislation both on access and benefit sharing, as well as biosafety. The public must be kept informed about the possible benefits and risks of Biotechnology;

(4) The ultimate challenge is the need for governments to recognize the potential and the opportunity and to invest in technology acquisition, human resource development and incentives to move research and industry forward if they are to gain benefit from the development of this technology; and

(5) Biotechnology is moving so fast in different directions and at different speeds (Figure 5). New technologies, unheard of a few years ago, such as transcriptomics and metabolomics and a host of others, are offering new opportunities for scientific development and industrial development. The opportunity is definitely there. The challenge is how to grab the opportunity in the most cost effective way.

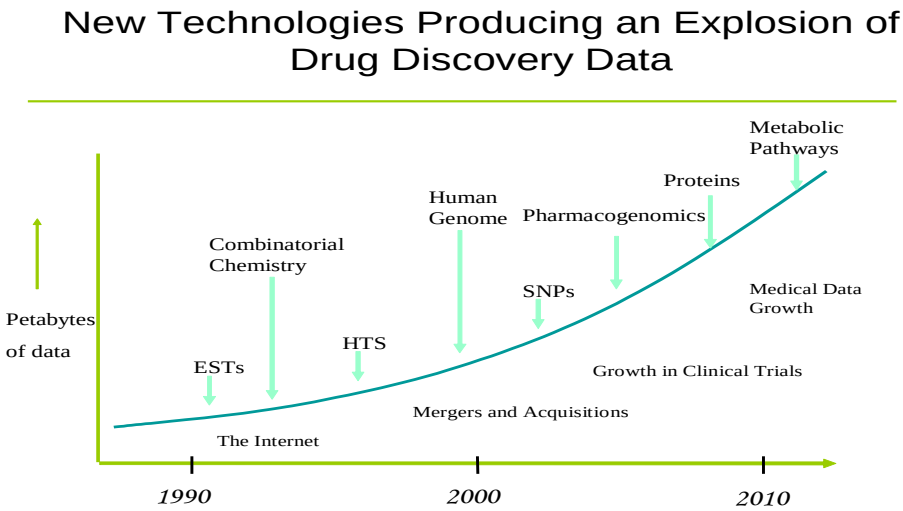


Figure 5. Drug discovery data.

5.3 Biotechnology potential for economic development

To realize the full potential of biotechnology as a frontline area of research and development with an overwhelming impact on society, Muslim countries have to nurture biotechnology at three distinct levels: Agriculture, Health, and Industry. The underlying agenda should be the utilization of research funds jointly and judiciously (*Shinwari et al. 2003*).

The biotechnology industry should include all the components that make use of the modern biological, rather than conventional techniques to develop commercial products for enhanced agricultural productivity; animal, and human healthcare; production of fine and value-added novel compounds and pharmaceuticals through genetically modified organisms; as well as food processing and environmental services. Moreover, the production of alcoholic solvents and compounds of industrial interest through fermentation processes is also regarded as biotechnology-based industry. Indeed, these processes fall under the category of conventional biotechnology and are important if raw materials are available in a country. While reviewing the scenario of the biotechnology industry in Muslim countries, this point has to be kept in mind.

The innovative capability of biotechnology is being looked upon as the pivot in maintaining/expanding the national share in the global economy. In the industrialized world, biotechnology has now revolutionized all major sectors of industrial activity. The exceptional dynamism of the science-technology interface is the main driving force of biotechnology. Under the present scenario, many advanced and sophisticated scientific techniques, technologies and methodologies are being employed daily not only in the industrialized world but also increasingly in the developing countries. This, however, by no means reflects the scientific and technological capacity of the developing world (*Tzotzos and Skryabin, 2000*).

To achieve significant success, biotechnological innovations have to be followed by a series of dynamic adjustments, both at institutional as well as at social levels, including technology transfer, financing, structural and strategic reorganization at enterprise level; improvements in skills by using educational schemes and also reorganization of science and technology policies.

In the developing countries like China, India, Brazil, Mexico and Pakistan, a working knowledge of the complex and multiple requirements that should be fulfilled to develop a vibrant, viable biotechnology-based industry, does exist.

Industrial sectors in many Muslim countries are growing at a very slow pace and relies mainly on an agro-based economy. Various Governments of Muslim countries have been encouraging the development of methodologies and institutions for acquiring the cutting-edge technologies like Biotechnology and Information Technology. However, the funding status has been such that support of expensive and high-priced technology has been financially prohibitive.

A major consideration for the need to invest in health sector biotechnology lies in the growing realization that most recent developments in the area of

vaccine and drug production are a direct result of investments in cell and molecular biology (*Hassan, 2001*). The unravelling of the human genome is set to lead to novel methods for evaluating and developing new drug products and targets employing advanced techniques of biotechnology and molecular medicine. These new products are rapidly replacing classic means of treatment and are predictably expensive. Slowly but surely, however, such products of genomics and biotechnology are replacing classic products and systems.

A notable example is the use of recombinant DNA technology to produce viral vaccines for example, like Hepatitis B and the formulation of drug products using DNA microarray chips. Already, India has embarked upon ambitious programmes in this field and has captured a major share of the pharmaceutical and biotechnology-based market. Failure or even lagging behind to invest in this field will result in Muslim countries becoming largely dependent upon imports for their health sector needs with a precarious supply system for vital products such as vaccines and other life saving drugs.

The greatest impact of biotechnology is in human health sector: 90% of all the biotechnology-based products on the world market are health related. Biotechnology is used for disease surveillance, diagnosis, treatment, and prevention. It permits the identification of disease agents where conventional means do not succeed, allows better tracking of pathogens, facilitates earlier detection of disease, and provides therapeutic products and processes.

Biotechnology is also used as a product base in the health industrial sector, and as an enabling technology in health sciences (*Evans and Relling 1999*).

In Canada, nearly 60 percent of Canadian biotechnology-based companies focus directly on health care. The industry is quite successful and employs over 8,000 people, mostly in knowledge-based positions. In recent years, its market capitalization has increased fivefold to US$10 billion; and its revenues have grown by over 45 percent in the same period. Three of Canada's biotechnology clusters (in British Columbia, Ontario and Quebec) rank among North America's top 20 centres by revenue and number of businesses. Canada ranks third in the world after the US and the UK in a US$20 billion industry that is expected to grow to US$50 billion by 2005.

Knowledge resource is one of Canada's recognized strengths. Biotechnology is a major part of the activities of a health research community that includes 30,000 investigators and technical personnel in 16 medical schools, 30 university departments, 75 research institutes, and numerous industrial firms producing pharmaceuticals, medical devices, fine chemicals, and novel foods.

5.4 Nanobiotechnology- a low-cost alternative?

Nanobiotechnology is a diverse field in which molecules and atoms are manipulated to create biomaterials, devices, and systems on the scale of one-billionth of a meter. Recent developments have resulted in a promising new method for detecting a wide range of genetic and pathogenic diseases, at the DNA level. The technique uses gold nanoparticles bound to DNA fragments,

which bind to DNA targets placed between two tiny electrodes. When the DNA probe and its target match, the gold particle closes the circuit and produces a detectable signal. Not only is this approach 100 times more sensitive than conventional diagnostic and detection methods (thereby by-passing the need for DNA amplification by PCR)- it also promises to be substantially more affordable, as much as 1000 times cheaper than then standard alternative (*Park et al. 2002*).

6 CONCLUSION

The history of human civilization has clearly demonstrated the pivotal role of science in the overall well-being and prosperity of human beings. There is no denying the fact that scientific discoveries do provide a double-edged sword whereby they can be an instrument both for destructive or for constructive purposes.

With the advent and progress of Internet access to information is no longer a problem. As a start, OIC states should remain in touch with the new developments. This will require an organized effort and also international cooperation. In addition, it is essential to spell out an implementation strategy in the OIC countries that will enable identification of specific plans, programmes and projects, with clearly defined tasks, estimates of necessary resources, and time targets. Creation of world-class facilities in carefully selected and nationally relevant fields should be undertaken, to enhance our international competitiveness in areas where we have strengths, opportunities, or natural advantages. *In order to encourage quality and productivity in science and technology, mobility of scientists and technologists between industry, academic institutions and research laboratories will be required.*

Women constitute more than half the population of the OIC countries. They must be provided significantly greater opportunities for higher education and skills that are needed to take up R&D as a career. For this, new procedures, and flexibility in rules and regulations, should be introduced to meet their special needs.

Science should move out of the research laboratories into private enterprises. Every effort should be made to achieve synergy between industry and scientific research. There has to be increased investments by industry in R&D in its own interest to achieve global competitiveness to be efficient and relevant. Efforts by industry to carry out R&D, either in-house or through outsourcing, should be supported by fiscal and other measures.

The process of globalisation is leading to situations where the collective knowledge of societies normally used for common good is converted to proprietary knowledge for commercial profit by a few. Action should be taken to protect our indigenous knowledge systems, primarily through national policies, supplemented by supportive international action.

In order to achieve significant success, biotechnological innovations need to be followed by a series of dynamic adjustments both at institutional as well as at social levels; including technology transfer, financing, structural and strategic reorganization at enterprise level (*Widdus 2001*); improvements in skills by using educational schemes and also reorganization of science and technology policies.

In developing countries like China, India, Brazil, Mexico and Pakistan, a working knowledge of the complex and multiple requirements that should be fulfilled to develop a vibrant, viable biotechnology industry, does exist. The real challenge is to take the existing knowledge and generate wealth.

A number of projects directed to specific sectors, locations-specific and based on natural resources, should be implemented. It is expected that these projects would generate employment opportunities in the rural areas, particularly for women. The biovillage concept (Swaminathan) would be significantly useful. Genetics counselling program would be expanded further.

For decision makers in the Muslim world, the real challenge is to make the right choices, define priorities and opt for very effective future strategies so that we can benefit from this newly acquired ability to manipulate the genetic material of living organisms. Suitable approaches can certainly lead to enhanced industrial and agricultural productivity. National Biotechnology strategy and Action Plan of Pakistan recently prepared by National Commission of Biotechnology can be used as a guideline.

At the level of the OIC, Muslim countries must link up with each other in the field of research, studies, expertise, information and biotechnology-related matters and work collectively to link the Islamic world with the rest of the world.

Unifying the Islamic perception of the production and use of biotechnology and sensitising the Muslim society about the definition of biotechnology, its applications, benefits, and dangers from a perspective that takes into consideration the ethical aspects of this technology, is also an important course of action that must be taken up by OIC-member countries.

REFERENCES

1. Dasilva, E. J. Biotechnology in the Islamic World, Nature Biotechnology. 1997. 15-733.
2. Delgado et al. Livestock to 2020. The Next Food Revolution:/ FPRI/FAO/ILRI. Food Agriculture and the Environment. Discussion paper 28. 1999.
3. Evans, W.E. and Relling, M.V. Translating functional genomics into rational therapeutics. Science 1999. 286: 487-491.
4. Hassan, M.H.A. Can Science save Africa? Science. 2001. 292: 1609
5. Malik, K.A. Nasim, A., Khalid, A.M. Biotechnology for Sustainable Development. National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan. 1995.
6. Nakashima, K.; Shinwari, Z.K.; Miura, S.; Sakuma, Y.; Seki, M.; Yamaguchi-Shinozaki, K.; and Shinozaki, K. Structural organization, expression and promoter activity of an Arabidopsis gene family encoding DRE/CRT binding proteins involved in dehydration- and high salinity-responsive gene expression. Plant Molecular Biology. 2000. 42 (4):657-665.
7. Nasim, A. Biotechnology for sustainable development. Science Vision. July-December 2001. Vol. 7(1-2):19-26.
8. Park, S.J. Taton, TA & Mirkin, C.A. Science. 2002. 295 (5559): 1503-06.
9. Proceedings of the Workshop on “Recent Advances in Biotechnology.” 1990. COMSTECH 3-Constitution Avenue, G-5/2 Islambad, Pakistan.
10. Shinwari, Z.K. Congruence between morphology and molecular phylogeneties in Prosartes (Liliaceae). 1995. Pak. J. Bot. 27(2): 361-369.
11. Shinwari, Z.K.; Nasim, A. and Rehman, A. Biotechnology: Opportunities and Challenges for OIC Countries (An Overview). 2003. COMSTECH-Islamabad-Pakistan.
12. Tzotzos, G.T. and Skryabin, K.G. 2000 Biotechnology in the Developing World and Countries in Economic Transition. CABI Publishing, Wallingford, UK.
13. Widdus, R. Public private partnerships for health: their main targets, their diversity, and their future directions. Bulletin of the WHO. 2001. 79: 713-720.
14. Zechendorf, B. Sustainable development: How can biotechnology contribute? Trends in Biotechnology. 17(6):219-225, 1999.

Biotechnology for Developing Countries: Challenges and Opportunities

SALLEH MOHD NOR*
and
P N AVADHANI
TropBio Research Sdn Bhd
Kuala Lumpur, Malaysia

1 INTRODUCTION

The history of human development is a very fascinating story. The present phase of our developmental history started with the industrial revolution when coal, the sunlight energy stored in the earth's crust, was mined and used to fuel the steam engine. Urban-based industries that started at that time uprooted traditional rural livelihoods and homes and created urban slums. Cement foundations were laid to create the urban metropolises. Scientists and engineers became the centre of development and their technologies and ideas were revered. Chemistry and physics played critical roles. The world of the atom and electron was unravelled and resulted in the atomic bomb. Chemists discovered new chemical processes in the understanding o our natural world, while physicists and engineers invented new modes of travel. Later while physicists and engineers sent Man to the moon, chemists were busy developing new products from a new hydrocarbon fuel, petroleum. New chemicals were made, plastics was invented and petro-chemical industries flourished. At the same time, flight became available to the common man, and the age of information technology has seen our lives changed in many ways. The computer has revolutionalised the way we live and communication has seen phenomenal development. They have changed the lives of everyone on this earth.

The 19th Century has been identified as the century of chemistry, during which numerous chemical discoveries were made, particularly the development of gunpowder. The 20th Century has been named the century of physics, although both chemistry and physics played important roles in both centuries. We are now into the 21st Century and already, it has been mentioned that this century is the century of biology or of the life sciences. During this century, human life is expected to change with developments in the biological sciences, which will be made possible by the integration of both chemistry and physics. With the foreseeable depletion of fossil fuels and the impending problems of the

* Secretary General, Academy of Sciences Malaysia.

global environment, the human race has to look to new ways of managing the environment and creating economic wealth in a more sustainable manner.

The biological resources of the world form the foundation of the ecosystems within planet earth, whether it is the sea or the land; whether it is deserts or mountain tops; whether is water or even the air. Biological life takes different forms, from microscopic microbes to large magnificent trees and animals. Some can travel fast while others are more docile or even stationery. The basic chemical process that supports life is photosynthesis, which converts light energy with the help of chlorophyll into starch and carbohydrate. Plants and trees form the greatest biomass of terrestrial ecosystems while fishes and corals form the bulk of marine ecosystems. Together all life on earth, including the human race, constitutes the global biodiversity wealth. All life depends on that biodiversity for survival. However, the use and consumption of non-biological and non-renewable products by the human race has increased over the last few decades, which resulted in numerous environmental and health problems.

2 BIODIVERSITY WEALTH

Biodiversity is the sum of all the different species of animals, plants, fungi and microbial organisms that live on planet Earth. Estimates of total species of life on Earth exceed 10 million and some estimates are even as high as 100 million. The greatest owners of biodiversity wealth are the developing tropical countries, where the largest diversity of plants and animals exist. Wilson (1992) in his book “The Diversity of Life,” gives an excellent description of the wealth and issues of global biodiversity. As an illustration of the relative diversity of life in different climatic regions of the world, he gives a comparison of breeding bird species in various countries:

Greenland -	56
Labrador -	81
Newfoundland -	118
New York State -	95
Guatemala -	469
Colombia -	1525

Some 30% of the world’s 9000 bird species occur in the Amazon basin and another 16% in Indonesia. The above is a clear illustration of the richness of tropical biodiversity. The difference in plant biodiversity between the northern temperate regions and the tropical regions of the world, are just as great. Wilson further states that vascular plants, which include the higher and lower plants, comprise 99% of land vegetation and out of nearly 250,000 species, about 68% occur in the tropics and subtropics, especially in the rainforest. The density of species can be phenomenal. Peter Ashton (in Wilson, 1992) is quoted as having found over 1000 species in ten selected one-hectare plots in Borneo, while

Alwyn Gentry found 300 species in each of two one-hectare plots in Peru. Compare these to only 700 native species in the whole of United States and Canada, including the species in the Florida everglades.

Even deserts, although appearing barren and dry, have life forms that are adapted to the extreme conditions of life in such dry and sandy habitat. Desert plants have evolved ways of conserving and efficiently using water available in limited quantity. Some flowering plants are very ephemeral, living for a short period only. Their seeds can remain dormant for long periods only to germinate, grow, bloom and complete their life cycle when that irregular rainstorm occurs. Woody desert plants have long root systems to look for deep water or spreading shallow roots that are able to quickly absorb any moisture from the occasionally rain or dew. They are specialists in many ways and these properties enable them to survive under difficult conditions.

3 UTILISATION OF BIODIVERSITY

The human race has depended on the biological wealth of the planet from the moment it came on earth. In the cycle of life, some organisms are food for others. All organisms need basic resources to continue to survive. Over the centuries, a complex web of life has been established. In a stable ecosystem, the various components are delicately and optimally balanced. Essential resources are constantly regenerated and they in turn, nourish various life forms including humans. However, when such balance is disturbed, living organisms loose their ability for survival. The advent and proliferation of the human species has created a situation somewhat close to this scenario. However, man with his intelligence has developed strategies, skills and tools to overcome the odds.

Food started with wild plants and animals before the discovery of fire when cooking became a norm. The transformation from being hunters and gatherers to an agrarian society has led to the development of agriculture as an industry. Using fertilizers and pesticides, hormones, breeding, and post harvest storage are some of the skills that have been developed. Wilson says that perhaps 30,000 species of plants have edible parts, and about 7,000 have been grown or collected as food, but only 20 species provide 90% of the world's food, and just three, wheat, corn, and rice, constitute more than 50% of that food. Cereals, pulses, oils, nuts, fruits and spices are some of the important food products derived principally from plants. The variety of the human diet has changed significantly and now processed foods are becoming common, but they are still derived from either plants or animals, which are part of the global biodiversity.

Traditional medicines depend largely upon plants and animal products, whether it is the rhinoceros horn or the ginseng or the thousands of various concoctions of the village medicine man. Modern nutraceuticals have evolved from the traditional medicines and now health foods and products derived from plants and other natural products fill the shelves of pharmacies all over the world. Traditions such as the Chinese traditional medicine, the Indonesian jamu,

the Indian ayurvedic medicine and numerous traditional medicines from Africa and Latin America, play an important role in the livelihood, health, economics and traditions of these peoples. They form an integral part of their culture and traditions over the centuries. Modern technologies, astute marketing and modern economics have made them more readily available to communities worldwide.

However, the main beneficiary of such traditional knowledge has been the developed world through the use of new and modern technologies owned by them. For example, the humble rosy periwinkle (*Catharanthus roseus*) of Madagascar is an inconspicuous plant with a pink five petaled flower. It produces two alkoloids, vinblastine and vincrastine, that are used to cure most victims of Hodgkin's disease and acute lymphocytic leukemia, two very deadly cancers. Income from the sale of these two substances exceed US$200 million per year. Yet, the owners of that resource, the people of Madagascar, have not directly benefited from their biodiversity wealth.

The Neem tree (*Azadarachta indica*) is an important plant in the annals of medicine and folklore of India. People have used the twigs to clean their teeth, cleared skin disorders with its juice, taken neem tea as tonic, cured pains, fevers, infections and other complaints with the leaves and used neem as environmental biodegradable insecticides. In short, the neem tree is the "village pharmacy."

There are numerous other examples such as caffeine from tea (*Camellia sinensis*), camphor from the camphor tree (*Cinnamonium camphora*), cocaine from the coca tree (*Erythroxylon coca*), menthol from mint (*Menta* species), penicillin from the Penicillium fungus (especially *Penicillium chrysogenum*), quinine from the Yellow cinchona (*Cinchona ledgeriana*), and taxol from the Pacific Yew (*Taxus brevifolia*). An insignificant looking weed, *Rawolfia serpentiana*, is the source of reseprine used for hypertension.

Many modern medicines are also derived from plants. Aspirin, the most widely used pharmaceutical, was first derived from salicylic acid discovered in meadowsweet (*Filipendula ulmaria*). In the United States a quarter of all prescriptions dispensed by pharmacies are extracted from plants, another 13% from micro-organisms and 3% from animals.

Thus, the human race has depended tremendously upon the plant biodiversity wealth for their livelihood, economic development, health and survival. Off course, animals have been an important component of their diets in many parts of the world through the domestication of such animals as chicken, ducks, cattle, sheep, goats and pigs. Besides meat, some of these animals supply milk and dairy products, which are important components of the human diet and the use of biotechnology in the dairy industry is well known. However, the range of animal products as food is relatively more limited than plants, and now there is an increasing movement to refrain from eating meat. Nevertheless, they continue to be an important part of the food industry.

4 BIOTECHNOLOGY

Biotechnology has been defined in many ways, such as:

- The scientific manipulation of living organisms to produce useful products;
- The manipulation of biological organisms to make products that benefit human beings;
- The industrial use of living organisms to manufacture food, drugs and other products;
- Any technological process where biological systems are utilized;
- Any technological application that uses biological system, living organisms, or derivatives thereof, to make or modify products or processes for specific use (as defined in the United Nations Convention on Biological Diversity); and
- The application of in-vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection. (as defined in the Cartegena Protocol on Biosafety).

However, the modern understanding of biotechnology is the use of the knowledge of biological systems to produce goods and services. The key is the use of living organisms to produce useful products with the use of various technologies.

Biotechnology as a science has existed since ancient times. Diverse strains of plants and animals were crossed or hybridised to produce greater variety and better quality organisms. The classic experiments of the monk, Mendel (1822-84), was the beginning of understanding breeding systems when he discovered the Laws of Inheritance through his controlled pollination of garden peas. Corn was one of the first food crops to have been cultivated and improved by breeding. The classical use of fermentation to produce cheese, yoghurt, alcohols and a range of other foods, is an ancient food technology. People from many cultures have used biological processes, in a controlled way, to produce foods, dyes, fuels, adhesives, paper and fertilizers. Biological processes have the advantage of being optimal in the usage of space, time and energy. Thus, the advantage of using biological systems to derive products including food is an attractive proposition.

In the 1960s, hybridisation and selective breeding was used to increase food production and the quality of wheat and rice. American scientist, Norman

Borlaug, who spearheaded the program, was awarded the Nobel Peace Prize in 1970, in recognition of the important contribution of increasing food to world peace. Zakri and Taeb (2002) give a brief but good history of the development of biosciences from the evolution of species to the development of modern biotechnology.

5 MODERN BIOTECHNOLOGY

The modern era of biotechnology started in 1953 when American biochemist James Watson and British biophysicist, Francis Crick, presented their double helix model of the DNA, which won them the Nobel Prize in chemistry in 1962. This was followed in the 1960s by the Swiss microbiologist Werner Arber's discovery of the special enzymes, called restriction enzymes, in bacteria, which can cut the DNA strands of an organism at precise points. Then in 1973, American geneticist Stanley Cohen and American Biochemist Herbert Boyer removed a specific gene from one bacterium and inserted it into another bacterium using restriction enzymes. This marked the beginning of recombinant DNA technology, commonly called genetic engineering. In 1977, genes from other organisms were transferred to bacteria, which eventually led to the first transfer of a human gene to *Escherichia coli* bacteria. The transgenic bacteria were able to produce a human hormone along side its own normal chemical compounds (Levine, 2002). The first DNA recombinant commercial product was human insulin for the treatment of diabetes in 1982.

Since then, modern biotechnology has developed by leaps and bounds, with a tremendous development of basic discoveries, new processes, new technologies, new techniques and new products. This has been made possible by the availability of advances in other sciences, especially physics, chemistry and with the support of computer technology, or more commonly known as information technology.

In the field of medicine, progress in molecular cellular biology and biotechnology has altered the traditional concept of drugs based upon organic chemistry. The progress of medical research has been summarized as the periods of the microbe hunters (1900-1920), the vitamin hunters (1920 -1940), the enzyme hunters (1940-1970) and the gene hunters (from 1970). The Human Genome Project, was a large global cooperative project undertaken by many laboratories and has resulted in the draft human genome published in 2001. This was a true milestone in medical research where over 30,000 genes were mapped. In addition to small compounds, proteins (biotech drugs), peptides, DNAs, RNAs (gene therapy) and cells (cell therapy) have been added to the drug catalogues. About 500 genes have been listed as drug targets until recently. However, the wealth of information provided by the human genome analysis has dramatically changed that picture and the number has increased dramatically to over 30,000 genes. They are replicated, recombined, repaired, spliced and translated into proteins in response to various stimuli. (Arai, 2002). Stem cell culture research is now a controversial topic with some countries banning such research while others are supporting it.

In the field of agriculture, biotechnology has been seen as the only hope for solving the problem of feeding the human population, which is predicted to increase to over 10 billion by the year 2050 from the current 6 billion. Moreover, the widespread occurrence of malnutrition with an estimated 50% of child death being due to malnutrition, offers new challenges to using biotechnology in increasing the quality of foods. As land and water resources are limited, productivity must be increased, and biotechnology is seen as the possible saviour. Over the past decades, modern biotechnology has changed the face of agriculture. Rapid large-scale clonal propagation through tissue culture has helped the production of large numbers of planting materials. New varieties of plants with new and beneficial traits and properties, such as resistance to various pests and diseases, have been produced. Genetically modified crops are now been planted in over 50 million hectares worldwide, compared to only a couple of million a few years ago, with genetically modified maize, cotton, tomato, soya bean and potato being the most widely planted crops. Biotechnology has helped reduce the use of agro-chemicals, water and fertilizers.

There are many examples in the use of biotechnology to improve crops. As rice is the staple crop of over 3.5 billion people in Asia, the success of producing the "golden rice" which was genetically engineered by Ingo Potrykus and Peter Beyer in 1999, to produce beta-carotene, without which the human body cannot produce vitamin A, may result in saving an estimated 100 to 150 million children in Asia who suffer from lack of vitamin A (Ola and D'Aulaire, 2002). Furthermore, plants are now looked upon as bio-factories, and bio-farming for enzymes and possible pharmaceuticals are being vigorously researched into. Developments in fisheries and animal husbandry have been just as phenomenal, culminating with the cloning of the sheep "Dolly."

Forestry has used biotechnology particularly in the area of mass propagation through *in vitro* plant propagation or tissue culture. This technology is not only important in the mass propagation of elite planting materials, but also in the conservation of threatened and endangered species. The development of genetic markers is also an important area for forestry. Due to the long maturity periods in forestry, use of biotechnology is both a challenge and an excellent opportunity that needs to be further developed (Krishnapillay, 2002)

The global environment has been a major area of concern and the recent World Summit on Sustainable Development in Johannesburg (Rio + 10) gave serious attention to addressing the many issues of the global environment and sustainable development. Environmental biotechnology has played and will continue to play an important role in ameliorating environmental degradation and pollution. The development of bioreactors, which rely on the metabolic versatility of mixed microbial populations, have enabled the treatment of domestic waste water and sewerage and the elimination of many water borne diseases. The proper management of landfills and proper composting have improved the disposal of solid wastes. With industrialization, the production of new and hazardous waste products have resulted in the use of biotechnological

systems that use biological catalysts to degrade, detoxify or accumulate contaminating chemicals through the process of bioremediation (Smith, 1996). Biotechnology has now been used in pollution clean-up, control and protection. Furthermore, advances in agriculture and forestry, with the use of biotechnology have reduced the input of chemicals into the soil and underground water, thus contributing to environmental protection. Possible development of saline resistant and water stress resistant plants can provide hope to green degraded and semi-arid lands of the world, and bring them into productivity.

Rifkin (1998) speaks of seven strands that make up the operational matrix of the Biological century based upon biotechnology, which he calls the Biotech Century.

(1) The ability to isolate, identify and recombine genes is making the gene pool available as the primary raw resource for future economic development. Recombinant DNA techniques and other biotechnologies allow scientists and biotech companies to locate, manipulate and exploit genetic resources for specific economic ends;
(2) The awarding of patents on genes, cell lines, genetically engineered tissue, organs and organisms, as well as the processes used, gives the market place the commercial incentives to exploit new resources;
(3) The globalisation of economics and trade has seen the development of a global life science industry beginning;
(4) The success in the mapping of the human genome has resulted in new technologies in genetic screening and other technological innovations;
(5) A spate of new scientific studies on the genetic basis of human behaviour and the new socio-biology that favours nature over nurture, which can support the cultural acceptance of the new biotechnologies;
(6) The development of the computer allows the organization and management of the masses of genetic information. Computational and genetic technologies are being fused together into a powerful new technology; and
(7) New ideas about nature provide the legitimising framework for the Biotech Century, by suggesting that the new economy and society are amplifications of nature's own principles and practices.

Rifkin concludes that genes, biotechnologies, life patents, the global life science industry, human-gene screening and surgery, the new cultural currents, computers, and the revised theories of evolution, are beginning to reshape the world.

6 BIOTECHNOLOGY IN BUSINESS

Modern biotechnology has resulted in a new breed of businesses based upon biology. Dolven (2000) mentioned that genetic discoveries and other advances in life sciences are set to turn biotechnology into big business, and that Asia will benefit. He mentioned that during the first quarter of year 2000, more than US$15 billion flowed into publicly listed biotech ventures in the United States. The U.S. National Institute of Health funds US$17 billion in research each year and the ten largest pharmaceutical companies spend over US$15 billion annually, mainly in U.S.A. and Europe.

Chong and Mutsuko (2000) mentioned that the mapping of the human and other genomes have generated high expectations and "buzz" about biotech ventures. Chong and Mutsuko also reported that funds had increased for research and countries from China to India are preparing themselves for the biotechnology boom. South Korea for example is expanding its biotechnology research with funds increasing by 43% in 1999 to US$142 million but still small compared to expenditures in Japan (US$ 4.6 billion) and the U.S.A. (US$18 billion). Singapore has its bioplan mapped out working to be a regional centre for clinical trials, including launching an ambitious Singapore Genomics Program to develop new drugs and customized treatments. Taiwan has similar plans and has set up incubators and research centres to create new products and spin off new biotech companies. Japan has set a goal to turn biotech into a US$234 billion business by 2010 and has embarked on aggressive plans towards that end.

There are currently an estimated 1500 bioventures in the United States, 1300 in Europe and 1200 in Asia. The bioventures in the U.S.A. have an estimated capital of over US$300 billion and an annual revenue in excess of US$30 billion. Biotechnology has been talked about as the "Next Big Thing" in Asia. The number of biotech start-up companies in Asia has grown to about 1200 in the last four years (FEER, 2002). South Korea has the largest number with 300, followed by Israel 200, Japan 130, China 100, Taiwan 100, Hong Kong 50 and Singapore 30. Malaysia has about 20 so far but has launched a program of development called the "BioValley" to promote biotech research and business development. Similarly, Singapore has set up a "BioPolis" to be the hub of its biotech development (Tan, 2002). On the other hand, Indonesia has planned the development of a "Bio-Island" on the island of Rempang, which is due to be launched in 2004 (Islamet, 2002). Besides attractive physical infrastructure, the Bio-Island will also be a tax haven for investors.

A special supplement to the journal Nature Biotechnology (Nature Biotechnology, 2000) produced an industry trends supplement where 18 industrial trends in biotechnology were listed. Platform technologies such as bioinformatics, data mining, signal transduction, pharmacogenomics, biochips, proteomics, combinatorial chemistry, xenotransplantation, tissue engineering and agricultural biotechnology are discussed. This clearly illustrates the huge spillover of the biotechnology into other industries, based upon electronics, the

computer, information technology, physics and chemistry. Eight diseases are also discussed including asthma, arthritis, cardiovascular diseases, cancer, malaria and drug discovery. The supplement discussed each platform technology and disease in terms of their historical development, the current state, selected companies involved in the industry, challenges to the industry and future directions. It is estimated that the total burden, direct and indirect, of the eight diseases mentioned was a staggering US$500 billion annually and the hope is that biotechnology will help solve some of the problems faced by these diseases. As an illustration of the market for some of the platform technologies, the market for biochip-based data gathering and analysis alliances was US$500 million in 1998 and is expected to grow to US$4 billion in 2002. Pharmacogenomics data gathering and analysis alliances are expected to grow from US$1 billion in 1998 to US$3.5 billion in 2002. In the field of tissue engineering, over 25 companies are involved in the business in 2000. The supplement further concludes that tissue engineering is a vibrant industry with a huge potential market. The field of agricultural biotechnology saw sales of transgenic crops being estimated to be US$1.6 billion in 1998 and rose to more than $2.2 billion in 1999.

Foo (2002) gives eight systematic factors for the development of successful biotechnology industries, and they are:

- Strong Government support;
- Strong research base;
- Intellectual property protection;
- An entrepreneurial culture;
- Access to infrastructure, network and clusters;
- Ability to form alliances;
- Global alliances; and
- Access to capital.

7 CHALLENGES AND OPPORTUNITIES FOR DEVELOPING COUNTRIES

In the recent past, scientific breakthroughs in biology opened unlimited and unimaginable opportunities to mankind. However, no opportunity is an unmitigated blessing and all such opportunities carry their risks and therefore challenges. Some of the risks and challenges are technological while others are socioeconomic. The latter pose greater threats and bigger challenges to developing countries. This does not mean that there are no technological challenges, but more often than not, these also tend to be socioeconomic in nature.

In the first place, many developing countries do not have the resources for the “state of the art” research. Hence, they are dependent on the so-called technology transfer from developed countries. This does not come cheap either.

Secondly, there is question of intellectual property rights. Unfortunately, while most of biological resources are located in the developing countries, present day technologies as well as the required developmental capital are available mostly in developed countries. Many people in the poor countries feel that they are being exploited. In many cases, they do have the knowledge for intelligent utilization of resources but it is not suitable for contemporary manufacturing and marketing. More over, present day practices require production on a scale that is not conducive for conservation. Thus ironically, developing nations loose out both due to having wisdom as well as lack of knowledge.

If we take the example of genetic engineering, which is at the forefront of biotechnology, we can see this conflict clearly with respect to opportunities as well as challenges. On the one hand, it provides tremendous opportunities in feeding the hungry world, in curing the sick and in providing a comfortable life to many poor and unfortunate people in the world, but the returns are more to the affluent. This by itself is not necessarily a bad thing for it shows there is something for everybody if only the world learns how to share resources and rewards. However, as per the norm of the day we will be anxious to reap the rewards as soon as possible and reluctant to share them. Thus, we end up with unsustainable and unstabilised products whose benefits in the long run may be questionable. At the moment, the transfer of genes is effected through vectors where there is a rapid turn over. This rapid turnover can destroy the stability. There are many genetic and environmental problems associated with such un-sustainability. Only time can tell the real value. It depends on how well we can use the opportunities and how rationally we can meet the challenges.

One of the basic issues to be addressed in developing countries is the issue of access to the genetic resources, which these countries are custodians of. The Bonn Guidelines on Access to Genetic Resources was adopted by the Conference of Parties of the UN Convention on Biodiversity (UNCBD) in April 2002. It provides guidelines for access to bioresources but most developing countries have not put in place proper legislation to encourage the use of bioresources while at the same time, protecting the country's rights to the benefits that may accrue. Issues of prior informed consent and mutually agreed terms are discussed, as well as intellectual property of traditional knowledge and farmers' rights. Unfortunately, some countries have taken an extreme protectionism stand and made it very difficult for researchers and potential investors to gain access to bioresources, while others are so lax that the possibility of abuse by collectors of bioresources may happen.

The other issue is the issue of biosafety. The Cartegena Protocol on Biosafety is the first protocol under the UNCBD and was adopted in January 2000. However, many developing countries have yet to develop their own laws on biosafety while at the same time, the import and consumption of genetically engineered foods continue to increase. Therefore, there is an urgent need for developing countries to institute appropriate legislation on access and benefit sharing, as well as biosafety. The public must be kept informed on what they are eating and how biotechnology can benefit them.

The greatest challenge is how to jump on the biotechnology business bandwagon without falling off. Research is expensive, long term and the technology belongs to the developed world. Specialized human resources in specific technological areas are lacking. At the same time, developing countries have the rich bioresources. Developing strategic alliances is the only way to gain access to technology while protecting the bioresources and ensuring some benefit. Sincere and honest relationships need to be developed between the institutions, companies and countries to ensure that unfair exploitation of one party by another does not result.

However, the ultimate challenge is the need for governments to recognize the potential and the opportunity and to invest into technology acquisition, human resource training and incentives to move research and industry forward. This is an urgent challenge to all governments of the developing countries, if they are to gain benefit from the development of this technology.

The technology is moving so fast in different directions and at different speeds. New technologies, unheard of a few years ago, such as transcriptomics and metabolomics and a host of others, are offering new opportunities for scientific development and industrial development. The opportunity is definitely there. The challenge is how to grab the opportunity in the most cost effective way. Universities must develop new teaching and research programs, specialized research institutions must be set up, networking and alliances must be developed, and human resources must be trained. Above all these, the right environment to create innovation with the right incentives must be put in place. The private sector must also play a leadership role. As an example, one of the greatest investors in biotechnology in Japan and Korea are the information technology giants as they see the opportunity knocking upon their doors.

8 CONCLUSION

The developing world had missed the opportunities offered by the information technology due to the lack of technical ability, funds and expertise. In general, we are merely users of the technology, while the wealth so created is largely reaped by multinationals in the developed world. However, unlike IT, developing countries have the rich bioresources of the world, which are the basic building blocks of the biotechnological industry. Practically all the centres of biodiversity are within the developing world, together with the traditional knowledge and historical cultures based upon these biological resources. Thus, developing countries must capitalize this rich bioresource capital for wealth creation and economic development. While strategic relationships and smart partnerships may be the only way to capture that potential, it need not always be between the developed and the developing world. There is now increasing, albeit limited, skilled human resources and adequate infrastructure in some developing countries. Biotechnology offers the best vehicle for bridging the gap between the haves and the have-nots, and for ameliorating the damage to our

global environment. Currently, biotechnology and related industries, offer tremendous opportunities for employment, human resource development, technological development, industrial development, wealth creation, social development and environmental protection. We call upon our political leaders to invest into this sunrise industry. We cannot allow this opportunity to pass by.

REFERENCES

1. Arai, Ken-ichi (2002). Paradigm Shift of Genomic Medicine by integrating Biotechnology, Information Technology and Nanotechnology. BioMalaysia 2002. Kuala Lumpur, Malaysia, 1-4 October 2002.
2. Chong, Tet Sieu and Murakami Mutsuko (2000). "The Buzz from Life". Asiaweek. October 6 2000.
3. Dolven, Ben (2000). Far eastern Economic Review 15 June 2000.
4. FEER (Far Eastern Economic Review) May and October 2002 issues.
5. Foo, Fatt Kah (2002). Opportunities in Biopartnering. BioMalaysia 2002. 1-4 October 2002, Kuala Lumpur Malaysia.
6. Islamet, Inez (2002). Development of Biotechnology in Indonesia. BioMalaysia 2002, 1-4 October 2002, Kuala Lumpur, Malaysia.
7. Krishnapillay, B. (ed.) (2002). Basic Principles of Biotechnology and their applications in Forestry. APAFRI, FRIM Malaysia.
8. Levine, Louis (2002). In "Biotechnology." Microsoft Encarta Online Encyclopaedia 2002. http://encarta.msn.com.
9. Nature Biotechnology, Supplement (2000). "Industry trends. A Snapshot of biotechnology's fastest moving fields." October 2000.
10. Ola, Per and Emily D'Aulaire (2002). The Quest for Golden Rice. Readers Digest June 2002.
11. Rifkin, Jeremy (1998). "The Biotech Century". Jeremy P. Tarcher/Putnam publication
12. Smith, John E. (1996). Biotechnology. Third Edition. Cambridge University Press.
13. Tan, Tin Wee. 2002. Life Sciences and Biotechnology Development in Singapore. BioMalysia 2002, 1-4 October 2002, Kuala Lumpur, Malaysia.
14. Wilson, Edward O. (1992). "The Diversity of Life" Published by W.W. Norton & Company, Inc. by arrangement with Harvard University Press.
15. Yoxen, Edward (1983). "The Gene Business". Pan Books Ltd.
16. Zakri, A.H. and M. Taeb. (2002). Emergence of Bio-age and its implication for Sustainable Development. Keynote address at the Second Conference of the Science Council of Asia. Kuala Lumpur, 13-15 May 2002.

Biotechnology: The Discovery of Disease causing Genes and the Treatment of Inherited Diseases in Man

S QASIM MEHDI*, ABDUL HAMEED,
MUHAMMAD ISMAIL, AIYSHA ABID
and
SHAGUFTA KHALIQ
Biomedical and Genetic Engineering Division
Dr A Q Khan Research Laboratories
Islamabad, Pakistan

1 ABSTRACT

Biotechnology is a multidisciplinary field in which a myriad of powerful new experimental techniques are applied to exploit biological systems for a variety of applications in industry, agriculture and the health sector. In recent years, biotechnology has come to be increasingly identified with the genomic revolution and genetic engineering. To describe the impact of these advances on medical research, a brief introduction to the principles would be in order.

Inherited characters are carried from parents to progeny by a chemical polymer called deoxyribonucleic acid (DNA). It contains all the information required to transform inanimate matter into living matter and nutriments into new cells and organisms- children or descendants. Almost all the DNA present in all eukaryotic cells is packaged in the nucleus in organised structures known as chromosomes. The chromosomes in every cell of an individual are the same. They distinguish each individual from every one else.

A new human is conceived with the fertilisation of an egg by a sperm. Thus, one of each of the 23 pairs of chromosomes of the new individual is contributed by the father and the other by the mother. The sex of the new individual depends on whether the father's sperm contributes an X (female) or a Y (male) chromosome. DNA in each chromosome is made up of a huge number of units called bases. These bases are of only four kinds: adenine (A), guanine (G), cytosine (C) and thymine (T). The sequence of these bases ultimately dictates every character from skin colour to the smallest biological function of the body.

DNA in the 23 pairs of chromosomes is made up of about three billion base

---

* Professor of Molecular Biology, and Fellow of the Islamic Academy of Sciences.

pairs (bp). Only 3-5% of this nuclear DNA codes for the synthesis of proteins that play a recognisable role in the activities of a living being. These DNA segments are called genes and it is estimated that 50,000 to 100,000 genes direct all human activities. The rest of the DNA is variously called "junk" DNA because its precise function is not known. However, this extragenic, non-coding DNA shows two major types of organisation: tandemly repeated (mini- and microsatellites) and interspersed. These DNA segments have been used to advantage in finding disease-causing genes as described later. Changes in this portion of DNA are also known to cause diseases (Fisher and Mayor, 1991).

DNA is also found in the mitochondria, which are small organelles in all cells. They are found outside the nucleus and are an important energy-producing powerhouse of the cell. The mitochondria are only inherited from the mother. Their 16,569 bp long circular DNA (mtDNA) is tiny compared to nuclear DNA. The complete sequence of human mtDNA has been elucidated (Anderson *et al.*, 1981).

The Human Genome Project (HGP) is one of the most ambitious undertakings of the twentieth century. At an estimated cost of US$3 billion, it aimed "to characterise all the human genetic material…. to discover all of the more than 50,000 human genes and to render them accessible for further biological study."

Historically, the Human Genome Project has progressed along with the introduction of powerful new technologies for DNA analysis and manipulation.

2 HUMAN GENETIC MAPS

(1) The first human genetic map based on Restriction Fragment Length Polymorphisms (RFLP) was published in 1987.
(2) High-resolution human genetic maps have been obtained using microsatellite markers.
(3) Considerable success has been achieved in producing maps of several individual human chromosomes.
(4) More than 60,000 genes and anonymous DNA markers have been mapped to specific locations on the human chromosomes
(5) A combined effort of the public sector HGP and Celera Genomics announced the sequence of the entire human genome in June 2000 (Macilwain, 2000).

Many disorders or diseases are caused by mutations in genes. "As our catalogue of human DNA variability becomes more complete, the first practical consequences will be improved abilities to predict disease risk" (Cantor, 1998). It has also been found that some genetically inherited diseases may be a consequence of a defective gene in one world population but not in another. This view is further supported by the discoveries in Pakistan, and in other countries,

that some of the genes responsible for deafness (Veske *et al.*, 1996; Scott *et al.*, 2001) and (night) blindness (Leutelt *et al*., 1995; Khaliq *et al*., 2000) etc. are different to those found in other parts of the world.

3 GENES AND DISEASES

An understanding of the molecular aetiology of inherited diseases requires that we find out everything about the gene(s) involved, their chromosomal locations, DNA sequence, expression control and finally the function of the protein product. For this, two approaches are possible. If the biochemical pathway and the lesion leading to the disease are known, then mutations can be examined in a gene or its product. For example, mutation in the haemoglobin molecule can be studied in haemoglobinopathies, or insulin in diabetes etc. This is often referred to as "forward" genetics. However, for many diseases such as cystic fibrosis or Duchenne muscular dystrophy etc., the underlying genetic defect was not known. In these instances the chromosomal location of the disease locus and positional cloning has proved to be successful. This process is known as "reverse" genetics (see Davies and Read, 1992).

Genetic linkage analysis is often the critical first step in identifying a disease- causing gene. The essence of this approach is to compare, within a family, the inheritance of a disease gene with specific DNA segments termed *markers.* Coinheritance of the disease gene with the DNA marker suggests that they are physically close, that is, linked on a specific chromosome. Linkage analysis provides an approximate position of the disease gene within the ***genome***.

4 LINKAGE ANALYSIS

Genetic linkage mapping observes the segregation of two or more non-allelic loci (disease traits and genes or genetic markers) during meiosis. During meiotic cell division, each pair of homologous chromosomes undergoes at least one recombination (crossover) between non-sister chromatids.

If two loci are physically located close together on the same chromosome, there is a greater chance that they will not be separated by random recombination events during meiosis and will appear "linked". To demonstrate linkage, representative markers are selected for each locus. Ideally these markers should be highly polymorphic within a given human population. This maximizes the probability that they will be different in the two copies of the chromosome that are inherited from the parents. It enables the markers to be used to demonstrate inheritance patterns and detect recombination events when typed in several members of a family. Loci that are adjacent will be inherited together permitting their proximity to be inferred (reviewed in Ott, 1991).

Table 1. Eye disease genes mapped by linkage analysis

Disease	Location	Gene/Protein
Anterior segment/corneal		
Autosomal dominant keratitis	11p13	PAX6
Congenital hereditary endothelial dystrophy	20p11.2	
Cornea plana	12q	
Granular dystrophy	5q	
Lattice dystrophy	5q	
Macular dystrophy	16q22	
Peters anomaly	11p13	PAX6
Posterior polymorphous	20q11	
Reis-Bucklers disease	5q	
Schnyder crystalline dystrophy	1p34.1-36	
Glaucoma		
Adult primary open-angle glaucoma	2 cen-q13, 3q21-24	
Aniridia	11p13	PAX6
Axenfeld-Reiger syndrome	4q25, 13q14	RIEG (4q25)
Iridogoniodysgenesis	4q25, 6p25	
Juvenile open-angle glaucoma	1q23-25	TIGR
Congenital glaucoma	2p21, 1p36	
Lens		
Cataract		
Congenital	16q22.1	CAM
Zonular pulverulent (Coppock disease)	1q21-25	CAE
Coppock-like disease	2q33-36	γ e-crystallin pseudogene
Anterior polar	2p25, 14q24	CAP
Marner disease	16q22.1	
Posterior polar	16q22.1	
Cerulean	17q24, 22q	
Ectopia lentis	15q15-21.3	
Marfan syndrome	15q15-21.3	
Myotonic dystrophy	19q13.1	
Weil-Marchesani syndrome	15q15-21.3	
Retina and viterous		
Leber congenital amaurosis	17p13	
Stickler syndrome	12q13.1-13.3	COL2A1
Wagner disease/erosive vitreoretinopathy	5q13-14	
Retinoblastoma	13q14	
Retinitis pigmentosa	More than 20 loci	7 genes
Macular dystrophy		

Table 1 (continued)

Disease	Location	Gene/Protein
Best	11q13	VMD2
North Carolina	6q14-16.2	MCDR1
Sorby	22q13-qter	TIMP3
Autosomal dominant radial drusen	2p16-21	
Fundus flavimaculatus /Stargardt disease	1p13	
Albinism	Many loci	
Von Hippel-Lindau syndrome	3p25-26	VHL/novel membrane protein
Optic nerve		
Leber optic neuropathy	mtDNA	LHON/NADH co-Q oxydoreductase
Kjer dominant optic atrophy	3q28-qte	
Eyelid		
Blepharophimosis-ptosis	3q21-24	
Oculopharyngeal muscular dystrophy	14q11.2-13	
Chronic progressive external ophthalmoplegia	mtDNA	
Congenital fibrosis of the extra ocular muscles	12p11.2-q12	
Neurofibromatosis type 1	17q11.2	NF1/neurofibromin
Other		
Congenital nystagmus	6p12	

Source: On-line Mendelian Inheritance in Man: http://www.ncbi.nlm.nih.gov/omim.

5 RECOMBINATION FRACTION (θ), GENETIC DISTANCE AND ORDER

Cross over events are more likely to occur between loci that are far apart than between two loci close together on the same chromosome (syntenic). Therefore the recombination fraction (θ), which is the probability of producing a recombinant, is related to the genetic distance separating the loci of the linked genes. These distances are measured in map units referred to as ‘centimorgans’ (cM), where 1 cM is equal to a 1% chance of recombination between two loci during a meiotic event. This simple relationship only applies when the genetic interval is too small to allow multiple crossover events, which in reality do occur between loci that are further apart. Therefore mapping functions that relate recombination fraction with genetic distance have been derived to take into account such multiple crossover events and also the phenomenon of interference.

Interference is the negative effect that an already existing crossover has on the formation of a second one nearby. The Kosambi mapping function (Kosambi, 1944), which takes interference and multiple crossover events into account, has been widely used in human genetic calculations since it produces more realistic map distance values.

6 CHOICE OF GENETIC MARKERS FOR LINKAGE ANALYSIS

An essential tool for performing genetic linkage analysis successfully is the use of a group of genetic markers that have multiple alleles (polymorphic) and have a known locus. Markers are inherited in the same manner as genes (the Mendelian pattern) and therefore can be traced through pedigrees. The more alleles a marker has, the more likely it will be that the maternal and paternal genotypes will differ and therefore, that the parental origin of a specific allele can be determine in the children.

The most common group of markers in use today are microsatellites, which are short tandem, repeat (STR) polymorphisms and single nucleotide polymorphisms (SNPs). Short tandem repeat polymorphisms are composed of strings of short repetitive base pair sequences (generally 1-4 bp) that vary in length between individuals. Of the mononucleotide repeats, runs of A and of T are very common and together account for about 10Mb or 0.3% of the nuclear genome. In contrast, runs of G and of C are rare. In the case of dinucleotide repeats, arrays of CA/TG repeats are the most common type of microsatellites and occur on an average at every 30 kb (Weber and May, 1989; Stallings, 1991). The CA repeats are equally distributed in the 5'- and 3'-untranslated regions and introns (the noncoding DNA segments) and are often highly polymorphic. CT/AG repeats are also common, occurring on an average once every 50kb of the genome. CG/GC dinucleotide, trinucleotide and tetranucleotide repeats are comparatively rare (Stallings, 1991).

The informativeness of a microsatellite marker is indicated by its polymorphic information content (PIC). The PIC value of a marker is calculated from the number of alleles and their frequencies in the population and is related to the mean repeat length of the marker (Weber and May, 1989). For use in linkage analysis, the PIC of a microsatellite must be >0.7.

The abundance, specificity of sites on each chromosome and ease of typing by polymerase chain reaction (PCR) has made microsatellites ideal genetic markers for linkage analysis. Furthermore, the advent of fluorescent labelling technology has enabled the typing of several markers in a single run (multiplex PCR) leading to lower costs and a greater degree of automation of marker typing (Ziegle *et al*., 1992).

The purpose of such a genome scan is to detect co-segregation between the disease locus, represented by the disease phenotype, and an allele of a DNA marker or gene. Establishing such segregation of a known marker locus with the disease phenotype will infer linkage between the two loci and therefore localise the disease trait to the region where the marker maps. Once linkage has been established, more DNA markers that map at close proximity to the disease locus are typed in the family to establish a haplotype, which is the combination of alleles at the linked locus. It is easy to identify a haplotype for the disease phenotype, as the markers are expected to co-segregate only in the diseased individual. Moreover any recombination (crossover) events that occur during meiosis in the affected parent would be reflected in the haplotype of the resulting offspring. Provided that the offspring still has the disease phenotype, this would lead to refinement of the disease locus.

7 STATISICAL EVALUATION OF LINKAGE

The conventional approach to evaluate whether two given loci are linked is to apply the maximum likelihood analysis (Morton, 1955). This estimates the "most likely" value of the recombination fraction (θ) as well as the odds in favour of linkage versus non-linkage. The ratio of likelihood or the "odds ratio" is the ratio of the probability of observing a segregation pattern of two loci if they are linked at a given recombination value (θ) where $\theta < 0.5$, versus the probability of such segregation if they are not linked ($\theta = 0.5$). This ratio is calculated at a series of recombination values (from $\theta = 0$ to $\theta = 0.5$). The logarithm of the "odds ratio" called the "lod score" (Z) is what geneticists normally use to report results of linkage analysis. These calculations can be summarised in the following equation:

$$Z(\theta) = \mathrm{Log}_{10}[L(\theta)/L(0.5)]$$

Where: $L(\theta)$ = the likelihood of obtaining the data if the two loci are linked and have a recombination fraction of θ; $L(0.5)$ = the likelihood of obtaining data when the two loci are unlinked. The recombination value that gives the maximum lod score (Z) is the best estimate of the degree of linkage between two loci. By convention, a positive lod score of 3 or higher is accepted as proof of linkage (i.e 1000:1 odds favouring linkage against no linkage), whereas a negative lod score of -2 or less is evidence against linkage (i.e. 100:1 odds no linkage against true linkage). If a marker generates a lod score between -2 and +3, it yields no useful information and is considered to be "uninformative." Several factors can influence the lod score, such as the certainty of clinical diagnosis, the size of the family being examined, the number of affected individuals and their location within the pedigree, the mode of inheritance, and the informativeness of the genetic markers used.

Table 2. LOD scores for the microsatellite markers analysed in the LCA/KC family

Marker	Recombination fraction								
	0.0	**0.01**	**0.05**	**0.1**	**0.2**	**0.3**	**0.4**	**Z_{max}**	**θ_{max}**
D17S1866	-0.21	-0.15	0.00	0.10	0.14	0.09	0.03	0.14	0.2
D17S849	2.63	2.56	2.31	2.00	1.35	0.74	0.24	2.63	0.0
D17S1529	∞	1.55	1.98	1.93	1.51	0.93	0.35	1.98	0.05
D17S829	3.21	3.14	2.84	2.46	1.70	0.94	0.29	3.21	0.0
D17S796	3.20	3.11	2.78	2.36	1.55	0.80	0.23	3.20	0.0
D17S960	1.18	1.32	1.42	1.29	0.87	0.43	0.11	1.42	0.05
D17S1796	∞	-3.20	-1.73	-1.08	-0.48	-0.19	-0.05	-0.05	0.4
D17S1353	∞	-1.22	-0.03	0.32	0.41	0.25	0.05	0.41	0.2
D17S1789	-1.42	-1.07	-0.58	-0.34	-0.13	-0.04	-0.01	-0.01	0.4

To achieve a Lod score of 3 a relatively large family, comprising at least 10-11 informative meioses, is required. Accurate diagnosis of disease status is essential and the number of meioses that can be included in the analysis may have to be reduced if the disease has a late age of onset and cannot be detected presymptomatically. The maximum lod score is also affected by the informativeness of the available markers. Therefore, linkage analysis is best suited to the study of extended, multigenerational pedigrees segregating fully penetrant autosomal dominant traits. If a single family cannot provide sufficient evidence for linkage, the sum of lod scores combined from more than one family can be used. In addition, the maximum lod score can only be obtained with a polymorphic marker that does not undergo recombination with the disease trait within the pedigree. In order to have a reasonable chance of finding such a marker a high-density genetic map is required, with markers mapped at regular intervals (10 cM or less).

Whilst the majority of kindred affected by autosomal recessive disease are small elsewhere in the world, large consanguineous families are relatively common in countries like Pakistan where cousin-marriage is culturally acceptable or even encouraged. These families are ideally suited to linkage analysis by homozygosity mapping. In homozygosity mapping it is assumed that both of an affected individual's disease alleles will have been inherited from a common ancestor and that both chromosomes will, therefore, have an identical pattern of marker alleles within the critical disease interval. This technique can be used to demonstrate linkage even in a small, inbred family so long as homozygosity in all affected individuals has only been observed at a single locus following a whole genome scan.

Once linkage to a single marker has been obtained, the critical genetic interval can be defined by genotyping the pedigree(s) with further genetic markers in the vicinity of the first, to identify key recombinant individuals. This "haplotype analysis" involves following the segregation of alleles, to determine whether each new marker has recombined with the disease locus or resides

within the critical interval bounded by the recombination events. The success of this approach is dependent on the number of informative recombinant individuals available, and on marker density and heterozygosity in the region. With the availability of high-resolution genetic maps covering most of the genome, fine mapping can now often delimit the disease region to only a few centimorgans. This is considered small enough to commence physical mapping and gene identification strategies.

Powerful statistical computer programmes like LIPED (Ott, 1974) and MLINK (Lathrop and Lalouel, 1984) are used for calculating lod scores for linkage analysis.

8 MAPPING OF GENES FOR RECESSIVE DISORDERS

Although conventional linkage mapping can be applied to recessive disorders, the following section describes an alternate approach:

8.1 Homozygosity mapping

Mapping of autosomal dominant disorders through traditional linkage analysis has been discussed previously. For mapping of recessive disorders a different approach which obviates the use of traditional family mapping is used. This is referred to as homozygosity mapping (Lander and Botstein, 1987). It requires polymorphic markers of high heterozygosity and DNA of affected children from consanguineous marriages. The method essentially involves the detection of the disease locus by virtue of the fact that the adjacent region will preferentially be homozygous by descent in such inbred children. The main advantage of homozygosity mapping is that it provides a way to map a recessive disease even when families with multiple affected individuals are scarce. When affected individuals from different families are used in the analysis, the disease is assumed to be homogeneous i.e. caused by mutation at a single locus.

Given the fact that dense maps of highly polymorphic markers are now available they facilitate the detection of homozygosity by descent, by detecting regions in which a contiguous stretch of markers are homozygous. Homozygosity mapping would specifically be performed as follows. To test the hypothesis that the disease gene maps to a given interval, the DNA of each affected inbred child is examined for homozygosity at say six consecutive loci, three on either side of the interval. Then for each possible outcome, the probability of it occurring due to linkage (P_1) and chance (P_2) is calculated. In which case the odds ratio in favour of linkage is P_1: P_2. A computer program called HOMMAP has been developed to do these calculations (Lander *et al*., 1987).

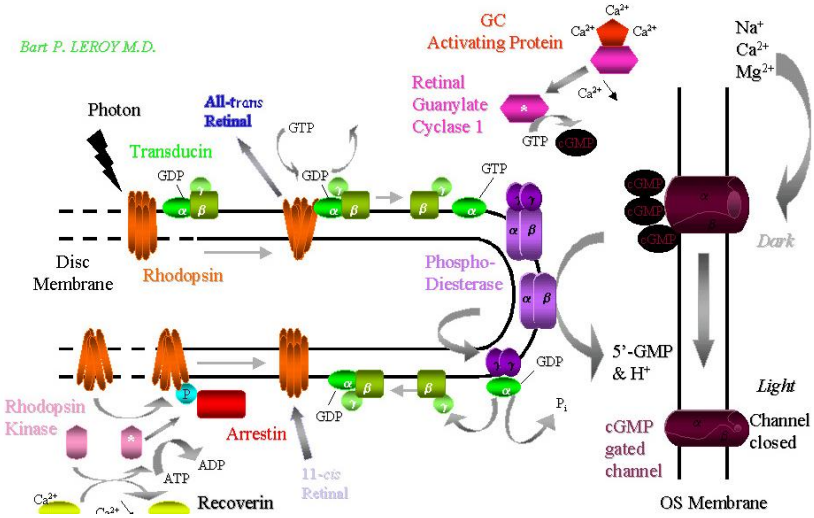


Figure 1. The Phototransduction Cascade.

8.2 The human genetic maps

The genetic map is the cornerstone of genetic linkage studies. Each gene has a "locus" or a specific location on a chromosome. Alternative forms of the same gene at its locus are called alleles**.** When both the members of a pair of alleles are identical, the individual is homozygous. When the alleles are distinct from each other, the individual is heterozygous. With very few and rare exceptions, the genes found on each chromosome have a specific order that is identical for all humans. Once a gene or marker is localised to a particular region on a chromosome, it is placed on a map. Therefore, the genetic map depicts the order of genes, genetic markers and other landmarks that compose the chromosomes.

As mentioned earlier, one of the aims of the International Human Genome Project has been the production of high-resolution genetic and physical maps. This would assist in localising and ordering genes and disease loci to chromosomal regions. Several international groups who produced a series of human genome maps with newly identified markers swiftly reached this target. Most of these were genotyped in a common set of reference families from CEPH (Centre d'Etude du Polymorphisme Humain; Dausset *et al.*, 1990) to establish linkage relationships between the markers. In 1992, two groups published linkage maps with an average resolution of 5 cM. The NIH/CEPH Collaborative Mapping Group (1992) created a linkage map with 1416 loci, 339 of which were microsatellites and 279 genes. Weissenbach and colleagues (1992) at Généthon, reported 814 novel CA repeat polymorphic markers spanning ~90% of the human genome in their "second generation linkage map of the human genome". The same group reported their 1993-94 linkage map (Gyapay *et al.*, 1994) which consisted of 1,267 new CA repeat markers and

were integrated into their previous 1992 map, presenting a total of 2,066 poly (CA) markers with ~50% of these spaced at an average distance of 1 cM or less.

The final Généthon map presents 5264 CA repeat markers with an average resolving power of 1.6 cM (Dib *et al.,* 1996). Other groups focused on integrating markers generated from several sources. The Cooperative Human Linkage Centre (CHLC; Murray *et al.* 1994) assembled an integrated map using data sets from the National Centre for Human Genome Research (NCHGR) Index Map Consortium and Généthon. This set also included tri and tetra-nucleotide repeat microsatellites, which resolve more clearly on polyacrylamide gels. A second integrated map was produced in 1994 (Murray *et al.,* 1994), which combined genotype data generated from the CEPH, Généthon and CHLC groups. This brought together 5,840 loci, most of which were microsatellite markers to give the map an average marker density of 0.7 cM.

These high-density gene maps have accelerated the pace of positional cloning efforts. The heterozygous polymorphic markers can be easily typed in families by PCR assay and reactions can be multiplexed together to reduce both time and effort. These maps have also benefited those groups interested in polygenic disease traits such as juvenile-onset diabetes, schizophrenia and multiple sclerosis (King, 1997). The markers can also be readily used as physical mapping reagents to provide a bridge between physical and genetic maps.

9 GENE IDENTIFICATION

A gene may be isolated by either a ***candidate gene*** approach or ***positional cloning*** approach. In the candidate approach, the disease gene is searched on the basis of the function of the protein involved, its amino acid sequence, or the underlying biochemical defect. Unfortunately, for most disorders, there is little information about the disease gene or its function. One therefore has to rely on the laborious positional cloning strategy, which require no information about gene function. A third approach that is gaining increasing popularity because it can expedite gene discovery is the ***positional candidate gene*** approach. This relies on a combination of the above two strategies, i.e., a gene is mapped to a chromosomal region by linkage analysis and then the attractive candidates in the area are surveyed. For example, when autosomal dominant retinitis pigmentosa (RP) was mapped to 3q21-24, the rhodopsin gene was already known to be in this area. The gene was examined and within the rhodopsin gene, mutations were found that cause retinitis pigmentosa. All genes located within the critical interval for a disorder automatically become *positional candidates*. Those genes that are specifically expressed in the tissue in question, or that possess a function relevant to the pathophysiology of the disease are viewed as the most likely candidates and are the first to be screened

for mutations in affected individuals. Genes from the critical region that do not appear to be tissue-specific or functionally related to the disease must also be examined. For example, the two X-linked RP genes, *RPGR* and *RP2*, are both ubiquitously expressed and unrelated to any previously identified retinopathy genes (Meindl *et al*., 1996; Schwahn *et al*., 1998).

When the detailed and accurate sequencing of the human genome and the location of the 50,000 to 100,000 human genes become known, the positional candidate strategy is likely to predominate to identify disease genes (Collins, 1995). With this ultimate objective, large scale sequencing and mapping of cDNAs has been carried out, leading to the publication of human transcript (gene and EST) maps and the deposition of thousands of EST sequences in the dbEST division of the publicly accessible Genbank electronic database (http:/www.ncbi.nim.nih.gov/).

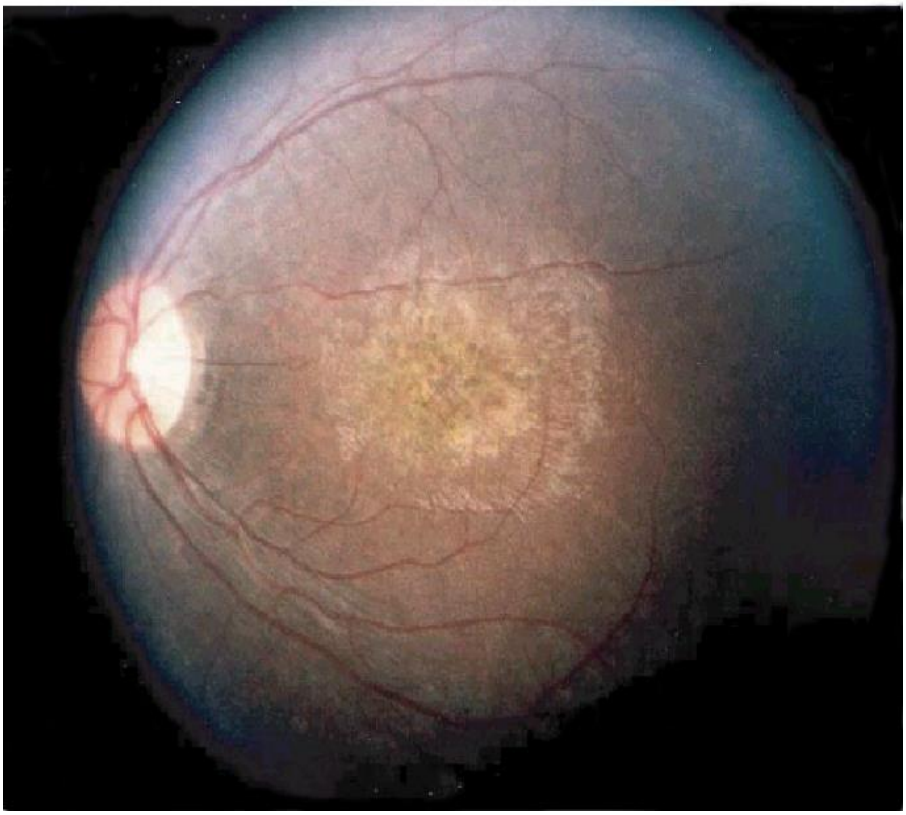


Figure 2. Fundus photograph of an RP patient.

I would like to briefly describe some of the work done in our laboratory in Pakistan with special reference to diseases of the eye. The human eye is a complicated organ made up of a variety of different tissue types. I shall not go

into details of the structure and function of the eye as it can be looked up in any ophthalmology textbook (e.g., Rosen and Rosen, 1997).

10 INHERITED OCULAR DISEASES

Of over 10,000 known genetic disorders that affect humankind, a disproportionately high number affect the eye. The total number of genes responsible for normal structure, function and differentiation of the eye is unknown, but the list of these genes is rapidly and constantly growing. A summary of some mapped human ocular disorders is given in Table 1. It can be seen that diseases of the retina are especially important because more than 100 diseases are due to some form of retinal dystrophy. Figure 1, for example, gives a cascade of biochemical reactions that are involved in vision. Genes express all of the large number of structural proteins and enzymes shown here and any dysfunction can lead to impaired sight or blindness. For the work described here, emphasis will be on the genetic aspects of ocular disorders.

11 GENETICS OF RETINAL DEGENERATIONS

The family of inherited ocular diseases that is collectively known as retinitis pigmentosa is a major cause of progressive retinal disease worldwide with a combined incidence of approximately 1 in 3500 (Bundey and Crews, 1984; Bunker *et al*., 1984). Figure 2 shows a typical fundus photograph of a patient suffering from retinitis pigmentosa. To put it simply, instead of being crystal clear as is normally the case, the area between the lens and the retina is cloudy.

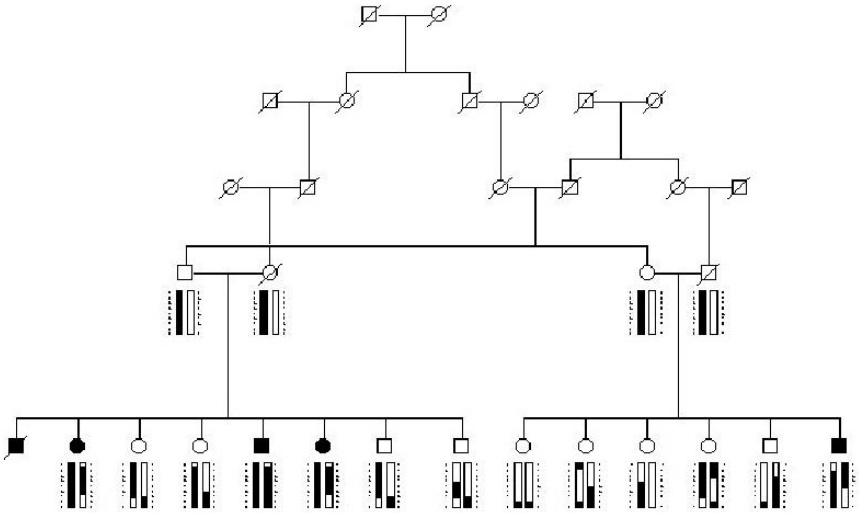


Figure 3. Pakistani family suffering from Leber Congenital Amaurosis with Keratoconus.

Hereditary retinal dystrophies and dysfunctions are an extremely heterogeneous group of diseases are in terms of clinical description and genetic cause. Many forms of these diseases now described, and a variety of partial and complete synonyms for the term “retinitis pigmentosa”, have been used in the literature (van Soest *et al*., 1999). The similarities that have led to the common grouping of these diseases are based on clinical symptoms, electroretinographic phenotype, and/or genetics. In addition to the typical forms of RP, there are a number of related or allied diseases. These may be similar by any of the three diagnostic criteria mentioned above. There are also syndromic forms of RP, in which the disease is present as a component of a multisystem disorder.

Table 3. Biomedical and Genetic Engineering Division (Oct. 2000)

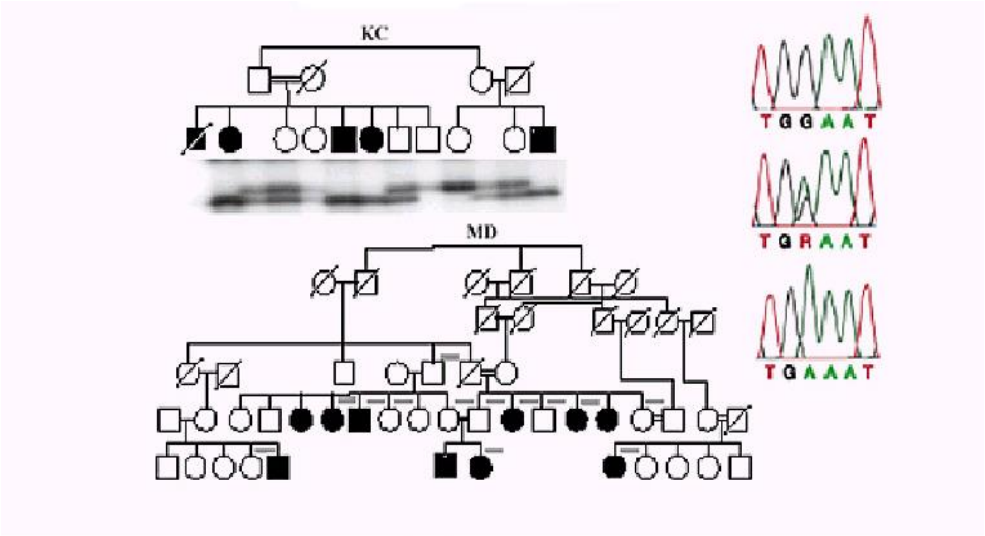
Pakistani Families ID	Locus	Candidate Gene
RETINITIS PIGMENTOSA & ASSOCIATED RETINAL DEGENERATIONS		
Macular Degeneration		
Keratoconous/LCA4	17pter	AIPL 1
PMK214-arRP	1q24-32	Phosducin
1RP-arRP	17q11.1-12	Ret GC
15RP-arRP	4q	(Guanylatecyclase)
16RP-adRP	3q21	?
18RP-adRP	19q	Rhodopsin (Ter-349-Glu)
20RP (cone-rod dystrophy)	6q	
24RP-arRP	6q11-13	Protein Kinase C gamma
28RP (cone-rod dystrophy)	1q	GABA-C receptor
		?
		?
CONGENITAL CATARACT		
3CCT	1q21-25	Connexin 50
4CCT	3q21-25	Phakinin
6CCT	1q21-25	Connexin 50
OTHER EYE DISORDERS		
MICROPHTHALMIA	14q32	?
NON-SYNDROMIC CONGENITAL DEAFNESS	21q22	Transmembrane protease

Recent reviews have summarized current research on a number of these diseases, including Leber congenital amaurosis (LCA; Perrault *et al*., 1999), Stargardt disease and fundus flavimaculatus (Rozet *et al*., 1999), macular degeneration (Zack, 1999), cone dystrophy (Simunovic and Moore, 1998) and the Refsum diseases (Verhoeven *et al*., 1998).

12 ANEXAPLE OF THE IDENTIFICATION OF A NOVEL DISEASE-CAUSING GENE IN A PAKISTANI FAMILY

Figure 3 shows the pedigree of a Pakistani family with several consanguineous marriages (double lines). The darkened squares (males) and circles (females) are patients. Each individual in this family was thoroughly examined by a qualified ophthalmologist. All patients are affected with Leber congenital amaurosis (LCA) and bilateral keratoconous (KC).

Blood samples were collected from all members of the LCA/KC family with informed consent and DNA purified from each sample was analysed as described above. Exclusion studies showed that no known locus was involved in causing the disease in this family, so a whole genome linkage analysis was performed. Table 2 shows the lod scores of the various markers that were used here and the disease locus was mapped to chromosome 17p13.1 between the microsatellite markers D17S849 and D17S960. The LCA in this family was designated LCA4 (Hameed *et al*., 2000). This region of the chromosome excludes the gene GUCY2D that was shown by Perrault *et al*. (1996), to be the cause of the disease in another study. However, mapping within the LCA4 region was the photoreceptor/pineal expressed gene AIPL1, which encodes the aryl hydrocarbon interacting protein like-1. This gene consists of six exons with alternate polyadenylation sites in the 3' UTR. The predicted protein product consists of 384 amino acids and the sequence includes three tetratricopeptide repeats which is a 34 amino acid motif found in proteins with nuclear transport or protein chaperon activity (Ma and Whitmore, 1997).



This nonsense mutation results into a truncated protein of 277AA instead of the normal 384 AA.

Figure 4. Pedigrees and mutation screen of AIPL1 in Pakistani families.

Using an amplimer from exon 6 and the 3'UTR of the AIPL1 gene as probe, Northern blot analysis identified mRNA molecules in total retinal RNA showing that this gene was functional in the retina. A week signal was also detected in skeletal muscle and the heart after prolonged exposure but not in the brain or cornea (Sohocki *et al*., 2000).

In order to ascertain that mutations in the AIPL1 gene were in fact responsible for the disease, DNA from patients in two Pakistani LCA (KC and

MD) families were sequenced. Figure 4 shows that a homozygous nonsense mutation (trp 278X, TGG to TGA) was present in all the patients causing a premature termination of the synthesised protein. The presence of the nonsense mutation at codon 278 leads to the synthesis of a truncated protein, which is 107 amino acids shorter than the wild type AIPL1. Sequencing of an unaffected control (TGG/TGG) is shown on the top right and is represented by a single upper band of the electropherogram under the unaffected individuals in the KC pedigree (Figure 4). The middle sequence shows a heterozygous G/A mutation at codon 278 and two bands while the patients have a homozygous (TGA/TGA) sequence and a single lower band in the electropherogram.

Thus we have discovered a new gene in which there is a homozygous nonsense mutation at codon 278 in all affected members of the Pakistani family. AIPL1 mutation was also found in an American family suffering from LCA. AIPL1 mutations are estimated to account for approximately 20% of recessive cases of LCA.

In addition to the discovery of the novel gene described above to be a cause of LCA, Table 3 is a summary of several other genes and loci in Pakistani families that have been identified in our laboratory for the first time.

13 CONCLUSION

Medical research in the future is expected to be the application of procedures such as antisense oligonucleotides (see Baker *et al*., 2001), gene therapy and stem cell therapy for curing diseases. Perhaps the most demanding aspect of gene therapy will be the availability of harmless vectors and the targeting of the corrective gene to the desired cell or tissue. There have been encouraging advances in stem cell therapy for the treatment of spinal cord injuries (McDonald *et al*., 1999) and diabetes (Lumelsky *et al*., 2001). Thus, the exciting future of medical biotechnology and genetic engineering research holds both promises and challenges.

REFERENCES*

1. Anderson S, Bankier AT, Barrell BG, de Bruijn, MH, Coulson AR, Drouin J, Eperon IC, Nierlich, DP, Roe BA, Sanger F, Schreier PH, Smith AJ, Staden R, Young IG. (1981). Sequence and organization of the human mitochondrial genome. *Nature*. 290:457-465.
2. Bundey S and Crews SJ. (1984). A study of retinitis pigmentosa in the City of Birmingham. I Prevalence. *J. Med. Genet*. 21:417-420.
3. Bunker CH, Berson EL, Bromley WC, Hayes RP, Roderick TH. (1984) Prevalence of retinitis pigmentosa in Maine. *Am. J. Ophthalmol.* 97:357-365.
4. Cantor CR. (1998). How will the Human Genome Project improve our quality of life? *Nat. Biotechnol*. 16:212-213.
5. Carr RE and Heckenlively JR. (1986). Hereditary pigmentary degenerations of the retina. In: Tasman W, Jaeger, EA, editors. Clinical Ophthalmology. Philadelphia: Lippencott, Williams, and Wilkins 3:1-28.
6. Collins FS. (1995). Positional cloning moves from perditional to traditional. *Nature. Genet*. 9:347-350.
7. Dausset J., Cann H., Cohen D., Lathrop M., Lalouel J. M. and White R. (1990). Centre d'Etude du Polymorphisme Humain (CEPH): collaborative genetic mapping of the human genome. *Genomics.* 6: 575-577.
8. Davies KE. and Read AP. (1992) Molecular basis of inherited diseases. 2nd edition, IRL Press, Oxford.
9. Dib C., Fauré P., Fizames C., Sanson D., Drouot N., Vignal A., Millasseau P., Marc S., Hazan J., Seboun E., Lathrop M., Gyapay G., Morissette J. and Weissenbach J. (1996). A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature.* 380:152-154.
10. Gyapay G., Morissette J., Vignal A., Dib C., Fizames C., Milasseau P., Marc S., Bernardi G., Lathrop M., Weissenbach J. (1994). The 1993-94 Généthon human genetic linkage map. *Nat. Genet*. 72: 246-339.
11. Hameed, S Khaliq, M Ismail, ND Ebenezer, T Jordan, SQ Mehdi, AM Payne, SS Bhattacharya. A novel locus for Leber congenital amaurosis (LCA) with anterior keratoconous mapping to chromosome 17p13. Am. J. Human Genetics. 65(4): 1408 (1999).
12. Khaliq S, A Hameed, M Ismail, SQ Mehdi, Bessant DAR, AM Payne SS Bhattacharya. A novel locus for autosomal recessive cone-rod dystrophy (CORD8) mapping to chromosome 1q12-q24. Investig. Ophthal. Vis. Sci. 41: 3709-3712 (2000).
13. Kosambi DD. (1944). The estimation of map distances from recombination values. *Ann. Eugen.* 12:172-175.
14. King MC. (1997). Leaving Kansas...finding genes in 1997. *Nat. Genet*. 15: 8-10.

* As listed by authors.

15. Lander ES and Botstein D. (1987). Homozygosity mapping: a way to map human recessive traits with the DNA of inbred children. *Science.* 236:1567-1570.
16. Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L. (1987). MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics.* 1:174-181.
17. Lathrop GM and Lalouel JM. (1984). Easy calculation of LOD scores and genetic risks on small computers. *Am. J. Hum. Genet.* 36:460-565.
18. Leutelt J, Oehlmann R, Younus F, van den Born LI, Weber JL, Denton MJ, Mehdi SQ, Gal A. (1995). Autosomal recessive retinitis pigmentosa locus maps on chromosome 1q in a large consanguineous family from Pakistan. *Clin. Genet*. 47:122-124.
19. Lumelsky N, Blondel O, Laeng P, Velasco I, Ravin R, McKay R. Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. Science. 292: 1389-1394.
20. Macilwain C. (2000). World leaders heap praise on human genome landmark. *Nature.* 405: 983-984.
21. McDonald JW, Liu X-Z, Qu Y, Liu S, Mickey SK, Turetsky D, Gottlieb D, Choi DW. Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. Nature Medicine. 5: 1410-1412.
22. Meindl A, Dry K, Herrmann K, Manson F, Ciccodicola A, Edgar A, Carvalho MR, Achatz H, Hellebrand H, Lennon A, Migliaccio C, Porter K, Zrenner E, Bird A, Jay M, Lorenz B, Wittwer B, D'Urso M, Meitinger T, Wright A. (1996). A gene (RPGR) with homology to the RCC1 guanine nucleotide exchange factor is mutated in X-linked retinitis pigmentosa (RP3). *Nat. Genet.* 13:35-42.
23. Morton NE. (1955). LODs past and present. *Genetics*. 140: 7-12.
24. Murray JC., Beutow KH., Weber JL., Ludwigsen S., Scherpbier-Heddema T., Manion F., Quillen J., Sheffield VC., Suden S., Duyk GM., Weissenbach J., Gyapay G. *et al*. (1994). A comprehensive genetic linkage map of the human genome. *Science.* 258: 67-85.
25. NIH/CEPH collaborative mapping group (1992) A comprehensive genetic linkage map of the human genome. *Science.* 258:148-162.
26. Ott J. (1974). Estimation of the recombination fraction in human pedigrees: Efficient computation of the likelihood for human linkage studies. *Am. J. Hum. Genet*. 26:588-597.
27. Ott J. (1991). Analysis of human genetic linkage. Johns Hopkins University Press, Baltimore.
28. Perrault I, Rozet JM, Gerber S, Ghazi I, Leowski C, Ducroq D, Souied E, Dufier JL, Munnich A, Kaplan J. (1999). Leber congenital amaurosis. *Mol. Genet. Metab*. 68:200-208.
29. RetNet: http://www.sph.uth.tmc.edu/Retnet/disease.htm

30. Rosen E and Rosen W., (1997). Ophthalmology. Cavendish Publishing Limited, The Glass House, Wharton Street, London WC1X 9PX. UK.
31. Rozet JM, Gerber S, Souied E, Ducroq D, Perrault I, Ghazi I, Soubrane G, Coscas G, Dufier JL, Munnich A, Kaplan J. (1999). The ABCR gene: a major disease gene in macular and peripheral retinal degenerations with onset from early childhood to the elderly. *Mol. Genet. Metab*. 68:310-315.
32. Scott HS, Kudoh J, Wattenhofer M, Shibuya K, Berry A, Chrast R, Guipponi M, Wang J, Kawasaki K, Asakawa S, Minoshima S, Younus F, Mehdi SQ, Radhakrishna U, Papasavvas M-P, Gehrig C, Rossier C, Korostishevsky, Gal A, Shimizu N, Bonne-Tamir B, Antonarkis SE. Insertion of B-satellite repeats identifies a transmembrane protease causing both congenital and childhood onset recessive deafness. Nature Genetics. 27: 59-63 (2001)
33. Schwahn U, Lenzner S, Dong J, Feil S, Hinzmann B, van Duijnhoven G, Kirschner R, Hemberger M, Bergen AA, Rosenberg T, Pinckers AJ, Fundele R, Rosenthal A, Cremers FP, Ropers HH, Berger W. (1998). Positional cloning of the gene for X-linked retinitis pigmentosa 2. *Nat. Genet.* 19:327-332.
34. Simunovic MP and Moore AT. (1998). The cone dystrophies. *Eye*. 12:553-565.
35. Sohocki MM, Bowne SJ, Sullivan LS, Blackshaw S, Cepko CL, Payne AM, Bhattacharya SS, Khaliq S, Mehdi SQ, Birch DG, Harrison WR, Elder FF, Heckenlively JR, Daiger SP. (2000). Mutations in a new photoreceptor-pineal gene on 17p cause Leber congenital amaurosis. *Nat Genet* 24:79-83.
36. Stallings RL., Ford AF., Nelson D., Torney DC., Hildebrand CE. and Moyzis RK. (1991). Evolution and distribution of (GT)n repetitive sequences in mammalian genomes. *Genomics.* 10: 807-815.
37. van Soest S, Westerveld A, de Jong PT, Bleeker-Wagemakers EM, Bergen AA. (1999). Retinitis pigmentosa: defined from a molecular point of view. *Surv. Ophthalmol*. 43:321-334.
38. Verhoeven NM, Wanders RJA, Poll-The BT, Saudubray JM, Jakobs C. (1998). The metabolism of phytanic acid and pristanic acid in man. *J. Inherit. Metab. Dis*. 21:697-728.
39. Veske A, Oehlmann R, Younus F, Mohyuddin A, Muller-Myhsok B, Mehdi SQ, Gal A. (1996). Autosomal recessive non-syndromic deafness locus (DFNB8) maps on chromosome 21q22 in a large consanguineous kindred from Pakistan. *Hum. Mol. Genet.* 5:165-168.
40. Weber JL. and May PE. (1989). Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am. J. Hum. Genet*. 44: 388-396.
41. Weissenbach J., Gyapay G., Dib C., Vignal A., Morissette J., Millasseau G., Vaysseix G. and Lathrop M. (1992). A second generation linkage map of the human genome. *Nature*. 359: 794-801.

42. Zack DJ, Dean M, Molday RS, Nathans J, Redmond TM, Stone EM, Swaroop A, Valle D, Weber BH. (1999). What can we learn about age-related macular degeneration from other retinal diseases? *Mol. Vis*. 5:30.
43. Ziegle JS, Su Y, Corcoran KP, Nie L, Mayrand PE, Hoff LB, McBride LJ, Kronick MN, Diehl SR. (1992). Application of automated DNA sizing technology for genotyping microsatellite loci. *Genomics.* 14:1026-1031.

Technical Aspects of Genetic Engineering: Current and Future Trends

NAIMA ALAMI-OUAHABI*
M LAHRICHI
and
A BELMAHI
Mohammed V University
Rabat, Morocco

1 ABSTRACT

Gene studies are emerging as key engine of science. In fact, scientists throughout the world are busy identifying and studying genes that control the entire spectrum of phenotypic traits in living organisms. Advances in Biotechnology and genetic engineering have proved to be a powerful tool in understanding gene functions, thus enabling scientists to discover and elucidate in a fast and efficient manner, the origin of disorders and pathogenesis in all living organisms. This paper provides background information on the basics of genetic engineering, along with various aspects of current molecular biology tools and techniques, as well as their applications in biological and biomedical research.

The paper, also discusses the current and future undoubtable advantages offered by these genetic engineering techniques, but it also points out to the limits, controversies and ethical dilemmas that have arisen with the recent and potential advances in biotechnology.

2 INTRODUCTION

2.1 From Mendel to Genetic Engineering

At the dawn of the twenty first century, the scientific community along with the society as a whole, recognize the major roles that genes play in shaping our lives, and the lives of all living creatures.

In fact, the classic Mendelian Genetics, that is now a century and a half old, has evolved, thanks to various arrays of research in: Genetics, Biochemistry, Bacteriology, Virology, Cellular Biology and even Chemistry, Physics,

* Department of Biochemistry and Molecular Biology, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco.

Engineering and Bio informatics, into a mature science offering powerful tools of study and analysis of different life processes. This science is now called Molecular Biology, Biotechnology, Genetic Engineering, Molecular Genetics or Recombinant DNA Technology.

2.2 Cells and Organisms

Cells, are structural and functional units of all living organisms. The smallest organisms, consist of single cells and are microscopic (procaryotes). Whereas larger organisms, are multicellular (eucaryotes). The human body, for example, contains at least 10^{14} cells. Multicellular organisms, contain many different types of cells, which vary in size, shape, and specialized function. Yet, each of these cells have:

- A Plasma membrane, that defines it's periphery;
- A Cytoplasm, composed of an aqueous solution, and a variety of insoluble suspended particles; and
- A nucleus, which is a membrane bounded particle, that contains the genetic materiel (DNA), and associated proteins (Figure 1).

While all eucaryotes, and most prokaryotes have DNA, as genetic material, some viruses (Retroviruses), have RNA as genetic material.

2.3 Chromosomes and genes

The DNA molecules containing the cellular genes, are commonly packaged into structures called chromosomes. Most bacteria and viruses, have a single chromosome. Eukaryotes usually have many (46 chromosomes in a human somatic cell).

A single chromosome, typically contains thousands of individual genes. The sum of all the genes and intergenic DNA, on all the different chromosomes of the cells, is referred to as the cellular genome .

2.4 Chromatin and nucleoid structure

The chromosomal material, is called chromatin. The chromatin, contains DNA, proteins, and a small amount of RNA. The DNA in the chromatin, is tightly associated with proteins called Histones, Histones, package and order DNA into structural units, called nucleosomes.

The nuclesome, is the fundamental organizational unit of the chromatin, and it contains DNA wrapped around a protein core of 8 histones (histone octamer). Six nucleosomes, are then packaged into a super helical solenoid, many solenoids are packaged into a solenoid loops, and many solenoid loops are condensed into chromosomal bands.

In fact, eucaryotic chromosomal DNA is packaged into a succession of higher order structures, that ultimately yield the compact chromosome (Figure 1).

Thus, the fundamental unit of information in living systems is the gene. A gene, is defined biochemically as that segment of deoxyribonucleic acid (DNA), or in few cases ribonucleic acid (RNA), that encodes the information required to produce a functional biological protein.

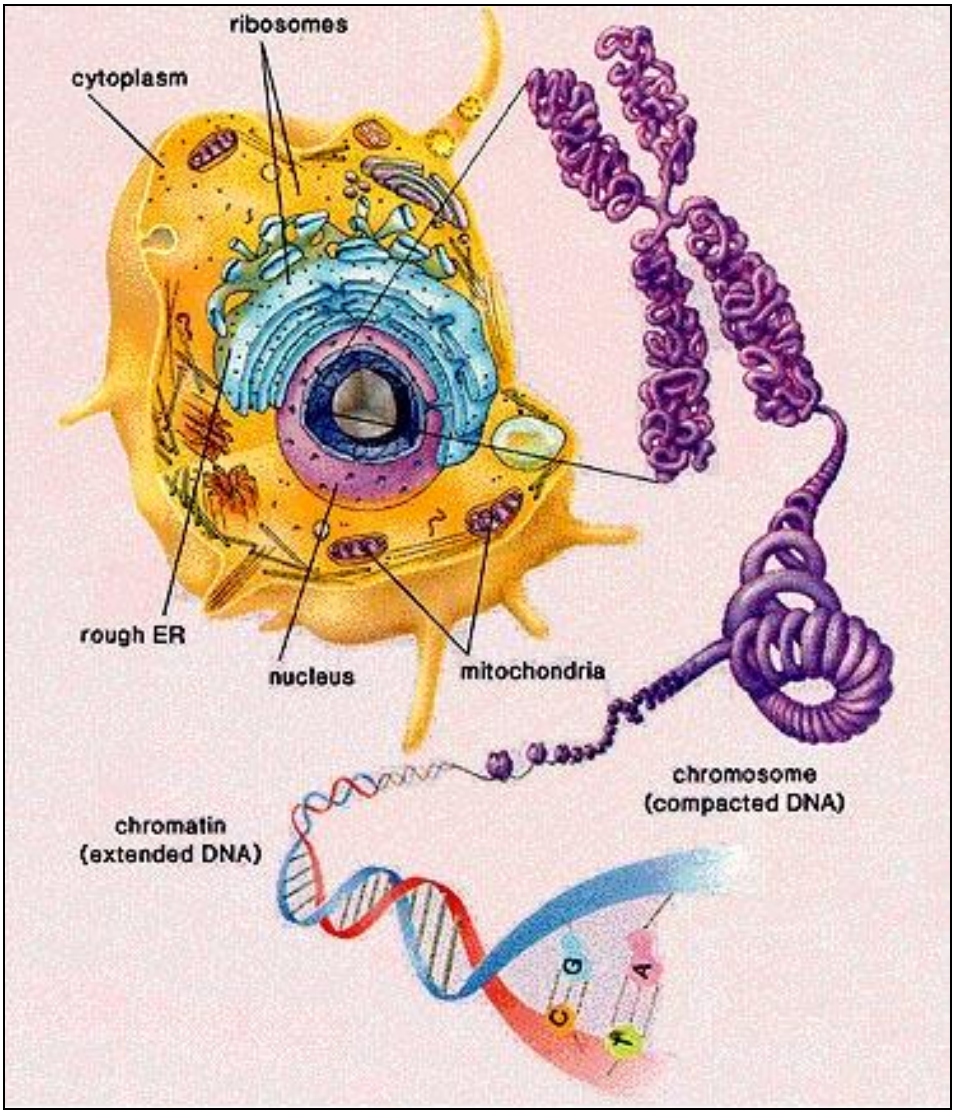


Figure 1. DNA location and structure (15).

The nucleic acids (DNA and RNA), are energy rich bio molecules, which are formed of units called nucleotides. The nucleotides, have three characteristic components:

(1) A nitrogenous base that derives of two parent compounds:

Purines	Adenine (present in DNA and RNA)
	Guanine (present in DNA and RNA)
Pyrimidines	Cytosine (present in DNA)
	Thymines (present in DNA)
	Uracil (present in RNA)

(2) A pentose: Ribose (in RNA)
Deoxyribose (in DNA)

(3) A phosphate or phosphoric acid H_3PO_4.

These components, are linked together to form nucleotides, that are joined by successive phospho diester linkage, thus forming the backbone or strands of nucleic acids (Figure 2).

2.5 DNA structure

Two strands of DNA, join together to form the DNA double helix. This double helix structure, has three characteristics.

(1) The two strands are antiparallel (head trail). The 5', 3' phosphdiester bonds run in opposite directions.
(2) The two antiparallel polynucleotide chains of double helical DNA, are complementary to each other by base pairing of Adenine with Thymine, and Guanine with Cythosine, based on Hydrogen bonding between appropriately spaced Oxygen and Hydrogen (Figure 2).
(3) These two characteristics, determine the tridimentional structural model of DNA.

The discovery of the structure of DNA by Watson and Crick, was a momentous event in classical genetics and in science in general. An event that gave rise to an entirely new discipline, known now as: Genetics engineering. This discovery, was the basis of our understanding of a cell's genetic information.

In fact, only four deoxyribonucleotides can be constructed, using the four purine and pyrimidine bases (Adenine, Guanine, Cytosine, Thymine). These 4 Nucleotides, form the repeating monomenic subunits of the informational macro molecule DNA. The number of possible permutations and combinations of four deoxynucleotides, is virtually limitless (as in the number of melodies possible with a few musical notes) (Figure 3).

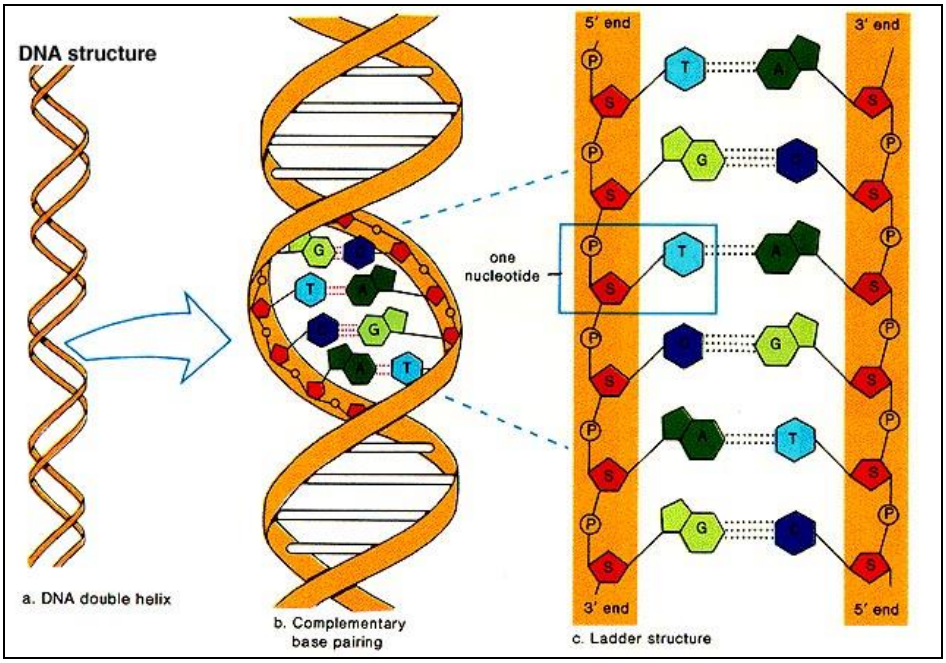


Figure 2. Nucleotide components of the DNA Double Helix (12)

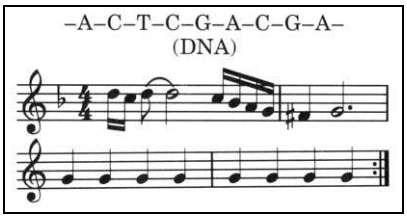


Figure 3. Limitless combinations of Nucleotides:
Subunits of genetic information sequences (21).

2.6 The genetic function and expression

A vastly improved understanding of DNA structure, inevitably led to questions about it's function. The structure itself, suggested how DNA might be copied so that the information contained therein, could be transmitted from one generation to the next. Understanding how the information in DNA was converted into functional proteins, became possible through the discovery of messenger RNA (mRNA) and transfer RNA (tRNA), as well as the solution of the genetic code.

In fact, the key to the organization of the genetic information contained in the DNA and specifying a protein, can be found in Codons (three successive nucleotides), and in the array or sequence of codons that constitutes a gene (an open reading frame or a coding sequence). While several codons serve as

genetic information for the amino acid sequence that will constitute a protein, other codons serve special functions in the open reading frame.

In fact, these special codons serve to initiate, or stop the genetic expression process (Figure 4).

These and other major advances, led to the central dogma of molecular genetics, which defines three major processes in the cellular utilization of genetic information.

- The first is replication: which involves the copying of parental DNA, to form daughter DNA molecules, having identical nucleotide sequences (Figure 5).
- The second is transcription: that is the process by which, parts of the coded genetic message in DNA are copied precisely, in the form of mRNA (Figure 6).
- The third is translation: in which the genetic message, coded in messenger RNA, is carried from the chromosome, to the ribosomes, then translated on these cytoplasmic sites, into a protein with a specific amino acid sequence (Figure 7).

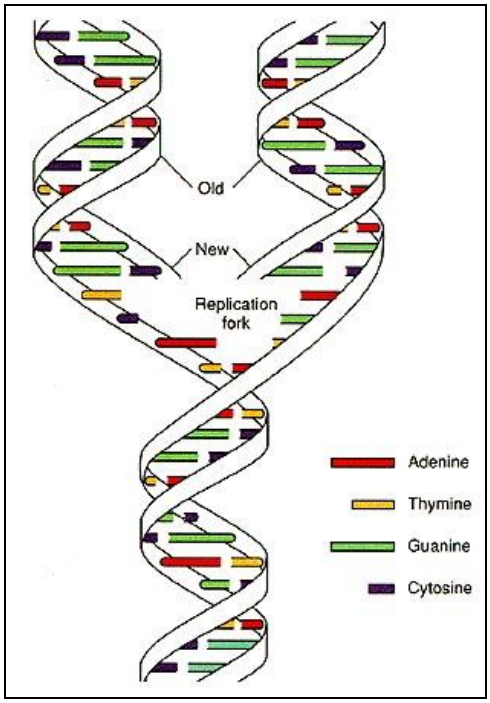


Figure 4. DNA Replication (15).

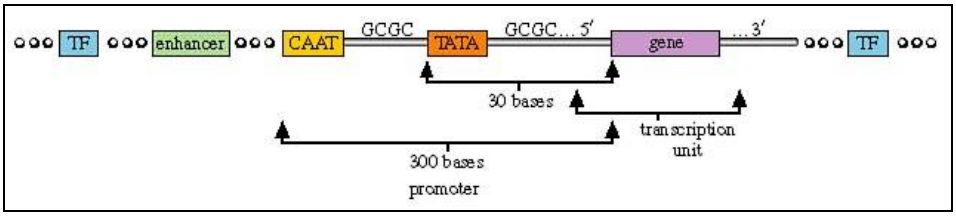


Figure 5. The transcription units and its control.

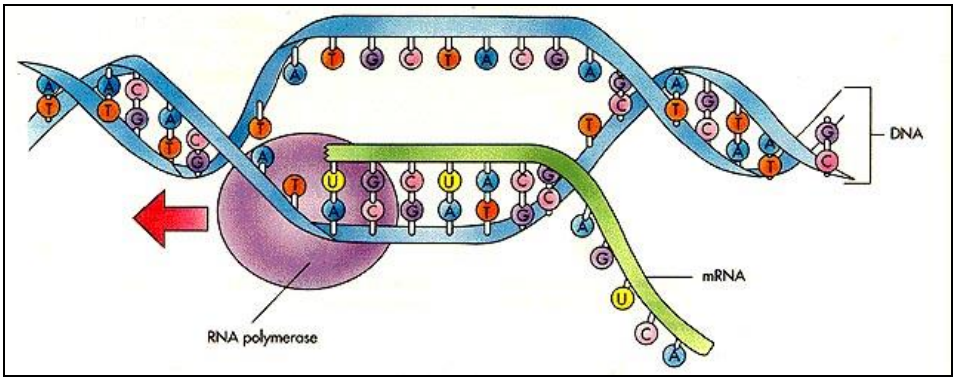


Figure 6. DNA transcription to mRNA (2).

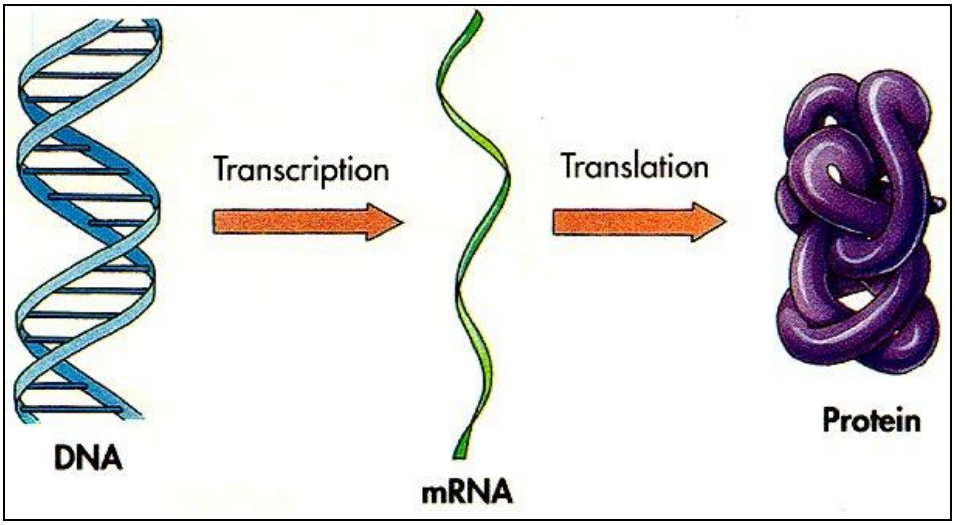


Figure 7. Central Dogma of Molecular Genetics (15).

2.7 Regulation of gene expression

Proteins, are the end products of the genetic expression processes. A typical cell, requires thousands of different proteins at any given moment. These proteins, must be synthesized in response to the cell's current needs, transported (targeted) to the appropriate cellular location, and degraded when the need has passed. To appreciate the central importance of protein synthesis to every cell, it can be enlightening to consider, that protein synthesis can account for up to 90% of the chemical energy of the cell, for all biosynthetic reactions.

Among 100 000 genes (3×10^9 nucleotide pairs of DNA) in the human genome, only a fraction are expressed at any given time. Some gene products, have functions that mandate their presence in very large amounts. Other gene products, are needed in much smaller amounts. Requirements for a given gene product, may also change with time. In fact, during development in a multi cellular eucaryote, some proteins that influence cellular differentiation, are present for only a brief time in a small subset of an organism's cells. The specialization of some cells for particular functions, can also dramatically affect the need for various gene products.

Thus, the regulation of gene expression, is a critical component in regulating cellular metabolism, and in orchestrating and maintaining the structural and functional differences, that exists in cells during development. Given the high energetic cost of protein synthesis, regulation of gene expression, is essential if the cell is to make optimal use of available energy.

Regulating the concentration of a cellular protein, involves a delicate balance of many processes. There are at least six potential points, at which the amount of protein can be regulated:

(1) Synthesis of mRNA transcript, from the DNA coding strands.
(2) Post transcriptional processing, and maturing of mRNA.
(3) mRNA degradation (turnover).
(4) Protein synthesis (translation).
(5) Post translational modification of proteins, that enables their structure to transform into an active and functional one.
(6) Protein degradation (Figure 8).

These regulating steps that are crucial for the cell, are regulated by the dynamic interaction between: nucleic acids (such as promoter sequences and particular strutres of the double helix), nuclear proteins (such as transcription regulatory factors), and enzymes (such as polymerases and topoisomerases). These DNA- Protein- enzymes interactions, may initiate, stop, activate or inhibit the genetic expression (Figure 9).

The understanding of the basics of genetic structure, expression and functions, permited great advances in various technical aspects of genetic engineering. These techniques, are becoming fundamental to the study, detection, and analysis of all processes of biological and biomedical science.

It is essential to know, that the performance and efficiency of Molecular biology techniques, is mostly due to a multitude of biological and biochemical tools such as:

Enzymes:	DNAase, Nuclease, Sequanase, Polymerase, Ligase, Phosphotase, Kinase, and endoclease.
Vectors:	Plasmids, Viral Vectors, or Yeast Vectors.

These crucial tools, have generally bacterial viral and sometimes vegetal origins, since their human analogues can be less accessible, or simply inexistent (such as bacterial restriction enzymes or viral reverse transcriptase).

Other more macroscopic tools, that are available thanks to biomedical engineering or Bio informatics, help increasing the speed and the efficiency of biotechnology.

In order to understand their principles and applications, let us now describe the most prominent tools and techniques of Genetic engineering.

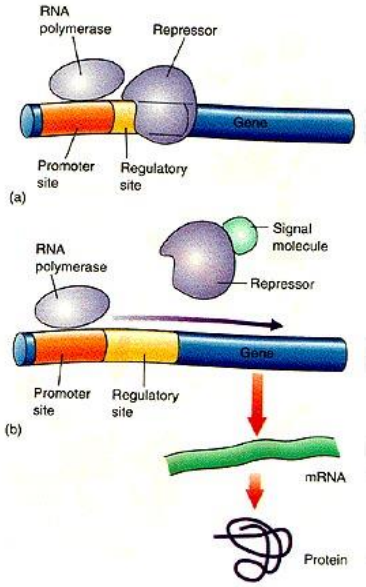


Figure 8. Gene regulation: promoter and transcription factors (21).

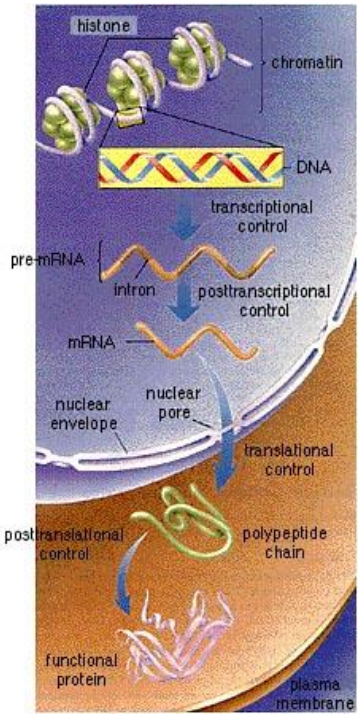


Figure 9. Control check points of genetic expression (12).

3 RECOMBINANT DNA AND GENE CLONING TECHNIQUES

3.1 The basics of DNA cloning

Cloning is defined as: making identical copies. It is a term that once was restricted to the procedure of isolating one cell from a larger population of cells, then allowing it to reproduce itself, to generate many identical cells. In such a way, sufficient quantities of a single cell type, were made available for study.

By analogy, DNA cloning, involves separating a specific gene or segment of DNA from it's larger chromosomes, and attaching it to a small molecule of carrier DNA (vector), then replicating it by selective amplification of that particular gene or DNA segment (1) (Figure 10).

Technical procedure:

Cloning a segment of DNA, either procaryotic or eucaryotic, entails six general procedures:

(1) In vitro extraction of DNA, using cell lysis, proteinases, nucleic acid isolation, and ethanol precipitation.
(2) In vitro cutting of DNA at precise locations, using sequence-specific endonucleases (Restriction enzymes), which act as molecular "scissor" of DNA.

(3) In vitro covalent joining of two DNA fragments, using DNA ligases.
(4) In vitro incorporation of a gene or a DNA segment of interest, into a small self-replicating DNA molecule, of viral or bacterial origin (viral DNA vector or a Plasmid vector). The resulting composite DNA, is called "recombinant DNA."
(5) Moving the recombinant DNA from the test tube into a host cell, that can provide the enzymatic machinery for DNA replication (or copying).
(6) Selecting or identifying those host cells that contain recombinant DNA, using selection markers (22).

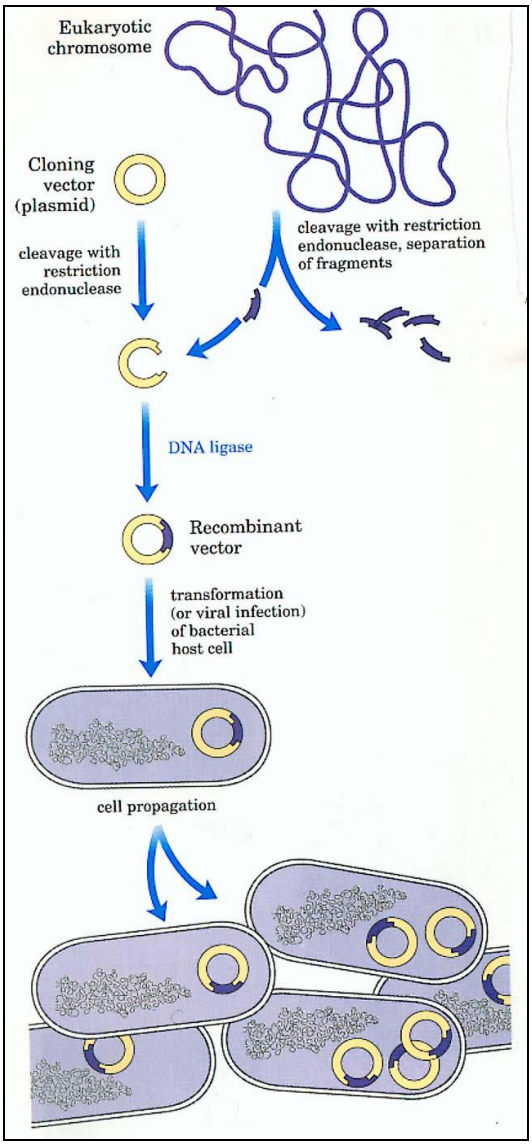


Figure 10. DNA cloning (21).

3.1.1 Host cells

The bacterium E. Coli, was the first organism used for DNA cloning and recombinant DNA technology (Figure 10). In fact, it is still the most common host cell, because it presents many advantages:

- its DNA metabolism and many other biochemical processes, are well understood.
- Many naturally occurring cloning vectors, such as bacteriophages and plasmids associated with E. Coli, are well characterized.
- And effective techniques, are available for moving DNA from one bacterial cell to another.

3.1.2 Restriction endonucleases and DNA ligases

They are a set of enzymes, particularly important to recombinant DNA technology. Restriction endonucleases, were made available by decades of research on nucleic acid metabolism. They are found in wide range of bacterial species, where they serve as protection, against intrusion of viral DNA in the bacteria.

In vitro, Molecular Biologists use these restriction enzymes, to cut or digest non methylated DNA at specific sequences, called restriction sites. These DNA digests, will generate a set of small fragments, called restriction fragments (Figure 11).

Some restriction endonucleases, cut both strands of DNA, as to leave no impaired bases on either end. These ends are often called “blunt ends”.

Other restriction enzymes, make staggered cuts on the two DNA strands, leaving at each end of each DNA strand, two or four impaired nucleotides. These ends are referred to as “cohesive ends” or “sticky ends”.

DNA ligases (contrary to the endonucleases), are a set of enzymes, used to sel DNA molecules together, such as joining a gene sequence to an unrelated cloning vector to form a recombinant DNA, also called, a recombinant chromosome, or a genetic construction.

3.2 Cloning vectors, selection markers, and transformation of host cells.

Cloning vectors, are used to carry and amplify inserted DNA segments. Three types of cloning vectors are commonly used in E Coli cloning:

Plasmids, Bacteriophages and Cosmids.

Plasmids, are circular DNA molecules, that replicate separately from the host chromosome. Naturally occurring bacterial plasmids, range in size from 5000 (5 kbp) to 40 000 (40 kbp) base pairs. Plasmids, can be introduced into bacterial cells, by a process called transformation: where the cells, incubated with the recombinant DNA, are subjected to a heat shock (rapidly shifting from $0^{o}C$ to $37\text{-}43^{o}C$). The selection of the host cells, that incorporated the recombinant plasmid DNA, is possible using selectable markers, which are

specific plasmid genes, that confer a specific antibiotic resistance to the host cells. Thus, only those cells that have been transformed with the recombinant construct, grow in presence of the antibiotic in the culture media. Many different plasmid vectors, suitable for cloning, have been developed, by modifying naturally occurring plasmids, yet they all must have, an origin of replication, an antibiotic resistance gene for selection, as well as endonuclease restriction sites (linker or polylinker site). Recombinant plasmids, can carry gene sequences, or DNA fragments, up to 15 000 bp (15 kbp).

Bacteriophages, are bacterial viruses, or phages, that grow in, and destroy the bacterial by lysis. As vectors, bacteriophages can carry DNA fragments up to 23 000 bp (23 kbp).

Cosmids, are DNA vectors, obtained by digestion of genomic DNA. It has a plasmid type replication, and can carry DNA fragments up to 45 000 bp (45 Kbp).

Other vectors contain, (in addition to the origin of replication, the antibiotic resistance gene for selection, and the restriction sites linker), an inducible regulatory sequence (promoter), that permits the expression of the gene of interest present in the recombinant construct, into the host cell. These vectors are called “Expression vectors” (22).

3.3 Constructing and screening of a DNA library

Isolating a gene from a cellular chromosome, required the preparing of thousands of digested DNA fragments, derived from the genome of a particular organism. Each fragment, is attached to a cloning vector. The DNA library, is then screened for the presence of a gene of interest, using a small labeled DNA sequence (probe). The probe, often presents homologies with the gene of interest, and hybridizes with it during the screening process. A purification and amplification of the DNA construct, is then performed using antibiotic resistance selection genes.

A DNA library can be made of, genomic DNA (Chromosomal DNA), or complementary DNA fragments (cDNA). The cDNA, is produced from purified mRNA, by using the enzyme reverse transcriptase (an RNA dependent DNA polymerase), which mediates the conversion of the genetic information, present in a single stranded molecule of RNA, into a double stranded molecule of DNA (22),(23).

3.4 Restriction mapping, enzyme cleavage of chromosomes

Most restriction endonucleases, cut DNA molecules in a site specific manner. As a result, they can be used to generate physical maps of chromosomes. These restriction enzymes digests, are of tremendous value in assisting researchers, to isolate small segments of chromosomes carrying genes, or other DNA sequence of interest. The restriction maps, are invaluable in DNA cloning and sequencing experiments.

The sizes of the restriction fragments, can be determined by polyacrylamide, or agarose gel electrophoresis. During DNA separation by electrophoresis, the DNA, which is charged negatively, migrates toward the positively charged electrode, providing an accurate estimates of the DNA fragments length (The rate of migration is inversely proportional to the length of DNA). Thus, the small fragments, are able to migrate through the gel, faster than the large fragments (20),(22).

There are two major approaches to restriction mapping, all of which depend on the use of polyacrylamide, or agarose gel electrophoresis, to obtain accurate estimates of DNA fragment size

(1) A sequential digest of chromosomes, with two or more different restriction enzymes.
(2) A partial digest of chromosomes, an incomplete digest, using only some of the restriction sites available to the enzymes(Figure 11).

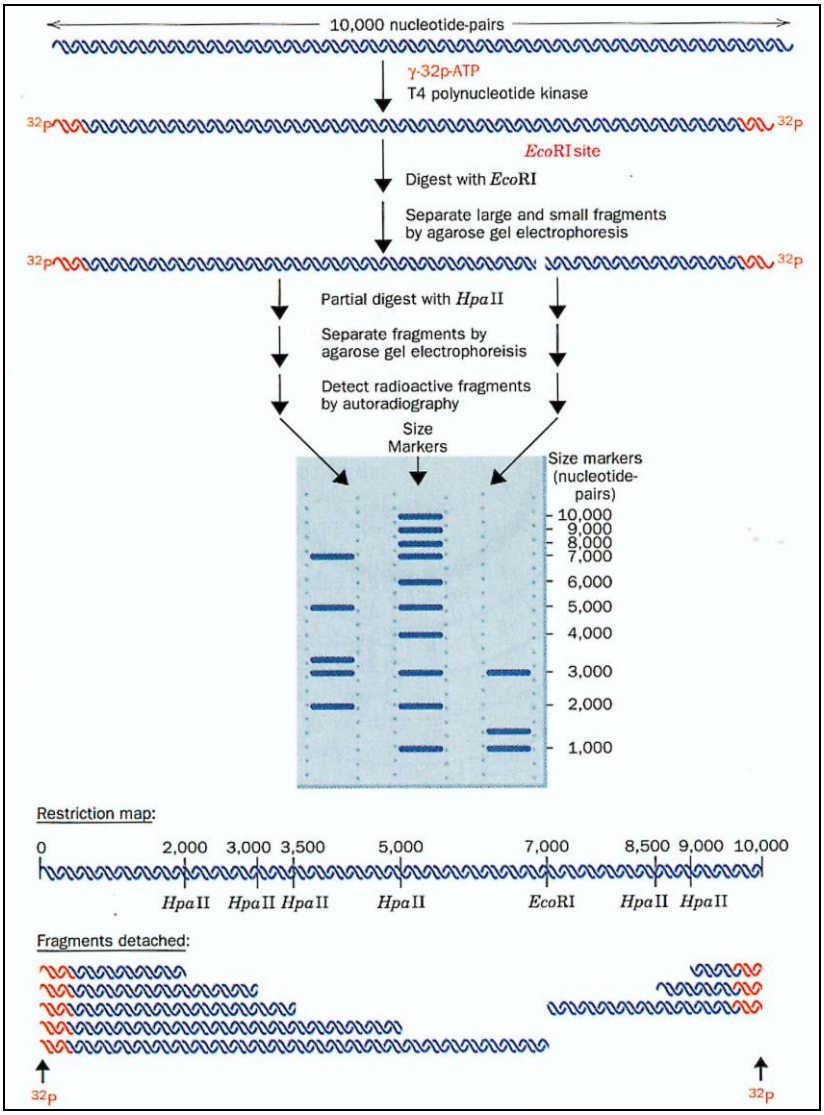


Figure 11. Chromosome restriction mapping (12).

3.5 Nucleotide sequencing, a fine structure chromosome mapping

The ultimate fine structure mapping of a gene, or a chromosome, is its nucleotide pairs sequence, complete with a chart of all nucleotide pair changes that alter the function of that gene or chromosome.

Prior to 1975, the thought of trying to sequence entire chromosomes, was barely conceivable. By late 1976, the entire 5 387 nucleotide long chromosome of bacteriophage ΦX174, has been sequenced. Then, the entire chromosomes of several viruses, including the complete 48 502 nucleotide pairs sequence of the circular form of the λ phage chromosome, and segments of eucaryotic

chromosomes, and tens of thousands of nucleotide pairs in length have been sequenced. Today, only three decades later, the map of 50 000 to 100 000 human genes has been completed. The entire $3x10^9$ nucleotide-pairs of the human genome, have been sequenced. This human genome project, was headed by Dr James D. Watson, the 1962 Nobel prize, and co-discoverer of the double helical structure of DNA. The project that started in 1988, and was expected to take 15 to 20 years, was actually completed in only 12 years.

Our present ability to sequence essentially any DNA molecule, is the result of four major developments :

(1) The discovery of restriction enzymes, and their use in preparing homogenous samples of specific segments of chromosomes.
(2) The improvement of gel electrophoresis procedures, to the point where DNA fragments that differ in length by a single nucleotide could be resolved.
(3) The development of gene cloning techniques, that greatly facilitated the preparation of large quantities of a particular gene or DNA sequence of interest, in order to use in sequencing studies.
(4) As well as the development of two different approaches, to the rapid sequencing of DNA molecules.

Both the two different sequencing methods, require a population of DNA fragments, that have one end in common (they all end with exactly the same nucleotide), and terminate at all possible positions at the other end.

These fragments, are separated on the basis of their length, by polyacrylamide gel eletrophoresis.

In both sequencing techniques, four separate parallel reactions are carried out, each of which generates a set of fragments, terminating at one of the four bases (A,G,C,T) in DNA.

- The Maxam and Gilbert procedure, uses four different chemical reactions, to cut DNA chains at As,Gs,Cs orCs+Ts.
- The second approach developed by Sanger and colleagues, uses an enzymatic procedure, and specific chain terminators, to generate four populations of fragments, that terminate respectively at, As,Gs,Cs and Ts. The 2’,3’ dideoxyribonucleocide triphosphates, used as chain terminators, are most commonly used in DNA sequencing studies. They are Nucleotides with no 3’OH, thus, when added to the end of a chain, no subsequent extension can be possible (1), (22).

After the DNA fragments are generated in the four parallel reactions, they are released from the template strands by denaturation. They can be separated by polyacrylamide gel eletrophoresis. Their positions in the gel is detected by autoradiography. The bands of the autoradiogramm, signal the labeled (radioactive or chemiluminescent) DNA restriction fragments. They produce a

ladder, defining the nucleotide sequence from the longest to the shortesr synthetised DNA chains.

The shortest fragment will migrate the lowest, and each successive band, will contain chains that are one nucleotide longer than the chain in the preceding band (Figure 12).

After the isolation and characterization of a genetic sequence, more interesting, is the study of gene expression, as well as the stage and localization of this expression

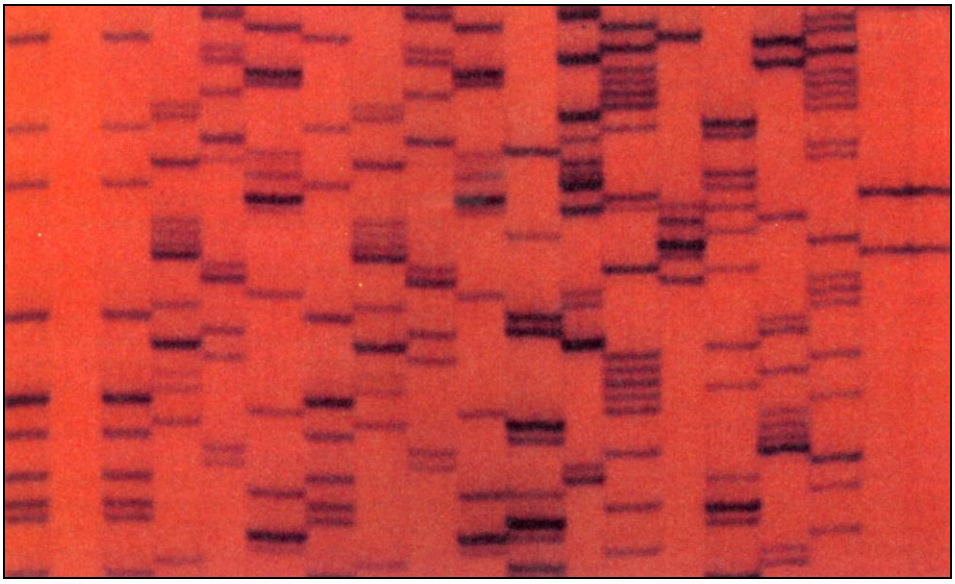


Figure 12. DNA sequencing (12).

3.6 Gene DNA analysis by southern blot hybridization (Figure 13)

Gel electrophoresis, provides a powerful tool for the separation of DNA, RNA, and Protein macromolecules. The essential feature of DNA southern blot is:

- The transfer of (the, negatively charged) DNA restriction fragments, that were separated by gel electrophoresis, to a (positively charged) nitrocellulose membrane;
- The DNA is then denatured, either prior to, or during transfer, using an alkaline solution;
- After the transfer is completed, the DNA is immobilized, and a labeled (radioactive or chemi luminescent) DNA “probe”, containing the sequence of interest, is hybridized or annealed with the complementary DNA sequence on the membrane;
- Nonannealed probe, is then washed off the membrane; and
- The transfer membrane, is then exposed to x-ray film, to detect the presence of the probe-labeled gene of interest (1).

3.7 RNA analysis by Northern blot hybridization

RNA molecules separated by agarose gel electrophoresis, can be similarly transferred, and analyzed, in the same way we described in DNA Southern blot (25).

3.8 Protein analysis by western blot techniques

Proteins are separated by polyacrylamid gel electrophoresis, in the presence of a denaturing SDS detergent (sodium dodecyl sulfate). After electrophoresis, the proteins can also be transferred from the gel to nitrocellulose, by using an electric current (western blotting or electro blotting). After transfer, a specific protein can be identified by immunodetection, using a primary antibody against the protein, and a secondary labeled antibody against the primary one (1).

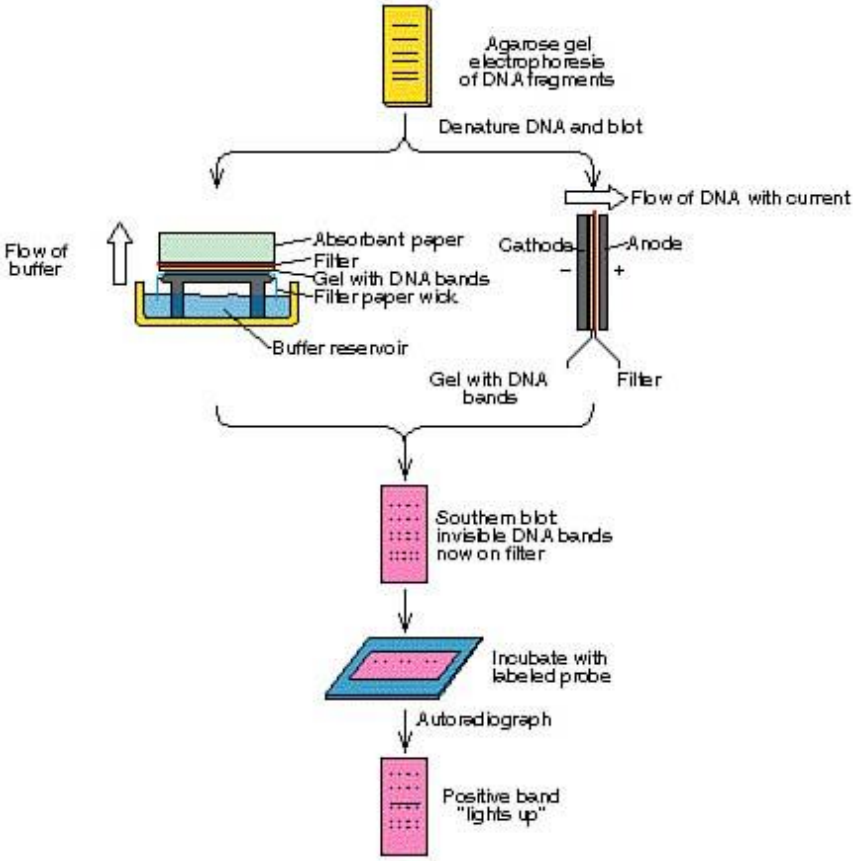


Figure 13. DNA Analysis by southern blot hybridization (22).

3.9 PCR or DNA amplification by Polymerase chain reaction (Figure 14)

The polymerase chain reaction, usually called PCR (invented by Karry Mullis in 1984) is an extremely powerful new procedure, that allows one to amplify (a million fold or more) a selected DNA sequence in a genome. PCR can be used: to clone a given DNA sequence in vitro, without using host cells, or DNA libraries during the cloning process.

The PCR procedure, involves using synthetic oligonucleotides (primers) complementary to two known sequences flanking the gene of interest. These primers, will initiate the in vitro enzymatic amplification of this DNA segment of interest.

The PCR procedure, involves three steps, repeated many times, to produce cycles of amplification:

Step 1 : DNA denaturation.

The genomic DNA containing the sequence of DNA template (to be amplified), is denatured by heating (temperature of 95-97°C).

Step 2 : Primer template hybridization.

The denatured DNA, is annealed to an excess of the synthetic oligonucleotide primers.

Step 3 : DNA polymerization.

Heat stable DNA polymerase (Taq polymerase), is used to replicate (copy) the DNA segment located between the flanking primers.

The denatured DNA, provides the required template function, and the primers, provide the free 3'OH end required for DNA chain extension.

The products of the first cycle of replication are then denatured, annealed to oligonucleotide primers, and replicated again with DNA polymerase.

This process is repeated through 25 or 30 cycles (which can take only a few hours when automated (1) (Figure 14).

The PCR amplification of DNA, is exponential, and sensitive enough, to detect as little as one DNA molecule in almost any type of samples.

PCR can also be used to amplify RNA extracted after converting it to cDNA by reverse transcriptase, we call this technique RT PCR (14).

3.10 RFLP and DNA finger printing (Figure 15)

DNA finger printing, is based on sequence polymorphisms, that occur in the human genome (and the genome of every other organism). Sequence polymorphisms, are slight sequence differences, (usually single base pair changes or point mutations), that occur from individual to individual, once every few hundred base pairs. Some of the sequence changes, affect recognition sites for restriction enzymes, resulting, in a variation, from individual to individual, in the size of certain DNA fragments, produced by digestion with a particular restriction enzyme. These size differences, are referred to as: Restriction Length Polymorphisms, or RFLPs. The identification of the RFLPs generated by restriction endonuclease digests, is possible using probes for

repeated sequences present in the human genome, called, mini satellite or micro satellite DNA.

Thus, RFLPs technique relies on:

- DNA extraction or amplification, depending on the amount of DNA available for analysis;
- DNA digestion, with restriction enzymes to generate RFLPs;
- Agarose gel electrophoresis, to separate the fragments;
- Southern blotting of DNA into a nitrocellulose membrane;
- Hybridization of the RFLPs with the labeled (radioactif or chemi luminescent) probe and autoradiography (14),(22).

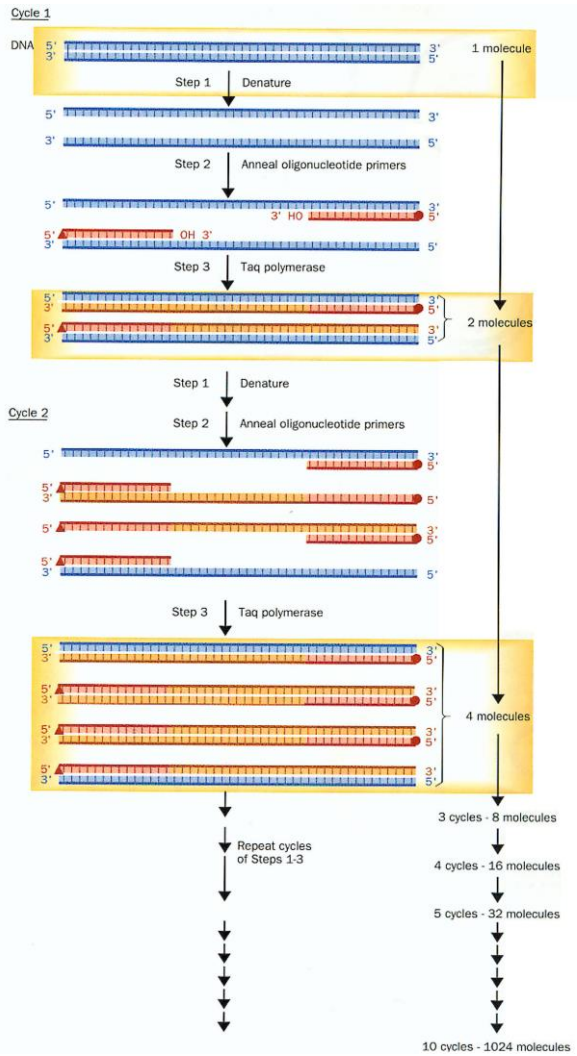


Figure 14. DNA amplification by polymerase chain reaction PCR (14).

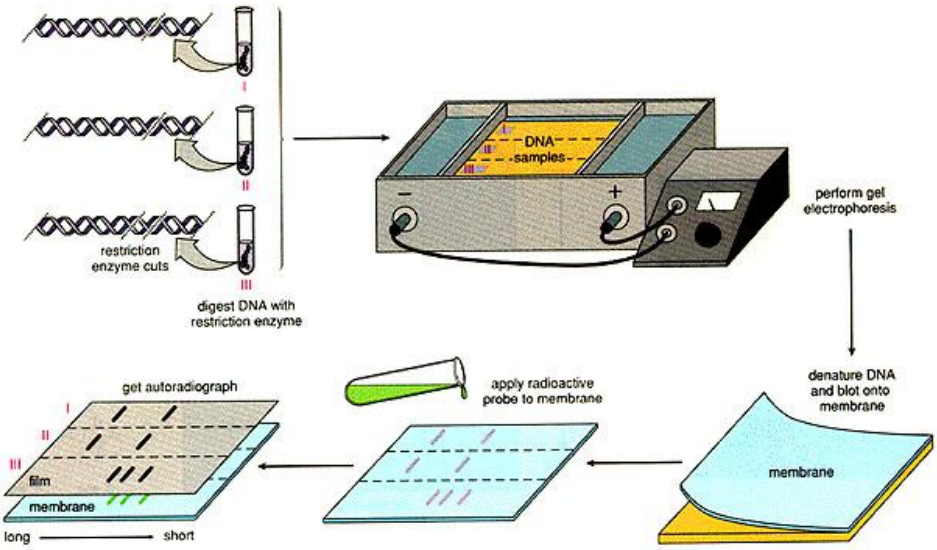


Figure 15. RFLP Analysis and DNA Fingerprinting (15).

3.11 In situ hybridization (Figure 16)

In situ hybridization, is a genetic engineering technique, used to detect and locate, specific DNA or RNA sequences, in tissues, or on chromosomes. A radioactive or fluorescent labeled DNA or RNA probe, homologous to the gene of interest, is applied to fixed tissue, or chromosome preparations. The labeled probe, hybridizes with the complementary sequences on the targeted gene. Fluorescent in situ hybridization is known as (FISH) technique (12).

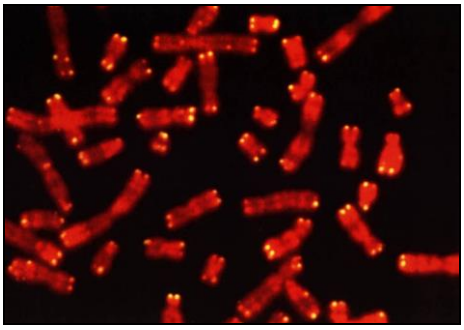


Figure 16. Fluorescent in situ Hybridization: FISH (12).

3.12 In vitro site specific mutagenesis

The recombinant DNA and gene cloning technologies described above, allow scientists to isolate, characterize, amplify, and analyse, any given gene from living organisms.

In addition, these methodologies, permit researchers, to modify or manipulate genes in vitro. In fact, we can now dissect genes at the nucleotide level, by changing one nucleotide at a time, and examining the effect of each change on the function of the gene or it's product (the protein). Such nucleotide changes, are accomplished by a procedure called "site specific mutagenesis", or "oligonucleotide directed site specific mutagenesis". This technique, uses the ability of the oligonucleotide primers to bind to the template DNA, even when they are not an exact sequence complement (partial complementarity or consensus complementarity hybridization).

Hence, this consensus binding, permits the incorporation of nucleotide changes such as, base substitutions, deletions, or additions, generating specifically mutated sequences. in eucaryotic cells(22).

3.13 Direct gene transfer into eucaryotic cells: Electroporation and Lipofection

Direst gene transfer, involves recombinant DNA introduction into eucaryotic cells, by transiently perturbing the cell membranes, and causing their permeability. This process can be accomplished using:

3.13.1 Lipofection

Lipofection, is a gene transfer technique, which uses liposomes. Liposomes are are small vesicle, artificially made from: phospholipides, DNA, proteins. Other materials such as recombinant gene constructs, can be enclosed within the liposome, and can be introduced into eucaryotic cells, using liposome fusion with the plasma membrane.

3.13.2 Electroporation

Electroporation, is a gene transfer technique, which uses a short pulse of high intensity electrical current to disrupt cell membranes, and render them temporarily permeable to DNA molecules (22).

3.14 Microinjection of DNA into fertilized eggs (Figure 17)

DNA microinjection, is at the basis of the production of transgenic animals. Integration of the microinjected DNA molecules in fertilized animal eggs, depend on the recombination mechanisms, of the organisms embryo itself.

The microinjected eggs, are surgically removed from the female parent, and are in vitro fertilized with sperm. The circular or linear DNA recombinant construction, carrying the gene of interest, is microinjected into the haploid male pronucleus of the fertilized egg. This injection is made, through a very fine tipped glass needle (12).

Since the injected DNA molecule, occurs early during embryogenesis, some germ line cells will carry the trans-gene. The progeny (G_0 generation) is almost

always a mosaic (hybrid). The mating of the G_0 generation with the G_1 progeny, result in complete transgenic animals, with all cells carrying the trans-gene.

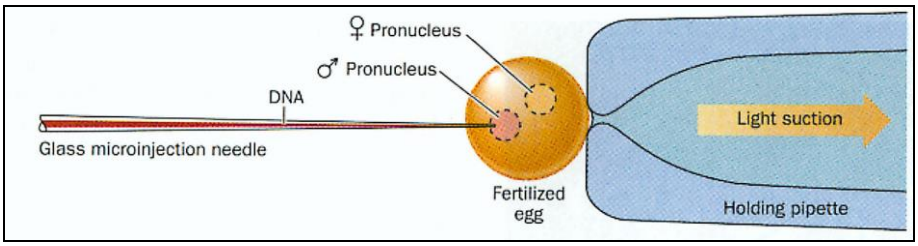


Figure 17. Recombinant DNA Microinjection in germ line cells (12).

3.15 Antisens RNA strategies

A new tool that has become possible with advances in recombinant DNA and gene cloning technologies, is the use of the so called "antisens RNA" (RNA which is unable to be translated). The method, simply involves, the synthesis of RNA molecules, that are complementary to mRNA produced by transcription of a given gene. The normal in vivo mRNA (sense mRNAthat), anneals with antisens RNA, to form a duplex. The antisens-RNA---mRNA complexe blocks translation of mRNA into a protein and thus, blocking genetic expression (2).

4 CURRENT AND POTENTIAL APPLICATION OF GENETIC ENGINEERING TOOLS AND TECHNIQUES

Decades of advances in various aspects of genetic engineering tools and techniques, led to numerous applications of this Biotechnology in Biological and Biomedical disciplines, such as: agriculture, criminology, industry, pharmacology, as well as preventive medicine, and early medical diagnosis.

Recombinant DNA techniques, (as described previously), such as gene cloning, sequencing, gene restriction mapping, southern, northern, and western blotting (of DNA, RNA and proteins), as well as in vitro mutagenesis were applied first, to study gene expression and function and to expand fundamental knowledge of biological processes.

Nonetheless, it was progressively clear to scientists, that the applications of genetic engineering techniques, can go beyond the fundamental studies and understanding of biological pathways.

Thus, Molecular Biology techniques, such as: recombinant proteins expression, PCR, RLFPs, DNA finger printing, in situ hybridization, RT PCR, direct gene transfer, transgenic organism, as well as antisens-RNA strategies, are becoming without doubt, the most powerful tool of analysis, diagnosis, and efficient solutions for the development of applied biological and medical fields.

4.1 Genetic engineering applied to pharmaceutics

Recombinant proteins expression and antisens RNA strategies

Genetic engineered proteins, or recombinant proteins technique, was developed: by inserting a gene of interest in an expression vector. The recombinant construct, introduced into a bacterial host, is amplified then extracted. Recombinant protein synthesis, proved to be a powerful technique, for producing large amounts of pure ,plant, animal, or human proteins in vitro. Hence, this technique, is now widely used in agricultural, and pharmaceuticals industry, as well as in human medical treatments(16, 28).

Thus, proteins, like human insulin, interferon, hormones, or anticoagulants are now available to patients, in large quantities, and a high degree of purification (1).

Other techniques, such as antisens-RNA strategies, are used in developing new drugs for methabolic diseases (eg. blocking the translation of an unwanted enzyme), or in plant genetic engineering (eg: preventing the production of enzymes that soften fruits and vegetables during long term storage) (17, 18, 19).

Table 1. Recombinant DNA products in medicine (21)

Product category	Examples/Uses
Anticoagulants	Tissue plasminogen activator (TPA) activates plasmin, an enzyme involved in dissolving clots; effective in treating heart attack victims.
Blood factors	Factor VIII promotes clotting and is deticient in hemophiliacs. Use of factor VIII produced by recombinant DNA technology eliminates the risks associated with blood transfusions.
Colony stimulating factors	Immune system growth factors that stimulate leukocyte production; used to treat immune deficiencies and to fight infections.
Erythropoietin	Stimulates erythrocyte production; used to treat anemia in patients with kidney disease.
Growth factors	Stimulate differentiation and growth of various cell types; used to promote wound healing.
Human growth hormone	Used to treat dwarfism.
Human insulin	Used to treat diabetes.
Interferons	Interfere with viral reproduction; also used to treat some cancers.

Interleukins	Activate and stimulate different classes of leukocytes; possible uses in wound healing, HIV infection, cancer , immune deficiencies.
Monoclonal antibodies	Extraordinary binding specificity is used in diagnostic tests. Also used to transport drugs, toxins, or radioactive compounds to tumors as a cancer therapy; many other uses.
Superoxide dismutase	Prevents tissue damage from reactive oxygen species when tissues deprived of O2 for short periods during surgery suddenly have blood flow restored.
Vaccines	Proteins derived from viral coats are as effective in "priming" an immune system as the killed virus more traditionally used for vaccines, but are safer. First developed was the vaccine for hepatitis B.

4.2 Genetic engineering applied in criminology and forensic medicine: PCR, southern blotting, DNA fingerprinting

while PCR, southern blotting, and sequencing, are frequently used as a rapid way for gene cloning, and expression studies in fundamental research, these recombinant DNA techniques, along with DNA finger printing, are progressively becoming the basis of forensic investigations. In fact, PCR and DNA finger printing, have become very praised in criminology, and are progressively replacing traditional fingerprinting. These techniques, are more valuable when the biological material collected from the scene of the crime (such as hair, blood, saliva, or semen strains), is a small amount .
As described previously, these techniques involves:

- Amplifying DNA, if the quantity is insufficient.
- Digesting DNA, with restriction endonucleases.
- Separating the restriction fragments, by gel electrophoresis.
- Southern blotting DNA into nitrocellulose membrane.
- Hybridizing the DNA with repetitive labeled probes.
- Exposing the hybridized membrane to x-ray film.
- Developing and analyzing the autoradiogramm (14).

Finally, comparing the RFLPs restriction fragment length polymorphism, of the samples found on the scene of the crime, with the RFLPs of the suspect, and the victim (Figure 18).

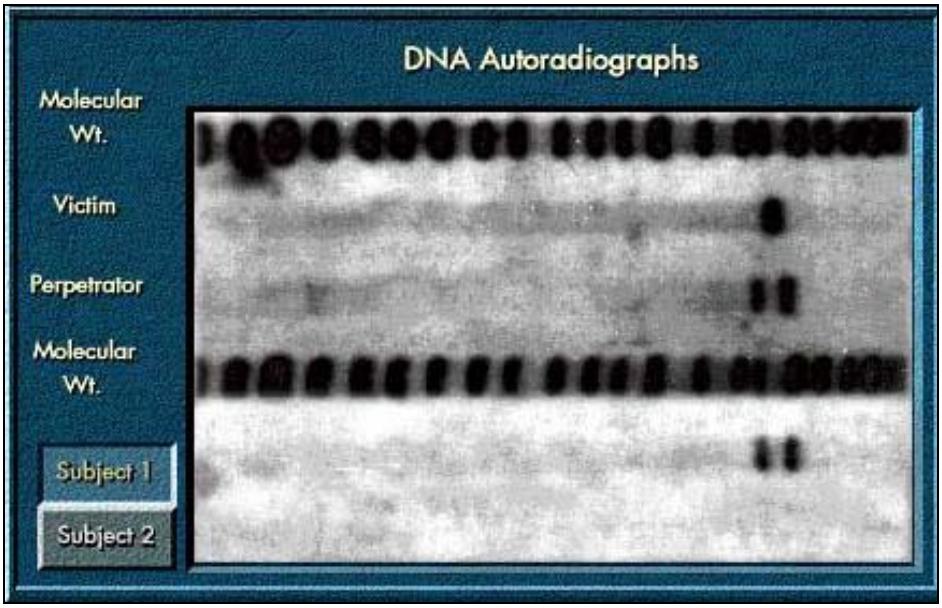


Figure 18. DNA Fingerprinting Analysis in criminology (15).

4.3 Genetic engineering applied to medical diagnosis and biomedical analysis

PCR, RFLPs and in-situ hybridization

PCR, has proved to be a powerful tool, for early medical diagnosis, and analysis, of different infectious diseases such as AIDS (HIV), or Hepatitis (HCV, HBV). RFLPs or restriction fragment length polymorphism (combined to PCR when needed), is as described previously a crucial tool in forensic medicine. In addition, RFLPs technique, makes it possible to identify different allelic forms of genes, detect point mutations on DNA sequences, and defective exons. Thus, RFLPs is a key technique for medical diagnosis of genetic diseases such as, Huntington's chorea, cystic fibrosis and sickle cell anemia (Figures 19, 20, and 21) ….,as well as the identification of cancer genetic markers. RFLPs is then very useful for preventive medicine, and early diagnosis, even at a fetal stage (prenatal diagnosis) (8, 9, 10).

Another genetic engineering technique, widely praised in medical analysis and diagnosis is: in-situ hybridization, especially fluorescent in-situ hybridization (FISH). FISH, is used in chromosome mapping, for it allows the study and detection of chromosome abnormalities, and can be complementary to RFLPs in preventive medicine (26).

Disease (Examples)	Gene	Frequency	Tri-nucleotide	Normal Number	Mutant Allele	Chromosome
Huntington's chorea	CH	1:10 000	$(CAG)_n$	11-34	42-100	4p16.3

A. Genetic diseases due to increased number of trinucleotides

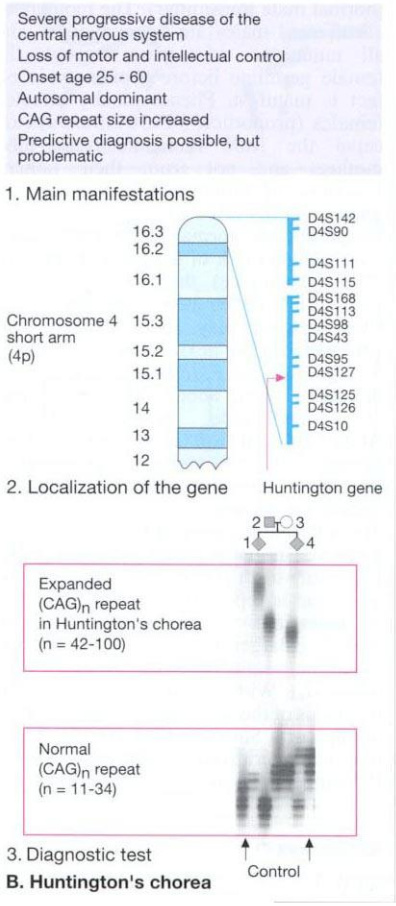


Figure 19. Genetic identification of Huntington disease.
(Atlas of Genetics. 1995; Thieme Medical Publishers Inc. New York).

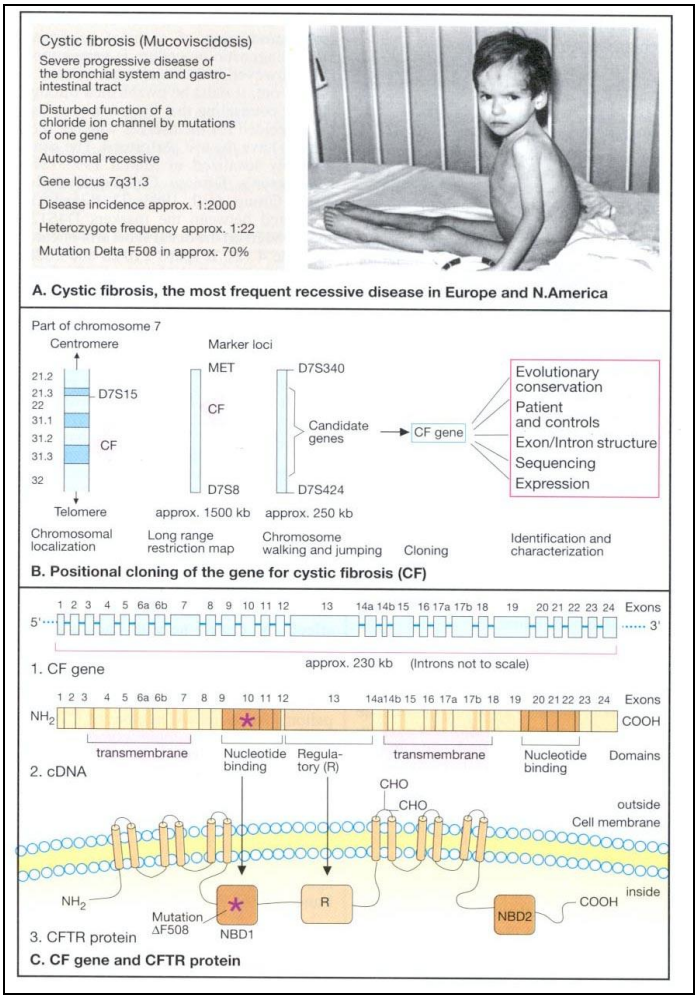


Figure 20. Genetic identification of Cystic Fibrosis.
(Atlas of Genetics. 1995; Thieme Medical Publishers Inc. New York).

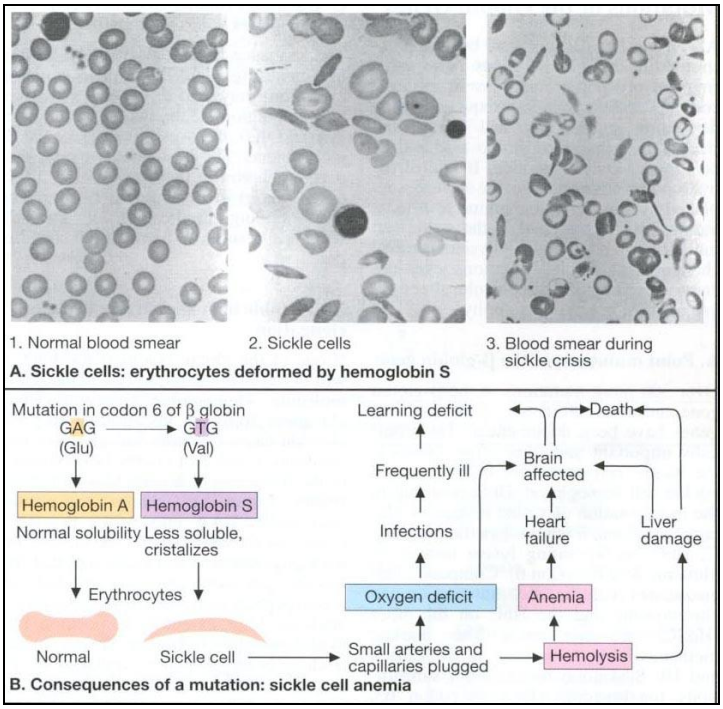


Figure 21. Molecular identification of Sickle Cell Anemia.
(Atlas of Genetics. 1995; Thieme Medical Publishers Inc. New York).

5 DISCUSSION

Current and future aspects of genetic engineering techniques

Advantages and potential hazards

It is obvious, that the current technical aspects of genetic engineering, present numerous advantages. In fact, these techniques, promise to enhance the efficiency, and quality of various facets of human life, in fields such as: research, pharmaceutics, agriculture, criminology, industry, medicine and biomedical analysis (3, 4).

Nonetheless, the limits of Biotechnology are still beyond reach, since Genetic Engineering research and development, are still coming up with wonderful techniques, that seem more skilful and ingenious than ever. Unfortunately, these techniques also raise controversies, and apprehensions, in the society as a whole (5, 6, 7).

Thus, Molecular Biologists, wondered whether these techniques might introduce potential hazards. The first controversy, which is considered a crusade by some organizations, is concerning genetically modified plants and organisms (GMOs) (Figure 22). These organizations, apprehend gene transfer of useful traits such as: insect and germ resistance, into plants that are destined to

consumers. They justify their fear, by the fact that the cloning and expression vectors used for gene transfer, may cause unknown side effects, that could be harmful to the consumer. Nonetheless, the use of sterile conditions, the extreme precision of constructing recombinant DNA, and the ability to map and sequence nucleotide by nucleotide the expression vectors before use, as well as the ability to cut unwanted fragments by restriction endonucleases, ensures a very high degree of protection against any potential hazard. In fact, techniques such as: Plant and animal genetic engineering, (GMO), are already used in plant genetic improvement, aiming to food security, and to avoid the use of toxic insecticides and herbicides in agriculture.

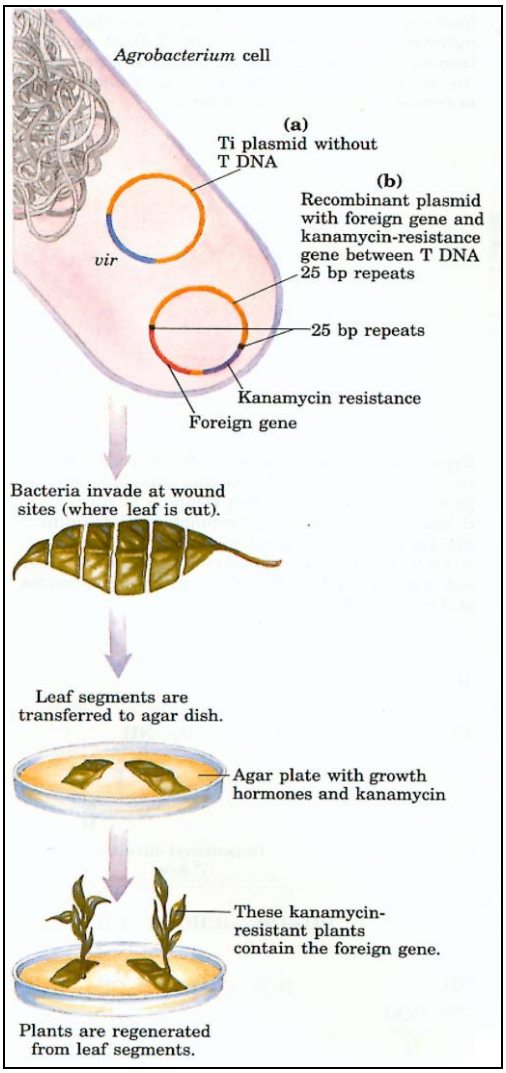


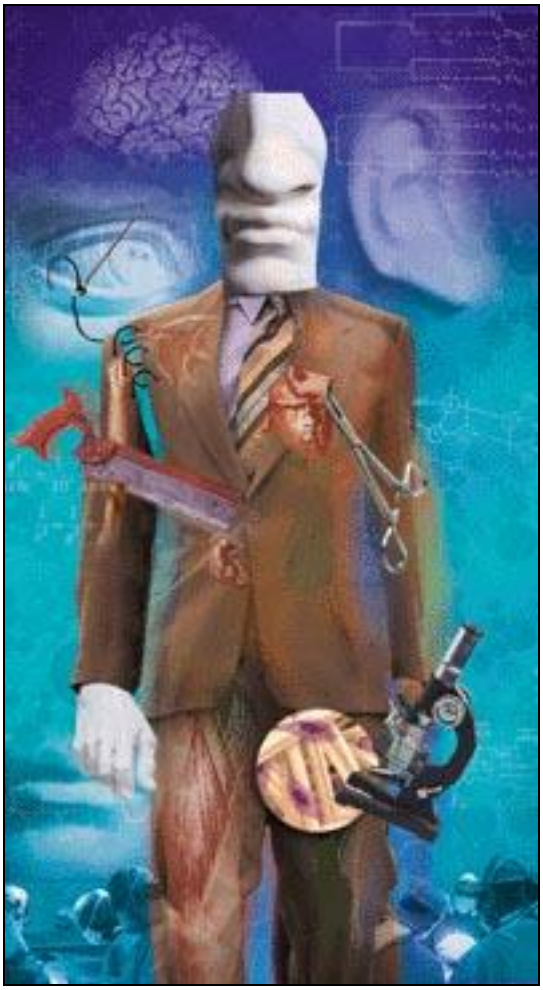
Figure 22. Genetically modified plants (12).

Tissue culture is now used to treat the burned. Organ cloning, is being developed for organ trans plants. Gene therapy, is also being developed to treat incurable genetic, and infectious diseases, along with cancer (26, 27).

The ethical dilemmas, among the scientific, political, legal, and social communities, about the use of genetic engineering techniques is growing, as the animal cloning has been completed, and the human cloning is eminent. The ultimate fear, is using human cloning (nuclear transplantation from a somatic cell to an enucleated oocyte), to genetically discriminate among human beings, or to genetically engineer and artificially selected super man, or to loose human freedom and intimacy by having all our genetic mutations, potential diseases and flaws, disclosed in the work place, or to insurance companies even before birth.

A more compromising and wise way to manage these apprehensions, is to understand that all great discoveries and advances in science have pros and cons, and that society can not stop science development, and justify it by potential misuses. Nonetheless, it's the role of the society as a whole, to use the advantages, as well as control, regulate and set legal limits for the potential hazards. We have to keep in mind, that thousands of incurable inherited diseases are catalogued to date, such as: various metabolic and genetic diseases, as well as numerous types of killer cancers (Phenylketonurea, Thalassemia, Sickle cell anemia, and Combined Immuno deficiencies).

A comforting thought, is that many incurable human diseases, could be prevented or treated, using recombinant DNA technology, and somatic cells gene therapy.



(Scientific American)

6 CONCLUSION

Genetic engineering technologies, are without doubt the most powerful tool ever developed in the field of Biological and Medical Science. The ability to carry out carefully designed genetic modifications of living cells, has many potential applications. Some of these applications, have already reached function. If used wisely, Genetic engineering promises to render humanity great services. If used haphazardly, and carelessly, genetic engineering could have negative impact on our society as a whole. Thus, while understanding, accepting, and applying genetic engineering advantages, we must set the ethical rules, and limits of their use, for the good of humanity.

AKNOWLEDGMENTS

I am indebted to Dr Mohammed Adad for invaluable help in informatics along with helpful criticism and comments. I am grateful to Lylia Adad Ms and Mr Adad (father) and Ms Nouzha Quasri for their patience and moral support.

REFERENCES*

1. Alami-Ouahabi. N *et al.* 1996. Mol Cel. Bio; 16(7): 3720-29.
2. Alberts. B *et al.* 1989. Molecular Biology of the Cell. Garland Publishing, NewYork.
3. Benton. D 1996. Trends in Biothechnology; 14(8): 261-72.
4. Carmichael. CG *and al*. 1995. HIV aids primary care handbook. Appelton and lange. Norwalk, Connecticut, USA.
5. Clemns. PR and Caskey. CT 1994. Eur Neurol; 34: 181-85.
6. Collins. FS and Galas. D 1993. Science; 266: 43-9.
7. Collins. FS 1992. Science; 256: 744-88.
8. Dry. S 2000. Nat Struct Biol ;7suppl: 946-49.
9. Eyster. KM and Lindahl. R 2001; S D J Med; 54(2): 57-61.
10. Friedmann. T 1994. Trends in Genetics; 10(6): 210-12.
11. Gabriel. SE *et al.* 1994. Science; 266: 107-14.
12. Gardner. EJ *et al.* 1991. Principles of Genetics. Wiley. J. ed. NewYork.
13. Georgopapadakou. NH and Walsh. TJ 1994. Science; 264: 371-80.
14. Innis. MA *et al.* 1990. PCR Protocols, a guide to methods and applications. Academic Press. Boston, USA.
15. Johnson. G 1996. Explorations in Cell Biology and Genetics. Brown Publishers.
16. Jordan. BR 1999. J. Biomed. Sci; 6(3): 145-50.
17. Kaul. PN 1998. Prog. Drug. Res; 50(9): 9-105.
18. Kay. MA *et al.* 1993. Science; 262: 117-19.
19. Kay. A *et al.* 1994. Trends in Genetics. 10(7): 253-57.
20. Kendrew. S and Lawrence. E 1994. Encyclopedia of Molecular Biology. Alden Press. Oxford.
21. Lehninger. AL *et al*. 1993. Principles of Biochemistry. Worth Publishers. NewYork.
22. Maniatis. T *et al*. 1989. Molecular cloning, a Laboratory Manual. Cold Spring Harbor Laboratory Press.
23. Muller. E and Alami-Ouahabi. N. 1993. Proc. Natl. Acad. Sci. USA; 90: 5818-22.
24. Rockey. DC 2000. Clin. Liver. Dis; 4(2): 319-55.
25. Thivierge. M and Alami-Ouahabi. N. 1993. J. Mol. Bio. Chem; 268(23): 17457-62.
26. Valbung. W *et al.* 1999. Ann. Med; 31(6): 430-1.
27. Vansteensel. MA *et al.* 2000. Proc. Soc. Ex. Biol. Med; 223(1): 1-7.
28. Vitullo. JC and Caskey. CT 1994. Eur Neurol; 34: 181-85.

* Compiled by authors but re-arranged by editors.

New Opportunities for Novel Drugs and Vaccines against HIV/AIDS based on the Structure and Functions of the HIV Accessory Proteins Nef and Vpr

AHMED ABDULLA AZAD*
Faculty of Health Sciences
University of Cape Town, South Africa

1 HIV/AIDS PATHOGENISIS

1.1 Introduction

With 3 million deaths, HIV/AIDS is now the fourth leading cause of death world wide, and ranks first among all infectious diseases, ahead of TB, Malaria and diarrhoeal diseases.

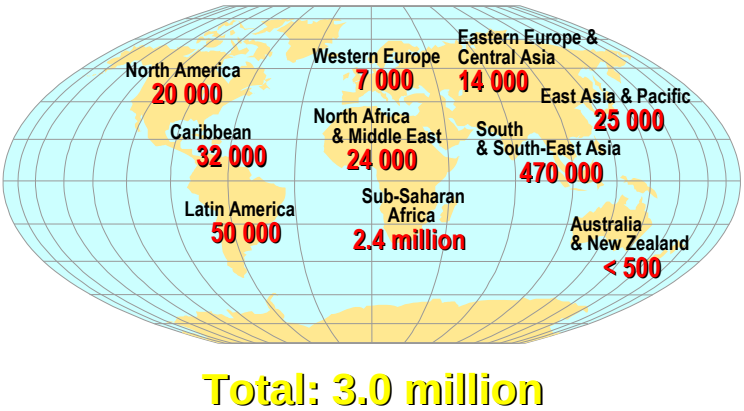


Figure 1. Estimated adult and child deaths from HIV/AIDS during 2000 (Source: UNAIDS).

* Professor of Biochemistry, and Director of Research, University of Cape Town, South Africa; and Fellow of the Islamic Academy of Sciences.

Of the 36 million people living with HIV/AIDS, 25,3m live in Sub-Saharan Africa, and 5.8m in South and South East Asia in developing countries which are least able to manage the epidemic or afford the costly combination of drugs that have helped to keep the infection under control in the affluent countries of the West.

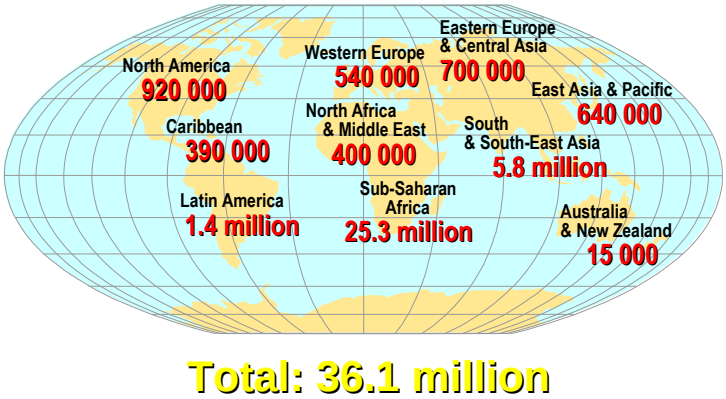


Figure 2. Adults and Children estimated to be living with HIV/AIDS as of end 2000 (Source: UNAIDS).

1.2 Current status of HIV vaccines

The best possible option for the developing world is prevention, and the availability of an affordable and effective preventive vaccine. In spite of almost twenty years of research, there is no vaccine in sight. Only a handful of candidate vaccines have gone into phase II clinical trials and none of these are likely to provide protection against strains prevalent in South Africa or India.

Vaccine research in South Africa is rightly directed to Clade C strains of HIV, and researchers in our own university are at the forefront of this effort. There is an urgency to produce a prototype Clade C vaccine for clinical trials and this will undoubtedly yield much needed information. However, HIV is even more variable than the influenza virus, and a vaccine against a single strain of HIV is unlikely to be protective against other strains even within the same clade.

In parallel with the very worthwhile efforts to produce a prototype Clade C vaccine for clinical trials, it is also very important to carry out basic research to find out all common conserved immunodominant regions of the different strains, and use this information to produce an engineered vaccine that is cross protective. There is also no effort in this country to produce a therapeutic vaccine for those people who are already infected.

Over 36 million people are now infected. Most of them are not even aware that they are infected, and will unknowingly pass on the virus to many more.

So the number of infected people will keep on rising for a very long time before it plateaus. In the absence of a vaccine to bring down the infection rate and for a long time into the future, the disease has to be managed by therapeutic intervention.

1.3 Currently used anti-retroviral therapy

Use of highly active anti-retroviral therapy (HAART) has resulted in a dramatic decrease in morbidity and mortality related to HIV infection in the developed world.

Short-term use is beneficial in reducing mother to child transmission and in emergencies and all efforts to bring down the treatment cost must be supported.

On long-term use there is treatment failure in over 50% of patients due to emergence of drug-resistant strains, and non-compliance due to severe side effects. HAART is also unable to eliminate virus reservoirs in the body. The combination of drugs is extremely expensive (up to US$ 15,000 per person per year) and simply not an option for developing countries as a life long therapy.

There is, therefore, an urgent need for drugs that are more affordable, less toxic more patient-friendly, and can do more than what currently used anti-retrovirals can do. The acceptance that HIV/AIDS is a chronic infection and has to be managed as such will allow the development of strategies that can slow progressions to full-blown AIDS.

1.4 Defining features of AIDS progression

The defining feature of AIDS, (which is the end stage of HIV infection) is the rapid decline in the number of $CD4^+$ T lymphocytes (the natural host for HIV), 98% of which reside in lymphoid tissues. Even during the so-called clinical latency period, when the number of $CD4^+$ cells in the peripheral blood remains steady and the amount of virus in the blood remains low, the situation is quite different in lymphoid tissues where there is extensive virus replication and immune cell depletion. During the course of HIV-infection, the architecture of the lymph nodes and other lymphoid organs is gradually but irreversibly destroyed due to the indiscriminate killing of all types of cells in the lymphoid tissues. This is what ultimately causes the severe immunodeficiency seen in AIDS patients. Similar cell depletion and tissue atrophy in the brain and in the central nervous system (CNS) causes AIDS dementia.

The majority opinion in the HIV-research community certainly favours the notion that HIV kills the cells that it infects. This does not adequately explain why not more than 1% of $CD4^+$ T cells are infected while almost all immune cells, including one that cannot be infected with HIV, are killed during the course of the disease. So what causes the killing of the immune cells that are not infected with HIV? The answer is simple. Most of the cell killing is caused by viral agents, which are produced inside HIV-infected cells on viral command, exported outside the HIV-infected cells where they seek out and destroy

uninfected bystander cells. Bystander cell death is absolutely dependent on HIV infection of $CD4^+$ T lymphocytes, and the killing agents are viral components. But while the virus finds ways to kill uninfected immune cells, which may endanger its survival, it finds very clever ways of keeping its own home, the HIV-infected cells, alive and intact.

1.5 Therapeutic strategies for slowing progression to AIDS

AIDS results from the failure of the immune system due to depletion of immune cells, destruction of lymphoid tissues, and prolonged survival of HIV-infected cells.

So an effective therapeutic intervention to slow progression to AIDS should:

(i) prevent the destruction of the immune system, and
(ii) promote and hasten death of HIV-infected cells

Research in my own laboratory has focused on identifying molecular targets responsible for elimination of immune cells, and prolonged survival of HIV-infected cells, and developing strategies to reverse this. This has been possible only through very focused basic and strategic research.

2 ROLE OF EXTRACELLULAR Nef AND Vpr IN THE DESTRUCTION OF THE IMMUNE SYSTEM

2.1 Role of extracellular Nef in destruction of immune system

Nef is an HIV protein that neither forms the structure of the virus, nor does it have any enzymic properties. It is, therefore, known as an accessory protein.

When present in the extracellular medium Nef (and N-terminal fragment of Nef) disrupt cell membranes and destroy all types of cells, including immune cells in the lymphoid tissues. Nef could, therefore, contribute to immune cell depletion. Nef is also the most abundant HIV protein in the CNS and could therefore contribute to AIDS dementia. The cell killing activity is resident in the N-terminal region of the Nef Protein.

Myr-GGKWSKSSVIGWPAVRERMRR-OH
2 9 16 22

Random *α-Helix* *Myr Nef$_{2-22}$*

Figure 3. Primary and Tertiary Structures of Nef N-terminus.

A number of studies have clearly shown that Nef (and N-terminal Nef) is present in extracellular medium and on the surface of HIV-infected cells.

When immunodeficient SCID mice are transplanted with human organs, such as the thymus, the human thymus is not rejected. When the human thymus is infected with wild type HIV, there is very severe depletion of $CD4^+$ T lymphocytes. This is not seen with HIV from which the *nef* gene has been deleted. This proves that the Nef protein is directly involved in the killing of $CD4^+$ T lymphocytes *in vivo.*

Mice cannot be infected with HIV. But in mice made transgenic for HIV *nef* gene alone the production of HIV Nef protein on its own causes T cell depletion, atrophy of lymphoid tissues, and severe AIDS-like pathologies. This clearly shows that HIV Nef protein alone can produce symptoms seen on infection of human lymphoid tissues with HIV.

Monkeys infected with SIV develop AIDS. This does not happen if the SIV *nef* gene is deleted. If the deleted SIV *nef* gene is replaced by HIV *nef* gene (SHIV), then the monkeys again develop AIDS showing clearly that HIV Nef is responsible for progression to AIDS. Other studies clearly implicate the Nef N-terminus in progression to AIDS.

These studies directly implicate Nef in progression to AIDS. Nef is, therefore, a very important molecular target for drug development.

2.1 Extracellular Vpr causes Apoptosis

Vpr is another accessory protein of HIV. When present in the extracellular medium, Vpr permabilises cell membranes, causes mitochondrial dysfunction and rapid collapse of mitochondrial membrane potential, and penetrates cells and localises in the nucleus. All this suggests that extracellular Vpr causes Aptopsis. This was confirmed by very sensitive assays. Extracellular Vpr causes apoptopic death of a wide variety of lymphoid and neuronal cells. Vpr is therefore an important molecular target for drug development.

Apoptosis (also known as Programmed Cell Death) is a mechanism used by living organisms to induce unwanted cells to commit suicide. Apoptosis is a normal feature of the differentiation and maturation of an organism and a key feature in developing organs and limbs by getting rid of excess cells. It also plays a very important role in the defence mechanisms of the body. Apoptosis is induced in cells when they are damaged or become dangerous to the body. Inhibition of apoptosis in injured cells will induce abnormal growth and cancers. Apoptosis is also a very important mechanism for getting rid of virus-infected cells. In this case, $CD8^+$ T lymphocytes or CTL will bind to infected cells and induce them to commit suicide. Failure of apoptosis will result in increased virus production and disease.

The cell killing function of Vpr is resident at the C-terminus of the Vpr protein.

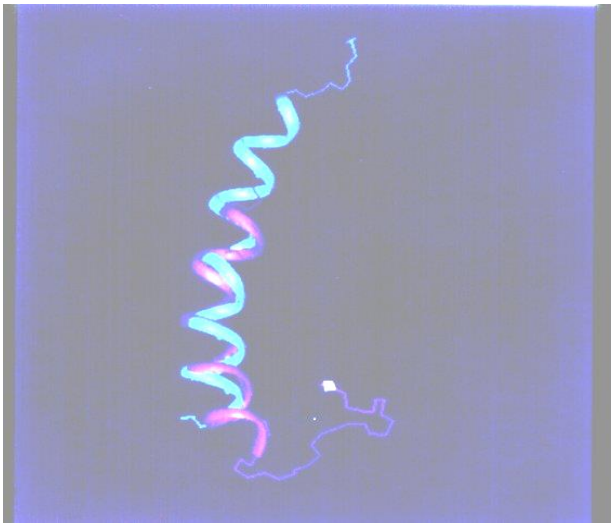


Figure 4. Primary and tertiary structures of Vpr C-terminus.

The cell killing function of Vpr is resident at the C-terminus of the Vpr protein.

3 ROLE OF INTRACELLULAR Nef IN PROLONGED SURVIVAL OF HIV-INFECTED CELLS

3.1 General

Earlier studies had shown that during HIV infection of lymphoid tissues cells that were undergoing apoptosis were rarely infected. On the other hand, HIV-infected cells were rarely apoptotic. This suggested that something within the HIV-infected cells were keeping them alive longer. Results from my own lab and other groups, strongly suggest that Nef could be responsible. Studies from other laboratories have shown that HIV proteins Vpr and Tat could also help to prolong the life of HIV infected cells.

3.2 Interaction of Nef with Host Cell Protein

Intracellular Nef (and N-terminal fragments) down regulate the surface expression of CD4 and IL2-R, which are involved in MHCII antigen recognition and cell proliferation, respectively, suggesting that Nef might interfere with cell signalling. Following this lead we identified four protein that bind to Nef and are inactivated. These proteins are all involved in signal transduction and Apoptosis. We also showed that intracellular Nef inhibits p53 and $p56^{lck}$-mediated apoptosis of $CD4^{+}$ T lymphocytes.

By inhibiting apoptosis of HIV-infected cells Nef could prolong their survival and thereby increase virus production. Inactivation of intracellular Nef should hasten death of HIV-infected cells. This could also have implications in the treatment of AIDS-related cancers.

3.3 Role of intracellular Nef in immune evasion:

There are a number of other ways by which intracellular Nef prolongs the survival of HIV-infected cells. One of this is the evasion of CTL-mediated apoptosis. (Define CTL).

> CTL is involved in eliminating virus after the initial infection. Studies involving a cohort of sex workers in Nairobi, long-term non-progressors, and discordant couples, clearly suggest that CTL is important in keeping HIV at bay. During the long clinical latency, when there is vigorous virus production in the lymphoid tissues, there is also constant generation of and persistence of HIV-specific CTL. So why does the existing HIV-specific CTL fail to eliminate virus and prevent progression to AIDS?

CTLs eliminate virus-infected cells by recognising MHCI molecules containing viral peptides on the surface of HIV-infected cells and kill them.

Intracellular Nef inhibits the surface expression of MHCI on the surface of HIV-infected cells. HIV-specific CTLs can no longer recognise the HIV-infected cells.

4 DRUG DEVELOPMENT

4.1 Novel drugs based on inhibitors of naturally occuring bee venom Mellitin

Besides structural similarity, Nef and Mellitin both disrupt cell membranes. So will a Mellitin inhibitor inactivate Nef? A small peptide inhibitor of Mellitin was found in a combinatorial library. This had some activity in inhibiting Nef-mediated cell death but it was extremely toxic and totally insoluble.

Using this Mellitin inhibitor as the starting point and doing analogue chemistry around it, we succeeded in producing a small number of novel compounds that had high anti-Nef activity and were water soluble and non-toxic.

These compounds inhibit Nef-mediated cell death and could be useful in preventing death of uninfected immune cells.

Some of these compounds inhibit the interaction between Nef and tumour suppressor p53 and $p56^{lck}$, which are involved in promoting apoptosis of $CD4^{+}$ T lymphocytes. It will be interesting to find out if these compounds reverse Nef-mediated inhibition of apoptosis and thereby hasten the death of HIV-infected cells. It will also be interesting to see if these compounds will allow MHCI to be displayed on the surface of HIV-infected cells so that these cells can be recognised and killed by CTL.

4.2 Bee Propolis inhibits Nef-mediated cell killing

If the bees produce highly potent venom, Mellitin, then what protects the bees from this toxin? Does the saliva of honeybees contain an anti-dote to bee toxin, and would this also inhibit Nef-mediated cell death? This prompted me to have a look at bee propolis which consists of salivary secretions of bees and a very heterogeneous mixture of resinous plant material.

Bee propolis, a natural product used in Chinese medicine, was highly active in inhibiting Nef-mediated cell death, and was water soluble and completely non-toxic. An added bonus was that it promoted lymphocyte growth and proliferation. We have succeeded in isolating a single peak of activity, but is may not be necessary to purify further.

This material is really worth subjecting to clinical trials as a cheap way to preserve the immune system.

This example illustrates the important point that natural products from the flora and fauna around us could prove just as valuable as those produced by using very expensive and sophisticated technologies. They could also be infinitely cheaper. It is, therefore, very important for countries in our region and other developing countries to scientifically assess our abundant biota for useful therapies.

Other therapeutic agents: such as DNA vaccines directed against Nef and Vpr "death domains" could also effectively prevent elimination of immune cells.

Our laboratory has also produced a new chemical entity (a dendrimer compound) *that* inhibits Vpr and Nef-mediated cell killing and also prevents virus from infecting cells.

5 THE RESEARCH TEAM

HIV-1 Nef and Vpr Research Team (Biomolecular Research Institute and CSIRO, Melbourne)

Molecular and Cell Biology

Ian Macreadie
Dean Hewish
Paul Failla
Sonia Sankovich
Laura Castelli
Cyril Curtain
Chianiah Arunagiri
Melinda Lowe
Karin Smith

MBCMR

Dale McPhee
Alison Greenway

Protein Chemistry

Ross Fernely
Anna Lucantoni
Nicole de Rozario
Thuong Nguyen

Mass Spectroscopy

Jeff Gorman
Bettina Ferguson

Synthetic Chemistry

Jonathan Baell
Barry Matthews

X-ray Crystallisation

Pat Pilling
Tom Garrett

NMR Spectroscopy

Ray Norton
Kevin Barnham
S Yao

Central Services

Large Scale Fermentation
Analytical Biochemistry
Oligonucleotide and Peptide Synthesis
DNA Sequencing

Team Leader

Ahmed A Azad

REFERENCES (HIV/AIDS)

Reviews

1. Azad. A. A. (2000). Could Nef and Vpr proteins contribute to disease progression by promoting depletion of bystander cells and prolonged survival of HIV-infected cells. *Biochem. Biophys. Res. Commun.* **267**, 677-685.
2. Azad, A. A. (2000). The possible involvement of HIV accessory proteins Nef and Vpr in bystander cell death and prolonged survival of HIV-infected cells. In "Advances in Animal Virology" pp. 297 – 307 Editors Shahid Jameel and Luis Villareal. Oxford and IBH Publishing Co. Pvt. Ltd, New Delhi and Calcutta.

Publications

3. Smith, J., **Azad, A.A.** & Deacon, N. (1992). Identification of two novel human immunodeficiency virus type 1 splice acceptor sites in infected T cell lines. *J. Gen. Virol.* **73,** 1825-1828.
4. Macreadie, I.G., Failla, P.P., Horaitis, O. & **Azad, A.A.** (1992). Production of HIV-1 Vpu with pYEULCBX, a convenient vector for the production of non-fused proteins in yeast. *Biotechnol. Lett.* **14,** 639-642.
5. Macreadie, I.G., Castelli, L.A., Failla, P.P. & **Azad, A.A.** (1993). Yeast-derived HIV-1 Nef protein lacks G-protein activities. *Mol. Biol. (Life Sci. Adv.).* **12,** 99-105.
6. Macreadie, I.G., Ward, A.C., Failla, P.P., Grgacic, E., McPhee, D. & **Azad, A.A.** (1993). Expression of HIV-1 *nef* in yeast: The 27 kDa nef protein is myristylated and fractionates with the nucleus. *Yeast.* **9,** 565-573.
7. **Azad, A.A.**, Failla, P.P., Lucantoni, A.C., Bentley, J.D., Mardon, C.J., Wolfe, A.G., Fuller, K., Hewish, D.R., Sengupta, S., Sankovich, S.E., Grgacic, E., McPhee, D. and Macreadie, I.G. (1994). Large scale production and characterisation of recombinant human immunodeficiency virus type 1 *Nef. J. Gen. Virol.* **75,** 651-655.
8. Curtain, C.C., Separovic, F., Rivett, D.E., Kirkpatrick, A., Waring, A.J., Gordon, L.M. & **Azad, A.A.** (1994). Fusogenic activity of amino-terminal region of HIV type 1 nef protein. *AIDS Res. Hum. Retroviruses.* **10,** 1231-1240.
9. Greenway, A.L., McPhee, D.A., Grgacic, E., Hewish, D.R., Lucantoni, A.C., Macreadie, I.G. & **Azad, A.A.** (1994). Nef27, but not the Nef25 isoform of human immunodeficiency virus-type pNL4.3 down-regulates surface CD4 and IL-2R expression in peripheral blood mononuclear cells and transformed cells. *Virology.* **198,** 245-258.
10. Greenway, A.L., **Azad, A.A.** & McPhee, D. (1995). Human immunodeficiency virus type 1 Nef protein inhibits activation pathways in

peripheral blood mononuclear cells and T-cell lines. *J. Virol.* **69,** 1842-1850.

11. Macreadie, I.G., Castelli, L.A., Hewish, D.R., Kirkpatrick, A., Ward, A.C. & **Azad, A.A.** (1995). A domain of human immunodeficiency virus type 1 Vpr containing repeated H(S/F) RIG amino acid motifs causes cell growth arrest and structural defects. *Proc. Natl. Acad. Sci. USA.* **92,** 2770-2774.
12. Macreadie, I.G., Castelli, L.A., Lucantoni, A.C. & **Azad, A.A.** (1995). Stress- and sequence-dependent release into the culture medium of HIV-1 Nef produced in *Saccharomyces cerevisiae. Gene.* **162,** 239-243.
13. Macreadie, I.G., Arunagiri, C.K., Hewish, D.R., Ward, A.C., White, J. and **Azad, A.A.** (1996). Extracellular addition of a domain of HIV-1 Vpr protein containing the amino acid sequence H(S/F)RIG causes membrane permeabilisation and death. *Mol. Microbiol.* **19,** 1185-1192.
14. Greenway, A., **Azad, A.A.**, Mills, J. & McPhee, D. (1996). Human immunodeficiency virus type 1 Nef binds directly to Lck and mitogen-activated protein kinase, inhibiting kinase activity. *J. Virol.* **70,** 6701-6708.
15. Arunagiri, C.K., Macreadie, I.G., Hewish, D.R. & **Azad, A.A.** (1997). A C-terminal domain of HIV-1 accessory protein Vpr is involved in penetration, mitochondrial dysfunction and apoptosis of human CD4+ lymphocytes. *Apoptosis* : **2**, 69-76.
16. Curtain, C.C., Lowe, M.G., Arunagiri, C.K., Mobley, P.W., Macreadie, I.G. & **Azad, A.A.** (1997). Cytotoxic activity of the amino-terminal region of HIV type 1 Nef protein. *AIDS Res. Human Retroviruses.* **13**, 1213-1220.
17. Barnham, K.J., Monks, S.A., Hinds, M.G., **Azad, A.A.** & Norton, R.S. (1997). Solution structure of a polypeptide from the N-terminus of the HIV protein Nef. *Biochemistry.* **36**, 5970-5980.
18. Macreadie, I.G., Lowe, M.G., Curtain, C.C., Hewish, D.R. & **Azad, A.A.** (1997). Cytotoxicity resulting from addition of HIV-1 Nef N-terminal peptides to yeast and bacterial cells. *Biochem. Biophys. Res. Commun.* **232,** 707-711.
19. Macreadie, I.G., Kirkpatrick, A., Strike, P.M. & **Azad, A.A.** (1997). Cytocidal activities of HIV-1 Vpr and Sac1P peptides bioassayed in yeast. *Protein and Peptide Letters.* **4,** 181-186.
20. Macreadie, I.G., Thornburn, D.R., Kirby, D.M., Castelli, L.A., de Rozario, N.L. & **Azad, A.A.** (1997). HIV-1 protein Vpr causes gross mitochondrial dysfunction in the yeast*Saccharomyces cerevisiae. FEBS Lett.* 410, 145-149.
21. Failla, P., Castelli, L.A., **Azad, A.A.** and Macreadie, I.G. (1997). Bactericidal properties of HIV-1 Vpr C-terminal sequences. *Prot. Pep. Lett.* **4**, 383-390.
22. Macreadie, I., Fernley, R., Castelli, L.A., Lucantoni, A., White, J. & **Azad, A.A.** (1998). Expression of HIV-1 *nef* in yeast causes membrane perturbation and release of the myristylated Nef protein. *J. Med. Sci.* 5,203-210.

23. Yao,S., Torres, A.M. Azad, **A.A.,** Macreadie, I.G.& Norton, R.S. (1998). Solution structure of peptides from HIV-1 Vpr protein that cause membrane permeabilisation and growth arrest. *J. Pep.Sci.* **4**, 426-435.
24. Yao, S., Azad, **A.A.,** Macreadie, I.G. and Norton, R.S. (1998). Helical structure of polypeptides from the C-terminal half of HIV-1 Vpr. *Prot. Pep.Lett*. **5**, 127-134.
25. Macreadie, I.G., Sankovich, S.E., Failla, P., Lowe, M.G., Curtain, C.C., Castelli, L.A. and **Azad, A.A.** (1998). Human immunodeficiency virus type 1 Nef protein causes death in stressed yeast due to determinants near the N-terminus and elsewhere in Nef. *Biochem. Mol. Biol. Int.* **46**, 277-286.
26. Sankovich, S.E., Koleski, D., Baell, J., Matthews, B., **Azad, A.A.** and Macreadie, I.G. (1998). Design and assay of inhibitors of HIV-1 Vpr cell killing and growth arrest activities using microbial assay systems. *J. Biomol. Screening*. **3**, 287-292.
27. Curtain, C.C., Lowe, M.G., Macreadie, I.G., Gentle, I.R., Lawrie, G.A. and **Azad, A.A.** (1999). Structural requirements for the cytotoxicity of the N-terminal region of HIV Type 1 Nef. *AIDS Res. Human Retroviruses*. **14**, 1543-1551.

Gene Therapy for the Treatment of Tumors: A Perspective

NAÏMA ALAMI-OUAHABI*
Mohammed V University
Rabat, Morocco

1 ABSTRACT

The ability to successfully introduce foreign genes into eukaryotic cells, has made possible a new approach to the treatment of human diseases.

In fact, gene therapy is now being used to revolutionize the twenty first century Medicine, for it upholds genuine and hopeful solutions to the treatment of genetic and infectious diseases, as well as numerous types of cancers.

This review, provides information on the genetic background of uncontrolled cellular proliferation that leads to cancer, as well as a summary of the status of gene therapy, in the treatment of tumors and malignancies unresponsive to conventional therapeutic interventions.

This paper also discusses the entry of gene therapy into the clinical testing arena, as well as the short comings along with the technical and ethical hurdles it needs to overcome for its wide spread in modern therapeutic and preventive medicine.

2 INTRODUCTION

Gene therapy, is the term used to describe the correction of a disease by genetic manipulation.

In fact, it involves the introduction of genetic material into eucaryotic somatic cells, in order to treat, inborn genetic, infectious or malignant diseases. This field is now becoming, a significant addition to the armamentarium of the clinician, faced with curing diseases.

The efficiency of gene therapy, has been, and still is being tested for the treatment of human diseases. Since the 1990s, the experiments went forward, with an unprecedented level of oversight and regulation by governments and scientific advisory committees.

---

* Department of Biochemistry and Molecular Biology, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco.

The key role that could be played by gene therapy, becomes more obvious when knowing that, of more than 3000 inherited diseases catalogued to date, only a few, such as phenyktonuria (a metabolic disease), are currently treatable. For many of these diseases, the missing or defective gene product (protein, enzyme, …), cannot be supplied exogenously as insulin is supplied to diabetics (26, 27, 28).

Most enzymes are unstable, and cannot be delivered in functional form to their sites of action in the body, at least, not in a form that provides for the ongoing enzyme activity, that is usually needed. Cell membranes, are not permeable to large macromolecules, such as proteins, thus, most enzymes, must be synthesized within the cell, in which their activity is required.

Treatment of inherited diseases, is largely restricted to cases where the product of the defective enzyme, is a small molecule that can be distributed to the appropriate tissues of the body, through the circulatory system.

For most other inherited diseases, somatic cell gene therapy offers the most promising approach to successful treatment.

In this paper, we will point out to gene therapy, in the treatment of genetic, infectious and metabolic diseases emphasizing on cancer and tumors gene therapy (8, 9).

2.1 Cancer cells

Compared to normal cells (Figure 1), cancer cells become malignantly transformed, so that they exhibit uncontrolled growth and invasive properties (Figure 2).

Depending on the cell type involved, they form a solid tumor (carcinoma, sarcoma), or cause various types of leukemia. Apart from their uncontrolled proliferation, and ability to metastasize (invade other tissues), there are numerous other differences, between cancer cells, and the normal cells from which they are derived. They often appear relatively undifferentiated, and express novel tumor antigens at the cell surface.

The development of a fully malignant cancer cell, is thought to be a multi step process, involving one or more DNA mutations, which alter, deregulate, or delete genes involved in the control of cell growth and differentiation (20).

(Science &Vie)

Figure 1. A diploïd somatic cell.

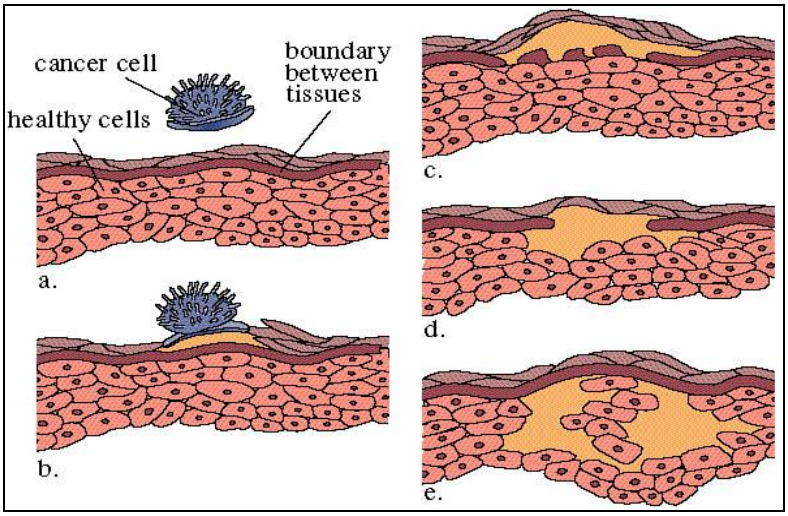


Figure 2. Steps of cancer spread (15).

2.2 Cell cycle

Growth requires an increase in cell mass, a duplication of the genetic material, and a cell division, ensuring that each progeny cell, receives an equal

complement of the genetic material necessary for the perpetuation of the cell line. These steps, occur in an ordered progression, during the cell life span, or cycle.

- Initially a diploid cell (containing 2 n chromosomes), undergoes a period of growth, and increase in cell mass. This is called G_1 phase (growth 1). For a cell requiring 24 hours for the complete cycle, the G_1 stage might take the first 10 hours, which are devoted to cell growth, and chemical preparation, for DNA synthesis.
- At a defined time, duplication of genetic material commences (DNA replication). During this DNA synthesis phase (S phase) of about 9 hours, the genetic material DNA of every chromosome is replicated.
- After the completion of DNA replication, the cell enters a second growth phase, called G_2 . This post DNA synthesis stage, might typically require about 4 hours, continuing until the onset of mitosis (M) (Figure 3).
- Two processes are involved in Mitosis, and are completed in one hour: First, the mitosis itself, consisting on the nuclear division. The second is, cytokinesis, implicating the changes in the cytoplasm, that includes proper division of the cell as a whole (Figure 4).

While all the stages of the cell cycle, are vital for its growth and differentiation. From a genetic point of view, two stages are crucial to the regulation and control of a normal cellular division (12).

First the S phase (duplication of the genetic information encoded in DNA). In fact, before a cell can divide, its DNA must be replicated completely and accurately. New copies of the two strands of DNA, are synthesized (polymerized) by DNA polymerases (nuclear enzyme) from deoxyribonucleotides. The sequence of the new DNA strands, is determined by base pairing precursors, to the unwound parental DNA strands before polymerization. Therefore, the sequence of the parental molecule is precisely copied. Fidelity of DNA replication, is ensured first, by immediate proofreading, using the DNA polymerases 3'→ 5' exonuclease activity, (DNA editing) (21).

Nonetheless, DNA repairs mechanisms, that occur during the phase G2 of the cell cycle, are critical as control points in cell proliferation. In fact, most of the alterations, and non silent mutations, that result on a lack of fidelity during DNA replication, may lead to DNA damage, causing unregulated gene expression. Thus, every altered cell, is destined to apostosis, or programmed cell death, in order to avoid ending as a mutated cell leading to carcinogenesis.

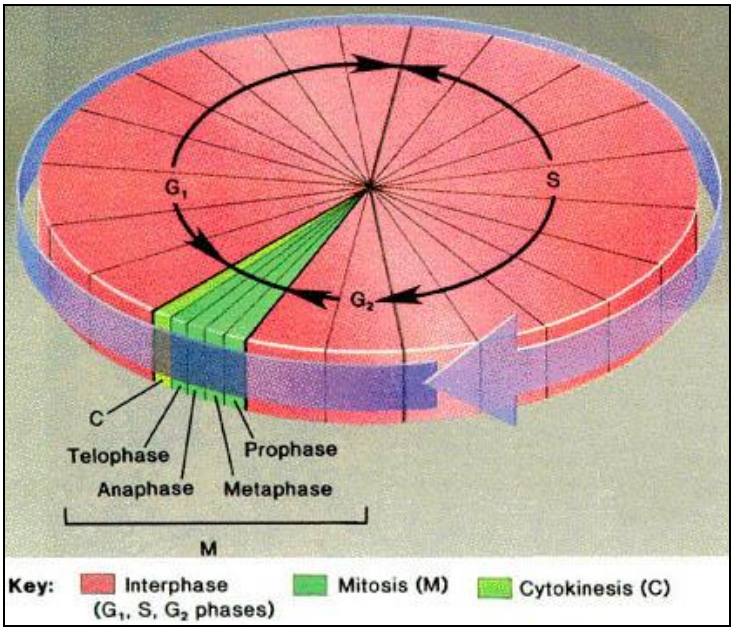


Figure 3. Somatic cell cycle (15).

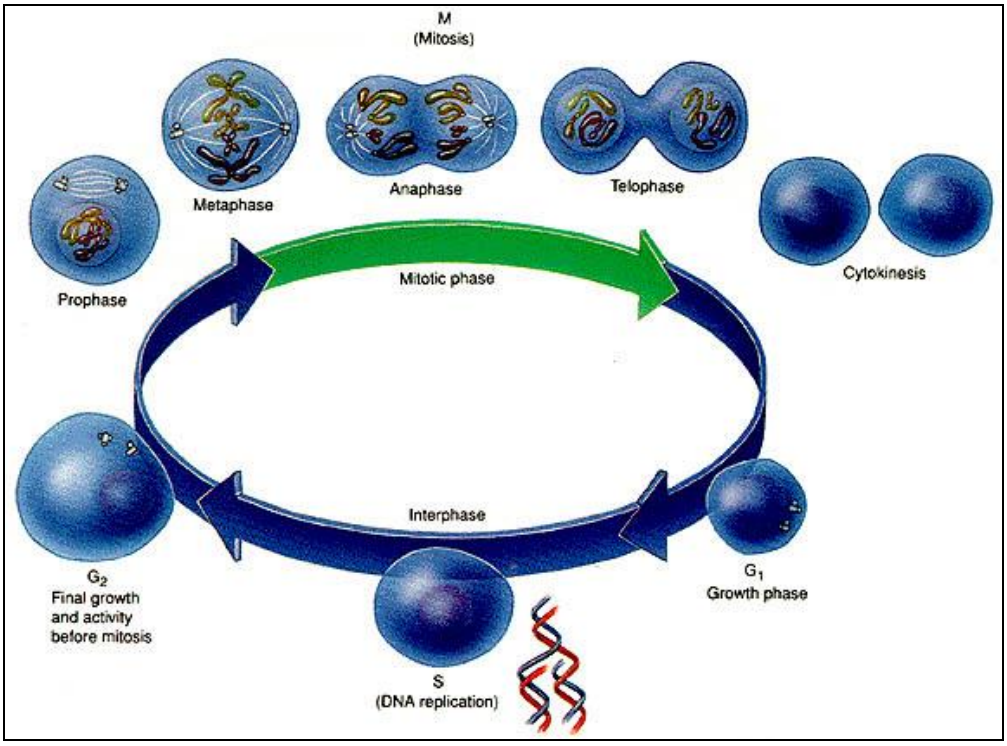


Figure 4. Principal steps of cell division and Mitosis (12).

2.3 The molecular basis of mutations

Mutation is a process whereby changes occur, in the quantity or structure of the genetic material of an organism. Mutations, are permanent alterations of the genetic material, which may lead to changes in phenotype. An organism, gene, or DNA sequence, ... in which mutations have occurred, is called a mutant. Mutations can involve: modifications of the nucleotide sequence (DNA) of, a single gene, many genes, or whole chromosomes (Figure 5).

Changes in a single gene, may be the consequence of:

- point mutations, which involve: the removal, addition, or substitution of a single nucleotide.
- A large number of nucleotide mutations, within a DNA sequence.
- Modifications of whole chromosomes, including structural changes, causing chromosome abnormalities, such as inversions or translocations.

Numerical chromosome mutations, may involve, multiples of the complete chromosome karyotype: termed polyploidy, or they may involve, deviation from the normal number of chromosomes, termed aneuploidy.

DNA sequence mutations, such as deletion, addition, or substitution (transitions, transversions), of a single “point mutation”, or multiple nucleotides in the DNA sequence, may affect the correct functioning of the product of the modified gene. Thus, affecting the outcome of the genetic expression which is the protein.

The extend of the effect, can however range from the undetectable, involving silent mutations: which have no effect on the protein amino acid sequence, to the severe effect, involving “missens” or “frame shift” mutations, leading to a defect, or a loss, of protein synthesis.

Different forms of a gene, which may be produced by mutations, are termed “alleles”. In diploid organisms, such as humans, chromosomes carry different, or similar alleles of a particular gene leading to a heterozygous, or a homozygous genotype for a particular locus. The dominant allele, is expressed phenotypically during development, while the recessive allele, is carried in the genome and stays silent. Co-dominant alleles, give rise to hybrid expression of the gene (2).

Somatic mutations, occur in diploid somatic cells, and are not transmitted to the sexually produced offspring. While inherited mutations, are genetic modifications, present in the germ cells (gametes), of sexually reproducing organisms, and can be transmitted to offspring. These inherited mutations, can be spontaneous (caused by errors in replication, or movements within the genome), or induced by external factors, such as physical or chemical mutation agents.

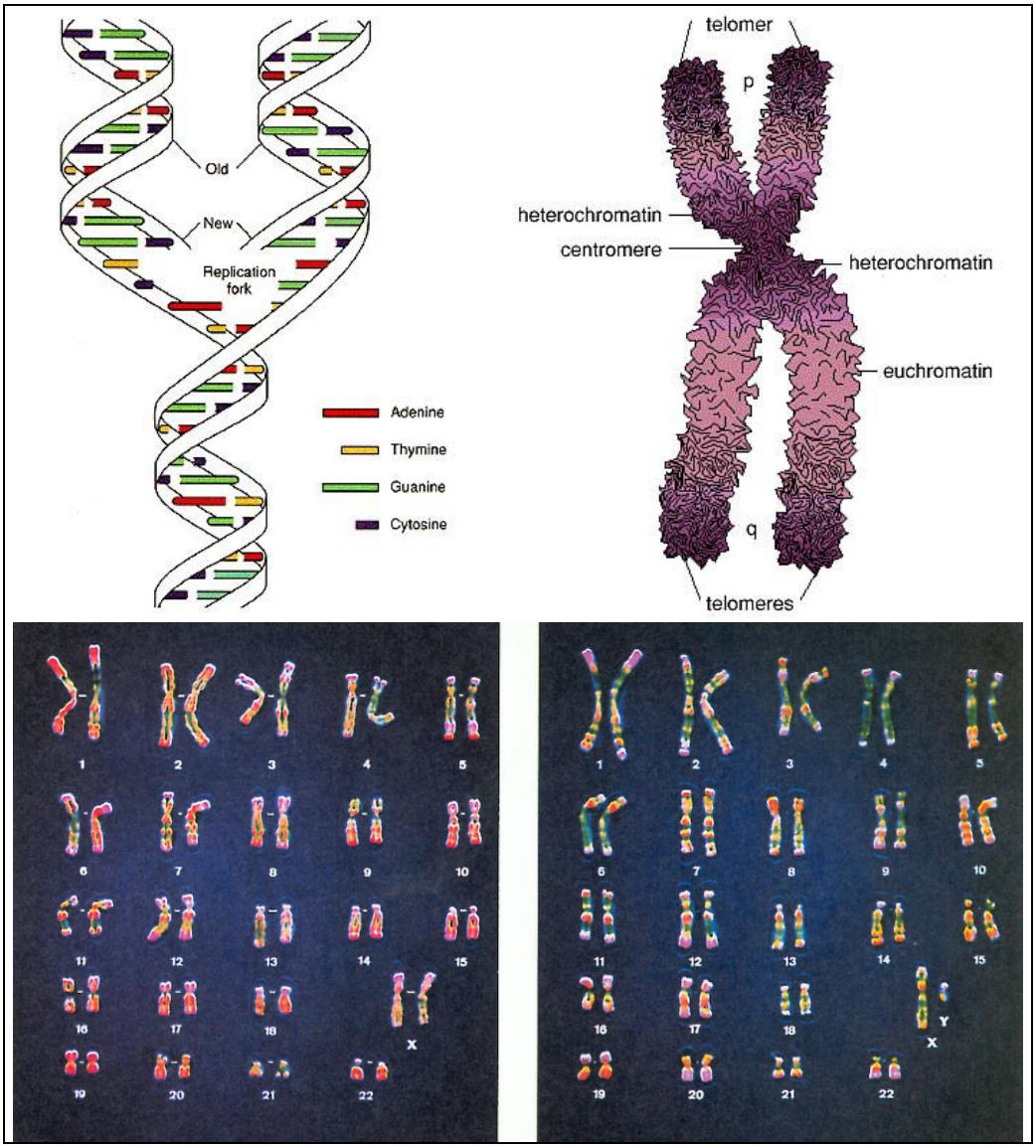


Figure 5. Genetic mutations: DNA sequence, Chromosome structure, and number (2).

2.4 Physical and chemical mutagenesis

Radiation, is the major physical agent that causes genetic mutations. In fact, there are non ionizing radiation such as Ultraviolet Rays, and ionizing radiation such as the high energy X-Rays, as well as Alpha, Beta, and Gamma rays, released by radioactive isotopes (^{32}P, ^{35}S, radium, cobalt 90 …). Whereas

chemical mutagene agents are mostly: Alkylating Agents, Nitrous Acid, Acridine Dyes, and Base Analogues (Figure 6).

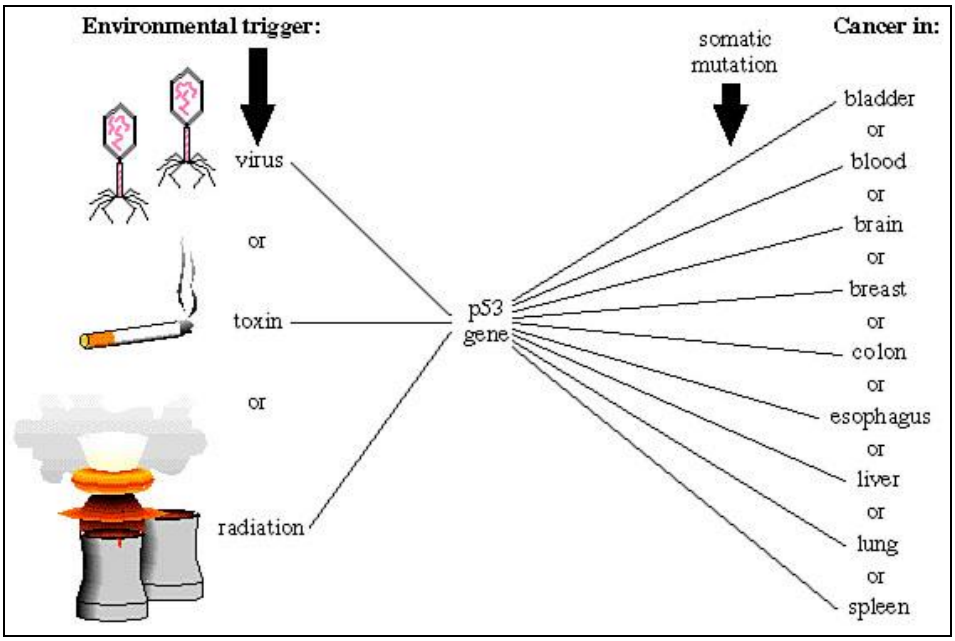


Figure 6. External Cancer Agents (15).

2.5 DNA repair mechanisms

As mentioned previously, inside every cell, DNA is constantly confronted to damaging mutations, whether afflicted by endogenous reactions (such as errors in DNA replication), or by environmental agents. This damage, interrupts the continuity of genetic information, and inhibits, prevents, or disturbs, the regulation of genetic expression (22). Thus, it is crucial for the cell to keep both somatic, and germ line mutations at a tolerable level, using DNA repair mechanisms. In fact, unrepaired damaging mutations, trigger the process of apoptosis, or programmed cell death, which is a sort of cellular suicide, that avoids the development of mutant cells, that could lead to corcinogenesis. While a few types of mutation, can be enzymatically repaired by direct reversal (without removing bases from the DNA), other mechanisms use base excision, nucleotide excision, or even strand break, to repair mutations .

2.6 Correlation between mutagenecity and carcinogencity

While some mutations, are considered to be a source of genetic diversity (eg. immuno-globulines or antibodies), and a good potential for an organism, to adapt to a changing environment. For many years, it has been recognized, that most of the strongly mutagenic agents, such as: ionizing radiation, ultraviolet

light, and chemicals, are carcinogenic (cancer inducing). Most geneticists, would agree that not only somatic mutations, but also inherited mutations, can cause cancer (Figure 7). In fact, cell division, is constantly under rigorous genetic control. Thus, a mutation in a gene, involved in the control of the cell cycle, can cause a loss of function, and a loss of the normal control of cell division, which is the common feature, of all of the many different types of cancer, and malignant cells (5, 6, 7). It is now clear, that mutations may lead to cancer when they occur in one or more of a battery of normal genes, referred to as the proto-oncogenes. These protooncogenes, may be modified (over expressed), by a variety of mutational changes, to produce the cancer causing oncogenes, of which more than 40 have been identified. Protooncogenes, play an essential part in the control of cell growth and differentiation. Thus, disruption of their normal activity by mutational events, may lead to the aberrant growth, characterizing cancer and tumors (24).

However, there are other types of genes called tumor suppressor genes, or anti-oncogenes (such as P^{53}), which contribute to cancer development, only when they are inactivated, or deleted. The normal forms of such genes, appear to suppress the development of tumors.

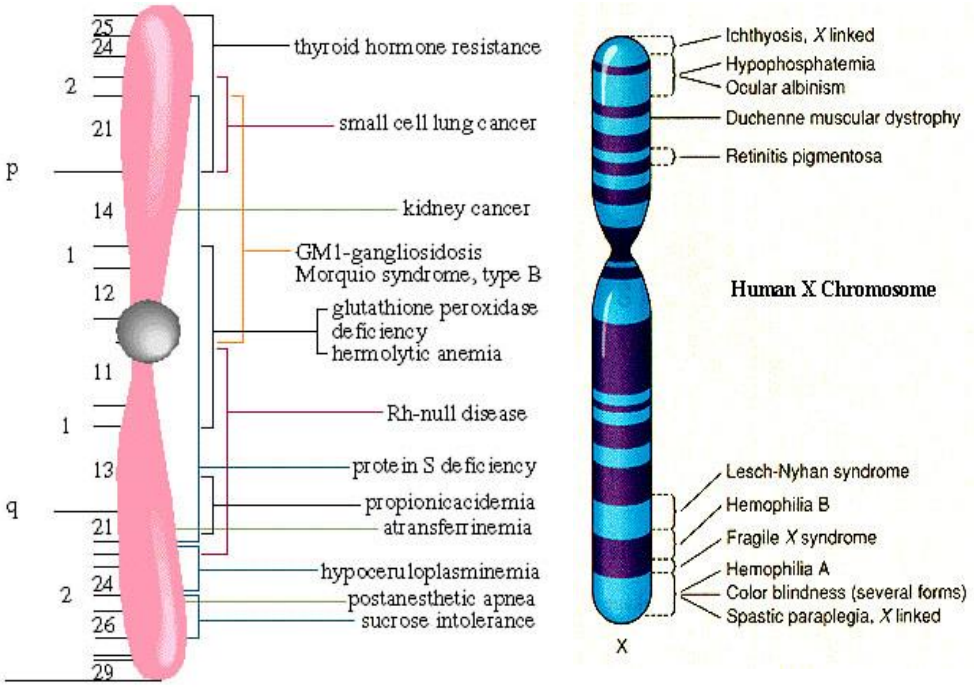


Figure 7. Correlation between mutagenecity, disease, and cancer (12).

2.7 Tumor inducing virsus, viral oncogenes

Most of our information about oncogenes, has come from studies of RNA tumor viruses (retroviruses), that store their genetic information in a single stranded RNA genome, and then convert it into a homologous double stranded DNA, using reverse transcriptase, after infecting the host cell. Nonetheless, the genomes of DNA tumor viruses, such as: polyoma virus SV_{40} (simian virus 40), and adenovirus, also contain oncogenes that are able to induce the uncontrolled neoplastic growth, of eucaryotic cells in culture. Studies of the diversity of retroviral oncogenes, were able to describe over 20 different retroviral oncogenes, and more remain to be discovered (Table 1). In total, including oncogenes of DNA viruses, and cellular origins, over 40 distinct oncogenes have been identified. It is now clear that the product of these oncogenes, play various roles in regulating cell division, in one or more cell types.
For example:

- The retroviral oncogene “V src” (rous sarcoma virus), encodes a membrane bound protein kinase.
- The “V sis” oncogene is closely related, to a polypeptide growth hormone called: Palatelet Derived Growth Factor (PDGF). The viral “V sis”, induces sarcomas, and transform fibroblasts to a neoplastic or tumerous cells. While the cellular normal proto oncogene “C sis”, is produced by platelet cells, and promotes healing, by stimulating cell growth at wound sites.

Table 1. Retroviral Oncogenes (2)

Oncogenes[a]	Virus	Host species	Function of gene product
abl	Abelson murine leukemia virus	Mouse	Tyrosine-specific protein kinase
Cbl	Cas NS-1 virus	Mouse	?
erbA	Avian erythroblastosis virus	Chicken	Analog of thyroid hormone receptor
erbB	Avian erythroblastosis virus	Chicken	Truncated version of EGF receptor[b]
Ets	E26 virus	Chicken	**Nuclear protein, unknown function**
Fes	ST feline sarcoma virus	Cat	Tyrosine-specific protein kinase
Fgr	Gardner -Rasheed feline sarcoma virus	Cat	Tyrosine-specific protein kinase
fms	McDonough feline sarcoma virus	Cat	Analog of CFS-l GF receptor[c]
Fos	FJB osteosarcoma virus	Mouse	**Transcriptional activator protein**
Fps	Fuginami sarcoma virus	Chicken	Tyrosine-specific protein kinase
jun	Avian sarcoma virus 17	Chicken	Transcriptional activator protein
Kit	HZ4 feline sarcoma virus	Cat	?
Mil (mht)	MH2 virus	Chicken	Serine/thr-specific protein kinase
mos	Moloney sarcoma virus	Mouse	Serine/thr-specific protein kinase
myb	Avian myeloblastosis virus	Chicken	Nuclear protein, unknown function
myc	MC29 myelocytomatosis virus	Chicken	Probably transcription regulator
Raf	3611 murine sarcoma virus	Mouse	Serine/thr-specific protein kinase
H-ras	Harvey murine sarcoma virus	Rat	GTP binding protein, GTPase activity
K-ras	Kirsten murine sarcoma virus	Rat	GTP binding protein, GTPase activity
Rel	Reticuloendotheliosis virus	Turkey	Cytoplasmic protein, unknown function
ros	UR11 avian sarcoma virus	Chicken	Tyrosine-specific protein kinase

Table 1. (continued)

Oncogenes[a]	Virus	Host species	Function of gene product
Sis	Simian sarcoma virus	Monkey	Analog of platelet-derived GF[c]
Ski	Avian SKV77O virus	Chicken	**Nuclear protein, unknown function**
Src	Rous sarcoma virus	Chicken	Tyrosine-specific protein kinase
Yes	Y73 sarcoma virus	Chicken	Tyrosine-specific protein kinase

[a] Oncogenes *fes and fps,* first isolated from retroviruses of cat and chicken, respectively, are homologous;; the same is true for *mil* and *raf.*
[b] EGF = epidermal growth factor.
[c] GF = growth factor; **CSF-l** (Colony Stimulating Factor -1) is a polypeptide growth factor that specifically stimulates the growth of macrophages; platelet-derived growth factor (secreted by platelet cells during clot formation) promotes growth of cells at wound sites, thereby enhancing the healing process.

Table 2. Mutational origin of c-ras oncogenes in cancer cells[a] (2)

TISSUE SOURCE	ras ALLELE	CODON		
		12	59	61
Normal	c-H-ras	G G C	G C C	C A G
human cells		gly	ala	gln
Normal	c-K-ras	G G T	**G C A**	C A A
human cells		gly	ala	gln
Normal	N-ras	G G T	G C T	C A A
human cells		gly	ala	gln
Human	EJ/T24-H-ras	C **T** C	G C C	C A G
bladder cancer		**val**	ala	gln
Human	HS242-H-ras	**G G C**	G C C	C **T** G
lung cancer		gly	ala	**leu**
Human	Calu-K-ras	**T** G T	G C A	C A A
lung cancer		**cys**	ala	gln
Human	SW480-K-ras	G **T** T	G C A	C A A
colon cancer		**val**	ala	gln
Human	LL-10-K-ras	**C** G T	?	?
lung cancer		**arg**		
Human	A1698-K-ras	**C** G T	?	?
bladder cancer		**arg**		
Human	PR371-K-ras	**T** G T	?	?
lung cancer		**lys**		
Human	PR310-K-ras	G G T	G C A	C A **T**
lung cancer		gly	ala	**his**
Human	SK-N-ras	G G T	G C T	**A** A A
Neuroblastoma		gly	ala	**lys**
Human	Mel-N.ras	G G T	G C T	**A** A A
Melanoma		gly	ala	**lys**
Human	SW127l-N-ras	G G T	G C T	C **G** A
lung carcinoma		gly	ala	**arg**

[a] Note that the nucleotide changes present in the oncogenic ras alleles and the corresponding amino acid changes in the mutant ras proteins are given in color print.

- Oncogenes “erb B”, and “fms”, encode proteins that are analogs, to the receptor of epidermal growth factors, and “CSF-1” growth factor. They stimulate macrophages growth, and differentiation, and are key components in transmembrane signaling.

- Oncogene “erb A” product, is an analog to the nuclear receptor, for the thyroïd hormone T_3.
- The largest group of oncogenes (including src), encode protein kinases, that phosphorylate tyrosine residues.
- The “ras” oncogenes, encode proteins, that bind GTP, and inhibit GTP ase activity. They may be analogous to proteins, called “G proteins”, that play a role, in the regulation of cyclic AMP level in cells. The functions of the “ras” gene products, are of particular interest, because considerable evidence implicates the involvement of mutant “ras” gene products, in several distinct types of human cancers.
- Finally, other oncogenes, such as Jun, fos, erb A, and myc, encode nuclear transcription factors, that activate the expression of specific genes, that are themselves, positive regulations (activators) of cell division (12).

In summary, proto oncogenes products are simply proteins, that play central roles in stimulating cell division. They appear to fit into 4 groups:

(1) Those that encode growth factors, (C-sis), or growth factor-receptors (C fms and C erbB).
(2) Those that encode GTP-binding proteins, with GTP ase activity (C h-ras, C k-ras, C n-ras).
(3) Those that encode tyrosine specific protein kinases (C abl, C fes, C fgr, C fps, C ros, C src, and C yes), or serine/threonine specific protein kinases (C mil, C mos, and C raf).
(4) Those that encode transcription regulators (C fos, C jun, C erb A, C myc, C myb, and C ets).

These proto oncogenes, and their protein products, can be altered by spontaneous or inherited mutations, chromosomal movements (eg. translocations), viral infections, or carcinogenic physical and chemical agents. Generally, alterations or mutations, transform proto oncogenes to oncogenes, encoding modified or overproduced proteins, that stimulate abnormal cell division (Tables 1 and 2).

2.8 Tumor suppressor genes

During tumor induction with acutely transforming retroviruses, the malignant character of transduced oncogenes (V onc), is expressed in the presence of their normal cellular analogs (C- onc). This implies, that viral sequences, are genetically dominant over their cellular counterpart, with respect to their ability, to promote tumerogenesis. Likewise, many tumor specific alterations of cellular protooncogenes, that occur independently of viral infection (by somatic point mutation or chromosome rearrangements), are also dominant in nature (3, 13). However, recent studies have demonstrated, that in addition to the protooncogenes that are malignantly activated, by dominant gain of function mutations, there also exists, a distinct class of genes, which promotes neoplasia,

upon loss of function. The activity of these genes, ensures non malignant phenotype. Thus suggesting that the chromosomes, harbor genetic material, that can defend the cell, by suppressing the malignant phenotype of the tumerogenic factors. These genes are called: tumor suppressor genes.

Evidence of the existence of tumor suppressor genes, emerged from studies of Retinoblastoma (a pediatric malignancy that occurs in two distinct forms).

Patients with heritable retinoblastoma (germ line mutation of RB), develop multiple tumors involving both eyes, and they transmit a gene of susceptibility to retinoblastoma (RB gene), to half their offsprings.

In contrast, patients with sporadic retinoblastoma, do not have a family history of the disease, and they only develop, an isolated tumor in one eye.

Molecular genetic studies, including the isolation of the RB gene, and its characterization, in patients with either the sporadic, or heritable disease, confirm that, inactivation of both RB alleles, is necessary for the formation of retinoblastoma (Figure 8).

Hence, the normal RB gene, serves as a tumor suppressor gene, that contributes to oncogenesis if it loses its function.

Recent studies, has led to the identification, of distinct tumor suppressor genes, in addition to RB, that play a role in other malignancies, by loss of function. These genes include:

- WT1, implicated in Wilm's Turner disease: a kidney tumor that occurs in children.
- NF1, implicated in Neurofibromatosis, malignant tumors of the nervous system (Figure 9).

Another tumor suppressor gene, is P53 (Figure 10). Loss of function or mutation of P53 activity, is the most common in genetic lesions observed in human neoplasia.

In fact, alterations in ¨the P53 gene, are frequently found in a number of different malignancies, including those responsible of human mortalities (such as: breast, colon and lung carcinomas) (Figure 11).

Moreover, germ line transmission of mutant P53 alleles, seems to be responsible for inheritence of Li-Fraumeni syndrome (LFS), which is a rare familial cancer condition. Individuals afflicted with LFS, are highly susceptible to a spectrum of different childhood, and adult neoplasmas.

Hence, loss of function of tumor suppressor genes, can promote formation of diverse tumors, and clearly contributes to significant proportion of human cancers. While if these tumor suppressor genes function normally, they play a crucial role in controlling unregulated cell division, thus, avoiding tumorigenesis.

These tumor suppressor genes, are believed to be key factors in triggering apoptosis, of mutated and defectious cells. As described previously, and contrary to necrosis, or cell death by senescence, apoptosis (programmed cell death or cell suicide), is the normal final destiny of a cell, which is modified

beyond repair. Mutant cells, that can escape from apoptosis, by a loss of function of tumor suppressor genes, are the cells that lead to carcinogenesis.

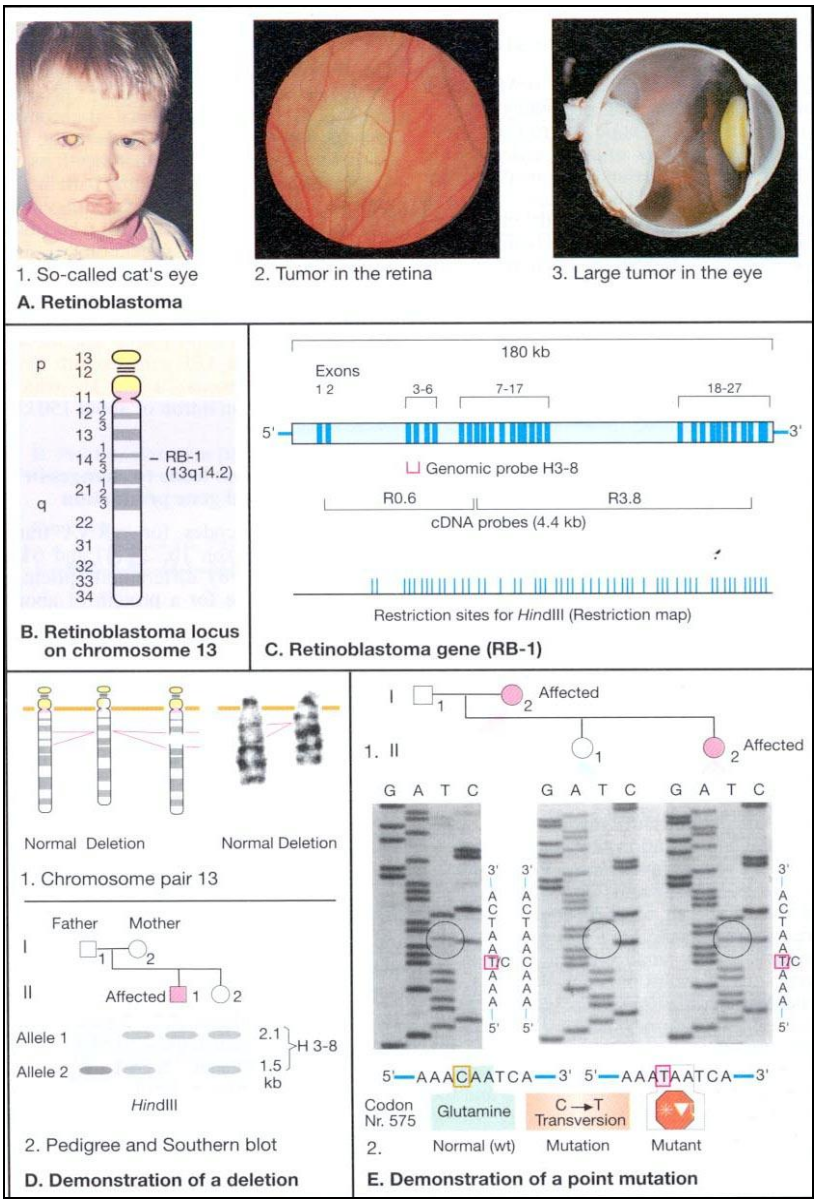


Figure 8. Molecular Genetic origin of Retinoblastoma loss of function of "RB" tumor suppressor gene.
(Atlas of Genetics. 1995; Thieme Medical Publishers, New York).

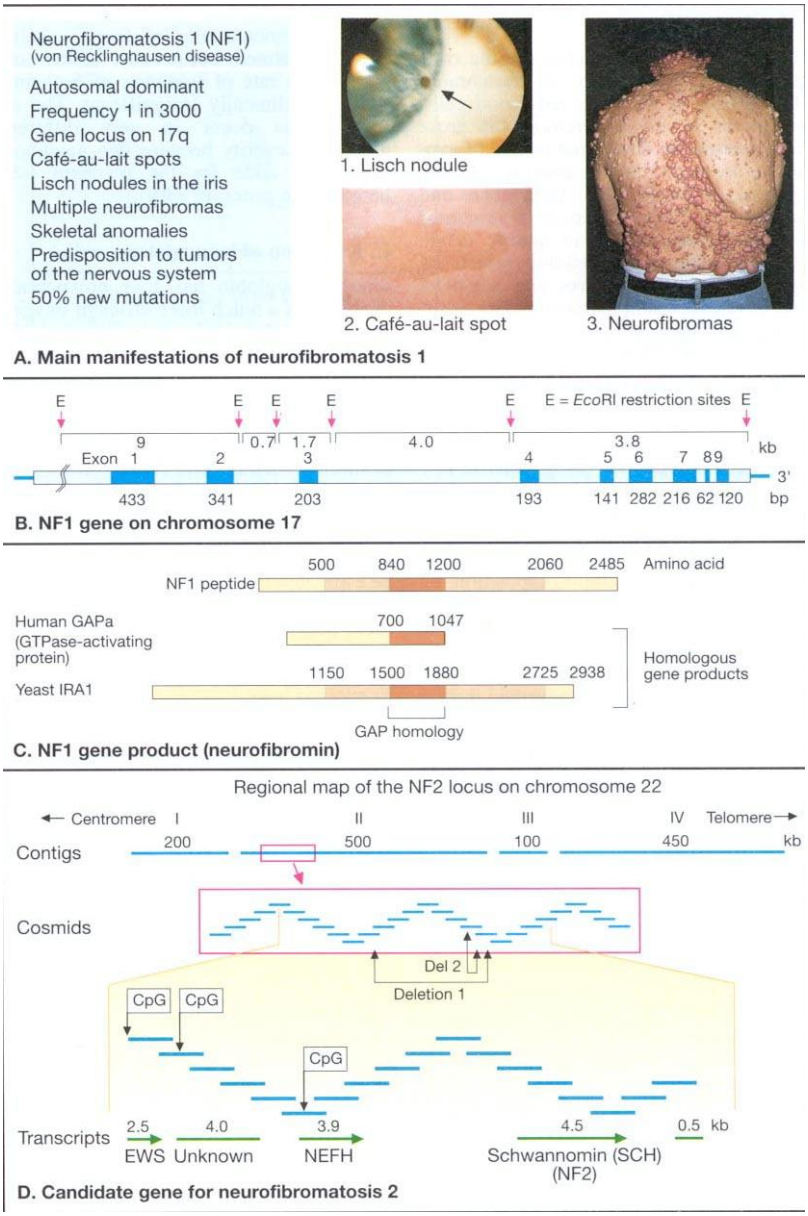
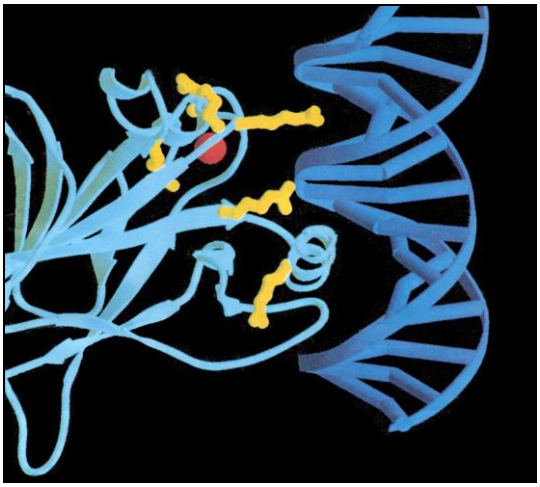
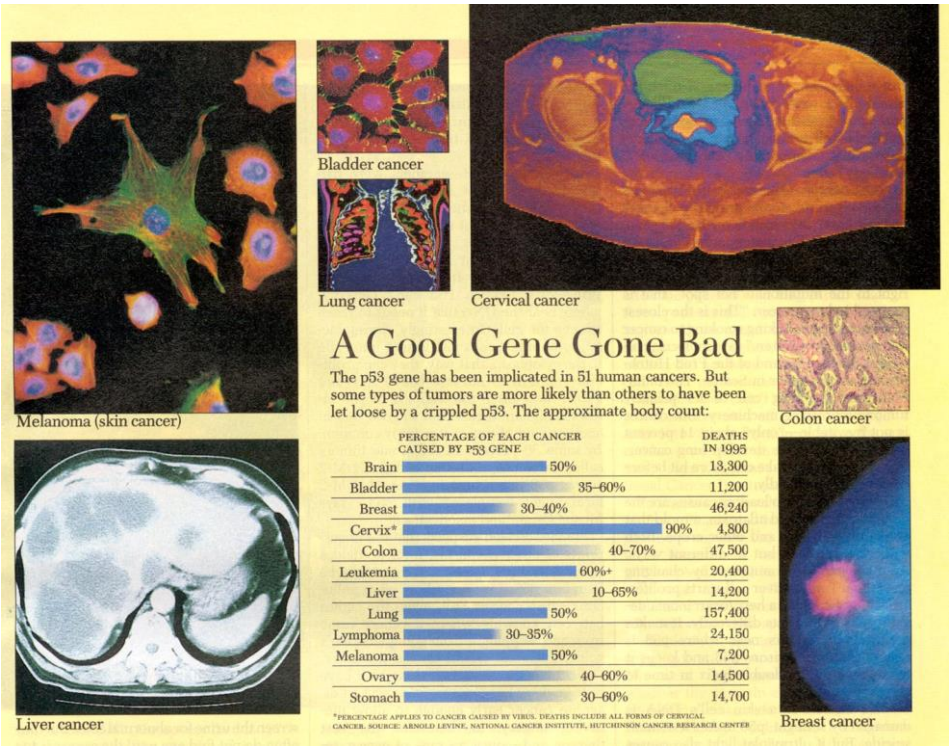


Figure 9. Molecular Genetic origin of Neurofibromatosis loss of function of NF1 tumor suppressor gene.
(Atlas of Genetics. 1995; Thieme Medical Publishers, New York).



(Newsweek)

Figure 10. Interaction of the P53 protein with the DNA double helix.



(Newsweek)

Figure 11. Genetic origin of cancer
Loss of function of "P53" tumor suppressor gene.

3 CONVENTIONAL CANCER THERAPY

While radiotherapy, chemotherapy, immunotherapy and surgery, have been the available treatments for tumors and cancers, thus saving the lives of numerous patients around the world. The limits, and side effects, of these treatments have always been clear to physicians. In fact, radiotherapy involves treatment of cancer cells, using ionizing radiation. While chemiotherapy, involves the use of synthetic chemicals, or poisons, that are analogs to cellular factors, that could inhibit topoisomerases, genetic expression, and cell proliferation. Immunotherapy, involves cancer treatment, using immunoglobulins or antibodies, that bind to cellular receptors, and provoque cell death, using their attached toxins activity. These therapeutic methods among others, have been under scrutiny, in order to optimize their use, and dosage, and to minimize, their side effects. These side effects, come generally from the fact that, these treatments are not specific to cancer cells, and that in trying to get rid of the tumors, they affect healthy cells, and disturb normal processes. Nonetheless, the worst fact, is that in many cases these therapies are helpless when they reach their limits, and are faced with incurable progressions of tumors, and generalization of cancer.

4 CANCER GENE THERAPY

Considering the side effects and limitations of the conventional cancer treatments, and considering the advances made in recombinant DNA technologies, (14) oncogenes description, and tumor suppressor genes studies, it's becoming more evident, that instead of using nonspecific synthetic chemicals to treat cancer. It could be more efficient, to use naturally occurring tumor suppressor genes, and antitopoisomerases genes, insert them into DNA vectors, along with tissue specific promoters, and introduce the genetic recombinant constructs into cancer cells (Figure 12). These recombinant genetic constructs, thanks to the tissue specific promoters, will permit the specific expression of the anticancer proteins, in target cells, without any effect, on the other non carcinogenic neighboring cells. This process of introducing genetic material into mammalian somatic cells, in order to treat malignancies is called: gene therapy.

4.1 Gene delivery systems

Although there exists several different approaches, for transferring genes into mammalian cells, the majority of clinical gene therapy trials, employ retroviral vectors, as the delivery strategy of choice.

Retroviral vector production systems, have been developed, and permit recombinant, non virulent, retroviral genomes, to be transmitted from a viral packaging cell line, to a target cell in the absence of the wild type virus. In order to accomplish this process, the recombinant viral genome has been engineered,

to contain only sequences, that are necessary for: production of the viral genome RNA, packaging of the viral genomic RNA into a virions, reverse transcription, and generation of double stranded DNA copy of the viral genomic RNA inside the target cell, as well as integration of the double stranded DNA copy of the recombinant virus into the chromatin of the target cell (10, 11).

There are specific non coding sequences in retroviral genome (promoters or regulatory sequences), that must be present (in cis). In addition, to these cis-acting sequences, there are certain retroviral coding sequences, whose protein products (gag, pol, env), must be provided in trans, for the gene delivery and expression to be successful (Figure 13).

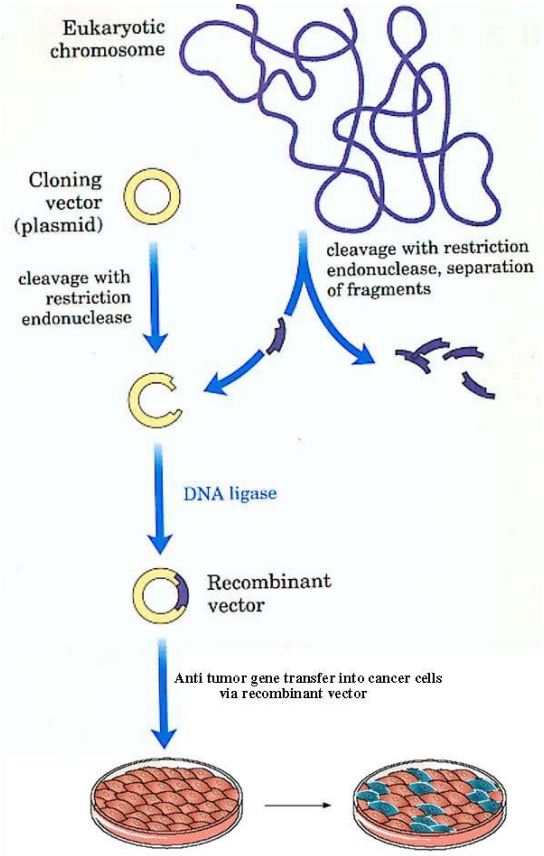


Figure 12. Cancer gene therapy.

These coding sequences, are expressed in the packaging cell line, in such a manner that their protein product, can be packaged into virions. Thus, providing the necessary components, for the packaging, and transmission, of the recombinant viral genome, but not for the coding sequences of the wild type.

The absence of the wild type viral sequences, results in transduced target cells, that are unable to sustain viral replication, preventing the spread of the

virus to other cells. Nonetheless, these transduced cells, will express the antitumor gene, delivered or carried by the vector. This feature of retroviral system, provides a safe, and efficient means of gene transfer (18, 19).

Other virus based gene transfer technologies exist, and are based on the use of hybrid or recombinant viruses, such as: adenoviruses, adeno-associated viruses (AAV), herpes virus, vaccina virus, polio virus, and RNA viruses. Thus far, only adenoviral systems, have had significant applications, in clinical setting as transfer viruses.

Adenoviral gene delivery systems, are based on the demonstration that adenovirus genomes, with deletion in E1 region, can be propagated in cells that express E1 genes. Subsequently, it was shown that replication defective adenovirus genomes, harboring foreign DNA sequences, could be replicated in cells, in which E1 proteins are supplied in trans, thus justifying the use of these vectors, in gene therapy applications.

Physical methods for the delivery of DNA into cells, is also being explored. The physical methods, include: lipofection, ligand DNA conjugates, adenovirus-ligand DNA conjugates, direct injection of DNA, or Liposome-DNA complexes, as well as other techniques, to directly deliver genes and gene constructs, into the cell for therapy (10).

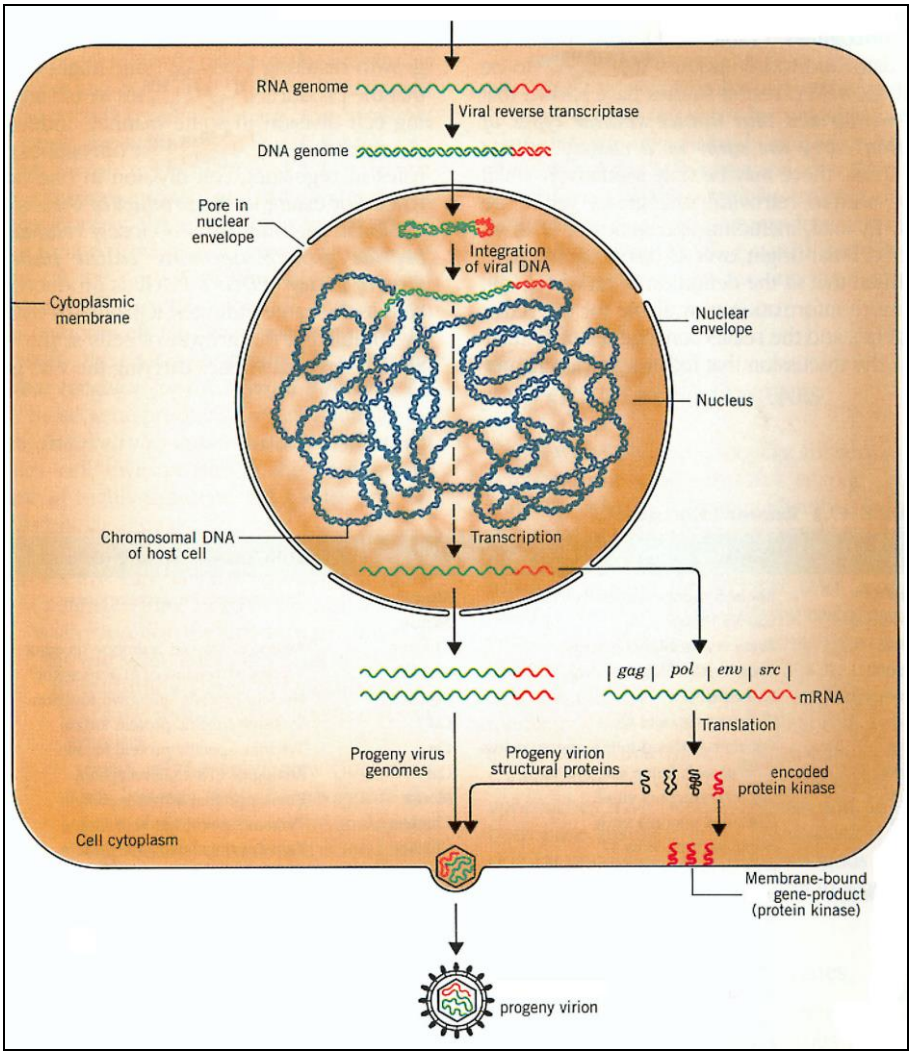


Figure 13. Gene delivery system via recombinant retroviral vectors (2).

4.2 Therapeutic gene trials for: cancer, as well as genetic, infectious and metabolic diseases

Thanks to these gene delivery systems, human gene therapy has progressed from speculation to reality in a short time. The first clinical gene transfer (only a marker gene) in an approved protocol, took place on the 22nd of may 1989, later, the first federally approved gene therapy protocols, for the correction of Adenosine DeAminase (ADA) deficiency, that causes an inherited, recessive Severe Combined Immunodeficiency Syndrome SCID, began on the 14th of September 1990. Few years later, there are tens of active clinical protocols, concerning tumor and cancer gene therapy (Table 3) as well as genetic, metabolic, and infectious diseases (Tables 4 and 5).

Table 3. Trials for Gene Therapy of Cancer (10)

Therapeutic gene	Target cell	Cancer	Investigator
TNF	TIL	Melanoma	Rosenberg (NIH)
TNF	Autologous tumor	Melanoma	Rosenberg (NIH)
IL-2	Autologous tumor	Melanoma	Rosenberg (NIH)
IL-2	Allogeneic tumor	Melanoma	Gansbacher (M-SKI)
IL-2	Allogeneic tumor	Renal	Gansbacher (M-SKI)
IL-4	Autologous fibroblasts and tumor	Melanoma	Lotze (University of Pittsburgh)
IL-4	Autologous tumor	Melanoma	Chang (University of Michigan)
IL-2	Autologous tumor	Neuroblastoma	Brenner (St. jude's)
GM-CSF	Autologous tumor	Renal	Simons (Johns Hopkins)
IFN-γ	Autologous tumor	Melanoma	Seigler (Duke University)
IL-2	Autologous tumor	SCLC	Cassileth* (University of Miami)
IL-2	Allogeneic tumor	Melanoma	DasGupta (University of Illinois)
HLA-B7	Allogeneic tumor	Melanoma	Sznol (NIH)
HSV-TK	Peritoneal metastasis	Ovarian	Freeman (Tulane)
HSV-TK	Tumor site in brain	Brain	Oldfield (NIH)
HSV-TK	Tumor site in brain	Brain	Culver (Methodist, Iowa)
HSV-TK	Tumor site in brain	Brain	Kun (St. jude's)
HSV-TK	Tumor site in brain	Astrocytoma	Raffel/Culver (USC)
HSV-TK	Tumor site in brain	Leptomingeal carcinomatosis	Oldfield/Ram (NIH)

Table 3. (continued)

Therapeutic gene	Target cell	Cancer	Investigator
Antisense (P53 and k-RAS)	Tumor site in lungs	NSCLC	Roth (M.D. Anderson)
Antisense (IGF-I)	Tumor site in brain	Brain	Ilan (Case Western Reserve)
HLA-B7[a]	Tumor sites in liver	Colorectal adenocarcinoma	Rubin (Mayo Clinic)
MDR-1	$CD34^+$ hematopoietic cells	Ovarian	Deisseroth (M.D. Anderson)
MDR-1	$CD34^+$ hematopoietic cells	Ovarian and breast	Hesdorffer (Columbia University)
MDR-1	$CD34^+$ hematopoietic cells	Breast	O'Shaughnessy (NIH)
HLA-B7 [a]	Metastasis	Melanoma	Nabel (University of Michigan)
HLA-B7 and β_2-microglobulin [a]	Metastasis	Solid tumors	Nabel (University of Michigan)

[a] Direct gene transfer into tumor via DNA-liposome complex.

Table 4. Trials of Gene Therapy for Genetic Disease (10)

Therapeutic gene	Target cell/tissue	Genetic disease	Investigator
ADA	$CD34^+$ cells/autologous cord blood	SCID	Blaese (NIH)
ADA	$CD34^+$ cells/PBL	SCID	Blaese (NIH)
Glucocerebrosidase	$CD34^+$ cells/PBL	Gaucher's	Karlsson (NIH)
Glucocerebrosidase	$CD34^+$ cells/PBL	Gaucher's	Barranger (University of Pittsburgh)
Glucocerebrosidase	$CD34^+$ cells/PBL	Gaucher's	Scheuning (FHCRC)
FACC	$CD34^+$ cells/PBL	Fanconia's anemia (C)	Liu and Young (NIH)
CFTR[a]	Airway	Cystic fibrosis	Crystal (NIH)
CFTR[a]	Airway	Cystic fibrosis	Wilson (University of Pennsylvania)
CFTR[a]	Airway	Cystic fibrosis	Welsh (University of Iowa)
CFTR[a]	Airway	Cystic fibrosis	Wilmott (Children's, Cincinatti)
CFTR[a]	Airway	Cystic fibrosis	Boucher (University of North Carolina)
CFTR[b]	Airway	Cystic fibrosis	Sorscher and Logan (University of Alabama)
LDL receptor	Hepatocytes	Hypercholesterolemia	Wilson (University of Pennsylvania)

[a] These trials will utilize a replication defective adenovirus vector to transduce the target cells.
[b] Gene delivery via cationic liposomes.

Table 5. Trials of Gene Therapies for AIDS (infectious disease)

Therapeutic gene	Target cells	Mechanism	Investigator
HIV-IT (v)	Autologous fibroblasts	Vaccine	Galpin (USC)
*rev*M10	Peripheral blood T cells	*Trans* dominant	Nabel (University of Michigan)
Hy-TK	Autologous CTL clones	Adoptive therapy	Riddell (University of Washington)
anti-5'-UTS ribozyme	Peripheral blood T cells	Inhibit replication	Wong-Staal (UCSD)
HIV IIIB *env/rev*	Intramuscular	Vaccine	Haubrich (UCSD)

4.3 NeoR/Til gene marking of therapeutic cell

Another effort is focused on new approaches, other than gene delivery systems, to test in cancer treatment clinical trials. These new approaches involve: immune system cells, known to be associated with tumors, called Tumor Infiltrating Lymphocytes TILs. These lymphocytes, have been modified to produce a protein, with demonstrated anti tumor activity, called Tumor Necrosis Factor TNF. When reintroduced into a cancer patient, the modified cells, migrate to the tumor, infiltrate cancer cells, and the TNF they produce facilitates tumor shrinkage. The tumor infiltrating lymphocytes TILs, could also be genetically modified, to produce and deliver cytokines (eg: interleikin 2), that stimulate the immune system to attack cancer cells. The transfer of gene marked immune cells TILs, into patients with advanced cancer, is now an approved genetic engineering treatment protocol (Table 6).

Table 6. Gene marker and therapy clinical protocols (7)

Protocol	Institute	Diseases	Date Initiated
Initiated: USAIRAC-approved			
Gene Marking			
NeoR/TIL	NIH, Bethesda, MD	Malignant melanoma	5/22/89
NeoR/bone marrow	St. Jude Children's Research Hospital, Memphis, TN	Pediatric AML	9/9/91
NeoR/bone marrow	St. Jude Children's Research Hospital, Memphis, TN	Neuroblastoma	1/16/92
NeoR/TIL	University of Pittsburgh, Pittsburgh, PA	Malignant melanoma	3/3/92
Gene Therapy			
ADA/peripheral blood T cells	NIH, Bethesda, MD	ADA-deficient SCID	9/14/90
TNF/TIL	NIH, Bethesda, MD	Malignant melanoma	1/29/91
TNF/tumor cells	NIH, Bethesda, MD	Advanced cancer	10/8/92
IL-2/tumor cells	NIH, Bethesda, MD	Advanced cancer	3/12/92
Initiated: International			
Gene Marking			
NeoR/TIL	Centre Leon Berard, Lyon, France	Malignant melanoma	12/12/91
Gene Therapy			
Factor IX/autologous skin fibroblasts	Fudan University and Changhai Hospital, Shanghai, China	Hemophilia B	12/3/91
ADA peripheral blood T cells + progenitor- enriched bone marrow cells	Scientific Institute, San Raflaele, Milan, Italy	ADA-deficient SCID	3/9/92

Table 6. (continued)

Protocol	Institute	Diseases	Date Initiated
Approved protocols			
Gene Marking			
NeoR/bone marrow	University of Texas, M. D. Anderson Cancer Center , Houston, TX	CML	
NeoR/hepatocytes	Baylor College of Medicine, Houston, TX	Liver failure	
NeoR/bone marrow	Indiana University, Indianapolis, IN	Adult AML and ALL	
NeoR/TIL	University of California at Los Angeles, Los Angeles, CA	Malignant melanoma and renal cell cancer	
Gene Therapy			
LDL receptor/ hepatocytes	University of Michigan Ann Arbor, MI	Familial hypercholesterolemia	
Herpes simplex, thymidine kinase/ Ovarian cancer cells	University of Rochester, Rochester, NY	ovarian cancer	
HLA-B7/melanoma in vivo	University of Michigan, Ann Arbor, MI	Malignant melanoma	
Herpes simplex thymidine kinase/ cytotoxic T lymphocytes	Fred Hutchinson Cancer Research Center and the University of Washington, Seattle, WA	AIDS	
ADA/bone marrow	Institute for Applied Radiobiology and Immunology (TNO) with University of Leiden, Leiden, the Netherlands	ADA-deficient SCID	

Table 7. Clinical trials involving gene marking of TILs (28)

Tumor target	Marker gene	Investigator
Melanoma	*neo*	Rosenberg (NIH)
Ovarian	*neo*	Freedman (Tulane)
Melanoma	*neo*	Economou (UCLA)
Renal carcinoma	*neo*	Economou (UCLA)
Melanoma	*neo*	Lotze (University of Pittsburgh)

Table 8. Targets for gene therapy (19)

Feasible approach			
Cell type	**Ex vivo**	**In vivo**	**Approaches being tested in clinical trials**
Hematopoietic progenitor cells	+	-	Ex vivo
Peripheral Lymphocytes	+	-	Ex vivo
Hepatocytes	+	+	Ex vivo
Fibroblasts	+	+	
Neuronal cells	+	+	
Keratinocytes	+	+	
Endothelial cells	+	+	
Skeletal muscle	+	+	
Pulmonary	-	+	In vivo
Epithelium			
Chondrocytes	+	+	
Cancer cells	+	+	Ex vivo and in vivo

Gene therapies used to combat cancer, fall into general categories designed to:

- Boost or create immunity to cancer cells: which involves, introducing one of several cytrokine genes into tumor cells, then reintroducing the genetically modified cells, that will serve as sites of cytokine production, an thus enhance immunity (Tables 3 and 6);
- Another way to induce immunity to tumor cells via gene therapy, is to increase the antigenecity of tumor cells. This approach has been taken, by introducing the HLA-B7 gene into malignant melanomas, by either retroviral vectors, or by DNA/Liposome complex. Tumor cells, that express the foreign HLA molecule, should trigger a vigorous allogenic immune

response at the tumor site, resulting in an immune response against specific tumor antigens, expressed by the malignant cells (Tables 3 and 6);

- A third approach that has been taken to a clinical setting, is the introduction of "suicide genes" directly into tumors. The gene that has been used for this approach is: the herpes simplex virus thymidine kinase or HSV-TK gene. Expression of this gene, render cells susceptible to killing by Ganciclovir. This drug is nontoxic to non modified human cells, but kills cells, carrying the HSV-TK gene, by converting the enzyme into nucleotide like precursors, that inhibit DNA synthesis. Because retroviruses, are capable of selectively transducing dividing cells, the preferential delivery of HSV-TK gene to tumor masses, can be accomplished by, stereotaxic injection of retroviral supernatant, or producer cells. The patients are then treated with Ganciclovir, to eradicate selectively, HSV-TK expressing tumor cells. Interestingly, experiments in animals, have indicated that not all tumor cells, need express HSV-TK, for this approach to completely eradicate the tumor mass. This "bystander" effect, may involve the ability of toxic metabolites, to cross gap junctions, between neighboring cells within the tumor (Tables 3 and 6);
- A rather similar strategy, but using different therapeutic genes, is the selective induction, of anti proliferative effects in tumor cells, by the introduction and expression, of genes encoding antisens RNA. This strategy involves, the synthesis of RNA molecules, that are complementary to mRNA, produced by transcription of tumor cell genes. The introduction of antisens RNA, turns off protein synthesis, and genetic expression in cancer cells, thus causing their death (Tables 3 and 6);
- Another approach to gene therapy for cancer, is to protect a given patient's hematopoietic system, from toxic effects, of high dose chemotherapy. The chemotherapy resistance gene, in clinical gene therapy trials, is MDR-1. This gene, encodes a protein (P glycoprotein), that is capable of serving as a multi drug efflux pump, to remove from a cell chemotherapeutic agents (eg: Taxol). However, the MDR-1 pump, is not effective against most alkylating agents, which are used in high doses chemotherapy regimes. Thus, limiting the usefulness of MDR-1 gene therapy, to specific chemotherapy applications. Additional genes, to endow cells of hematopoeitic lineages, with selective resistance to chemotherapeutic agents and radiations, are under consideration for clinical gene therapy applications (Tables 3 and 6); and
- Gene studies, are still giving gene therapy new tools, to use in it's fight against cancer. In fact, scientists are cloning genes that act as anti topoisomerases, thus, blocking the unwinding of chromosomal DNA, and causing an inhibition of genetic transcription. The general blockage of genetic expression, leads to cancer cell death.

In fact, Alami-Ouahabi *et al*. cloned, expressed, and characterized an antitopoisomerase I nuclear factor called tsHMG (Figure 14). TsHMG, is

believed to be implicated in super condensing the spermatid chromatin (Figure 15), as well as the inhibition of genetic transcription (Figure 16). The in vitro studies, showed the anti topoisomerase I activity of tsHMG, while the sequence analysis studies, showed that the structure of a segment of tsHMG protein, is an analog to anti tumor drugs used in chemotherapy, such as: Netropsin and Hoechst 33258. These results, suggest that tsHMG, could be a potential anticancer protein, that might be used in gene therapy, using tissue-specific gene promoters, to target specific cancer cells. transecting tsHMG into breast cancer culture cells, is still underway, to verify the in situ effect of tsHMG.(1).

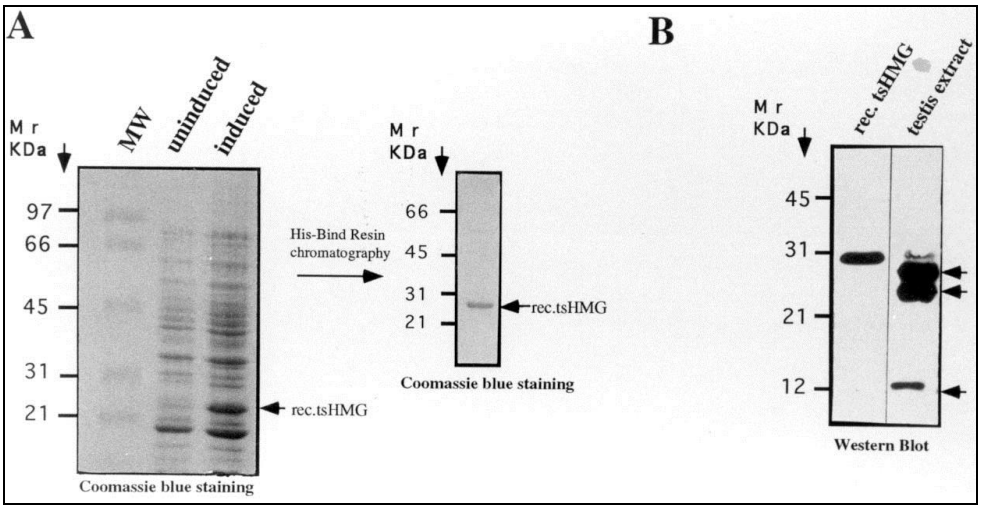


Figure 14. Purification of recombinant tsHMG protein and western blot analysis.

A potential new natural anti cancer agent;
N. Alami-Ouahabi et al. 1996. Mol. Cel. Biol, 16(7); 3720-29.

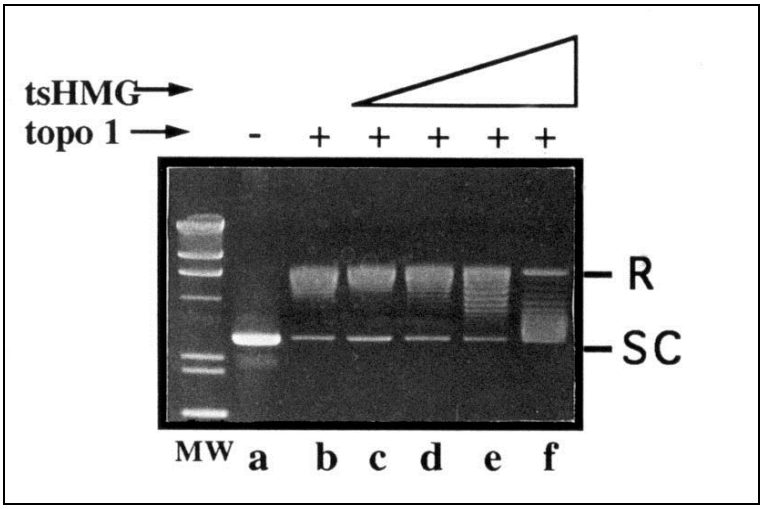


Figure 15. DNA topological activity of recombinant tsHMG protein.
N. Alami-Ouahabi *et al*. 1996. Mol. Cel. Biol., 16(7); 3720-29.

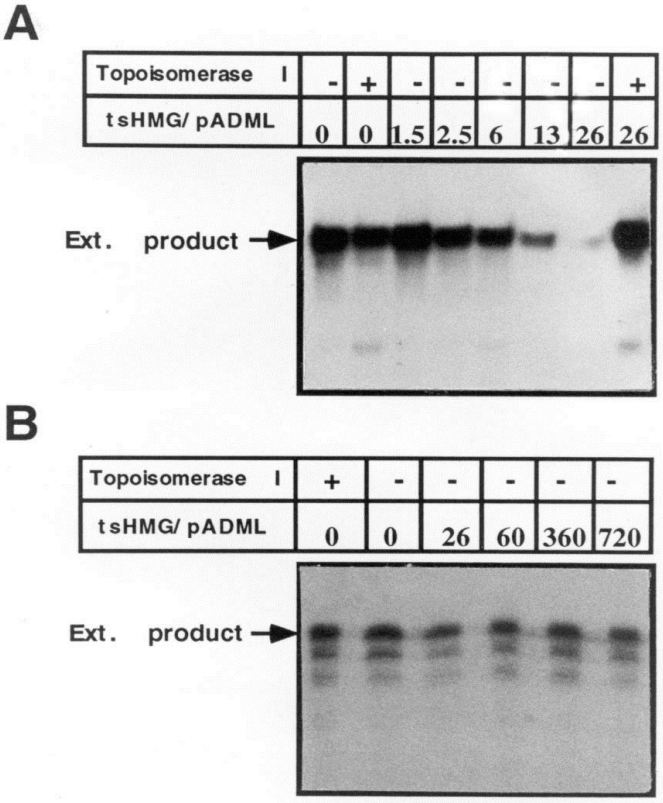


Figure 16. Inhibition of genetic transcription by tsHMG.
N. Alami-Ouahabi *et al*. 1996. Mol. Cel. Biol, 16(7); 3720-29.

5 DISCUSSION

5.1 Future prospects

Gene therapy, is in its way to becoming the strategy of choice, for a wide variety of clinical settings. Improvement in the efficiency of gene transfer into target cells, and the maintenance of expression, from relevant transferred genes must occur. Efficient gene transfer, will require further research, to improve delivery systems, and vector constructions. As well as a better understanding, of the Molecular Biology of cell targeting, and cell division. Improvement must also occur, in the expression of the introduced gene. In fact, research into tissue-specific cellular regulatory regions, or tissue-specific promoters, As well as viral enhancers of gene expression, could be of great help, in an accurate targeting, and an optimal quantity expression, of the anti tumor agents.

Safety considerations of these clinical protocols, are the first concern, whenever human gene therapy is discussed. Until now, side effects from gene transfers, have not been observed, pathology as a result of gene transfer, has not been found, and there has been no malignancies observed as a result of a replication defective retroviral vector. Still, improvement has to be made, to ensure the safe and wide use of gene therapy in all aspects of clinical medicine, instead of it's use only when conventional treatments fail.

5.2 Ethical and social implications of gene therapy

The ethical and social implications of somatic cell gene therapy, have been discussed by united states and European advisory committees, and there is now a general consensus, that somatic cell gene therapy for the purpose of disease treating is a serious and ethical therapeutic option. However, considerable controversy exists, as to whether or not, germ line gene therapy would be ethical, the issues, are both medical and philosophical.

The medical concern, is that genetic manipulations of the germ line, could produce damage in future generations. We still understand very little about how the human body works. Thus, Altering the genetic information in a patient's cell, before he is born, may produce unexpected problems. Besides the medical arguments, there are a number of philosophical, ethical, and theological concerns.

For instance:

- Do we have the right to manipulate an enfant genome, before his birth?
- Does the concept of informed consent, have any validity for patients who do not yet exist? And at what point, do we cross the line into "playing God?"

The feeling of many observers, is that germ line gene therapy, should not be considered, until much more is learned from somatic cell gene therapy. There is also considerable concern, about using gene transfer, to insert genes into

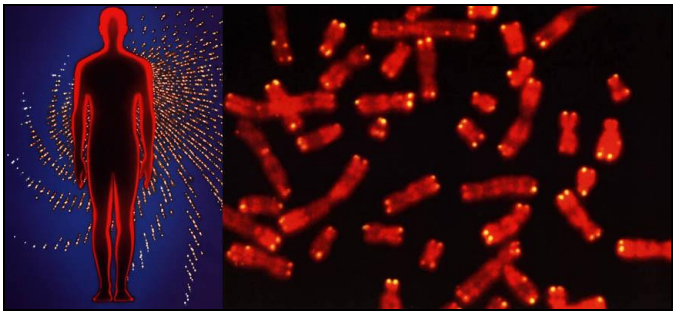
humans, for the purpose of enhancement, to improve desired characteristics and phenotypes.

But then other observers might also ask the society:

- Doesn't conventional medicine, already practices in vitro fertilization, and prenatal testing for genetic disorders, in a fetus?
- Doesn't conventional medicine, already corrects some defects in a new born, without the infant's consent?
- Doesn't conventional medicine, already practices organ transplant surgery?
- Aren't esthetic surgery, and hormone treatments, already used to improve the phenotypes, as well as the level of activity and comfort in humans?

6 CONCLUSION

In my point of view, gene therapy is, and will be, a powerful tool available to researchers, physicians and patients, in order to improve a great deal the quality of the human life and health. Nonetheless, it's the role of ethical committees, social, theological and scientific advisories representing their society, including the advisories of our Islamic countries, to set the limits, for the applications of gene therapy. Thus, trying to wisely consider, their social, ethical, and medical needs, in order to find an equilibrium, between following the advances in science and medicine in one hand, and the ethical, moral and spiritual well-being of the people.



7 AKNOWLEDGMENTS

I am indebted to Dr Mohammed Adad for his invaluable help along with his helpful criticism and comments. I am grateful to Ms Lylia Adad and Mr Adad (father) and Ms Nouzha Quasri for their patience and moral support.

REFERENCES

1. Alami-Ouahabi. N *et al.* 1996. Mol Cel. Bio; 16(7): 3720-29.
2. Alberts. B *et al.* 1989. Molecular Biology of the Cell. Garland Publishing, NewYork.
3. Benton. D 1996. Trends in Biothechnology; 14(8): 261-72.
4. Carmichael. CG *et al.* 1995. HIV aids primary care handbook. Appelton and lange. Norwalk, Connecticut, USA.
5. Clemns. PR and Caskey. CT 1994. Eur Neurol; 34: 181-85.
6. Collins. FS and Galas. D 1993. Science; 266: 43-9.
7. Collins. FS 1992. Science; 256: 744-88.
8. Dry. S 2000. Nat Struct Biol ;7suppl: 946-49.
9. Eyster. KM and Lindahl. R 2001; S D J Med; 54(2): 57-61.
10. Friedmann. T 1994. Trends in Genetics; 10(6): 210-12.
11. Gabriel. SE *et al.* 1994. Science; 266: 107-14.
12. Gardner. EJ *et al.* 1991. Principles of Genetics. Wiley. J. ed. NewYork.
13. Georgopapadakou. NH and Walsh. TJ 1994. Science; 264: 371-80.
14. Innis. MA *et al.* 1990. PCR Protocols, a guide to methods and applications. Academic Press. Boston, USA
15. Johnson. G 1996. Explorations in Cell Biology and Genetics. Brown Publishers.
16. Jordan. BR 1999. J. Biomed. Sci; 6(3): 145-50.
17. Kaul. PN 1998. Prog. Drug. Res; 50(9): 9-105.
18. Kay. MA *et al.* 1993. Science; 262: 117-19.
19. Kay. A *and al*. 1994. Trends in Genetics. 10(7): 253-57.
20. Kendrew. S and Lawrence. E 1994. Encyclopedia of Molecular Biology. Alden Press. Oxford.
21. Lehninger. AL *et al.* 1993. Principles of Biochemistry. Worth Publishers. NewYork.
22. Maniatis. T *et al.* 1989. Molecular cloning, a Laboratory Manual. Cold Spring Harbor Laboratory Press.
23. Muller. E and Alami-Ouahabi. N 1993. Proc. Natl. Acad. Sci. USA; 90: 5818-22.
24. Rockey. DC 2000. Clin. Liver. Dis; 4(2): 319-55.
25. Thivierge. M and Alami-Ouahabi. N 1993. J. Mol. Bio. Chem; 268(23): 17457-62.
26. Valbung. W *et al.* 1999. Ann. Med; 31(6): 430-1.
27. Vansteensel. MA *et al.* 2000. Proc. Soc. Ex. Biol. Med; 223(1): 1-7.
28. Vitullo. JC and Caskey. CT 1994. Eur Neurol; 34: 181-85.

The Candidate Genes of Cardiovascular Diseases: Study of the Cholesterol Ester Transfer Protein and of the Apolipoprotein E Polymorphism

O. KABBAJ, N. LAHLALI-KACEMI, A. TOUZANI, Y. BAMOU, N. ALAMI, M. HASSANI and M. LAHRICHI*

1 ABSTRACT

The cardiovascular diseases constitute the principal cause of morbidity and mortality in industrial countries. A country like Morocco is not escaping this plague. The factors of risk which most studied in the development of this disease are hypertension, obesity, dyslipoproteinemiae and diabetes. Theses factors of risk are partially from genetic origin.

The development of molecular biology techniques is contributing to evidence a plethora of candidate genes. In this study, we are interested in the apolipoprotein E (apo E) and the cholesterol ester transfer protein (CETP) in which the polymorphism influence the concentration of the lipidic parameters, in particular the one of cholesterol.

We have determined the apo E genotype in the Moroccan population. The repartition of different alleles is as follow: 11% for ε4, 84% for ε3 and 5% for ε2. There exists a correlation between the apo E alleles and the concentration of lipidic parameters. So the concentrations of total cholesterol, triglycerides and the apolipoprotein B (apo B) are higher at porter's of ε4 allele than at porter's of ε3 alleles. Otherwise, we measured the CETP concentration and we found that its rate is influencing the one of cholesterol. We are discuss the obtained results concerning the apo E and the CETP in relation with the others polymorphism involved in the lipidic metabolism, arterial hypertension, thrombosis and leukocytic adhesion, without forgetting the gene-gene interaction and gene-environment interaction.

* Laboratory of Biochemistry, Chemistry, Biology and Molecular Biology, Faculty of Medicine and of Pharmacy, Mohammed V University, Rabat, Morocco.

2 INTRODUCTION

The cardiovascular diseases constitute the main cause of morbidity and mortality in industrial countries. In order to mention only the case of the United States, 42% of deaths are caused by the cardiovascular diseases of which the half by coronary diseases. The cost is estimated at US $ about 260 billion (1). The most studied factors of risk in the development of these diseases are hypertension, obesity, dyslipoproteinemia and diabetes.

Some countries like Morocco doesn't escape to this plague. A recent investigation achieved by the Ministry of the health in Morocco allowed to put a prevalence of 24.5 % of the arterial hypertension in a Moroccan population aged of 30 years and more (2). In the same way, this prevalence is of 18.8% in Sousse (Tunisia) in an aged population of 20 years and more (3) and of 11.1% in a population besides of 10 years to the Southwest of the Arabia Saoudite (4).

Most these risk factors are partially of genetic origin. The development of the molecular biology techniques is contributing currently to evidence of a plethora of candidate genes. The most studied genes could be classified in 5 groups (Table 1) (5):

- The genes of lipidic metabolism;
- The genes of hypertension;
- The genes of homocystein metabolism;
- The intervening genes in the mechanism of thrombosis formation; and
- The intervening genes in the phenomenon of leukocytes adherence.

Table 1. The five groups of genes candidates of cardiovascular diseases, according to C. Pallaud *et al*. (5)

Panel of markers included in the multilocus assay	
Gene	Polymorphism(s)
Lipid Metabolism	
Apolipoprotein B (apo B)	Thr 71 Ile, Arg 3500 Gln
Apolipoprotein CIII (apo CIII)	T (-625) Del, C (-482) T, T (-455) C
	C (1100) T, C (3175) G, T (3206) G
Apolipoprotein E (apo E)	E2/E3/E4 (variation at codons 112 And 158)
Cholesteryl Ester Transport Protein (CETP)	Ile 405 val, Asp 442 Gly
Lipoprotein Lipase	T (-93) G, T (-39) C, Asp 9 Asn, Asn 291 Ser, Ser 447 Term
Paraxonase (PON)	Gln 192 Arg
Hypertension (renin-angiotensin system)	
Angiotensin Converting Enzyme (ACE)	Ins/Del in intron 16
Angiotensin type 1 Receptor (AT1R)	A 1166 C
Angiotensinogen (AGT)	Met 235 Thr
Homocystein Metabolism	
Methylentetrahydrofolate reductase (MTHFR)	C 677 T, 692
Cystathionine Beta Synthase (CBS)	Ile 278 Thr, 68 bp insertion in exon 8, Gly 307 Ser, Ala 114 Val, Arg 125 Gln, Glu 131 Asp
Thrombosis	
Factor V	Arg 506 Gln (leiden mutation)
Glycoprotein Platelet IIIa (GPIIIa)	Leu 33 Pro
Fibrinogen	G (-455) A
Leukocyte adhesion	
Endothelial Leukocyte Adhesion molecular (ELAM)	G 98 T, Ser 128 Arg, Leu 554 Phe

Our laboratory is interested since a long time to the study of lipid and lipoprotein metabolism in different pathological situations (diabetes, malnutrition….).

In this study, we will present some results concerning:

- The cholesterol ester transfer protein (CETP); and
- The apolipoprotein E (apo E).

The concentration of the CETP and the apo E polymorphism influence the rate of the plasma lipidic parameters and particularly the one of cholesterol, who is the principal "accused" in cardiovascular diseases development.

3 THE CHOLESTEROL ESTER TRANSFER PROTEIN (CETP)

The CETP is a plasma protein that assures the exchange between the neutral lipids: the esterified cholesterol and the triglycerides. Because the VLDL and the LDL are rich in triglycerides, and that the HDL are rich in esterified cholesterol, the CETP assures the neat transport of cholesterol esters since the HDL toward the VLDL and the LDL.

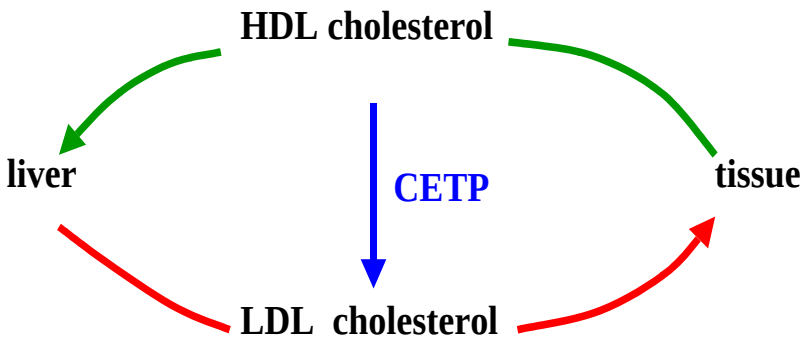


Figure 1. Role of the CETP in the way of cholesterol return toward the tissue.

The CETP is therefore implied in the way of esterified cholesterol return toward the tissue and is thus a potential candidate in atherosclerosis development (Figure 1).

3.1 CETP and lipidic parameters

We determined the CETP in 90 patients with hypercholesterolemiae compared to 22 healthy controls (Table 2).

Table 2. The CETP and the lipidic parameters in hypercholesterolemic patients compared to healthy controls

	Patients (n = 90)	Controls (n = 22)	
CETP %	29.75 ± 5.18	24.8 ± 4.5	p<0.005
Total cholesterol (g/l)	1.76 ± 0.46	1.37 ± 0.14	p<0.01
Triglycerides (g/l)	1.00 ± 0.69	0.5 ± 0.01	p<0.01
LDL cholesterol (g/l)	1.11 ± 0.21	0.71 ± 0.131	p<0.01
HDL cholesterol (g/l)	0.57 ± 0.09	0.51 ± 0.09	N.S

We note that the CETP is higher in hypercholesterolemic patients; the hypercholesterolemia touches the total cholesterol and the LDL cholesterol, whereas the rate of HDL cholesterol doesn't vary significantly.

These results lead us to follow the variation of these same parameters for 6 months in another patients group (Table 3).

Table 3. Evolution of CETP and some lipidic parameters in hypercholesterolemic patients before treatment

	A	B	C
CETP (%)	151 ± 127,5	96 ± 78,83	89 ± 54,39*
Total cholesterol (g/l)	1,76 ± 0,46	1,76 ± 0,36	1,51 ± 0,22*
LDL Cholesterol (g/l)	1,11 ± 0,21	0,77 ± 0,37	0,71 ± 0,18*
HDL Cholesterol (g/l)	0,57 ± 0,10	0,58 ± 0,13	0,65 ± 0,06
Triglycerides (g/l)	1,11 ± 0,69	0,94 ± 0,82	0,79 ± 0,58*

(A), three months (B) and six months (C) of treatment. * P<0.05 *vs.* A.

The CETP decreases after three months of treatment but this reduction become significant only after a period of 6 months. The same variations are observed in the level of total cholesterol and of LDL cholesterol. Whereas the HDL cholesterol didn't undergo a significant variations. So an elevated concentration in CETP drags an elevated concentration in LDL cholesterol and explain at least partly the role of the CETP in the atherosclerosis development.

Some other studies confirmed these results, in particular, the study led in China by Zhuang et al. (6) who showed that CETP concentration is more elevated in patients having survived to a myocardial infarction compared to the control population (Table 4).

Table 4. CETP in the myocardial infarction (6)

	Patients	Controls
CETP (mg/l)	1,98 ± 1,68	1,45 ± 1,40*

* p< 0,01.

In addition of the effect of CETP concentration, recent studies using molecular biology techniques put in evidence the CETP polymorphism. The most polymorphisms studied are the variation of Ile 405 Val and Asp 451 Gly (7). A study achieved in Finland showed that these polymorphisms influence the thickness of intima determined by ultra sonography (7).

3.2 Roles of the CETP in the lipidic metabolism

The roles of the CETP are complex and varied (8). The CETP intervenes in very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) metabolism. CETP intervenes also in the cholesterol transfer between the lipoproteins and the cells.

3.2.1 Role of the CETP in VLDL and LDL metabolism

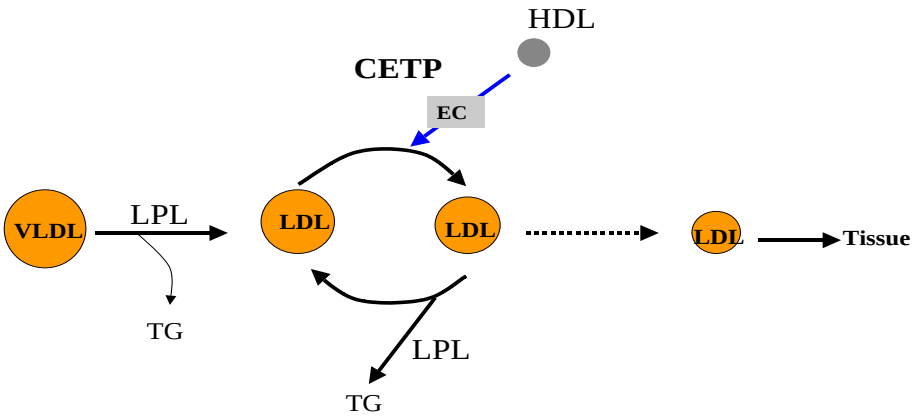


Figure 2. Role of the CETP in the VLDL and LDL metabolism.

The conversion of the VLDL in LDL implies the hydrolysis by lipoprotein lipase of besides 95% of triglycerides. The LDL receives esterified cholesterol of the HDL by CETP action. The LDL enriched in esterified cholesterol goes toward tissues.

3.2.2 Role of CETP in HDL metabolism

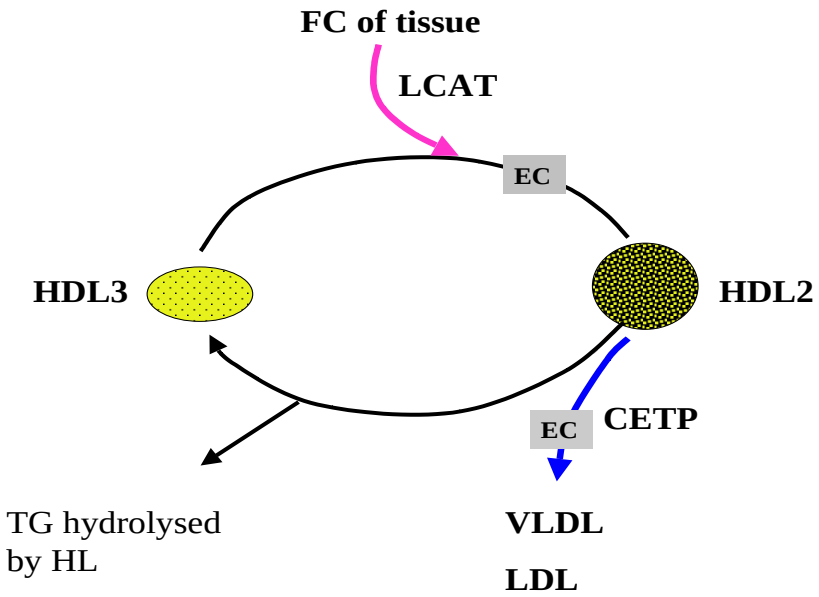


Figure 3. Role of CETP in HDL metabolism.

Free cholesterol proceeding from tissues are esterified by the lecithine cholesterol acyl transferase (LCAT). Native HDL, poor in cholesterol or HDL3 captures this esterified cholesterol. Thoses HDL3 enriched in esterified cholesterol are transformed in HDL2 with a bigger diameter. The HDL2 could deliver a part of their esterified cholesterol toward the VLDL and the LDL. Finally the HDL2 are going to lose their cholesterol and their triglycerides under the effect of the hepatic lipase (HL) and transforms again in HDL3.

In vivo, the role of the CETP in HDL metabolism was studied in the rat where CETP activity is low (9) and in the rabbit where CETP activity is elevated. The injection of CETP in the rat drags the disappearance of the esterified cholesterol rich HDL whereas in the rabbit, its increases the concentration of the esterified cholesterol rich HDL.

3.2.3 Role of the CETP in esterified cholesterol transfer between lipoproteins and cells.

The CETP is also capable to transfer some lipids between lipoproteins and liposomes and between rough and smooth endoplasmic reticulum (10).

In conclusion, CETP provokes a change of the distribution of esterified cholesterol between different plasma lipoproteins. It is known that the development of atherosclerotic lesions depends further of the way of which the cholesterol is left between the lipoproteins than total concentration of

cholesterol in the plasma. This shows the effect of the CETP in atherosclerosis development.

4 THE APOLIPOPROTEIN E (APO E)

The apo E is a protein formed by one chain of 299 amino acids. This apoprotein is synthesized by many tissues in particular the liver (90%) and the brain but also by some other cells as the monocytes and the macrophages (11).

The apo E human gene is polymorph with the existence of 3 alleles ε2, ε3 and ε4 coding for 3 proteic isoformes E2, E3 and E4. The molecular bases of this polymorphism are substitutions in 112 and 158 apo E gene codon. Three major proteins results of this polymorphism: E2 (Cys 112/ Cys 158), E3 (Cys 112/ Arg 158) and E4 (Arg 112/ Arg 158) with six genotypes, three homozygotes (E2/ 2, E3/ 3, E4/ 4) and three heterozygotes (E2/ 3, E2/ 4, E3/ 4) (12) (Figure 4).

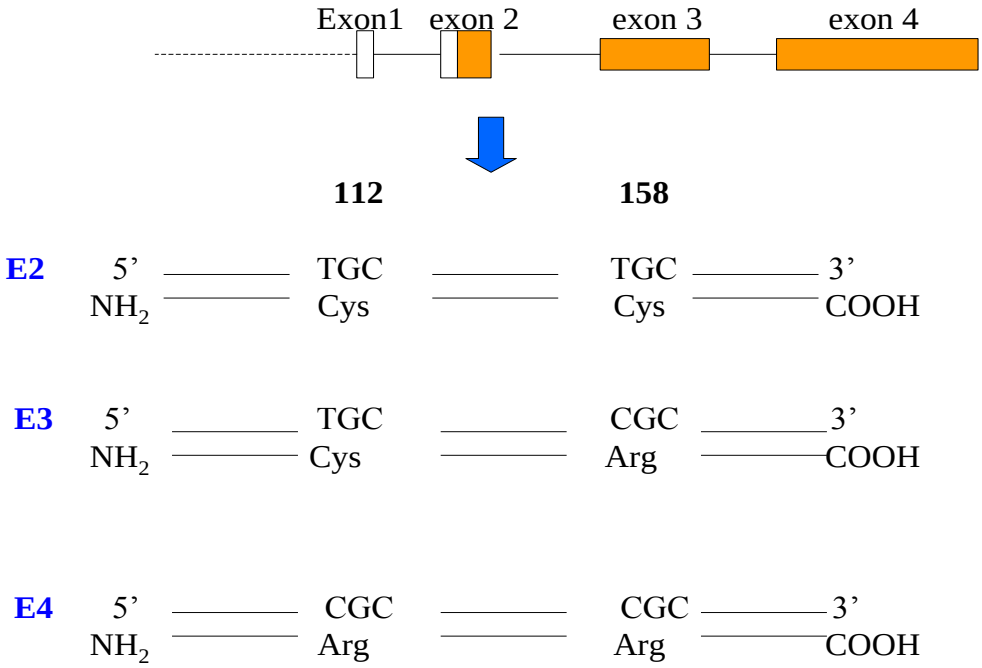


Figure 4. The apo E polymorphism and the 3 apo E isoforms (13, 14).

We determined the distribution of the different alleles of the apo E in our population and shown the influence of this polymorphism on the lipidic parameters concentration implied in the cardiovascular diseases development and particularly the cholesterol, the apo E and the apo B.

4.1 Genotype and allelic frequency in a Moroccan population

We determined the apo E genotype in a Moroccan healthy population of 168 subjects all disconcerted sex (Table 5).

Table 5. Apo E genotype frequency in our Moroccan population

Genotype	E4/4	E4/3	E4/2	E3/3	E3/2	E2/2
Total (n=168)	1	31	1	114	20	1
Genotype frequency	0,64	19,90	0,64	67,30	12,80	0,64

We notice that E3/3 genotype is the most frequent, followed by E4/3 genotype and E3/2. However the genotypes E4/4, E4/2 and E2/2 are the rarest. Seen the low number of E2/E2, E4/E2 and E4/E4 genotype, we consecrated our statistical study to the allelic frequency and compared the results to those obtained in other populations (Table 6).

Table 6. Allelic frequency in the Moroccan population compared to the other countries

Allelic frequency			
Populations studied	ε4	ε3	ε2
Our population	0,11	0,84	0,05
Morocco (15)	0,10	0,86	0,04
Cyprus (Greek) (16).	0,07	0,88	0,05
Spain (17)	0,10	0,85	0,04
France (18) (19)	0,12 0,08	0,80 0,79	0,08 0,13
Germany (20)	0,15	0,77	0,08
Suede (21)	0,20	0,72	0,08
Ireland of North (22)	0,22	0,66	0,12
Finland (23)	0,24	0,70	0,06
China (24)	0,07	0,86	0,07
Japan (25)	0,11	0,87	0,04
Southern Korea (26)	0,10	0,85	0,05

Our results show that the apo E allelic frequency in Morocco is comparable to the one of Japanese, Korean populations and of southern Europe population like France and Spain, with the exception of the Greek Cypriotes at who the ε4 allele has the lowest frequency. On the other hand the frequency of this allele is more elevated in Finland 24%, 22% in Sweden and 20% in Northern Ireland. This frequency decreases in Europe from the North toward the South. The ε3 allele is the majority isoform in all the studied populations since his frequency is about 70%. The ε2 allele is least frequent.

4.2 Relation of the apo E allele and the physiological data as well as the lipidic parameters

The relations between the different apo E alleles, the physiological data as well as the lipidic parameters are summaries in Table 7.

Table 7. Relation between apo E alleles, the physiological data and the lipidic parameters

	Allele		
Parameters	ε4	ε3	ε2
Subjects number	33	165	22
Age (years)	35,30 ±10,80	31,90 ± 9,40	31,00 ± 7,40
Body masse index (BMI)	26,04 ± 3,54	25,36 ± 3,98	26,36 ± 4,50
Apo E (mg/l)	29,84 ± 9,41	31,42 ± 9,42*	38,89 ± 9,51**
Total cholesterol (mmol/l)	5,08 ± 1,33**	4,63 ± 1,01**	4,34 ± 0,69
Triglycerides (mmol/l)	1,03 ± 0,70*	0,81 ± 0,49	0,85 ± 0,49
Apo Al (g/l)	1,38 ± 0,22	1,38 ± 0,22	1,41 ± 0,18
Apo B (g/l)	0,80 ± 0,24**	0,69 ± 0,21	0,64 ± 0,15

The carriers of the ε2 allele have apo E concentrations more elevated than the carriers of the other alleles and lower cholesterol and apo B concentrations. The ε4 allele carriers have a lower apo E concentrations, but a significantly higher cholesterol, triglycerides and apo B concentration than the ε2 and ε3 allele carriers.

For these reasons, the ε4 genotype is considered as a risk factor in the cardiovascular diseases. Indeed, different studies confirmed these results, in particular the one of Lenzen H.J. (27) and Nieminen M. S. (28) who showed that the ε4 allele carriers have a high total cholesterol and high LDL cholesterol concentration, and develops coronary diseases verified by angiography. On the contrary the ε2 genotype seems to play a protective role in the cardiovascular diseases. Schachter et al., in 1994 (29) reports a prevalence of ε2 allele in hundred-year-old subjects. It would exist therefore an interrelation between the apo E polymorphism, the state of health and the cardiovascular diseases (Figure 5) (30, 31).

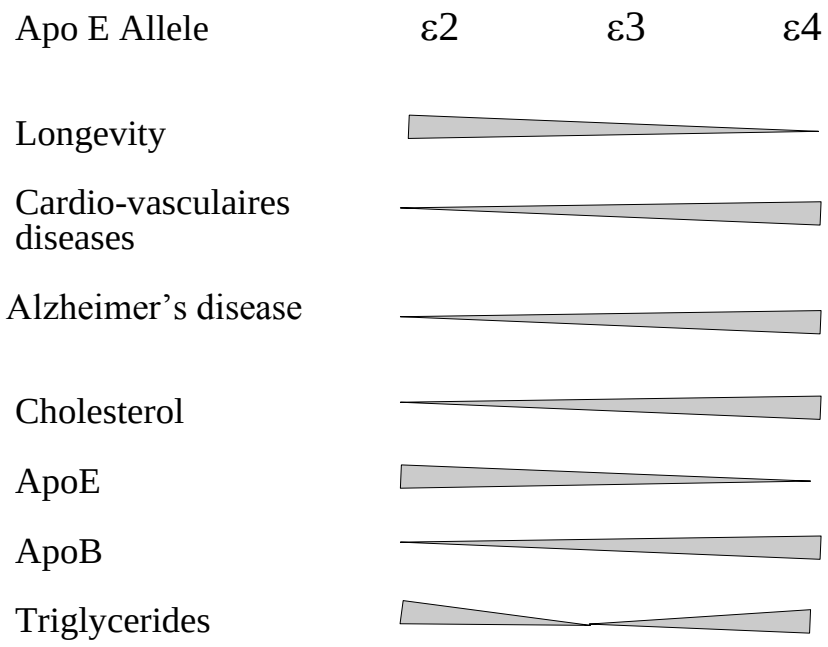


Figure 5. Relation between the apo E polymorphism, the state of health and the lipidic parameters (30, 31).

4.3 Apo E action mechanism

Apo E serves as ligand to several cell receptors, in particular the receptor of LDL, who controlling the removal of apo E-rich lipoproteins (VLDL, chylomicron remnants, and intermediate-density lipoproteins). The three common apoE isoforms have different affinities for the LDL-receptor. Because of their structure, apo E3 and E4 have the same affinity for this receptor, whereas apo E2 shows defective binding activity, corresponding to 1% of that of the two other isoforms. VLDL and remnants containing apo E2 are slowly removed from the plasma and induce an up-regulation of the liver LDL-receptor and thus a low concentration of plasma cholesterol. VLDL-apo E4 particles are removed faster from plasma than VLDL–apoE3 particles, inducing a down-regulation of the LDL-receptor. VLDL apo E4 phenotype is thus associated with a higher concentration of circulating cholesterol. The effect of apo E polymorphism on the lipidic parameters is bound to the affinity of every isoform for the lipoprotein receptors (12) (Figure 6).

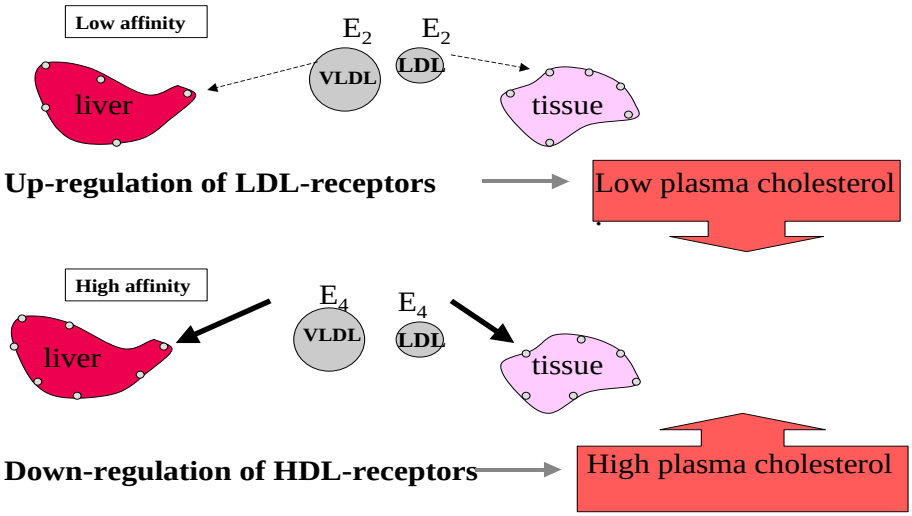


Figure 6. Apo E action mechanism.

Beside the polymorphism of the apo E, some other genes are implied in cardiovascular diseases development, as the genes of ACE, MTHFR (C677T) fibrinogen (G455A) CETP (Ile 405 Val) and apo CIII (C3175G). The most important polymorphisms are those concerning the apo CIII, CETP, MTHFR and fibrinogen.

Recent works concerning familial studies using different genetic markers and their correlation with the thickness of the carotid intima and media showed that the carotid is submissive to a genetic control. So the genetic factors control per 30% the thickness of the intima and media of the right carotid and per 33% the one of the left carotid (32)

5 CONCLUSION

The results of this study, show that the CETP concentration and the apo E polymorphism influence plasma lipidic parameters rate particularly the one of cholesterol. The people having an elevated CETP as well as those having the ε4 allele presents an elevated concentration in LDL cholesterol which is the principal "accused" in cardiovascular diseases development. This implies that these two parameters are cardiovascular diseases risk factors. However this doesn't want to say that all the people having an elevated CETP and/or the ε4 allele is going to develop cardiovascular diseases. Indeed, all the individuals don't have the same genetic predisposition, but environmental factors among which it is necessary to compile the food regimes and the fashion of life contributes extensively to release genetic mutations. The excess of tobacco or

alcohol, the rich regimes in animal fat, the use of pesticides or other poisonous products the pollution of the water, of the air or foods, some habits of sedentary life and the absence of physical exercises. All of these factors have capacity of provoking genetic mutations and of trigger cardiovascular diseases or some others pathologies with genetic predisposition as the breast and prostate cancers, hypertension or diabetes. The interaction gene-environment is fundamental but the interaction gene-gene plays also an important role. This explains that the predisposition to cardiovascular diseases is individual and their exist a several factors predisposing to those diseases.

REFERENCES

1. American Heart Association. Cardiovascular disease statistics. 1998.
2. Ottmani S, Chaouki N, Lahmouz F, Saad A, Tazi A, El Ourdighi N, Mahjour J. Prévalence de l'hypertension artérielle chez les personnes âgées de 30 ans et plus de la préfecture de Rabat. Bulletin épidémiologique 1998;34:2-10.
3. Ghannem M, Hadj Frej A. Epidemiology of hypertension and other cardiovascular disease risk factors in the urban population of Soussa, Tunisia. Eastern Mediterranean Journal 1997;3:472-479.
4. Abolfotouh M.A, Abu-Zaid H.A.H, Abdel Aziz M. Prevalence of hypertension in South-Western Saudi Arabia. Eastern Mediterranean Hearth Journal 1996;44:417-426.
5. Pallaud C, Maurice M, Cheng S, Grow M, Aguillou D, Sass C, Siest G, Visvikis S. Multilocus approch to cardiovascular risk. Scand J Clin Lab Invest 1999;Suppl (230):168-176.
6. Zhuang Y, Wang J, Qiang H, Li Y, Lui X, Li L, Chen G. Serum cholesterol ester transfer protein concentrations in healthy chinese subjects and cardiovascular disease patients. Clin Chim Acta 2001;305:19-25.
7. kakko S, Tamminen M, Paivansalo M, Kauma H, Rantala A O, Lilya M, Reunanen A, Kesaniemi Y A, Savolenien M J. Cholesteryl ester transfer protein gene polymorphisms are associated with carotid atherosclerosis in men. Eur J Clin Invest 2000;1:18-25.
8. Touzani A. Contribution à l'étude des variations de composants lipidiques sériques lipidiques et lipoprotéiques au cour du diabète insulino-dépendant. 1995. Université Sidi Mohamed Ben Abdellah, Faculté des sciences Dhar-El- Mahraz, Fes, Maroc. Thesis.
9. Walden C, Robert M S, Knop M D. Sex differences in the effect of diabetes on lipoprotein, triglyceride and cholesterol concentrations. N.Engl.J.Med 1984;31:953-999.
10. Hashimoto S, Morton R E, Zilversmit D B. Facilitated transfert of cholesteryl ester between rough and smooth microsomal membranes by plasma lipid transfer protein. Biochem biophys Res Commun 1984;120 (2):586-592.
11. Basu S K, Ho Y K, Brown M S, Bilheimer D W, Anderson R G W, Goldstein J L. Biochemical and genetics studies of the apoprotein E secreted by mouse macrophages and human monocytes. J Biol chem 1982;257:9788-9795.
12. Siest G, Pillot A, Bailly A.R, Leninger-Muller B, Steinmetz J, Galteau M M, Visvikis S. Apolipoprotein E: An important gene and protein to follow in laboratory Medicine. Clin Chem 1995;41/8:1068-1086.
13. Das H K, McPherson J, Bruns G A P, Karathanasis S K, Breslow J L. Isolation, characterisation and mapping to chromosome 19 of the human apolipoprotein E gene. J Biol chem 1984;260:6240-6247.

14. Paik Y, Chang D J, Reardon C A, Davies G E, Mahley R W, Taylor J M. Nucleotide sequence and structure of the human apolipoprotein E gene. Proc Natl Acad Sci 1985;82:3445-3449.
15. Valveny N, Esteban E, Kandil M, Moral P. Apo E polymorphism in Spanish and Moroccan populations. Clin Genet 1997;51:354-356.
16. Cariolou MA, Kokkofitou A, Manoli P, Christou S, Karagrigoriou A, Middleton L. Underexpression of the apolipoprotein E2 and E4 alleles in the Greek Cypriot population of Cyprus. Genet Epidemiol 1995;12:489-497.
17. Adroer R, Santacruz P, Blesa R, Lopez-Pousa S, Ascaso C, Oliva R. Apolipoprotein E4 allele frequency in Spanish Alzheimer and control cases. Neurosci Lett 1995;189:182-186.
18. Bailleul S, Couderc R, Landais V, Lefevre G, Raichvarg D, Etienne J. Direct phenotyping of human apolipoprotein E in plasma: application to population frequency distribution in Paris (France). Human Heredit 1993;43:159-165.
19. Siest G, Visvikis S, Herbeth B, Gueguen R, Vincent-Viry M, Sass C. Objectives, design and recruitment of a familial and longitudinal cohort for studying gene-environment interactions in the field of cardiovascular risk: the Stanislas cohort. Clin Chem Lab Med 1998;36:35-42.
20. Uterman G, Kindermann I, Kafarnif H, Steinmetz A. Apolipoprotein E phenotypes and hyperlipidemia. Human Genet 1984;65:232-236.
21. Eggertsen G, Tegelman R, Ericsson S, Angelin B, Berglund L. Apolipoprotein E polymorphism in a healthy Swedish population : variation of allele frequency with age and relation to serum lipid concentrations. Clin Chem 1993;39:2125-2129.
22. Sheehan D, Bennett T, Cashman K. Apolipoprotein E gene polymorphisms and serum cholesterol in healthy Irish adults: a proposed genetic marker for coronary artery disease risk. Ir J Med Sci 2000;169:50-54.
23. Ehnolm C, Lukka M, Nikkila E, Uterman G. Apolipoprotein E polymorphism in Finnish population: gene frequencies and relation to lipoproteins concentrations. J Lipid Res 1986;27:227-235.
24. Shen X, Xia Y, Sasse C, Visvikis S, Siest G. Association of apolipoprotein E polymorphism and concentration with serum lipids and apolipoprotein level in the Chinese from Shanghai. Clin Chem Lab Med 1998;36:615-619.
25. Tsukamoto K, Watanabe T, Matsushima T, Kinoshita M, Kato H, Hashimoto Y, Kurokawa K, Teramoto T. Determination by PCR-RFLP of apo E genotype in a Japanese population. J Lab Clin Med 1993;121:598-602.
26. Kim YS, Paeng JR, Woo JT, Kim SW, Yang IM, Kim JW, Kim KW, Choi YK. Apolipoprotein E genotypes of normal and hyperlipidemic subjects. J Korean Med Sci 1993;8:262-266.
27. Lenzen H J, Assman G, Buchwalsky R, Shule H. Associated of apolipoprotein E polymorphism, low density lipoprotein cholesterol and coronary disease. Clin Chem 1986;32:778-781.

28. Nieminen M S, Mattila K J, Aalto Setala K, Kunsi T, Kontuba K, Kaupinen-Malselin R, Ehnolm C, Jauhianen M, Vall M, Tasknien M R. Lipoproteins and their genetic variation in subjects with and without angiographically coronary artery disease. Arterioscler Thromb 1992;12:58-69.
29. Schatter F, Faure-Delanef L, Guenot F, Rouget H, Froguel P, Lesveur-Ginot L. Genetic associations with human longevity at the apo E and ACE loci. Nat Genet 1994;6:29-32.
30. Louhija J, Miettinen H E, Kontula K, Tikanen M J, Miettinen T A, Tilvis R S. Aging and genetic variation of plasma apolipoproteins. Relative loss of the apolipoprotein E4 phenotype in centenerians. Arterioscler Thromb 1994;14:1084-1089.
31. Kervinen K, Savoleinen M J, Salokannel J, Hynnuien A, Heikikinen J, Ehnolm C. Apolipoprotein E and B polymorphism longevity factors assessed in nonagenarians. Atherosclerosis 1994;105:89-95.
32. Visvikis S, Sass C, Pallaud C, Grow M A, Zannad F, Siest G, Erlich H A, Cheng S. Familial studies on the genetic of cardiovascular diseases: the Stanislas cohort. Clin Chem Lab Med 2000;38:827-932.

Advances in Cancer Gene Therapy

SAIMA N WAQAR and M ANWAR WAQAR*
Department of Biological and Biomedical Science
The Agha Khan University
PO Box 3500, Karachi 74800
Pakistan

1 ABSTRACT

With recent advances in recombinant DNA technology, together with increased understanding of the molecular biology and immunology of cancer, researchers quickly seized upon the potential for gene therapy in the treatment of cancer. Unlike conventional therapies, cancer gene therapy promises specific targeting of cancer cells and hence fewer toxic effects and greater potential of cure. The molecular basis of cancer involves activation of dominant oncogenes and inactivation of tumor suppressor genes. Gene therapy requires a vector, such as retrovirus- and adenovirus- derived ones, for efficient transfer and expression of the transgene in the cancer cells. It also requires introduction of therapeutic genes. These include gene replacement of mutated tumor suppressor genes, and genetic immunomodulation by introducing cytokine genes into cancer cells or lymphocytes, thus stimulating a cytotoxic immune response against the tumor. Introduction of prodrug genes such as wild-type p53 appears to sensitize tumor cells to chemotherapy or radiation therapy and induce apoptosis of these cells; this is known as suicide induction. Despite the growing number of cancer gene therapy clinical trials that have been reported, barriers still remain before gene therapy gains widespread clinical application. Indeed, the twenty-first century will be the era of gene therapy and molecular pharmacology.

2 INTRODUCTION

Conventional cancer therapy aims to destroy the tumor while sparing the normal host tissue, however, both chemotherapy and radiotherapy are riddled with technical problems posed by tumor invasion and metastases. Furthermore, with chemotherapy, drug resistance and low therapeutic ratio are a significant limitation (1).

There has been a vast improvement in our understanding of the molecular biology of cancer with activation of dominant oncogenes and inactivation of tumor suppressor genes. Cancer at the cellular and tissue level, metastases, host-

* Professor of Biochemistry, and Fellow of the Islamic Academy of Sciences.

tumor interaction, and mechanisms of tumor evasion of host control are also being extensively studied (2). A variety of novel strategies for treating tumors by gene therapy have been developed in recent years (2,3). Cancer gene therapy is the transfer of nucleic acids into tumor or normal cells to eliminate or reduce tumor burden by direct cell-killing, immunomodulation, or correcting genetic errors to reverse the malignant state (4). Genes can selectively inhibit the metastatic potential of cells, such as nm 23, fibronectin receptor, β-actin and E-cadherin (1). Human cancer cells contain mutations at multiple loci and rather than addressing these, a logical approach is to introduce genes coding prodrug metabolizing enzymes that have the potential to kill cancer cells. (5) Alternatively, normal host cells (eg. Bone marrow) may be protected from the toxicity of chemotherapy by transfecting chemoprotective genes into them (4,5). DNA can be introduced into target cells cultured *in vitro* prior to placing those cells back into the body or directly into target cells *in vivo* agents that facilitate the transfer of genes to recipient cells are called vectors (4). A significant limitation is the ability to transfer therapeutic genes uniformly in all tumor cells *in situ* (3). To increase cell killing within tumors and render metastatic disease manageable, new strategies will be needed to deliver therapeutic genes systemically or to induce systemic antitumor responses following local therapy (3). There are still many technical difficulties to overcome, but recent advances in molecular and cellular biology will soon play an increasing role in clinical practice, particularly in the treatment of cancer (1). This short review will explore and critically review the most widely studied approaches to cancer gene therapy, their limitations and future developments.

3 GENE TRANSFER VECTORS

An essential prerequisite of gene therapy is the efficient and stable delivery of the transferred DNA into the target cell (6). Vectors need to be engineered that will target specific cell types, insert their genetic information into a safe site in the genome, and be regulated by the normal physiological signals (1). To date, the perfect vector does not exist. Vectors available include replication defective viruses, liposomes, or a delivery complex consisting of nucleic acid conjugated to ligands. (Refer to Tables 1 & 2).

Table 1. Vectors for gene transfer

Viral	Non-viral
Retroviruses	Calcium phosphate precipitation
Adenovirus	Liposomes
Adeno-associated virus	Electroporation
Herpes simplex virus	Injection of naked plasmid DNA
Vaccinia virus	DNA-protein conjugates
Poxviruses	
Baculoviruses	

In the first human clinical gene study, viruses, being naturally occuring genetic vectors (6) (Table 2), were employed to deliver corrected genes *in vitro* into target host cells (7). Viral vectors are rendered replication defective, so they can carry genes into cells, but cannot reproduce themselves (7). The genetic material of viruses may be either RNA (ribonucleic acid) or DNA (deoxyribonucleic acid). Retroviruses, a form of RNA virus, convert their RNA to DNA in the cell they infect, which is then inserted into the host cell genome (7). Their carrying capacity can be exploited to transfer foreign genes into a variety of cells such that they become integrated and expressed in a predictable and stable manner: this is known as transduction (7,8). However, retroviral vector systems have several inherent disadvantages: complexity of preparation, poor target cell selectivity (8) and a transduction capacity limited to actively dividing cells, necessitating *ex-vivo* gene transfer. (4,6,7,8). Furthermore, as retroviruses integrate themselves randomly into the genome, there is a small, but finite risk of insertional mutagenesis (6), if they insert into vulnerable promoter (regulatory) regions of DNA, triggering oncogenes or inactivating tumor suppressor genes thus facilitating malignant transformation of target cells. (4,6,7,8). The potential for production of replication-competent (helper) virus may be minimized by careful design of the packaging cell lines (6). The size of the gene inserted in wild-type retroviral vectors is limited to 8 kilobases, though this is more than enough for most gene therapy applications (1,9). The currently achievable titres (10^7) are low compared to what will be needed for large tumors (1). Retroviral vectors derived from the Moloney murine leukemia virus (MMLV) are currently the gene transfer vehicles of choice (1). Recently, vectors derived from human immunodeficieny virus type 1(HIV-1) have shown great promise as they can transduce nondividing cells, making them suitable for host tissues such as muscle, brain and lung; the biosafety of these HIV-based vectors understandably requires scrutiny (1).

DNA viruses can only accept a limited number of foreign nucleotides, can infect a narrow range of cell types, and do not insert their own genetic material into the cell's chromosomes (1,7). The latter eliminates the risk of insertional mutagenesis but may necessitate multiple injections into the target tissue (1). Two promising agents, adenovirus and Adeno-associated virus, have the advantage of being able to achieve a very high titre that may allow the direct introduction of genes *in vivo* (10). Adenoviruses are double-stranded DNA viral vectors with advantages over retroviral vectors, including a high efficiency of infection of both dividing and non-dividing cells, and the ability to package larger segments of DNA; recently the wild-type adenoviruses have had all their structural genes removed, allowing proviral DNA of up to 35 kb to be inserted (1,4). Furthermore, they can be engineered to infect specific cells. The major disadvantage of these is that they induce antiviral immune responses (4). Moreover, adeno-associated virus integrates at a single site in the genome, thus avoiding concern over insertional mutagenesis that is associated with retroviruses (10). Herpes simplex virus (HSV) is another double-stranded DNA viral vector, with the ability to package up to 30 kb of foreign DNA (4). It

infects neurons and has the ability to establish latent infection in the brain, hence its potential in gene therapy of brain tumors (1). The primary problems with this vector are the cytopathic nature of HSV, potential for neural toxicity and difficulty maintaining long-term expression of inserted genes (1,4). Vaccinia viruses, poxviruses and baculoviruses are under investigation as gene therapy vectors (1,4).

Table 2. Characteristics of commonly used vectors

	Insert size	Cell division	Expression	Advantages	Disadvantages
Viral vectors Retrovirus	5-7 kb	Necessary	Permanent	Integrates into genome of target cell	Insertional mutagenesis; requires dividing cells for transduction
Adenovirus	7-35 kb	Unnecessary	Transient	Relatively high transduction into normal and tumor cells; easy production at high titres	Little cell specificity of viral transduction Local tissue inflammation and immune response
Adeno-associated virus	2-4 kb	Unnecessary	Long-term	High transduction efficiency into muscle and brain	Insertional mutagenesis; difficulties with production; do not work in all organs
Non-viral vectors Liposome	No limit	Unnecessary	Transient	Broad cell range of transfection; less immunogenicity; cheap and no risk of insertional mutagenesis	Low efficiency of transfection, little target cell specificity
Direct Injection of DNA	No limit	Unnecessary	Transient	Safe and inexpensive; simple; little immunogenicity	Low efficiency, gene transfer limited to cells near injection site; lack of cell specificity
Protein DNA complex	No limit	Unnecessary	Transient	Cell specificity; Large-size DNA transfer possible	Low efficiency; immune response to protein complexed with DNA

There are non-viral physical methods available by which genes can be directly delivered to target cells *in vitro* and *in vivo*, including coating of DNA in liposomes or a precipitate of calcium phosphate, both of which increase the efficiency with which DNA traverses the cell membrane (6). Liposomes are positively charged lipid membranes that complex with DNA; fusion of the liposome complex with negatively charged cell membranes results in transfer of DNA into target cells (4). Liposomes are easy to prepare, non-toxic, biodegradable agents (7), which do not induce an immune response: however they have low efficiency of gene transduction, compared to viral vectors (4). They have been used to insert HLA-B7 gene into tumors to heighten their immunogenicity and recognition by T-lymphocytes. (1,7,10). Electroporation is an important method that allows the passage of naked genetic material into the target cell by using electrical currents to disrupt the cell membrane (1). It allows the use of naked DNA, eliminating the need for viral packaging systems. (1) The DNA is not incorporated into the genome and hence long-term expression is not achieved, however, the gene needs only to be expressed for a sufficient time to cause death of target cells in tumors, unlike in inherited genetic disorders, where lifetime maintenance of the gene is desirable (1). Injection of naked plasmid DNA is another method under investigation, with lesser concerns of generation of immune response and insertional mutagenesis, but they have low levels of gene expression of short duration (4). However, this method of delivery is limited only to cells near the injection site acquiring the DNA with no tissue targeting; another problem is the inability to transduce a large number of cells (1). This method is being exploited for cancer vaccines (1). Initial work on protein-plasmid DNA complex vectors have demonstrated target cell selectivity and increased gene-tranduction (4).

4 STRATEGIES OF GENE THERAPY

Malignant transformation of cells is a result of a series of mutations in several different genes; these genes may be broadly classified as proto-oncogenes and tumor suppressor genes. Proto-oncogenes encode proteins that participate in the pathways of normal cellular proliferation; mutation of these genes transforms them into oncogenes (6). Tumor suppressor genes negatively regulate cell proliferation and promote cell differentiation (6). Mutations in proto-oncogenes and deletions in tumor suppressor genes thus constitute the molecular basis of cancer (6). Oncogenes are genetically dominant, implying that mutation of one of the two alleles is sufficient to trigger neoplastic transformation, whereas tumor suppressor genes function in a recessive manner and require deletion or inactivaion of both alleles for this (6). Therefore, the rational approach of gene therapy is to replace tumor suppressor genes or inactivate oncogenes.

Table 3. Current clinical applications of cancer gene therapy

Hematological	Acute leukemia Chronic myelogenous leukemia Non-Hodgkin's lymphoma (NHL) Multiple myeloma
Lung and pleura	Small cell carcinoma (SCC) of lung Non-small cell lung cancer (NSCLC) Malignant mesothelioma
Gastrointestinal	Liver cancer Colon cancer
Genito-urinary	Prostate cancer Bladder carcinoma Renal carcinoma
Gynecological	Breast cancer Ovarian cancer
Others	Brain tumors Malignant melanoma

5 TUMOR SUPPRESSOR GENE REPLACEMENT THERAPY

Gene replacement therapy is the delivery of a mutated or missing tumor suppressor gene to tumor cells (3,6). In addition to the induction of cell death (apoptosis), the goals are the production of changes in growth, behavior, invasiveness and metastatic ability (3) TP53 is the most frequently mutated gene in human cancers (4,11,12) and is thought to play a crucial role in malignant transformation due to its critical involvement in cell cycle control and apoptosis signaling (4) and angiogenesis (the development of a tumor's blood supply) (3). Large clinical trials with adenoviral-based p53 transfection are under way (11). Transduction of cancer cells with p53 inhibits cell growth and induces apoptosis in several tumor models, including lung, prostate, ovarian cervical, head and neck and breast (3,11) (Table 3). Furthermore, it increases sensitivity to chemotherapy, whose cytotoxic effects are presumed to be dependent on induction of apoptotic cell death (3,11,12). It also suppresses tumor angiogenesis (3) and decreases metastasis; thus improving long term survival (12). Combinations of p53-based gene therapy with other components involved in apoptosis, such as tumor necrosis factor-related apoptosis lignand (TRAIL)/APO2L, or agents neutralizing tumor-promoting anti-apoptotic signals

such as humanized anti-growth factor antibodies, should further improve the effectiveness of cancer treatment in the future (11).

Retinoblastoma gene(Rb) codes for a nuclear phosphoprotein, p105Rb, crucial in the differentiation and replication of undifferentiated cells. Loss of Rb function is associated with retinoblastoma, osteosarcoma, small cell lung carcinoma, bladder and prostate carcinoma and breast cancer (13,12) The products of other tumor suppressor genes such as p16 have been shown to suppress tumor growth in animal models (1). With DCC, reintroduction of the normal tumor suppressor genes resulted in suppression of tumorigenicity and growth rates, but not complete reversion to untransformed phenotype (6).

However, despite encouraging results of interventions directed against tumor suppressor genes, many conceptual problems remain; carcinogenesis is a multi-step process and a large number of target genes are clearly known to induce or maintain malignancy (6,12). Multiple replacements of different genes may be needed to arrest the growth of tumors, and all tumor cells must be transduced to result in a cure (3,4) In some cancer cells, tumor suppressor genes may play a less important role in determining the aberrant phenotype, the failure of Rb gene to revert prostate cancer is a prime example (12). Suppression of metastatic growth through p53 gene therapy has been attempted for breast cancer with liposome vectors but this approach lacks tumor targeting (3). To be clinically useful, highly efficient and targeted gene delivery vectors must be developed (14).

6 TARGETING OF ONCOGENES AND ANTISENSE THERAPY

This involves turing off the functions of oncogenes, either by correcting the mutant genetic sequence itself or by interfering with its expression (12). The latter involves the use of ribozymes or antisense therapy (Figure 1). Ribozymes are naturally occurring pieces of RNA that act as enzymes and perform numerous functions. Ribozymes are designed to target specific mRNA encoding the oncogene product through complementary base pair hybridization. The enzymatic activity of the ribozyme cleaves the target mRNA thus preventing its translation into protein (4). An anti-fos ribozyme completely reversed the multi-drug resistant phenotype of human ovarian carcinomas and an anti-ras ribozyme altered a human melanoma phenotype *in vitro* (12).

Antisense therapy is based on tumor targeting of oligonucleotides that are complementary to messenger RNA encoding the oncogene product. By base pairing with the messenger RNA, they prevent the translation and expression of the oncogene product and make the bound oncogene's mRNA susceptible to breakdown by normal cellular enzymes (nucleases) (4,6). Antisense DNA oligonucleotides are synthesize in reverse orientation to the complementary nucleic acid strand and by binding directly to the DNA template, they interfere with transcription (4). Antisense oligodeoxynucleotides against c-myb, c-muc,

H-c-ras, brc/abl, PCNA, and EGF-related growth factor have been shown to inhibit cell growth in a variety of cell types *in vitro* (12).

As in gene replacement strategies, ribozymes and antisense approaches require transduction of all tumor cells. Moreover, ribozyme genes or antisense sequences must be expressed for long enough to down-regulate specific genes that result in tumor cell killing (4).

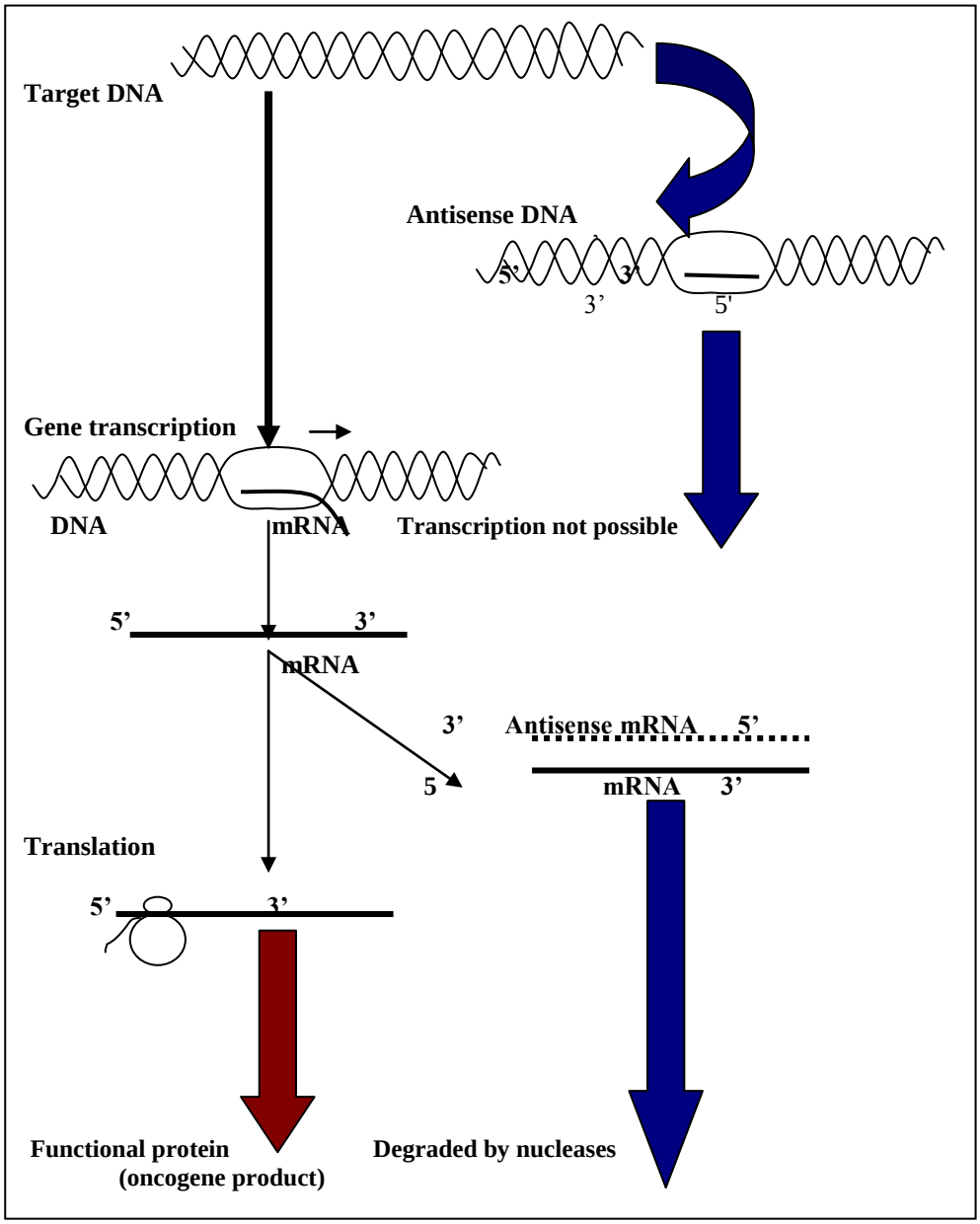


Figure 1. Mechanism of antisense therapy.

7 SUICIDE GENE THERAPY

Suicide gene therapy is defined as the transduction of a chemosensitization gene encoding an enzyme that transforms a nontoxic form of a drug (a "pro-drug") into a toxic substance (3,12). Restriction of the expression of the enzyme in tumor cells allows conversion of the prodrug to its toxic form only at the tumor site. Locally high levels of drug at the tumor site enhance killing while limiting systemic toxicity (12,13).

The E. coli cytosine deaminase gene (CD) converts 5-fluorocytosine to 5-fluorouracil, a chemotherapeutic agent. This combination produces a bystander effect, whereby more cells die than are transduced; this results from passive transfer of fluorouracil in the intracellular space (3,4). 5-Fluorocytosine can be given at very high doses with little toxicity (2) (Figure 2). Suicide gene therapy with CD has been successful in animal models with hepatic metastases of GI tumors, for which 5-fluorouracil is commonly used (3). However, the specificity with which the CD gene can be delivered to a tumor site is low. Delivery of CD to specific sites and the use of tissue-specific promotors are a focus of work.

Human thymidine kinase (tk) catalyzes the phosphorylation of thymidine and a limited number of structurally related analogues (5). In contrast, herpes simplex virus tk(HSV-tk) phosphorylates a variety of analogues, including ganciclovir and acyclovir (5). By introducing HSV-tk into tumors and treating systemically with ganciclovir, selective targetting of tumor cells is possible (5). The phosphorylated nucleoside analogues are incorporated into replicating DNA of tumor cells and stop the elongation of the replicating DNA strand and cell death follows (5,15). It also causes a bystander effect due to intracellular transfer of phophorylated nucleoside via gap junctions into neighbouring tumor cells (4, 5, 15). Non-transduced cells endocytose debris containing phosphorylated gancyclovir from dying cells; and an induced immune response leads to tumor killing (3,12). It is being evaluated for possible treatment of localized brain tumors, liver metastases, peritoneal-based metastases, and mesotheliomas (3). However, the bystander effect, though positive is unpredictable. Also there are difficulties in transduction by vectors that need to be overcome. The immunosuppressive effects of the dosages needed for tumor regression currently limit efficacy (5).

As with p53 gene therapy, both the CD and HSV-tk systems sensitize cancer cells to radiation, suggesting possible combination therapies to control advanced tumors (3).

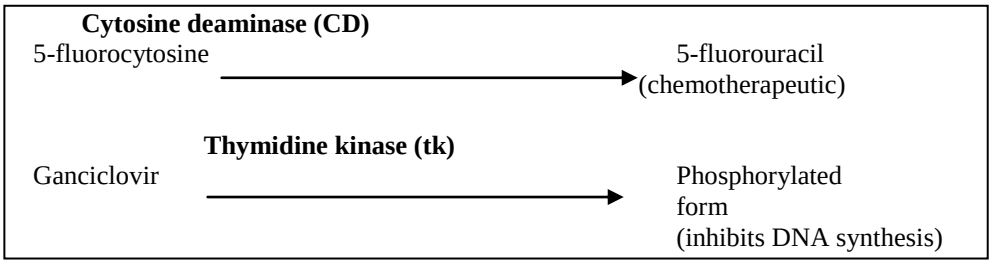


Figure 2. Most widely studied applications of suicide gene therapy.

Other examples are the E. coli gpt gene which works on the pro-drug 6-thioxanthine and the human gene for a specific form of P-450 which heightens the sensitivity of tumor cells to cyclophosphamide (5).

Furthermore, genes can be mutated to provide better protection for host tissues, such as the bone marrow, against the toxicity of specific chemotherapeutic agents. Expression of the mutant enzyme could render sensitive tissues, such as bone marrow, more resistant to specific cytotoxic agents, for instance genes encoding dihydrofolate reductase (DHFR) transfected into host tissues would protect against the toxicity of methotrexate, similar is the case with thymidylate synthetase (TS) gene and the chemotherapeutic agent 5-fluorouracil (5).

8 IMMUNOMODULATORY GENE THERAPY

Cytokines are key modulators of host immune response to tumors but have systemic side effects and very short half-lives (8,13). Vectoring of genes encoding cytokines with cells that home specifically on tumors results in antitumor response with minimal side-effects (8).

Active cancer immunotherapy depends on antigens encoded by tumor cells being sufficiently distinct from self-antigens to induce an immune response leading to tumor eradication (8) Melanoma and renal cell carcinoma possess some inherent immunogenicity that may be enhanced by immunotherapy; similarly tumor vaccination of non-immunogenic cancers of the prostate, pancreas, colon and small cell carcinoma of the lung seems promising (2,8,15) .

Immunotherapy induces cellular immune responses to metastatic lesions by vaccinating the patient against that specific cancer. Tumor cells are modified ex vivo with a cytokine gene. These cells are irradiated to prevent further cell division before being transplanted back into the patient, thus generating a systemic immune response against tumor-specific antigens (3). Genes that have been tagged as candidates for this strategy include IL2, GM-CSF, IFN-g and TNF (3,7,15). Vaccinia virus has been used to develop an immune response to carcinoembryonic antigen (CEA) (10).

The success of immunotherapeutic strategies relies on the identification of tumor-specific antigens, on the capability of the immune system to recognize these specific antigens and attack tumor cells with which these antigens are associated (3). Vaccination of subcutaneously placed tumors generates a

superior response to deeper-seated ones; similarly the response to small tumor burden is better than that to established metastatic disease (3,8). This approach may be useful for the treatment of minimally residual disease or for vaccinating individdduals at high risk of developing cancer (10).

Alternatively, the tumor surface antigen can be modified by introducing co-stimulatory molecules so that tumor cells are more efficiently recognized by the host's immune system (3). The most widely studied co-stimulatory molecule is B7, which interacts with CD- 28 receptor on T-cells to enhance T-cell activation.(3,7,15) Furthermore, co-vaccination with IL-2 markedly enhances immunological activity and leads to systemic immunity in addition to inhibition of tumor growth (4). A potential limitation is that generation of an effective T-cell response requires complex interaction of multiple cell-surface molecules (10).

Direct injection into the tumor of IL-12 augments T-cell and NK-cell activity, induces INF-g production and promotes T cell differentiation into Th1 cells, thus suppressing tumor growth through cell-mediated immunity (3).

Combining a suicide approach, such as HSV-tk and ganciclovir with IL-2 transgene therapy is another promising area under study (7,15). This inhibitory action is short-lived and seems to be mediated through CD8+ T-lymphocytes (3). However, addition of GM-CSF results in long-term antitumor activity. It is hypothesized that HSV-tk activity induces rapid necrosis, thus generating large amounts of tumor antigen; GM-CSF aids in more efficient presentation of the antigen, while IL-2 stimulates T-cell proliferation (3).

Another approach is to use tumor-infiltrating lymphocytes(TIL) as targets for gene therapy in order to increase their anti-tumor efficacy.(12,15,16).

9 GENETIC PROFILING

Individuals respond differently to chemotherapy because of inherited variations in genes involved in drug metabolism. Increasingly rapid techniques are being developed to identify and study specific variants of genes. This has heralded an era of pharmacogenetics: making genetic profiling of individuals a component of high-dose chemotherapy strategies. Two genes, CYP3A4 and CYP3A5, appear to be associated with poorer survival in breast cancer patients and poorer response to cyclophosphamide.

10 CONCLUSION

In conclusion, cancer gene therapy promises specific targeting of cancer cells, fewer toxic side effects and greater potential for cure. Despite the growing number of cancer gene therapy clinical trials that have been reported barriers remain before gene therapy gains widespread clinical application. Many cancers may not be curable by single modality therapy. Carefully selected combination of strategies may be more efficient. Indeed, the 21st century will be the era of gene therapy and molecular pharmacology.

REFERENCES

1. Kouraklis, G. Gene therapy for cancer: from the laboratory to the patient. Dig-Dis-Sci 45(6): 1045-52, 2000.
2. Hersh, E M; Stopeck,-A-T. Advances in the biological therapy and gene therapy of malignant disease. Clin-Cancer-Res. 3(12 Pt 2): 2623-9, 1997.
3. Hall, S J; Chen, S H; Woo, S L. The promise and reality of cancer gene therapy. Am-J-Hum-Genet. 61(4): 785-9. 1997.
4. Weichselbaum, R R; Kufe, D. Gene therapy of cancer. Lancet. 349 Suppl 2SII10-2, 1997.
5. Encell,L P; Landis, DM; Loeb, LA. Improving enzymes for cancer gene therapy. Nat-Biotechnol. 17(2): 143-7, 1999.
6. Dorudi, S; Northover, J M; Vile, R G. Gene transfer therapy in cancer. Br-J-Surg. 80(5): 566-72 1993.
7. Jenkins, J; Wheeler, V; Albright, L. Gene therapy for cancer. Cancer-Nurs. 17(6): 447-56, 1994.
8. Weber, C E. Cytokine-modified tumor vaccines: an antitumor strategy revisited in the age of molecular medicine. Cancer-Nurs. 21(3): 167-77, 1998.
9. Vile, R G; Russell, S J. Retroviruses as vectors. Br-Med-Bull. 51 (1): 12-30, 1995.
10. Zwiebel, J A; Su, N; MacPherson, A; Davis, T; Ojeifo, J O. The gene therapy of cancer: transgenic immunotherapy. Semin-Hematol. 30 (4Suppl 4): 119-28; discussion 129. 1993.
11. Zeimet, A G; Riha, K; Berger, J; Widschwendter, M; Hermann, M; Daxenbichler, G; Marth, C. New insights into p53 regulation and gene therapy for cancer. Biochem-Pharmacol. 15; 60(8): 1153-63 2000.
12. Yu M, Advances in cancer gene therapy. MJM 2:93-106, 1996.
13. .Gutierrez, A A; Lemoine, N R; Sikora, K. Gene therapy for cancer. Lancet. 339(8795): 715-21 1992.
14. Friedmann, T. Gene therapy of cancer through restoration of tumor-suppressor functions? Cancer. 70(6 Suppl): 1810-7 1992.
15. Culver, KW. Clinical applications of gene therapy for cancer. Clin-chem. 40(4): 510-2, 1994.
16. Rosenberg, S A. Immunotherapy and gene therapy of cancer. Cancer-Res. 51(18 Suppl): 5074s-5079s 1991.

Biotechnology in the Palm Oil Industry: The Experience of the Malaysian Palm Oil Board (MPOB)

AHMAD PARVEEZ GHULAM KADIR*
SITI NOR AKMAR ABDULLAH
and
S RAVIGADEVI
Malaysian Palm Oil Board
PO Box 10620, 50720 Kuala Lumpur, Malaysia

1 ABSTRACT

Malaysia is the largest producer of palm oil in the world, accounting for 50% of total world production. It is a major income earner and contributes significantly to the economy of the country. The current scenario of shortage of manpower and arable land requires that the return per unit area be increased in order to maintain profitability. Fluctuating commodity prices is also a problem. MPOB has identified genetic engineering as a promising technology to overcome the above limitations. Genetic engineering, with all the advantages over conventional breeding, could be used to produce transgenic oil palms with value-added fatty acids and novel metabolites. Establishment of a reliable transformation and regeneration system is essential for genetic engineering. Transgenic oil palm has been successfully produced using the biolistics method, which now makes it possible to improve the oil palm through genetic engineering. MPOB's primary goal of oil palm genetic engineering is to increase oleic acid content. Besides increasing oleic acid, the programme has targeted the following products: stearic acid, ricinoleic acid, palmitoleic acid, lycopene and biodegradable plastics (polyhydroxybutyrate). â-ketoacyl ACP synthase II, palmitoyl acyl ACP thioesterase, stearoyl ACP desaturase, oleoyl CoA desaturase and α- and β-lycopene cyclase were identified as important genes for some of the above targets. Studies were carried out on fatty acid biosynthesis, gene and promoter isolation, vector construction and improvement of transformation methods, all of which have contributed towards getting closer to realizing the above genetic engineering targets. The latest achievements on

* Advanced Biotechnology and Breeding Centre, Malaysian Palm Oil Board, PO Box 10620, 50720 Kuala Lumpur, Malaysia; *parveez@mpob.gov.my*

genetic transformation, biochemical studies, gene isolation and vector construction will be elaborated. The use of the *Arabidopsis* transformation system for evaluating transformation vectors will also be discussed.

2 INTRODUCTION

Palm oil, produced in tropical countries is the second largest source of edible oil in the world (Scowcroft, 1990). It is used as a cooking oil in many parts of the world. It is also used in the making of other food products like shortenings, margarines (Sudin, *et al*., 1993) and spreads (Pantzaris, 1993). In addition to the cost benefit and its multiuse, palm oil has also been proven to be nutritious. Studies have indicated that palm oil lowers serum cholesterol levels to the same degree as sunflower oil that is rich in unsaturated fatty acids (Chen and Fan, 1992; Qureshi *et al*., 1991). Palm oil does not increase the plasma cholesterol or LDL, low-density lipoprotein, where increased level could be harmful (Heber, *et al*., 1992; Manorama and Rukmini, 1992; Sundram *et al*., 1992). On the other hand, it increases the HDL, high-density lipoprotein, which protects against heart disease (Sundram, 1993; Kritchevsky, 1992). Furthermore palm oil has anti-tumour effects (Kritchevsky *et al.*, 1992; Carroll and Parenteau, 1992) especially with the presence of high levels of vitamin E, tocopherols and tocotrienols (Nesaretnam *et al*., 1992; Tan, 1992).

The challenge that the oil palm industry will face in the 21st century is the ability to maintain profitability in the face of labour shortage and limited land resources. At present, palm oil contributes to around 20% of world oils and fats production. It is envisaged that the demand for oil will grow faster then the rise in supply (Oil World Annual, 2001). By the year 2020, it is expected that nearly 26% of the world's oils and fats demand will be met by palm oil. It is anticipated that palm oil will capture approximately 50% of the world's oils and fats trade (Rajanaidu and Jalani, 1995). Due to this projected demand, it is important to increase the yield of oil palm as well as to improve the palm oil quality at a faster rate than has been achieved by conventional breeding (Parveez, 1998).

Present oil palm planting materials are derived from a narrow gene pool, which restricts the introduction of new traits via conventional breeding techniques. The long generation time (approximately 7-10 years), and the open pollinated behaviour of oil palm contribute to the slowness of conventional genetic improvement methods besides requiring large amounts of planting area. All the above limitations make oil palm an ideal crop for the use of genetic engineering tools in its improvement. Development of oil palm tissue culture techniques, combined with the ability to transfer genes of interest into elite germplasm are attractive tools with which to overcome the slowness of genetic improvement in this perennial crop. Additionally, genetic engineering can source genes from any plant, animal, bacteria, fungus or virus species (Gasser and Fraley, 1992). Approximately, four to five years are required to produce

transgenic plantlets carrying a new trait from initial date of explant culture. Taking into account the requirement of back-crossing in conventional breeding, genetic engineering could save 80 - 90% of the time required for introducing a new gene/trait into oil palm (Parveez, 1998).

The main goal of the MPOB genetic engineering programme is to change oil quality and in particular to increase oleic acid content (Cheah *et al*., 1995) which can be used as feedstock for oleochemical industries (Pryde, 1983). Increasing stearic acid is another target. Besides increasing oleic and stearic acids, production of novel high value products such as palmitoleic acid, lycopene (carotenoid), ricinoleic acid and biodegradable plastics has been targeted (Parveez *et al*., 1999).

Genetic engineering of the oil palm requires the development of several tools and input from different disciplines such as biochemical studies, gene and promoter isolation and genetic transformation. This paper will discuss the progress that has been made on the above disciplines.

3 BIOCHEMICAL STUDIES

3.1 General

The fatty acid biosynthetic pathway is generally similar in all plants and it involves repeated incorporation of two-carbon units derived from malonyl-CoA to elongate the fatty acid chain to around 16 or 18 carbon (Stumpf, 1984). The reaction is initiated by acetyl-CoA. Malonyl Co-A is derived from acetyl-CoA by a carboxylase reaction catalyzed by acetyl CoA carboxylase (ACCase). The main enzymes involved in fatty acid biosynthesis are collectively known as fatty acid synthase (FAS) and are localized in the plastids. The synthesized fatty acids are attached to a cofactor, acyl carrier protein (ACP). The ACP is cleaved from the fatty acids by specific thioesterases before they move out from plastids and are esterified to CoA. Further elongation and desaturation occur in the microsomes before the fatty acids are either returned to the plastid or incorporated into triacylglycerols (in the microsome). Although the mechanism of fatty acid biosynthesis is similar in all crops, the regulation is different as evident from the different fatty acid profiles of different crops (Sambanthamurthi *et al*., 2000).

In order to tailor fatty acid composition of palm oil, the regulation of fatty acid biosynthesis in the oil palm has to be clearly defined. Since the primary aim of the oil palm genetic engineering programme is to decrease palmitic acid and increase oleic acid, a pertinent question to be answered was why palmitic acid accumulates in the oil palm resulting in 44% palmitic acid content in palm oil. Based on the fatty acid composition of palm oil and the fatty acid biosynthesis pathway common to all plants (Figure 1), the following postulation was made:

(1) â-ketoacyl ACP synthase II (KAS II) activity is rate-limiting in the oil palm mesocarp resulting in a “bottleneck” of palmitic acid;

(2) Thioesterase activity towards palmitoyl ACP is very high resulting in the release of palmitic acid;
(3) Oil palm mesocarp contains an active stearoyl ACP desaturase. Thus most of the stearoyl ACP formed is effectively desaturated to oleic acid. Increasing stearoyl ACP desaturase is unlikely to increase oleic acid (unless the stearoyl ACP pool is increased) (Cheah *et al*., 1995).

Therefore, two approaches were considered for channelling palmitic acid further along the pathway to produce more oleic acid: i) stimulate KAS II activity, and ii) reduce thioesterase activity towards palmitoyl ACP. Therefore the biochemistry of KAS II and acyl ACP thioesterase was investigated.

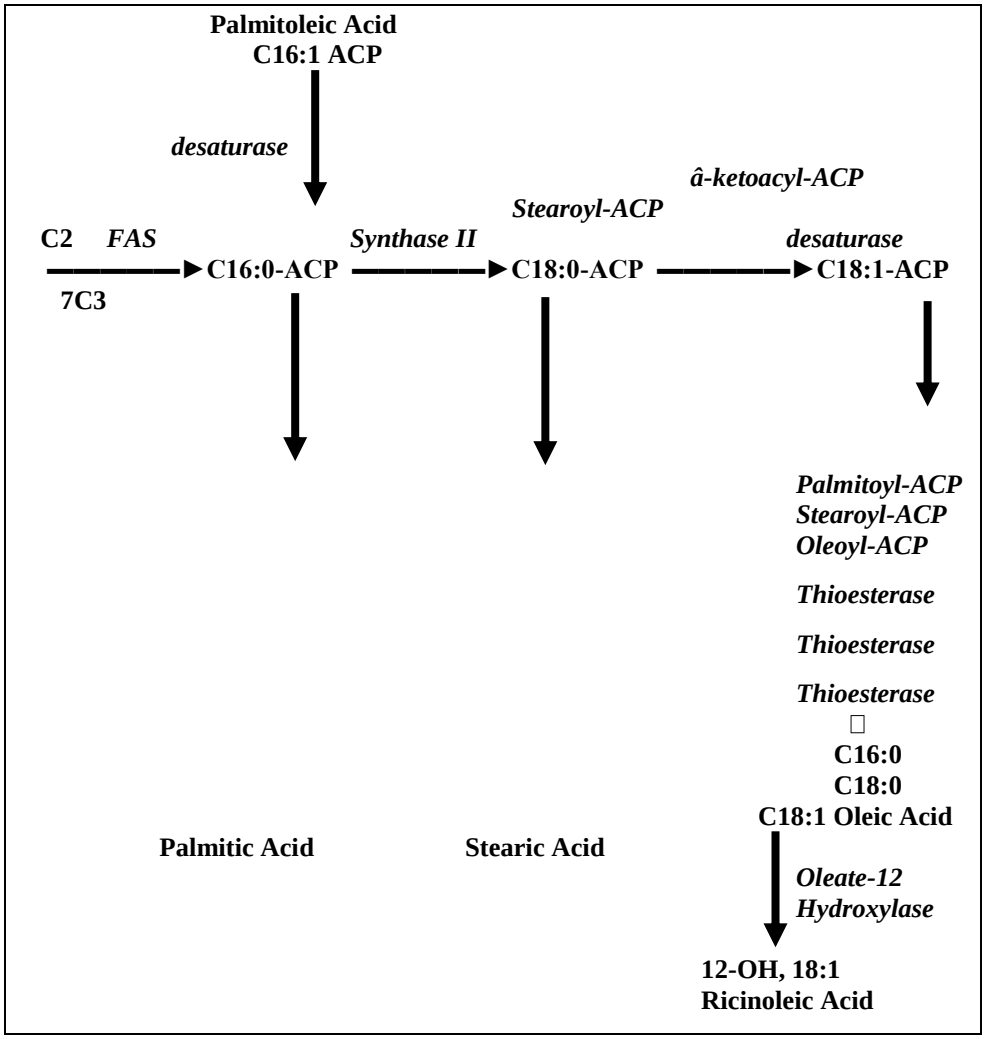


Figure 1. Possible reactions involved in the modification of products of fatty acid synthetase.

3.2 â-ketoacyl ACP synthase II (KAS II)

As was hypothesized earlier, limited KAS II activity is one of the main factors contributing towards high palmitic acid. Increasing KAS II activity would increase stearoyl ACP that would subsequently be desaturated to oleic acid. The relationship between KAS II activity and the level of unsaturation was studied in mesocarp of *E. guineensis* and *E. oleifera* as well as their hybrids. The results showed strong positive correlation between KAS II activity and C18 unsaturated fatty acids (C18:1 + C18:2 + C18:3) (Umi Salamah, 1995; Sambanthamurthi *et al*.,1996a). Furthermore, it was also shown that desaturase activity was not limiting as increased KAS II activity did not result in a build-up of stearic acid but an increase in unsaturated C18 fatty acids. Interestingly, the level of C16:0 was negatively correlated to the level of C18:1. This negative correlation confirmed that palmitic acid accumulation is controlled by KAS II activity. The higher the KAS II activity the higher the amount of palmitic acid that could be channelled to oleic acid.

3.3 Acyl ACP thioesterases

In most plants, the major product of fatty acid biosynthesis in plastids is oleic acid. In all plant species examined so far, a thioesterase highly active towards oleoyl ACP has been described which presumably ensures that oleic acid is released from ACP and exported out of the plastid. Plants that accumulate medium chain fatty acids such as the California bay and *Cuphea* were shown to express a medium chain specific acyl ACP thioesterase (Pollard *et al*., 1991; Davies *et al.*, 1991). Therefore the presence of palmitoyl ACP thioesterase activity in oil palm mesocarp was investigated.

Crude oil palm mesocarp extract was assayed for thioesterase activity against different acyl ACP substrates. Maximum activity was obtained with palmitoyl ACP (Sambanthamurthi and Oo, 1990; Abrizah, 1995). Thus, palmitic accumulation in the oil palm mesocarp could be attributed to chain termination by the action of palmitoyl ACP thioesterase. The oil palm mesocarp also showed high oleoyl ACP thioesterase activity.

Since the oil palm mesocarp exhibited high thioesterase activity towards both palmitoyl ACP and oleoyl ACP, it was important to determine if the activities were on the same or on different proteins. The thioesterases were partially purified and shown to be two separate proteins (Abrizah, 1995). This was fortuitous as it meant one could decrease palmitoyl ACP thioesterase activity independently of oleoyl-ACP thioesterase and hence reduce palmitic acid levels without reducing oleic acid levels. Similarly one could increase oleic acid by increasing oleoyl ACP thioesterase activity without increasing palmitoyl ACP thioesterase activity.

The biochemical studies identified and confirmed KAS II and palmitoyl ACP thioesterase as the main target enzymes for manipulation to produce high oleic acid at the expense of palmitic acid. Other enzymes that could contribute

towards high oleic acid are stearoyl ACP desaturase, oleoyl CoA desaturase and oleoyl ACP thioesterase.

The biochemical studies identified the target genes to be isolated. Appropriate tissue specific expression of the introduced gene was also necessary. Thus, in addition to isolating above genes, isolation of mesocarp specific promoter to ensure that the target genes are expressed in the mesocarp during oil synthesis is also essential.

4 GENE ISOLATION

4.1 Isolation of desaturase genes

Plants have two main types of desaturases, a soluble plastid Δ9 stearoyl ACP desaturase (C18) and membrane-bound desaturases. Full-length clones of Δ9 stearoyl ACP desaturase have been isolated from a 15-week mesocarp cDNA library (Siti Nor Akmar *et al*., 1997; 1999). Analysis of the clones showed that there were at least two members of the Δ9 stearoyl ACP desaturase gene family in the oil palm. The coding sequences of the genes were almost identical. However, their 3' untranslated sequences were different. Northern analysis using gene specific probes that were constructed based on the 3' untranslated sequence showed that one clone, designated pOP-SN16 was highly expressed in different tissues and at different stages of development. It was expressed in tissues including mesocarp, kernel and leaf. It is thus highly likely that these genes have house-keeping functions, such as in membrane lipid synthesis. The other clone, pOP-SN19, was more specific in its expression. Its expression correlated with oil synthesis in oil palm mesocarp (Parveez *et al*., 1999).

E. oleifera mesocarp contains more unsaturated fatty acids than *E. guineensis*. It was of interest to compare the Δ9 stearoyl-ACP desaturase between the two species. Stearoyl-ACP desaturase cDNA clone *E. oleifera* was isolated using the Δ9 Stearoyl-ACP desaturase gene from *E guineensis* as a probe (Siti Nor Akmar, 1999). However, it was found that the nucleotide sequence of the isolated *E. oleifera* cDNA clone had remarkable homology with that of *E. guineensis* with identities exceeding 99%. This suggests that the sequence of Δ9 stearoyl-ACP desaturase is very well conserved throughout the evolution of these two species.

4.2 Isolation of Acyl carrier protein genes

The period of active oil synthesis in mesocarp begins around 15 weeks after anthesis (WAA), and terminates at about 21 WAA (Oo *et al*., 1985). Certain enzymes or proteins involved in fatty acid biosynthesis would be most active at the period of oil synthesis while the regulatory proteins, which are involved in switching on or increasing the level of expression of the genes coding for these enzymes, may be present at the start or just prior to the period of active oil synthesis. Acyl carrier protein is one such protein that is temporal specific for

oil synthesis. Besides acting as the temporal specific marker, the ACP transit peptide could also be used for targeting enzymes into chloroplasts or plastids.

Efforts were thus directed towards isolation of the oil palm ACP cDNA clones prior to isolation of genomic clones for the identification of the promoter sequences. Using a partial rice ACP gene (C1555, a gift from the Rice Genome Programme, Japan) as probe, several partial clones and one full length clone of ACP were isolated by screening a 15-week mesocarp cDNA library by plaque hybridization (Rasid *et al.*,1999). All the clones isolated were sequenced. The results pointed to the presence of at least two members of the ACP gene family in the oil palm. One of the partial clones designated pACP1 had a 3' untranslated non-coding region which was entirely different from that of the full length clone (pACP3) and the other partial clones. The coding region of pACP3 was identical to those of the partial clones except for pACP1 which showed only 93% homology (with pACP3). pACP3 sequence showed approximately 70% homology to barley and wheat ACP sequences. The full length clone was 701 base-pairs (bp). The open reading frame (ORF) comprised 144 amino acid residues of which 61 amino acid residues constitute the transit peptide and 83 amino acids make up the mature protein. Full length ACP cDNA clone from *E. oleifera* also has been isolated and analyzed (Rasid *et al*.,2001)

4.3 Isolation of Acyl-ACP Thioesterase genes

In the effort to isolate full-length sequence of acyl-ACP thioesterase, two sequence specific primers were designed (ACO1 and ACO2) based on a 130-base pair thioesterase sequence from the oil palm mesocarp. Using PCR, a 750bp fragment from 15-week mesocarp cDNA library and 800-bp fragment from 17-week mesocarp cDNA library were consistently obtained when using ACO1 and T7 primers (which would give 3'-end sequence). When using ACO2 and T3 primers (which would give 5'-end sequence), a 700bp fragment from 15-week cDNA library and 350bp fragment from 17-week cDNA library were obtained. ACO2 and T3 indicated that there were two clones in the library that extended as far as the ACO1/2 region, and that there were no clones present with inserts longer than 1500bp. The 750bp and 800bp fragments were used to probe 15-week mesocarp cDNA library. Three plaques hybridized to both 3' and 5' probes. These cDNA clones were purified by further rounds of screening and later transformed into *E. coli* DH5L. Digestion analysis showed inserts approximately 1450bp and derived from a single cDNA clone. Sequence analysis showed the cDNA (designated pHA-3) to be incomplete, extending from the coding region to the poly(A) tail, with the 5' end of the insert located within the transit peptide sequence. The 3' region is 360bp long. This sequence corresponding to the mature protein is 66% identical to *Cuphea hookeriana*, Ch FatB1, and 35% identical to *Garcinia mangostana*, Garm FatA1, indicating that this clone belongs to the FatB (16:0) (Kinney, 1998) type of acyl-ACP thioesterase. A full-length *Fat* B thioesterase gene has been isolated. The gene was over-expressed in *E. coli* and the recombinant protein was used to test for

acyl-CoA hydrolyzing activity. The cloned enzyme was able to hydrolyse medium-chain and long-chain acyl-CoAs (Abrizah *et al*., 2000).

4.4 Isolation of KAS II gene

Isolation of the KAS II gene was extensive using various strategies and currently a near full length KAS II clone has been isolated from oil palm mesocarp (Umi Salamah, Pers. Comm. in: Siti Nor Akmar *et al*., 2001). The information on KAS II in the database is very limited and varies from one species to another. Multi directional approaches are currently being carried out in order to isolate and confirm the full-length sequence of the KAS II gene.

4.5 Oleoyl-CoA desaturase

The pathway of fatty acid biosynthesis in plants from malonyl-CoA up to oleic acid operates in the plastid. Desaturation of oleic acid requires CoA as a cofactor and takes place in the cytosol. This reaction is catalysed by the action of oleoyl CoA desaturase. The higher correlation between KAS II and linoleic acid (C18:2) compared to oleic acid (C18:1) in oil palm mesocarp (Sambanthamurthi *et al*., 1999) suggests that increasing the activity of KAS II may result in the increased conversion of oleic acid to linoleic acid. Genetic manipulation for increased oleic acid may thus require the down regulation of this gene by antisense approach. Efforts are in progress to isolate this gene from the oil palm (Siti Nor Akmar *et al*., 2001).

5 ISOLATION OF TISSUE SPECIFIC PROMOTERS

5.1 Mesocarp specific promoters

In order to genetically engineer the oil palm for producing high oleic acid, the target genes need to be specifically expressed in the mesocarp. Isolating mesocarp specific gene(s) and subsequently its promoter sequences were thus an important requirement of the genetic engineering programme. Multiple method for isolating mesocarp specific cDNA clones have and are being carried, namely differential screening (Siti Nor Akmar *et al*., 1995), substractive hybridization (Siti Nor Akmar *et al*., 1996), differential display (Nurniwalis and Siti Nor Akmar, 2000) and most recently using DNA microarray (Siti Nor Akmar, *et al*., 2001).

In efforts to isolate the mesocarp specific promoters, a cDNA library of oil palm mesocarp was constructed using size-fractionated mRNA from 15 week mesocarp (Siti Nor Akmar *et al*., 1995). About 200,000 cDNA clones were obtained and 60% of them were recombinant based on β-galactosidase assay. Insert sizes of 0.4-2.5 kilo-basepairs (kbp) were obtained.

Mesocarp specific cDNA clones were screened using subtracted cDNA probes. Subtracted cDNA probes were prepared by hybridizing labelled first strand 15-week mesocarp cDNA to an excess of 5-week mesocarp mRNA. The

probes were used to obtain highly abundant and developmentally specific cDNA clones. Eight putative developmental specific clones were identified (Siti Nor Akmar *et al*., 1996). A mesocarp-specific gene was isolated and the gene was found to be highly abundant in mesocarp tissue at 15 weeks after anthesis. No expression was detected in other tissues (Siti Nor Akmar, 1999).

The Universal Genome Walker Kit was used to isolate the promoter for the above gene. The promoter was attached to a green fluorescence protein (GFP) gene and showed expression only in mesocarp and not in leaf (Siti Nor Akmar *et al.*, 2001; Zubaidah and Siti Nor Akmar, 2000). This data further confirm the mesocarp specific properties of the isolated promoter.

5.2 Kernel-specific promoter

Oil palm kernel is rich in medium chain fatty acids (mainly C12). Besides modifying the fatty acid in the mesocarp, kernel fatty acid may also be targeted for producing some useful novel fatty acids. A cDNA clone encoding the glutelin gene has been isolated through sequencing random cDNAs (EST) from oil palm cDNA libraries and transcript profiling. The full-length cDNA sequence of this gene of about 1.6 kb was obtained by 5'-RACE using total RNA from the kernel. The coding sequence shares 94% with one of the two glutelin gene sequences (accession nos. AF193433 and AF261691) submitted by Cha and Shah to the public data base. Southern analysis revealed that there are several copies of the glutelin gene in the oil palm genome (Siti Nor Akmar and Cheah, 2000). Northern analysis showed that it is highly expressed in the kernel and endosperm but no expression was detected in the embryo. Since the endosperm is the site of oil synthesis, the promoter of this gene would be suitable for controlling expression of introduced genes for modification of kernel oil composition (Siti Nor Akmar and Cheah, 2000).

Genomic clones have been isolated using the genome walker approach and further used to isolate the promoter sequence. A 2.0 kb PCR product was amplified and is being cloned and sequenced (Siti Nor Akmar *et al*., 2001).

6 TRANSFORMATION STUDIES

6.1 General

A major pre-requisite for the success of a genetic engineering programme is the development of techniques for the stable introduction of manipulated genes into the oil palm. Genetic transformation process involves the uptake of naked DNA (e.g. a gene of interest) by competent cells, followed by integration into the chromosome, and subsequent expression to produce the gene product. The process starts with the penetration of DNA into a cell through the cell wall and plasma membrane. The DNA eventually penetrates into the nucleus. Among the various transformation methods available, *Agrobacterium*-mediated and microprojectile bombardment (biolistics) are the most reliable.

Biolistics, although the best transformation method for monocots, suffers from one main limitation that the efficiency of obtaining stable transformation has been very low. Estimates of the efficiency of stable transformation over transient expression have been around 0.1-2.0% (Gordon-Kamm *et al.*, 1990; Bower and Birch 1992). Success in the production of transgenic plants via biolistic transformation depends on the optimized physical and biological parameters affecting DNA delivery, availability of suitable promoters, appropriate selectable marker genes, an effective selection agent, and a reproducible regeneration protocol (Parveez, 1998).

6.2 Biolistic method

Penetration of DNA into cells requires the optimization of physical and biological parameters that affect the entry of DNA. In oil palm, the conditions for delivering DNA into embryogenic calli have been optimized. Independent experiments were carried out to study the effects of each parameter and its variables on transient expression. All the variables used in these experiments were found to be significantly different except for vacuum pressure, bombardment number and genotype (Parveez *et al*., 1997; 1998).

Five constructs carrying different promoters: Emu (based on Adh1), maize Ubiquitin1, rice Actin1, CaMV 35S and maize Adh1 were evaluated to identify the most suitable promoter for use in oil palm. Significant effects on transient GUS gene expression were demonstrated by each of the different promoters tested (Chowdhury *et al.*, 1997). The effectiveness of kanamycin, geneticin (G418), neomycin, hygromycin antibiotics and the herbicide Basta™ as selection agents to inhibit growth of oil palm embryogenic calli was evaluated. Of the five compounds tested, hygromycin and Basta™ were found to be most suitable as selection agents for oil palm as they could stop the growth of embryogenic calli at lower concentrations (Parveez *et al*., 1996).

Upon successful optimization of the above parameters, stable transformation experiments for producing transgenic oil palm were initiated using the identified optimum parameters. Bombarded embryogenic calli were exposed to 40 and 80 mg/l of Basta™ after 1 or 3 weeks. It was found that there were no significant differences in the number of resistant embryogenic calli produced per plate when selected at different concentrations and time after bombardment. The presence of transgenes in resistant embryogenic calli was confirmed by PCR and Southern analysis (Parveez and Christou, 1998). Transgenic embryogenic calli were later successfully regenerated into whole plants and their transgenic status verified by PCR, Southern hybridization and protein analysis by thin layer chromatography (Parveez, 1998; 2000). Recently the transgenic plants, which are more than three years old, have been subjected to leaf painting and shown to be resistant to herbicide Basta™. This is the first reported success in the production of transgenic oil palm free from chimerism. This success makes feasible any plan to manipulate oil palm by genetic engineering.

6.3 Pollen-mediated transformation

The employment of pollen as a gene transfer vehicle is attractive as it circumvents the need for tissue culture. In addition, the procedure is known to be effective for monocots as well. Successful delivery of DNA using this approach has been reported in maize (Ohta, 1986), rice (Luo and Wu, 1988) and rye (de la Pena *et al*., 1987).

Pollen of both *E. guineensis* and *E. oleifera* were incubated with gene constructs carrying the GUS reporter gene. Transient expression of the GUS gene was observed, albeit at a very low frequency. The major impediment to this technique was the presence and persistence of exogenous microbes on the pollen. This problem is likely to contribute towards the low frequency of transformation as the microbes are capable of degrading the incoming DNA (Cheah, Pers. Comm).

Sterilization of the pollen reduced contamination. However, it also resulted in a reduction of pollen germination. An alternative approach was to use pollen from unopened spikelets. Although these pollen grains showed a lower contamination level, germination level was also low. Delivery of DNA, either using microprojectile bombardment or electroporation has so far resulted in very low transformation efficiencies based on transient expression. Further optimisation of DNA delivery conditions and probably the use of a stronger promoter may be required for increasing transformation efficiency. High efficiency of transformation is required not only because the stable transformation rate is expected to be low but also to compensate for the lower germination rate (Parveez and Rasid; 1998).

6.4 Development of an agrobacterium-mediated transformation method

Progress of this method on oil palm is still at an early stage. Immature embryos aged 11-12 w.a.a. were transformed with pCAMBIA 1301 (*gusA* and *hpt* genes driven by cauliflower mosaic virus 35S promoter) (Roberts *et al*., 1997) using Sonicated Assisted *Agrobacterium*-mediated Transformation (SAAT) method (Santarem *et al*., 1998). Cell wall disruption caused by ultrasonic energy may aid in the production of certain phenolic signals in the plant cells, which would enhance the accessibility of putative cell-wall binding factor to the bacterium. To date this technique enhances the transient transformation efficiency 30 to 45 % more than the non-sonicated technique, however the actual efficiency is still low and inconsistent (Parveez *et al*., 2000).

6.5 Utilization of GFP as selectable marker gene

Production of transgenic plants based on green fluorescent protein (GFP) gene as a selectable marker offers several advantageous (Leffel *et al*., 1997). The system requires no antibiotic or herbicide selection. The transformed cells can be visualised under UV or blue light without addition of any cofactors or substrate. It is also non-destructive. Bombardment of six plasmid constructs, carrying different version of GFP gene and driven by different promoters, into

oil palm embryogenic calli was carried our successfully. Transient expression of GFP in oil palm embryogenic calli has been observed (Na'imatulapidah and Parveez, 2000). Some indication of stable transformation of GFP gene in oil palm embryogenic calli was obtained for two out of the six plasmids tested. Proliferation and regeneration of transgenic oil palm expressing GFP is in progress. Possible toxic effect of the GFP protein on oil palm embryogenic calli was recently observed. Experiments to confirm the above effect is on going using Basta™ selection and organelle targeted approach (Na'imatulapidah and Parveez, 2001).

7 GENETIC ENGINEERING OF OIL PALM

7.1 Production of high oleate transgenic oil palm

Two approaches were considered for channeling palmitic acid to produce more oleic acid: i) stimulation of KAS II activity by overexpressing the KAS II gene and ii) reducing thioesterase activity towards palmitoyl ACP by introducing an antisense copy of the palmitoyl-ACP thioesterase gene (Parveez *et al*., 1999).

Based on the proposed approach to produce high oleate transgenic oil palm, four transformation vectors have been constructed driven by constitutive promoters The four constructs were pUbiSADN (full-length Δ9 stearoyl-ACP desaturase gene driven by maize ubiquitin 1 promoter), p35SSADN (full-length Δ9 stearoyl-ACP desaturase gene driven by cauliflower mosaic virus 35S promoter) and pCB302-AT1 and pCB302-AT2 (full-length antisense palmitoyl-ACP thioesterase gene driven by cauliflower mosaic virus 35S promoter with different enhancers) (Figure 2). Transformation of the full-length antisense palmitoyl-ACP thioesterase and Δ9 stearoyl-ACP desaturase gene constructs is in progress. Embryogenic calli and suspension cultures of oil palm have been transformed with the constructs via co-bombardment. Selection of transgenic oil palm embryogenic calli resistance to herbicide Basta™ has been initiated. Fatty acid composition of the selected embryogenic calli and later regenerated transgenic plants will be carried out once enough embryogenic calli and plants are obtained. Evaluation of the constructs in *Arabidopsis* has also been initiated.

PUbiSADN	**UBI**	**BAR**	**NOS**	**UBI**	**FL-DES**	**NOS**
p35SSADN	**UBI**	**BAR**	**NOS**	**35S**	**FL-DES**	**NOS**
pCB302-AT1	**PNOS**	**BAR**	**NOS**	**352E**	**FL-AsTE**	**NOS**
pCB302-AT2	**PNOS**	**BAR**	**NOS**	**35Ω**	**FL-AsTE**	**NOS**

Figure 2. Schematic diagram of the plasmids used for transforming oil palm.

*Note: **352E:** CaMV35S promoter with double enhancer; **35Ω:** CaMV35S promoter with a translational enhancer; **Ubi:** maize ubiquitin promoter; **As:** anti-sense; **Bar:** basta resistance gene; **TE:** palmitoyl ACP thioesterase; **DES:** Δ9 stearoyl ACP desaturase; **NOS:** napoline synthase poly A terminator; **PNOS:** napoline synthase promoter: **FL:** full-length.*

The ultimate goal of this project is to increase the oleic acid content in the mesocarp of oil palm, where the oil is being synthesized. Construction of the palmitoyl-ACP thioesterase, â ketoacyl-ACP synthase II (when the full-length gene is made available) and Δ9 stearoyl-ACP desaturase gene transformation vectors driven by the mesocarp-specific promoter (Siti Nor Akmar et. al., 2001) is in progress. Once the constructs are made available, transformation of the constructs into oil palm embryogenic calli will be carried out until transgenic plants are produced. Fatty acid analysis can only be carried out once the plants produce fruits as the expression is targeted in the mesocarp.

7.2 Production of high stearate transgenic oil palm

Oil palm contains an active Δ9 stearoyl-ACP desaturase which effectively desaturates steoryl-ACP into oleoyl-ACP which is cleaved into oleic acid (Figure 1). Interrupting the activity of Δ9 stearoyl-ACP desaturase should reduce the conversion of stearate to oleate. Therefore, by introducing an antisense copy of the Δ9 stearoyl-ACP desaturase gene, stearic acid would accumulate in the oil palm. Knutzon and colleagues (1992a) altered rapeseed oil content through the antisense expression of a stearoyl-ACP desaturase gene to produce a high-stearate oil suitable for use as a natural margarine. The expression of stearoyl-ACP desaturase gene driven by a seed-specific promoter has led to an increase in the stearate content from 1.8% by weight (normal) to 39.8% in the seed of the transgenic plants with a concomitant reduction of oleate. The high stearate producing plants were morphologically normal and yielded normally (Knutzon *et al.*, 1992b). Manipulating oil palm for high stearate oil content will give rise to new applications. High stearate oil is useful as a cocoa butter substitute. In addition, stearic acid can also be used in personal care products such as lotions, shaving cream and rubbing oils (Parveez *et al*., 1999).

Four transformation vectors have been constructed (driven by constitutive promoter) for the production of high stearate transgenic oil palm. The four constructs are pPAsUbiSADN (partial-length antisense Δ9 stearoyl-ACP desaturase gene driven by maize ubiquitin 1 promoter), pPAs35SSADN (partial-length antisense Δ9 stearoyl-ACP desaturase gene driven by cauliflower mosaic virus 35S promoter) pAsUbiSADN (full-length antisense Δ9 stearoyl-ACP desaturase gene driven by maize ubiquitin 1 promoter) and pAs35SSADN (full-length antisense Δ9 stearoyl-ACP desaturase gene driven by cauliflower mosaic virus 35S promoter) (Haliza *et al*., 2001) (Figure 3). Transformation of the two partial-length antisense Δ9 stearoyl-ACP desaturase gene constructs is in progress. Embryogenic calli and suspension cultures of oil palm have been transformed with the constructs and some Basta™ resistance embryogenic calli has been produced. Efforts to regenerate the resistant embryogenic calli have been initiated. Transformation of the two full-length antisense Δ9 stearoyl-ACP desaturase gene constructs has just been initiated. Fatty acid composition of the

selected embryogenic calli will be carried out once enough embryogenic calli are obtained. Evaluation of the constructs in *Arabidopsis* has also been initiated.

pPAsUbiSADN	**UB1**	**BAR**	**NOS**	**UBI**	**P-AsDES**	**NOS**
pPAs35SSADN	**UBI**	**BAR**	**NOS**	**S5S**	**P-AsDES**	**NOS**
pAsUbiSADN	**UBI**	**BAR**	**NOS**	**UBI**	**FL-AsDES**	**NOS**
pAs35SSADN	**UBI**	**BAR**	**NOS**	**35S**	**FL-AsDES**	**NOS**

Figure 3. Schematic diagram of the plasmids used for transforming oil palm for high stearic acid.

*Note: **Ubi:** maize ubiquitin promoter; **As:** anti-sense; **Bar:** basta resistance gene, **DES:** Δ9 stearoyl ACP desaturase; **NOS:** napoline synthase poly A terminator; : **P:** partial length ; **FL:** full-length.*

The final objective of the project is to increase the stearate content in the mesocarp. Construction of the full-length antisense Δ9 stearoyl-ACP desaturase gene transformation vector driven by the mesocarp-specific promoter (Siti Nor Akmar *et al*., 2001) is in progress. Once the constructs are made available, transformation of the constructs into oil palm embryogenic calli will be carried out until transgenic plants are produced. Fatty acid analysis can only be carried out once the plants produce fruits as the expression is targeted in the mesocarp.

7.3 Production of high palmitoleic acid transgenic oil palm

Palmitoleic acid is produced by desaturation of palmitic acid. It is envisaged that Δ9-stearoyl-ACP desaturase which acts mainly on stearic acid could also use palmitic acid as substrate to produce palmitoleic acid (Figure 1). Therefore, increasing stearoyl-ACP desaturase activity may result in the accumulation of palmitoleic acid. Palmitoleic acid is an important fatty acid for pharmaceutical applications. It has been shown to have anti-thrombotic effects, which can help in preventing stroke (Parveez *et al*., 1999). A previous study on oil palm protoplasts showed that *E. guineensis* protoplasts can synthesise up to 30% of palmitoleic acid in the total lipids (Sambanthamurthi *et al.*, 1996b). This result illustrates the palm's inherent capability to produce high levels of the acid.

Construction of two transformation vectors driven by constitutive promoter has been carried out successfully for the potential production of palmitoleic acid in oil palm. The two constructs are pUbiSADN (full-length Δ9 stearoyl-ACP desaturase gene driven by maize ubiquitin 1 promoter) and p35SSADN (full-length Δ9 stearoyl-ACP desaturase gene driven by cauliflower mosaic virus 35S promoter) (Figure 2). Transformation of both constructs is in progress. Embryogenic calli and suspension cultures of oil palm have been transformed and some Basta^{TM} resistance embryogenic calli has been produced. Efforts to regenerate the resistant embryogenic calli have been initiated. Fatty acid

composition of the selected embryogenic calli will be carried out once enough embryogenic calli are obtained.

7.4 Synthesis of ricinoleic acid

Oleic acid is converted to ricinoleic acid by the enzymatic action of oleate 12-hydroxylase (Figure 1). Introduction of the oleate 12-hydroxylase gene into oil palm could lead to the synthesis of ricinoleic acid. Ricinoleic acid is an important industrial fatty acid. It has medium to high value industrial uses in products ranging from cosmetics and pharmaceuticals to polymers and high grade lubricants (Parveez *et al*., 1999).

Construction of the full-length oleate 12-hydroxylase gene transformation vector driven by the constitutive and mesocarp-specific promoters (Siti Nor Akmar *et al*., 2001) is in progress. Once the constructs are made available, transformation of the constructs into oil palm embryogenic calli will be carried out until transgenic plants are produced. Fatty acid analysis will be carried once enough calli are obtained or once the plants produce fruits for the mesocarp-targeted construct.

7.5 Production of biodegradable thermoplastics

In bacteria, PHB (polyhydroxybutyrate) is derived from acetyl-coenzyme A by a sequence of three enzymatic reactions. The first enzyme of the pathway, *3-ketothiolase* catalyses the reversible condensation of two acetyl-CoA moieties to form acetoacetyl-CoA. *Acetoacetyl-CoA reductase* subsequently reduces acetoacetyl-CoA to D-(-)-3-hydroxybutyryl-CoA, which is then polymerized by the action of *PHB synthase* to form PHB (Anderson and Dawes, 1990). Following demonstrations of the production of both PHA and PHB in plants, such as *Arabidopsis thaliana* (Poirier *et al.*, 1992 and Nawrath, *et al.*, 1994) and rapeseed (Mitsky *et al.*, 1997), it now appears possible to produce biodegradable plastics on a large scale in plants. Introduction of these genes into oil palm may lead to the accumulation of PHB in oil palm tissues. Both PHB and related polyhydroxylkanoates (PHA) are renewable sources of biodegradable thermoplastic materials. Due to its properties, it is environmental-friendly as compared to synthetic plastics (Parveez *et al*., 1999).

The metabolic engineering programme to produce biodegradable plastics PHB and PHBV is being carried out in collaboration with the Massachusetts Institute of Technology (MIT), USA. Cloning of PHB genes for transformation has been designed such that all the three genes involved in PHB synthesis will be driven by a different promoter. A second construct substituting *phbA* gene with *bktB* gene as proposed by MIT collaborator for accumulating PHBV instead of just PHB alone has also been designed (Masani *et al*., 2000). The plastid targeting sequence from oil palm ACP gene (Rasid *et al*., 1999) is being used in making the chimeric gene constructs because studies in *Arabidopsis* suggested that higher yield can be obtained when PHB production is confined to the plastids compared to production in the cytosol (Nawrath *et al*., 1994). Both

constructs have been successfully made in a binary vector, flanked by matrix attachment regions of tobacco, using maize ubiquitin, cauliflower mosaic virus 35S and rice actin promoters (Masani *et al*., 2001). Transformation of both constructs into oil palm embryogenic calli and suspension cultures has just been initiated. Another two constructs from MIT collaborator, carrying all the genes driven by maize ubiquitin promoter has been transformed into oil palm embryogenic calli and suspension culture. Selection of Basta™ resistance embryogenic calli has just been initiated.

The oil palm mesocarp is expected to have a high flux through acetyl CoA, the substrate for PHB, as it is also the substrate for fatty acid biosynthesis. Therefore construction of the PHB vector using the mesocarp specific promoter will be carried out to direct the production of PHB into the mesocarp. However, it is expected that the fatty acid synthesis will be reduced due to substrate competition.

7.6 Transgenic oil palm synthesizing carotenoids

Oil palm is one of the richest sources of α- and β-carotene. However, very little is known about carotenoid biosynthesis in the oil palm. Knowledge of this pathway will allow for diversion of the pathway to other valuable metabolites as well as for further increase in α- and β-carotene. Separation of carotenoids extracted from oil palm mesocarp from 16-20 w.a.a. showed that the isomers of α- and β-carotene were the major carotenoids of oil palm. An interesting observation was that oil palm mesocarp has very little or no lycopene (Kaur and Sambanthamurthi, 2000). Lycopene is a more valuable nutraceutical than α- or β-carotene. Since both α and β-carotene are produced from lycopene by the action of α- and β-lycopene cyclase, down regulation of these cyclase genes via antisense approach would result in accumulation of lycopene. Efforts are in progress to isolate the cyclase genes. Study of their expression at gene and protein levels will improve our understanding on the possible regulatory roles of the genes.

Lycopene has been shown to reduce the prostate cancer risk (Gann *et al*., 1999). The consumption of carotenoids in human diet provides a source of pro-vitamin A (Bartley and Scolnik, 1995). In addition, there are also other significant health benefits that are attributed to carotenoids. This is associated with their activity as antioxidants and their anticancer properties (Canfield, 1995; Mayne, 1996). Carotenoids also have many industrial applications as food and feed additives, in cosmetics and pharmaceuticals.

7.7 Development of an *Arabidopsis thaliana* transformation system

In this study the development of *Arabidopsis thaliana* transformation system was carried out for use as a model to evaluate oil palm transformation vectors which then will be used for transforming oil palm. The vectors will be used to modify oil palm via genetic engineering for various targets or products.

Production of transgenic *Arabidopsis thaliana*, ecotype "Columbia" has been developed successfully. The *Arabidopsis thaliana* were transformed via "floral-dip" transformation method. *Arabidopsis thaliana* seeds were transformed with *gusA* reporter gene and *bar* (Basta™ resistant) selectable marker gene.Transformed seeds were selected by growing on wet compost containing Basta™ and spraying with Basta™. Basta™ resistant plants were subjected to GUS assay or PCR analysis to prove the transgenes integration and expression (Parveez, 2001).

8 CONCLUSION

Genetic engineering has proven to increase income and production in many plant species. Improving oil palm genetically is important for the industry to remain competitive in the future. The success in producing Basta™ resistant transgenic oil palm makes other efforts to genetically engineer oil palm possible. Biochemical studies and the isolation of key genes and mesocarp specific promoter are other milestones towards genetically modifying oil palm for improved oil quality and novel fatty acids biosynthesis. The main target for oil palm genetic engineering is increasing oleic acid at the expense of palmitic acid. Other targets of interest include stearic acid, palmitoleic acid, ricinoleic acid, lycopene and thermoplastics. With the establishment of a transformation system and isolation of important genes, efforts are now focused towards constructing transformation vectors with the required combination of genes (both in sense and antisense orientation) and transforming it into oil tissues for transgenic plant production. Most of the targeted products will be directed into the mesocarp, the oil rich tissue, and therefore the isolation and use of mesocarp specific promoter is important. It is targeted that the first filed trial of transgenic oil palm with high oleic acid will be carried out in 2007.

ACKNOWLEDGEMENT

The authors thank the Director General of MPOB for permission to present this paper. We also like to thanks Dr. Ariffin Darus, Deputy Director General of MPOB for critically reviewing this paper. We would like to acknowledge the contributions of Dr. Cheah Suan Choo, Mr. Zamzuri Ishak, Dr. Ahmad Tarmizi Hashim, Dr. Umi Salamah Ramli, Mrs. Abrizah Othman, Dr. Mohd Arif Abd. Manaf, Mr. Omar Rasid, Ms. Haliza Haris Fadillah, Mr. Abd. Masani Mat Yunos, Mrs. Na'imatulapidah Abdul Majid, Mrs. Zubaidah Ramli, Ms. Nurniwalis Abd. Wahab, Ms. Jane Sonia Kaur, Mr. Andy Yip and Ms. Chan Pek Lan and all the assistants in Transformation, Gene Expression and Metabolics Laboratories at MPOB to the MPOB Genetic Engineering Programme.

REFERENCES*

1. Abrizah, O (1995). Penulenan Separa dan Pencirian Enzim Asil ACP Tioesterase daripada Mesokarpa Buah Sawit (*E. guineensis*). *MSc thesis* submitted to Universiti Kebangsaan Malaysia.
2. Abrizah, O; Lazarus, C, Fraser, T and Stobart, A K (2000). Cloning of palmitoyl-acyl carrier protein thioesterase from oil palm. *Biochemical Society Transactions 28 (6):*619-622.
3. Anderson, AJ and Dawes, EA (1990). Occurrence, metabolism, metabolic role and industrial uses of bacterial polyhydroxylalkanoates. *Microbiological Reviews 54*: 450-472.
4. Bower, R and Birch, RG (1992). Transgenic sugarcane plants via microprojectile bombardment. *Plant Journal 2* :409-416
5. Bartley, GE and Scolnik, PA (1995). Plant carotenoids: Pigments for photoprotection, visual attraction and human health. *Plant Cell* 7: pp. 1027-1038.
6. Canfield, LM (1995). β–carotenoid metabolites: Potential importance to human health. *Malaysian Oil Science Tech*. 4: pp. 43-46.
7. Carroll, KK and Parenteau, HI (1992). A suggested mechanism for effects of diet on mammary cancer. *Nutrition Res*. 12:S159-S161.
8. Cheah, SC; Sambanthamurthi, R; Siti Nor Akmar, A; Abrizah, O; Manaf, MAA, Umi Salamah, R and Parveez, GKA (1995). Towards genetic engineering of oil palm (*Elaeis guineensis* Jacq.). In J.C. Kader and P. Mazliak (eds.) *Plant Lipid Metabolism.* Netherlands: Kluwer Academic Publishers, pp. 570-572.
9. Chen, XS and Fan, WX (1992). Studies of utilization and health effect of palm oil in China. *Nutrition Res*. 12:S23-S29
10. Chowdhury, MKU, Parveez, GKA and Saleh, NM (1997). Evaluation of five promoters for use in transformation of oil palm (*Elaeis guineensis* Jacq). *Plant Cell Reports 16:*277-281.
11. Davies, HM; Anderson, L, Fan, C and Hawkins, J (1991). Developmental induction, purification, and further characterization of 12:0-ACP thioesterase from immature cotyledons of *Umbellularia californica*. *Archives of Biochemistry and Biophysics 290* : 37-45.
12. De La Pena, AH, Lorz, H and Schell, J (1987). Transgenic rye plants obtained by injecting DNA into young floral tillers. *Nature 325:* 274-276.
13. Gann, PH; Giovannucci, E; Willett, W; Sacks FM, Hennekens, CH and Stampfer MJ (1999). Lower prostate cancer risk in men with elevated plasma lycopene levels: results of a prospective analysis. *Cancer Res*. 59:1225-1230.
14. Gasser, CS and Fraley RT (1992). Transgenic crops. *Scientific American*. June :62-69.

* As listed by authors.

15. Gordon-Kamm, WJ; Spencer, TM; Mangano, ML; Adams, TR; Daines, RJ; Start, WG; Obrien, JV; Chambers, SA; Adams, WR; Willets, NG; Rice, TB; Mackey, CJ; Krueger, RW, Kausch, AP and Lemaux, PG (1990). Transformation of maize cells and regeneration of fertile transgenic plants. *Plant Cell 2:*603-618.
16. Haliza HF; Masri MM; Ho CL, Masani AMY and Parveez GKA (2001). Construction of antisense desaturase gene transformation vectors for the production of high stearate transgenic oil palm. *Proc. 2001 MPOB International Oil Palm* Congress, 20-23 August, Kuala Lumpur, pp. 723-734.
17. Heber, D; Ashley, JM; Solares, ME, Wang, HJ and Alfin-Slater, RB (1992). The effect of a palm oil enriched diet on plasma lipids and lipoproteins in healthy young men. *Nutrition Res.* 12:S53-S59.
18. Kaur, JS and Sambanthamurthi R (2000). Preliminary studies on carotene profiles in the developing oil palm mesocarp. *First Malaysia MIT Biotechnology Partnership Programme (MMBPP) Symposium.* Kuala Lumpur, 16-18 November 2000.
19. Kinney, AJ (1998). Manipulating flux through plant metabolic pathways. *Current Opinion in Plant Biology 1*: 173-178.
20. Knutzon, DS; Thompson, GA; Radke, SE; Johnson, WB, Knauf, VC and Kridl, J C (1992a). Modification of *Brassica* seed oil by antisense expression of a stearoyl-acyl carrier protein desaturase gene. *Proc. Natl. Acad. Sci. USA. 89*: 2624-2628.
21. Knutzon, DS; Thompson, GA; Radke, SE; Knauf, VC, Svee, ER and Kridl, JC (1992b). Engineering stearate levels in canola oil through the use of antisense stearoyl-ACP desaturase gene constructs. In *Proceeding. of the 1992 Miami Bio/Technology. Winter Symp. Miami*. pg. 102.
22. Kritchevsky, D. (1992). Palm oil in lipid metabolism. *Nutrition Res*. 12:S31-S41.
23. Kritchevsky, D, Weber, MM and Klurfeld, DM (1992). Influence of different fats (soybean oil, palm olein or hydrogenated soybean oil) on chemically-induced mammary tumors in rat. *Nutrition Res*. 12:S175-S179.
24. Leffel, SM, Mabon, SA and Stewart CN (1997). Applications of green fluorescent protein in plants. *Biotechnol 23:* 912-918.
25. Luo, ZX and Wu, R (1988). A simple method for the transformation of rice via the pollen-tube pathway. *Plant Molecular Biology Reporter 6 (3):* 165-174.
26. Manorama, R and Rukmini, C. (1992). Serum lipids of rats fed crude and refined palm oil in high cholesterol and cholesterol free diets. *Nutrition Res*. 12:S93-S103.
27. Masani, AMY; Na'imatulapidah, AM; Ho, CL; Omar, RA, Harikrishna, K and Parveez, G K A (2000) Transfer or polyhydroxybutyrate (PHB) genes into oil palm for the production of biodegradable plastics. *First Malaysia-MIT Biotechnology Partnership Programme (MMBPP) Symposium,* Kuala Lumpur, 16-18 November.
28. Masani, AMY, Ho, CL and Parveez, GKA (2001). Construction of PHB gene expression vectors for the production of biodegradable plastics in

transgenic oil palm. *Proc. 2001 MPOB International Oil Palm* Congress, 20-23 August, Kuala Lumpur, pp. 694-711.

29. Mayne, ST (1996). Beta-carotene, carotenoids and desease prevention in human. *FASEB J.* 10: pp. 690-701.
30. Mitsky, TA; Taylor, NB; Gruys, KG; Hao, M; La Vallee, BJ; Colburn, SM; Slater, SC; Rodriquez, DJ; Tran, M; Thorn, GM; Wu, Z; Shah, DT; Valentin, HE; Houmiel, KL; Gunter, CA; Mahadeo, DA; Reiser, SE, Kishore, GM and Padgette, SR (1997). Production of biodegradable plastics in plants. *Abstracts of the 5th International Congress of Plant Molecular Biology*, Singapore, 21-27 September 1997. No. 302.
31. Na'imatulapidah, AM and Parveez, GKA (2000). Preliminary study of GFP expression in oil palm. *Proceeding of 12th National Biotechnology Seminar*, Lumut, Perak, 12-15 November 2000, pp. 367-369.
32. Na'imatulapidah AM and Parveez GKA (2001) Towards the production of transgenic oil palm expressing *gfp* gene. *Proc. 2001 MPOB International Oil Palm* Congress, 20-23 August, Kuala Lumpur, pp. 686-693.
33. Nawrath, C, Poirier, Y and Somerville, C (1994). Targeting of the polyhydrxybutyrate biosynthetic pathway to the plastids of *Arabidopsis thaliana* results in high levels of polymer accumulations. *Proc. Natl. Acad. Sci. USA 91*: 12760-12764.
34. Nesaretnam, K, Khor, HT, Sundram, K, Chong, YH, Ganeson, J, Gapor, A. (1992). The effect of vitamin E tocotrienols from palm oil on chemically induced mammary carcinogenesis in female rats. *Nutrition Res.* 12:879-892.
35. Nurniwalis, AW and Siti Nor Akmar, A (2000). Identification of differentially expressed gene(s) in the oil palm leaf and mesocarp tissues using differential display. *Proceeding of 12th National Biotechnology Seminar*, Lumut, Perak. , 12-15 November 2000, pp. 487 – 489.
36. Oil World Annual (2001). ISTA Meilke GmbH, Germany.
37. Ohta, Y (1986). High efficiency genetic transformation of maize by a mixture of pollen and exogenous DNA. *Proc. Natl. Acad. Sci. USA. 83*: 715-719.
38. Oo, KC; Teh, SK, Khor, HT and Augustine, S H (1985). Fatty acid synthesis in the oil palm (*Elaeis guineensis*): Incorporation of acetate by tissue slices of the developing fruit. *Lipids 20*: 205 B 210
39. Pantzaris, TP (1993). Trends in yellow fats consumption in EEC. *Palm Oil Dev*. 18:3-7.
40. Parveez, GKA, Chowdhury, MKU and Saleh, NM (1996). Determination of minimal inhibitory concentration of selection agents for oil palm (*Elaeis guineensis* Jacq.) transformation. *Asia-Pacific Journal on Molecular Biology and Biotechnology 4*: 219-228.
41. Parveez, GKA, Chowdhury, MKU and Saleh, NM (1997). Physical parameters affecting transient GUS gene expression in oil palm (*Elaeis guineensis* Jacq.) using the biolistic device. *Industrial Crops and Product 6*: 41-50.

42. Parveez, GKA (1998). Optimization of parameters involved in the transformation of oil palm using the biolistic method. *Ph.D thesis*, Universiti Putra Malaysia.
43. Parveez, GKA and Christou, P (1998). Biolistic-mediated DNA delivery and isolation of transgenic oil palm (*Elaeis guineensis* Jacq) embryogenic callus cultures. *Journal of Oil Palm Research 10:* 29-38.
44. Parveez, GKA and Rasid, O (1998). Production of transgenic oil palm (Elaeis guineensis Jacq) - Progress and Future consideration. Presented at 8th FAOBMB Congress, 22-26 November, Kuala Lumpur.
45. Parveez, GKA, Chowdhury, MKU and Saleh, NM (1998). Biological parameters affecting transient GUS gene expression in oil palm (*Elaeis guineensis* Jacq.) embryogenic calli via microprojectile bombardment. *Industrial Crops and Product 8*: 17-27.
46. Parveez, GKA; Sambanthamurthi, R; Siti Nor Akmar, A; Rasid, O, Masri, MM and Cheah SC (1999). Production of transgenic oil palm – Current success and future considerations. *Proceedings of the 1999 PORIM International Palm Oil Congress (Agriculture).* Arifin D, Chan, K.W. and Sharifah, S.R.S.A (eds.), pp. 3-13.
47. Parveez, GKA (2000) Production of transgenic oil palm (*Elaeis guineensis* Jacq.) using biolistic techniques. In *Molecular Biology of Woody Plants* Volume 2 (Eds: Jain, S. M and Minocha, S.C.). Kluwer Academic Publishers, Netherlands, pp. 327-350.
48. Parveez, GKA; Rasid, O; Alizah, Z; Masri, MM; Majid, NA; Fadilah, HH; Yunus, AMM and Cheah, SC (2000). Transgenic Oil Palm: Production and projection. *Biochemical Society Transactions 28 (6):* 969-972.
49. Parveez GKA (2001). Development of *Arabidopsis thaliana* transformation: A model system for evaluating oil palm transformation vectors. *Proc. 2001 MPOB International Oil Palm* Congress, 20-23 August, Kuala Lumpur, pp. 712-722.
50. Poirier, Y; Dennis, DE, Klomparens, K and Somerville, C (1992). Polyhydroxy-butyrate, a biodegradable thermoplastic, production in transgenic plants. *Science 256*: 520-523.
51. Pollard, MR; Anderson, J; Fan, C, Hawkins, J and Davies, MH (1991). A specific acyl-ACP thioesterase implicated in medium-chain fatty acid production in immature cotyledons of *Umbellularia californica. Archives of Biochemistry and Biophysics 284*: 306-312.
52. Pryde, EH (1983). Utilization of commercial oilseed crops. *Econ. Bot*. 37: 459-477.
53. Qureshi, AA; Qureshi, N; Wright, JJK; Shen, S; Kramer, G; Gapor, A; Chong, YH; Dewitt, G; Ong, ASH, Peterson, D and Bradlow, BA (1991). Lowering of serum cholesterol in hypercholesterolemic humans by tocotrienols (palmvitee). *Am. J. Clin. Nutr.* 53:1021S-1026S.
54. Rajanaidu, N and Jalani, BS (1995). World-wide performance of DXP planting material and future prospects. *In* Proceedings of 1995 PORIM National Oil Palm Conference. - Technologies in Plantation, The Way

Forward. 11-12 July 1995. Kuala Lumpur: Palm Oil Research Institute of Malaysia, pp.1-29.

55. Rasid, OA, Cheah SC and Mohd Arif A M (1999). Isolation and Sequencing of cDNA Clones Coding for Oil Palm (*Elaeis guineesis*) Acyl Carrier Protein (ACP), *Journal of Oil Palm Research (Special Issue)*: 88-95.
56. Rasid, OA, Mohd. Arif, AM and Cheah, SC (2001). Isolation of cDNA clones coding for oil palm (Elaeis oleifera) acyl carrier protein (ACP). Paper presented at *The 11th Scientific Meeting of MSMBB*. Hyatt Regency Saujana, Subang, Selangor, May 14-16, 2001.
57. Roberts, C; Rajagopal, S; Wei, Y; Nugroho, S; Smith, L; Tuan, N; Ravi, KS; Vijayachandra, K; Patell, V; Harcourt, R; Dransfield, L; Desamero, N; Slamet, I; Keese, P, Kilian, A and Jefferson, R (1997). A comprehensive new set of modular vectors to allow both routine and advanced manipulations and efficient transformation of rice by both *Agrobacterium* and direct gene transfer methods. *General Meeting of The International Programme on Rice Biotechnology*, Malacca, Malaysia, Rockefeller Foundation, USA, pg. 178.
58. Salmiah, A (1994). Palm based oleochemicals and their uses. In A. Ariffin *et al* (eds.) *Selected readings on palm oil and its uses*. Rev. ed. Kuala Lumpur: PORIM, pp. 160-182.
59. Santarem, ER; Trick, HN, Essig, JS and Finer, JJ (1998). Sonication-assisted Agrobacterium-mediated transformation of soybean immature cotyledons: optimization of transient expression. *Plant Cell Reports 17:* 752-759.
60. Sambanthamurthi, R; Abrizah, O and Umi Salamah, R (1996a). Towards understanding the biochemical factors that affect oil composition and quality in the oil palm. In PIPOC-ISOPB International Conference on Oil and Kernel Production in Oil Palm, Kuala Lumpur, 27-28 September 1996.
61. Sambanthamurthi, R; Parman, SH and Noor, MRM (1996b). Oil palm (*Elaeis guineensis*) protoplasts: isolation, culture and microcallus formation. *Plant Cell, Tissue and Organ Culture 46*: 35-41.
62. Sambanthamurthi, R and Oo KC (1990). Thioesterase activity in the oil palm (*Elaeis guineensis*) mesocarp. In *Plant Lipid Biochemistry, Structure and Utilization*. (Eds. P. J..Quinn and J.L. Harwood) Portland Press Ltd. London, pp. 166-168.
63. Sambantthamurthi, R, Abrizah, O and Umi Salamah R (1999). Biochemical factors that control ol composition in the oil palm *Journal of Oil Palm Research (Special Issue):* 24-33.
64. Sambanthamurthi, R; Parveez GKA and Cheah SC (2000). Genetic engineering of oil palm. *In advances in Oil Palm Research*. Yusof Basiron, Jalani B. S. and Chan K. W (Eds). Malaysian Palm Oil Board.
65. Scowcroft, WR (1990), New fats and oils through biotechnology. INFORM. 1:945-951
66. Siti Nor Akmar, A, Shah, FH and Cheah SC (1995). Construction of oil palm mesocarp cDNA library and the isolation of mesocarp-specific cDNA

clones. *Asia Pacific Journal of Molecular Biology and Biotechnology* 3, *No. 2:* 106-111.

67. Siti Nor Akmar, A, Murphy, DJ and Cheah, SC (1996). Isolation of mesocarp-specific cDNA clones from the oil palm by subtractive hybridization. *Proc. 1996 PORIM International Palm Oil Congress, Agriculture Conference*, pp. 552-556.
68. Siti Nor Akmar, A, Murphy, DJ and Cheah, SC (1997). Isolation and characterization of stearoyl- ACP desaturase genes for the oil palm, *Abstracts of the 5th International Congress of Plant Molecular Biology*, Singapore, 21-27 September 1997.
69. Siti Nor Akmar, A (1999). Structure and regulation of stearoyl-ACP desaturase and metallothionein-like genes in developing fruits of the oil palm (*Elaeis Guineensis). PhD Thesis*, University of East Anglia.
70. Siti Nor Akmar, A; Cheah, SC; Aminah, S; Leslie, CLO; Sambanthamurthi, R and Murphy, DJ (1999). Characterization and regulation of the oil palm (*Elaeis guineensis)* stearoyl-ACP desaturase genes. *Journal of Oil Palm Research (Special Issue):* 1-17.
71. Siti Nor Akmar, A and Cheah, SC (2000). Isolation and characterization of kernel-specific glutelin gene from the oil palm. *Sixth International Congress of Plant Molecular Biology*. Quebec, Canada, 18-24 June 2000.
72. Siti Nor Akmar, A; Sambanthamurthi, and Parveez GKA (2001). Progress in genetic engineering of oil palm. *Proc. 2001 MPOB International Oil Palm* Congress, 20-23 August, Kuala Lumpur.
73. Stumpf, PK (1984). Fatty acid biosynthesis in higher plants. In Numa S (ed) *Fatty acids metabolism and its regulation*. Elsevier Sci. Publ, Amsterdam, pp 155-179.
74. Sudin, N; Sahri, MM, Kun, TY and Oh, F (1993). Modification of palm kernel oil and their fractions for margarine. *Palm Oil Dev*. 18:1-3.
75. Sundram, K; Hornstra, G, Houwelingen, ACV and Kester, ADM (1992). Replacement of dietary fat with palm oil : effect on human serum lipids, lipoproteins and apolipoproteins. *British J. of Nutrition.* 68:677-692.
76. Umi Salamah, R (1995). Kajian aktiviti dan penulenan enzim β-ketoasil ACP sintase II di dalam mesokarpa kelapa sawit. *M.Sc. Thesis*, Universiti Kebangsaan Malaysia.
77. Zubaidah, R and Siti Nor Akmar, A (2000). Application of biolistic method for analysing mesocarp- specific promoter. *Proceedings of the 12th National Biotechnology Seminar.* Lumut, Perak, 12-15 November 2000, pp. 436 – 437.

Use of Biotechnology and Molecular Tools for Crop Improvement and Sustainable Agriculture

AHMAD S ISLAM*
University of Texas
Austin, TX 78713, USA
and
ZEBA I SERAJ**
University of Dhaka, Dhaka, Bangladesh

1 INTRODUCTION

Not long ago, plant breeders used visual traits, dominant or recessive, to identify individuals carrying a gene of interest in a population. In case of recessive and polygenic traits, it was necessary to raise a large population, which involved a lot of cost, required a large area to grow, and continuous effort to maintain and screen the population for the trait of interest. Screening in many cases was difficult, particularly when there were genotype X environment interactions. With the availability of DNA-based markers such as RFLP, RAPD, AFLP, micro- and mini satellites of more common genomes, it is now possible for breeders to pinpoint the precise location of the gene(s) of interest. This technique termed marker-aided selection (MAS), is in turn applied to rapidly screen a population for identifying genes conditioning the expression of important agronomic traits. These recently discovered molecular tools have accelerated the breeding program making it possible to reach the breeding objective within the stipulated time. Molecular knowledge of pathogen resistance genes has enabled MAS to be applied to pyramid different genes into the same cultivar, providing more durable resistance. DNA markers have been recently used to introgress improved traits resulting in better yield, quality and early maturity in cultivated rice from Oryza rufipogon, a wild relative of rice.

Use of transformation to improve plant resistance so that less pesticide and fungicides are used has been successful for some crops and is the target aimed at for others. Transgenics for improved quality products such as vitamin A and iron enriched rice, plants tolerant to abiotic stresses are desirable goals to be reached, particularly for developing countries where either arid or semiarid environment dominate or inundation of arable land by flood water is of frequent

* Fellow of the Islamic Academy of Sciences.
** Department of Biochemistry and Molecular Biology, University of Dhaka, Bangladesh.

occurrence. The recent report of cultivars growing in heavily contaminated aluminium soil by exudation of organic acids through their roots, is yet another example of ways to create cultivars for inhospitable soil.

The production of transgenics by targeting genes of interest to chloroplasts rather than to nuclear genomes should also be embarked upon with all the emphasis it needs. This is because it eliminates position effects, foreign genes are expressed in many copies, multiple genes can be introduced, and there is no danger of foreign gene transfer to pollen. However, this technique, which has been successfully applied in tobacco only, remains to be fine-tuned for other crops.

The important molecular tool, which has been essentially developed to counter the objections of anti-GM group comprises using genes other than those for antibiotic resistance. Some non-toxic marker genes of this category are: beta glucuronidase, xylose isomerase, phosphomannose isomerase, firefly luciferase or jellyfish green fluorescent-protein. The nature of the marker gene determines whether transformed cells would metabolise the specific sugar: xylose or mannose or would show colour, luminescence or fluorescence respectively.

Sustainable agriculture is only possible if the use of pesticides is drastically reduced. GM crops resistant to pests offer minimum use of pesticides; bioengineered salt-, drought and heavy metal- tolerant crops will open up marginal land for cultivation of crops. Identification of genes on a large scale, by use of tools such as functional genomics, EST libraries, DNA microarrays, proteomics and large scale generation of mutants will help identify many important genes which will contribute to enhanced abiotic stress resistance.

2 CROP IMPROVEMENT THROUGH PLANT BREEDING PRACTICES

Not long ago, plant breeders used a visual trait, dominant or recessive to identify individuals carrying a gene of interest in a population. For instance, the presence of petal spot in the F_2 and subsequent progeny of the cross, *Gossypium hirsutum* × *G barbadense* served as a guide to breeders whether the gene controlling the petal spot and those of traits of interest closely linked to it, were transmitted to the offspring. If genes imparting disease and pest resistance were tightly linked to a visible trait, screening would be easy. If not, it would be necessary to infect the soil with fungal spores, to test for a soil-borne disease or to inoculate the aerial parts, if those were the target of the pathogen. Seeds saved from resistant individuals were planted to raise the following generation; the process was continued for 3-4 generations till there was no more segregation in the population for the gene conferring resistance to the disease under investigation. In case of recessive and polygenic traits, it was imperative to raise a large population, assign a large area to grow the population, maintain and screen the population for the trait of interest; all of which added to the expense of breeding. Screening in many cases was extremely difficult, particularly when

the expression of the trait was dependent upon genotype-environment interactions.

Interested readers who want to know more about relationships between morphological traits and crop resistance to insects may refer to an article by Stein (1991). He describes a number of morphological characters linked to or implicated in conferring resistance to insect attacks. The traits he mentions in his article are: variations in colour, shape, hairiness, thickening of cell walls, surface wax, anatomical adaptations and presence of plant defensive compounds. For instance, cabbage worm is less attracted to red coloured *Brassica* species (cabbages, broccoli, and related species). Cucumber beetles do less damage on reddish coloured varieties of leaf lettuce. Regarding shape, they noted that, rounded turnips were less damaged by turnip maggots. Another study showed that onions with leaves having a narrow angle of contact are more attractive to thrips than those with looser leaves.

Visual markers are too few in order to be of universal use in breeding programs. In addition, there are a number of traits such as yield, aroma, salt-, submergence- and drought tolerance and sometimes disease resistance that are controlled by polygenes i.e., by a number of genes. Expression of these genes may have additive or subtractive effects. Therefore, these are also referred to as quantitative genes and do not follow Mendelian gene inheritance patterns. Genes that condition the expression of quantitative characters are called, "quantitative trait loci", in short QTL. Their expression is not easy to score. Breeders, therefore, find it extremely difficult to select individuals merely by visual observations. Moreover, to obtain lines with all the desired genes (contributing to the trait of interest) combined in non-segregating individuals is (a) labour intensive, (b) environment dependent, (c) time consuming because selection of individuals in advanced generations need to be carried out in a large population covering an extensive field area.

There is another category of genes such as "clock" genes that cannot be studied or analysed by inspection of visual characters. The expression of these characters is dependent upon diurnal fluctuation of light, water and temperature and as such, conventional methods including visual observations are inadequate to identify them. For instance, the stomatal aperture opening during the day and closure during the night or drooping of leaves of some leguminous species following sunset, are some of the phenomena controlled by clock genes. No conventional methods are known to monitor their functioning. Identification and cloning of such genes is extremely important for breeding crops in drought-prone areas. It is worth mentioning here that a gene controlling guard-cell functions in *Arabidopsis* has been recently reported by Wang XQ *et al.* (2001).

Thus, breeders were looking for a set of new tools to assist in the selection of desired progeny without having to visually recognize linked characters, which are limited in number.

3 MARKER ASSISTED SELECTION (MAS)

3.1 General

DNA markers that flank a gene of interest can be identified. Any piece of DNA can theoretically be used as a marker. Therefore, there are virtually hundreds of markers available, which can be linked to a trait of interest. This process came to be known as marker-aided selection (MAS). Seedlings of the selected progeny from a particular cross are screened for the trait of interest, e.g. resistance to a disease in the field. DNA markers are identified from the resistant progeny and these markers are obviously absent in susceptible plants. In this way, markers are selected to tag the genes of interest. If multiple genes are involved in resistance and separate markers are identified to each, these can be used in combining multiple genes into one genotype to give it a broad-based level of resistance. In some cases, if the gene is known, specific DNA within this gene can also be used as a marker for presence of the gene.

In other words, the presence of the DNA markers can indicate the presence of the gene of interest in the progeny and can be detected at the seedling stage without being influenced by environmental fluctuations. MAS is specially valuable in case of perennial timber plants and fruit trees that take many years to flower and fruit, and in evaluating disease and pest resistance ability in a population.

The technique also enables plant breeders to distinguish heterozygotes from homozygotes. Once the lab facility is developed to undertake MAS-related projects, the subsequent cost on running the experimental field and the ancillary staff to maintain it, is greatly reduced. MAS has the power to reduce the whole operational time to release a variety by 2-3 years.

DNA-based molecular markers thus provides a powerful tool to plant breeders to accelerate a breeding programme. DNA markers have been recently used to introgress improved traits resulting in better yield, quality and early maturity in cultivated rice from *Oryza rufipogon*, a wild relative of rice (Xiao *et al.* 1998).

3.2 Some important molecular markers

3.2.1 General

However, identifying the gene for a particular character from the huge amount of DNA within an organism is a voluminous task, and it becomes more complicated in case of polygene-controlled quantitative traits such as yield and resistance to biotic and abiotic stresses. For detailed information about various kinds of molecular markers, readers are referred to a number of recently published articles (Gupta *et al*. 1999, Gupta and Varshney 2000, Gupta *et al*. 2001a, Gupta *et al*. 2001b). Some important molecular markers belonging to three different generations are listed below:

3.2.2 Restriction fragment length polymorphism (RFLP)

A DNA molecule, when cut at specific sites by restriction enzymes, generates fragments of different sizes in their digest. When mutation occurs, the length of fragments changes because of the alteration of recognition sites. Fragments of different lengths derived from original and mutant types can be separated by means of electrophoresis on polyacrylamide. It reveals differences in the sites between genotypes of individuals in the crossing populations.

Although RFLP is reliable, and it is easy to detect differences between different genotypes, it is labour intensive and therefore a time-consuming and costly operation. The technique can be conveniently used to detect co-dominant genes in the population. Application of this technique revealed that the two dominant loci (markers) on chromosome 4 and 7 in rice are linked to blast resistance in F7 recombinant population of the cross: susceptible *indica* x durably resistant *japonica* variety (Wang GL *et al.* 1994).

3.2.3 Random amplified polymorphic DNA (RAPD)

Markers are generated by random polymorphic DNA sequences using 10-base-pair primers and the PCR (polymerase chain reaction) technique. Compared to RFLP, it is a faster process and does neither require Southern blots nor DNA hybridisation. It generates a high level of polymorphism, thereby displaying differences in the sites at which DNA is amplified by PCR. Using both RFLP and RAPD, the GM2 (gall midge) gene in rice, which confers resistance to biotype 1 of gall midge, was mapped on chromosome 4 (Mohan *et al*. 1994). One disadvantage of this technique is that RAPD markers are dominant and heterozygotes cannot be distinguished from homozygotes.

3.2.4 Amplified fragment length polymorphisms (AFLPs)

It is a DNA fingerprinting technique involving the specificity of restriction enzyme digestion and the quick amplification of special fragments by PCR. It is a variation of RAPD in that it does not require prior knowledge of sequence information but is much more specific. In AFLP, only a fraction of small fragments is analysed, following selective PCR amplification. It is a powerful, reliable and reproducible PCR-based technology that requires only a minimal amount of starting DNA. It has been used to map QTLs linked to submergence tolerance in rice (Nandi *et al.* 1997). Another major gene *Saltol* was tagged to chromosome 1 between two flanking AFLP markers (Gregorio 1997).

3.2.5 Expressed sequence tag (EST)

Base sequence data of expressed genomic regions are essential to develop EST. Markers are themselves directly associated with functional genes. EST studies help in assessing functional relatedness between various genotypes

3.2.6 Simple sequence repeats (SSRs) or microsatellites

SSRs are stretches of 1 to 6 nucleotide units repeated in tandem and randomly spread in eukaryotic genomes. SSRs are highly polymorphic due to elevated mutation rates affecting the number of repeat units. Such length-polymorphisms can be easily detected on high-resolution gels (e. g. sequencing gels), by running PCR amplified fragments. The latter are obtained using a unique pair of primers flanking the repeat differences in repetitive DNA sequences. One disadvantage of this method is that the flanking primers need to be identified first. It is convenient for genomes such as rice where already sufficient sequence information is available (McCouch *et al.* 1997).

3.2.7 Single nucleotide polymorphism

It is a kind of polymorphism represented by point mutations due to change of a single nucleotide. Point mutations occur frequently and as such there are a large number of potential SNP markers that can be used to map QTLs. Another variation of this marker is indel polymorphisms (IDPs). Both gel-based and non gel-based methods are being used to detect these abundant polymorphisms. However, adequate sequence information is necessary. In case of gel-based methods, specific primers are designed which would cause amplification of a positive allele due to exact match of primer. Mismatches in primer design and/or DNA sequence fail to give amplification of a negative allele. In case of non gel-based methods, appropriate regions are amplified and then mismatches detected by techniques such as denaturing HPLC (high performance liquid chromatography) or MALDI TOF MS (Matrix-assisted laser desorption/ionization time of flight mass spectrometry). Both these techniques are extremely sensitive and can detect mismatches from a few down to only one nucleotide.

4 HOW HAVE THE DIFFERENT INTERNATIONAL AGRICULTURE RESEARCH INSTITUTES UTILIZED THIS TECHNOLOGY TO ACCELERATE THEIR BREEDING PROGRAMS?

4.1 General

The various International Agriculture Research Institutes, particularly those funded by the Consultative Group for International Agriculture Research (CGIAR) have profitably utilized this technology for crop improvement. For instance, CIMMYT, IRRI, have demonstrated clearly how this technology helped them reach their objectives much faster and more effectively compared to the traditional techniques. Here are some examples:

- Pyramiding of genes against bacterial blight in rice (Yoshimura *et al.* 1995; Huang *et al.* 1997);

- Cyst nematode resistant soybean (Concibido *et al.* 1994; Danesh *et al.* 1995);
- Scald resistance variety in barley (Abott *et al.* 1995; Schweizer *et al.* 1995; Graner and Tekauz 1996); and
- Enhancement of durability of resistance by incorporating multiple resistance genes in a single variety of wheat (Li *et al.* 1999; Nelson *et al.* 1997 Seyfarth *et al.* 1998; Singh *et al.* 1998; Spielmeyer *et al.* 1998).

4.2 Achievements of International Rice Research Institute (IRRI)

Applying MAS technology, scientists in IRRI have mapped (www.cgiar.org/irri):

- A major gene for salt tolerance on chromosome 1, coding for a protein of 140 amino acids;
- A major gene for P-deficiency tolerance on chromosome 12 linked to RFLP markers (RG9 and RG241);
- A major gene for submergence tolerance on chromosome 1 and 9;
- A major QTL associated with Zn deficiency on chromosome 5 flanking microsatellite RM167 and RM87;
- QTLs associated with aluminium toxicity on chromosome 1 and 12; and
- Two loci associated with iron toxicity tolerance on chromosome 1, the first one flanked by RG146 and RG345 and the second by RG810 and RG331.

4.3 IRRI's efforts to develop efficient but less costly molecular markers

Taking into consideration the huge cost involved to set up a sophisticated modern molecular biology lab, IRRI has been concentrating to develop low cost MAS technology to achieve the same purpose towards screening populations against: salinity, submergence, iron toxicity, aluminium toxicity, phosphorus deficiency and elongation ability tolerance etc.

4.4 A multinational collaborative cocoa-breeding program using MAS

4.4.1 Background

Cocoa is a plantation crop that is grown extensively in the region of West Africa and parts of Latin and South America. Recently, cocoa production reached an all-time low on account of heavy loss of this crop by a multitude of diseases such as witches' broom and frosty pod. The huge loss of this crop has prompted the cocoa-growing countries to launch a multinational collaborative projects using MAS technology. Scientists that traditionally work with cacao at institutes in Brazil, Costa Rica, Trinidad, Ecuador, the United Kingdom and the USA joined hands to work in this project**.** Using molecular markers, scientists will first direct their attention to genetically fingerprint all accessions in the major cacao germplasm collections in the Americas.

The fingerprinting will be carried out in the laboratory of Dr James A Saunders in Beltsville, Maryland. The next phase of the work will be taken up

by researchers in Miami, USA; using EST and microarray technology, they will work to identify the genes involved in disease resistance reactions.

4.4.2 The role of Islamic Academy of Sciences

IAS may play a catalytic role in establishing such a multinational collaborative project on the use of MAS for development of superior and disease- and pest resistant varieties of crops that are unique to some of the Islamic countries. Some of these crops such as date palm are prone to excessive drought and some like jute are susceptible to flood.

In this connection, it may be mentioned that in January 2000, COMSTECH held an important meeting of a number of Islamic countries to discuss ways and means for applying biotechnology in boosting up agricultural produces. The consensus in the concluding session was to select a crop of major importance to the majority of the Islamic countries and use biotechnology for its improvement in terms of quality and yield instead of handling a number of crops with no substantial achievement. Date palm was the choice. A project on the improvement of date palm through tissue culture by the use of molecular markers was prepared by a team of Muslim scientists headed by a leading biotechnologist of Saudi Arabia, Dr Nasser Al-Khalifah. It was submitted to the Islamic Development Bank (IDB). The Islamic Academy of Sciences may use its influence to find donors to fund this project because of its greater chances of success both for increasing the quality of date palm as well as its enhanced productivity.

5 PRODUCTION OF GENETICALLY MODIFIED CROPS

5.1 General

The technology to improve plant resistance genetically by means of transformation by inserting alien genes so that less pesticide and fungicides are used will remain targets to be achieved for specific crops in developing countries. This strategy has already been successfully used for crops like corn, potato, cotton and some fruits in developed countries (James, 1998). Estimates for the year 2001 indicate that about 130 million acres worldwide are planted with transgenic crops. This represents about 19% of cultivated land all over the world.

Transgenics for improved quality product such as vitamin A and iron fortified *indica* rice grains are very important for developing countries where chronic malnutrition leads to blindness and other deficiency diseases. IRRI is working on the transfer of the vitamin A and iron-fortifying genes from Taipei 309 (www.cgiar.org/irri) into *indica* rice cultivars popularly grown in South and South-East Asia.

Abiotically stressed soils are a major hindrance to further increases in crop productivity. The recent reports of cultivars, growing in heavily contaminated

aluminium soil by means of exudation of organic acids through their roots, is an excellent example (De la Fuente *et al.* 1997) of a way to create cultivars for inhospitable soils (Ma *et al.* 2001). Ground breaking success has also been achieved in production of salt tolerant tomato and *Brassica* by use of single genes, which was previously thought not to be possible (Zhang and Blumwald, 2001; Zhang *et al*. 2001).

5.2 Transgenics using chloroplasts

The production of transgenics by targeting genes of interest to the chloroplasts rather than to nuclear genomes has recently proved to be an area of great interest. Alien genes, when incorporated randomly into the chromosomes of the host plant, are silenced (inactivated), a phenomenon called 'position effect', thus thwarting the very objective of the breeder. Position effects can be eliminated by inserting foreign genes into the host chloroplasts, which have one single, small circular DNA. This technique has three other great advantages, one of which is the amplification of the foreign gene by a factor of 10,000 or more because that figure represents the number of the chloroplasts present in each host cell. The second advantage is that genes from chloroplasts are NOT transmitted to pollen (Daniell *et al*. 2001), thereby allaying the fears of those who apprehend that super-weeds would result following the introduction of GM crops that contain genes for herbicide resistance. The third advantage is that multiple genes, e.g., those of a metabolic pathway can be inserted, since chloroplast genes are expressed like bacterial operons where a single promoter allows transcription of multiple genes. It must be mentioned here that this technology has so far been tried successfully in tobacco only and hopefully this powerful tool can be profitably applied in other crops also.

5.3 Avoidance of any toxic gene while developing a transgenic line

An important molecular tool has been to use genes other than those for antibiotic resistance (Elborough and Hanley, 2001). Although there is no definite proof, many people are concerned that there might be a danger of creating "super bugs," if GM food containing antibiotic resistance genes are consumed. Use of some alternative selectable marker genes have been reported which confer positive selection pressure or enhance the performance of transformants over that of normal plants. These are phosphomannose isomerase (PMI) gene and the xylose isomerase (XI) gene. The PMI has been isolated from *E. coli* and transformed plant tissues expressing this gene can grow on a culture medium containing the sugar mannose as the only source of carbon. Untransformed tissues can maintain their size but lacking utilizable carbon, do not grow further. The gene confers no advantage in the natural environment, where plants are self-sufficient in carbon derived from the atmosphere using photosynthesis and so cannot contribute to the generation of a superweed. Other carbohydrates that do not support plant growth can be used similarly when there exists a non-plant enzyme that can convert them into a form usable by the plant.

For example, xylose and the xylose isomerase gene isolated from *Thermoanaerobacterium thermosulfurogenes*. Non-toxic screenable markers include beta glucuronidase, firefly luciferase or jellyfish green fluorescent-protein (GFP). GFP has the added advantage that the screening for transformants can be done non-invasively, without destroying the sample. Observation however requires special cameras, which would allow the fluorescence to be observed.

5.4 The advent of functional genomics (Miflin, 2000)

With publications of the full sequence of *Arabidopsis* and major efforts for publication of sequences of other major crops, such as maize and rice, the next research thrust will be the analysis of gene function. Such gene identification in turn would create a repertoire of useful genes, which could be used as desired. The first approach is usually to search for homology with other known genes. A second approach is to carry out a functional analysis in which the gene is either over-expressed or activity reduced by transformation with the gene. A number of approaches to gene suppression have been developed such as co-suppression or anti-sensing of a defined sequence. However, transformation of specific sequences can only deal with relatively small number of genes. Populations of mutants, in which a series of gene knockouts occur, are being developed to deal with the identification of a large number of genes. These are then screened for changes in phenotype, which are then traced back to the specific DNA that has been mutated. A fourth approach to understanding the function of specific sequences is being done by looking at their expression under a range of different conditions. For example, Ruan *et al.* (1998) have measured the expression, during plant development of 1400 *Arabidopsis* cDNA sequences using microarray technology. This approach is invaluable for the study of traits being controlled by multiple genes such as abiotic stress sensitivity or tolerance, since such stresses cause major changes in metabolism. Therefore, microarray technology is a potentially powerful tool for gene discovery, functional analysis and the understanding of genetic regulatory networks and gene interaction.. It can be defined as a new way of studying how large numbers of genes interact with each other and how a cell's regulatory networks control vast batteries of genes simultaneously. The method uses a robot to precisely apply tiny droplets containing functional DNA to glass slides. Researchers then attach fluorescent labels to DNA from the cell they are studying. The labelled probes are allowed to bind to complementary DNA strands on the slides. The slides are put into a scanning microscope that can measure the brightness of each fluorescent dot; brightness reveals how much of a specific DNA fragment is present, an indicator of how active it is.

Yet another important tool for studying the function of proteins in a technology for which the world proteomics has been coined. The word proteomics combines the word protein with omics such as used in the word genomics. One definition of proteomics is the quantification of all proteins

expressed at any individual time in a cell. Functional proteomics may be defined as the determination of the function of all the proteins encoded by an organism's entire genome. Structural proteomics is the determination of the structures of proteins, which may have multiple functions that can only be identified in the context of their interactions with other proteins, DNAs, small molecules, cofactors, etc. The basic technique involved in proteomics is two-dimension-gel electrophoresis [2D-gel]: for separation of crude protein mixtures. The advent immobile preformed IEF strips and systems for simultaneous running of the 2D gels have increased the efficiencies of protein purification and identification as well as added reproducibility and sensitivity.

In the past ten years, two further scientific developments in very different fields have resulted in a new approach to solving protein structure, which has revolutionized proteomics. One is the development of easy-to-use, yet high-performance mass spectrometers. Techniques, such as MALDI (Matrix-assisted laser desorption/ionization) that have high resolution, sensitivity and accuracy have allowed the measurement of proteins and the peptides generated from the digests of these proteins, followed by fragmentation of these peptides, from as little as a few femtomoles of proteins. This leads to quick identification of proteins. The second is, the automation, efficient and fast techniques for DNA sequencing. The human genome project as well as bacterial, animal and plant genome sequencing has provided huge databases that can be searched by feeding into these programs data such as molecular weight [MW], isoelectric point [pI], hydrophobicity index, amino acid sequence or composition of the protein being tested.

6 CONCLUSION

Sustainable agriculture is only possible if the use of pesticides is drastically reduced. GM crops resistant to pests offer minimum use of pesticides; bioengineered salt-, drought and heavy metals tolerant crops will open up marginal land for cultivation of crops in areas where hardly anything grows now. Such crops can also be bred using marker-aided selection techniques. Identification of genes on a large scale which is now possible due to tools such as functional genomics, EST libraries, DNA microarrays, proteomics and large scale generation of mutants will help identify those which contribute to enhanced abiotic stress resistance and increased yields (Stuber *et al.* 1999; Miflin 2000). Thus, on the one hand biotechnology and molecular tools will enhance crop yield with less yearly fluctuation of crop productivity and on the other ensure economic viability for the farmers, keep environment safe by causing least disturbance to soil structure and topography.

ACKNOWLEDGEMENT

The authors are grateful to Dr Gregory Clark at the Department of Molecular Cell and Developmental Biology, University of Texas, Austin, Texas, and to Dr R H Sarker of the Department of Botany, University of Dhaka, Bangladesh, for their critical scrutiny of the manuscript and offering helpful suggestions.

REFERENCES*

1. Abott DC, Lagudah ES, Brown AHD (1995). Identification of RFLP's flanking a scald resistance gene on barley chr. 6. *J. Hered*. 86:152-154.
2. Concibido, VC,. Denny RL, Boutin SR., Hautea R, Orf JH, Young ND (1994) DNA marker analysis of loci underlying resistance to the soybean cyst nematode (*Heterodera glycines*). *Crop Sci*. 34:240-246.
3. Henry Daniell, Streatfield SJ, Wycoff Keith (2001) Medical Molecular Farming: Production of antibodies, biopharmaceuticals and edible vaccines in plants. *Trends in Plant Science* 6:219-226.
4. Danesh D, Young, N, Concibido, V, Orf, J (1995) Physical mapping of DNA markers near a major soybean cyst nematode resistance locus. *Soybean Genetics Newsletter* 22:273-277.
5. Elborough KM and Hanley SZ (2001) Emerging Technologies in Plant Biotechnology. Information Systems For Biotechnology News Report, February 2001, pp. 2-4.
6. Fuente De la, JM, Ramirez-Rodriguez V, Cabrera- Ponce JL, Herrera-Estrella L. (1997) Aluminum tolerance in transgenic plants by alteration of citrate synthesis. *Science* 276, 1566-8.
7. Graner A and Tekauz A (1996). RFLP mapping in barley of a dominant gene conferring resistance to scald. *Theor. Appl. Genet*. 93:421-425.
8. Gregorio, GB (1997) Ph.D. Thesis, University of Philippines at Los Banos. Tagging salinity tolerance genes using Amplified Fragment Length Polymorphism (AFLP).
9. Gupta PK, Varshney RK, Sharma PC, Ramesh B (1999) Molecular markers and their application in wheat breeding. *Plant Breeding* 118: 369-390.
10. Gupta PK and Varshney RK (2000) The development and use of microsatellites markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica* 113:163-185.
11. Gupta PK, Roy JK, Prasad M (2001a) Single nucleotide polymorphisms (SNPs): a new paradigm in molecular marker technology and DNA polymorphism detection with emphasis on their use in plants. *Curr. Sci*. 80: 524-533.
12. Gupta PK, Varshney RK, Prasad M (2001b) Molecular markers: principles and Methodology. In: "Molecular Techniques in Crop Improvement." (Jain, SM, Ahloowalla BS and Brar DS eds.) Kluwer Academic Publishers, Netherland (Press).
13. Huang N, Angeles Er, Doming J, Magpantay G, Singh S, Zhang G, Kumaradivel N, Bennett J, Khush GS (1997). Pyramiding of bacterial blight resistance genes in rice marker aided selection using RFLP and PCR. *Theor. App. Genet*. 95:313-320.
14. James Clive (1999). Global review of commercialized transgenic crops. ISAAA Briefs No. 21. Also see www.isaaa.org/publications.

* As listed by authors.

15. Li WL, Faris JD, Chittoor JM, Leach JE, Hulbert SH, Liu DJ, Chen PD, Gill BS (1999). Genomic mapping of defence response genes in wheat. *Theor. Appl. Genet*. 98:226-233.
16. Ma Jian Feng, PR Ryan and Delhaize E (2001) Aluminium tolerance in plants and the complexing role of organic acids. *Trends in Plant Science* 6: 273-278.
17. McCouch, SR, Chen X, Panaud O, Temnykh X, Zu Y, Cho YG, Huang N, Ishii T, Blair M (1997) Microsatellite marker development, mapping and applications in rice genetics and breeding. Plant Molecular Biology 35:89-99.
18. Miflin B (2000) Crop improvement in the 21st century. *J. exptl. Bot*. 51:1-8.
19. Mohan M, Nair S, Bentur JS, Prasada Rao U, Bennet J (1994). RFLP and RAPD mapping of the rice Gm2 gene that confers resistance to biotype 1 of gall midge. *Theor. Appl. Genet* 87:782-788).
20. Nandi SP, Subudhi K, Senadhira D, Manigbas NL, Sen-Mandi S, Huang N (1997).
21. Mapping QTL's for submergence tolerance in rice by AFLP analysis and selective genotyping. *Mol. Gen. Genet*. 255: 1-8.
22. Nelson JC, Singh RP, Autrique JE, Sorrels ME (1997). Mapping genes conferring and suppressing leaf rust resistance in wheat. *Crop. Sci*. 37:1928-1935.
23. Ruan Y, Gilmore J, Conner T(1998). Towards *Arabidopsis* genome analysis: monitoring expression profiles of 1400 genes using cDNA microarrays. *The Plant J*. 15:821-833.
24. Schweizer GF, Baumer M, Daniel G. Rugel H , Roder MS (1995). RFLP markers linked to scald resistance gene *Rh 2* in barley. *Theor. Appl. Genet*. 90: 920-924.
25. Seyfarth R, Feuillet C, Keller B (1998). Development and characterization of molecular markers for the adult plant leaf rust resistance genes *Lr 13* and *Lr 35* in wheat.
26. Slinkard AE (ed). Proc. 9th Int. Wheat Genet Symp. Vol 3. University Extension press, Saaskatoon, Saskatchewan, Canada, pg. 154.
27. Singh RP, Mujeeb-Kazi A, Huerta-Espino J (1998). *Lr 46*: a gene conferring slow-rusting resistance to leaf rust in wheat. *Phytopathology* 88:890-894.
28. Spielmeyer W, Robertson M, Collins N, Leister D, Schulze-Lefert P, Seah S, Moullet O, Lagudah ES (1998). A superfamily of disease resistance gene analogs is located on all homoeologous chromosome groups of wheat. *Genome* 41:782-788.
29. Stein, Dan, 1991, "Morphological mechanisms of crop resistance to insects", Vol. 11, No. 2, Summer 1991, pp. 15. (See also his web site: http://www.eap.mcgill.ca/MagRack/JPR/JPR_11.htm).
30. Stuber CW, Polacco M, Lynn Senior M (1999). Synergy of empirical breeding, marker-assisted selection and genomics to increase crop yield potential. *Crop Sci*. 39:1571-1583.

31. Wang GL, Mackill DJ, Bonman JM, McCouch SR, Champoux MC, Nelson RJ (1994) RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. *Genetics* 136:1421-34.
32. Wang XQ, Ullah H, Jones AM, Assmann SM (2001) G protein regulation of ion channels and abscisic acid signaling in Arabidopsis guard cells. *Science* 15:292:2022-3.
33. Xiao Jinhua, Li Jiming Li, Silvana Grandillo, Ahn Sang Nag, Yuan Longping, Tanksley Steven D, McCouch Susan R. (1998) Identification of Trait-Improving Quantitative Trait Loci Alleles From a Wild Rice Relative, *Oryza rufipogon***.** *Genetics* 150: 899 - 909.
34. Yoshimura, S, Yoshimura A, Iwata N, McCouch SR, Abenes M, Baraoidan M, Mew TW, Nelson RJ 1995. Tagging and combining bacterial blight resistance genes in rice using RAPD and RFLP markers. *Molecular Breeding* 1:375-387.
35. .Zhang H-X and Blumwald E. (2001). Transgenic salt tolerant tomato plants accumulate salt in the foliage but not in the fruits. *Nature Biotechnology* 19: 765–768.
36. Zhang H-X, Hodson J, Williams JP, and Blumwald E. (2001). Engineering salt-tolerant *Brassica* plants: Characterization of yield and seed oil quality in transgenic plants with increased vacuolar sodium accumulation. *Proceedings of the National Academy of Sciences, USA* 98: 12832–12836.

Application of RT-PCR Technology for Detection of Bluetongue and Epizootic Hemorrhagic Disease Viruses

IMADELDIN E ARADAIB*
University of Khartoum
Sudan
and
BENNIE I OSBURN**
University of California Davis USA

1 ABSTRACT

A multiplex reverse transcriptase (RT) polymerase chain reaction (RT-PCR)-based assay, for simultaneous detection and differentiation of bluetongue (BT) virus (BTV) and epizootic hemorrhagic disease (EHD) virus (EHDV) in cell culture and clinical samples, was developed. Two pairs of primers (B1 & B4) and (E1 & E4) were designed to hybridized to non-structural protein 1 (NS1) genomes of (BTV-11) and that of (EHDV-1), respectively. The multiplex RT-PCR-based assay utilized a single tube-PCR amplification in which EHDV and BTV primers were used simultaneously in a multiplex format. The BTV primers generated a 790 base pair (bp) specific PCR product from RNA samples of BTV serotypes 1, 2, 4, and 16; whereas EHDV-4 and EHDV- 318; or total nucleic acid extract from non infected baby hamster kidney (BHK) cells failed to demonstrate the 790-bp specific BTV PCR product. Likewise, the EHDV primers produced a 387 (bp) specific PCR product from RNA samples of EHDV serotypes 4 and 318, but not from BTV serotypes 1, 2, 4 and 16; or from total nucleic acid extract of BHK cell controls.

Two pairs of nested primers (B2 & B3) and (E2 & E3), internal to the annealing sites of primers (B1& B2) and primers (E1& E2), produced a 520-bp specific BTV and a 224-bp specific EHDV PCR products from BTV and EHDV first amplification products, respectively. These nested amplifications increased the sensitivity of the RT-PCR assay and confirmed the specificity of the first amplified EHDV or BTV PCR products. The described multiplex RT-PCR-based assay could be used to facilitate rapid detection and differentiation

* Department of Medicine, Pharmacology and Toxicology; Faculty of Veterinary Science; University of Khartoum; PO Box 32; Khartoum North; Sudan.

** Department of Pathology, Microbiology and Immunology; School of Veterinary Medicine; University of California Davis; 95616; USA.

of Sudanese BTV and EHDV serotypes and to provide a valuable tool to study the epidemiology of these orbivirus infections in susceptible animal populations.

2 INTRODUCTION

Epizootic hemorrhagic disease (EHD) virus (EHDV) and Bluetongue (BT) virus (BTV) are insect-transmitted double-stranded (ds) RNA orbiviruses in the family Reoviridae [1,2]. BTV and EHDV infect domestic, captive and free-ranging ruminants [3,4,5,6,7,8,9]. Cattle are susceptible to infection but the association of EHDV or BTV with clinical hemorrhagic disease is rare [10,11,12,13,14]. Twenty-five serotypes of BTV [15] and ten serotypes of EHDV [16] are recognized worldwide. Of these orbiviruses, only BTV serotypes 2, 10, 11, 13 and 17 and EHDV serotypes 1 and 2 are present in North America. Clinical hemorrhagic disease in deer populations and restrictions on the sale of livestock and associated germplasm in the international markets are of concern to wildlife managers and dairy producers [14]. In addition, clinical signs and pathological lesions induced by BTV in susceptible animals are indistinguishable from those induced by EHDV and hence these viral infections are of interest to veterinary diagnosticians [17,18]. Therefore, the development of a rapid, reliable, reproducible, sensitive, specific and inexpensive diagnostic assay for simultaneous detection and differentiation of these hemorrhagic infections among susceptible North American deer population would be advantageous [19,20]. In a previous report, we described a single BTV PCR amplification based on NS1 genome of BTV-10 [21,22,23]. A nested PCR, based on NS1 genome sequence analysis of BTV-10, was also developed and evaluated for detection of BTV in the biting midge [24]. The NS1 genome of BTV-17 was cloned and sequenced [25]. The sequence analysis showed that this genome has a 97% nucleic acid identity compared to the other members of BTV serogroup [26]. A digoxigenin-labeled RNA probe derived from NS1 of BTV-17 hybridized with at least 24 serotypes of BTV in cell culture [27]. Recently, we have developed PCR-based assays for detection of EHDV serogroup and serotypes in cell culture and tissue samples [28,29,30,31]. A multiplex RT-PCR for simultaneous detection and identification of United States serotypes of EHDV-1 and EHDV-2 was also reported recently [32]. Although these BTV and EHDV PCR-based detection assays proved highly sensitive and specific, they do require individual testing of each submitted sample for the presence of EHDV or BTV ribonucleic acids. This limitation renders these PCR-based detection assays rather expensive and time consuming. In the present study, we describe a reproducible, sensitive, and specific assay for simultaneous detection and differentiation of North American BTV and EHDV, closely related orbiviruses, in cell culture and clinical samples using a multiplex PCR amplification technology.

3 MATERIALS AND METHODS

3.1 Virus and cells

The North American prototypes serotypes of EHDV (EHDV-1 and EHDV-2), the five BTV prototype serotypes 2, 10,11, 13, and 17 present in the United States (Arthropod-Borne Animal Disease Research Laboratory, Laramie, WY.) were studied. All viruses were propagated on confluent monolayers of BHK-21 cells as described previously [11]. The infectious material was harvested and centrifuged at 1,500-x g for 30 min at 25° C and the cell pellet was used for the dsRNA extraction.

Viral nucleic acid extraction from infected BHK cell monolayers

The BTV and EHDV dsRNAs were extracted from the infected cells as previously described [3]. Total nucleic acid was ethanol-precipitated. Viral dsRNA was purified by differential lithium chloride precipitation, and resuspended in 100 ul double distilled water, and quantified using a spectrophotometer at 260 nm wave length [22].

3.2 Clinical Samples

Blood samples were collected from previous studies of EHDV and BTV experimentally infected Calves [10,32] and from BTV and EHDV seronegative calves. The processed blood samples were inoculated on BHK-21 cell monolayers for virus isolation (VI) as described previously [10] and serotyped by plaque inhibition test [33] Viral nucleic acid extraction from the clinical samples was as previously described [22]. Briefly, 250 ul of processed blood or spleen was digested with SDS and proteinase K (Boehringer Mannheim, Indianapolis, IN.). The sample was phenol extracted twice. Total nucleic acid was ethanol precipitated and resuspended in 20-ul of double distilled water. Five microliters of the resuspended nucleic acid was employed in the PCR assay.

3.3 Primer selection for PCR amplification

Two pairs of BTV primers (B1&B4) and (B2&B3), and 2 pairs of EHDV primers (E1&E4) and (E2&E3) were designed based on the nucleotide sequences of genome segment 6 of BTV serotype 11 (BTV-11) and that of EHDV serotype 1 (EHDV-1). The BTV primers (B1 and B4) were designed for amplification of the first BTV PCR product. Primer B1 included bases 71-90 of the positive sense strand of (BTV-11) NS1 genome (5)-GATTACGCAAATGCCACGAG*(3).*Primer B4 included bases 841-860 of the complementary strand (5)-GGTGTAATGGAAATTCACCT*(3)*. Primers B1 and B4 would result in amplification of a 790 bp BTV specific PCR product. The BTV internal primers (B2 and B3) were designed for the nested PCR amplification. B2 included based 311-330 of the positive sense strand of (BTV-

11) NS1 genome (5)-TACGAGGAGGATGTCGAAGG(3). B3 included bases 811-830 of the complementary strand (5)-TTCCGAAGAGCTGTTGTACA(3). Primers B2 and B3 would result in a 520-bp BTV specific PCR product internal to the annealing site of B1 and B2.

The EHDV primers (E1 and E4) were designed for amplification of the first EHDV PCR product. Primer E1 included base 175-194 of the positive sense strand of (EHDV-1) NS1 genome (5)-TCGAAGAGGTGATGAATCGC(3). Primer E4 included bases 543-562 of the complementary strand (5)-TCATCTACTGCATCTGGCTG(3). Primers E1 and E4 would result in a 388-bp EHDV specific PCR product. The internal EHDV primers (E2 and E3) were designed for the nested EHDV PCR amplification. E2 included bases 216-235 of the positive sense strand of EHDV-1 NS1 genome. (5)-CATGCGGCATATAGATTGGC. E3 included bases 421-440 of the complementary strand (5)-GTCATCTAGCACGATGCGTG(3). Primers E2 and E3 would result in a 225-bp EHDV specific PCR product internal to the annealing site of E1 and E4.

All primers were synthesized on a DNA synthesizer (Milliigen/Biosearch, a division of MilliporeBurlington, MA) and purified using oligo-pak oligonucleotide purification columns (Glen Research Corporation, Sterling, VA.) as per manufacturer's instructions.

3.4 Multiplex Polymerase chain reaction

BTV Primers (B1, B4) and EHDV primers (E1 &E4) at a concentration of 20-micromole\ liter each, were pooled. One ul of 80-mM methyl mercuric hydroxide was used to denature a mixture of 5 ul of the target nucleic acid and 4ul of the pooled (BTV&EHDV) primers. After a 10 minutes incubation at room temperature (25 ^{0}C). 10 ul of neutralizing mixture, containing 1 ul of 1M 2-mercaptoethanol, 1 ul of 20 U/ul RNAse inhibitor, and 2ul of each dNTPs (dATP, dTTP, dCTP, dGTP) was added. Immediately following neutralization, 8.8 ul of a reverse transcriptase mixture, containing (2.7 ul of 10X PCR buffer, 5 ul of 25 mM $MgCl_2$, 1 ul of 50 U/ul M-MLV reverse transcriptase) was added. The reaction was incubated at 42 C for 30 min followed by 5 min at 99 C. The reaction was then quenched on ice. Finally, a PCR mixture containing (7.3 ul of 10 X PCR buffer, 8 ul of 25 mM $MgCl_{2,}$ and 5.0 U of Taq DNA polymerase) was added. Double distilled water was added to each PCR tube to obtain a total volume 100 ul.

The thermal cycling profiles were as follows: a 2-min incubation at 95^0 C, followed by 40 cycles of 95^0 C for 1 min, 55^0 C for 30 sec and 72° C for 45 sec, and a final incubation at 60° C for 10 min. Thermal profiles were performed on a Techne PHC-2 thermal cycler (Techne, Princeton, NJ.). For the nested amplification of BTV or EHDV PCR product, 2 ul of the first amplification product were transferred to a PCR tube containing amplification buffer. For each PCR reaction, the amplification buffer consisted of (10 ul of 10X PCR buffer; 10 ul Mg Cl_2 of 1.5 mM concentration; 4 ul of primers (B2&B3) and (E2&E3) at a concentration of 20 micromole/ liter; 8 ul of dNTPs including

ATP, TTP, GTP, CTP; 1 ul of Taq DNA polymerase (Perkin Elmer Corporation, Norwork, CT) at a concentration of 5.0 units/ ul. Double distilled water was added to each PCR tube to obtain a total volume of 100 ul. The PCR reaction mixture was covered with a drop of mineral oil and the PCR tubes were replace in the thermal cycler for another 40 cycles at the same temperature per cycles described above.

Following amplification, 20 microliters from each PCR reaction containing amplified product were loaded onto gels of 1.5% SeaKem agarose (FMC Bioproduct, Rockland ME) and electrophoresed. The gels were stained with ethidium bromide, and the PCR products were visualized under UV light.

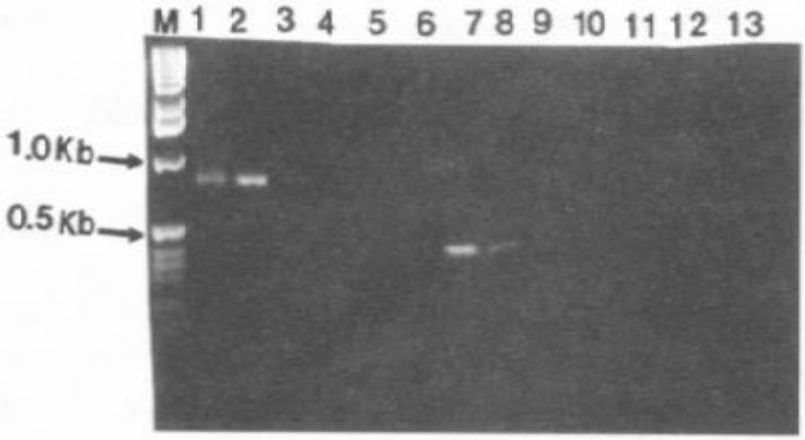
4 CONCLUSION

The EHDV specific 388-bp PCR product was visualized on ethidium bromide-stained agarose gels from $\geq$ 100 fg RNA of EHDV serotypes 1 but not from BTV serotypes 2, 10, 11, 13 and 17 or total nucleic acid extract from BHK cell control. The BTV specific 790-bp PCR product was visualized on ethidium bromide-stained agarose gel from $\geq$ 100 pg RNA of BTV serotypes 11 but not from EHDV serotypes 1 or BHK cell control (Figure. 1A). Whereas the nested amplification detected 0.1 fg of BTV-11 or EHDV-1 RNA target (Figure. 1B).

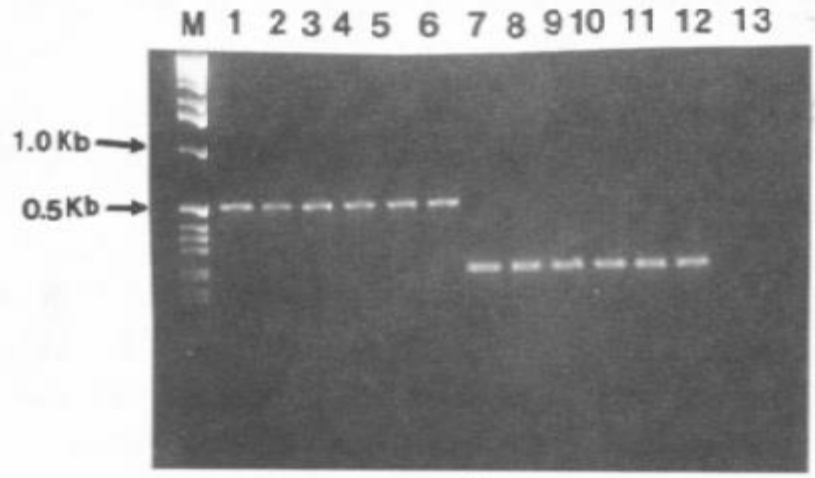
Using 1 pg of BTV or EHDV target, the 520-bp BTV specific and the 387-bp EHDV specific PCR products were detected in all North American BTV and EHDV serotypes with ethidium bromide-stained agarose gels (Figure 2).

In a reconstitution experiment, total nucleic acid extracted from non-infected BHK cell culture was spiked with 2 ul of BTV and EHDV RNAs at a concentration of 1.0 pg/ul each. Application of the spiked sample to the multiplex PCR resulted in simultaneous detection of the 790 bp BTV specific and the 388 bp EHDV-specific PCR products. The nested 520 bp BTV-specific and the nested 225-bp EHDV specific PCR products were successfully amplified using BTV (B2&B3) and EHDV (E2&E3) internal primers (Figure 3).

The nested 520 bp BTV-specific and the nested 225 bp EHDV-specific PCR products were detected directly from clinical samples including unfractionated lysed blood from calves experimentally infected with BTV and EHDV serotypes. Blood from uninfected calves failed to produce amplification products (Figure 4). All BTV or EHDV serotypes, which were PCR positive, were also positive by conventional virus isolation and serology as mentioned previously [11,33].



A



B

Figure 1. Sensitivity of the BTV-EHDV multiplex polymerase chain reaction (PCR)-based assay for simultaneous detection of bluetongue virus serotype 11 (BTV-11) and epizootic hemorrhagic hemorrhagic disease virus serotype 1 (EHDV-1).

A-First amplification showing visualization of the specific 790-bp BTV PCR product and the specific 387-bp EHDV PCR product on ethidium bromide-stained agarose gel. Lane M: molecular weight marker; lanes 1-6: (BTV-11) 10 pg, 1.0 pg, 100 fg, 10 fg, 1.0 fg, and 0.1 fg of BTV RNA, respectively. Lane 7-12: (EHDV-1) 10 pg, 1.0pg, 100fp, 10fg, 1.0fg. and 0.1fg of EHDV RNA Lane 13: non-infected BHK-21 total nucleic acid extract.

B-Nested amplification from the first amplification product showing visualization of the 520-bp specific BTV PCR product and the 225-bp specific EHDV PCR product from as little as 0.1 fg of BTV and EHDV RNA, respectively.

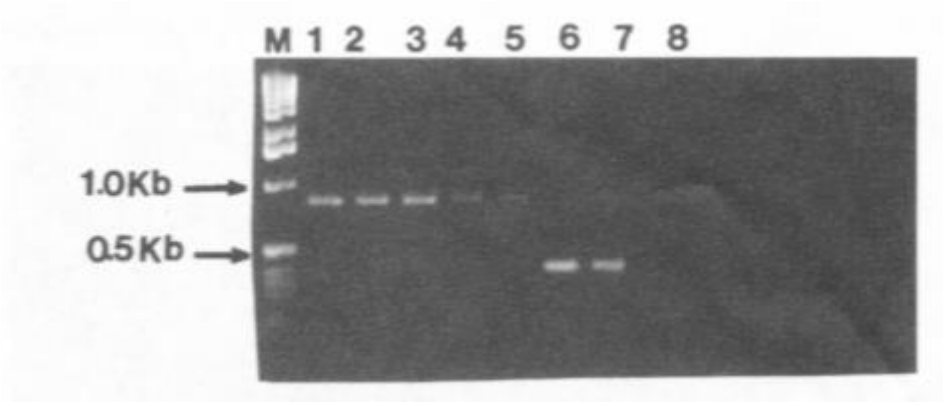


Figure 2.Simultaneous detection and differentiation of the nested 520-bp BTV specific and the nested 224-bp EHDV specific amplification products on ethidium bromide-stained agarose gel. Lane M: molecular weight marker; Lane 1: Total nucleic acid extracted from non infected BHK-21 cells spiked with 1.0 pg of BTV and 1.0 pg of EHDV RNAs.; Lane 2: 1.0 pg BTV RNA; Lane 3: 1.0 pg EHDV-1 RNA; Lane 4: non-infected BHK cell control.

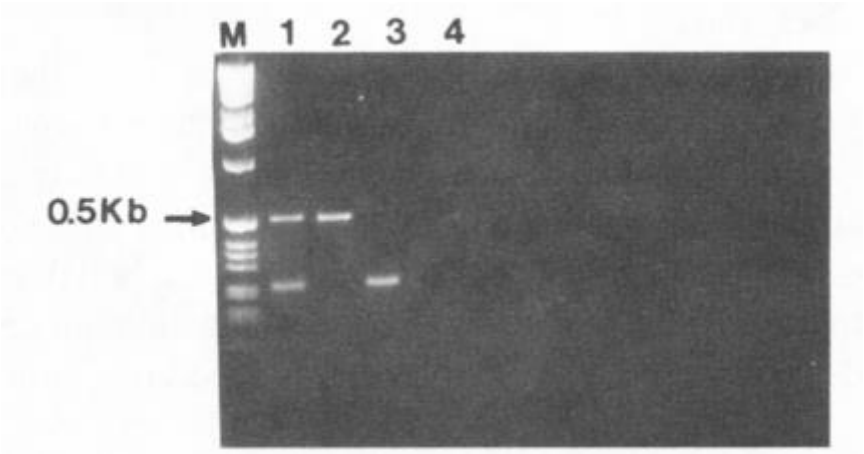


Figure 3. Detection of the nested 520-bp BTV specific and the nested 225 EHDV specific PCR products from clinical samples. Lane M: molecular weight marker; Lane 1-4: unfractionated lysed blood from calves experimentally infected with BTV-10, BTV-11, BTV-13, and BTV-17; Lane 5-6: unfractionated lysed blood from calves natural infected with BTV-10 and BTV-11; Lane 7-8: unfractionated blood from calves experimentally infected with EHDV-1 and EHDV-2; Lane 9-10: unfractionated lysed blood and spleen suspension from EHDV-2 naturally infected calves; Lane: 11-12 unfractionated blood from uninfected calves.

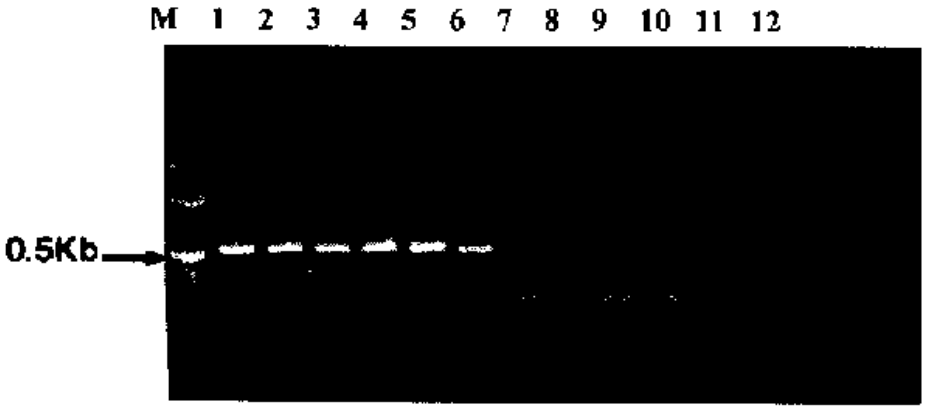


Figure 4. Blood from uninfected calves failed to produce amplification products.

5 DISCUSSION

The described multiplex PCR-based assay, using primers derived from NS1 genome of BTV-11 and NS1 genome of EHDV-1, reproducibly and specifically detected North American BTV and EHDV RNAs in infected cell cultures and clinical samples. Selection of the primers was based on the observation that the non-structural proteins genomes of these orbiviruses require small number of infected cells to produce positive hybridization signal compared to other nucleic acid probes [11,20,28,34]. In the present study, the multiplex PCR assay was optimized to detect and differentiate between BTV and EHDV RNA targets. This was achieved by using a pool of BTV and EHDV primers in a single-tube PCR amplification. The EHDV and BTV primers were used simultaneously in a multiplex format.

Using ethidium bromide-stained agarose gel, the sensitivity of the BTV\ EHDV multiplex PCR assay was found to be at least 100 fg of EHDV or BTV RNAs (equivalent to 6x 10^3 BTV or EHDV viral particles [21,30]. Nested amplification of the first BTV or EHDV amplification products, using internal primers, resulted in increased sensitivity up to a level of 0.1 fg of BTV or EHDV RNAs, equivalent to 60 viral particles [21,30]. This level of sensitivity is comparable to or even more sensitive than virus isolation [21,11,35). Excellent correlation of results from agarose gels indicated that diagnosis of EHD and BT infections could be based on visualization of the first amplified PCR products on an ethidium bromide-stained agarose gel, since it is a simple procedure, which requires only 1 hour after amplification. The nested amplification is necessary to confirm the nucleic acid identity of the first amplified PCR products of EHDV and BTV [30].

Simultaneous detection and differentiation of BTV and EHDV will simplify the assay, save time and above all save on cost, because each clinical sample

will be tested once instead of individual testing for BTV and EHDV viral nucleic acids. Because of its reliability, reproducibility, rapidity, sensitivity and specificity; the PCR assay described in this study would be advantageous in epidemiological investigations where field isolates can be tested to determine the prevalence and frequency of orbivirus infections in susceptible animal populations, and can also be used for evidence of viral incursion in a particular geographical region during a sentinel study.

In the present study, nested amplification should increase the sensitivity of the PCR-based assay by at least 1000 times, particularly when the concentration of the RNA in the sample is less than 100 fg. In PCR assays, the use of a nested (internal) PCR product as a probe in chemiluminescent hybridization assays to confirm the specificity of the PCR product and/or to increase the sensitivity of the PCR assay is well documented. [10,11, 13,21,22,24,28,29,30]. In this study, the non-radio labeling procedure of the probe removes the hazardous and cumbersome radioactive laboratory procedures of working with ^{32}P or ^{33}P.

Successful amplification was also obtained from whole blood received from calves experimentally infected with BTVor EHDV serotypes. The BTV and EHDV were isolated on BHK-21 cell monolayer from all North American BTV and EHDV serotypes tested. The isolates were identified by plaque inhibition test [33].

This multiplex RT-PCR assay could be used as supportive diagnostic techniques to the lengthy cumbersome conventional virus isolation procedures, currently used for detection of BTV and EHDV and could be validated for the purpose of export regulation to certify animals free of BTV and/or EHDV infections.

Nested amplification is an extremely sensitive procedure and contamination may be a problem. To avoid contamination, extraction of EHDV and BTV dsRNAs should be carried out in separate rooms using aerosol resistant tips Negative and positive controls should be included in each PCR amplification to estimate the lower limit of specificity and the higher limit of sensitivity.

In conclusion, the modification described in this communication indicated that the described multiplex PCR-based assay provides a simple, rapid, sensitive, specific, reliable and inexpensive method for simultaneous detection and differentiation of BTV and EHDV infections in cell culture and clinical samples.

Further studies are currently under way to determine the capacity of the described multiplex PCR-based assay for direct detection of dual infections with BTV and EHDV in clinical samples from naturally infected animals, and to evaluate its potential as a sensitive and specific diagnostic assay through comparison with current diagnostic techniques used for detection of these orbiviruses.

REFERENCES*

1. Borden EC, Shope RE, Murphy FA. (1971) Physicochemical and morphological relationships of some arthropod-borne viruses to bluetongue virus-a new taxonomic group. Physicochemical and serological studies. J Gen Virol 3: 261-271.
2. Fenner F, Pereira HG, Porterfield JS. (1974) Family and generic names for virus approved by the international committee on taxonomy of viruses. Intervirology 3: 93-194.
3. Hoff GL, Trainer DO. (1974) Observation on bluetongue and epizootic hemorrhagic disease virus in white-tailed deer. J. Wildlife. Dis 10**:**25.
4. Karstad L, Winter A, Traine RDO. (1961) Pathology of epizootic hemorrhagic disease of deer, Am J Vet Res 22: 227-230.
5. Maclachlan NJ, Jagels G, Rossitto PV, Moore PF, Heidner HW. (1990) The pathogenesis of experimental bluetongue virus infection of calves. Vet Pathol 27:223-229.
6. Shope RE, Macnamara LG, Mangold R. (1995) Report on the deer mortality, Epizootic hemorrhagic disease of deer, New Jersey Outdoors 6: 17-21.
7. Shope RE, MacNamara LG, Mangold R. (1960) A virus-induced epizootic hemorrhagic disease of the Virginia white-tailed deer (*Odocoileus virginianus*) J Exp Med 111: 155-170.
8. Work, TM, Jessup DA, Sawyer MM. (1992) Experimental bluetongue and epizootic hemorrhagic disease virus infection in California black-tailed deer. J Wildlife Dis 4: 623-628.
9. Jessup DA. (1985) Epidemiology of two orbiviruses in California's native wild ruminants: Preliminary report. In bluetongue and Related Orbiviruses, ed. T. Barber and M. Jochim. Progress in Clinical and Biological Research 78: 53-65. Alan Liss, Inc., New York, USA.
10. Aradaib, IE, Sawyer, MM, Osburn BI. (1994) Experimental epizootic hemorrhagic disease virus infection in calves: virologic and serologic. J Vet Diagn Invest 6:489-492.
11. Aradaib IE, Akita GY, Pearson JE, Osburn, BI. (1995) Comparison of polymerase chain reaction and virus isolation for detection of epizootic hemorrhagic disease virus in clinical samples from naturally infected deer. J Vet Diag Invest 7: 196-200.
12. Maclachlan NJ, Osburn BI. (1983) Bluetongue virus-induced hydroencephalopathy in cattle Vet Pathol 20: 563-573.
13. Mohammed MEH; Aradaib IE, Mukhtar MM, Ghalib HW, Riemann HP, Oyejide A, Osburn, BI. (1996). Application of molecular biological techniques for detection of epizootic hemorrhagic disease virus (EHDV-318) isolated from a sentinel calf in Central Sudan. Vet Microbiol 52:201-208.

* As listed by authors.

14. Osburn BI., Aradaib IE., Schore CE. (1994) Comparison of Bluetongue, and epizootic hemorrhagic disease complex. Proceedings of the 13th Int Symp World Associ Veterinary Microbiologist, Immunologist and Infectious Disease Specialist, Perugia, Montova, Italy, pp. 219-224.
15. Davies FG, Mungai JN, Pini A. (1992) A new bluetongue virus serotype isolated in Kenya. Vet Microbiol 31: 25-32.
16. Gorman B.M. (1992) An overview of the orbiviruses. In Bluetongue, African horse sickness and related orbiviruses, eds Walton TE, and Osburn BI. CRC Press, Boca Raton, Florida, pp. 335-347.
17. Metcalf HE, Ludeke AJ, Jochim JO. (1992) Epizootic hemorrhagic disease virus infection in cattle. Bluetongue, African horse sickness and related orbivirus, ed. Walton, TE, Osburn BI, CRC press, Boca Raton, Fl, 222-237.
18. Pearson JE, Gustafson GA, Shafer AL, Alstad AD. (1992) Diagnosis of bluetongue virus and epizootic hemorrhagic disease. In: Bluetongue, African horse sickness and related viruses, eds Walton TE., Osburn, BI. CRC press, Boca Raton, Florida, pp. 533-546.
19. McColl KA, Gould AR. (1991) Detection and characterization of bluetongue virus using the polymerase chain reaction. Virus Res 21: 9-34.
20. Wang L.F, Dio R.H., Osburn B I. (1989) Cloning and sequencing of NS1 genome of bluetongue virus serotype 17. Nucleic Acid Res. 17:8002.
21. Akita GY, Chinsangaram J, Osburn BI. (1992) Detection of Bluetongue virus serogroups by PCR. 1992. J Vet Diagn Invest 4: 400-405.
22. Akita GY, Glenn J, Castro AE, Osburn BI. (1993) Detection of bluetongue virus in clinical samples by polymerase chain reaction. J Vet Diagn Invest 5: 154-158.
23. Dangler CA, Demattos CA, Demattos CC, Osburn BI. (1988) Identifying bluetongue virus ribonucleic acid sequences by the polymerase chain reaction. J Virol. Methods 28: 281-292.
24. Wilson WC, Chase CCL. (1993) Nested and multiplex polymerase chain reaction for the identification of bluetongue virus infection in the biting midge, Culicoides variipennis. J Virol. Methods. 45: 39-47.
25. Hwang GY, Chiou JF, Yang YY, Li JKK. (1993) High sequence conservation among the United State bluetongue viruses cognate M2 gene, which encode the non-structural NS1 tubule protein. Virology 192: 321-327.
26. Roy P, Gorman BM. (1990) Bluetongue virus. Current Topics in Microbiol and Immunol 62: 1-200.
27. Brown CC, Myer RF, Grubman MJ. (1993) Use of a digoxigenin-Labeled RNA probe to detect all 24 serotypes of bluetongue virus in cell culture. J Vet Diagn Invest 5: 159-162.
28. Aradaib IE, Wilson WC, Cheney W, Pearson JE, Osburn, BI. (1995) Application of the polymerase chain reaction for specific identification of epizootic hemorrhagic disease virus serotype 2. J. Vet Diagn Invest 7: 388-392.
29. Aradaib IE, McBride JW, Wilson WC, Osburn BI. (1995) Specific

identification of epizootic hemorrhagic disease virus serotype 1 by PCR. Arch of Virol 140: 2273-2281.

30. Aradaib I.E, Wilson WC, Schore CE, Mohammed MEH, Yilma TD, Cullor JS, Osburn BI. (1998) PCR detection of North American and Central African isolates of epizootic hemorrhagic disease virus based on genome segment 10 of EHDV serotype 1. Clini Microbiol 36: 2604-2608.
31. Aradaib IE. (2001) RT-PCR-based for detection of Sudanese isolates of epizootic hemorrhagic disease virus. Sudan J Veterinary Science & Animal Husbandry (Accepted).
32. Aradaib I.E, Karrar AE, Ibrahim KEE, Yilma TD, Cullor JS, Osburn BI. (1998) A multiplex PCR for simultaneous detection and identification of United States serotypes of epizootic hemorrhagic disease virus. Sud. J Vet. Sci. Anim. Husb. 37: 1-12.
33. Stott JL, Barber TL, Osburn BI. (1978) Sereotyping bluetongue virus: a comparison of plaque inhibition and plaque neutralization methods. Proc 21st Annu Meet Am Assoc Vet Lab Diagn, pp. 399-410.
34. Mecham JO, Dean VC. (1988) Protein coding assignments for the genome of epizootic hemorrhagic disease virus. J Gen Virol 69: 1255-1262.
35. Wade-Evans AM, Mertens PPC, Bostock JC. (1991) Development of polymerase chain reaction for the identification of bluetongue virus in tissue samples. J Virol Methods 30: 15-24.

Biotechnology Research and Development in Tunisia

RADHOUANE ELLOUZ*
Centre de Biotechnologie de Sfax (CBS)
Sfax, Tunisia

1 ABSTRACT

The paper will focus on Biotechnology development in Tunisia. It will cover the different aspects:

- Organisation of the Research System;
- Human Resources, Education and Training;
- Research and Development; and
- International cooperation.

Regarding research organization, the State Secretariat of Research and Technology has published in January 1996, the first law devoted to Research and Development. Since then, human resources, education, research activities within laboratories created in many institutions have been developed.

Two main institutions; the Pasteur Institute (Tunis) or (IPT) and the Biotechnology Centre (Sfax) or (CBS) are directly involved in research and development in Biotechnology. Some other laboratories, within universities, are just starting a limited activity in this area. Some research activities in the field of industrial microbiology will be reported and the transfer to the industry of the achieved results outlined.

2 RESEARCH ORGANISATION

The Secretariat of State for Research and Technology, created in 1991, plays a major role in the coordination of all activities related to research and development (R&D). The first law devoted to research was published in 1996. It aims to:

(1) Specify that research and technology development activities should be carried out within:

* Professor of Biochemistry and Director General of CBS.

- *Laboratories or research units (having their own budget); and*
- *Pilot plant units.*

(2) Set up new structures for coordination and evaluation of research activities particularly:

- *The Scientific Research and Technology Council, to be headed by the Prime Minister, with all the departments involved in R and D, being member.*
- *The National Committee for Research Evaluation, which is an independent body.*

(3) Give a real boost to the Research Budget (RB)- increasing the RB /GNP ratio (Table 1).

(4) Give high priority to the following research sectors: Biotechnology, Computer Sciences, Information Technology, and Telecommunications.

3 HUMAN RESOURCES

According to the national policy objectives related to Biotechnology (BT), the government decided to:

- *Create new academic institutions. Some were launched at the beginning of this academic year 2001/2002; and*
- *Re-enforce the existing education and training institutions by updating their scientific programmes and upgrading their BT infrastructures.*

4 RESEARCH ACTIVITIES

4.1 Research objectives

The objectives of the national biotechnology strategy in terms of research and development is to primarily focus on a few specific areas:

(1) Human and animal health

- *Diagnosis of infectious diseases as well as genetic diseases using different techniques mainly genetic engineering technology;*
- *Contribution to the study of functional genes involved in hereditary diseases; and*
- *In-vitro fecundation techniques.*

(2) Agriculture

- *Study and Production of Bioinsecticides and bacteriocins of Bacillus thuringiensis;*

- *Production of Transgenic potato plants resistant to PVY; and*
- *Search of genetic markers responsible of durum wheat resistance to salt and drought stresses.*

(3) Production and applications of industrial enzymes.

(4) Biological wastewater treatment:

- *Study of biological systems involved in the biodegradation and biotransformation of recalcitrant compounds;*
- *Anaerobic wastewater treatments, namely isolation and characterisation of new strains able to degrade phenolic compounds and detergents; and*
- *Development of a process for margin treatment.*

4.2 Biotechnology programmes

4.2.1 General

Tunisia has developed a relatively important programme in industrial enzymes. This programme started with a project oriented to the development of a process for glucose syrup production.

4.2.2 Glucose syrup production process

The sugar consumption in Tunisia is estimated as the following:

- 230,000 tons of sucrose, 200,000 tons are imported; and
- 10,000 tons of glucose syrup (DE 42) are also imported

Out of the 230,000 tons, 35,000 tons are used for soft drinks, which could be substituted by fructose syrup.

In order to cover our need for glucose syrup (10,000 tons) and to substitute a part of imported sucrose for fructose syrup, Tunisia has developed a production process of glucose and fructose syrups.

Taking in consideration the deficit in the wheat and corn production (sources of starch), Tunisia has developed this process by using a sub-product of wheat grinding (gruel), which contains approximately 60% of starch.

This process has been developed in the laboratory and at a pilot plant scale (1ton/day).

To transform starch to glucose, enzymes such amylase, pullulanase and glucoamylase are needed. If we want to transform glucose to fructose, we also need the glucose isomerase.

For this reason and to use enzyme technology for other applications such as detergents, textile industries, starch industry…, we developed programmes to study and produce: amylases, pululanases, glucose isomerase, cellulases and pectinases. Table 2 shows the world market of industrial enzymes.

4.2.3 Studies carried out on glucose isomerase

4.2.3.1 Background

- D-glucose isomerase (GI) is a key enzyme used for the production of sweet high fructose syrups;
- Referring to the industrial standard conditions, the conversion of glucose to fructose is generally done at 60°C with a pH between 7 and 9. In this case, only 42% of fructose is obtained due to the catalytic equilibrium between glucose and fructose;
- To shift this equilibrium towards fructose, the temperature must be increased. However, most of the commercial GIs work at high pH and the isomerisation at high temperature and pH, generates the formation of secondary reactions and undesirable bitter sub-products such as D-psicose (Hiromichi *et al.*, 1995). Several thermostable glucose isomerases have been described, nevertheless, the optimal pH of these thermostable glucose isomerases is generally over neutrality.

4.2.3.2 A new thermostable glucose isomerase from a thermophilic Streptomyces sp. SK strain, with a lower optimal pH has been isolated from a Tunisian soil of hot springs

The study (Table 3) shows that the enzyme was stable at 80°C for more than 5.5 hours, while at 90°C its activity declined with a half-life of approximately 5hours.

4.2.3.3 Molecular cloning of the GI (xyla) of Streptomyces SK strain

- A genomic library was performed in *E.coli* using a multicopy cloning vector;
- This library was screened by using as probe, the *XYLA* of *S.olivochromogenes; and*
- We succeeded in cloning a 6.5 kb DNA fragment encoding the *XYLA* of *Streptomyces SK* strain.

AA Sequences analysis

The XYLA SK protein consists in 386 aa with a theoretical molecular weight of 42,702 Da (Table 4). XYL A SK presents the highest percentage of hydrophobic aa. This could contribute to the flexibility of the enzyme and consequently to its tolerance of high temperature and a large range of pHs.

Comparison between XYLA SK and XYLA of S.olivochromogenes

The highest homology (94%) was found with XYLA of *Sterptomyces olivochromogenes*. The comparison of the characteristics of these two enzymes showed that:

- The XYLA of S.olivo: pH_{op}: 7-9 and a T_{op} of 80°C;
- The XYLASK: pH_{op}= 6.5 and a T_{op}= 85°C;
- The comparison of the aa sequences showed: there are only 18 different aa out of 386 aa between these two enzymes Table 5; and
- We establish that 10 over 18 aa of *S. olivochromogenes XYLA* are substituted for aa hydrophobic aa in the XYLA SK protein.

For this reason, we assume that there is a relationship between thermostability and tolerance to lower pH on the one hand, and the rate of hydrophobicity on the other hand.

5 INTERNATIONAL COOPERATION

Tunisia is developing cooperation, in the field of Biotechnology, on a bilateral or multilateral basis mainly withFrance, Germany, Portugal, Japan, USA, India, Lebanon, Morocco, Algeria and Egypt. She also has co-operation agreements with the EU, FAO, WHO, and ICG.

6 CONCLUSION

The Tunisian Policy in the Biotechnology Development is clearly defined and based on:

- Strengthening human resources;
- Increasing the research budget;
- Developing research centres, laboratories and research units;
- Setting the **"Biotechnology Evaluation Committee"** (An independent national assessment body);
- Reinforcing training programmes; and
- Paying attention to the regulations issues (IPR,GMO….).

Table 1. Research Budget (RB)/Gross National Product (GNP)

Year	1991	1996	2001	2004
RB/GNP (%)	0.28%	0.35%	0.5%	1%

Table 2. World market of enzymes (US$ million)

Applications	1983	1995	2005
Food	180	460	606
Detergent	172	432	1110
Textile	29	70	90
Paper Industry	1.1	2.7	11
Tanneries	11.5	28.5	36
Others	6.4	6.8	58
Total	400	1000	1900

Table 3. Thermostability of GI SK

	Residual activity (%)					
Time (min)	30	90	150	210	270	330
Temperature (°C)						
80	100	100	100	100	100	100
90	100	98	97	94	53	40

Table 4. XYLA SK exhibits a large homology with those of other *Streptomyces*.

XYLA of	Identity %	% of Hydro. a.a.
Streptomyces sp SK	100.00	47.4
S.olivochromogenes	94.0	45.0
S.violaceoniger	92.49	46.1
S.albus	91.97	45.0
Thermus thermophilus	58.81	46.1
B.subtilis	13.21	46.6
E.coli	10.62	46.9

Table 5. Comparison of the aa sequences of SSK ans S.oliv

Pos	40	62	69	71	100	102	109	132	152	175	195	279	327	332	336	346	348	356
S.SK	**D**	**A**	**A**	**V**	**A**	**A**	**A**	**V**	**A**	**I**	**I**	**W**	**E**	**A**	**Q**	**L**	**A**	**Y**
S.olv	**E**	**S**	**S**	**I**	**D**	**G**	**D**	**T**	**V**	**T**	**V**	**Y**	**Q**	**S**	**E**	**V**	**E**	**F**
		♠																

REFERENCES

1. Drocourt D., Bejar S., Calmels T., Reynes J.P. and Tiraby G. (1988). Nucleotide Sequence of the Xylose Isomerase Gene, *Streptomyces Violaceoniger*. Nucleic Acids Res. 16: 9337.
2. Tiraby G., Bejar S., Drocourt D., Reynes J.P., Sicard P.J., Farber G.K., Glasfeld A., Ringe D., and Petsko G.A. (1988). Genetic, Enzymatic And Crystallographic Studies of the Xylose Isomerase of two *Streptomyces.*
3. Bejar S., Belghith K., Gargouri R. and Ellouz, R., (1995). Construction of New Strain of *Streptomyces Violaceoniger* has strong constitutive and stable Glucose-Isomerase activity. Biotechnology Letters 16:1259-1264.
4. Belguith K. And Bejar S. (1998). A Thermostable Glucose Isomerase having a low acting ph: Study of activity, Molecular Cloning and Nucleotide Sequence of Corresponding Gene. Biotechnology Letters 20: 553-556.

Development of the Biotechnology Sector: The Iraq Perspective

SAMI A AL-MUDHAFFAR*
Academy of Sciences
Baghdad, Iraq

1 ABSTRACT

Iraq occupies a very diversified geographic and climatic zone and assumes a great deal of importance. However, its wealth is under UN sanctions. Its health, and agricultural problems are diverse.

For almost twenty years (1970-1990), its biotechnological activities have sought to address various problems of agriculture and medicine. In recent years (1990s), these activities have started in a very limited way, to use some of new developments in basic biotechnology such as molecular biology and genetic engineering.

Various centres and departments are involved in the national biotechnology research systems in Iraq. The biological and agricultural research centres also integrate such activities into their research programmes.

Presently, these centres implement different types of programmes (long term and short term) in agriculture and medicine, for the generation, development and transfer of Biotechnology. Major components of these programmes include development of technology transfer facilities and establishment of international collaboration in the field of Biotechnology.

Medical biotechnology in Iraq, as a whole, demands strong collaboration in order to reduce the present gap between Iraq and developed countries. Agricultural biotechnology, on the other hand, seeks to increase food crop resistance to insects and diseases, utilize a wide variety of techniques, including biological control and various forms of biotechnology, such as tissue culture, molecular genetics and genetic engineering.

This paper analyses major activities of biotechnology in health, agriculture and basic science in Iraq; and offers general background information on the development of biotechnology. It examines the current state of biotechnology research and potential thereof, and suggests an outline for the strategies of the future of Biotechnology in Iraq.

* Professor of Biotechnology and Fellow of Islamic Academy of Sciences; Minister of Education (June 2004).

2 INTRODUCTION

Iraq, which has already entered the biotechnology era, whether on purpose or by chance, will inevitably suffer the consequences of the widespread use of such techniques in industrialized countries. Therefore, the question is no longer whether such a move is desirable, but more how can it best be put into use.

Currently, the people of Iraq are suffering from a poor quality of life because of UN sanctions, which have resulted in:

- Food shortage and malnutrition;
- Unsafe drinking water;
- Improper sanitation system; and
- Poor health care.

This is why Iraq needs to have at least a basic level of skills to define and implement policies in biotechnology and the other technologies. Biotechnology alone will not feed Iraqis or give them better health, but it would be irresponsible not to see it as one of the available tools. A certain number of social, cultural or institutional conditions also have to be satisfied before technical success can be converted into economic and social progress.

3 HISTORICAL ASPECTS OF BIOTECHNOLOGY IN IRAQ

Biotechnological activities were known to ancient Iraqis in Sumer and Babylon. For instance, fermentation processes and perfume extraction were very well-known to them.

It has been reported that the first experiment in scientific research was performed in the palace of the king of Babylon. The experiment was carried out by two palace ladies who prepared perfumes by distillation from plants (steam distillation).

Cheese making is believed to have started in the Tigris-Euphrates valley (in present day Iraq), approximately 8,000 years ago.

In the early 1970s' scientific infrastructure construction began and several research activities were planned and set-up.

Most of these biotechnologal activities were limited to traditional methods to serve their needs; i.e. industrial fermentation, soil microbiology and bioconversion of waste products.

The government showed its interest in providing support to biotechnology by offering to host the first Arab Conference on Genetic Engineering in 1984. As a consequence of that meeting, the Council of Scientific Research established the "Genetic Engineering Centre," which became responsible for all research and development in this field. Iraq then signed an agreement of affiliation, through its Scientific Research Council, with the International Centre of Genetic Engineering (ICGEB).

Since then, and during the early 1990s research in agricultural biotechnology, especially dealing with production of Biocides and Biofertilizers and Tissue Culture Techniques was carried out by the various research centres.

The Ministry of Higher Education, during the 1990s, established new centres for genetic engineering research. These centres were primarily involved the application of basic genetic engineering particularly in the following fields:

- **Molecular biology:** for enzyme production, industrial and clinical diagnosis;
- **Cell biology:** for animal cytogenetics (chromosome and gene mapping); and
- **Microbial genetics:** for the production of useful microbial compounds through genetically improved strains.

Iraq through a new council of biotechnology at the University of Saddam has been monitoring international development in biotechnology in order to apply some of these techniques within the country's research institutions. As a result, the council has developed plans for biotechnology and genetic engineering in the sectors of health, agriculture and basic biotechnology. The major components of this plan are:

- Generation, transfer and development of biotechnology;
- Development of trained personnel;
- Strengthening of research and technology transfer facilities; and
- Establishment of international and regional collaboration in the field of biotechnology.

4 APPROACHES OF BIOTECHNOLOGY IN IRAQ

4.1 General

Most biotechnological activities, which are applied in Iraq, are limited to traditional methods and serve their needs through:

- Fermentation;
- Antibiotic industry;
- Single cell protein;
- Plant biotechnology:
  - ✓ *Tissue culture;*
  - ✓ *Soil fertility, through biological activities;*
  - ✓ *Increasing food production through plant cell-culture;*
  - ✓ *Bioconversion of waste for food and feed ingredients.*

4.2 Fermentation

During the early 1970s, Iraq established traditional fermentation industries, baker's yeast production, ethanol, acetic acid, acetone, butanol and citric acid production. In 1970, a factory for making bakers yeast from sugar beet molasses

was established with plans for the production of compressed yeast. This industry faced many problems, especially in dried yeast production:

- There are abundant sources of raw materials for fermentation in Iraq. Large quantities of hydrocarbons, and carbohydrate by-products (molasses) and lignocellulose waste are found;
- Fermentation of food crops like dates, which enjoy comparatively large market sizes, does not receive sufficient attention. Local research on bioreactors is therefore needed to support the development of new processes and improve the performance of food industry in Iraq. Bioreactors also play a key role in the production of enzymes used in the beverage, detergent and leather industries.

4.3 Antibiotics industry

The antibiotics industry in Iraq started in 1970 with the production of penicillin and tetracycline. Tetracycline production continued until 1980. This industry was discontinued for economical and technical reasons as a result of sanctions. Research and development programs may have a positive effect for restarting this bioindustry and the production of different types of antibiotics.

As the pharmaceutical industry in Iraq is directed to satisfy the local market needs, appropriate biotechnologies must be developed against various diseases that are endemic.

4.4 Single-cell protein

A research and development programme for single-cell protein (SCP) production at pilot plant level started in Iraq in 1982. The production of SCP from methanol using local and imported strains of Candida Utilis, was investigated. The research plan included an economic feasibility study and the assessment of technological and nutritional aspects of SCP under local conditions. Then, another pilot plant was established to utilize date syrup for the production of bakers yeast and SCP.

The most important achievements of this programme were the establishment of pilot research facilities, training of personnel, the nutritional assessment of available commercial SCP products and the isolation of several methanol-utilizing bacterial cultures.

4.5 Plant biotechnology

4.5.1 General

Plant biotechnology applications in Iraq include soil fertility (nitrogen fixation) using yeast strains in a mixed culture and cell conversion. Scientists have carried out research on nitrogen fixation by grain legumes. The main objective of this research was to increase the yields of grain legumes while decreasing the input of inorganic nitrogenous fertilizers.

4.5.2 Tissue culture

- In view of the importance of the date palm in Iraq, a tissue culture laboratory was established in 1979 at the Agriculture and Water Resources Research Centre in Baghdad. Another laboratory was initiated at the Genetic Research and Biotechnology Scientific Research Centre for the improvement of plant production. Tissue culture laboratories were also established at the Universities of Basrah and Mousl and also at the Ministry of Agriculture. The latter worked in collaboration with FAO through regional centre for date-palms in the near east and north Africa. The major objective was the commercial propagation of plants by in vitro techniques;
- In 1982, the vegetative micropropagation through tissue culture was carried out as a promising technique. However, future research is needed to early following and lack of uniformity of the closed plant; and
- Date palm propagation by tissue culture was also implemented at research institutes and universities in Baghdad and Basrah. Other species such as lettuce and potatoes were propagated by tissue culture at the department of Biology of Mosul University.

4.5.3 Other activities

- Research at the Faculty of Agriculture and Biology in the Nuclear Research Centre was concentrated on the study of a sexual embryoensis techniques and the induction of mutations by mutagenic agents.
- Agricultural and forest residues in Iraq are considered renewable resources that can be utilized by biotechnologal means for the production of food, fertilizers and fuels.

5 BIOTECHNOLOGY IN IRAQ SINCE THE IMPOSITION OF SANCTIONS

As a result of sanctions, many fields in public health system were affected including critical areas such as food security and nutrition, water resources, women's health, children health, national health emergencies, hospital care, humanitarian donations and international co-operation. Scientific fields were also affected such as Oncology, Cardiology, Nephrology, Endocrinology, Ophthalmology, diagnostic testing of blood, scientific information and medical education, pharmaceutical and biotechnology inputs, etc.

In agriculture, the UN sanctions prevented the import of fertilizers. Shortages in production of crops led to the deterioration in the Iraq's population nutritional intake (daily caloric intake).

- Iraq is facing three main threats: food supply, health services and environmental protection. Millions of people in Iraq suffer from poverty and poor health due to UN sanctions;

- The food security situation in Iraq is unique; it is not related directly to biotechnology problems. Genetically altered seeds are not necessarily needed to feed Iraq. This view rests on two critical assumptions, which we question: 1) Poverty is not due to a gap between food production and growth of population, and 2) Biotechnology is not the only or best way to increase agricultural production;
- Iraq is not able to undertake effective agricultural biotechnology research for its own urgent needs without the scientific support of developed countries. Iraq needs more investment in developing appropriate agricultural biotechnology; and
- Iraq is basically an agricultural country. Approximately 80% of the total area is devoted to cereal production, mainly wheat, barley, rice and corn. The remaining 20% includes a wide variety of crops, date-palm, citrus, tobacco, cotton and others. In general the average production level of all the important crops in Iraq is very low compared to those in developed countries. In the last 10 years, plans have been organized by the Iraqi government with cooperation of agricultural scientists to improve both the quality and quantity of crops per capita, using proper machinery and equipments, fertilizers and suitable control measures.

6 APPLICATIONS OF BIOTECHNOLOGY IN IRAQ IN THE EXPERIMENTAL STAGE

6.1 Animal production

The use of biotechnology in animal production in Iraq has occurred, in the field of reproduction, animal health, feeding, nutrition, growth and production.

In the field of reproduction, new biotechnologies such as embryo transfers, in vitro fertilization, cloning and sex determination of embryos have been studied experimentally for different types of livestock at the faculty of Agriculture and Biology in the Nuclear Research Centre. Animal health can be improved with new biotechnology methods at experimental stage of diagnosis, prevention and control of animal diseases.

Biotechnology in animal nutrition concentrates on improvement of feed; enzymatic treatment, the decreasing of the antinutritional factors in certain plants, such as legumes, which are used as feed.

6.2 Plant production

At present, more traditional aspects of biotechnology such as the followings are used:

6.2.1 Tissue culture

The application of tissue culture does not require very expensive equipment. This technology was applied in Iraq to improve local varieties of food and crops.

6.2.2 Pest and weeds

In Iraq, most of the land is affected by many weeds that cause big losses in agricultural crops. Since 1970s several herbicides were used to control the weeds of corn, cotton and vegetable fields.

6.3 Biocontrol programmes used for controlling pests

The Plant Production Research Centre, State Board for Agricultural Research, College of Agriculture, Baghdad and Mosul Universities, Agriculture and Biology Research Centres and Iraq Atomic Organization are the main research centres in Iraq, which carry out different research studies on the biology, taxonomy and control of pests. These research centres successfully adopted control measures on the most important pests attacking crops and vegetables by applying chemical and agricultural methods. Promising results were obtained from many plant extracts against pests, by inhibition pest life cycle.

In Iraq, most of the agricultural lands are subjected to weeds growth, causing big losses to the agricultural crops. Many research workers have however confirmed the positive results of the weed control measures that resulted in increase of the yield of the crops.

Researchers started to evaluate herbicides since 1965, and in the mid seventies, herbicides were applied to control the weeds in wheat, rice, corn, cotton, potatoes and tomatoes. Increased support is needed to expand research designed to develop new herbicides that are not likely to pollute ground water and that provide reliable control.

Several centres are involved in biocontrol programs such as:

- Agricultural and Biological Research Centre (Baghdad);
- State Board for Agricultural Research / Ministry of Agriculture Baghdad; and
- College of Agriculture / Baghdad and Mosul Universities.

Commercially, research centres introduced two biocontrol mutant fungi. Both fungi were successfully applied to control plant parasitic nematodes and soil born fungi on vegetables and citrus.

State Board for Agricultural Research successfully adopted several control measures on the most important pests attacking field crops, vegetable and fruit trees, by applying chemical, biological and agricultural methods. Many insect growth regulators, biocontrol agents, fungi and plant extracts were experimentally applied on small and large-scale fields. Most of the research

studies of the graduate students in the department of plant protection concentrated on the biological control, and plant extracts.

At the present time, UN sanctions which have been imposed on Iraq since 1990, destroyed most of biological control programs and Iraq is facing a lack of well trained personnel, and shortage facilities. As a result, the first generation biotechnologies used in Iraq such as insect resistance, herbicides resistance, are not easy to apply any more.

6.4 Health biotechnology

- Several centres are interested in carrying out research on health biotechnology. The following may be mentioned:

  (a) Saddam Centre for Cancer and Medical Genetics Research (SCCMGR);
  (b) Institute of Biotechnology and Genetic Engineering for Graduate Students, University of Baghdad;
  (c) Department of Genetic Engineering, College of Science, University of Baghdad; and
  (d) Genetic engineering departments in a number of universities.

- SCCMGR is engaged in several lines of in health related biotechnological activities:

  (a) Cloning of tetanus toxic gene into tumour cells;
  (b) Preparation of tumour cell lines in vitro for gene therapy technique;
  (c) Preparation of restriction enzymes vectors for gene therapy; and
  (d) Studies on disorders of mitocondrial DNA in muscular dystrophy.

- Several biochemists have participated in various research projects that deal with diagnosis, and monitoring of several types of tumours.

7 BIOTECHNOLOGY FOR THE FUTURE

7.1 Development of biotechnology applied to food and health to face basic human needs in Iraq

7.1.1 Background

The need for the application of biotechnology to face the basic needs regarding food and health in Iraq is real. There are different approaches such as the development of plant biotechnology, biotechnology applied to livestock production and biotechnology applied to food processing.

So, the suggested components of biotechnology plan include:

- Micropropagation: through for example tissue culture for multiplication;
- Genomics: the molecular characterization of all species;

- Bioinformatics: the assembly of data from genomics analysis into accessible forms;
- Diagnostics: the molecular characterization and identification of pathogens;
- Molecular breeding: the identification and evaluation of desirable traits in breeding programmes with the use of marker assisted selection
- Transformation: the introduction of single genes conferring potentially useful traits into crops, livestock, fish and tree species;
- Vaccine technology: use of modern immunology to develop recombination DNA vaccine for control of lethal diseases;

Plans for future biotechnology research should be formulated through several priorities:

- Food security through the increase and improvement of agricultural production. Breeding for higher-yielding plant varieties and improve nutritive development values. Pest and pathogen-resistant genotypes and conservation of plant genetic diversity;
- Production of pharmaceuticals for the extraction of biologically active plant substances;
- Immunology: Production of vaccines and monoclonal antibodies; and
- Use and recycling of agricultural products for the production of ethanol, acetone, butanol and methanol.

7.1.2 Food security

The applications in agricultural biotechnology in Iraq have the promise of bringing about the much-needed requirements in agricultural production, such as enhancing resistance/tolerance to abiotic stresses (drought and salinity) and providing options for better rotation to conserve natural resources.

Iraq is neither in the process of testing genetically enhanced products in number of crops, nor commercial products are in the market to date. Many technological advances are not visible in the farmers' fields in Iraq but in the future are expected to provide ways to improve crops in a precise and fast manner. Use of functional genomics to address complex traits, marker assisted breeding to ensure presence of key genes, improving nutritional quality, and managing natural resources better by use of efficient monitoring tools, are areas in which Iraq must be an active participant so that specific needs of food security are achieved.

7.1.3 Agricultural production

Despite the importance of health and industry sectors, the suggested priorities in the plan should have great emphasis on agrobiotechnologies for two reasons; 1) Research on plants for crop improvement directly relates to specific ecological conditions predominant, whereas biotechnology applications for industry and human health are more difficult in Iraq, 2) Preliminary data indicate that most biotechnology research activities in Iraq relate to agriculture.

The following classes of agricultural biotechnology are suggested to be used in Iraq in the future:

- Gene transfer technologies, which provide transgenic plant, resistance to many pests pathogens, herbicide as well as resistance to stress such as temperature, drought and salinities;
- Non transgenic biotechnological approaches for improving the efficiencies and effectiveness of conventional plant breeding methods; and
- Technologies for better monitoring of natural resources and environment.

Additional suggestions for the implementation of the plant are the following:

Plant biotechnology

- ✓ The establishment of biological treatment plants for sanitary wastewater and utilization of the treated wastewater for landscaping and agriculture;
- ✓ The establishment of commercial production of inoculants such as Rhizobium and the development of efficient methods for recycling agricultural waste such as beet molasses and maize and rice straw. Research should be carried out on the use of biofertilizers to increase rice yields in Iraq;
- ✓ The use of biotechnological techniques for the development and improvement of bio-insecticide for control of plant pests;
- ✓ Increasing protein content of rice by the application of biotechnological techniques. Rice is one of top five cereals grown in Iraq. It has unique capabilities and can grow in stress conditions, and it is a crop of choice for Iraqi people. Rice has received comparatively less attention for research in general;
- ✓ The creation of date-palm clones resistant to disease, and the application of tissue culture to improve date-palm varieties;
- ✓ Future research is needed to overcome the difficulties related to early flowering and lack of uniformity of cloned plants; and
- ✓ Production of secondary metabolites by tissue culture, the selection of plant cell lines for stress tolerance to salinity and drought and also the production of virus-free potatoes planting material; and the micropropagation of plant.

Animal Biotechnology

Biotechnological application in livestock and fish production and the adoption of embryo culture to improve local animal breeds through embryo transfer technology, pre-implantation and embryo freezing.

Microbial Biotechnology

- ✓ Microbial biotechnology for ethanol production from sugar by-products and methanol production from agro-industrial wastes;

- ✓ Microbial genetics: elimination or degradation of pollutants transformation of cellulolytic nitrogen fixers; construction of Saccharmyces cervisiae strains capable of cellulose, cellobiose or lactose consumption;
- ✓ Proper technology to convert biomass into biofuel and biogas to convert agricultural biomass and animal droppings into biofuel and manure; of the various wastes used for biomass production. Rice straw is one of the possible applications;
- ✓ Bioconversion of lignocellulosic wastes to protein-enriched fermented materials followed by the production of microbial biomass from the hydrolyzed cellulose product; and
- ✓ The use of bacterial treatment for the removal of oil and chromium; and also in the nitrification-denitrification process to remove ammonia.

7.2 Health biotechnology

7.2.1 General

Medical Biotechnology in Iraq, as a whole, will demand a strong collaboration in order to reduce present gap between developed countries and Iraq to achieve this aim:

- A centre of bone marrow transplant should be established in one of the hospitals. This requires the availability of experts, equipment and materials; and
- Iraq is facing cancer and genetic diseases, hence research projects in gene therapy should be initiated.

7.2.2 Pharmaceutical industry

The pharmaceutical industry in Iraq should be expanded and developed so as to meet at least the local requirements. Biotechnological techniques should be introduced.

7.2.3 Environment

- Applications of natural occurring organisms (e.g. yeast, fungi and plants) should be used to convert hazardous substances in soil;
- Using microorganisms pollutants from sewage systems to clean up industrial sites; and
- The use of biotechnology to avoid pollution is of increasing importance such as the use of bioreactors to treat hazardous products.

7.2.4 Forensics

Applications of technologies to forensic science.

7.2.5 Bioinformatics

This new discipline (biology and computing), which will be the core of biology in the 21st century, should be used for measuring and monitoring thousands of genes at one time. Computer-aided bioinformatics will stimulate future developments in the pharmaceutical industries.

7.3 Cooperation with international agencies

- Co-operation with Islamic and International agencies and countries is required;
- Well-trained scientists from Arab and Islamic countries, directly involved in the training and transfer of various biotechnologies, should be interacted with;
- Post-graduate short training courses sponsored by international organizations such as the United Nations Educational, Scientific and Cultural Organization (UNESCO) should be organized in the various fields of biotechnology;
- Training of medical personnel in bone marrow transplantation, to help in gene therapy especially for cases of leukemia and lymphoma.
- Broadening the biotechnology base is a must for characterization, collection and conservation of germplasm that is already in gene bank collections around the world; and
- Strengthening capabilities/developing projects/visits/ and training programs of mutual interest to all participating countries in the following areas of biological control:
    - ✓ Exchange of biotic agents on a case to case basis;
    - ✓ Mass production of host insects and natural enemies;
    - ✓ Biological suppression of crop pests by developing joint projects;
    - ✓ Computerization of information and networking research organization in different countries; and
    - ✓ Training in different aspects of biological control.

7.4 Establishment of Islamic Biotechnology Center

In the light of what has been presented in this paper, one would come to the conclusion, that an Islamic Biotechnological Centre is really needed. Islamic countries which have long experiences in biotechnology could greatly help in establishing such a centre.

7.5 Ethical issues related to biotechnology

The Islamic world needs to have sharp opinions on various current issues related to biotechnology and genetic engineering such as genomics, human cloning and genetically modified organisms.

REFERENCES

1. Alian W., (2000), GMOs in developing countries, Ag. Biotech Net. Vol. 2 July, ABN 052.
2. Altiere M.A., (1999), "Ten reasons why biotechnology will not ensure food security, protect the environment and reduce poverty in the developing world", Food first (Institute for food and development policy), U.S.A.
3. Anderson P.P., (2001), The Australian, Edition 1, page 31.
4. Anderson P.P. and Cohen M.J., (2000), "Biotechnology and the CGIAR", paper prepared for the presentation at the international conference on "Sustainable Agricultural in the next Millennium. The Impact modern of biotechnology on developing countries."
5. Anderson P.P., (1999), "Biotech and The Poor," in the Washington Post, October 27, page A31.
6. Anuradha M., (1999), Redefining security in the new world order. Food first (institute for food and development policy), September 1, pp. 1-6.
7. Biotechnology, Industry Studies (2000), The Industrial College of the Armed Forces, pp. 1-20.
8. Chen R.S. and Kates R.W., (1994), Food Policy, Vol. 19, pp. 192-208.
9. Ehrlich P.R., Ehrlich A. and Daily G.C., (1993), "Population and development review," Vol. 19, pp. 1-32.
10. Essmat E., (2000), A powerful instrument in the response to human basic health need, WCS Abstracts Forum I and II: 1.3 Science in Response to Basic Human Needs.
11. FAO, (1999), The state of food insecurity in the world, Rome, FAO.
12. FAO, (1996), Rome Declaration on World Food Security and World Food Summit Plan of Action, World Food Summit, 13-17.
13. Graham S.P., (1997), "The prohibition of biological weapons-current activities and future prospects," International Review of the Red Cross, No. 318, p. 267-281.
14. Hamdan Y. and Villalobos V.M., (2000), "Status and prospects of biotechnology in the Near East and North Africa," Chapter 16, pp. 1-12.
15. Hans B. and Patrick C., (1995), "New Strategic Priorities," Washington Quaterlym p. 116.
16. Hildebrand D., (2001), The New "Smart" Sanctions on Iraq (Acommentary), Director, Inter-Church Action, June 8, citizen concerned for the people of Iraq, pp. 1-6.
17. IBS Activities (2001), Isnar biotechnology service activities, pp. 1-12.
18. Iris H. and Marie C., (2000), "The Case of the Biological and Toxin Weapons Control Regime. Questions and Compliance: Arms Control and Disarmament Cluster," pp. 1-9.
19. Juune G., (2000), Biotechnology: the impact on food and nutrition in developing countries, (Dr. Gerd Juune is a Professor of International Relations at the University of Amsterdam, and editor of the Biotechnology and Development Monitor).

20. Kates R.W., (1996), “Ending Hunger: Current Status and Future Prospects,” Consequences: Vol. 2, pp. 1-11.
21. Menon K.K.G., (1993), “Food security in developing countries,” in Biotechnological Applications for Food Security, pp. 57-66.
22. Ritcheson P.L., (1997), Proliferation Scope, Prospects and Implications, IVWC Review, pp. 1-4.
23. Utah State U., Biotechnology Center, Making cheese using modern biotechnology, (2000), pp. 1-8.
24. Zehr U.B., (1998), Broadening the technological base-biotechnology and genetic resources, Maharashtra Hybrid Seeds Co. Limited, India, pp. 1-7.

Present Status and Future Prospects of Biotechnology in Sudan with Special Reference to Veterinary Medicine

HASHIM M ELHADI*
and
IMADELDIN E ARADAIB**

1 ABSTRACT

Sudan is the largest country in Africa. The animal resources constitute one of the major national sources of income. It is, therefore, becoming increasingly necessary to provide the control measures for these animal populations against various diseases. To improve herd health monitoring, production of vaccines against contagious and infectious diseases biotechnology activities were initiated to facilitate vaccination programs. Thus, biotechnology was first applied in vaccine production in 1930. Vaccines were produced locally for immunization against diseases of veterinary importance. At this particular point in time, at least eleven viral and bacterial vaccines were produced in Sudan. The vaccines are produced in the Central Veterinary Research Laboratory, Soba. Attempts to produce additional vaccines against parasitic infections are in progress.

Recombinant DNA vaccines are now being produced against viral and parasitic diseases. With the surge of new techniques in cellular immunology and molecular biology, even complex organisms like Schistosomes became potential candidates for vaccine production. Schistosomiasis is an endemic disease in many parts in the Islamic world including Sudan. Comparative studies were conducted on irradiated, killed and recombinant *Schistosoma mansoni* subunit vaccine expressing the glutathione-s-transferease (GST) gene in *E.coli* and yeast expression vectors. These studies were conducted at the faculty of veterinary science, University of Khartoum, Sudan, in collaboration with the Department of Medical Helminthology, Pasteur Institute, France (Aradaib *et al*., 1988).

Theriogenology shows rapid expansion in biotechnology. Artificial

* Department of Physiology, Faculty of Veterinary Science, University of Khartoum, Sudan; and Fellow of the Islamic Academy of Sciences.

** Department of Medicine, Pharmacology and Toxicology; University of Khartoum, Sudan.

insemination (AI) was recognized by animal breeders a long time ago. The Ministry of Animal Resources established the National Artificial Insemination Centre in 1976. Subsequently, semen collection, evaluation and insemination of synchronized cows were made possible locally through importation of good quality semen. Currently, there is an on-going research for artificial insemination project to improve the genetic make up and productivity of the Sudanese Nubian goats. A research team at the faculty of veterinary science, University of Khartoum, conducts the work. The embryo transfer (ET) was started in 1983 in bovine. Successful delivery of a full term calf from ET was reported in 1984, at the Gezira University farm. Nuclear transfer (NT) experiments were conducted both in mice and in cattle. Some of the teaching staff of the department of theriogenology, faculty of Veterinary Science of the University of Khartoum have become experts in NT. The Department of Theriogenology houses a well-equipped laboratory for AI and ET. A modern laboratory for NT is expected to be fully installed in the near future.

Nucleic acid amplification technology was also applied to the area of molecular diagnostics and forensic medicine. Mitochondrial cytochrome-b gene (Mtcyt-b) PCR-based assay was developed for detection of animal derived contaminants in processed human food and in animal feed ingredients. This was done to address possible contamination of animal feeds with other animal protein sources, an important issue with the concerns associated with spread of bovine spongioform encephalopathy (BSE) in Europe, since bovine meat by-products were recycled as cattle feed. Similar studies are currently underway to evaluate the potential of the Mtcyt-b PCR for detection of pork or swine-derived products in processed human food, as accidental pork consumption is not uncommon, although prohibited in the Islamic religion. These studies are conducted in the Molecular Biology Laboratory of the Department of Medicine, Pharmacology and Toxicology, Faculty of Veterinary Science, University of Khartoum. Future prospects of biotechnology in Sudan with special emphasis on veterinary medicine are discussed in the following section.

2 PRODUCTION OF VACCINES

2.1 General

Production of vaccines against diseases of veterinary importance was started earlier in (1930) at the Central Veterinary Research Laboratory (CVRL), Animal Resources Research Corporation, Ministry of Science and Technology.

Recent research was focused on development of vaccines against parasitic diseases of public health importance. Among these diseases, Schistosomiasis was received special attention. The disease is caused by different species of Schistosomes. *Schistosoma bovis*, is a major veterinary problem in Sudan. (Hussein, 1968; Bushara *et al.*, 1978; Saad *et al.*, 1980; Aradaib, 1988 and

Aradaib *et al.*, 1993). The economic importance of the disease is attributed mainly to losses occurring in animals 6-30 months of age and is mainly due to morbidity, mortality, retarded growth, liver condemnation and poor subsequent reproductive performance.

2.2 Irradiation-attenuated *Schistosoma bovis* vaccines

Irradiated *S.bovis* cercarial or schistosomular vaccines sensitized the host to schistosome antigen without acquisition of a patent infection (Hussein and Bushara, 1976). At least partial immunity to infection can be induced in laboratory animals by using irradiated schistosomular vaccine). In cattle, *S.bovis* infestation can be partially prevented by prior immunization with homologous irradiated cercariae or Scistosomulae. Irradiated cercarial schistosomular vaccines have shown their greatest promise in the control of bovine schistosomiasis; Experimental immunization of zebu calves with *S.bovis* cercariae irradiated at 3 or 20 K rad induced partial protection against homologous challenge as judged by parasitological and hematological parameters (Bushara, 1978; Aradaib, 1988; Aradaib, 1995). Under field conditions in the endemic area of the White Nile province of the Sudan a single intramuscular injection of 10,000 cercariae of *S.bovis* irradiated at 3 kilo-rad induced a 60% reduction in worm recovery and 80% reduction in faecal egg counts. Improved survival and body weight in the vaccinated calves were also recorded. The protection lasted at least 40 weeks (Majid *et al.*, 1980). However, the shorter life span of the normal cercariae or the shistosomula necessitates the need for expensive cryopreservation of the vaccine. In addition, concerns about the pathological lesions, which may be produced by the irradiated vaccines, limit their use as effective vaccines against schistosomiasis (Aradaib, 1994). Moreover, thousands of worms were recovered from the immunized calves at the time of perfusion (Bushara *et al*., 1978; Majid *et al*., 1980, Bushara, 1984 and Aradaib, 1988). This indicated that the irradiated *S.bovis* vaccines induced immunity against challenge infection by suppressing worm fecundity without causing any lethal effect on worm viability.

2.3 Non-living (dead) vaccines

Unfortunately, experimental immunizations with dead schistosome antigens have been largely unsuccessful. In previous studies, we demonstrated that Nubian goats treated with immunopotentiating drugs such as levamezole (L-tetramezole) developed partial protection to infection with *F. gigantica*. The protection was reflected by reduced worm recovery at necropsy and by development of less severe hepatic lesions (Goraish *et al*., 1988). Primary infection of sheep and cattle with *F.gigantica* provided partial protection against *S.bovis* challenge. In our laboratory, extensive immunization experiments were conducted in the bovine species. Zebu calves were injected with schistosome antigen prepared from adult worms of *S.bovis* in an attempt to stimulate resistance to challenge with this trematode parasite. Unfortunately, our immunization experiments proved

unsuccessful. There was no significant difference between the vaccinated and the control groups as determined by fecal and tissue egg counts, worm recoveries and hematological parameters (Aradaib *et al.*, 1993). In addition, *S.bovis* whole-egg antigen did not protect zebu calves against experimental schistosomiasis using homologous challenge as judged by parasitological and hematological parameters (Aradaib *et al.*, 1994). Protection was not achieved in spite of the detection of strong immune responses to both adult schistosomes and whole-egg antigens using the agar gel diffusion (AGID) or the enzyme-linked immunosorbent assay (Aradaib, 1988).

2.4 Recombinant DNA vaccines

The surge of new techniques in cellular immunology and molecular biology has made possible the development of potential candidate vaccine antigens from complex organisms including schistosomes. A range of defined vaccine antigens has been identified (Simpson and Cioli, 1987). Two of the antigens which have shown vaccine potential in experimental *S.mansoni* are glutathione-s-transferase (GST) and (GP38). The *S. man*soni GST (smGST), developed by Capron and his co-workers, is an enzyme of a molecular weight of 26-28 K Dalton in all species of schistosomes including *S.bovis* (Balloul *et al.*, 1987). The smGST shares cross-reactive epitopes with other schistosome species including *S.hematobium*, *S.bov*is and *S.jap*onicum (Balloul *et al.*, 1987 and Trottein *et al*., 1992). The smGST partially protected mice and baboons against homologous challenge. Protection is provided mainly by affecting both worm viability and worm fecundity (Boulanger, 1992). In previous studies, zebu cattle were immunized with a recombinant smGST expressed in *E. coli* or yeast. Using western blots and the enzyme linked immunosorbent assay (ELISA), immunoglobulin G (IgG) antibodies to smGST were detected in sera from the vaccinated calves, but not in sera from control calves. However, the specific IgG antibodies from *S.bovis* infected calves did not recognize the smGST as determined by western blots or ELISA. It was suggested that structural variations between *S.bovis* and *S. mansoni* GST might be responsible for this finding (Aradaib, 1988). This assumption has been confirmed by Trottein *et al.*, (1992) who reported inter-species variation between schistosomes 28 KDa glutathione-S-transferases by demonstrating structural difference within the immunologically essential regions of schistosome 28 KDa GSTs. Therefore, it was concluded that the primary antibody response induced by smGST in vaccinated calves might not have an association with protection against *S.bovis* challenge (Aradaib *et al*., 1994). Subsequently, adult *S.bovis* were recovered from experimentally infected cattle and used for preparation of native *S.bovis* GST (sbGST). Zebu calves immunized with sbGST developed partial protection against homologous challenge under laboratory conditions. The protection was due to production of specific anti-sbGST IgG that has a lethal effect on worm fecundity, and thereby inducing suppression of egg production, as determined by fecal and tissue egg counts. However, no

significant difference was observed in worm recoveries of the vaccinated animals or their controls. It is hoped that the next decade will probably see considerable improvement in bovine schistosomiasis vaccines development. A schistosomicidal vaccine, which can elicit antibodies, that have a lethal effect on *S.bovis* worm viability is yet to be found. At the present time, the sbGST would probably replace the current cumbersome time consuming procedure required for the preparation of irradiated vaccines. Because of the structural variation within the immunologically essential regions of schistosomes 28-K Da GSTs, the smGST may not protect cattle against *S.bovis* infection. Nevertheless, because of cross-reactive epitopes between schistosome GSTs, the possibility of developing a broad-spectrum schistosomicidal vaccine there exists.

3 HERIOGENOLOGY

Historically, animal breeders have sought to select and increase the frequency of desired traits within the population. In farm animals the rate of such selection schemes is limited by both the generation interval and reproductive efficiency. Nuclear transfer as a technique for production of single genotype animals can be used to multiply animals of desired genetic traits faster. Additionally, there is both scientific and economic incentive to produce clones. In research, truly identical individuals would be perfect controls for all types of studies. On a commercial level, identical individuals would enhance marketing of livestock, as more uniform product could be sold. Moreover, management of a group of identical individuals would be easier, as all would respond similarly to management changes. But the implications of cloning technology go much further, opening up new avenues of research and development. At this particular point in time, however, nuclear transfer procedure is considered the most powerful tool for production of transgenic animals. It allows the application of modern molecular techniques for the precise genetic modification, presenting the opportunity for gene knockouts, gene replacement etc. Until recently, in farm animal species, transgenic animals have been produced by the pronuclear injection technique. This method has a number of disadvantages because after the gene has been added, not all of the cells within an animal contain the gene (mosaicism), thus production of required phenotype and germ line transmission of the trait may require the generation of several transgenic lines. In contrast animals produced by nuclear transfer are derived from a single nucleus. Therefore, mosaicism is eliminated and germ line transmission is ensured. In this respect multiple copies may be produced allowing production of medicinally useful pharmaceuticals, for example, human factor IX, organ or tissue donation, and production of animal models for human diseases such as cystic fibrosis. (Nour, 2001).

4 MOLECULAR DIAGNOSTICS

Conventional methods for isolation and identification of infectious agents represent the most accurate diagnosis for an active microbial infection. However, these methods are tedious, laborious and time consuming. Serology is useful to identify a previous infection in epidemiological studies. Molecular diagnostic techniques including the use of monoclonal antibody (Mab) in competitive enzyme linked immunosorbent assay (Celisa); Cdna probes and nucleic acid amplification technology, commonly known as polymerase chain reaction, (PCR) may improve the existing diagnostic techniques used for detection and identification of specific infectious agents. These emerging techniques provide the basis for better understanding of the epidemiology of diseases of veterinary importance. In addition, the application of biotechnology in diagnostic veterinary medicine will enhance herd health monitoring, vaccination and control programs, and will facilitate detection of active microbial infections during an outbreak of the disease among susceptible wildlife and domestic livestock (Aradaib, 1994).

Beside our laboratory at the faculty of Veterinary Science in Khartoum, well-equipped molecular laboratories are also located at the Institute of Endemic Disease, University of Khartoum and the National Council for Research, Ministry of Science and Technology. The scientific research in these institutions is mainly focused around the molecular epidemiology of endemic diseases including malaria and leshmaniasis. Extensive experiments are currently conducted to study the genetic diversity among different isolates of leshmania parasie, the cause of cutaneous and visceral lishmaniasis. For the last 10 years, the major thrust of research efforts was directed towards the improvement of the existing techniques used for diagnosis of viral and parasitic diseases. Extensive studies were conducted on diagnosis of Orbiviruses, including bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) and palyam serogroup Obivirus infections. Similar studies were conducted to evaluate molecular diagnostic techniques for detection of *Schistosoma bovis* infection in cattle (Aradaib, 2001). We were able to use monoclonal antibodies (Mab) in competitive ELISA (Celisa) for detection and specific identification of EHDV-1 and EHDV-2 (Aradaib *et al.*, 1994).

The serological techniques mentioned previously, despite their advantage in detecting infected individuals, are complicated by cross-reactions between EHDV serogroups and the other members of the Orbivirus genus. Although the use of monoclonal antibodies (Mab) in competitive ELISA (Celisa) technique has improved sensitivity and specificity at the serotypes level, this technique is applicable only to blood samples (serum) and requires at least 10-14 days post infection for the production of specific antibodies by the susceptible host (Aradaib *et al.*, 1994).

Complementary DNA (Cdna) probes are stable and are more commonly used as probes in Northern blot hybridization of RNA. Cdna probes are used in southern blot hybridisation. The Cdna probe of a particular segment could be

used as a fragment of the segment or as a full-length genome. The Cdna could also be synthesized by cloning in a vector [44]. The cloning procedure is tedious, laborious and time consuming. Recently PCR-generated probes has been described and used for detection of EHDV serogroup (Aradaib, 1995; 1996; 1998). A PCR-generated 224 bp Cdna probe derived from genome segment 6 of EHDV-2 (Alberta strain), representing 15 % of the full length genome, was used for chemiluminescent hybridization of the blots [1].

Nucleic Acid Hybridisation (NAH) assay using Cdna probes could be used for detection of viral nucleic acid. The probe could be labelled with radioactive material such as ^{32}P or ^{125}I [21]. The nonradiolabeled procedure of the probe removes the hazardous and cumbersome laboratory procedure of working with radioactive materials. The enzyme labelling procedure of working with non-radiolabled chemiluminescent hybridisation assay has been described [1,2,3,13]. The use of the peroxidase-labeled Cdna probe removes the hazardous and cumbersome laboratory procedure of working with radioactive labelling techniques. The dot blot hybridisation technique, using PCR-generated Cdna derived from genome segment 6 of EHDV-2 (Alberta strain), showed positive hybridisation signals with RNA from EHDV-1, EHDV-2 and EHDV-318. No hybridisation signal was detected from total nucleic acid of the BHK-21 cell control (Aradaib *et al.*, 1996). Because of its safety and rapidity, this technique readily lends itself to use in developing countries, such as Sudan, for the detection of EHDV in cell culture. However, one of the disadvantages of dot blot hybridisation is that the technique is incapable of direct detection of EHDV RNA in clinical samples from infected animals. Hence, a prior amplification step by RT-PCR technology is deemed necessary.

EHDV RT-PCR has been successfully applied for detection of RNA viruses by addition of a complementary DNA (Cdna) synthesis step using reverse transcriptase (RT) enzyme, before cyclic amplification by PCR [1]. This RT-PCR-based assay for detection of EHDV serogroup in cell culture and clinical samples, using primers derived from NS1 genome sequence analysis of EHDV-2 Alberta strain [3, 13].

This EHDV RT-PCR was compared with different virus isolation (VI) procedures from a variety of tissue samples. The results indicated that the sensitivity of the previously described EHDV-PCR assay is comparable or more sensitive than VI method in BHK-21 cell culture or ECE. In addition, the EHDV RT-PCR assay could provide a superior diagnostic alternative to replace the current cumbersome and time-consuming virus isolation procedures [11]. Serogrouping and topotyping of United States and Sudanese isolates of EHDV were also compared using EHDV RT-PCR (Aradaib *et al*., 1996).

The recently reported EHDV serogroup-specific PCR-based assay using well-characterized serogroup-specific primers derived from segment 6 of EHDV-2, which codes for NS1, reproducibly and specifically detected RNA from EHDV-1 and EHDV-2 in cell culture and a variety of biological specimens [1, 13, 16]. The serogroup-specific 387 bp PCR products, visualized on ethidium bromide-stained

agarose gel or detected with chemilumiescent hybridisation, were obtained from the EHDV RNA sample used in this study. This confirms the ability of the PCR assay to detect new serotypes of EHDV from different continents using the same serogroup primers. The EHDV PCR assay provides an attractive diagnostic alternative to the lengthy and cumbersome conventional virus isolation procedures.

Studies on experimental EHDV infection demonstrated that cattle could amplify the virus, as determined by conventional virus isolation (VI) and PCR technology, and will become seropositive as determined by the serotype-specific EHDV Celisa [2]. Using VI and PCR assay, similar results were obtained during evaluation of EHDV infection in sentinel cattle from the San Joaquin l valley of California [19]. Thus, cattle can provide virus for insect transmission to more susceptible wild ruminants, such as the white-tailed deer populations, where a fatal disease and high mortality may occur. Widespread application of the molecular biological techniques described in this study should facilitate rapid detection and epidemiological investigation of EHDV outbreaks among susceptible ruminants in the Mediterranean and the African continents.

Specific identification of EHDV serotype 1 (EHDV-1), in cell culture or tissue samples, was described using RT-PCR-based assay [12]. Similar study was conducted to demonstrate specific identification of EHDV-2 in cell culture and clinical samples [11]. This RT-PCR was based on nucleotide sequences of genome segment 2, which codes for viral structural protein 2 (VP2). This nucleotide sequence of this genome was found to be variable among cognate genes of different serotypes of EHDV serogroup. No cross amplification product or hybridisation signals were detected between serotype-specific EHDV-1 and EHDV-2 PCR-based detection assays. In addition, using RNA from EHDV-318, the described serotype-specific (EDDV-1) or (EHDV-2) assays failed to produce the specific PCR products. This result confirms the variability of the nucleotide sequences of genome segment 2 (L2) among cognates of different EHDV serotypes [33]. Further studies are needed to determine the complete nucleotide sequence of L2 of EHDV-318. The L2 nucleotide sequence would be advantageous for serotype-specific identification of EHDV-318 using PCR-based detection assay.

The use of nested PCR removes the hazardous and cumbersome radioactive laboratory procedures of working with ^{32}P or ^{33}P [1,2, 16]. Details for the nested PCR protocol was previously described [15].

A Multiplex Polymerase Chain Reaction was developed. In this type of PCR different primer pairs derived from different EHDV serotypes are used in a single-tube PCR amplification. The multiplex PCR-based assay, using primers derived from NS3 genome of BTV-10 and NS1 genome of EHDV-2, reproducibly and specifically detected BTV and EHDV RNAs in infected cell cultures. The multiplex PCR assay was a simple procedure that utilized a single amplification in which EHDV and BTV primers were used simultaneously. The level of sensitivity could be increased using a more sensitive chemiluminescent

hybridisation techniques [1, 2, 16, 17, 18].

Simultaneous detection and differentiation of orbivirus serogroups will simplify the assay, save time and above all save on cost, because each clinical sample will be tested once instead of individual testing each specific viral nucleic acids. Because of its rapidity, sensitivity and specificity, the multiplex PCR assay would be advantageous in epidemiological investigations where field isolates can be tested to determine the prevalence and frequency of orbivirus infections in susceptible animal populations, and can also be used for evidence of viral incursion in a particular geographical region. The multiplex PCR assay could be used for export regulation to certify animals free of Orbivirus infections [37]. Nucleic acid amplification technology, using PCR or RT-PCR, would revolutionize detection of microbial infection and would serve as the bases for future diagnostic investigation.

5 ANIMAL FEED INDUSTRY

Recently, epidemiological studies have implicated animal feed containing ruminant-derived protein, such as meat and bone meals, may contain the etiologic agent that causes transmissible spongioform encephalopathy (TSE) including bovine spongioform encephalopathy (BSE), commonly known as mad cow disease. Animal protein derived from ruminant tissues is not generally recognized as safe for use in ruminant feed as stated by several Governmental officials. Regulatory guidelines are being proposed that will either ban or severely restrict the feeding of ruminant proteins back to ruminants (Aradaib *et al.*, 1999b). Therefore, regulatory officials must currently rely on an honour system or upon a paper trail for verifying none of animal proteins, particularly ruminant proteins, are present in the feed. To address the void in scientific regulatory monitoring, a means of rapid sensitive and reliable verification, that ruminant proteins are not present in ruminant feeds, is urgently needed in order to implement Hazard Analysis and Critical Control Points (HACCP) system. However, recently we have reported a rapid diagnostic assays for documenting the presence or absence of ruminant derived proteins in feed stuffs using polymerase chain reaction (PCR) amplification technology (Aradaib *et al*., 1999b). We were able to develop a rapid diagnostic method for detection and specific identification of sheep meat and sheep-derived products in processed human food or in animal feed concentrates (Aradaib *et al*., 1999c). A nested PCR amplification technology for specific identification of beef or bovine-derived products was also described (Aradaib *et al*., 2001). Differentiation of sheep and goat meat in processed human food was made possible through direct sequencing techniques and digestion of the PCR products with restriction endonuclease enzyme (Chikuni *et al*., 1994). The development of a rapid diagnostic test for detection and specific identification of goat meat or goat-derived products in commercial animal feed was recently reported (Aradaib *et*

al., 2001). In addition, the development of a multiplex PCR amplification technology for simultaneous detection and specific identification of the animal species utilized in processed food or animal feed would be advantageous.

Nested and multiplex polymerase chain reaction (PCR)-based assays, for simultaneous detection and ruminant species-specific identification, including (cattle, sheep, goat), utilized in commercial cattle feed concentrates or in feed additives are urgently needed. It is worth mentioning that, the application of biotechnology in diagnostic veterinary medicine offers an excellent opportunity for rapid detection of animal diseases, where molecular biological techniques could be used to identify the causative agents of diseases (Aradaib *et al*., 1999a). In addition, the surge of new techniques in cellular immunology and molecular biology plays an important role in human food and animal feed industry. These techniques have been applied to authenticate the animal species utilized in processed food or animal feed (Aradaib,1999b; Aradaib, 1999c; Aradaib, 1999d). Nucleic acid hybridisation, sequencing of oligonucleotides and DNA amplification technology have been successfully applied for species identification in human food (Aradaib *et al.*, 1999b) and animal feed (Aradaib *et al*., 1999c). In our laboratory, a lot of research efforts have been conducted to describe a reliable, rapid, sensitive and specific method that could be used for specific identification of ruminant-derived products in ruminant feed concentrates. The development of a reliable diagnostic assay, that could be used to trace the source of animal contaminants, which is associated with transmission of an emerging infection in a susceptible animal population, is urgently needed.

6 CONCLUSION

It is proposed that the nested and multiplex PCR protocols could be used as simple and rapid methods for detection and identification of ruminant-derived products in commercial animal feed concentrates or feed ingredients. In addition, these PCR assays provide scientific information that could support future policy decisions based upon import/export regulations to the animal feed industry and will also provide a means for verification of the ruminant protein status of specified feeds or feed ingredients.

REFERENCES*

1. Aradaib, I.E. 1988. Serologic studies on bovine schitosomiasis, MVSc thesis, University of Khartoum, Sudan.
2. Aradaib, I.E.; Abbas, B.; Bushara, H.O. and Taylor, M.G. 1993. Evaluation of *Schistosoma bovis* Adult Worm Extract for Vaccination of Calves. Preventive Veterinary Medicine 16: 77-84.
3. Aradaib, I.E.; Akita, G.Y. and Osburn, B.I. 1994. Detection of epizootic hemorrhagic disease virus serotype 1 and 2 in cell culture and clinical samples using polymerase chain reaction. Journal of Veterinary Diagnostic Investigation 6: 143-147.
4. Aradaib, I.E;, Sawyer, M.M. and Osburn, B.I. 1994. Experimental epizootic hemorrhagic disease virus infection in calves. Journal of Veterinary Diagnostic Investigation 6: 489-492**.**
5. Aradaib, I.E.; and Osburn, B.I. 1995. Bovine Schistosomiasis vaccines development: current status and future prospects. Fourth International Veterinary Immunology Symposium**,** Davis, California, USA.
6. Aradaib, I.E, Abbas, B.; Osburn, B.I.; Bushara, H.O. and Taylor, M.G. 1995. ELISA for bovine Schistosomiasis vaccine evaluation: A preliminary report. Journal of Ciencia Rural 24: 563-566.
7. Aradaib, I.E; Omer, O.H; Abbas, B.; Bushara, H.O.; Elmalik, K.H.; Saad, A.M.; Osburn, B.I. and Taylor, M.G. 1995. *Schistosoma bovis* whole-egg antigen did not protect zebu calves against experimental schistosomiasis. Preventive Veterinary Medicine 21: 339-345.
8. Aradaib, I.E.; and Osburn, B.I. 1995. Evaluation of a recombinant *Schistosoma ma*nsoni 28-Kilodalton antigen for vaccination of zebu calves against *S. bovis* infection. Vaccine 13: 130.
9. Aradaib, I.E.; and Osburn, B.I. 1995. Vaccination of cattle against bovine schistosomiasis: Present status and future prospects: A Review. Preventive Veterinary Medicine 22: 285-291**.**
10. Aradaib, I.E.; Akita, G.Y; James, E. Pearson; Osburn, B.I.; 1995. Comparison of polymerase chain reaction and virus isolation for detection of epizootic hemorrhagic disease virus in clinical samples from naturally infected deer. Journal of Veterinary Diagnostic Investigation 7: 196-200.
11. Aradaib, I.E., Abbas, B., and Osburn, B.I. 1995. Experimental *Schistosoma bovis* infection in cattle. J Ciencia Rural 24: 99-105.
12. Aradaib, I.E.; Wilson, W.C.; Cheney, C.W., Pearson, J.E. and Osburn, B.I. 1995. Application of the polymerase chain reaction for specific identification of epizootic hemorrhagic disease virus serotype 2. Journal of Veterinary Diagnostic Investigation 7: 388-392.
13. Aradaib, I.E.; McBride, J.W.; Wilson, W.C. and Osburn, B.I. 1995. Development of polymerase chain reaction for specific identification of

* As listed by authors.

epizootic hemorrhagic disease virus serotype 1. Archives of Virology 140: 2273-2281.

14. Aradaib, I.E.; Elabbas M. Abdelmageed; Hassan, S.A. and Riemann, H.P.1995. A review on the diagnosis of *Schistosoma bovis* infection in cattle. J. Ciencia Rural 25: 493-498.
15. Aradaib, I.E.; Mohammed, M.E.H.; Mukhtar, M.M.; Hassan, S.A.; Ghalib, H.W. and Osburn, .I. 1997. Serogrouping and topotyping of Sudanese strains of epizootic hemorrhagic disease virus using polymerase chain reaction. Comparative Immunology, Microbiology and Infectious Diseases. 20:211-218.
16. Aradaib, I.E.; Brewer, A.W. and Osburn, B.I. 1997. Interaction of epizootic hemorrhagic disease virus with bovine erythrocytes in vitro. Comparative Immunology, Microbiology and Infectious diseases. 20: 281-283.
17. Aradaib, I.E.; Ahmed, S.; Ibrahim, K.E.; Karrar, A.E.; Cullor, J.S.; Osburn, B.I.; 1998. A multiplex PCR for simultaneous detection and identification of United States serotypes of epizootic hemorrhagic disease virus. The Sudan Journal of Veterinary Science and Animal Husbandry 37: 1-12.
18. Aradaib, I.E.; Kabour, M.; Karrar, A.E.; Ibrahim, K.E.; Cortopassi, G.A.; Osburn, B.I.; Cullor, J.S.; 1998. Application of nucleic acid amplification technology for detection of ruminant-derived contaminants in animal feed concentrates. The Sudan Journal of Veterinary Science and Animal Husbandry 37: 13-26.
19. Aradaib, I.E.; Karrar, A.E.; Ibrahim, K.E.; Cortopassi, G.A., Osburn, B.I.; Cullor, J.S.; 1998. Specific identification of sheep meat or sheep-derived products based on ovine mitochondrial cytochrome-b genome sequence analysis. The Sudan Journal of Veterinary Science and animal husbandry 37: 105-112.
20. Aradaib, I.E., Mohammed, M.E.; Schore, C.E., Willson, W.C.; Cullor, J.S. and Osburn, B.I. 1998. PCR detection of North American and Central African Isolates of epizootic hemorrhagic disease virus based on genome segment 10 sequence analysis of EHDV-1. Journal of Clinical Microbiology. 36: 2602-2608.
21. Aradaib, I.E.; Schore, C.E.; Cullor, J.S. and Osburn B.I. 1998. A nested PCR for detection of North American isolates of Bluetongue virus based on NS1 genome sequence analysis of BTV-17. Veterinary Microbiology. 59: 99-108.
22. Aradaib, I.E; Ibrahim, K. E. E.; Karrar, A. E.; Osburn, B.I.. 1999. A review on the diagnosis of orbivirus infection in Veterinary Medicine. The Sudan Journal of Veterinary Science and Animal Husbandry 38: 1-29.
23. Aradaib, I.E. 1999. A multiplex Polymerase chain reaction (PCR)-based for simultaneous detection and identification of bluetongue and epizootic Hemorrhagic disease viruses. The Sudan Journal of Veterinary Science and Animal Husbandry 38: 39-43.

24. Aradaib, I.E**;** Karrar, A.E.; Abdalla, M. A. 2000. Application of biotechnology in diagnostic Veterinary Medicine. The Proceedings of the 9th Conference of Veterinary Medicine, Assiut, Egypt PP, 362-385.
25. Aradaib, I.E.; Moderos, R ; Ibrahim, K.E.; Osburn, B.I**.** 2000. Evaluation of epizootic Hemorrhagic disease virus infection in sentinel cattle from the San Joaquin Valley of California. The Sudan Journal of Veterinary Science and Animal Husbandry 39: 1-14.
26. Aradaib, I.E.**;** Cortopassi, G.A.; Ibrahim, K.E.E.; Karrar, A.E.; Osburn, B.I.; Cullor, J.S. 2001. Specific identification of goat meat or goat-derived products using mtcyt-b PCR amplification technology. Journal of Science and Technology 1 (1): 1-12**.**
27. Aradaib, I.E. 2001. Detection of *Schistosoma bovis* infection: Current status and future prospects. The Journal of Science and Technology 1 (1): 165-183.
28. Aradaib, I.E. 2001. Detection and species-specific identification of ruminant-derived products using mtcyt-b PCR amplification technology. The Sudan Journal of Veterinary Science and Animal Husbandry. (In press).
29. Aradaib, I.E.; Cortopassi, G.A.; Ibrahim, K.E.E.; Karrar, A.E.; Osburn, B.I.; Cullor, J.S**.** 2001. A simple and rapid method for specific identification of beef or bovine-derived products in processed human food or in animal feed concentrates. Comparative Immunology, Microbiology and Infectious Diseases (Accepted).
30. Aradaib, I.E.; Smith, W.S; Cullor, J.S; Osburn, B. I. 2001. Development and evaluation of a multiplex RT-PCR for detection and differentiation of North American Bluetongue and epizootic hemorrhagic disease viruses. Comparative Immunology, Microbiology and Infectious Diseases. (In press).
31. Balloul, J.M; Grzych, J.M.; Piercec, R.J. and Capron, A. 1987. A purified 28,000 dalton protein from Schistosoma mansoni adult worms protects rat and mice against experimental schistosomiasis. The J. of Immunology, 138: 3448-3453.
32. Balloul, J.M.; Sondermeyer, P.; Dreyer, D.; Capron,M.; Grzych,J.M.; Pierece R.J.; Carvallo,D.; Lecocq, J.P. and Capron,A. 1987. Molecular cloning of a protective antigen of schistosomes. Nature, 326: 149-153.
33. Boulanger, D., Ried, G.D., Sturrock,I., Wolowezuk, I., Baloul J.M., Grezel, D., Pierce,R.J., Otino, M.F. Cuerret,S., Grimaud, J.A. Butterworth,A.E. and Capron, A. Immunization of mice and baboons with the recombinant SM28GST affects both worm validity and fecundity after experimental infection with Schistosoma mansoni. Parasite immunology 13:473-490.
34. Bushara, H.O.; Hussien, M.F.; Saad, A.M; Taylor, M.G.; Dargie, J.D.;Marshal, T.E. and Nelson, G.S. 1978. Immunization of calves against

Schistosoma bovis using irradiated cercariae or schistosomula of S. bovis. Parasitology, 77: 303-310.
35. Bushara, H.O., Bashir, M.E.N., Malik, K.H., Mukhtar, M.M., Trottein, F., Capron, A. and Taylor, M.G. 1993. Suppression of Schistosoma bovis egg production in cattle by vaccination with either glutathione S-transferase or keyhole limpet hemocyanin. Parasite immunology 15: 383-390.
36. de Mattos C.C., de Mattos C.A. Osburn BI: 1989, Recombinant cDNA probe from bluetongue virus genome segment 10 for identification of bluetongue virus. J Vet Diagn Invest 1:237-241.
37. Goraish, I. A.; Abdelsalam, E.B.; Tartour, G.; Abbas, B. Aradaib, I.E. 1988. The effect of Levamezole (L.Tetramizole) treatment on the susceptibility to Fasciola gigantica infection in goats. Revue de Elv. Med Vet. Pay Trop., 41: 283-287.
38. Gorman B.M.: 1992, An overview of the orbiviruses, Proc. of the 2nd International Symposium on bluetongue, African horse sickness and related Orbiviruses 335-347.
39. Hammami S., Osburn B.I.: 1992, Analysis of genetic variation of epizootic hemorrhagic disease virus and bluetongue virus field isolates by coelectrophoresis of their double-stranded RNA. Am J Vet Res 53:636-642.
40. Hoff G.L., Trainer D.O.: 1974, Observation on bluetongue and epizootic hemorrhagic disease virus in white-tailed deer . J. Wildl Dis 10:25.
41. Huismans H., Bremer C.W., Barber L.T.: 1979, the nucleic acid and proteins of epizootic hemorrhagic disease virus. Onderstepoort J Vet Res 46: 95-104.
42. Hussien, M.F. 1973. Animal schistosomiasis in Africa: A review of Schistosoma bovis and S. matthei. Veterinary Bulletin 43: 341-347.
43. Hussien, M.F. and Bushara, H.O. 1976. The investigation on the development of an irradiated vaccine for animal schistosomiasis. In nuclear techniques in animal production and health. Austria: International Atomic Energy Agency. 421-431.
44. Maddison, S. E. ; Slemenad, S.B.; Chandler, F.M. and Kagan, I.G. 1978. Studies on putative adult worm-derived vaccine and adjuvant for protection against Schistosoma mansoni. J. of Parasitology. 64: 986-993.
45. Majid, A. A.; Bushara H.O.; Saad, A.M.; Hussien, M.F.; Taylor, M.G.; Dargie, J.D. Marshal, T.F.de c.; and Nelson G.S. 1980. Field testing of an irradiated Schistosoma bovis vaccine. American J. of Trop. Med. and Hyg. 29: 452-455.
46. Majid, A.A.;Marchall, T.F.; Husein,M.F.; Bushara, H.O.and Taylor, M.G. and Nelson, S.G.; Dargie,J.D. 1980. Observations on cattle schistosomiasis in the Sudan, a study in comparative medicine, 1. Epizootilogical observations on Schistosoma bovis in the White Nile Province. American J of Tropical Med and hyiegene, 29:435-441.
47. Metcalf H.E., Leduke A.J., Jochim J.O.: 1991, Epizootic hemorrhagic disease virus infection in cattle. Proceedings of the second international

symposium of Bluetongue, African horse sickness and related orbivirus.

48. Mohammed, E.H.M., Mellor, P.S., 1990. Further studies on bluetongue and bluetongue-related Orbiviruses in the Sudan. Epidemiology and infection 105: 619-632.
49. Mohammed, M.E.H.; Aradaib, I.E.; Mukhtar, M.M.; Ghalib, H.W.; Riemann, H.P.; Oyejide, A.; Osburn, B.I.; 1996. Application of molecular biological techniques for detection of epizootic hemorrhagic disease virus (EHDV-318) recovered from a sentinel calf in Central Sudan. Veterinary Microbiology 52: 201-208.
50. Mohammed, M.E.H.; Mukhtar, M.M.; Ibrahim, K.E.E.; Karrar, A.E.; Osburn, B.I. Aradaib, I.E. 1999. RNA genome profiles of Sudanese isolates of palyam virus serogroup using PAGE and agarose gel electrophoresis. The Sudan Journal of Veterinary Science and Animal Husbandry 38: 30-35.
51. Nel L.H., Huismans H.: 1990, A comparison of different genome segments of epizootic hemorrhagic disease virus as serogroup specific probes. Archive of Virology 110: 103-112.
52. Nettles V.F., Hylton S.A., Stallknecht O., Davidson WR: 1991, Epidemiology of epizootic hemorrhagic disease in wildlife in the USA. Proceedings of the 2nd international symposium on bluetongue, African horse sickness and related orbiviruses, pp. 238-248.
53. Osburn, B.I.; Aradaib, I.E.; Schore, C.; 1994. Comparison of bluetongue and epizootic hemorrhagic disease complex. Proceedings of the 13th International Symposium of the World Association of Veterinary Microbiologist, Immunologist and Specialists in Infectious Diseases (WAVMI), Mantova, Italy.
54. Pearson J.E., Gustafson G.A., Shafer A.L., Alstad AD: 1992, Diagnosis of bluetongue virus and epizootic hemorrhagic disease. Proceedings of the 2nd international symposium on bluetongue, African horse sickness and related viruses, pp.533-546.
55. Rabenau, H., Ohlinger, V., Anderson, J., Cinatl, J., Wolf, W., Forst, W., Mellor, P., Doerr, H.w., 1993. Contamination of genetically engineered CHO-cells by epizootic hemorrhagic disease virus (EHDV). Biochemicals 21: 207-214.
56. Saad, A.M.;Hussien, M.F.; Dargie, D.G.; Taylor M.G.; and Nelson, G.S. 1980. Schistosoma bovis in calves: the Development and Clinical Pathology of primary infections. Research in Veterinary Science, 28: 105-111.
57. Shope R.E., Macnamara L.G., Mangold R.: 1955, Report on the deer mortality, Epizootic hemorrhagic disease of deer, N. Jersey outdoor s 6: 17-21
58. Trottein, F., Godin, C., Pierce, R.J., Sellin, B., Taylor, M.G., Gorillot, I.,

Silva, M.S.Lecocq, J.P. and Capron, A. 1992. Iner-species variation of schistosome 28-KD glutathione S-transferases. Molecular and biomedical parasitology, 54:63-72.

59. Venter E.H., Viljoen G.J., Nel L.H., Huismans H., Van Dijk A.A.: 1991, A comparison of different genomic probe in the detection of virus-specified RNA in Orbivirus-infected cell, J of Virol Methods 32: 171-180.
60. Voller, A., Bartltte, A., and Bidwell, D.E. (1976). Enzyme immunoassay for parasitic diseases. Transaction of the Royal Society of the tropical Medicine and Hygiene 70: 98-106.
61. Wilson W.C., Fukusho A., Roy P.: 1990, Diagnostic complementary DNA probes for genome segment 2 and 3 of epizootic hemorrhagic disease virus serotype 1. Am J Vet Res 51: 855-560.

Arsenic Contamination of Drinking Water in Bangladesh

S Z HAIDER*
Department of Chemistry
Dhaka University, Dhaka 1000
Bangladesh

1 ABSTRACT

Arsenic in ultratrace levels is ubiquitously present in all living systems including man as an essential nutrient element and is always present below the detection limit. The estimated human requirement is about 20 microgram per day. Slight excess in human system is easily excreted by continued intake of arsenic free water. The persistent presence of arsenic in the human body leads to cumulative effect and the element concentrates in hairs, nails, the liver, kidney and urine. The initial symptoms of arsenic poisoning is observed in the palms of the hands and soles of the feet due to ingestion of excessive amount and imbalance in the excretion-ingestion ratio. The cellular metabolism of the epidermal and subcutaneous cells leads to arsenicosis in the long run and the ultimate health hazards give rise to the carcinogenic conditions and slow death. The mechanism of arsenic toxicity through food chains still remains a mystery. The paper describes the possible sources of arsenic in the drinking water and the chemistry involved in the release of arsenic in the ground water in a large number of hand operated tube wells. Some aspects of the remedial measures have been discussed and proposals for research for the study and mitigation of the arsenic problem have been suggested.

2 INTRODUCTION

During early 1980s, a number of cases of arsenic poisoning in human beings were detected at the School of Tropical Medicine, Calcutta, India. Later on, a large number of patients suffering form arsenicosis were identified at the School of Environmental Studies, Jadavpur University, Calcutta. The search indicated consumption of arsenic contaminated water extracted with tube wells from ground sources. In early 1990s, the laboratories of Public Health Engineering Department, Government of Bangladesh, reported presence of arsenic in ground

* Professor of Chemistry, and Fellow of the Islamic Academy of Sciences.

water above the recommended level. Chronic arsenicosis cases were reported from north-west Bangladesh adjoining the affected areas of West Bengal in India. The arsenic content of the hand operated tube wells was found to be sometimes as high as 2 ppm as compared to the WHO recommended level of arsenic of 0.05 ppm for developing countries and only 0.01 ppm for the technologically advanced countries as in the USA.

During 1970s, after the disastrous surface water pollution immediately after the great cyclone and storm surges, the Government of Bangladesh with assistance from international organisations, augmented the use of shallow tube wells in the rural areas to provide safe drinking water to avoid the epidemics of cholera and diarrhoeal diseases causing the death of thousands of people and domestic animals. The testing for the presence of arsenic was not included then as a part of routine water quality protocol. The Department of Public Health and Engineering made a preliminary survey and it was reported that a large number of the tube wells (about 30%) tested were contaminated with arsenic of concentrations above the recommended level of 0.05 ppm. About 30 million people were at risk of being exposed to arsenic poisoning.

The time required for the symptoms of arsenic poisoning in human being varied from 2 to 6 years of continued ingestion. The children and women show arsenicosis more readily. The palms of the hands and the soles of the feet appear to be initially affected showing dark spots on the skin which subsequently hardens. In the long run formations of lesions occur and when more pronounced the symptoms lead to cancerous condition. There is no known cure for acute arsenic poisoning. However, it is claimed that initial stages of arsenicosis is a reversible process and the affected people may show signs of removal of the black spots on continuous intake of arsenic-free water.

3 SOURCES OF ARSENIC IN BENGAL DELTA

3.1 Background

The sources of arsenic in the Bengal Delta comprising most of Bangladesh and a part of West Bengal in India are definitely geogenic. The Himalayan region has been formed mostly by marine sedimentary rocks that are subjected to the fluvial actions of the large rivers. These rivers have brought down the mineral constituents including arsenic with the aquifers in the sedimentary environment of the active delta. Arsenic is a pathfinder element and occurs in association with silver, gold, platinum, copper, nickel etc. in the mineral zones. The geogenic origin of arsenic appears to be initially of magmatic sulphides and iron ores. The average arsenic contents in volcanic region is 200 ppm. Other locations contain only 2 ppm.

3.2 Arsenic in soil and water

Arsenic in soil of granite origin indicates concentration of about 4 ppm. However, the sedimentary rock soils contain 20 - 30 ppm of arsenic. The arsenic species appear to be concentrated in the soil aquifers with clay, silt, sediment and fine sands. The release of arsenic in water may be possibly due to geochemical redox conditions and also due to the biogeochemical interactions in the soil-water interface. The plant residues in soil tend to solubilize arsenic compounds and arsenic becomes enriched in the humus horizon of the soil. The iron poor environment increases the mobility of arsenic resulting in the detectable dispersion trains in the stream water of the aquifers.

Concentration of arsenic in the ground water depends on the process of soil formulation (pedogenesis) and the interactions of physico-chemcial processes containing inorganic and organic contents at the soil-water interface. Mobilization of arsenic in groundwater at a depth of about 100 meters in the forms of As(III) and As(V) in the aqueous phase arises due to the processes of oxidation-reduction, precipitation, dissolution, absorption-desorption, arsenic-speciation and organic-biochemical interactions. The processes depend on the factors of pH and E_h and other geochemical actions. In most cases the groundwater source of acrsenic coexists with iron and sulphur. On disintegration of the solid phase, the dominant arsenic species at pH range 6-9 are:

$$\mathbf{As(V) \rightarrow H_3AsO_4,\ H_2AsO_4^-,\ HASO_4^{2-},\ AsO_4^{3-}}$$
$$\mathbf{As(III) \rightarrow H_3AsO_3,\ H_2AsO_3^-,\ HASO_3^{2-},\ HAsO_2}$$

The corresponding soluble salts are also present. Retention and release of arsenic depend on the chemical properties of the sediment (particularly those containing iron and aluminium oxides). Arsenic is removed into the aquifer by adsorption and by precipitation. Arsenic removal from sediment by multiple regression analysis using As(III) species and Fe/As ratio are given by the relationship:

$$\mathbf{\%\ As(III)\ removal\ \ R = C[initial\ As(III)]^m \times (Fe/As)^n \times (pH)^p}$$

c, m, n and p are the empirical constants.

$$\mathbf{c = 4.44,\ \ m = 0.17 \pm 0.01,\ \ n = 0.34 \pm 0.01,\ \ p = 0.33 \pm 0.04}$$

R^2 has been found to be 0.95.

In natural sediments, the reactions of arsenic with hydrated oxides occur.

$$\mathbf{4FeAsS + 13O_2 + 6H_2O \rightarrow 4FeSO_4 + 4H_3AsO_4}$$
$$\mathbf{M\text{-}OH + H_2O \rightarrow M\text{-}OH_2^+ + OH^-}$$
$$\mathbf{M\text{-}OH_2^+ + H_2AsO_4^- \rightarrow M\text{-}OH_3 + HAsO_4^{2-}}$$
$$\mathbf{M\text{-}OH + H_2AsO_4^- \rightarrow M\text{-}HAsO_4 + H_2O}$$

MnO_2 coated sediments produce oxidation to As(V) and / or adsorption on the surface.

$$HAsO_2 + MnO_2 \rightarrow MnO_2HAsO_2$$
$$(MnO_2).HAsO_2 + H_2O \rightarrow H_3AsO_4 + MnO$$
$$H_3AsO_4 \rightarrow H_2AsO_4^- + H^+$$
$$H_2AsO_4^- \rightarrow HAsO_4^{2-} + H^+$$
$$(MnO_2).HAsO_2 + 2H^+ \rightarrow H_3AsO_4 + Mn^{2+}$$

The sediments in the cases of flooded soil become enriched in arsenic under both the oxidative and anaerobic conditions.

Obviously phosphates compete with arsenates for adsorption. In fact inorganic insoluble phosphates have been used effectively for adsorption of various species including radioactive products.

3.3 Aresenic in soil bacteria

Arsenite (AsO_2^-) in presence of soil bacteria is converted to methyl arsonic acid ($CH_3OH.As.O.OH$) which on further reaction forms dimethylarsinic acid. Dimethyl arsinic acid ($CH_3OH.As.O.OH_3$) which is also known as cacodylic acid on further reaction with bacteria is converted to dimethylarsine ($CH_3HAs.CH_3$). Dimethyl arsenic is oxidised to dimethylarsinic acid or cacodylic acid, which reacts with fungi to produce trimethylarsine $(CH_3)_3As$. Trimethylarsine is oxidized back to dimethylarsinic acid. These transformations of arsenic species take place at soil-water interface under biogeochemical environment.

3.4 Arsenic in soil aquifer interface

Arsenic in soil aquifer locations in Bangladesh and Nepal are shown in Table 1, and the iron content of the soil has also been given.

Table 1. Arsenic soil - aquifer*

Location	As in ground water/ ppm	Depth in feet	As in soil/ ppm	Iron in soil/ ppm
Faridpur	1.60	80	12.20	130
Faridpur	0.27	175	9.00	184
Ganges sediment	-	surface	40.90	260
Jamuna sediment	-	surface	17.20	142
Jamuna suspended sediment	-	surface	14.30	150
Sonargaon	0.68	6	12.20	264
Sonargaon	1.02	115	40.10	262
Birganj (Nepal)	0.20	5	25.20	288
Birganj (Nepal)	0.16	5	21.40	202

* *Source: S. S. Ullah, Jahangirnagar University, Bangladesh.*

It is rather interesting to note that arsenic contamination of ground water in the Bengal Delta could not be detected prior to the 1980s. The water table of the aquifers brought about geochemical changes in the components of the groundwater apparently due to human interferences in the flow of the water system in the region and excessive extraction of ground water both by the shallow pumps and deep tube wells for human use and for agriculture. Arsenic species existed in the stable state, mostly insoluble in water. But now it is estimated that more than 75 million people, large number of animals, vegetation and food crops production are exposed to the poisoning effects of arsenic in Bangladesh and India.

3.5 Mineralogical examinations of soil samples

The fragmented soil, minerals, coarse sand, fine sand and clay have been subjected to examination. Maximum concentration of arsenic was present in coarse grains of sand. The arsenic-rich fragments were examined by x-ray diffraction, x-ray fluorescence analysis, scanning electron microscopy, electron probe microanalysis and microprobe mass spectral analysis, by several workers. The phase analysis of microstructures indicated the presence of pyrite grains, iron oxide phase, quartz inclusions, white calcite phase and also woody fragments. Phases such as magnetite, hematite, quartz, pyrite and rozenite were also identified.

The existence of fram-boidal pyrite has been reported in the Bangladesh sediment of iron oxyhydroxide. The sulphate produced is easily reduced to elementary sulphur or further to H_2S by sulphate reducing bacteria and therefore groundwater is deficient in sulphate content. Therefore, the hypothesis for the presence of arsenic in groundwater appears to be rather hazy. The probability of interfacial inclusion of arsenic with iron pyrite is indicated since the presence of the arsenopyrite (FeAsS) phase shall have chemically balanced correlation between iron, sulphur and arsenic. The laser microprobe mass spectra analysis showed that the spectra of arsenic-rich sediment samples were almost identical with that of iron pyrite. However, there was no correlation of the ratios of arsenic and iron concentrations in the groundwater samples with the depth of the sediments at any place of the study area. It was also confirmed that arsenic and iron concentrations in the groundwater from any source have no chemical correlation at all. The location of the Himalayan region north of the Indian subcontinent appears to have been formed by the marine sedimentary rocks. The fluvial actions of the rivers originating from the Himalayas have brought down the mineral constituents including arsenic both in the ground and surface water of sedimentary environment. The West Bengal surface sand also has been found to be rich in arsenic and the mineralogical study indicated the presence of goethite, FeO(OH) and possibly limonite, $Fe_2O_3.Fe_2(OH)_3$ as well. So it is not only the groundwater but also surface water of all origins that has trace arsenic in the deltaic region.

Due to different fluvio- depositional history of the aquifers in the same area the arsenic species appear to be concentrated in clay, silt and fine or coarse sand. The soil aquifer may become contaminated with arsenic possibly due to geochemical redox conditions. The percentage of arsenite in the aquifer is comparatively much less than the arsenate content.

The plant residues in soil tend to solubilize arsenic compounds to a certain extent and arsenic appears to become somewhat enriched in the humus horizon of the soils. The low mobility is connected with coprecipitation as AsO_4^{3-} with limonite and possibly as $FeAsO_4$ (scorodite).

4 DIVERSION OF NATURAL FLOW OF WATER IN THE REGION OF BANGLADESH

As mentioned before the arsenic problem surfaced in the region of Bangladesh and West Bengal, during 1980s. Before that, symptoms of arsenicosis were not known although people used water from ground sources or the pond and river sources. It is the human interference with the natural flow of water in the entire deltaic region that appears to be responsible for the emergence of arsenic in the extracted water. In water or surface water, there is always an equilibrium both in the wet and dry seasons due to the biogeochemical activities of the soil, and that is why arsenic content has never been found to increase to the danger level as has happened during the last 2 decades. The soil acted as interfacial phase and the discharge and recharge of water during the wet and dry seasons never altered the quality of water to such extent as to cause excessive release of arsenic in the aquifer.

5 PROPOSALS FOR STUDY AND RESEARCH

5.1 General

If arsenic becomes concentrated in sediments, the question arises how this arsenic is mobilized from sediment to groundwater. It is therefore suggested that simulated experiments by competent scientists and engineers be carried out at appropriate locations taking all precautionary measures to ascertain the real situation. All conceivable factors responsible for arsenic mobilization in the groundwater artificial streams have to be adopted. The study of mobilization of arsenic in sedimentary aquifers as mentioned before may possibly be due to the creation of new geochemical environment on account of large-scale extraction of water and may be associated with compaction of the sub-soil layers. The geochemical effects of such anthropogenic activities need to be proved by experiments under the simulated conditions with all the component factors which are responsible for the arsenic contamination in order to dispel the water related controversy.

5.2 Pollution of the rivers

It is well known that the river Ganges is one of the most polluted rivers in the world. The toxic wastes are discharged into Bangladesh during the monsoon season. Outbreak of diseases such as diarrhoea, dysentery, typhoid, cholera, kidney problems and skin diseases are rampant during the high flood discharge. Whether the arsenic content of the aquifers in the Ganges delta is altered or not remains to be studied both during the normal flood and high flood monsoon seasons. The arsenic levels of aquifers for seasonal variation need to be compared. The surface water pollution must be also thoroughly investigated particularly with regard to the adverse effects on biodiversity and ecological damage concomitant with the discharge of toxic wastes into Bangladesh region.

5.3 Study of the Redox process

The oxidation-reduction processes in the groundwater of both the shallow and deep tube wells waters in contact with the soil system need to be studied on a comprehensive scale since these processes have been considered to be the cause of arsenic release in the form of water-soluble species in the groundwater.

6 MITIGATION MEASURES AND TESTING CAMPAIGN

The mitigation measures for the removal of arsenic on domestic scale has been claimed to be successful by introducing various types of filters for absorption of arsenic. However, community scale measures need the establishment of water treatment plants for long-term study.

Moreover, the disposal of arsenic contaminated sludge needs serious consideration to convert the wastes to have harmless effect on man and the environment.

The field tests for the detection of the presence of arsenic in water developed by various agencies do not produce reliable results because of the wide variations. Cross-checking of analytical results is necessary with different kits so as to detect at least 0.01 ppm arsenic, the level recommended by WHO for the advanced countries.

The Public Health Engineering Department of the government of Bangladesh has set up a plan under "Bangladesh Arsenic Mitigation Water Supply Project" in order to supply arsenic-free water for drinking purpose. Initial stages of the project deal with testing of water samples of tube wells scattered all over the country both in the rural areas and townships. For the purpose of testing water samples, test kits have been employed mainly for field-testing for arsenic and other parameters on the spot. Some testing kits have also been produced locally. A large number of small-scale laboratories will be established at selected spots in remote places for the testing of water quality. Training programmes for testing of water samples and capacity building for arsenic analysis need to be strengthened.

Simultaneously alternative safe water systems are being promoted in the form of extraction by very deep tube wells, surface water, collection of rain water and household filters.

7 CLINICAL STUDIES

The clinical studies and the effective methods of treatments for patients suffering from arsenicosis is a virgin field of medical research. It has been reported that the arsenicosis patient at the initial stages of the ailment can be cured by continuous intake of arsenic free water. People suffering from arsenic lesions may need suitable treatment with chelating agents incorporated in the form of lotion so that arsenic is chelated in the form of water soluble species which may be washed off with water without producing harmful effect on the skin of the patients. This aspect also deserves to be tested. Patient identification, counselling, medical help and epidemiological studies may be undertaken for alleviation of arsenicosis.

8 CONCLUSION

A project covering some important aspects of arsenic problems related to global climate change may be undertaken on an international scale for the welfare of mankind.

The correlation between nutritional status of people and vulnerability to arsenic poisoning via food crops is another aspects of long-term study. The seasonal variations are also another area that could be looked at. The effects of various environmental parameters may possibly play an important role in the variations of arsenic concentrations in the groundwater regime under different seasonal conditions.

REFERENCES

1. D. Chakraborti, *et al.*, Bulletin of WHO, 70(4), 481-485, 1992.
2. S. S. Ullah, S. Z. Haider, J. Bang. Acad. Sci, 22(1), 143-147, 1998.
3. D. Chakraborti, *et al.*, The Analyst, 119(12), 168-170, 1994.
4. D. Chakraborti, *et al.*, The Analyst, 120(3), 917-924, 1995.
5. D. Chakraborti, *et al.*, The Analyst, 120(3), 643-650, 1995.
6. S. S. Ullah, *et al.*, Proc. Intl. Workshop on Environmental Biogeochemistry, Jawaharlal Nehru University, New Delhi, Dec 13-18, pp 175-180, 1998.
7. S. Z. Haider, Proc. Isl. Acad. Sci., (Water in the Islamic World: An Imminent Crisis – Editors: M Ergin, H D Altinbilek, and M R Zou'bi), IDB, UNESCO, IAS, Jordan, pp. 253-261, 1994.
8. D. K. Barua, Marine Geology, 91, 243-253, 1990.
9. S. Z. Haider, FACS Newsletter, 2, 27-30, 1987.
10. T. R. Chowdhury, *et al.*, Book on Arsenic, Chapman & Hall, 91-111, 1997.
11. B. K. Mandal, *et al.*, Current Science, 72(2), 114-117, 1997.
12. D. Chakraborti, *et al.*, Current Science, 74(4), 346-355, 1998.
13. R. K. Dhar, *et al.*, Current Science, 73(1), 48-59, 1997.
14. D. Chakraborti, *et al.*, The Telegraph, Calcutta, March 29, 1999.
15. D. Chakraborti, *et al.*, The Daily Star, Dhaka, April 29, 1997.
16. T. F. Bridge and M. T. Hossain, The Independent, Dhaka, January 21, 2000.
17. R. Nickson, *et. al.*, Nature, 345, 338, 1998.
18. M. M. Adel, The Independent, Dhaka, January 25, 2000.
19. S. A. Majumder, BCBSNA Newsletter, USA, October 16, 1997.

Common Characteristics of the Required Strategy in Biotechnology in Islamic Countries: Educational, Scientific and Economic Aspects

FAIQ BILLAL*
Islamic Educational, Scientific, and Cultural Organisation (ISESCO)
Rabat, Morocco

1 ABSTRACT

Biotechnology holds tremendous promise for the future in Islamic countries. As the present biotechnological programmes and policies being pursued in the Islamic countries are not capable of producing tangible results, it is necessary to draft an appropriate strategy for the development, adaptation and implementation of biotechnologies in Islamic countries, consistent with their capacities, aspirations, priorities and opportunities. This paper reviews the present status of biotechnology and describes strategies in various important areas. The key elements necessary in drafting a biotechnological strategy have been highlighted. These include education and manpower development, strengthening the necessary infrastructure for the commercialisation of biotechnologies, promotion of R&D capability and its applicability especially in agriculture and health as per the needs of ISESCO-Member States. Emphasis has also been placed on drafting bio-safety regulations and examining the ethical implications of new technologies from an Islamic point of view. The international co-operation, which always provides a new life to the national efforts in the development process, has also been emphasized.

2 BIOTECHNOLOGY IN ISLAMIC COUNTRIES: AN OVERVIEW

Islamic countries, although differ considerably in Gross Domestic Product and technological capability, can all be categorised as developing countries as far as science and technology is concerned. The S&T indicators available show that human development index of Islamic countries is extremely low, scientific and development staff in all Islamic countries is not more than few hundred thousands whereas the technical and supporting staff directing the research is

* Director (Science); Islamic Educational, Scientific and Cultural Organization (ISESCO), Rabat, Morocco.

15-20 times lower than industrialized countries. The number of universities in Islamic countries and scientific departments is far less than the required to produce the desired human resource. Research establishments, which are also few and far between as compared to developed countries, are facing acute financial shortages. Financial resources are mostly confined to public financing whereas private financing is rare or non-existing. Science and technology being pursued with the aforementioned status is unable to bring any tangible progress in the coming decades. As a result Islamic countries will drift farther behind the rest of the world in scientific and technological capabilities.

Some Islamic countries have already defined biotechnological policies and are undertaking steps to concretise them like Malaysia, Morocco, Pakistan, Indonesia, Egypt and Iran. Islamic countries, with reference to development of biotechnologies fall in three main categories. First, countries with limited interest, facilities and some programmes in conventional biotechnology but no national policy on biotechnology (most of the African Islamic countries fall in this category). Second, countries with a national policy mainly with programmes in conventional biotechnology and some linkages to industrialized countries. Third, countries with a national policy and research programmes in conventional as well as modern biotechnology with small collaborative linkages to industrialized countries in acquisition of technologies. However, we see no real progress in establishing effective international linkages in both public and private sectors or in commercialising technologies. Many of these countries lack capacities to adapt biotechnologies to local conditions. The biotechnological status in Islamic countries has also a major share of the individual efforts of biotechnologists and genetic engineering experts resulting in some high quality research being conducted and success in marketing some products, at local levels.

3 FUTURE POTENTIAL OF BIOTECHNOLOGY IN ISLAMIC COUNTRIES

There are various technologies, which have immense potential for development in Islamic countries if promoted in an appropriate manner. Some Islamic countries are pursuing policies in information technology vigorously. The realization of the benefits of IT by the public consolidated the understanding and dedication of decision-makers therein to develop the missing infrastructure necessary for promotion of information technologies. These efforts resulted not only in providing immense facilities in urban areas but also in rural and remote communities. Similarly, biotechnologies, which have been utilized by the finance investors and scientific investigators in the industrialized countries, to obtain immense commercial benefits, have enormous potential for development in Islamic countries. The industrialized countries invested heavily in the biotechnologies due to commercial benefits related to it. Private companies also came forward and invested a lot. These heavy investments strengthened the

already available infrastructure to produce efficient results. The biotechnology revenues collected in USA increased by 16% in 1999 to US$18.6 billion. 1283 biotechnologies companies spent US$9.9 billion on research and development in 1999. Similarly Canada, Japan and Europe have showed equally impressive growth. In Islamic countries, mostly scientists realized the potential when the industrialized countries had already achieved immense success. The efforts made by them in the absence of adequate supportive infrastructure failed to utilize the results for commercial benefits or even solving their own problems. This does not mean that the potential available in development of biotechnologies is difficult to utilize. In fact, boosting the biotechnologies is not like boosting other sciences. Biotechnology development needs careful planning to foster investment in infrastructure, R&D and commercialisation to derive economic benefits.

There is a dire need to draft a strategy for the development of biotechnology in Islamic countries that may encompass; the preparation of research and development programmes consistent with the needs and priorities, strengthening a supportive biotechnological education system, development of the qualified human resource, establishment of biotechnological centres with high level research and innovative capacities and the development of necessary infrastructures to ensure commercialisation of biotechnologies. The strategy should address the present situation and bring future developments in view of rapidly changing environment.

4 ROLE OF GOVERNMENTS IN PROMOTION OF BIOTECHNOLOGIES

4.1 General

One of the crucial factors in development process is transformation of government vision into reality. Authorities in the Islamic countries always proclaim worthy goals for promotion of the biotechnology, however, these promises wax and wane off with time without achieving any tangible results. This is due to fact that realization of the potential never coincides with realization of the tasks lying ahead. Usually, economic planners have wrong expectations that investments in biotechnology research will start producing results in a short period of time and when the results do not start coming, the confidence in the national scientific capacities is shaken and biotechnological programmes relegates into a low priority in the national planning process. It is, therefore, of prime importance to create a strong linkage between legislative and executive action through legislation in order to make it obligatory to take an effective course of action. Second, it is necessary to include biotechnologists in the decision making process so that biotechnology goals are accorded appropriate priority in national economic plans for development.

Some Islamic countries due to financial constraints are not able to meet even their immediate national demands. However, with a careful strategy, it is possible to mobile funds from the whole Islamic community and financial bodies to help them develop their own capacities in the utilization of these technologies at least for the welfare of their own people.

4.2 Key elements necessary for the strategy

- Urging the governments to adopt legislation to help achieve time targeted goals and to ensure financial support for biotechnological development;
- Setting up of national representative bodies to review the implementation mechanism of biotechnology policies in each country;
- Promoting the involvement of biotechnologists in the decision making process; and
- Mobilizing the support of international financial institutions in general and Islamic institutions in particular for the promotion of biotechnologies in the least developed countries.

5 STRENGTHENING HUMAN RESOURCE IN BIOTECHNOLOGY

5.1 General

Creation of a knowledgeable human resource is the only means to achieve economic progress. The education status in Islamic countries is at present not adequate to support biotechnological development. Various efforts are being made to promote higher education in Islamic countries. In the light of the rapid advancement in biotechnology it is necessary for Islamic countries to introduce biotechnology education from primary to higher levels and to strengthen the laboratory facilities to provide hands-on training.

One of the serious problems is the lack of the qualified and skilled manpower like researchers, technicians and support staff. Biotechnology like other scientific fields is an experimental endeavour. Hands-on training and research experience helps to promote research. But there is dearth of training institutes in Biotechnology in Islamic countries.

5.2 Key elements necessary for the strategy

- Strengthening biotechnological education and its introduction from the primary level;
- Ensuring provision of world-class education in biotechnology through continuous curricula and textbooks updating as per changing demands and fostering teaching facilities;
- Establishment of biotechnology training centres and laboratories in the various Islamic countries to develop trained manpower of international standards;

- Creation of Islamic central fund for development of appropriate human resource in biotechnology;
- Continuous updating of the research skills through training courses and workshops especially in new biotechnologies; and
- Drafting of necessary policies for women resource development and encouraging their role in the promotion of biotechnologies.

6 INCREASING GENERAL AWARENESS OF BIOTECHNOLOGY

6.1 General

Today, access to knowledge is synonymous with development, well-being and quality of life. One of the major challenges being faced by Islamic countries is to make science and technology an essential part of the culture of the people. Various programmes for popularisation of science and technology are being pursued in Islamic countries. However, in spite of all these efforts, adequately educated people in urban areas or even the science graduates are ignorant of the biotechnological developments taking place around the world. Enhancing general awareness of biotechnology and its economic viability may help to create a political, social and economic climate in favour of biotechnology. As new information and communication technologies are providing direct benefit to the people even in the rural and remote areas and increasing their understanding of the value thereof, Biotechnology knowledge from basics of gene technology to details of its commercialisation should be promoted, as it may help to attract entrepreneurs and industrialists, which are of prime importance in promotion of these technologies.

6.2 Key elements necessary for the strategy

- Establishment of interactive centres for collection and dissemination of information, monitoring and advice, facilitation of access to new technologies;
- Promotion of public understanding of biotechnologies utilizing information and communication technologies, mass media and Internet facilities; and
- Publication of simplified books for general public understanding as well as attracting the entrepreneurs.

7 PROMOTION OF RESEARCH AND DEVELOPMENT

7.1 General

Research activities, which play a vital role in the development of new technologies, are limited in Islamic countries. The number of researchers in Islamic countries is far below international standards. It is necessary to

strengthen this critically important resource. Strategies in biotechnological education and promotion of qualified manpower will create qualified scientists and engineers of international standards. There is a need to establish biotechnological research centres with well-equipped laboratories of international standards to utilize this qualified manpower to continue with high quality research. Research in food and agriculture, forestry (cloning of tree from tissue culture) in biomedical sciences (recombinant drugs, vaccines and diagnostic kits), in energy sector (production of energy from biomass), in combating pollution (through use of enzymes, microbes and plants) and in bioelectronics (the sensitivity of biological components, such as cells and monoclonal bodies) have immense potential for meet the immediate demands in Islamic countries.

7.2 Key elements necessary for the strategy

- Establishment of international biotechnological research centres for Islamic countries, to perform research of international standard in specified areas and provide hands-on training;
- Strengthening the biotechnological research laboratories in Islamic countries to international standards;
- Creation of Islamic biotechnological researcher groups and experts panel to determine priorities, jointly address problems of common concerns and foster cooperation among them in vital areas of scientific research;
- Wide dissemination of research findings through the publication of research journals, proceedings of research seminars and research directories;
- Raising allocation for biotechnological research and inciting public and private sector to invest in research and development; and
- Provision of expert advice by renowned scientists to needy Islamic countries to determine their priorities in biotechnological research and policies.

8 FOOD AND AGRICULTURE

8.1 General

The need of applications of biotechnology in the field of food security and agriculture is greatest in Islamic countries as the economies of most of them are dependent on agricultural. There are millions of people who do not have access to sufficient food to meet their own demands. Malnutrition is playing a significant role and is a major cause of death of millions of children each year. Deficiencies in micronutrient are widespread. An Agribiotechnological revolution is taking place in the industrialized world for economic gain. Such a revolution is needed in Islamic countries to meet human and environment challenges. The benefits of biotechnology may be extended to millions of poor

people living in the rural areas, who are dependent on local food production. Poor farmers who are mainly dependent on their income from agriculture lose large portions of their crop production due to pathogens and pests. Through agribiotechnologies, it is possible to produce specific resistance to pathogens and pests. Increased production may reduce threat to natural rural ecosystem and may help conservation of biodiversity.

8.2 Proposed key elements for the strategy

- Identify, monitor and assess opportunities and challenges in agriculture and food industries to draft appropriate policies;
- Encourage research leading to the introduction of biotechnologies in agriculture and food production and processing; and
- Facilitating biotechnology research programmes suitable to adaptation in the local environment.

9 HEALTH AND MEDICINE

9.1 General

Biotechnology-derived products have the potential to positively change our society, from improving health care to provision of useful medicines. Biotechnologies have provided a growing range of new and better treatments for common diseases. Hundreds of biotechnological drug products and vaccines are already on the market. These medicines have provided us with protection from various diseases like cancers, heart disease, diabetes, multiple sclerosis, obesity etc. Biotechnologies have enabled production of hundreds of medical diagnostic kits that detect disease earlier, thereby enabling us to take precautionary measures in time. In the near future biotechnologies may enable us the prediction and early prevention of many diseases. These developments are expected to bring radical changes in the health industry. It is expected that over the next two decades, biotechnologies in medicine research will produce spectacular breakthroughs and enormous opportunities.

In Islamic countries, there are millions of people suffering due to disease and poor health. Modern pharmaceuticals, depend heavily on imported drugs and technology. Due to high costs of these drugs most of the population still relies on traditional plant-derived drugs. Apart from modern biotechnologies in production of new products, plant derived drugs offer an interesting potential as a resource for local industry and a substitute for costly pharmaceutical imports. The market potential for herbal drugs is in the range of US$45billion. This is due to shifting of consumers' preference from chemicals towards plant-derived drugs. Pharmaceutical companies are continuously seeking new compounds for medicine to improve their competitiveness. There are some successful

achievements in biotechnologies applied to drug and vaccine production and public health in Islamic countries.

9.2 Key elements necessary for the strategy

- Adoption of necessary measures for the promotion of biotechnologies in medicine and production of useful chemicals to ensure health and safety of people;
- Prioritisation of biotechnology in order to cater for immediate needs, and to perform targeted research in public health and medicine; and
- Utilization of easier technologies in plant derived medicines and drugs.

10 APPLICATION AND UTILIZATION OF BIOTECHNOLOGIES

10.1 General

Investments in manpower resources development and the building of research facilities can bring socio-economic benefits only if research results are exploited in the commercialisation of biotechnologies. This capability initiates a systematic process for development and strengthening of other support factors (development of private firms, first rate universities and even consumer demand). Failure to utilize research results undermines the goals of any policy for economic growth. Due to immense potential in biotechnological research and success of the new products and processes in various disciplines like health care and agricultural production, the biotechnology companies and researchers have increased their investments many times. During 2000, there was more money invested in biotechnology firms than in the previous five years combined. The business models have matured and the tools of biotechnology research are refined to the extent that the industry is ready for unprecedented dramatic growth.

In Islamic countries considerable amount of research has been conducted but it has not resulted in real commercial successes. Promotion of the research capacities in biotechnologies requires higher investments, which always remains crucial for developing countries due to their other priorities. Furthermore, technology utilization and marketing are faced with several problems, constraints and limitations. In fact, transfer of scientific research results from scientific laboratories to the industries is a subtle and complex process, which requires development of necessary support systems, which are usually absent in developing countries. We may say that this is one of the weakest points in the development of technology. Technologies, which produce dazzling results in the industrialized countries, when transferred to developing countries, in the absence of this support system, fail to be adopted locally and become an economic liability.

The linkage between R & D institutions and industries need to be strengthened. The scientific research institutions should work closely with private-sector manufacturing and service firms to harness its natural wealth into sustainable economic growth. We need to develop the technical skills required for the commercialisation of technologies. There is a dearth of risk capital and technologies generated indigenously are usually not mature and proven and in many cases have to compete with those imported from abroad. The data and information regarding the nature of R&D results should receive due publicity to attract entrepreneurs and there should be channels and mechanisms for diffusion of the technologies generated indigenously.

10.2 Key elements necessary for the strategy

- Preparation and implementation of special policies and programmes for the transfer of scientific research results to the production sector;
- Measures to enhance close cooperation between university, scientific research institutes and industries especially for the promotion of technological partnership between scientists and entrepreneurs;
- Encouraging the public and private sectors to create biotechnological research laboratories and institutes serving as an interface between scientific research and high biotechnological enterprises;
- Provision of financial incentives to scientists in conducting appropriate research and provision of risk capital for conversion of the results to technological capability; and
- Strengthening capacities in management of Intellectual Property in biotechnology.

11 BIO-SAFETY REGULATIONS AND ASSESSMENT PROCEDURES

11.1 General

Biotechnological products, which may be commercialised, are at the stage of infancy in Islamic countries. Though there are some risks associated with new products, processes or genetically modified organisms, the benefits that could be yielded therefrom cannot be ignored. Therefore, bio-safety regulations in adopting biotechnological products and genetically modified organisms (GMOs) should be formulated with care. Gene transfers are unnatural and may create natural imbalance. Emergence of new viruses that may be more lethal, or agricultural products, to which humans may become allergic cannot be ruled out. This may lead to transnational companies relocating the risky part of their biotechnological research to developing countries, where loss of biodiversity and soil degradation may be already present.

Some Islamic countries have already drafted their bio-safety protocol or in the process of drafting it. It is necessary for the Islamic countries to adopt a bio-

safety protocol to safeguard the people and the environment from any emergency due to any new biotechnological product.

11.2 Key elements necessary for the strategy

- Development of a bio-safety protocol to control the technology and protect the people and the environment from harmful effects;
- Establishment of bio-safety information network and advisory services to provide technical assistance and awareness guidelines;
- Training of researchers and local people in the safe handling of genetically-modified organisms; and
- Establishment of regional groups for provision of new guidelines and to make arrangements for routine reciprocal exchanges.

12 ETHICAL IMPLICATIONS OF BIOTECHNOLOGY

12.1 General

Unguided application of biotechnological techniques may pose serious threat to the ethical and Islamic values of our society. While promoting application of new technologies, it is equally necessary for us to debate these issues, assess realistic risks and adopt a clear policy to provide necessary guidelines to the scientists and public in the light of Islamic Shari'a. We must not ignore the moral and ethical dimensions while drafting a Biotechnological Strategy for the Islamic world. It is important to analyse and monitor special biotechnological regulations in the light of ethical values. We must listen to community, scientists, researchers and religious scholars through various forums. At present, there is no Islamic Ethics body.

The First Meeting of the Consultative Council for Implementation of the Strategy for the Development of Science and Technology has recommended establishment of the Islamic Ethical Body. Necessary initiatives have already been taken in this regard.

12.2 Key elements necessary for the strategy

- Establishment of an Islamic Ethical Committee, consisting of the representatives of all the Member States to study with great care various new technologies from the ethical, Islamic and Shari'a point of view to ensure respect of the Islamic principles;
- Discussion forums for consulting and liaising with community leaders regarding new biotechnologies and encouraging public contribution towards preparation of guidelines;
- Provision of clear guidelines on various important topics to enable the scientists to work with confidence; and

- Ethical aspect should be an integral part of the education and training of all scientists.

13 STRENGTHENING COOPERATION AND COORDINATION

13.1 Coordination among Islamic countries

Another area, which needs immediate attention in terms of drafting an appropriate strategy, is our weaknesses in joint scientific and technological collaboration with other countries in general and among Islamic countries in particular. International collaboration in scientific activity has become one of the powerful determinants of success and is considered one of the key elements in national scientific policy. It is not possible for any single country to achieve, on its own, any significant or meaningful scientific and technological progress and development. Biotechnology, which is more dependent on sharing of knowledge and expertise, needs active collaboration with foreign scientists to provide access to resources otherwise unavailable. Islam has already bounded all Islamic countries in a relationship of brotherhood. We may consolidate solidarity among us and utilize it for our mutual benefits. Cooperation in biotechnology can be achieved through creation of communication networks by forming scientific affiliations, regional alignments and international groupings. The strategy should be aimed at the promotion of close cooperation among the Islamic countries and consolidation of understanding among biotechnologists.

13.2 Key elements necessary for the Strategy

- Sharing of knowledge and experiences between laboratories in the Islamic countries, which are at the frontier of the biotechnological research;
- To promote communication among colleagues with similar interests to avoid repetition of work and consolidate their efforts; and
- Promotion of technical cooperation among Islamic countries and initiation of joint biotechnological programmes on bilateral and multilateral basis.

13.3 Cooperation with industrialized countries

Due to the process of globalisation, the countries of the world are already coming closer to each other. Now, anything happening in one part has it repercussions in other parts of the world. We cannot have a peaceful world with large disparities among its peoples. Inequalities should be bridged up and developments should be considered on the global scale. In this new millennium, industrialized countries, which have already utilized natural resources of the world, should realize that they do not live alone, they should not regard the technologies only as a way of making money. They should now come forward to help the least developed countries with knowledge and skills to enable them to utilize the benefits of new technologies for the welfare of their own people.

This is not only a moral obligation but reflects on security, peace and economic progress of the whole world.

13.4 Key elements necessary for the strategy

- Mobilization of technological support from industrialized countries through international scientific forums, to the least developed countries enabling them to face their own challenges;
- Enhancing the biotechnological programmes of international scientific bodies and organizations; and
- Establishment of specialized assistance programmes for developing countries in identifying biotechnological needs, priorities and strengthening of their capabilities.

14 CONCLUSION

As a consequence of rapid pace of new discoveries and innovations, bio-technologies are expected to bring enormous benefits and gains to socio-economic development. The current science or biotechnology policies pursued in most Islamic countries are not sufficient to bring any breakthrough unless special time target goals are fixed, practical measures are taken and realistic plans are drafted and implemented. A strategy at the Islamic world level for the development of biotechnologies is a necessity and all Islamic countries should support this process. The financial institutions in the world in general and in Islamic countries in particular should come forward to support biotechnological development. Governments in Islamic countries cannot continue with low priorities to research and development. It is a time to think and act seriously and bring a change in our national priorities. Decision-makers must have to realize that the potential lies in the promotion of biotechnologies. The scientific community must not remain a spectator and needs to raise its voice, so as to force a change of direction.

Education reforms should support the biotechnological developments and should be supplemented with enhancing of general awareness thereof.

Biotechnological research in the various fields is very promising, like effective agricultural and food production, treatment and prevention of disease, the satisfaction of our energy needs and the protection of environment. It is also necessary to take account of the ethical challenges posed by the development of bio-technologies. International cooperation will have an important role to play in the practical application of such technologies. In addition to consolidating solidarity among Islamic countries, international collaboration with industrialized countries and international scientific organizations should be sought for this purpose. A healthy balance in planning all these factors is necessary for drafting an appropriate strategy for the development of biotechnology in the Islamic countries.

REFERENCES

1. Strategy for the Development of Science and Technology in the Islamic Countries, Pub: ISESCO, Rabat, October, 2000.
2. Science Agenda-Framework for Action: Resolution of WSC99, Budapest, Hungary, 26 June-1st July 1999.
3. Status of Scientific Research in Islamic Countries, Pub: ISESCO, Rabat, October 2000.
4. Biotechnologies in Developing Countries: Present and Future, Volume 1:Regional and National Survey, Albert Sasson, Pub: UNESCO, 1998.
5. Higher Education in Islamic Countries "Current Situation and Future Perspectives," Pub: ISESCO, Rabat, October 2000.
6. Science for the Twenty-First Century: A New Commitment. World Science Conference Document, Pub: UNESCO, 2000.
7. An Analytical Study on Problems and Issues of Transfer of Scientific Research Results to the Production Sector. Dr. S. M. Junaid Zaidi, Pub: ISESCO, Rabat, October, 2001.
8. Biotechnologies in Developing Countries: Present and Future, Volume 2: International Cooperation, Albert Sasson, Pub: UNESCO,1998.

Genetic Engineering and the Ecobalance

MUNIR OZTURK*
and
IFFET IREM UZONUR**
Biology Department, Fatih University, Büyükçekmece 34900
Istanbul, Turkey

1 ABSTRACT

Scientific knowledge is the source of technological development. Developments in science and technology have lately resulted in the birth of a new discipline called Genetic Engineering. This is expected to become a distinguishing power of the 21st century together with Ecology. Their revolutionary status is determined by the range of their applications involving a wide spectrum of human needs. The discoveries made in the field of Genetic Engineering so far and the potential discoveries are enormous, exciting and encouraging, but the consequences are really complicated and instead of progress they may lead to a disaster. Pollution from Genetic Engineering could lead to disasters far worse than those caused by accidents in nuclear and chemical installations. The release of a genetically engineered microorganism lethal to humans could result in the end of humanity. The harmful effects associated with the use or release of genetically engineered organisms is not well known as yet. Not all of these organisms may be harmful, and not all are likely to proliferate in the environment.

We have always been dependent on the environment for our survival. Ecology oriented activities have never harmed or threatened any organism or crop. The developments in science and industrial revolution changed all that. These activities have brought the world to the sharp edge of environmental crisis. Today's environmental deterioration does not originate from science and technology or lack of information, trained people or money, rather our life-styles that depend on anthropocentric views and such basic beliefs that nature exists only for human use. It is bad science that has let the world down and caused the major problems we now face. Applications of science and technology can change the environment for better or for worse. However a

---

* Professor of Ecology at Fatih University (2001); and Fellow of the Islamic Academy of Sciences; Ege University (2004).

** Lecturer of Molecular Biology and Genetics, Biology Department, Fatih University.

peaceful global welfare society cannot be constructed if our mental progress fails to catch up with scientific and technological progress in the space age. We should try to re-introduce science back into our cultural stream and learn to integrate our cultural values into our scientific activities.

2 INTRODUCTION

Our universe with more than 600 million solar systems within a galaxy is infinitely large and we need high-speed carriers with high technology to roam about in the outer space. For this purpose progress in technology, culture and development is a pre-requisite. Knowledge of information technology will play a great role in this connection to the extent that it will be a new experience and challenge for the human society. As a matter of fact knowledge is considered science only when it is in the public domain (Ziman 1969). In the 21st century this new experience will come to forefront in the fields of ecology, biotechnology, recycling, environmental protection, new materials like super alloys for super conductivity and energy such as; clean and inexhaustible energy in place of nuclear plants and fossil energy. It will be particularly worth noting that since late 80's, we have been dominated by tremendous growth of innovations in "*Computer Technology*" and "*Genetic Engineering.*" These fields resemble each other in the sense that they are based on the same digital technique, with the "*Computer code"* having series of numbers or "*DNA-code*" with series of bases.

Science is a historical complex body of knowledge with different procedures used at different times, and is defined as a self correcting activity, with a curiosity, specific critical attitude, criteria and coherent subject matter, differing only in scope and approach to many other human activities (Boulter 1999). Scientific thoughts arise through inspiration, dreaming, and personal creativity or by separating important from unimportant. Doubt and uncertainty are parts of scientific attitude. They form the best way to solve some problems; in particular management of our surroundings, food production, disease control and other biological innovations. The strength of science lies in its freedom from dogma, authority and preconceived values. It is characterized by different levels but all ending up with a theme of reductionism, which has been over stressed to the extent that some branches not dealing with bottom-up search for fundamental mechanism like ecology have been insulted. However, science is not the only way to the truth of the world. Many interrelations about living organisms are outside its present scope. Science has been accepted as infallible till recently but some non-beneficial applications have uprooted this view. As such, scientists are often accepted as impertinent by the public in view of their social considerations. An interference with nature has changed some scientists to the extent that they are disrespectful and lack care. This is also due to the fact that scientists sometimes lose control, starting things without realizing the

consequences. Basically the reason for this is that science changes, evolves and can only reach the approximate point.

The history of science clearly shows that it is very difficult to predict where new findings will lead. Applications of science can change our surroundings for good as well as for bad. We blame science and technology for this but all innovations will be impossible without that particular individual; the **"*human being*".** We, the humans, are unique among all creatures because of our power for reasoning. This makes our behaviour undetermined. We never think of the attitudes and ethical side of our societies towards the nature. We achieve great successes but commit big mistakes as well. It basically stems from our life-styles. Our anthropocentric thinking leads us to feel that everything around us is only for human use, which is more a crisis of mind and spirit. Here, we need greater consideration in the larger dimension where science and spirit can meet. The complex web of environment was used carefully by traditional societies till the industrial revolution, afterwhich the changes started. Today, the world is on the sharp edge of an environmental crisis not experienced before (Figure 1). In order to build an environmentally stable future, we need a broader vision. Under present circumstances a society satisfying its needs without jeopardising the prospects of future generations is a sustainable one. Our planet needs this action during next few decades. If we do not succeed in our attempts, "economical decline" will start feeding on "environmental degradation" and vice-versa. This will cause social disintegration. During the last century morality collapsed together with our souls. Do we expect something better for the 21st century, which is expected to be the century of genetic engineering and ecology. In this paper we will try to present the facts concerning these two fields i.e., DNA coded "*genetic engineering"* and organism coded "*ecology*."

3 GENETIC ENGINEERING

3.1 Achievements

In our new millennium, genetic engineering is taking over every aspect of our daily lives. The possible benefits promised to humankind are limitless. There is something to satisfy everyone. For those morally concerned about inequality and human suffering, it promises to feed the hungry with genetically modified crops able to resist pests and diseases and to increase yields. For those who despair of the present global environmental deterioration, it promises to modify strains of bacteria and higher plants that can degrade toxic wastes or remove heavy metal contaminants. For those interested in sustainable agriculture, it promises to develop greener, more environmentally friendly transgenic crops that will reduce the use of pesticides, herbicides and fertilisers. The real revolution is expected in the realm of human genetics. Plans to uncover the entire genetic blueprint of the human being will enable geneticists to diagnose, in advance, all the diseases that an individual will suffer in his or her lifetime,

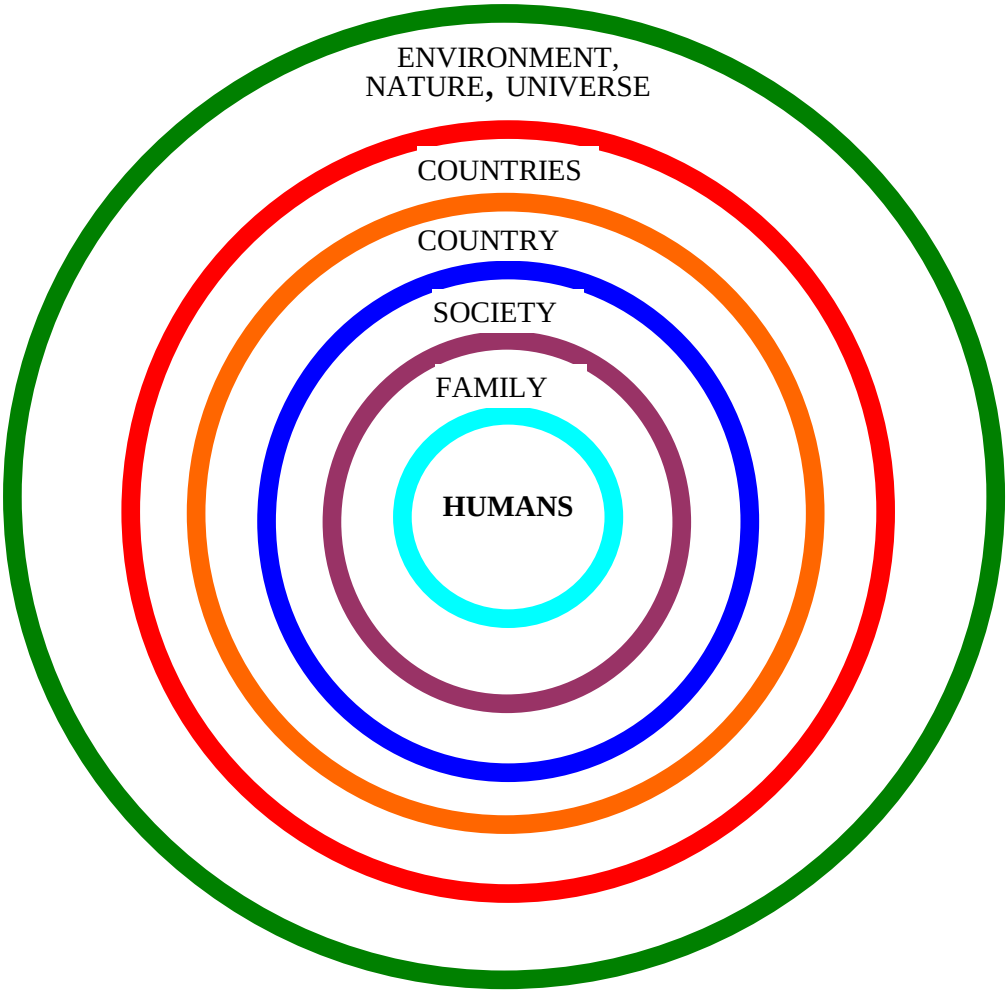


Figure 1. Interrelationship hoops for Man and the environment.

even before the individual is born, or even as the egg is fertilised *in vitro*. A whole group of specific drugs required for individual genetic needs could be designed to cure all diseases. The possibility of immortality is dangling from the horizons, as the "longevity gene" is isolated.

It started only few years back when BST-milk from cows fed genetically engineered bovine growth hormone to boost milk yield (Epstein 1990). An avalanche of products arriving, or soon to arrive, at the supermarkets are tomato puree, rapeseed oil, soybean, maize, sugar beet, squash, cucumber. Although developing a transgenic plant with a stable genome usually takes years to get to the open field, today we see that genetic engineering has left the laboratories. There is no large-scale consumer boycott for the products containing transgenic soya.

Studies on genetic engineering and biotechnology have very old roots dating back to the time of Mendel, but impetus in its advancement came from 1953 onwards after Watson and Crick discovered molecular configuration of DNA, which resembles a ladder with two side components deoxyribose sugar and phosphate, the steps are bases; adenine, thymine, guanine and cytosine. This DNA determines the organism but is referred to as one-way ticket only in which DNA is the cause and the organism is the effect, such thinking is completely opposite to ecological one.

"Genetic modification," we are told, is simply the latest in a "seamless" continuum of biotechnologies practised by human beings since the dawn of civilisation, from bread and wine-making, to selective breeding. The significant advantage of genetic modification is that it is much more "precise," as genes can be individually isolated and transferred as desired. Such spectacular achievements as protein synthesis, artificial synthesis of the functional DNA, production of insulin from bacterial cells and construction of functional artificial chromosome, have been obtained (Akbar 1986). Biologists can split up specific segments of DNA using restriction enzymes or use ligases to incorporate DNA bits in to plasmids, i.e. extra chromosomal DNA particles. This cloning then gives us a bio-product needed, which could be a new variety with highest acceleration of reproduction span and immune to diseases. N fixing bacterial gene cloning through plasmids and their insertion into wheat, corn, rice will allow these crops to fix N from the air. So no N fertilizers will be needed. A salt tolerant DNA has been produced which can be used for agricultural production of crops on such degraded soils. Somatostatin hormone is effective against insulin dependent diabetes as well as acute pancreatitis. Its chemically synthesized form is expensive whereas genetically engineered production has become extremely cheap. Similarly, interferon, effective against hepatitis, influenza, some cancer types is also expensive in natural form but can be produced very cheaply through bacteria. Bacterially produced insulin too is under trial as well as treatment of dwarfism. Genetically engineered microorganisms produce urokinase enzyme useful in dissolving blood clots. Hormone thymosin α-1 can be used to treat brain and lung cancer. In

immunology, hereditary modifications are followed to increase resistance of organisms to diseases. Vaccines for diseases; measles, polio and malaria are developed using recombinant DNA methods. Gene therapy is used now against thalassemia, a hereditary blood disease (Tadmouri *et al*. 1998, 2000, 2001).

Fermentation technology industry is using genetic engineering on a large scale for producing antibiotics, ethanol, enzymes, sugars, food products and flavouring agents with least pollution. Industries that are involved in plastics, resins, flavours, perfumes, synthetic rubbers, pesticides are benefiting much now from genetic engineering, so this field is accepted as the new alchemy with possibilities to change a basic organism into genetic gold (Popper *et al*. 1998; Parades-Lopez 1999).

The most popular transgene apart from antibiotic resistance marker genes has been for herbicide resistance, which have now been put in soybeans, maize, wheat, fodder/sugar beet and chicory. These allow the crop to be sprayed with herbicide only if weeds are becoming a problem, without killing the crop. Other common transgenes is for insect resistance, for example; *Bacillus thuringiensis* produces some 150 insect toxins, a mixture of which is widely used in organic agriculture to avoid insect attacks. The gene coding for the toxic proteins have been isolated and transferred to crop plants such as maize, potato and cotton. If insects eat the leaves they are killed. Another transgene prevents softening in tomatoes allowing them to be ripened on the plant whilst reaching the supermarket sufficiently firm for sale. Some of the other transgenes on trial include leaf-roll virus resistance, insect resistance from snowdrop and modified starch content in potatoes; altered lignin content in poplar; modified oil content, reduced pod shatter, fungal tolerance and male sterility in rape; insect resistance in tomatoes; and Arctic turbot antifreeze protein genes in strawberries. Terminator technology is accepted as an important achievement in genetically modified crops, where the embryo in the germinating seed dies as a result of the presence of a toxic transgene, thus rendering the saved seed useless for cultivation. Marker genes for antibiotic resistance are widely used to allow selection at the test tube stage of organisms, which have successfully taken up the transgenic organism (Timmons *et al*., 1996; Koskella and Stotzky 1997; Tapp and Stotzky 1998). Whilst some markers correspond to antibiotics rarely used in medicine like kanamycin in sugar beet, others are for antibiotics that are in common use.

A serious worldwide soil erosion problem is caused by tillage and soil compaction, resulting from heavy machinery and fossil fuel combustion, which ends up in loss of fertility. Roundup ready soybeans have been advertised as a good solution to this problem.

3.2 Risks

The risks *inherent* to genetic engineering, particularly as misguided by an outmoded and erroneous worldview that comes from bad science are many. Many previous technologies have proved to have adverse effects unexpected by

their developers e.g., DDT and chlorofluorocarbons. Warnings of unexpected effects on agriculture and biodiversity, of the dangers of irreversible "genetic pollution," genetic discrimination and the return of eugenics have been marginalized. Transgenic animals, plants and seeds, are taken freely by geneticists of developed countries from the Third World, as well as human genes and human cell lines from indigenous people. What harmful effects might turn out to be associated with the use or release of genetically engineered organisms. So far, scientists have identified a number of ways in which genetically engineered organisms could potentially adversely impact both human health and the environment (Mikkelson *et a*l., 1996).

The potential harms to health are new allergens in the food supply, production of new toxins, bioaccumulation of toxic metals, and enhancement of the environment for toxic fungi. Genetic engineering might be used to produce decaffeinated coffee beans by deleting or turning off genes associated with caffeine production. But caffeine helps protect coffee beans against fungi. Beans that are unable to produce caffeine might be coated with fungi, which can produce toxins.

The potential environmental harms are; increased weediness, gene transfer to wild or weedy relatives, change in herbicide use patterns, squandering of valuable pest susceptibility genes, poisoned wildlife, and creation of new or worse viruses.

There is much concern about transgenic insect and herbicide resistant crops, e.g. maize which contains an ampicillin resistance gene. Ampicillin and antibiotics closely resembling it are still of therapeutic value and a transfer of the ampicillin resistance gene from maize forage to livestock gut mycoflora and thence to humans would render the antibiotic useless. Crops with antibiotic resistance markers could pose a greater threat if consumed directly by humans. Similarly herbicide resistance gene in soya and glufosinate resistant oilseed rape is said to be of great advantage to the farmer because he can bring down the herbicide use up to one-third, thus reducing his herbicide costs through an environmentally favourable weed controller as well as with flexibility to treat weeds only when needed and rotate crops more easily. However, an unidentified component from this group has recently been found to react with DNA in mice, thus pointing to its possible mutagenicity and hence carcinogenicity.

A gene flow from herbicide resistant oilseed rape to wild relatives like mustard, wild turnip and wild radish may result in new herbicide resistant invasive species, costly for the farmer and potentially damaging to the environment because more toxic herbicides will be needed to get rid of them. We know that accidental and deliberate movement of alien species round the world has already created hundreds of invasive species. In the meantime using herbicide year after year exerts a selective pressure on weed populations resulting in the evolution of more resistant weeds, where the options are; either stronger, more toxic herbicide or organic farming based on traditional methods

of weed control. Weeds support insect populations and both are food for birds. As such use of more toxic herbicides could prove environmentally harmful by destroying our wildlife. A recent laboratory study has shown that death rates of beneficial insects feeding on herbivorous insects, are doubled when feeding on insects that have eaten GM plants (Springett and Gray 1992). In a study nearer to natural conditions, potatoes containing a snowdrop insecticidal gene were found to grow less well than controls when both were exposed to aphid attack (Heaf 1998; Hilbeck *et al.* 1998). The cause was traced to a fall in numbers of ladybirds, an aphid predator. The plants need ladybirds too. Little is known yet of the ecological impact of modified crop toxins on bees producing honey (Eady *et al.*, 1995).

Everybody knows that with time, target insects develop resistance to toxins, even if the refuges are planted around the area. However, it is said that genetic engineers have a gene library of many genes, enough to keep ahead of the resistance problem for future. Although consuming an actual 'foreign' gene in food is not yet widely thought to be dangerous but the dangers from long term consumption of GM crops are not fully understood. They contain a group of 150 toxin genes and could generate enough allergenicity as well as toxicity, if not now maybe as an after effect in the years to come. A reduction in fuel and emissions seems to shift the situation from petrochemicals to agrochemicals.

Genetic engineering bypasses conventional breeding by using the artificially constructed vectors to multiply copies of genes, and in many cases, to carry and smuggle genes into cells. The insertion of foreign genes into the host genome has long been known to have many harmful and fatal effects including cancer. Recent outbreaks in some diseases are believed to have originated from horizontal gene transfer from the pathogens. What is the connection between horizontal gene transfer and genetic engineering? Genetic engineering is a technology designed specifically to transfer genes horizontally between species that do not interbreed. It is designed to break down species barriers and overcome the species' defence mechanisms. The technology thus contributes to an increase in the frequency of horizontal gene transfer of those genes that are responsible for virulence and antibiotic resistance, and allow them to recombine to generate new pathogens (Syvanen 1994). Geneticists have now found that the presence of antibiotics typically increases the frequency of horizontal gene transfer 100-fold or more. The horizontal gene transfer previously thought to be confined to bacteria alone now is said to involve practically all species of animals, plants and fungi. It is possible for any gene in any species to spread to any other species, especially if the gene is carried on genetically engineered gene-transfer vectors. Transgenes and antibiotic resistance marker genes from transgenic plants have been shown to end up in soil fungi and bacteria. These microbial populations in the environment serve as the gene-transfer highway and reservoir, supporting the replication of the genes and allowing them to spread and recombine with other genes to generate new pathogens (Syvanen 1987; Ho and Tappeser 1997). The "crippled" laboratory strains of bacteria and

viruses are reported not to survive when released into the environment, but evidence shows they can survive quite well and multiply, or go dormant reappearing after acquiring genes from other bacteria. DNA is said to be easily broken down in the environment but it can remain in the environment and even survive rigorous boiling, and readily finds its way into the bloodstream, and into the cell in the body, where it can insert itself into the cell's genome, and create genetic disturbances.

Genetic engineering is not that simple and precise as cutting and pasting with a word processor. Since favourable genetic mutation is of rare occurrence, we need millions of micro-organisms to be handled at a time for developing herbicide resistant mutant bacterium. Cloning allows the desired gene to be pinpointed, but getting it into the crop plant is more miss than hit. During these applications how many copies of the transgene go in or where they end up in the plant genome is unknown. Transgenesis can end up with loss of entire chromosomes in the host genome. *Agrobacterium tumefaciens*; which causes plant tumours; smuggles the gene past defence mechanisms which is the same as infection by a retrovirus inserting itself into an organism's genome, sitting there sometimes for years before taking part in a disease process.

This field is accepted as a continuation of greed in human beings to control the living world on his planet for the time period of his life. Certainly a few genetically modified species might rapidly supplant the existing worldwide crop diversity necessary for withstanding disease and climate changes, thereby leaving the food supply wide open to sudden catastrophic loss. Some of the leading scientists point out that pollution from genetic engineering, has created a 'gene smog' (Sloan and Powers 1986; Heaf 1998), which is potentially more dangerous than radioactive fallout or chemicals. These scientists are accepted as alarmist by their colleagues, but *genetic engineering* is inherently hazardous. It could lead to disasters far worse than those caused by accidents in nuclear installations, because genes can replicate indefinitely, spread and recombine. For this reason the release of a genetically engineered micro-organism that is lethal to humans could well spell the end of humanity (Ho 2001).

One is left to wonder why, if the products are as safe and wonderful as claimed, they could not be segregated, as organic produce has been for years, so that consumers are given the choice of buying what they want. Recently the term 'copy gene' has been applied to overcome human psychology that if we ever have human genes in a food plant it would not be cannibalism to eat it. What will happen when interfering with nature and revealing the genetic code of species and then introducing human genes into animals, and animal genes into vegetables e.g., if a pork gene is transferred to food plants, will it not counter certain religious beliefs - in comparison with the much more fundamental questions of eugenics? It took nearly 300 trials to get one success “Dolly,” but no mention is made of the vast majority of the embryos that failed. Is that ethical? If it can be done on sheep, does it mean it cannot be done on human beings? Are we nearer to cloning human beings? The fact stays that once

a rogue organism gets into the wild it can reproduce and spread beyond geographical boundaries spelling death to thousands of human beings.

Rotblat who won the Nobel Prize in 1995, after battling against nuclear weapons for years says; “My worry is that other advances in science may result in other means of mass destruction.”

3.3 Bad science and big business

The fact that makes genetic engineering dangerous is the close alliance between “ science” and “commerce.” When bad science starts working together with big business for quick profit it changes the fate of good science, which promises to work for the benefit of mankind. It is bad science that has let the world down and caused major problems. However, in reality science is nothing more, and nothing less, than a system of concepts for understanding nature and for obtaining reliable knowledge that enables us to live sustainably with nature, but there can be *bad science* that ill-serves humanity. Some believe that science can never be wrong, it is neutral and value-free, from its application, technology, which can do harm or good, although it can be misused. Science can sometimes be wrong. The distinction between science and technology is deceptive, especially in the case of an experimental science like genetics, and almost all of biology, where the techniques determine what sorts of questions are asked and hence the range of answers that are important, significant and relevant to science.

Science is seen to be beyond reproach, as it is the "laws" of nature. The same goes for "technology" - the application of science. Risk assessments are to do with the technology, leaving the science equally untouched. Technology can be bad for your health, but not science. Bad science is unquestionably bad for one's health and well-being, and should be avoided at all costs. Science is, above all, fallible and negotiable, because we have the choice, to do or not to do. Scientists dominated by the reductionist view see the world as bits and pieces, and deny that there are organic wholes such as organisms, ecosystems, societies, and communities. For them nature and fellow human beings are as objects to be manipulated and exploited for a gain.

4 ECOLOGY

4.1 General

Genetic engineering has a central-concept, the DNA-molecule, which dwells in the nucleus of the cell. Ecology too has a central-concept but this concept i.e., *ecosystem*, is regarded as a periphery concept, leading scientists towards vast surroundings. The ecosystem is not limited within strict borders. It 'floats away,' always interacting with a bigger surrounding. There is a tendency of integration and synthesis where we try to create friendship with the outer world. This

tendency has developed mainly in the science of ecology. Biotic and abiotic components of our environment show a hierarchical arrangement among the components and a state of equilibrium exists at each level, with time and space acting as limiting factors on these levels. For example a small pond forms a small aquatic ecosystem. This pond is surrounded by terrestrial ecosystems - forests, grasslands and mountains. These ecosystems are interwoven with each other. There is a continuous flow of matter, energy, information and organisms between the surrounding ecosystems and the pond. Looking deeply on the pond makes us realise that we are a part of these ecosystems like the beads of a chain. Each bead is beautifully placed and an interrelationship exists among these beads in a most balanced fashion. These beads are acting under the orders of a prescribed and unchangeable course of law. The air we breath was a little while ago part of our environs. If we limit our environs to the border of our skin, this is only an illusion, a view coinciding with that of Dawkins (1989) that genes have made us, body and soul, to serve one single purpose: gene-reproduction and we are simply gene-carriers, survival-machines, blindly programmed to fulfil those gene-transmission-strategies (Skaftnesmo 1998).

Every ecosystem is more or less open, continually exchanging matter and energy with its surroundings but each has a life of its own, a life which follows quite distinct laws. The pond we have in front of us shows distinct polarity between its different depths in the water. It has three zones; the shoreline- and surface-zone followed by the intermediate zone and the bottom-zone. Synthesising activity, building up new organic material through photosynthesis is observed in upper layers, which makes these layers a 'life-pole' for the pond. At the bottom, a disintegrating, analysing activity dominates, resulting in the decomposition of organic material which can be regarded as a 'death-pole' because organic material built up through the life-processes changes into inorganic, mineralised state here. These layers accommodate producers (plankton / green plants), consumers (crustaceans, insects and fishes) and decomposers (mainly fungus and bacteria), interacting continuously in some sort of circulation process. Consumers use the biomass of the producers and the substances produced reach decomposers, which takes care of the last part of the circulation. Latter mineralise the substances to nutrition-components taken up by the producers. The system is *homeostatic* showing a seasonal balance around specific mean-values. This reminds us of an image of an *organism*! The ecosystems too are like organisms with a central characteristic concept of *homeostasis*. A complex relationship exists between the organs and the organism as well as between the individual organisms and the ecosystem. Neither ecosystems nor organisms can be understood by simple linear causality. There is a correlation-causality working hand in hand with a network of relationships and feedback-processes like beads in a chain, so we need to study the way these beads are interconnected with each other and to the system on the whole.

The field of ecology then basically appeals to an inner activity of wholeness-recognition. Central-concept of the ecosystem is the 'face' we see. This ability of recognition can be cultivated. As against this, the sciences like genetic engineering that use analytical-fragmenting and linear-causal way of reasoning, stimulate towards decay and destruction, with an orientation directed towards the centre. The way of reasoning here is one way linear expressed through the hunt for simple chains of causes and effects.

DNA* → *RNA* → *PROTEIN* → *CELLS/TISSUES* → *ORGAN* → *ORGANISM

In natural sciences the tradition has been to take notice of the complexity as a whole in the field of social and historical relations. Ecology has been following this tradition. Thus the aim of ecology lies in the circle rather than in the line (Figure 2).

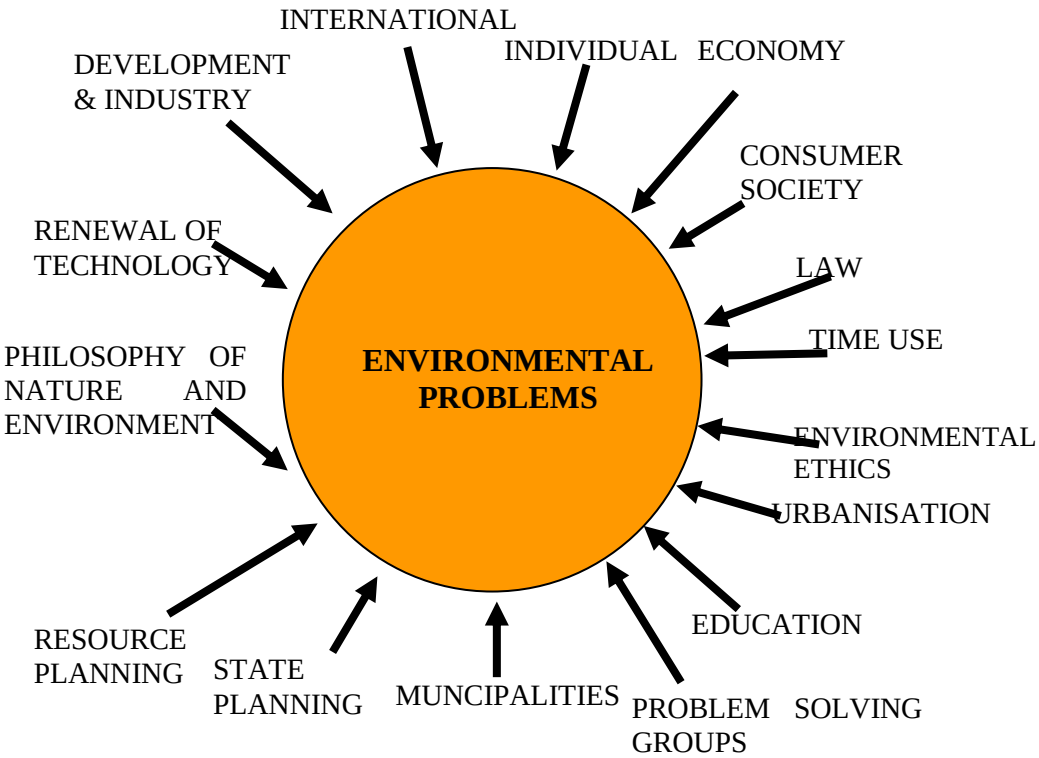


Figure 2. Factors affecting environmental problems.

4.2 Ecology vs genetic engineering

Ecology is accepted by everyone as a good science, because ecology, has not yet harmed or threatened anything abiotic or biotic, but has dealt with a study of the conditions for the maintenance and sustainability of the organism and the ecosystem. Genetic engineering developed with such sciences as agriculture and medicine. Its activities can potentially be saving and protecting life - under certain circumstances. Inspite of this it is regarded as something dangerous, because it can also be potentially a threat to life. This is why there is far more scepticism towards the field of genetic engineering than towards the field of ecology. But still, it is genetic engineering that makes the profits although regarded by some scientists as environmental Russian roulette. Our elders used to say that our past is written in our genes, now it seems that our future lies there too. According to Watson (2000), “We used to believe that our fate was written in the stars, now we know that it's in our genes." What a strange situation! An ecological insight is what we need for our survival but it is not a profitable business. When we look at a tree it appears to us as a whole, but when we begin to examine the leaves, branches, flowers and fruits one by one we eventually become doubtful. Our vision of the tree is one of wholeness resulting in a spontaneous image on our mind.

5 CONCLUSION

In the age of global interdependence, demographic outbursts will expand the needs for development, as such our millennium could be an age of mass displaced persons derived from lack of peace, violation of human rights, and land degradation. The best solution for this will be progress in human minds as well as science and technology. Different approaches can be conceived without making any moral judgements. We need to look at the dangers that come out when, while defining science, a single tendency becomes totally sovereign in education and research. A one-sided investment in genetically oriented thinking will stimulate a technologically oriented science imprinted with reductionism. The phenomena of organic and spiritual nature will thus disappear and this branch will become unscientific.

A progress in human minds will include spiritual progress. Latter cannot be achieved by simple developments in genetic information in many generations. Relying on mutations and self-organisations will slow down our spiritual progress. This will in turn affect our mental progress that serves as the backbone for a peaceful global welfare society. Scientists must accept the challenge to reintroduce science back into mainstream culture and learn to integrate such wider cultural values into their scientific activities.

The main problem is that we are mixing up the motive for healthy performances, because we cannot differentiate well between “ethical” and “unethical” themes. Ethics is a state of our souls, embodying all our actions bad

or good, ugly or beautiful. We always forget about the soul, which includes psychological and spiritual aspects. In fact ecocentric solution of the problems will be easier if we include the soul. Genetic engineering and ecology can thus be shown as:

$$\textit{DNA} \longrightarrow \textit{ORGANISM} \rightleftarrows \textit{ENVIRONMENT}$$

In this ‘headline,’ the first part depends on a give and take relationship determined by a completely blind DNA, which is deaf towards any feedback-messages. There seems no possibility that the organism could have influence upon the DNA. In the second part the headline, organism is intimately correlated with its environs. It is both formed and is actively forming it. So we can say that the characteristic of the gene-approach is a simple linear atomistic model, directed towards the centre, whereas the characteristic of the ecological approach is holistic, directed towards the periphery. Our own consciousness wakes up when it meets something that is quantitative and centre-directed. But this is not the only way to be a human being.

Gene technology is strongly promoted by the groups who argue that it is the best way to combat world food shortage on a demographically overloaded planet earth. When we question gene technology, we raise once again many old issues: intensive agriculture; pesticide and herbicide use; and the power of the big companies. We cannot deal with new or old issues without cultural freedom and the possibility of pluralism.

The threefold nature of human beings has made their society three-fold. First is the cultural/spiritual sphere, which develops properly only when freedom prevails. Second, is the rights domain dealing with laws and their making, being recognised where equality between human beings exists. Third, is economic life demanding a feeling of brotherhood for or solidarity with one's fellow human beings. This presupposes power-free dialogue between all parties. Undoubtedly using wealth for manipulating public opinion by contacting people in the upper circles of a state and arranging placement of full page letters in the press to permit use of gene technology for the sake of hunger will be an injustice to *Homosapiens.*

Food is a cultural/spiritual matter as much as it is one which belongs to the economic and rights spheres and we would expect to see freedom in the cultural sphere. Food production, especially with modern technologies such as genetic engineering raises ethical issues. Whether something is acceptable or not acceptable is a moral, not a scientific judgement. Yet it seems that already political decisions have been made about genetic engineering without adequate cultural involvement in the decision-making.

Gene technology is only a little more than 20 years old and we can expect that more research including unravelling the entire genomes of target plants will greatly improve the precision of gene insertion. Even if gene insertion into a plant's DNA works, the gene can still be switched off by gene silencing. To

dismiss it for poor technical performance now would be like rejecting space technology for railway technology. The question posed here is that we do tolerate the cars which kill hundreds of people every day all over the world. Currently genetic engineering is not welcomed in agriculture but we know that traditional breeding also created giant forms in wheat and maize long back.

As with human health risks, all the potential harms of genetic engineering to the environment have not been identified at length. The answer to that question depends on how well scientists understand the organism and the environment into which it is released. At this point, biology and ecology are too poorly understood to be certain that question has been answered comprehensively. Once the potential harms are identified, the question becomes how likely are they to occur. The answer to this question falls into the arena of risk assessment. Having identified a list of possible harms that might occur as a result of using or releasing genetically engineered organisms, the next question is how likely are any of these to occur? Like the original "brainstorming" of potential harms, the answer to this question depends greatly on how well the organisms and their interaction in the environment are understood. Risks must be assessed case by case as new applications of genetic engineering are introduced. In some circumstances, it is possible to assess risks with great confidence. This can be complicated because they involve numerous assumptions, and could include full environmental impact assessment statements.

ACKNOWLEDGEMENTS

We are highly indebted to Prof. Mehmet Ergin for encouraging us to prepare this review article. Our sincere thanks go to the Islamic Academy of Sciences for providing financial support to one of us (M. Ozturk) to participate in the meeting.

REFERENCES

1. Akbar, K. F. (1986), Biorevolution and the Islamic World. Science, Technology and Development 5, (1) pp. 29-34.
2. Boulter, D., (1999), Public Perception of Science and Associated General Issues for the Scientist. Phytochemistry, 50 pp. 1-7.
3. Dawkins R. (1989), The Selfish Gene, Second Edition, Oxford University Press.
4. Eady, C., Twell, D., and Lindsey, K., (1995), Pollen viability and transgene expression following storage in honey. Transgenic Research Vol. 4. pp. 226-231.
5. Epstein, Samuel S. (1990), Questions and answers on synthetic bovine growth hormones. International Journal of Health Services, Vol. 20, No. 4, pp. 573-82
6. Heaf D., (1998), Beans Means Genes Transgenic crops and food: a threat to cultural freedom? *New View*, pp. 35-39.
7. Hilbeck, A. (1998), Effects of transgenic Bt corn-fed prey on mortality and development time of immature *Chrysoperla carnea.* Environ. Entomol. 27(2), 460- 489.
8. Ho, M.W. and Tappeser, B. (1997), Potential contributions of horizontal gene transfer to the transboundary movement of living modified organisms resulting from modern biotechnology. In Transboundary Movement of Living Modified Organisms Resulting from Modern Biotechnology: Issues and Opportunities for Policy-Makers (K.J. Mulongoy, ed.) pp. 171-193, International Academy of the Environment, Switzerland.
9. Ho, M. W. (1997) The Unholy Alliance *The Ecologist* Vol. 27 No 4. July/August.
10. Koskella, J. and Stotzky, G., 1997, Microbial Utilization of Free and Clay-Bound Insecticidal Toxins from Bt and Their Retention of Insecticidal Activity after Incubation with Microbes. Applied and Env. Microbiology, Sept. 1997, p. 3561-3568.
11. Mikkelson, T., (1996), The Risk of Crop Transgene Spread. Nature, 1996, 380:31.
12. Octavio Paredes-Lopez (Ed) (1999), Molecular Biotechnology For Plant Food Production, Culinary and Hospitality Industry Publications Services.
13. Popper, S., C. Wagner, and E. Larson. (1998), *New Forces at Work: Industry Views Critical Technologies*. Santa Monica, CA: RAND.
14. Skaftnesmo, T., (1998), Ecology versus Genetics Two Opposite Tendencies within the Sciences of Life in *Naturforvaltning - veier til handling*, edited by Knut Arild Melbe, published by Vett & Viten, Oslo.
15. Sloan, A.E. and M.E. Powers (1986), A perspective on popular perspections of adverse reactions to foods. Journal of Allergy and Clinical Immunology, 78: 127-133.

16. Springett, J. A. & Gray, R.A.J. (1992), Effect of repeated low doses of biocides on the earthworm Aporrectodea caliginosa in laboratory culture. Soil Biol. Biochem. 24:1739-1744.
17. Syvanen M. (1987), Molecular Clocks and Evolutionary Relationships: Possible Distortions Due to Horizontal Gene Flow. J. Mol. Evol. 26: 16-23.
18. Syvanen M., (1994), Horizontal Gene Transfer: Evidence and Possible Consequences. Annu. Rev. Genet. 28: 237-61.
19. Tadmouri G.O., Tüzmen S., Ozçelik H., Ozer A., Baig S.M., Senga E.B., and Basak A.N., "Molecular and Population Genetic Analyses of b-Thalassemia in Turkey," *American Journal of Hematology*, Vol. 57, pp. 215-220, 1998.
20. Tadmouri G.O., Garguier N., Demont J., Perrin P., and Basak A.N., "History and Origin of b-Thalassemia in Turkey: Haplotype Diversity of b-Globin Genes," *Human Biology*, in press, 2000.
21. Tadmouri G.O., and Basak A.N., "b-Thalassemia in Turkey: A Review of the Clinical, Epidemiological, Molecular, and Evolutionary Aspects," *Hemoglobin*, 2001 May; 25(2): 227-39.
22. Tapp, H. and Stotzky, G., 1998, Persistence of the Insecticidal Toxin from Bt subsp. Kurstaki in Soil. Soil Biology and Biochemistry, Vol. 30, No. 4, p. 471-476.
23. Timmons, A.M., (1996), Risks from transgenic crops. Nature Vol 380. p 487.
24. Watson, James D. (2000), A Passion for DNA: Genes, Genomes, and Society Cold Spring Harbor Laboratory Press, USA.
25. Ziman J.M., (1969), Information, communication, knowledge. Nature. 25;224(217):318-24.

Conservation and Utilization of Genetic Resources: An Issue for the Developing World

MOHAMED B. E. FAYEZ*
National Research Centre
Cairo, Egypt

1 ABSTRACT

After many centuries of neglect and many decades of systematic usurpation in recent history, the genetic resources of developing countries are receiving attention and beginning to be accorded protection by international law. There has prevailed until now a simmering conflict, with many manifestations, over the genetic resources, which reside mostly and in the greatest variety, in the South. There are countless cases of bio-piracy that affected not only the natural biodiversity and farmers' germplasm, but extended to bio-knowledge, innovations and practices of local communities. Supported by the explicit pronouncements of the Convention on Biological Diversity, governments of developing countries are entitled – in fact committed – to enforce respect for and legitimate access to and utilization of local genetic resources. A prime duty of governments that now emerges as both timely and pragmatically to be upheld is the disciplined scientific recording and meticulous documentation of national possessions of genetic resources and traditional knowledge. There is clearly considerable latitude for cooperation in this pursuit between neighbouring countries with similar ecosystems.

2 THE PAINFUL REALITIES

As the biological age progresses and fills our lives with bounties unlimited, a few biological realities need to be addressed. An estimated 90 per cent of the world's biological resources are to be found in developing countries (1). Over countless centuries, peoples of these countries accumulated knowledge about the use of many of these resources for food and medicine. Their knowledge-based experiences multiplied and diversified as a result of original innovations in the arts of agriculture, hybridization and breeding at the hands of observant

* Emeritus Research Professor, and Fellow of the Islamic Academy of Sciences.

and hard-working farmers at a level, and in the arts of extraction, biological testing and medication at the hands of serious scholars and pharmacists at another level.

It was this inter-generational, collective and cumulative knowledge that has fed, healed and clothed the world for many centuries (2). The benefits continue to be reaped. In the field of medicine, there is the estimate that three-quarters of the plants that yield active ingredients for prescription drugs came to the attention of researchers because of their use in traditional medicine (3). A similar quantitative correlation is to be found between the active chemicals currently isolated from higher plants that have established therapeutic application and the indicative traditional use of the plants from which they were originally derived (4).

Estimates also exist that set the current value of the world market for medicinal plants which benefited from the knowledge of indigenous peoples at US$ 43billion (5). The value of the international seed industry of crop varieties improved and developed by traditional farmers is estimated to be US$15 billion (5). Other natural products, which similarly owe their current market value to traditional knowledge of peoples of the developing world, include a large diversity of perfumes, sweeteners, bio-pesticides, fabrics and cosmetics (6).

The paradox that has persisted and continues to live with us is that the creativity of local communities is grossly treated differently from the creativity of corporate interests. Only the latter is accorded value and reward, and receives protection by national and international law. For countless decades, bio-prospectors have collected samples of plant material and documented – then later utilized for commercial ends – traditional medicinal uses and improved crop traits developed by local communities. The knowledge thus collected, more often than not under well-organized schemes, is then exploited by multinational corporations to develop highly profitable drugs as naturally occurring chemical entities, or in the form of synthetic copies of the natural products. According to one estimate, if only a 2 per cent royalty were levied on genetic resources that had been developed by local innovators in the South, the North would owe more than US$2 billion in royalties for medicinal plants alone (1).

The problem of freely tapping the biological diversity of the South is easily compounded and sometimes clouded. This occurs through the modifications or improvements introduced by the corporations to the source materials, which are classified as ‘inventions,’ and for which intellectual property rights are claimed to protect the end product or the process that leads to it. The gap between the source material and the end product thus widens, as does the gap between the originator/innovator and the commercial actor. What is to be noted, as an outstanding historical development during the past half-century, is the advent of intellectual property-impacting international instruments, which affected the biological scene to an extent previously unexperienced by humanity. The examples of the valuable natural plant resources turmeric, neem, basmati rice, kava, barbasco, squill, endod, quinoa, joublie, among many more, are still vivid

in the memory of many peoples of the South. They have been the object of many usurpation incidents that occurred only in recent years.

3 THE NEW WORLD LEGAL SYSTEM

There are serious threats that there will be more legitimisation of the private appropriation of community-based resources and knowledge, which could undermine indigenous and local communities. This would also give the North legal rights to plunder the biological heritage of the Third World by permitting the patentability of traditional medicines and crops which the Third World peoples have discovered and nurtured and kept as public domain knowledge for millennia. Every country in the developing world can easily cite several examples of its plant genetic resources of natural origin, which have found way to the developed countries to be highly profitable items of commerce in the drug and seed industries, or in other areas of commercialisation. What is particularly alarming is that the process of illicit appropriation is now enshrined in international law, and that the peoples of the Third World are forced to buy back resources that were originally taken from them.

We make here a direct reference to the World Trade Organization's Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS), of the 73 articles of which, Article 27.3(b) has been the most controversial and elicited the largest number of widely divergent comments. A WTO-sponsored world function was to be held in 1999 specifically for reviewing this very TRIPS Article, but it never saw light until today. Following is the text of this Article.

"27.3. Members way exclude from patentability:

> *(b) Plants and animals other than micro-organisms, and essentially biological processes for the production of plants and animals other than non-biological and microbiological processes. However, members shall provide for the protection of plant varieties either by patents or by an effective 'sui generis' system or by any combination thereof. The provisions of this subparagraph shall be reviewed four years after the date of entry into force of the WTO Agreement."*

The controversy over this Article arises from the fact that it addresses the highly sensitive question of life forms and their patentability. The grounds for this controversy stem not purely from commercial considerations, but include important legal, ethical, environmental and social elements; the more frequently addressed among which are the following:

(1) A patent bestows upon the title-holder exclusive rights of ownership and commercial exploitation, which mean preventing third parties from the acts of making, using, offering for sale, selling, or importing or other distribution of the subject matter of the patent. When these rights extend to

living matter (plants and animals), it could easily amount to the appropriation from the South of genetic resources constituting part of the natural biodiversity, as well as traditional knowledge and local experiences that had accumulated over many centuries. Nowhere in the TRIPS Agreement is there any express concern about the means by which a patent applicant has obtained the knowledge and materials which form the basis of the 'invention,' or about the identity of the legitimate owners or originators of such knowledge or materials;

(2) The TRIPS Article 27.3(b) obligates countries to allow patenting of micro-organisms and microbiological processes. This, together with a loose interpretation of inventiveness[*] in national patent offices, accelerates patenting of genetic resources in the lower forms of life, and further stimulates appropriation of the biological assets of developing countries by the corporate interests of the North;

(3) Another concern of relevance is the absence of a consensus definition of the term 'micro-organism.' While it is a matter of objection in many circles of the developing, and also developed world that in principle natural life forms should be patentable, there may be a tendency to allow the patentability of only genetically modified organisms (bacteria, fungi, algae, protozoa or viruses). There is at the same time strong objection to expand the definition of the term to cover living tissues, isolated cells, cell lives, organs, or DNA obtained from higher organisms, including human beings. The contemporary advances in biotechnology are producing every day new concepts and new products, which may fall under the expanded definition and for which the originators, claiming their findings as inventions, are demanding patent protection as a step to be followed by commercial exploitation. The opponents are willing to admit these achievements only as important scientific discoveries for which the originators are to be fully honoured and credited in the world scientific literature; and

(4) A word needs also to be said about the moral and ethical concerns, harboured in many quarters in the developing world, over the extension of patents – as permitted under TRIPS Article 27.3(b) – to life forms. Many religious and cultural traditions in and outside the Muslim World regard such extension to living organisms as intrinsically wrong. The claim violates the belief in the divine Creator and offends the fundamental doctrine that life is a gift of the Creator and a shared inheritance of human kind. The patenting of life forms "marks a significant further step in the larger process of the commodification of life" and the "reduction of the value of life and nature to the merely economic" (7). Many groups worldwide are concerned that patents underpin developments in genetic engineering that risk disturbing a complex pattern of interrelationships in the natural world that we still only partially understand (8).

---

[*] Inventiveness, or inventive step, is one of the three criteria of patentability; the other two are industrial applicability and newness (or novelty).

To a large extent, communities in most developing countries welcome interest in their knowledge and possessions of genetic resources, but only as long as their rights are respected as holders (or authors) of such knowledge and resources. They are, and quite justifiably, opposed to the 'mining' of their knowledge and resources by commercial firms or even by scientists who fail to acknowledge their role or to recognize that they are entitled to benefits flowing back to them. These communities, moreover, see the shortcomings of the intellectual property laws, as forged in compliance with the World Trade Organization rules, since these fail to specifically address their rights in the protection of their inter-generational knowledge or even their own biodiversity.

At the same time, it must be conceded; the TRIPS Agreement does not in fact prevent creative interpretations that maintain its basic tenets of intellectual property rights while allowing for additional non-conflicting rights. This is a reference to the opportunity open for providing in the national legislation for the protection of traditional knowledge and of the genetic resources peculiar for the local ecosystem; the Agreement on TRIPS contains no explicit language to the contrary. While this is true of the TRIPS Agreement, the total membership of which now is 143 countries, the same matter receives explicit support and straightforward legally-binding provisions in the Convention on Biological Diversity (CBD), the membership of which is 175 countries.

4 A WORLD AGREEMENT FOR THE PROTECTION OF BIODIVERSITY

The CBD, concluded in Rio de Janeiro in 1992 and entered into force in 1993, has the aim of "conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources, including by appropriate access to genetic resources and by appropriate transfer of relevant technologies." The Convention (8):

- ✓ Recognizes the sovereign rights of states over their biological and genetic resources;
- ✓ Stipulates that access to genetic resources can only occur on mutually agreed terms and with the prior and informed consent of states, unless states have otherwise determined;
- ✓ Requires signatories to protect and promote the rights of communities, farmers and indigenous people's vis-à-vis their customary use of biological resources and knowledge systems;
- ✓ Requires each party to endeavour to facilitate access by other parties for environmentally sound use;
- ✓ Requires transfer to be on terms which recognize and are consistent with the adequate and effective protection of intellectual property rights, and aims to enable developing countries, which provide genetic resources, to

have access to technology which makes use of those resources, on mutually agreed terms, including technology protected by patents and other intellectual property rights;

- ✓ Requires the equitable sharing of benefits arising from the commercial use of communities' biological resources and local knowledge; and
- ✓ Asserts that intellectual property rights must be supportive of and not run counter to the objectives of the CBD.

The CBD is thus clear and definitive in providing for the protection of traditional knowledge of relevance to biodiversity. However, it is the position of many experts that, for realistic implementation purposes, a necessary step is the institution of an international system, which recognizes the value and standing of the knowledge, innovations and practices of local communities. Protection and intellectual property right issues are important to them, as they often feel threatened in the absence of an internationally recognized system that secures this protection. A concrete first step in ensuring benefits for use of this knowledge would be to make the granting of intellectual property rights subject to disclosure of information on 'prior informed consent,' and the origin of the biological resources used in the patent application (9).

Despite the basic drive of the Convention as related to genetic resources, enabling regulated access to, and the fair and equitable sharing of benefits arising from using those resources, there has remained one unresolved, but a critical area. This relates to the future of the germplasm collections that have been acquired before the entry into force of the Convention (in 1993). It has been a relentless effort by industry in the North, particularly in the seed and biotechnology companies, to ensure minimal regulation over access to genetic resources, and to exclude existing ex situ germplasm collections from any new access regulation or benefit sharing schemes. There thus remains a loophole in the Convention. These materials, quite ubiquitous in the developing countries, are to be found, for example, in agricultural seed (gene) banks, microbial collections and botanical gardens. They have been freely collected from the wild and from farmers' fields, mostly in developing countries. They have brought over the past few decades billions of dollars in profit to the seed and pharmaceutical industry of the North countries, which had free access to all the collections.

The imbalance will be redressed only if an international CBD-related scheme is created that is based on the principle that States have sovereign rights over natural resources within their territory, and hence access to such resources should be regulated, and fair and equitable sharing of the benefits arising from their commercial exploitation should be provided for. Expectedly, considerable resistance to such scheme will be staged in order to maintain a *status quo*, thereby keeping, or continually keeping the enormous *ex situ* collections of germplasm outside the perimeters of the Convention objectives and regulation.

According to a relatively recent report (8), there exist over 1300 gene banks that hold over six million accessions, largely as a result of the wide degree of

access to plant genetic resources for food and agriculture historically. Many of the largest gene banks in the world, including those in Europe, North America and in the Consultative Group on International Agricultural Research system (CGIAR), have policies of unrestricted availability to *bona fide* users. The same is true with the gene banks located in developing countries. The collections of the CGIAR, a publicly-funded institution, are held in trust for the benefit or all mankind by the UN Food and Agriculture Organization, to be used to support research of interest to developing countries. However, there have been instances of material from the CGIAR system, which found its way to commercial breeders in industrialized countries who have tried to patent it (8) as inventions of their achievement.

5 CONCLUSION

It appears highly purposeful to act at the national levels, and collectively at sub-regional and regional levels, to minimize the chances of foreign appropriation of local plant genetic resources and valued traditional knowledge. Such unlawful appropriation, having become known as bio-piracy, threatens to assume even more serious dimensions than hitherto experienced. A first step to discourage bio-pirates is to plan and implement programmes for the documentation of national possessions in terms of natural biodiversity components and of traditional knowledge, and to publish such information in an orderly fashion to be part of the scientific literature. This act corresponds to the patenting of inventions, and is a formal disclosure of the knowledge subject matter and declaration of its rightful owners – for all to know and hopefully to respect.

REFERENCES

1. Oxfam Report, "Fatal Side Effects: Medicine Patents Under the Microscope," website www.oxfam.org.uk/cutthecost (2001).
2. G. S. Nijar, "Legal and Practical Perspectivs on Sui generis Options," report of the Third World Network, April 1999.
3. Gray, "Between the Spice of Life and the Melting Pot: Biodiversity Conservation and its Impact on Indigenous Peoples," International Working Group for Indigenous Affairs, document 70, 1991.
4. N. Farnsworth, *et al.*, "Medicinal Plants in Therapy", Bulletin of the World Health Organization, pg. 965, 63, 1985.
5. Gray, "Indigenous Peoples and the Marketing of the Rainforest," The Ecologist, vol. 20, No. 6, 1991.
6. S. Brush, "Indigenous Knowledge of Biologial Resources and Intellectual Property Rights: the Role of Anthropology, "American Anthropologist, 95(3), pg. 653, 1993.
7. D. Bruce and A. Bruce, "Engineering Genesis. The Ethics of Genetic Engineering in Non-human Species", Earthscan, pg. 229, 231, 1998.
8. G. Tansey, "Trade, Intellectual Property, Food and Biodiversity," Quaker Peace & Service, London, *cf.* http://www.quaker.org/quno.
9. Third World Network paper "UN Moves to Protect Biodiversity and Traditional Knowledge," http://www.twnside.org.sg.

Some Educational Activities in Biotechnology in Iraq

M. A-H. KADHIM*
and
A. E. YOUSIF**
Saddam University
Baghdad, Iraq

1 ABSTRACT

The paper deals with a developing country's needs in the fields of biomedical engineering and the medical sciences.

An extended definition of the biomedical engineering field is given together with the roles that can be played by medical engineers in a developing country such as Iraq.

The special and difficult circumstances of Iraq, being forced to enter into two major wars, followed by an economic and scientific embargo (now in its eleventh year) (2001) produced the need for biomedical engineers in the fields of clinical health, biomedical design and also for scientific research. It was decided that universities should take the lead in preparing professionals as no external help is anticipated. To this end, two structurally different biomedical engineering departments were established in 1997; one (Medical engineering) at the college of engineering at Saddam University leading to the degrees of BSc and MSc, together with a biotechnology department at the college of science; and the other at the college of engineering at the University of Baghdad leading to the degree of BSc. A brief review of these departments is given together with future ambitions for the development of this branch of technology.

* Member of the Iraq Academy of Sciences; and Dean of Engineering, Saddam University, Baghdad, Iraq.

** Head of Medical Engineering Department, Saddam University, Baghdad, Iraq.

2 INTRODUCATION

2.1 Definitions

2.1.1 Biotechnology

It is the manipulation of biological organisms to make products that benefit human beings. Biotechnology contributes to such diverse areas as food production, waste disposal, mining, and medicine.

Although biotechnology has existed since ancient times, some of its most dramatic advances have come in recent years. Modern achievements include the transfer of a specific gene from one organism to another (by means of a set of genetic engineering techniques known as transgenics); the maintenance and growth of genetically uniform plant –and animal– cell cultures, called clones; and the fusing of different types of cells to produce beneficial medical products such as monoclonal antibodies, which are designed to attack a specific type of foreign substance.

The first achievements in biotechnology were in food production, occurring about 5000 BC. Diverse strains of plants or animals were hybridised to produce greater genetic variety. The offspring from these crosses were then selectively bred to produce the greatest number of desirable traits. Repeated cycles of selective breeding produced many present-day food staples. This method continues to be used in food-production programs.

The modern era of biotechnology has its origin in 1953 when American biochemist James Watson and British biophysicist Francis Crick presented their double–helix model of DNA. This was followed by the Swiss microbiologist Werner Arber's discovery in the 1960s of special enzymes, called restriction enzymes, in bacteria. These enzymes cut the DNA strands of any organism at precise points. In 1973, American geneticist Stanley Cohen and American biochemist Herbert Boyer removed a specific gene from one bacterium and inserted it into another using restriction enzymes. This event marked the beginning of recombinant DNA technology, commonly called genetic engineering. In 1977, genes from other organisms were transferred to bacteria. This achievement eventually led to the first transfer of a human gene, which coded for a hormone, to *Escherichia coli* bacteria.

Although the *transgenic* bacteria (bacteria to which a gene from a different species has been transferred) could not use the human hormone, they produced it along with their own normal chemical compounds.

In the 1960s an important project used hybridisation followed by selective breeding to increase food production and quality of wheat and rice crops. American agriculturalist Norman Borlaug, who spearheaded the program, was awarded the Nobel Peace Prize in 1970 in recognition of the important contribution that increasing the world's food supply makes to the cause of peace.

Today biotechnology is applied in various fields. The field of medicine employs some of the most dramatic application in biotechnology.

2.1.2 Bioengineering

It is the application of engineering principles and design procedures to medical problems. Sub disciplines include biomechanical, biochemical, and bioelectrical engineering.

2.1.2.1 Biomechanical engineering

In this field, the musculoskeletal system of the human body is viewed as a mechanical structure that can undergo certain motions and stresses. Thus, it includes the analysis of human gait and the investigation of stresses on flesh and bone during accidents. Biomechanical engineering is also concerned with the flow of blood, the mechanics of respiration, and energy exchange in the living human body.

Application range from the development of protective automotive safety belts to the design and operation of heart-lung machines. An important early development was the iron lung, which permitted some victims of poliomyelitis to survive. Biomechanical engineering also forms the basis of reconstructive developments such as artificial implants and artificial limbs. For example, special artificial arms, driven by small electric motors and operated by bioelectrical signals from muscles, have given children born without limbs a nearly normal appearance and enough mobility for them to function socially and vocationally. Artificial hearts are also under development; since 1982 many patients have been treated with such devices, with varying success .

2.1.2.2 Biochemical Engineering

This subdiscipline concerns the chemical interactions between the human body and artificial materials that, implanted into life tissue, may provoke adverse reaction or rejection. For example, woven acrylic artificial arteries were developed to prevent the rapid clotting of blood in artificial blood vessels. Capsules of silicone were developed to protect implanted electric equipment, such as cardiac pacemakers, and to permit their integration into live tissues. Perhaps the most dramatic biochemical engineering feat, however, has been the design and construction of the artificial kidney for patients with incurable kidney diseases.

2.1.2.3 Bioelectrical engineering

This area of biomedical engineering concerns bioelectric activity, which encompasses the nervous system and regulates most life processes. The bioelectrical engineer assists this regulation and uses bioelectric signals for diagnostic purposes. Developments in such engineering led to the invention of the pacemaker, the defibrillator, and the electrocardiograph. The pacemaker is a device that senses and restores the normal contraction rhythm of the heart muscle, by electro stimulation, in heart-disease victims. The defibrillator sends

a powerful but controlled electric current through the body of a patient whose heart has stopped, and restores normal heart action. An electrocardiograph records, through electrodes placed on the skin, the wave form and the frequency of heart action. Transmitting electrocardiograms to central computers and diagnostic centres enable physicians to form a diagnostic network. The monitoring of many other bioelectric functions by means of electrodes plays an important part in surgical recovery rooms and intensive–care units.

2.2 Biomedical engineering

Although biomedical engineering is currently enjoying great popularity, its roots actually go back many centuries.

The sixteenth century (A.D.) marked the beginning of modern medicine, with men like Leonardo Da Vinci (1452–1518). Medicine, during earlier medieval times and even further back, developed very slowly.

Da Vinci (artist, engineer, and scientist) founded (in a way) the science of anatomy, and studied the principles of the motions of bones and muscles in extensive detail. Later, Vesalius, (1514-1564) advanced in 1543 human anatomy, which initiated exuberant new scientific activity in medicine and transformed surgical practice.

Physical sciences began to develop in the Renaissance era, and started to have an effect on medicine. Sanatorios (1561-1636) applied new measurement techniques to the human body and by careful weighing of body inputs and outputs, was able to show that “insensible perspiration” occurs. This laid the foundation for the study of metabolism. Sanatorios also developed a crude fever thermometer and a rudimentary device for determining pulse rates.

The science of physiology began to blossom during the same era. William Harvey (1578-1657), an English physiologist, discovered that blood is pumped in a circuit by the muscular contraction of the heart and pointed out the differences in directions of flow in the arteries and veins.

During the seventeenth century, new theories of how the body works appeared. The important ones dealt with “iatrophyics,” “iatrochemistry” and “vitalism.” Borelli (1608-1679) of Italy propounded the first view, which explains animal activity on a mechanical basis.

Hales (1677-1761), an English physiologist, investigated the dynamics of the circulatory system by tying tubes into the arteries and veins of animals and manometrically measuring the pressures at various points. Hales also measured circulation rules and velocities.

Studies of electrical phenomena in the body were made by men such as Galvan (1737-1798) and Volta (1745–1827), who both showed, for example, that muscles can be made to contract by application of external currents.

Clinical medicine was aided during this period with the development of the first (good) pulse watch, the first safe and reliable thermometer, and with the invention of the stethoscope by Laënnec in 1819. Public health conditions of cities were remarkably improved such as the covering of sewerage systems and

draining of open wetlands. It was about this same time that there occurred a strong movement to build many important hospitals.

Jean Poiseuille (1797-1869), a French physiologist, studied blood flow dynamics extensively and was the first to derive the laminar flow law that bears his name. He was also the first to investigate the viscometric properties of blood. Another physiologist who likewise deduced a fundamental physical law was the German Adolf Fick (1829-1901), discoverer of the law of diffusion in liquids.

Great advances in diagnosis and treatment followed the discovery of X- rays by Röntgen in 1895 and of radium by the Curies in 1898. Advances in surgical procedures in the last half–century were too numerous to mention here (many of these, resulted from the fact that World Wars I and II provided surgeons with a vast number of battlefield patients, on whom daring new techniques were justified.

Special mention should be made, however, of the development of implantable devices of various kinds (e.g. metal and plastics) and of artificial organs. Stainless steel has been used extensively to repair joints and bones, and tantalum metal was used in wire or mesh for many purposes (e.g. strengthening vertebrae). Ceramic materials are commonly used to replace jawbones and lip joints. Plastics such as Orlon and Dacron, fashioned into tube form, have been implanted in place of arteries. Nylon thread is a widely used surgical material. Other plastics (clear ones) have found use in artificial corneas for eyes. Silastic, a silicon rubber that has an enormous range of flexibility and consistency, has been employed as imitation body fat in breasts, cartilage in ear and nose repair, and as the main material for artificial heart devices.

Artificial organs are a comparatively recent development. The first artificial kidney treatment was carried out on a dog by Able, Rountree, and Turner in 1913. In 1943, the first applications to humans were made. Heart – lung devices, which oxygenate blood outside of the body and pump it back to the patient, were developed and used in the early 1950. The use of artificial heart valves and implantable partial hearts has also been relatively recent, occurring since 1960.

Many of the problems confronting health professionals today are of extreme importance to the engineer because they involve aspects to device and systems analysis, design, and practical application – all lie at the heart of processes fundamental to engineering practice. These medically relevant design problem can range from very complex large-scale constructs, such as the design and implementation of automated clinical laboratories, multiphase screening facilities (i.e. centres that permit many tests to be conducted), and hospital information systems to the creation of relatively small and “ simple” devices, such as recording electrodes and transducers that may be used to monitor the activity of specific physiological processes in either a research or a clinical setting.

They encompass the many complexities of remote monitoring and telemetry and included the requirement of emergency vehicles, operating rooms, and intensive care units.

Since biomedical engineering involves applying the concepts, knowledge and approaches of virtually all engineering disciplines (e.g. electrical, mechanical, and chemical engineering) to solve specific health- related problems, the opportunities for interaction between engineers and health care professionals are many and varied.

Biomedical engineers may become involved for example, in the design of a new medical imaging modality or development of new medical prosthetic devices to aid the disabled. Although what is included in the field of biomedical engineering is considered by many to be quite clear, many conflicting opinions concerning the field can be traced to disagreements about its definition. For example, consider the terms biomedical engineering, bioengineering, biological engineering and clinical (or medical) engineering, which are defined in the Bioengineering Education Directory [(2)].

This brief review demonstrates that the history of bio-medical engineering is a long one, and that present–day efforts are but an extension or advances on previous breakthroughs.

The term biomedical engineering appears to have a rather comprehensive meaning. Biomedical engineers apply, electrical, chemical, optical, mechanical, and other engineering principles to understand, modify or control biological (i.e. human and animal) systems. When biomedical engineers work in a hospital or clinic he should perhaps be called a clinical engineer. However, this theoretical distinction is not always observed in practice since many professionals working in hospitals today continue to be called biomedical engineers or medical engineers.

The breadth of activity of biomedical engineers is significant. The field has moved significantly from being concerned primarily with the development of medical devices to include a more wide – ranging set of activities. As illustrated in Figure1, the field of biomedical engineering now includes many new career areas. These areas include:

- Application of engineering system analysis (physiologic modelling, simulation, and control to biological problems);
- Detection, measurement, and monitoring of physiologic signals (i.e. biosensors, and biomedical instrumentation);
- Diagnostic interpretation via signal-processing techniques of bioelectric data;
- Therapeutic and rehabilitation procedures and devices (rehabilitation engineering); and
- Devices for replacement or augmentation of bodily functions (artificial organs).

3 BIOMEDICAL ENGINEERS

Biomedical engineering essentially involves training three types of individuals:

(a) The clinical engineer in medical health care;
(b) The biomedical design engineer for industry; and
(c) The research scientist.

The first type and the most common, may be called the "problem solver." This engineer maintains the traditional service relationship with medical professionals who originate a problem that can be solved by applying the specific expertise of the engineer.

For this problem-solving process to be efficient and successful, some knowledge of each other's language and a ready interchange of information must exist. Biomedical engineers must understand the biological situation to apply their judgment and contribute their knowledge toward the solution of a given problem as well as to defend their methods in terms that medical professionals can understand. If they are unable to do this, they would not merit the biomedical prefix.

The second, rather rare, type may be called the "technological entrepreneur" (most likely a biomedical design engineer in industry).

These individuals bridge the gap between that of the medical professional or physician and current technological trends. They examine some portion of the biological or medical front and identify areas in which advanced technology might be advantageous. They pose their own problem and then proceed to provide the solution, at first conceptually and then in the form of hardware or software. Finally, these individuals must convince the medical community that they can provide a useful tool because, contrary to the solution in which problem solvers find themselves, the entrepreneurs activity is speculative at best and has no ready- made customer for the results. Because of the nature of their work, technological entrepreneurs should have a great deal of engineering and medical knowledge as well as experience in numerous medical systems.

The third type of biomedical engineer, " the research scientist" is, most likely, found in academic institutions and industrial research laboratories, and is primarily interested in applying engineering concepts and techniques to the investigation and exploration of biological processes. The most powerful tool at their disposal is the construction of an appropriable physical or mathematical model of the specific biological system under study. Through simulation techniques and available computing machinery, they can use these models to understand features that are too complex for either analytical computation or intuitive recognition. In addition, this process of simulation facilitates the design of appropriate experiments that can be performed on the actual biological systems. The results of these experiments can, in turn, be used to amend the model. Thus, increased understanding of a biological mechanism results from this iterative process.

Each of the roles envisioned for the biomedical engineer requires a different attitude and a specific degree of knowledge about the biological environment. However, each engineer must be a skilled professional with a significant expertise in engineering technology. Therefore, in preparing new professionals to enter this field at these various levels, biomedical engineering educational programs are continually being challenged to develop curricula that will provide an adequate exposure to end knowledge about the environment without sacrificing essential engineering skills.

4 THE IRAQ CASE

4.1 Background

Iraq went through two wars (1980-1988 and 1991) that were followed by economic and scientific sanctions that affected almost all walks of life, in particular, healthcare, and the educational system. However, it made the Iraqi people self-dependent and also self-sufficient.

A report issued by the ministry of health states that 1,290,543 very young, and elderly persons died since sanctions were implemented on 6 August 1990 up to the end of February 2000, due various diseases and lack of medicine and medical-care equipment.

Table (1), shows the number of deaths in children less than five years of age due to various diseases, whilst Table (2) shows the number of deaths in adults over fifty years of age. The comparison in both tables was made between two months, February 1989 and February 2000. Table (3), gives the total number of deaths caused by the economic sanctions due to various selected reasons, as a comparison between the months: August 1990 and December 1999 for various age groups.

Table (4) shows the monthly percentage birth rate of children weighing less than 2 ½ kg during the economic sanctions period 1990-2000.

Table (5) shows the percentage reduction in major medical operations conducted during the period of the sanctions from 1989 to 2000.

Table (6) shows the effect of sanctions on medical laboratory tests conducted, also during the sanctions period.

It is clearly seen from the data given in the foregoing tables that the effects of the sanctions have caused great deterioration in all aspects of the health care system. It is therefore obvious that medical instrumentation and equipment, which are usually obtained, from industrially developed countries have suffered greatly due to both lack of renewal and spare parts. This lead universities and the medical profession to try to establish biomedical engineering departments at certain Iraqi universities to take the lead in training engineers in this urgently needed field, particularly as it is almost impossible to send scholars abroad to study due to the present difficult circumstances.

Table 1. Death of children less than five years of age

	Disease	Period	
		February 1989	February 2000
		Number of deaths	Number of deaths
1	Diarrhoea	104	1377
2	Pneumonia and respiratory tract infection	158	3002
3	Malnutrition	94	2560
Total		356	6936

Table 2. Death of adults above fifty years of age

	Disease	Period	
		February 1989	February 2000
		Number of deaths	Number of deaths
1	Heart diseases and hypertension	71	593
2	Diabetes mellitus	90	589
3	Malignant disease	318	1868
Total		479	3050

Table 3. Total number of deaths, during the embargo, for different selected reasons

Year	Age groups		Total
	Less than 5 years	Above than 5 years	
1989	7110	20224	27334
1990	8903	23561	32464
1991	27473	58469	85942
1992	46933	76530	123463
1993	49762	78261	128023
1994	52905	80776	133681
1995	55823	82961	138784
1996	56997	83284	140281
1997	58845	85942	144787
1998	71279	88760	160039
1999	80044	97439	177483
(Jan) 2000	7608	7999	15607
Total	523,682	784,206	1,307,888

Diagnostic reasons

Below 5 years of age	Above 5 years of age
1. Acute respiratory infections. 2. Diarrhoea and enteritis. 3. Malnutrition.	1. Heart diseases. 2. Hypertension. 3. Diabetes mellitus. 4. Kidney diseases. 5. Liver diseases. 6. Malignant diseases.

Table 4. Percentage of child-births less than 2.5kg in weight during the embargo period

Year	Monthly rate %
1990	4.5
1991	10.8
1992	17.6
1993	19.7
1994	21.1
1995	22.05
1996	22.60
1997	23.02
1998	23.81
1999	24.04
January 2000	24.8

Table 5. Effect of embargo on major medical operations

Period of time	Monthly rate	% reduction on 1989
1989	15125	-
1990	8668	-34
1991	6507	-57
1992	5477	-63.7
1993	5202	-65.6
1994	4679	-69.1
1995	4492	-70.3
1996	4417	-70.8
1997	4357	-71.19
1998	4322	-71.4
1999	4442	-70.6
January 2000	4051	-73.2

Table 6. Effect of embargo on medical laboratory examinations

Year	Monthly rate	% reduction on 1989
1989	1494050	-
1990	1091230	-27
1991	635446	-57
1992	589952	-60.5
1993	576225	-61.4
1994	531384	-64.4
1995	501095	-66.4
1996	500036	-66.53
1997	499579	-66.56
1998	498989	-66.6
1999	515506	-65.4
January 2000	506545	-66.1

4.2 An Iraqi solution

As a humble contribution from the academic society to reduce the heavy burdens of sanctions on both the healthcare and educational systems, the College of Engineering at Saddam University took the initiative in establishing a Medical Engineering department in 1997. A study plan was prepared that leads to the degrees of BSc and MSc. The various facilities in the colleges of medicine and engineering of the university were utilized in organizing the various courses and the use of various laboratories. Practical training of the students was achieved by using the facilities of the various specialized hospitals in the City of Baghdad, together with the maintenance centre of bio-medical equipment. The study plan is based on the credit-hours system adopted by all courses at Saddam University and it consisted of fixed percentages of pure science subjects (Mathematics, Computer Programming, Physics, Chemistry, Biology) and Basic Medical Sciences (Anatomy, Histology, Cell Biology, Physiology, and Pathology). Electronic engineering, Mechanical Engineering, and Chemical Engineering subjects, together with an appropriate percentage of general Humanity subjects were also included. The study plan is spread over four years leading to the degree of BSc in Medical Engineering, and a further two years for the degree of MSc. The sixth year is spent on the preparation of the MSc thesis.

The College of Science at Saddam University, also established a three years BSc course in the field of Biotechnology based also on the credit-hour system. The study plan consisted of fixed percentages of pure science subjects (Mathematics, Physics, Chemistry, Biology, Computer programming) and fixed percentages in Bio-Technology subjects (plant biology, Animal Physiology, Cell-biology, Genetics, Genetic Engineering, Microbial Taxonomy, Cell genetics, Medical Microbiology, food microbiology, Molecular genetics,

Microbial physiology, Development Biology, Fermentation technology, Immunology, etc) together with appropriate percentage of humanity subjects.

The College of Engineering at the University of Baghdad followed on, when realizing the acute need for such specialized fields, and established a BSc course in Biomedical Engineering, which differs in its structure to that established at Saddam University. In this course, students are accepted after finishing their second year in Electronic, Mechanical and Computer Engineering to then continue their studies for a further three years in the department of Biomedical Engineering.

However, the course subjects were mainly in engineering fields with little emphasis on basic medical sciences as was the case at Saddam University. This system graduates engineers who are mainly trained to deal with bio-instrumentation and medical equipment but perhaps lacking the ability to work seriously with the medical profession.

The plan of study for the BSc degree course offered at Saddam University in medical engineering is given in Appendix (A). It can be seen that it consists of the following approximate percentage of various fields of studies:

(a) 25% Basic medical sciences;
(b) 25% Basic mechanical sciences;
(c) 25% Basic electronic sciences;
(d) 15% Basic sciences; and
(e) 10% Humanities.

Appendix (B) shows the BSc study plan of the BSc degree course offered at the university of Baghdad. It is readily seen that the course is biased towards subjects in bioelectronics and less so towards subjects belonging to biomedical engineering especially in the basic medical science subjects.

Appendix (C) shows the study plan for the BSc degree course in the biotechnology department of college of science at Saddam University. It should be mentioned here that it is the only course of its kind given by an Iraqi university.

It is readily appreciated that the main subjects enlisted in this plan study fall into four main categories i.e.: medical basic sciences, bioelectronics, biomechanics, and biomaterials. It is expected that the student pursuing such studies will learn about the following:

- The importance of anatomy, physiology, histology, and cell biology, in the biomedical engineering field;
- Cell biological theory and types of organic compounds and other elements found in cells;
- The calculation of the internal osmolarity and ionic concentrations of a model cell at equilibrium;
- The description of the five major components and functions of five organ systems; circulatory, respiratory, nervous, skeletal, and muscular;
- The process used to build a mathematical physiological model;

- Qualitative description of a saccadic eye movement and the explanation of the pulse-step saccadic control signal;
- Muscle operation using a non-linear and linear muscle model;
- How the brain controls the saccadic eye movement system and the major neural sites;
- The growing area of cellular therapies;
- The cellular dynamics underlying tissue function;
- The importance of stem cells in tissue function;
- The cellular fate processes;
- The cellular communication processes;
- The parameters that characterize the tissue microenvironment and how to approach mimicking them into vitro; and
- The issues that one encounters when delivering cellular therapies to patients.

Regarding the subjects of biomechanics and biomaterials, it is expected that the student will be able to:

- Understand the application of engineering kinematics relations to biomedical problems;
- Understand the application of engineering kinetic relations to biomedical problems;
- Understand the application of engineering mechanics relations to orthopaedic structures;
- Use MATLAB to write and solve biomechanical static and dynamic equations;
- Study the viscoelastic properties of tissues;
- Understand how kinematics equations of motion are used in clinical analysis of human gait;
- Understand how kinetic equations of motion are used in clinical analysis of human gait;
- Explain how biomechanics applied to human gait is used to quantify pathological conditions, to suggest surgical clinical treatment, and to quantify their effectiveness;
- Define different classes of materials;
- List failure modes of different materials;
- List mechanical tests and determine which are appropriate for a given application;
- Understand the considerations for selection of appropriate materials for specific applications;
- Describe the biological responses to implanted materials;
- Define in appropriate biological responses;
- Determine correct biocompatibility testing protocols;

- Define a fluid, both physically and mathematically with special emphasis on characteristic parameters that distinguish one type of fluid from another;
- Quantify physiologic mechanisms that produce flow in cardiovascular channels, with special emphasis on human heart and the cardiac cycle;
- Develop field equations that account for conservation of mass, energy, and momentum in fluid dynamic systems;
- Apply the field equations to formulate and solve problems in biofluid mechanics, with special emphasis on flow through the cardiovascular system; and
- Identify some basic physiologic measurements that are responsible for controlling flow through the human cardiovascular system.

Regarding the subjects that fall within the biomedical equipment, it is expected that the student will be able to:

- Describe the different clarifications of biomedical sensors;
- Calculate the half–cell potentials generated by different electrodes immersed in an electrolyte solution;
- Describe the electrodes that are used to record the ECG, EEG and EMG and those that are used for infra cellular recordings;
- Describe how displacement transducers, airflow transducers, and therimstors are used to make physical measurements;
- Describe how blood gases and blood pH are measured;
- Describe how enzyme–based on microbial biosensors work and some of their uses;
- Explain how optical biosensors work and describe some of their uses;
- Describe the components of a basic instrumentation system;
- Describe a variety of circuits that use operational amplifiers to condition analog signals and calculate the effect that these circuits have on the signal that is being monitored;
- Determine the transfer function for cascaded simple circuits;
- Explain the effects of different filters (low–pass, high-pass, band-pass, and band-stop) on analog input signals;
- Describe some of the ways in which noise is handled when designing instruments;
- Design a differential biopotential amplifier system;
- Explain how analog signals are converted into digital signals;
- Describe the components of a computer;
- Describe the role of computers in medical instrumentation;
- Describe the different origins and types of biosignals;
- Distinguish between deterministic, periodic, transient , and random signals;
- Explain the process of analog to digital conversion;
- Define the sampling theorem;

- Describe the main purposes and uses of the wavelet and Fourier transforms;
- Define the Z– transform;
- Explain the basic concepts and advantages of fuzzy logic; and
- Describe the basic concepts of artificial neural network.

The above are brief examples of the usefulness of some of the courses given in the Medical engineering department.

The biotechnology department of the college of science of Saddam University allows the student to be able to:

- Define biotechnology and important terms that are used in biotechnology, and describe important events in the history of biotechnology;
- Discuss the relationship between DNA, mRNA, and proteins;
- Describe the basic techniques that are used in biotechnology;
- Describe therapeutic uses of biotechnology; and
- Describe the human genome project and its potential effects on health care.

5 CONCLUSION

The paper described the effects of the embargo on Iraq on the biotechnological, bioengineering and medical engineering activities.

As part of the total effort to balance these negative effects, the field of education was taken as a case study. In total, three attempts were described; medical engineering and biotechnology at the University of Saddam and bioengineering at the University of Baghdad. It was shown that such efforts contribute to bridging the gap between developed nations and Iraq, a country burdened with sanctions.

BIBLIOGRAPHY

1. Edexcell Foundation Web Site:
   http://web.ukonline.co.uk/webwise/spinneret/edexcel/biotechnol/defbio.htm
2. National Institute of Health Bioengineering Consortium
   http://www.becon.nih.gov/bioengineering_definition.htm
3. Biomedical Engineering Society
   http://www.bmes.org/
4. Various reports of the Ministry of Health, Republic of Iraq, 1999, 2000 and 2001.
5. Various reports, Departments of Biotechnology and Genetic Engineering, Saddam University and University of Baghdad, 1999, 2000 and 2001.

Appendix (A)

BSc STUDY PLAN IN
THE MEDICAL ENGINEERING DEPARTMENT
COLLEGE OF ENGINEERING
SADDAM UNIVERSITY

FIRST YEAR

AUTUMN QUARTER

	Subject	Theory (Hours)	Practical (Hours)	Credits (Hours)
1	National Culture	2	-	2
2	English Language I	2	-	2
3	Computer Programming I	1	2	2
4	Mathematics I	4	-	4
5	Physics	3	3	4
6	Engineering Drawing I	1	3	2
7	Chemistry I	4	2	5

WINTER QUARTER

	Subject	Theory (Hours)	Practical (Hours)	Credits (Hours)
1	National Culture	2	-	2
2	Chemistry II	1	3	2
3	Computer Programming II	1	2	2
4	English Language II	2	-	2
5	Mathematics II	4	-	4
6	Engineering Drawing II	1	3	2
7	Biochemistry I	2	3	3
8	Anatomy I	2	3	4
9	Cell Biology	3	3	4

SPRING QUARTER

	Subject	Theory (Hours)	Practical (Hours)	Credits (Hours)
1	National Culture	2	-	2
2	Biochemistry II	2	3	3
3	Computer Programming III	2	2	3
4	Mathematics III	4	-	4
5	Engineering Drawing III	1	3	2
6	Workshops	-	3	1
7	Anatomy II	3	4	5
8	Material Science	2	-	2
9	English Language III	2	-	2

SECOND YEAR

AUTUMN QUARTER

	Subject	Theory (Hours)	Practical (Hours)	Credits (Hours)
1	National Culture	2	-	2
2	Sociology	1	-	1
3	Biochemistry III	2	4	4
4	Anatomy	2	6	4
5	Physiology	4	4	6
6	Histology	2	3	3
7	Medical Equipment	1	3	2

WINTER QUARTER

	Subject	Theory (Hours)	Practical (Hours)	Credits (Hours)
1	National Culture	2	-	2
2	Philosophy	1	-	1
3	Biochemistry IV	3	4	5
4	Anatomy	3	6	5
5	Physiology	4	4	6
6	Histology	2	3	3
7	Medical Equipment	1	2	2

SPRING QUARTER

	Subject	Theory (Hours)	Practical (Hours)	Credits (Hours)
1	National Culture	2	-	2
2	Arabic Language	1	-	1
3	Clinical Chemistry	2	3	3
4	Anatomy	3	6	5
5	Physiology	4	4	6
6	Histology	2	3	3
7	Medical Equipment	1	2	2

THIRD YEAR

AUTUMN QUARTER

	Subject	Theory (Hours)	Practical (Hours)	Credits (Hours)
1	National Culture	2	-	2
2	Element of Management	1	-	1
3	Strength of Materials	3	2	4
4	Electronics I	3	2	4
5	Engineering Mechanics I	3	2	4
6	Programming I	3	2	4
7	Pathology I	4	4	6

WINTER QUARTER

	Subject	Theory (Hours)	Practical (Hours)	Credits (Hours)
1	National Culture	2	-	2
2	Communication Skills	1	-	1
3	Biomechanics I	3	2	4
4	Electronics II	3	2	4
5	Engineering Mechanics II	3	2	4
6	Programming II	3	2	4
7	Pathology II	3	4	5
8	Applied Medical Sciences	-	2	1
9	Workshop Technology	-	3	1

SPRING QUARTER

	Subject	Theory (Hours)	Practical (Hours)	Credits (Hours)
1	National Culture	2	-	2
2	Physiology	1	-	1
3	Biomechanics II	3	2	4
4	Electronics III	3	2	4
5	Engineering Mechanics III	3	2	4
6	Programming III	1	2	2
7	Pathology III	4	4	6
8	Applied Medical Sciences	-	2	1
9	Medical Equipment	2	2	3

FOURTH YEAR

AUTUMN QUARTER

	Subject	Theory (Hours)	Practical (Hours)	Credits (Hours)
1	Electronics IV	3	2	4
2	Communications I	2	2	3
3	Mechanical Sciences	2	-	2
4	Biomechanics I	2	-	2
5	Biomedical Engineering Skills	-	2	1
6	Applied Medical Sciences	1	3	2
7	Research Project	-	6	2
8	Control I	2	2	3
9	Friction and Wear	2	-	2

WINTER QUARTER

	Subject	Theory (Hours)	Practical (Hours)	Credits (Hours)
1	Electronics V	3	2	4
2	Communications II	2	2	3
3	Engineering Design I	3	2	4
4	Biomaterials	2	-	2
5	Engineering Skills	-	2	1
6	Applied Medical Sciences	1	3	2
7	Research Project	-	6	2
8	Control II	2	2	3

SPRING QUARTER

	Subject	Theory (Hours)	Practical (Hours)	Credits (Hours)
1	Electronics VI	3	2	4
2	Communications III	2	2	3
3	Engineering Design II	3	2	4
4	Spectral Analysis	2	-	2
5	Medical Equipment	1	2	2
6	Engineering Applied Science	1	3	2
7	Research Project	-	6	2
8	Environmental Engineering	2	-	2

Appendix (B)

UNIVERSITY OF BAGHDAD
COLLEGE OF ENGINEERING
STUDY PLAN
BSc IN BIOMEDICAL ENGINEERING

	3rd Year Subject	Unit	1st Semester			2nd Semester		
			Theo.	App.	Lab.	Theo.	App.	Lab.
1.	Numerical Engineering Analysis	4	2	1	1	2	1	1
2.	Analytical Mechanics	6	3	1	1	3	1	1
3.	Material science	4	2	-	-	2	-	-
4.	Microelectronics & Solid State Devices	4	2	1	-	2	1	-
5.	Engineering Electronic Circuit Design	4	2	1	-	2	1	-
6.	Signal & Communication	4	2	1	-	2	1	-
7.	Anatomy I	8	3	-	2	3	-	2
8.	Electronic Circuit Lab.	2	-	-	3	-	-	3
9.	National & Social Culture	4	2	-	-	2	-	-
	Total	40	18	5	7	18	5	7
			30			30		

+ 4 weeks summer training.

	4th Year Subject	Unit	1st Semester			2nd Semester		
			Theo.	App.	Lab.	Theo.	App.	Lab.
1.	Anatomy II	8	3	-	2	3	-	2
2.	Control System Design	4	2	1	1	2	1	-
3.	Integrated Optics & Laser	4	2	1	-	2	1	1
4.	Ultrasonic Microwave and X-ray	6	3	-	-	3	-	-
5.	Neurology	4	2	1	-	2	1	-
6.	Biochemistry and Biophsiology	4	2	1	-	2	1	-
7.	Bioelectromagnetic	4	2	1	-	2	1	-
8.	Microprocessor & Micro Computer	4	2	-	1	2	-	1
9.	Medical Measurements	2	-	-	3	-	-	3
	Total	40	18	5	7	18	5	7
			30			30		

+ 4 weeks summer training.

UNIVERSITY OF BAGHDAD
COLLEGE OF ENGINEERING
STUDY PLAN
BSc IN BIOMEDICAL ENGINEERING

	5th Year Subject	Unit	1st Semester			2nd Semester		
			Theo.	App.	Lab.	Theo.	App.	Lab.
1.	Medical Instrumentation & System	4	2	1	-	2	1	-
2.	Physiological Control System	6	3	1	-	3	1	-
3.	Medical Engineering System	6	3	1	-	3	1	-
4.	Medical Imaging	6	3	1	-	3	1	-
5.	CAD Analysis of physiological Signal	6	2	-	2	2	-	1
6.	Artificial Limbs	4	2	-	2	2	-	1
7.	Medical Engineering Lab	2	-	-	3	-	-	3
8.	Clinical Engineering	4	2	1	-	2	1	-
9.	Project	2	-	-	3	-	-	3
	Total	40	17	5	8	17	5	8
			30			30		

+ 4 weeks summer training.

Appendix (C)
BIOTECHNOLOGY DEPARTMENT
COLLEGE OF SCIENCE
SADDAM UNIVERSITY
STUDY PLAN 2000/2001

FIRST YEAR

AUTUMN QUARTER

	Subject	Theory (Hours)	Practical (Hours)	Credits (Hours)
1	National Culture	2	-	2
2	English Language I	2	-	2
3	Geology	2	-	2
4	Computers I	2	3	3
5	Mathematics I	3	-	3
6	Physics I	3	3	4
7	General Chemistry	2	3	3
8	Biology I	3	3	4

WINTER QUARTER

	Subject	Theory (Hours)	Practical (Hours)	Credits (Hours)
1	National Culture	2	-	2
2	English Language II	2	3	3
3	Computers II	2	3	3
4	Physics II	3	3	4
5	Organic Chemistry I	3	3	4
6	Biology II	3	3	4
7	Biotechnology	2	-	2

SPRING QUARTER

	Subject	Theory (Hours)	Practical (Hours)	Credits (Hours)
1	National Culture			
2	English Language III	2	-	2
3	Computers III	2	3	3
4	Mathematics III	3	-	3
5	Biotechnology III	2	-	4
6	Plant Biology	3	3	4
7	Animas Physiology	3	3	4
8	Organic Chemistry II	3	3	4
9	General Chemistry II	2	3	3
10	Physics III	2	3	3

SECOND YEAR

AUTUMN QUARTER

	Subject	Theory (Hours)	Practical (Hours)	Credits (Hours)
1	National Culture	2	-	2
2	Communication Skills	1	-	1
3	General Microbiology	3	3	4
4	Fermentation Technology I	3	3	3
5	Immunology	3	3	4
6	Genetics I	3	3	4
7	Cell Biology	3	3	4

WINTER QUARTER

	Subject	Theory (Hours)	Practical (Hours)	Credits (Hours)
1	National Culture	2	-	2
2	Arabic Language	1	-	1
3	Fermentation Technology II	2	3	3
4	Microbial Taxonomy	3	3	4
5	Genetics II	3	3	4
6	Biochemistry	2	3	3

SPRING QUARTER

	Subject	Theory (Hours)	Practical (Hours)	Credits (Hours)
1	National Culture	2	-	2
2	Philosophy	1	-	1
3	Medical Microbiology	3	3	4
4	Food Microbiology	3	3	4
5	Molecular Genetics	2	3	3
6	Biochemistry	3	3	4
7	Microbial Physiology	3	3	4
8	Development Biology	3	-	3

THIRD YEAR

AUTUMN QUARTER

	Subject	Theory (Hours)	Practical (Hours)	Credits (Hours)
1	National Culture	2	-	2
2	Element of Management	1	-	1
3	Plantation of Animal Tissues	2	3	3
4	Biochemistry	2	3	3
5	Vaccination Techniques	3	2	3
6	Bimolecular Science	3	-	2
7	Viruses	2	3	3
8	Environmental Microbiology	3	-	2
9	Research Project I	-	-	2

WINTER QUARTER

	Subject	Theory (Hours)	Practical (Hours)	Credits (Hours)
1	National Culture	2	-	2
2	Genetic Engineering	3	3	3
3	Implantation of Genetic Tissues	2	3	3
4	Immunological Chemistry	2	3	4
5	Microbial Poisson	3	3	3
6	Bimolecular Science	2	-	2
7	Microbial Industry	2	-	2
8	Research Project II	-	-	2

SPRING QUARTER

	Subject	Theory (Hours)	Practical (Hours)	Credits (Hours)
1	National Culture	2	-	2
2	Genetic Engineering in Nucleus	2	-	3
3	Microbial Poisson	3	3	4
4	Plasmas	3	3	4
5	Clinical Genetics	3	3	4
6	Antibiotics	3	3	4
7	Research Project III	-	-	2

Islam and Biology*

MUHAMMAD AL-HABIB IBN AL-KHOJA
Secretary General, Islamic Fiqh Academy
Jeddah, Saudi Arabia

1 INTRODUCTION

In this presentation, I hope to adequately address the main, not all, constituent elements of this topic. In this respect, there is no doubt that attention should be directed to three main points which constitute the windows that overlook the reality of our main theme, reveal its essence and demonstrate the interest of Islam in it.

The first main point is the divine instruction to us to deeply think and meditate over the magnitude of this universe and the living things dwelling in it, in a bid to discern their secrets and to duly appreciate the profound wisdom and rationale underlying their creation, with the resulting most certain facts that make man's conceptions and intellectual capacities transcend materialism and raise him high towards sublime unlimited expanses.

The second main point is to show Arab and Muslim contributions to the establishment and advancement of biological sciences throughout the period of Muslim renaissance.

The third main point is to define Islamic contemporary legal stand towards some medical courses of action and practices concerning new treatment issues resulting from scientific developments and advances.

2 LEARNING IN ISLAM

If one scrutinizingly looks into these points, one will find in the first of them plentiful constructive implications and lofty goals all of which emanate from the general denotation of the first Qur'anic word to be sent down as a heavenly revelation, i.e. when Almighty God said:

> *Read in the name of the Lord and Cherisher Who created man, out of a mere clot of congealed blood: Read and thy Lord is Most Bountiful, He who taught (the use of) the Pen, taught that which he knew not. (Sura 96, Verses 1-5).*

* Translated from the original Arabic by S. Odeh. Reviewed and edited by M. R. Zou'bi.

Thus science, knowledge and civilization are the best aspirations of man in search for which he beseeches help through the kind care of God. God has educated His Apostle in the best way, for He said to him: *Read in the name of your Lord and Cherisher*. Thus it is He whose help is sought for the achievement of the knowledge and learning pursued through reading. Learning and teaching are natural and even necessary for any human civilization and progress. Thus Islam has accorded a high status to learning by making the latter a sort of worship and an article of faith. It is a blessing bestowed by God on man whereby he discovers the Creator's originality by means of the miraculous creation and wonderful dexterity. In this, there is an intimation to this living being, his nature, structure, distinctive qualities, qualifications, gifts and capacities. All of these are discussed in biological science, lifting the veil and cover from both sight and insight.

God has stressed these blessings through the talent of teachableness and learning. The Almighty has also emphasized them through the secrets of man's creation. He also singled man out by honouring him through His imperative injunction to man to read, alluding by that to benefits accruing from the pen without which no religion can be established and no life can smoothly go on. Indeed, thanks to the pen, people have been able to come out of the darkness of ignorance to the light of knowledge and learning. It is also the basic instrument of writing down branches of learning, recording wisdom, accurately putting down news, codifying the scriptures revealed by God over and above whatever totalities and partialities encompassed by the pen and using the pen as an instrument for grabbing what is known and looking for what is unknown.

3 BRANCHES OF BIOLOGY

A careful look into biological science would reveal that it is divided into dozens of specializations such as: embryology, medical sciences, anatomy, physiology, zoology, botany, pathology, therapeutics etc.

These specializations are as numerous, diversified and complicated, as they are useful, feasible and fruitful. Under such circumstances, however, one should not feel confused and lost with the continuously size, growth and complexity of scientific knowledge, because such scientific discoveries may profoundly affect one's life, and even life in general as we know it. This would only whet one's appetite for more knowledge and learning. It is for this reason that the Holy Qur'an urges us in the following verses to exercise contemplation and meditation, and carry out research and study:

> *On the earth are Signs of assured Faith, as also in your own selves,: will ye not then see? And in heaven is your Sustenance, as (also) that which ye are promised. (Sura 51, Verses 20-22).*

These verses offer scenes and food for thought to those who contemplate. In them they would sense conceptions and cognitions that are suitable for and

harmonious with all spheres, environments, circumstances and conditions. This is what one of the scholars meant when he said: "Qur'anic texts are capable of giving a certain stock of knowledge to every soul, mind and conception: each as much as it can receive and tolerate. For the more elevated the level of man is in learning, the wider his knowledge and experience are; and the more acquainted with the secrets of the universe and the soul he is the greater the share and stock and the more diversified his Qur'anic provision will be. This, by the way, explains one of the secrets of differences in Qur'anic exegesis books and commentaries in terms of precision, comprehensiveness, meanings and aims."

The Qur'an itself has stated this fact openly where Almighty God says:

> *Say: If the ocean were ink (wherewith to write out) the words of my Lord, sooner would the ocean be exhausted than would the words of my Lord, even if we added another ocean like it, for its aid. (Sura 18, Verse 110).*

For earth, this planet where God has settled us and charged us with the development there of has been most perfectly prepared and equipped for life to receive and accommodate such life in an unparellelled way. Provisions for nourishment are stored up in this earth for the living beings and organisms which inhabit this earth, fly in its skies, traverse its waters, hide in its caves and caverns or hide in its subterranean tracks and cavities. All these ready provisions, simple as well as complex and viable in all forms and species, are prepared to meet the needs of these countless living beings.

But man is certainly the greatest miracle on this earth. When his heart becomes heedless of belief (in God) and is deprived of the blessing of certainty, he becomes simply distracted from numerous facts within himself. He forgets his own value, the mysteries lying within his very being, the secrets of his physical and psychological construction and all the apparent as well as hidden secrets.

But when he comes to his senses and meditates over the wonders of his own self, he will meet with astonishing and perplexing enigmas. He is confronted with the secrets of the formation, distribution, functions, and methods of performance of his limbs and organs. He will be faced with the harmony, collaboration, full and exact responsiveness of all his body's systems. Then he is made to see the secrets of sex and the role it plays in his procreative and genetic life, the secrets of the cell from which he is formed and which carries the entire stock of human characteristics. He is bewildered at his conception of material and mentally comprehensible things, how they are conceived, memorized and remembered. These are only a few samples of the wonders of God's creation whose explanation and the explanation of things similar to them we find in the facts, ramifications, issues, proems, totalities and partialities of biological science.

4 BIOLOGY IN THE QUR'AN

The Qur'an is replete with cosmic signs all of which it has mentioned and drawn attention to. Many references to biological sciences can also be found therein. Some of them are related to the creation of man, to the species of animals or to the realm of plants. All of these give hints to scientific facts inherent in man's creation, which leads us to explanations that reveal to indications noted by science and confirmed by experimentation and tests. Examples are found when Almighty God says:

> *Man did we create from a quintessence of clay; then we placed him as a drop of sperm in a place of rest, firmly fixed; then we made the sperm into a clot of congealed bleed; then of that clot we made a (foetus) lump, then we made out of that lump bones and clothed the bones with flesh; then we developed out of it another creature. So blessed be God the Best to create. (Sura 23, Verses 12-14).*

God also says:

> *It is He who brought you forth from the wombs of your mothers when you knew nothing and He gave you hearing and sight and intelligence and affection that you may give thanks (to God). (Sura 16, Verse 78).*

Here is a move from demonstrating the phases of creation to means with which human beings have been equipped to help them in the fields of knowledge and perception.

In this respect, discussion begins with the sense of hearing before the sense of vision, both of which are the most important instruments whereby man gets acquainted with his surroundings. Hearing is a blessing that is greater and more important than vision, highly significant as this latter may be. The function of the hearing sense precedes that of vision. The hearing sense starts to early function when the foetus is still in its mothers womb i.e. in the fifth month of pregnancy. Vision, on the other hand, starts functioning after birth. Vision or sight concentration starts only after the infant is six months old. This is a scientifically confirmed fact. Then mention has been made of conception, i.e. mental conception that only comes as a result of sensuous and mental cognitions. It is thus the road to learning items of knowledge and acquisition of experience. It would be appropriate here to cite the following Qur'anic verse:

> *It is God who creates you and takes your souls at death; and of you there are some who are sent back to a feeble age so that they know nothing after having known (much): for God is All-Knowing, All-Powerful. (Sura 16, Verse 70).*

What is clearly pointed out and carefully noted by the first major point are the animal and plant realms. It mentions a part thereof and pauses there to explain their constituent elements, roles in life and benefits with which God has

distinguished them. This is repeatedly stated as we can discern in numerous Qur'anic verses that speak about animals, e.g. Sura (2) {al-Baqarah}, Verse 175; Sura 24 {al-Nur}, Verse 45; Sura 6 {al-An'am}, Verse 38; Sura 16 {al-Nahl}, Verses 5-7 and 14. Then it points out to other Qur'anic verses about plants such as Sura 13 {al-Ra'd} Verses 3-4; and Sura 6 {al-An'am} Verse 99.

5 MUSLIM CONTRIBUTION TO BIOLOGY

Because these fields are so numerous and diverse, we will content ourselves in this brief account with glimpses related to anatomy, medical sciences, and fields of treatment with herbs and plants. This should be sufficient for merely drawing attention and making reference to some relevant points.

One of the most important issues of biological science that is striking to the researcher in the age of Islamic renaissance is anatomy, which is a *sine qua non* for the qualification of medics. In his book "Mihnat al-Tabib" (test of the physician) al-Razi says: that the first questions asked to a medical student are about anatomy and physiology, and whether he is well versed in the authored works of ancient people. If he is not well-versed, then there will be no need to test him about disease. Razi went on to say that a physician, in order to discern internal organ ailments, should first know the essence of such organs through anatomy. This is confirmed by Abul-Qasim Khalaf ibn al-Abbas al-Zahrawi who says: "A surgery practitioner has to acquire adequate practical experience in anatomy in order to have a good idea about physiology; a good knowledge of bones (orthopedics), nerves (neurology) and muscles, their numbers and outlets, veins, holding and stabilizing muscles and positions of their outlets; because a person who is ignorant of what we mentioned above is liable to commit mistakes which may prove fatal to other." An obligatory textbook for surgery graduates was Galen's work on surgery.

Early Muslim Arab celebrities in this field include Abu Bakr Al-Razi, Abu al-Qasim al-Zahrawi, Ibn Sina, al-Hasan Ibn Al-Haytham, Ibn Al-Nafis, Abdul-Latif al-Baghdadi, Ibn Zuhr and others.

They translated the basic original works of this science from Greek and other languages and added to that their experimentations and experience and added to that their own original contributions and pioneering discoveries that made them paragons and outstanding examples to be followed. Most of the works of these Arab and Muslim worthies were translated into Latin and other tongues.

In medical science they gave exact definitions to diseases and described as a state to which the body exposed where a limb or an organ in human body suffers from partial or total failure. Their definition of medicine came through Ibn Sina who said that medicine is a science whereby the conditions of human body are known in terms of what is healthy and then loses health. This science aims at keeping the body healthy or restoring it to its former healthy condition in case it suffers from any ailment. In pre-Islamic times, it may be noted, medicine was restricted to what the Arabs learned from Persians and Indians,

and the knowledge and experience they had acquired about drugs and herbs. This branch of knowledge was known as divination and prediction. Meanwhile their treatment was confined to cauterisation, cupping, and bloodletting (venesection).

Arabs and Muslims had various and effective methods in disease diagnosis. In his “al-Hawi” (the comprehensive book) al-Razi called for feeling the pulse, observation of temperature, shivering, face congestion, eyes, respiration, and nails. In the Bimaristan (hospital) Ibn Sina paid special attention to urine testing and observation of colour, purity and smell. He recommended careful observation of the patient, writing down notes and hanging the data plate on the patient’s bed.

During the Muslim era these medical gains were reinforced by traditional borrowings transmitted to Muslims by translation from Greek books. This new material was distinguished in both scientific and therapeutic fields. A number of medical scientists appeared in the Umayyad period and comprised, among others; Athal, al-Hakam of Damascus, Abdul-Malik al-Kenani and the Christian Abu Bakr of Damascus.

During the Muslim scholarly upswing in the Abbasid era, sciences and various branches of learning prospered. Contact took place under the caliphate empire between Arab conquerors and inhabitants of Muslim provinces. The caliphs invited the best physicians to Baghdad and other provincial capitals of the empire.

A galaxy of medical scientists dedicated themselves to and perfected several specializations and developed special talents therein. This led them to discoveries that often enabled them to criticize, correct and comment on earlier authored works. This led to the appearance of anatomists and other medics who devoted their attention to a particular branch of medicine such as surgery, ophthalmology, paediatrics, gynaecology and obstetrics, anaesthesia, resuscitation and psychiatry.

6 MUSLIM CONTRIBUTION TO PHARMACOLOGY

Care for plants also received a good deal of care from them. There books in that subject showed translations from Indian, Greek, Persian and Nabatean sources, most significant of which were the books of Discorides and Galen. In their expositions and commentaries they added their own earning views and experiments to what they had translated.

Pharmacists were at first known as herbalists in the east as well as in the west of the empire, because they dedicated themselves to studying herbs and extracting drugs therefrom. This clearly demonstrated the relation between medicine and botany. For nine tenths of the widely circulated medicines were made of plants or plant essences. The Arabs here did not confine themselves to the theoretical side in botany but worked also for development of agriculture in their lands inside the Arabian Peninsula and in its neighbouring conquered

countries by introducing new species and plant drugs not known to the Greeks such as tamarind, camphor, saffron, rhubarb, senna and embolic beside round fruits of citron and orange which they brought from Europe.

They acquired more and more knowledge of plant of various types through the species they come to know from the conquered countries like Egypt, Iraq, Persia, North Africa and Arab Muslim Spain.

In plant taxonomy they had a wide variety of methods that can be sought on their relevant sources.

Moreover, they were instrumental in spreading agricultural plant terminology in Europe outside Arab countries as could be evidenced from the public botanic garden established by Abdul-Rahman I near Qurtuba (Cordova) who brought to it the best stocks of seeds from Syria and other Asiatic countries. He planted the first palm tree in Europe. The plants that were transferred by the Arabs to Andalusia and Sicily in particular comprised saffron, grapes, rice, apricots, melons, roses and jasmine.

From Egypt they brought to it broad beans and onions, and from China mulberry and radish. From India they brought cucumber and spices. All this was then recorded by the gardens of Rusafa, Zahra, Zahirah, Tulaytial (Toledo) and Ishbiliya (Seville), which gave high credit to the Arabs, because they displayed high talent and dexterity in agriculture and ingenuity in organization of irrigation, drainage and water distribution.

If we go back to the theoretical side in medical science we notice that during the early Abbasid period Muslims directed their attention to books of pharmacology and botany, which they translated into Arabic. One of the first works from which Muslim scientists gained knowledge was Discorides's book in botany which was entitled "Materia Medica."

Relevant sources confirm that Arab and Muslim scientists developed this science after copying its basic origins from Greek tradition. Thanks to their efforts, experiments and researches, there was a transfer of a very wide range of medical and aromatic herbs and plants from the east to Europe. Along with them were transferred numerous plant technical terms and names which amounted to nearly 80 in number.

A good number of botanists stood out including: Ahmad Ibn Dawud Abu Hanifah Al-Daynawari (d. 895 A.D.), Ibn Sina (1037), Al-Baghdadi (1231), Ibn Abi Al-Fadl Al- Suri (1241), Ibn Al-Rumiyyah (1239), Ibn Al-Bitar 1248 and Dawud Al- Antaki (1600).

In this connection, many scientists returned recently to medical herbs for purposes of treatment and therapy. Lauding the benefits of herbalism, Dr Simon Mills said: "Through pharmaceutical testing of a number of easily available plants in most Muslim countries, it will be clear how to obtain alternatives and merits that make these plants preferable to other corresponding modern medical drugs."

7 MEDICAL ETHICS

For an individual who is committed to a straight-forward life and who abides by Muslim rules in his behaviour and dealings with others, and who bounds to moral rules of decorum and ethical values in terms of his general behaviour and the health care methods he follows, and the treating physician who take seriously the code of ethics and the burden of trust, and the heavy weight of responsibility, and therefore, always observes God with regard to the patients he handles, devoted to duty and realizing that he has a uphold the sublime values prescribed by Islam for man whom God has surrounded with a strong fence of guarantees indicated by Qur'anic verses and Hadiths, both (the individual and the treating physician) realize that the Holy Qu'ran was not brought down to teach people to receive medication, while Prophet Mohammad (PBUH) was neither a diviner, a soothsayer nor a medical doctor who pursued medical treatment as a profession and taught it to people. But Qur'an and Sunnah worked hand in hand to preach Islam, to uphold its creed and to make people abide by its law. Later on, both of them spread the civilization and culture of this faith and clearly showed people the concepts of Islam, which encompass main entity and position on this earth and his function in the universe. This fact indeed gives health a profound and fully integrated sense, which sick people as well as medics, even Muslims among them, have hardly stuck to.

This present world via which we pass on to our hereafter is full of types of health and disease, bliss and ordeal. We cannot obtain good therein or even a moderate minimum of good unless we have a wakeful conscience and crystal clear paths of right, and only when integrity, consistency and steadfastness are free from fluctuation and confusion resulting from inducements, motives, affecting factors and propensities. Thus a true believer is always thinking of his Lord, and carefully and critically looking upon his own work. Owing to diseases and their causes and because of the circumstances surrounding him in this life, one is compelled to ward off and escape such inconveniences. Thus both the physician and patient have to behave in accordance with the Sharia (Muslim canon law) standards. Both have always to be careful and to calculate sagaciously, and to stand fast in the face of anything that might happen to them or when they embark on any kind of work. They have to subject that to observance of Sharia rules, and abide by the rules of religion. Accordingly anything made permissible by Sharia may be done and anything banned by Sharia must be avoided and neglected in favour of anything that is better, more correct, more virtuous and has a safer outcome. To achieve that, there are alternatives that if one earnestly seeks or searches for one will certainly find.

In God's Book and in His Apostle's Sunnah, people will find answers to whatever causes confusion to them, especially if the issue is explicitly addressed in either or both these venerated sources. If people fail to find that and want to find out the reality they should look carefully into what they are about to do: if it is righteous, they can go ahead with it, and if it is vicious they should avoid it. Al-Nawwas Ibn Sam'an asked Prophet Muhammad (PBUH) about

righteousness and sin and the Prophet replied: “Righteousness is good conduct and sin is what is working up in your chest and you don’t like people to know of it.”

If the subject is a novelty pertaining to sin, and is illegal (Haram) whether its rises from the patients’ or the doctors’ behaviour, people of knowledge and learning must be consulted. This is a matter which needs inquiry and needs independent judgement (Ijtihad) coming from accredited legal bodies such as Fiqh (Jurisprudence) academies and Fatwa (formal legal opinion) committees and the like. In our present time many are the issues raised in this context including ideological, social, economic and medical questions. They need concerned people to arrive at God’s judgement with regard to them, and to show what stand Islam takes.

8 ISLAM’S VIEW OF CERTAIN CONTEMPORARY MEDICAL QUESTIONS

8.1 General

Faqihs (religious scholars) have worked towards giving discretionary or independent judgements regarding various complicated and numerous contemporary issues such as: deciding death scientifically as well as legally, postmortem examination, blood transfusion, use of wine and narcotics in treatment of some diseases, organ transplantation, methods of child bearing such as artificial insemination, new techniques of generation and human cloning. Members of the (Fiqh) academy examine the case, and experts and specialist doctors, and an accredited group of Faqihs are asked to state their views in writing. All answers concerning the presented issue are placed before the academy council members. Then the academy decision is made showing the legal ruling if the studies submitted were adequate and if they emanated from a complete and full discussion. Two examples may be given in this regards: organ or limb transplantation and human cloning.

8.2 Organ or limb transplantation

Man’s need for such intervention comes with cases of disease or physical incapacity and because of the ailment or incapacity that may result from such cases or when it is desired to get rid of some prenatal or organic deformities. This practice was known to people since earliest times.

Evidence of legality of such medical behaviours and action rose from the incident when Prophet Muhammad (PBUH) restored the eye of Qatadah Ibn Al-Nu’man, the hand of Mu’awwath Ibn Afra and the hand of Habib Ibn Yassaf to their natural places, all of which were, miraculous feats performed by the Prophet. This is a sort of limb or organ, transplantation, which confirms the possibility of this practice. Transplantation dates back to a long time ago. It was

carried out by a medic called Maysa in 1770 who transferred the tendon of the middle finger's extensor muscle to the index finger.

Surgery specialists and a number of scientists and thinkers discussed the issue of organ and limb transplantation. The entire topic was comprehensively placed before Fiqh (jurisprudence) academies and Ifta (delivery of Fatwas or formally legal opinions) bodies and individual as well as academy Fatwas were issued with regard thereto.

In the Fourth Session of the International Islamic Fiqh Academy, held during the period Jumada Al-Thaniya 18-22, 1408 A.H.; February 6-11, 1988 A.D. Resolution No. 1 on organ transplantation was issued as follows:

(1) An organ may be transferred from one part of the human being's body to another part, after having made sure that the benefit expected from this operation out-weighs the harm resulting therefrom, and provided that this process is performed to replace a lost organ, to restore its normal shape or function, to repair a defect or to remove a deformity that would cause a psychological or physical discomfort to the person concerned.

(2) An organ may be transferred from a person's body to another person if this organ is automatically renewed like blood and skin. In this case due observance must be given to the fact that the donor is fully qualified and legally competent and that relevant legal conditions are fulfilled.

(3) It is permissible to benefit from a part of the organ that was removed for remedying an ailment of another person. An example is taking the cornea of a person when the eye is removed for a pathological reason.

(4) It is forbidden to transplant an organ, which is indispensable for one's life like the heart from one living person to another.

(5) It is forbidden to transplant an organ from a living person when the removed of such organ disrupts a basic function in this person's life even if it is not an absolute necessity for the donor's survival, like transplantation of both eye corneas, but if transplantation partially disrupts a basic function, this will be subject to discussion and further consideration as explained in the eighth paragraph.

(6) An organ may be transferred from a dead person to a living person whose survival depends on that organ or when the integrity of a basic function of his body depends on that, on condition that the deceased or his heritors after his death permit such transplantation, or subject to the approval of the Muslim's top authority if the dead donor is unknown or has no inheritors.

(7) It must be taken into account that agreement on the permissibility of organ transplantation in the cases stated above is subject to the condition that the process is not to be carried out through sale of the organ because sale of human organs is not allowed at all. But payment of money by the beneficiary in a bid to obtain the required organ, when that is necessary, or

as a reward or by way of acknowledgement and gratitude is a subject to discretion and consideration.

(8) Anything except for the aforesaid cases and situations but relevant to the essence of this issue is subject to discussion and careful examination, and should be presented for further study and deliberation at a future session and in the light of medical data and Sharia rules and regulations.

8.3 Human cloning

In its Tenth Session held on Safar 23-28, 1418 A.H./ June 28-July 3, 1997 A.D., the (Fiqh) Academy Council discussed the issue of human cloning. Jurisprudential and scientific studies on the subject were surveyed and discussed together with the resolution finally adopted by the World Health Organization (WHO) at the meeting of the Scientific and Ethical Group on the issue of human cloning on April 25, 1997. The Academy Council adopted the following resolutions:

(1) Human cloning must be banned through both the aforementioned methods or any other method leading to human proliferation.

(2) If any disregard for the legal ruling stated in paragraph "1" above takes place, the impacts of such cases should be fully discussed to know the legal rulings with regards to them.

(3) All cases are banned where a third party is made to intrude on marital relation whether this third party is an uterus, an ovule, a sperm, or a body cell for cloning.

(4) It is legally permissible to adopt cloning and genetic engineering techniques in the field of microbiology, botany and zoology, but within the framework of legal controls and in a manner that serves public interest and wards off harm.

(5) Muslim countries must enact laws and make regulations needed for blocking direct and indirect channels before local and foreign agencies, research institutions and experts in any attempts, to make Muslim countries open to cloning experimentations and to promoting such experimentations.

(6) Joint follow up and monitoring by the Islamic Fiqh Academy and the Islamic Organization of Medical Sciences of cloning the scientific new developments therein, and control of its terminology, must be pursued. At the same time, symposia and colloquiums required for exposition of Sharia rulings related to cloning have to be organized.

(7) Calling for setting up specialized committees comprising experts and Sharia scholars to lay down ethical checks in the field of biological researches to be adopted in Muslim countries.

(8) Calling for establishment and support of scientific institutes and institutions which conduct researchers in biological sciences and genetic engineering in other fields than human cloning, and in accordance with Sharia checks, so that the Muslim world may not remain in a state of dependency on others in this field.

(9) Authentication of dealing with new scientific developments through an Islamic outlook and urging the media to adopt the outlook based on belief in dealing with such issues, avoiding their use in a manner that contradicts with Islam, and enhancement of public opinions awareness to stand on a firm ground before taking any particular stand in due response to what Almighty God says:

When there comes to them some matter touching (public) safety or fear, they divulge it. If they had only referred it to the Apostle, or to those charged with authority among them, the proper investigators would have tested it from them (direct). (Sura 4, Verse 83).

9 CONCLUSION

Thus the attitude of Islam towards biological science becomes clear. It is an attitude based on encouragement of a comprehensive knowledge of all aspects of this science, acquaintance with its realities and exploration of its secrets. It is also an attitude that calls for contribution to and participation in the ramifications of this science through transmission, translation, research, authorship, discovery and amendment in both theoretical and practical spheres. This entails discussion of newly presented issues or theories in biology to weigh and measure them within the norms of ethics, values, and human principles. Moreover, these issues and discoveries have to be subjected, in terms of their essence and as regards the behaviors of both patients and medics, to legal rules and jurisprudential principles, which would safeguard human dignity, observe the aims of Sharia, keep away evils, and serve public welfare.

The Twenty First Century is a Century of Biology: Prospects and Challenges*

MUHAMMAD AL-MUKHTAR ESSALAMI
Tunis, Tunisia

1 ABSTRACT

Background

In the twentieth century, the world achieved, more progress in various fields of learning than it had done throughout its long history.

Human learning has gone deep into probing the secrets of the universe thereby bestowing a distinguished position on physical sciences in the century we bade farewell to only some months ago.

With a wider outlook into scientific horizons, biology has come to the fore and scientists vehemently addressed themselves to the structure of man himself and to the plant and animal nutritive elements that comprise his build-up. New wider horizons have been opened all of which constitute evidence both from the universe and from man himself testifying to accurate creation. In this context Almighty God says: *Soon we will show them Our Signs in the furthest regions (of the earth) and in their own souls until it becomes manifest to them that this is the Truth.*

And just as science has attained full knowledge of the atom and the contents thereof in the material world, it has also reached out in biology to the smallest constituent of the cell: i.e. the genes, and managed to read their letters. By that means, man knew the genetic sack which contains the entire program of life. Following that, techniques have evolved into the ability of affecting the genes in terms of linkage, separation, transference and arrangement. As a result, genetic engineering came to existence. In this context, it cannot be denied that these scientific and technical gains pose great challenges for the Muslim world in the following fields:

Plants

Genetic engineering affects plants in several respects (quantitative output, qualitative improvement in taste, form and nutritional value and protection against what leads to decrease in production and returns).

As the Muslim world is still far away from achieving self-sufficiency and is still relying on others in production of its food, which adversely affects its

* Translated from the original Arabic by S. Odeh. Revised and edited by M. R. Zou'bi.

economic growth and decision-making, it has become inevitable that it should endeavour to attain a good grasp of the scientific and technical level in plant genetic engineering.

Since the aim of such techniques is to serve man's interest, we must not offer for nutritional purposes the genetically modified products until we have made sure that they are good enough and suitable for human consumption.

Animals

With regard to animals starting from mammals and ending up with Bacteria, scientists have reached out DNA inside the cell and made it reveal its secrets and then they impacted the chromosomes carrying the genes, which hold the genetic sack. For example, it has become possible to spread a gene that provides milk, meat or eggs, protects from certain diseases or has a better taste. Thereby the animal will change into a type that provides wealth and supplies food at a lesser price.

In this situation, the Muslim world with its limited financial and food resources has been provided with prospects enabling it to overcome the limitations of its present situation. It is therefore necessary that we have scientist, technicians, laboratories and researchers that enable us to be independent in this sphere and thus secure more food at less cost and consequently have a better health.

In this respect the Muslim *Ummah* (community) has to adopt an approach of cooperative and integrative planning where channels are open between scientific and research communities.

Man

With regard to man, science has become able to read the human genome this year (2000). This is certainly a remarkable gain the effects of which cannot be defined at the beginning of this century. But while opening so many doors, it also has objects of caution in practice that are to be subjected to ethical norms constituting a safety valve that safeguards human dignity. This should not overlook individual acceptance; for human innate character can be erratic and can be sound. Reliable controls are, therefore, those emanating from absolute right.

2 INTRODUCTION

Some months ago we said goodbye to the 20th century. In that century, human learning become wider and deeper. It achieved an accuracy and a diversity on a wider scale than achieved ever before by humanity throughout its long history and encompassed land, sea, space and planets; men, animals and plants. Every branch of human learning had a share of manifestation and human control

which man has harnessed for the purposes he intended and thereby was able to arrive at the mysteries hidden for many long centuries.

The most significant branch of science that achieved great progress and considerable success that changed the character of civilization in that century was the Physical Sciences. These sciences which ramified into sub-branches each of which became theoretically almost independent by itself, although in practice it embraced and affected many sciences, clearly leaving its offshoots and its fingerprints either patent or concealed in the development of these sciences.

Applications became complicated and have been amalgamated with technological application. They have been different in objectives and aims. Some took the course of destruction while others opened unlimited prospects for human welfare, shedding light an and elucidating the norms of the universe. Thus the twentieth century was the century of the Physical Sciences.

Steven Weinberg, a Noble laureate of 1979, says that the ultimate goal of physics is to discover a theory that embraces the entire physical and material world.

This identification of the ultimate goal of scientists' efforts, i.e. arriving at an all-embracing theory shows that, through theories confirmed by experiments and applications, that the world is predominated by order, not by chaos, and by harmony, not by discord. This is confirmed by what Almighty God says: "No want of proportion will thou see in the Creation of (God) Most Gracious"[(1)]. In fact physics during the 20th century achieved so many discoveries, which emphasized this cosmic order and its harmony. This has been manifested in the theory of the time dimension with the relativity with which Einstein attained a level proved by all subsequent experiments and applications, Planck's Quantum Theory or the atom structure theory derived from the feats of previous thinkers, which came to the conclusion that it was one single system. All these discoveries and applications have brought scientists considerably closer to the conception of this theory that encompasses all the aspects of the universe. With every scientific step forward this theory comes to bear witness that the Holy Qur'an cannot possibly come from any other source than God.

Following is an interesting dialog between Bill Moyers, a journalist who was for sometime in charge of the White House press relations, and Steven Weinberg who first voiced the Big Bang Theory.

Q: If you feel relatively certain about the hypotheses related to all the first three minutes of the universe, can you tell what you believe about the last three minutes?

A: We are trying. This is an interesting scientific point, because it is linked with astronomic observations going on now. All is linked to the quantity of material currently existent in the universe. If it is sufficient, expansion of the world will stop one day, and counter-motion will begin. In that case we shall have contraction and everything will disappear in a horrible crushing.

Q: Will this take place after about 50 billion years?

A: This is a good guess; for when we know more exactly about the density of material in the world we will then be able to tell exactly when the Big Crushing will take place. On the other hand we may discover that no sufficient material is available to cause a contraction. The galaxies are getting farther from one another and then everything will be frozen and completely dead forever.[(2)]

In the scientific answers to these questions we find corroboration to what Almighty God says: *With power and skill did we construct the Firmament: for it is we who create the vastness of space.* [(3)] This draws the attention of humanity to the expansion of the sky through the capacity of the Great Creator. Secondly, all beings: great or negligible, are bound to vanish. It will not be a gentle vanishing or a slow death but a horrible crushing. Almighty God says: *The convulsion of the Hour (of Judgement) will be a thing terrible.*[(4)]

What is certain is that the cognitive level which humanity has been destined to attain does not qualify it (humanity) to define the hour of this crushing. Almighty God says: "They ask thee about the (final) Hour-when will be its appointed time? Say: The knowledge thereof is with my Lard (alone): None but He can reveal as to when it will occur. Heavy were its burden through the heavens and the earth. Only all of a sudden will it come to you." [(5)]

These scientific predictions and the theories and applications that preceded them all converge to proclaim what has also comes in the Holy Qur'an: *Soon will we show them Our Signs in the (furthest) regions (of the earth), and their own souls, until it becomes manifest to them that this is the Truth.*[(6)]

The formulation of this ayah (Qur'anic verse) has come about in such a way as to be revealed by its inimitability. For this verse has carried a promise that God will show His Signs to the world first in the distant regions of the earth and secondly in man's own soul, which is what has actually happened. For physics discoveries about the mysteries of furthest regions have come in a way not conflicting with the Qur'an. This was followed by discoveries in man's own soul. Knowledge has investigated the base of material and the very small particles of which it consists. This has been established hypothetically and arithmetically and through a number of proofs. Then humanity comes into inlets from which it gets to know about man and the minutest details of his structure and the plant and animal elements that comprise his body.

3 MAN'S LIFE AND ITS STATUS IN ISLAM

3.1 General

Anyone who traces what has been sent down by divine revelation will inevitably come to realize that the Divine Law's ramifications are brought together within certain general rules which guide the Muslim scientists to

distinguish what has been in conformity with this religion from what has not; that every problem or issue which faces man during his life comes within these ascertained rules; and that the sublimest of these rules are those regarded by Islam as an religious necessity. One of these necessities is life preservation.

Some writers confine this rule to forbidding murder and legalizing retribution. This is one aspect, or the negative side of Islam's care for life. The positive aspect, on the other hand, is enabling every individual to have what preserves his or her life. In other words, it is food, drink, clothing, shelter and health care.

3.2 Man's need of food

3.2.1 General

Food is a basic constituent factor of life. For when an infant is born, life will be entirely dependent on milk. With its progressive growth, its bodily needs become more diversified to enable it perform its task in this life.

Every mouth's potentially open to maintain man's life and activity. Consequently, scientists in the last century after the alarm sounded by Malthus Corly in the nineteenth century, hastened and struggled to make the world, and particularly decision makers, sense that the world will suffer from the disaster of progressive or accelerating growth of population, and that the world resources will fall short of the needs of its population, and that solution lies in birth control campaigns which have been adopted by governments with varying degrees of extremism or moderation. This has been followed by the growing old age particularly in the economically comfortable world. An imbalance has arisen between active manpower living in the productive stage of life and the rest of consumers who are unable to produce because they are either two old or too young. The United States, by the way, is distinguished for being the only country, which has been careful to regularly provide its society with new young blood. It never completely shut the door in the face of all types of immigrants and herein perhaps lies the secret of its excellence in all fields.

Along with this trend, however, there has been a counter-current, which based its argument on:

(a) Two centuries have elapsed since Malthus sounded his alarm, and disaster has not befallen our globe. In fact many food stuffs like dairy products have accumulated to a degree that exceeded the needs of people although the latter's consumption was doubled as compared with the consumption in the previous century; and

(b) Whenever man faces a challenge, he usually responds in a manner that neutralizes such challenge and maintains man's capacity to remain as God's vicegerent in this world. He is in a continuous toil and struggle. He is meeting constant challenges and is showing a remarkable capacity to cope with them.

3.2.2 Genetically modified foods

Interference with genes comes within this framework of challenge where man has proved to be successful throughout human history. God has provided a parable in the Qur'an about multiplication of production when he says: "It (the grain of corn) growth seven ears, and each ears has a hundred grains." [(8)]

Science has interfered with the genes of plant stocks in a manner that multiplied production in quantity, improved in quality and protected agriculture from damaging pests. In this respect, the most important factor was the amount of water needed for the plant to ripen. All this has become an actual fact and not mere hopes entertained by humanity.

These products have started to fill the markets, and it seems that man has only to sing the song of triumph and success and to be hopeful of a future that promises humanity to get rid of hunger, which has posed a serious threat for it. This success also helped the farmer who often had his work and toil ruined by numerous types of fungi, bacteria and insets which destroyed his crops. Crops that also suffered from parasitic plants that devoir humility and the nutritive ingredients of the soil.

But, as Prophet Muhammad –peace be on him- has taught us, good opportunities may come and are mishandled or misunderstood and may turn into evil. Al-Bukhari, Muslim and other hadith scholars narrated on the authority of Abu Said Al-Khadri that Prophet Muhammad said: "I am worried about you with regard to worldly splendors (i.e. wealth, security etc.)" whereupon a man asked him whether good can beget evil. The prophet replied that good produces only good results but wealth is like a delicious plant which may stimulate the appetite of animals and make them eat too much, which could cause their death, while if they only eat a reasonable amount, it will benefit and nourish them.

What is noticeable about scientific advancement achieved in the genetically modified agricultural products is that such progress was an outcome of generous expenditures offered by giant international companies where science and money worked together to bring about such beneficial results.

However their goal was not to promote human welfare and feed the hungry but to obtain satisfactory returns for the money they spent. This is neither forbidden nor objectionable. The problem in fact has risen from hasty application of research findings without making sure that what they have arrived at is not harmful for men or animals. For example Mad Cow Disease, which killed some people and caused the destruction of very large numbers of flocks of cattle and sheep, thereby aggravating environmental pollution over and causing heavy losses to farmers and even to livestock resources of the world at large. This disease did not result from genetic modification but from undue haste in marketing new types of animal feed before making sure of safe consequences. To put it differently, it was a result of running after accumulation of wealth within the shortest possible period. The same may be said about pharmaceutics.

An American journal said that a company has modified genes in the tomato plant whereby the fruit could be stored for a long time without rotting. Researches have cost the company five million dollars. This species of tomatoes is currently being sold at a price three times higher than ordinary tomatoes. Of course, storing tomatoes, which do not putrefy is a revolutionary development in dealing this type of vegetables, normally cause producers to incur heavy losses if they could not sell the tomatoes in time. But does this modified product maintain its original natural properties? Is it safe for future human use and without any adverse impact on human organs and body systems? Have all the precautions based on studies and experiments been taken? These are important questions that call for reassuring answers.

In this context, the US National Academy of Sciences has published a report which says that crops modified through molecular and cellular process do not constitute any threats which are different from those caused by conventional genetic methods of the same qualities. In February 1992, the White House made a statement to the effect that genetically engineered products should not be subjected to additional federal measures because they do not constitute any serious danger.

Genetic modification, it should be said, is still a very young science. It is, therefore, bound to follow the steps of other techniques and sciences. They will become more diverse and complex with the passage of time. What will happen in the future is not necessarily as safe as what has happened so far. For this reason, it is a must to agree upon scientific norms to which each genetically modified product is to be subjected and that it should be supervised in accordance with these rules by governmental labs far from any interference on the part of producers.

The decision taken so far that "Genetically Modified" should be written on the modified material, does not, to my mind, protect consumer for two reasons:

(i) Media effect on individuals' choices is formidable. We have seen that tobacco and cigarette manufacturing companies have been made to write, "This is harmful to your health" on cigarette packets. Despite this long-standing and explicit warning, smoking has not been curtailed. Probably, the contrary is true; and

(ii) The people who carefully follow up or trace what is written on the commodities they purchase are only a minority, particularly in the developing world where illiteracy is still high. It is not illiteracy that is only related to reading and writing, but also cultural illiteracy. For even literate people do not care for reading what is written on the foodstuffs or medicines they buy.

3.2.3 A proposed course of action

This world to which we belong and the productive agricultural resources of which are meager compared to its population, has definitely to come forward to

the arena of plant genetic modification by allocating generous funds for research and experimentation to achieve tangible results and be certain that the product is useful and free from any likelihood of danger. If genetic modification in tomatoes has been so costly to the researching company, and if world resources are so limited and poverty is so prevalent, the most likely outlet is cooperation among Muslim countries in this sphere by dividing fields of research and exchanging experience and results.

To adopt and master these techniques is a national, human and religious duty because it is concerned with human life preservation. This if determination, money and mutual collaboration are present, intelligence is not restricted to any particular race, colour or location. If what we have just mentioned is available, the Muslim world will be able to overcome this big challenge to its independent decisions. Anyone who depends on others for his food will be lacking in free choice, dignity and independence.

Some years ago, an American biologist wrote that the future of economic welfare in the USA lies in the genetically modified plants, which the world has no choice but to import from the USA. He claimed that scientific secrets in this field were more important than military ones.

The question thus remains despite such thinking, which is unfortunately in harmony with the materialist civilization that regards money as a top priority in the field of human relations, of how can we effectively meet this challenge?

4 GENETIC MODIFICATION OR GENETIC ENGINEERING IN ANIMALS

4.1 General

Inside the animal cell, scientists have discerned the smallest part which is the basis of life, i.e. the DNA Deoxy-ribonucleic-acid. They have found it to be an unusual world which they ardently dedicated themselves to reading and decoding. Six highly developed countries allocated billions of dollars to knowing the chromosomes which vary in number and which amount to 46 among human beings, in an endeavour to read the genes thereof.

After having known the effect of every gene on the shape and production of the animal, scientists managed to plant a gene from one animal in another with which it has been integrated according to high technologies and pursuant the exact and accurate estimate of creation to change the production of the animal within which this new gene has been integrated to the situation conducive to achievement of the aim for which this process has been carried out.

Genes have been modified in the upper and lower species of animals. Scientists have modified the virus to make it carry genes with which to break into the fortress where DNA lies. They have modified microbes to make them more resistant and defy antibiotics or to make them more effective. They have also modified the genes of the animals on which man relies for his food. Dairy

or milk producing animals have had their genes modified with a gene from some cows that produce large quantities of milk or those with less fat in their milk. Scientists have moreover modified the genes that accelerate the growth of animals thereby producing a maximum possible amount of meat within a minimum possible time. In addition, they have modified the animals' genes making them more resistant to disease. This, needless to say, constitutes a tangible gain for cattle breeders, a step that brings about plentiful food and cheap prices. This in turn would make it possible for low-income people get a fully integrated diet.

Furthermore, they changed animal products with genes that enter pancreas to activate its insulin secretions after the pancreas has stopped functioning. Researches are also going on to overcome diseases that have proved to be incurable or costly to treat like some types of cancer where complete cure can be achieved through gene plantation.

4.2 Related challenges

Some biotechnology and genetic engineering techniques are pushed towards generalized applications by a financial power and avidity to hoard wealth. For example, when Rosaline Institute announced its global achievement, its shares in the stock market rose by 13% in one single day. But running so fast after this goal entails a variety of risks as we have shown in interference with plant genes. It is, therefore, mandatory to take the following precautions:

(a) Experiments have to be made on reactions resulting from eating the genetically modified meat and dairy products. Such experiments should prove that there is no danger resulting from long-term consumption of these foodstuffs; because their effects may not be known unless they are checked continuously and for a long time;

(b) Genetic modification must not be spread to include all flocks and cattle's and entail extermination of other stocks, because history of animal epidemics has shown that some diseases are quicker to eradicate than others. It is therefore, mandatory that mankind keeps various stocks in order to protect man from threats which he does not know until they have actually taken place; and

(c) Some lower animals can be genetically modified to make them more ferocious, resistant and capable of seriously hurting mankind. In this respect what has been revealed about biological weapons, their relatively uncomplicated process of production, and the devastating damage they cause to human tissues and the debility they bring about makes it necessary for all nations to mobilize and harness their efforts to stop experiments in this horrible arena, and control relevant labourites in a reassuring way.

The representatives of the OIC science community may appeal for the proclamation of an international covenant that commits all individuals,

corporations and governments to completely forbid any experiments leading to production of genetically modified microbes, viruses or bacteria that may be destructive to any human organ. During this summer (2001) we have learnt that two scientists have defied entire humanity by conducting forbidden tests in the high seas far away from national territorial waters. This a transgressive action because seas, rivers and land have been created for all people and nobody can claim that he has an exclusive right to a place where he can do what endangers mankind as a whole. Almighty God says in this respect: "*And he has subjected to you as from Him all that is in the heavens and on earth*" [(11)]. This is as far as the world at large is concerned. As for the developing or Third world of which the Muslim world is a part, it must take the following measures:

(i) Allocate resources that enable it to achieve self-reliance in the field of genetic modification, and not to be content with dependency on the economically and scientifically advanced world, which always gives top priority to economic profit and furtherance of its hegemony in dealing with others; and

(ii) Laboratories are to be spread all over the Third world countries which, in turn, must maintain complete mutual cooperation and exchange of experience and results.

Western scientists, it must be noted, have succeeded in their experiment only through cooperation between their international labs and scientists, and building on the results worked out by others in various specializations.

5 AN AMBIGUITY

Some contemporary interested people think that Sura 4 (Al-Nisa) of the Holy Qur'an prohibits scientific intervention with the genetic sack of animals. In this respect Almighty God says: *He (Satan) said (to God): I will take of Thy servants a portion marked off; I will mislead them, and I will create in them false desires; I will order them to slit the ears of cattle and to deface the (fair) nature created by God.* [(12)] These people believe that this Qur'anic verse shows outwardly that slitting cattle ears and changing the natural creation of animals is forbidden because it is a part of the portion marked off by Satan to lead them a stray from the path of God.

To understand the Qur'an according to apparent meaning is an approach that runs counter to what God has told us about the real nature of this Book which tells people seven times to meditate when reading it: *(Here is) a Book which We have sent down unto thee, full of blessings that they may meditate and that men of understanding may receive admonition* [(13)]. The Qur'anic verses in Sura (4) have come in succession to make people aware of the loopholes through which Satan can enter to delude people and lead them away from the path of God. Satan prepared himself for leading them a stray by making them turn away from

the clearly manifest track of monotheism to that of dark doubt or confusing obstruction. Then Satan revealed some of the features of its deception and delusion. It mentioned some methods known in the time of Prophet Muhammad like slitting the ears of cattle, and depriving people of the ability to benefit from them and only to dedicate them for their gods. Meanwhile God's approach is based on creating animals for the use of man. Satan, on its part, told its followers that if a she-camel gives birth to ten camels, its ear will be slit as a sign that it is sacred and belongs to the gods and therefore cannot be ridden and its milk cannot be utilized. The same applies to a sheep which given birth to seven lambs. If the last-born happens to be a male it is slaughtered and consecrated for the temples of their deities, but if it is a female it will be left for these deities. These are features of deep infiltration of polytheism into their gains. This is not, by the way, harmonious with working for upgrading the quality of offspring; for it does not lead to corruption in faith or in universe. Ibn Atiyyah says in his commentary: "This verse does not enjoin any harmful change but allows any change leading to improvement and betterment."

6 MISHANDLING AND MANIPULATION OF CREATION

Scientific success and desire to be ahead of others for discovering unfamiliar and new things has led some researchers to try and construct new animals through planting the genes of some into others to change their shape and qualities. Some even tried to mix human beings with animals. This is an attempt that must be completely stopped and all manipulators thereof have to be prevented from such attempts.

7 GENETIC ENGINEERIGN IN MAN

The theme of the 11th IAS Conference is "Biotechnology and Genetic Engineering for Development in the Islamic World." As the success of development is measured by its ability to change human situations for the better, it is obvious that taking care of these techniques in terms of their relation is the supreme goal. These techniques, in so far as they illustrate scientific achievement as it is hoped to be shortly realized, lie within the realm of man's protection from congenital hereditary deformities and treatment of many diseases, because a physically and mentally sound person is the foundation stone of development.

The largest gain in this field has been what was announced this year i.e. that reading life books and discovery of the genes have been accomplished (Sequencing the Human Genome – Editors).

Two things remain to be achieved in this weighty subject:

(a) From a technical standpoint …the application still practised by human intelligence and the discovery of techniques based on them will continue for a long time to come; and

(b) From an ethical standpoint in practice… when the secret of the gene is discovered announcing the near or distant future destinies, or announcing on undeniable fact that affects life and human relations.

It might be difficult to fully investigate these issues but we can refer to some potential situations that might arise:

(i) Genetic tests may prove that the tested child cannot possibly be the husband's son. Will the medical doctor announce this or not?

(i) If the doctor discovers that genetic test has shown that a gene indicates that the person it belongs to will suffer from cancer, a back deformity, a particular heart disease or any other handicap, and medicine at the time of discovery is unable to extend any protections, will this be announced to the patient to make him apprehensive of the inevitable end, thereby delivering him to intolerable worries? Or will it be withheld from the person directly concerned? And if it is withheld from the latter, will the secret be divulged to a family member? And if it is hidden from this family member will the medic enter it in the health file that belongs to each one of his patients? If he entered it, will he be sure that others will not be able to see it such as a physician who may be deputizing for him or when he (the immediately involved doctor) dies and is succeeded by others?

(iii) If the genetic test is conducted for the benefit of an Islamic or other insurance company, will the medic make public his discovery? And what will the case be if the potential peril is likely but not inevitable?

In his valuable paper which was a scientific research moulded in an elegant style [(15)], Dr Hassan Hathut was keen on citing numerous potential perils.

8 CONCLUSION

Governments and scientific bodies in Muslim countries must cooperate to control the techniques whereby man has opened the door towards knowing his/our self. These techniques, however, are to be surrounded with a fence of ethical norms which cannot be decided by one single scientist or scholarly sage, but a collective Islamic effort that respects man without victimizing him, does not transgress what God has forbidden and cautioned against. Its goal must be to help people attain a better and more perfect life.

REFERENCES

1. Sura 67 (al-Mulk), Verse 3.
2. The “Dialog” Magazine, p. 31, No. 3, 1990.
3. Sura 51 (al-Dhariyat), Verse 47.
4. Sura 22 (al-Hajj), Verse 1.
5. Sura 7 (al-A’raf), Verse 187.
6. Sura 41 (Fussilat), Verse 53.
7. “Dialog” Magazine p.60 No. 4, 1991.
8. Sura 2 (al-Baqarah), Verse 261.
9. Fathul-Bari 6396, part 14, p.21.
10. Created Beings and Genetic Engineering, Dr. Salih Abdul Aziz Krayyim, p. 11. A paper presented to the symposium entitled “Genetic and Genetic Engineering” (Islamic Medicine, Kuwait) 1998.
11. Sura 45 (al- Jathiyah), Verse 13.
12. Sura 4 (al-Nisa), Verse 119.
13. Sura 38 (Sad), Verse 29.
14. “Al Muharrar al-Wajiz” part 4, p. 232.
15. “Reading the Human Genome,” Prof. Dr. Hassan Hathut, the symposium entitled “Genetics, Genetic Engineering and Human Genome,” Organisation of Islamic Medicine, Kuwait, 1998.

Ethical Issues of Genetic Engineering: An Islamic Perspective

ANWAR NASIM*
COMSTECH Secretariat
Constitution Avenue
Islamabad, Pakistan

1 ABSTRACT

In this paper, some of the recent developments in such areas as cloning, gene therapy, prenatal diagnostics, stem cell research, genetic screening and other related advances in human reproductive biology would be highlighted from a geneticists' viewpoint. A search for answers to these extremely complex questions also leads to other issues such as the patenting of DNA fragments, genetic pre-screening for insurance and employment, screening of human populations for early diagnosis and the individual's right to privacy. There are specific issues related to the human genome project, somatic and germ cell gene therapy, and organ transplantation that also warrant a detailed discussion.

It may not possible to discuss all these examples at great length, and only one or two examples can be dealt with to illustrate the basic approach. For this purpose the current research and ethical debate that surrounds the stem cells will be used as a focal point.

At the outset, one needs to state that for Muslims the two major sources of guidance are the Holy Qur'an which is a holy book revealed by God to the Holy Prophet Muhammad (PBUH) and; the Sunnah which represents the sayings and the way of life of the Holy Prophet Muhammad (PBUH). It is in the light of these above two sources that all ethical dilemmas have to be examined.

The most recent studies in the field of stem cell research will be used as an example to illustrate the possible future approaches that need to be used to deal with ethical issues and other related questions.

The major breakthroughs in molecular biology and genetic engineering have raised many serious legal, ethical and social questions. That is why it was not surprising that from the very beginning, the Human Genome Project assigned nearly 3 per cent of the total budget for a committee to examine the ethical, legal and social implications (ELSI) of this research.

---

* Geneticist, and Fellow of the Islamic Academy of Sciences.

2 GENETIC ENGINEERING

Genetic engineering is a laboratory technique used by scientists to manipulate the DNA of living organisms.

DNA is the blueprint for the make up of an organism. The organism relies upon the information stored in its DNA for the management of every biochemical process. The life, growth and unique features of the organism depend on its DNA. The segments of DNA, which control specific features or functions of an organism, are called genes.

Molecular biologists have discovered many enzymes, which can manipulate the structure of DNA in living organisms. Some of these enzymes can cut and join strands of DNA. Using such enzymes, scientists learned to cut specific genes from DNA and to build customized DNA using these genes. They also learned about vectors, such as viruses, which can infect a cell and insert themselves into DNA the host.

With the discovery of the double helical structure of DNA by Watson and Crick in 1953, a new era of molecular biology started. Since then new techniques to cut, join, recombine, introduce into host cells, sequence, synthesize genes in the laboratory have all been developed. These techniques collectively have enormously enhanced the ability of geneticists to manipulate the genetic material.

With this knowledge, scientists started to build vectors, which incorporated genes of their choosing and used the new vectors to insert these genes into the DNA of living organisms. Genetic engineers believe they can improve the foods we eat by doing this. The global debate that surrounds the eating of genetically modified foods and the possible consequences for human health is also an issue that will need to be dealt with by Muslim scientists.

3 HUMAN GENOME PROJECT (HGP)

The Human Genome Project simply stated has been the goal to obtain the sequence of three billion bases that constitute the Human Genome. Human struggle in coping with the challenges posed by such diseases as cancer, Alzheimer, diabetes and cardiovascular disorders has been going on for centuries. The sequencing of the Human Genome will become a historical milestone in the history of science and will have a major impact on future medical research. (The genome inspiration, The News, 19 July 2000).

HGP is armed with the exact makeup of every human gene, scientists would be able to generate new medicines, dovetail drugs to suit every individual on Earth, unravel the complexities of the mind and understand our physiology as never before. The most critical issue of social implications of the Human Genome is the privacy, confidentiality and the fairness in the use of the genetic information. Confidentiality, on the other hand, is the right of an individual to

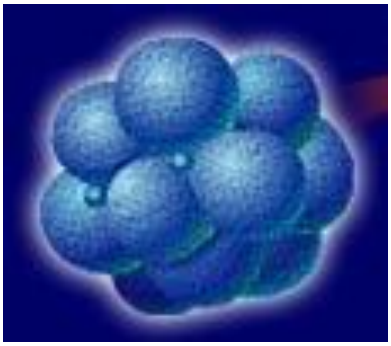
prevent the disclosure of certain information that was originally disclosed in a confidential relationship.

4 HUMAN CLONING

There are major practical and ethical objections to cloning human beings. Based on current knowledge, the efficiency of embryonic development after nuclear transfer is so low, and the chance of abnormal offspring is so high, that experimentation of this sort on human beings would be achieved with great risk to the offspring. Moreover the deliberate generation of cloned human beings could infringe upon the dignity and integrity of human individuals. From an Islamic point of view, cloning a human being would be an unethical and reprehensible act.

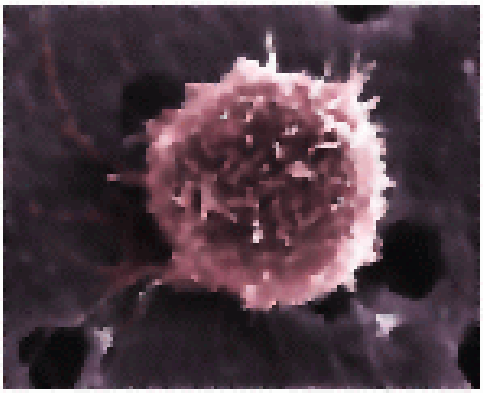
The only exception to this would be in case of infertile couples. Infertile women will be able to have children by having cloned embryos implanted in them. Also, at the same time for couples, it would eliminate the hassle of going through ordeals of mental and physically painful procedures even with the small chance of having children. The justification for therapeutic cloning needs to be very critically discussed among Muslim scholars.

All lines of Muslim thought converge on a dynamic concept of the universe. Thus Islam rejects a static view of the universe and regards it as always changing and evolving. According to the Qur'an, change is one of the greatest signs of God. It is explicitly implied in the verse; *Every day has its own glory*. *Ijtihad* gives us the opportunity of flexibility in terms of addressing this creative activity, which requires a consensus among Mulsim scholars.



"How do cells become specialized to form the different organs in the body? Human development begins when a sperm cell fuses with an egg cell. This initial fertilized egg, although it is only a single cell, is able to form an entire human being. This cell starts to divide into additional cells, which at this early stage are all able to produce a complete organism. These cells are therefore called totipotent, meaning they have total potential to produce all cell types present in a living human. As development proceeds and an embryo forms, these cells become pluripotent, meaning they have potential to become many different kinds of cells but can no longer give rise to a complete embryo. Later in development, through a process called cell differentiation, these pluripotent cells eventually give rise to the different and more specialized kinds of cells in the body and the different organs begin to form."

5 STEM CELL RESEARCH



Stem cells are cells that have not gone through the process of cell differentiation and therefore have the potential to give rise to many different kinds of specialized cells. The current sources of stem cells include embryos (which, consist of pluripotent cells) and foetal tissue. In addition, some recent evidence suggests that even adults have a small number of mulitpotent cells that can be isolated and can later differentiate into various cell types."

"One source of stem cells is from embryos that were formed from a process called in vitro fertilization (IVF). IVF involves taking eggs from the woman, fertilizing them in the laboratory with her partner's sperm and transferring the resulting embryos back to her uterus 2-6 (usually 2 or 3) days later. This is a technique that has been used by doctors for some time, where eggs are removed from a woman after stimulation of the ovaries, and the isolated eggs are then fertilized by sperm cells in the laboratory. The fertilized eggs are allowed to divide for a few cycles and are then implanted into the woman's uterus, where a normal pregnancy can then take place. The purpose of this technique is to allow couples that cannot normally have children to be able to reproduce. The technique is not that efficient however, and so doctors usually produce several embryos, hoping that at least one will be able to implant correctly in the uterus and start growing. The remaining embryos are either frozen for later use or are destroyed. Recently, scientists found that they can take these embryos at the stage before they are implanted into the uterus (within 1-5 days after fertilization), and remove pluripotent cells from them. These cells can then be grown and divided on dishes in the laboratory, and then theoretically used to produce all kinds of tissues, from liver cells to heart muscle cells to brain cells."

Research on stem cells has much value both for scientific understanding of human development and for its potential to treat human disease. Many experiments on the effectiveness and safety of new drugs or treatments could be done on cell lines derived from stem cells, instead of having to experiment on humans. In addition, stem cells might be used to produce liver cells that can then be formed into a functioning liver and

transplanted into patients with liver failure. This would solve the current problem of organ shortage and could also solve the problem of immune rejection of organs. Stem cells might be used to create cells that produce insulin, which can then be transplanted into patients with type I diabetes. In short, stem cells have the potential to cure many diseases from liver disease, to diabetes, to Alzheimer's and Parkinson's disease, to heart disease, to spinal cord injury.

To make this potential of stem cells a reality, much research needs to be done. To do this research, scientists need an ample supply of stem cells. Controversy arises here because the main source stem cells are embryos used for in vitro fertilization. By removing cells from these embryos, scientists are essentially destroying the embryo, which could have otherwise gone on to develop into a child. Perhaps if research was limited to using only these already existing embryos, it would be more acceptable than if embryos were created and destroyed specifically for the sake of acquiring stem cells."

Another source of stem cells could be the adults. Some research has shown that even adult humans have a small number of cells that are multipotent, meaning they have the potential to become several different types of specialized cells. The best example of this is cells from the bone marrow. These cells have long been known to be able to produce the different types of blood cells, from white blood cells to red blood cells to platelets involved in blood clotting. Just this past year, a group of researchers showed that some rare bone marrow cells can also be triggered to form fat, cartilage, bone, and muscle. Additional research can theoretically be done on ways to make these multipotent cells become pluripotent; in other words to somehow trigger these cells to go in reverse and become less specialized, and then allow them to differentiate into many kinds of cells. Inspite of this interesting research on adult stem cells, it appears that stem cells derived from adults will not be as versatile as stem cells from embryos. Firstly, adult stem cells will not be able to provide cells for all kinds of tissues, and in addition they are difficult to isolate because they are so rare in the body. Thus, adult stem cells do not hold as much promise as do stem cells from embryos."

In Islam, research on stem cells made possible by biotechnical intervention in the early stages of life, is regarded as an act of faith in the ultimate will of God, as long as such an intervention is undertaken with the purpose of improving human health. The teachings of Islam cover all fields of human activity: spiritual and material, individual and social, educational and cultural, economic and political, national and international. In Islam infertility and its treatment with the unforbidden is allowed and encouraged. It is essential if it involves the preservation of procreation and treatment of infertility in one partner of the married couple. There are different modalities available for the treatment of infertility for both the male and the female partners depending upon the cause of the infertility. Some of these modalities had been practiced for hundreds of years and never were of ethical concern: medical therapy, hormonal therapy, corrective and reconstructive surgery for male or female infertility, are some of these modalities. These treatments were not of major

ethical concern because they did not separate the bonding of the sexual act as a result of lawful marriage from the process of reproduction.

6 ISLAM AND GENETIC ENGINEERING

In Islam, for a man and a woman to have a child, they must be *legally married.* Thus, if a normally infertile married couple is able to procreate by in vitro fertilization or artificial insemination, there is no obstacle to doing so. The condition is that *no sperm other than her lawful husband's* must be introduced into a woman's abdomen, and no sperm and ovum belonging to an unmarried couple should be combined in in vitro fertilization.

This is an extremely important point since this clearly relates to the ultimate objective or purpose of any undertaking. Islamic teachings place a great deal of emphasis on "Neyyat" (intentions), Allah's Apostle said, *"The reward of deeds depends upon the intentions and every person will get the reward according to what he has intended.*" Production of stem cells at the commercial level is an industrial setup, with the purpose of exploiting others for one's own commercial and monetary benefit, is obviously something that Islam will not approve of. However, limited and defined supplies for curing serious diseases will be acceptable. In any such situation a careful risk/benefit analysis becomes essential including the analysis of the motive.

It is just not possible to review all the ethical issues in one article. The important thing is to outline the basic approach and guiding principles, which can be used to address the questions that come up from time to time. Table 1 summarizes the Islamic view on some of the questions that are currently being debated.

Table 1. Islam's views on a number of issues related to Biotechnology and Genetic Engineering Research

Issue	Islamic point of view
Gender selection	Gender selection is interference in the deeds of the Creator and Islam will never grant us the right to choose gender. Islam teaches us to be contented with the Will of God.
Patenting of human DNA	Research of a commercial nature is not allowed.
Human cloning	The cloning of humans, males or females, would be a disaster for the world. This would be the case whether the aim was to improve quality, select the offspring, which is smarter, stronger, braver, healthier or more beautiful, or if the aim was to increase the number in order to increase the population. This would be a cause of evil. It is *Haram* and not allowed.
Embryonic stem cell	Issue is still under debate.

Table 1. (continued)

Surrogate motherhood	Since marriage is a contract between the wife and husband during the span of their marriage, no third party should intrude into the marital functions of sex and procreation. Surrogate motherhood is not acceptable because the newborn will not be aware of his mother as well as his lineage. *"None can be their mother except those who gave birth".* (58:2)
Gene therapy	From a Muslim perspective, human gene therapy should be restricted to therapeutic indications. Somatic cell gene therapy is encouraged, as it should lead to remedy and alleviation of human suffering. However, enhancement genetic engineering or eugenic genetic engineering would involve changing God's creation, which may lead to an imbalance of the whole universe and must therefore be prohibited. Gene therapy to manipulate hereditary traits such as intelligence, stupidity, stature, beauty or ugliness may unbalance the life of man.
Infertility therapeutic cloning	In Islam infertility and its treatment is allowed and encouraged. It is essential if it involves the preservation of procreation and treatment of infertility in one partner of the married couple.
Prenatal diagnosis	If the decision not to transfer is performed to protect the mother's life or health or because of a foetal anomaly incompatible with life, it is acceptable.
Human cloning	Based on current knowledge, the efficiency of embryonic development after nuclear transfer is so low, and the chance of abnormal offspring so high, that experimentation of this sort on human beings would be of great risk to the offspring. Moreover the deliberate generation of cloned human beings could infringe upon the dignity and integrity of human individuals. We would regard cloning a human being as an unethical act.
Artificial insemination with donor sperm	In Islam artificial insemination by donor is absolutely forbidden because it is by a donor and not by the woman's husband.
Artificial insemination with husband's sperm	There is no problem in the artificial insemination by husband (AIH) as it is done between a lawfully wedded couple.
Genetic screening	For detection of genetic abnormalities and possible cure of birth defects would be acceptable.
Genetic counselling	Acceptable.

7 WHAT THE FUTURE HOLDS!

It is important to realise that knowledge is growing rapidly. The rate at which information is being generated has indeed no parallel in earlier human history. The ease with which one can access available information is just as impressive. It has been estimated that whereas the total knowledge pool will double in six to seven years, for life sciences such doubling time is estimated to be nearly three years. This new scenario provides both an extremely rich and almost unmanageable store of data but also a serious challenge for an effective and meaningful approach towards a focused discussion.

Every other day newspapers carry a new story dealing with human cloning. So where will this all end? How to control and curb such initiatives specially when individuals launch a new project. A recent news item is being reproduced here below. (Box 1).

Box 1. A press cutting

Human Cloning Pregnancy Revealed

Rome, April 5: Italy's Medical Association is considering disciplinary action against fertility specialist Severino Antinori amid reports that one of his patients is eight weeks pregnant with a clone.

"One woman among thousands of infertile couples in the (cloning) programme is eight weeks pregnant," Antinori was quoted as saying at a meeting in the United Arab Emirates.The news was reported by the online edition of Britain's New Scientist magazine. If confirmed, it would represent the first human cloning pregnancy, New Scientist wrote.

The practice has been banned in many countries, including Italy and the United States, amid experts' warnings of the high risk of still births and severe birth defects in any babies born alive. Last August, Antinori and Dr Panos Zavos, a former University of Kentucky researcher, said they planned to transfer DNA from the nuclei of living cells into human eggs to create a human embryo, which would then be implanted into a woman's uterus.

Their announcement drew worldwide criticism. Friday's report caused an immediate uproar in Italy.

Experiments on sheep, mice and other mammals have suffered high rates of embryo loss and early death.

Antinori's office in Rome declined to comment on Friday's reports but Richard Gardner, a British expert on therapeutic cloning, told New Scientist the pregnancy would be "grossly irresponsible given the current state of knowledge, even aside from any ethical issues."

The most important aspect of this discussion is promoting an open, free and frank dialogue between research scientists and religious scholars. It is really crucial that these two groups clearly understand their respective points of view.

8 CONCLUSION

It is important to emphasize that moral pronouncements and ethical values are intimately related to the beliefs, values and composition of any society. Within the Muslim world consisting of nearly twenty percent of the total human population, the ethical issues will obviously be examined in the light of the teachings of Holy Qur'an. For Muslims there are two major sources of guidance, firstly the holy book which gives a complete code of life including economic social, legal and ethical principles and secondly Hadith, which represents the sayings and the way of life of the holy prophet (PBUH).

It is somewhat unfortunate that the Muslim world has never taken any serious initiative to put in place the infrastructure needed to address these questions. A number of international symposia going as far back as 1993 when a seminar on Ethical Implications of Modern Researches in Genetics was held in Doha, Qatar, attempted to address these important issues. Recently COMSTECH has taken an initiative to establish an international committee on bioethics to address these complex ethical issues from an Islamic perspective.

All ethical, legal and social questions always need to be examined in the unique and highly specific social scenario of a society. The onus for this kind of in-depth analysis is upon our academicians, religious scholars and scientists. It is also important to appreciate that the required formal infrastructure for such dialogue and discussion is presently lacking in the Muslim world.

The questions raised for Europe and North America such as discrimination by insurance companies and employers based on genetic testing though are important issues for them may not necessarily be the ones that deserve highest priority for us.

The socio-economic situation demands that in view of these breathtaking scientific developments the situation is examined in our own societies. Addressing these question requires a continuing dialogue, debate and discussion between academicians, religious scholars and researchers within Islamic countries.

REFERENCES

1. Nasim, A. (1993), Ethical Implications of Modern Researches in Genetics. Proceedings of Seminar organized by the Islamic Educational, Scientific and Cultural Organization and the World Islamic Call Society with the cooperation of the Faculty of Science, University of Qatar. Doha, Qatar.
2. Nasim, A. (1995), Genetic Manipulations, Biotechnology and Ethical Issues: New Challenges for the Muslim Scholars. International Conference on Science in Islamic Polity in the Twenty-first Century (March 26-30) pp. 98-110
3. Nasim, A. (1997), Ethical Issues of the Human Genome Project: An Islamic Perspective. Bioethics in Asia: Proceedings of the UNESCO Asian Bioethics Conference, 3-8 Nov. pp. 209-214.
4. Nasim, A. (2002), Ethical Issues: An Islamic Perspective. Presented in the workshop on Asian Genomics: Cultural Values and Bioethical Practices, at Leiden, The Netherlands, 28-29 Mar.
5. Nasim, A. (1992), Genetic Engineering State-of-the-Art. A monograph prepared for ISESCO.
6. Serour, G.I. and Omran, A. (1992), Genetics, Ethics and Human Values. Genome Mapping, Genetic Screening and Gene Therapy: Ethical Issues. (XXVIth CIOMS Conference), Geneva, (The Tokyo and Inuyama Conference).
7. Serour. G.I. (1992), Proceeding of The First International Conference on Bioethics in Human Reproduction Research in the Muslim World, 10-13 Dec. 1991, IICPSR, Cairo, Egypt, pp. 37-39.
8. Serour, G.I. (1997), Islamic Perspectives on Genetic Technology and Information Use. (Ed) J. Kegley "Genetic Knowledge : Human Values and Responsibility", Lexington, Kentucky, USA.
9. Serour, G.I. (1998), Human Cloning, its Applications and Ethical guidelines. Paper presented at the Conference on "Cloning Dilemma" held in Dubai, UAE, April 4-5.
10. Serour, G.I. (2000), Ethical Implications of Human Embryo Research. Publication of the Islamic Educational, Scientific and Cultural Organization – ISESCO.
11. Ronald M. Kline, (2001) Whose blood is it, anyway? Scientific American. Vol. 284 Number 4.
12. Muzammil Siddiqi, An Islamic Perspective on Stem Cells Research. www.islamicity.com.
13. Hamid K. Ahmed. Human Genome Social and Ethical Dimensions.

Convergence and Divergence between Religious Thought and Rational/Scientific Thought in Islam: A Preliminary Survey

MAZHAR M QURASHI*
Pakistan Association of History & Philosophy of Science
Islamabad, Pakistan

1 ABSTRACT

The proper resolution of ethical science-related issues, e.g., in the biomedical fields, requires a corresponding understanding of the inter-relation between Science and Islam. In most revealed religions, there appears to have been initial religious support for rational thought and science, as an exploration of the creator's purpose, frequently followed by later antagonism. The purpose of the present paper is to present a brief synoptic picture of the relationship between religious thought and scientific or rational thought in Islamic culture, which incorporated knowledge of medicine, physical sciences and philosophy from various cultures. Starting with a chronological picture of a selection of the outstanding scholarly and scientific personalities of the first three centuries Hijri, the analysis shows the ups and downs represented by the Mu'tazila, a group of rationalists and then Al Ghazali, the most outstanding of the 'Ash'arites, who recognized medicine, but suspected the physical sciences and endorsed the sufi lifestyle.

The assessment covering the 14 centuries has been made on a linear rating of 100% for complete divergence or opposition and 0% for complete convergence. The assessment is based on the comments given in four relevant papers amongst those presented at the "International Conference on Science in Islamic Polity in the 21st Century," held at Islamabad, in 1995.

In view of the present importance of the interaction between science and Islamic thought, in the biological as well as physical sciences, it is desirable to study this in further detail and attempt to develop a working strategy for proper interpretation of ayaat and ahadith dealing with scientific and technological topics. Further studies are being planned.

* Professor of Physics, and Fellow of the Islamic Academy of Sciences.

2 INTRODUCTION

From the earliest times, man has been impressed and intrigued by the happenings around him and has always wanted to know how and why these occur. Mythology was developed mostly to cover this immediate lack of knowledge, and so religious myths were frequently diametrically opposed to the later scientific explanation. These myths took a long time to die. The prevailed religious have all tried successively to "get rid of false gods" and fake notions and so have often been associated with a basic rationalism.

The Qur'an (the last of the revealed Books), in numerous verses, invites us to see, to observe to ponder, and think over the wonders of nature created by Allah (subhanahu wa ta'ala) through his divine powers and thus calls us to recognize Him as the one and only Creator and Nourisher. On the one hand, He tells us that the sun and the moon follow precise paths according to the laws laid down by Him, e.g., Sura Yasin, 38-40, and on the other hand, He tells us again and again that He makes and sends the clouds to give us rain (Al Nur, 49; Al Furqan, 48), which nourishes the dead earth (Al 'A'raaf, 57, Al Furqan, 49) and that He makes the crop to grow out of the seed (Al 'A'raaf 57, 58; Al 'An'am, 95).

Not only this, but Ibrahim (peace be upon him) tells us that Allah nourishes him when he needs it and He heals him when he falls ill (Al Shu'araa, 78-80) – so that, biological phenomena are seen as more *directly* under Allah's control: a clear reflection of the present large uncertainties of our knowledge of biological processes, whether of growth or sustenance or of healing. Even though the healing arts may be considered the oldest, yet the uncertainties are still probably the highest. Moreover, today's ethical issues in biomedical problems and biotechnology require the exercise of considerable depth of Islamic knowledge[1].

For any serious study concerning the inter-relation between the sciences and Islamic religious thought, it is essential to begin with the course of development of all knowledge, whether religious, sociological or philosophical/scientific, in the early phase of Islamic history, when these individual facets of knowledge were actually emerging. Only then is it possible to form a clear picture of the nature and extent of the interactions between these three facets.

The energy and enterprising endeavours of the early Muslims have been seen to be the result of their endeavours to live their lines according to the dictates of the Qur'an and the Sunnah of Muhammad (PBUH) and bring others into this fold. "Let us be clear that science and technology are the offsprings not of cut and dry propositions which can be spelled from the Qur'an, the Holy Book of Islam, but that book in itself engenders a kind of ethos in the soul-life of those who make a deep study of it[1]. And it is this that that enables them to look upon nature and nature's ways in a perspective where the human mind is apt to discover the secret of its operation and learn to encase it in the form of a mathematical formula, which could then be utilized for applying to a variety of instances for controlling the forces of nature and exploiting them in the service of man. That is why perceptive students of the history of development of

scientific thought have noticed that the Muslim science arose as a result of the Qur'anic impulse in history."

3 THREE PARALLEL ACTIVITIES

Early Muslims took up three more or less parallel, but distinct activities during the first two centuries of Islam. These are:

(1) Giving to the nations they came in contact with the message of Islam, i.e., obedience to Allah;
(2) Acquiring knowledge from these nations and developing it; and
(3) Codifying the details of the message of Islam.

The third activity may have begun with Imam Ja'far Sadiq around 100 A.H., while records of the second began early in the 2nd century Hijri, with names like Al Fazari, Yaqub ibn Tariq, and a rough chart (with time-scale) of some outstanding figures in these two activities is shown in Figure 1 for the 2nd, 3rd and 4th centuries Hijri.

It is at once apparent that the activity of gathering and developing knowledge gained momentum gradually and seems to have lagged behind and followed that of codification of Islam and its rules and regulations, by something like a century. There has of course been some sort of interaction between these two, as the Mosques were the original source of both these. The sciences were generally classified by various Muslim scholars, usually following Farabi's (d. 950) scheme, in which physics was placed next to metaphysics in the category of philosophical sciences, while arithmetic, geometry, optics, music, etc., were considered only documentary sciences. So, the natural sciences were a part of philosophy – indeed the sciences were called natural philosophy in the middle-ages in Europe and even until relatively recent times-witness the usual research degree of PhD for scientific research.

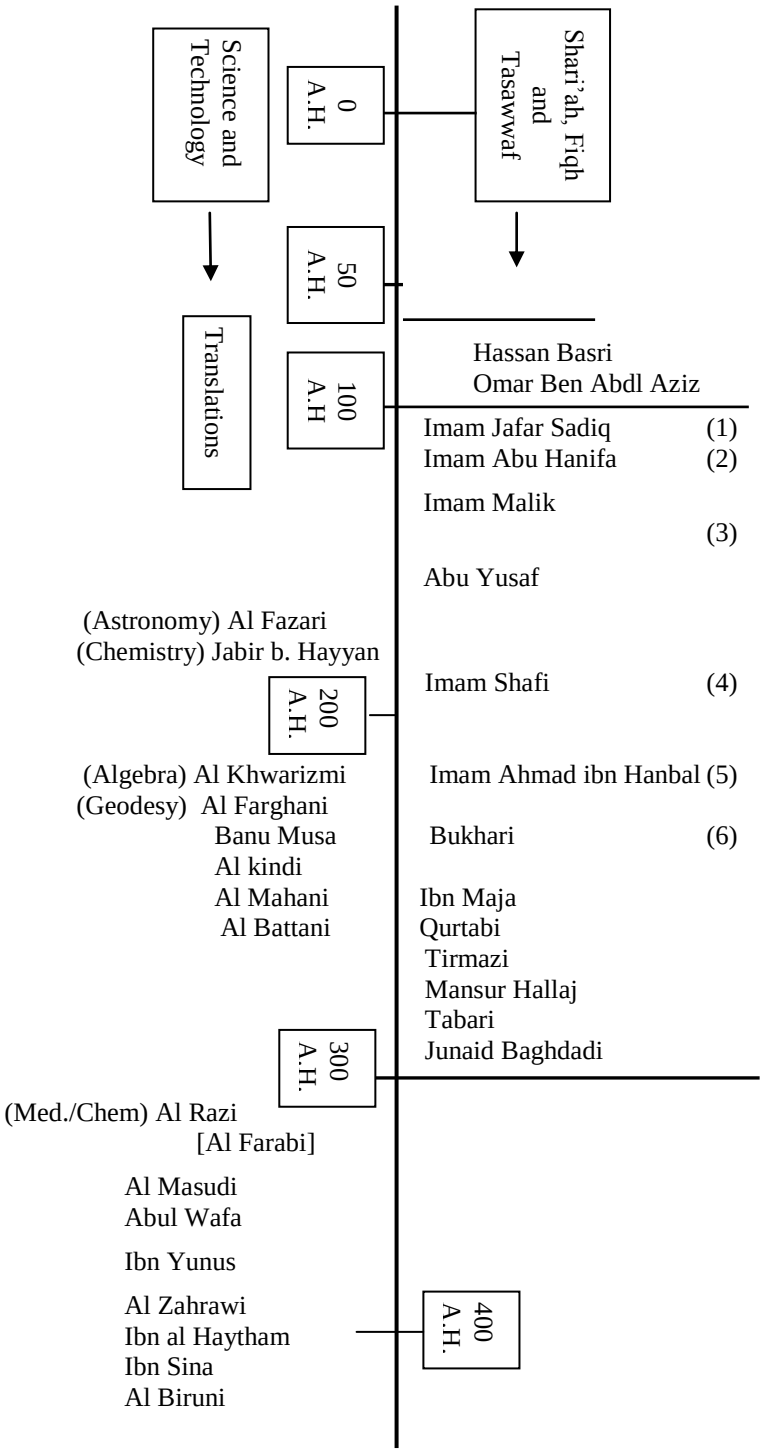


Figure 1. Outline chronology.

Medicine (or Tibb) was classed by itself, as an important and essential accomplishment, and has for centuries been associated, as "Tibb Unani" (Ionian Medicine), with Islamic religious scholars, many of whom even today practice it. This relationship is useful in view of the ethical problems being thrown up by modern medicine and biotechnology.

4 RELATION BETWEEN THE TWO STREAMS

4.1 Background

Islamic religious thought has, of course, concerned itself right from the start with medicine and biology, as well as the physical sciences. In view of the above-noted interaction between philosophy and Muslim orthodoxy, even the philosophical sciences were frequently not accepted as legitimate fields of study. In most revealed religions, there was initial religious support for rational thought and science as an exploration of the Creator's purpose, but this was frequently followed by antagonism later, as in the West. In the light of the modern ascendance of science and the scientific culture, it is thus desirable to study, at some length, the history of the relationship between religious thought and scientific/rational thought in the Islamic context. Islamic religious thought has, of course, concerned itself right from the start with medicine and biology, as well as the physical sciences.

The purpose of this short paper is to provide a base for further studies and analyses that may shed light on the best way to mould these relationships for the future development of the Islamic way of life, in order to provide abiding solutions and peace for all mankind. Accordingly, we begin with analysis of the synoptic chronological picture of some of the outstanding scholarly and scientific personalities of the first three centuries Hijri, presented in Figure 1.

4.2 The *Mu'tazila*

Almost right at the beginning, we encounter the confrontation between the Mu'tazila and some Muslim theologians. "When the Greek sciences were introduced (mainly) by the Abbasi Caliphs in the 9th century (2nd/3rd century Hijri), the Mu'tazila, a group of rationalist Muslim thinkers, developed a theology (Ilm-al Kalam) to defend Islam against possible conflict between Muslim faith and the pagan Greek philosophical ideas. The Abbasi Caliphs, Mamun and Mu'tasim (favoured this and) tried to impose the Mu'tazili Kalam by force. For various reasons, this attempt failed. Consequently antagonism against philosophical science came to be solidified in Muslim society. The later Abbasi Caliphs tried to correct the situation by appeasing the theologians, e.g., Caliph Mu'tadid Billah proscribed writing and selling of books on philosophy in 279 H. Afterward, philosophy was condemned continually by Muslim scholars. For instance, Ghazali (d. 1111), Ibn al Salah (d. 1243) and Soyuti (d. 1505) wrote books refuting philosophical sciences[3]. Al Ghazali, although a

pioneering intellectual, later adopted the path of Sufism*. In fact, as noted by M.H. Gilani[4], the founders of all four orders of Sufis appear to have been directly influenced by Al Ghazali's sufistic experiences and writings.

Nearly all of four present-day orders (silsila) of Tasawwuf ultimately link their reasoning with what Al Ghazali has written; so there is a tendency to deny observational sciences.

5 ESTIMATION OF DIVERGENCE AND CONVERGENCE BETWEEN RELIGIOUS THOUGHT AND RATIONALISM OVER THE CENTURIES

There are people, in the Islamic West, who wrote vigorously in defence of philosophy and science, notably Ibn Rushd (d. 1198) and Ibn al Khatib (d. 1374). Thus, we find an interesting situation wherein the streams of rationalism and so-called religious orthodoxy were both running strongly and in competition, even in the 5th to the 8th centuries Hijri. Accordingly, a preliminary effort has been made to assess the degree of convergence and divergence between religious thought and rational/scientific thought in various periods, by looking at the outstanding personalities of the period. The assessment is based on a linear rating-of 100% for complete divergence or opposition and 0% for complete convergence. The results, using the comments given in four relevant papers[3-7] among those presented at the 1995 "International Conference on Science in Islamic Polity in the Twenty First Century," are collected together here as Table 1. (Use has also been made of a book on Shah Waliullah[8].) Even a quick look at these enables one to make rough estimates of the degree of convergence or divergence between religious and rational thought on a linear rating of 0 to 100% for complete divergence or opposition. There values are plotted as hollow and solid circles in the graph of Figure 2 from 700 to 1900 i.e. 1st century Hijri to 13 century Hijri; the hollow circles are for less than 50% divergence. The horizontal scale in Figure 2 shows centuries (A.D.), while the vertical extends from 0% (no divergence) up to 100% (complete divergence). The circled for less than 50% divergence are shown as hollow.

A smooth graph, as shown by the thick line in Figure 2, can be drawn to pass more or less evenly between the plotted points (solid circles) with an average deviation of ± 10%. The graph apparently swings between a minimum of about 20% divergence and a maximum of about 80% ivergence. Three such complete cycles (or swing-swangs) can be seen between 800 A.D. and 2,000 A.D., i.e., 200 A.H. to 1400 A.H. We discuss these results briefly below.

---

* It must, however, be noted that Al Ghazali in his "Al Munqidh min-al-Dalal (pp.21-22) had clearly stated that a problem is created by the ignorant friend of Islam who thinks that religion needs to be helped by denial of all sciences and their relevant knowledge, … so that the believer in these sciences, on the basis of definite observations, concludes that Islam is based on ignorance and denial of (observed) facts.

Table 1. Significant extracts from 4 papers presented at the 1995 Islamabad Conference on "Science in Islamic Polity in the 21st Century"

Moin Farooqi (India)	**Khalid Masud** [Islamic Studies, Pakistan]	**Qaiser Mushtaq** [Scientist, Pakistan]	**Ahmed Y. Ozmere** (Turkey)
– On pp. 380-1, he. quotes from S. Kocabas (1993): "In fact, a weary drastic change about the concept of knowledge (IBm) had taken place in the history of Islamic Thought viz" Ibn al din and Ibn ad dunya" …. No indication of any such categorization in the Qur'an or the Sihah Sitta …. Was introduced in subsequent centuries and planted deep in Muslim minds in the 11th century. – Its chief exponent was Al Ghazali, says M. Farooqui----	– Mu'tazilites Books on Philosophy prescribed (892) – 'Ash'arites – Al Ghazali (d. 1111 H.)	– The historical conflict between Mu'tazilites and 'Ash'arites was finally won by the 'Ash'arites…. This led to the development of Taqlid ….	
while – G. Sarton (1975) says: "Could it not be simply that Islam ceased to develop because it had reached the limit of its growth? The phenomenal growth of Islam was perhaps a case of intellectual precocity, rather than one of true intellectual greatness". … "Instead of balancing external circumstances, it is more rational to believe that it's the genius itself which failed"	– Ibn Rushd (d. 1198) imprisoned Ibn al-Salah (d.1243) – Ibn Taymiyya (d. 1328) – Ibn al Khab (d.137) condemned by Qadi as being a heretic – A quote from al Shatibi (d. 1385) – Suyuti (d. 1505) – Katib Celbi (d. 1656) – Rasala Hamidiyya demonstrated the conflict between science & religion. – **Note 1**: Rational Sciences were a part of Dars-i-Nizami in India in 18th century A.D. – **Note 2**: Sir Syed Ahmd Khan's movement for modernization.	– The principal cause of the intellectual stagnation … was the rise of rulers and governments who were pre-occupied with pursuits other that building schools of learning and science.	– In 1470, Mehmet Fateh inaugurated a university around the Mosque of his name. – Murad III approved Taqi-al Din's project to build an Observatory on the heights of Galata. – Fatwa by Saleh Zaki (Shyakhul Islam); Observatory destroyed in 1580. – Some Technological inventions, e.g. flight around 1650 and submarine in 1719. – Another reason for the decline of scientific activity … is the so-called "closure of the doors of Ijtihad".

6 DISCUSSION

A study of convergence and divergence, in Figure 2, between Islamic thought and rationalism brings forth three conclusions, which can be summarized briefly as follows:

(1) The situation has been far from static: there have been possible 3 cycles between 800 and 2000 A.D., i.e., one every 400 years (from A.H. to 1400 A.H.), swinging between 80% convergence and 80% divergence between the two. The effects of the 2nd peak of divergence seem to have lasted all through the Ottoman Empire.
(2) The work of Da'wah, on which Al Ghazali placed great emphasis, seems to be in between these two activities, being a mix of Shari'ah and Tazqiah of the inner self (or sufism/mysticism).
(3) Finally, we may note that, from time-to-time, various Muslim scholars have lamented the fact that Muslim scholars have given up the study of science in favour of Fiqh, Shari'ah, etc., to become civil servants, Qadis, lawyers, officials, etc.

Coming to recent times, we find that Sir Syed Ahmad Khan, the ardent 19th century Muslim protagonist of science and education was on terms of friendship with Maulana Qasim Nanotavi, the founder of Darul-Uloom Deoband, who never subscribed to the ulama's labelling of Sir Syed Ahmad as a Kafir. Mere recently, Maurice Bucaille[9], in his book "The Bible, the Qur'an and Science", has repeatedly pointed out the correspondence of the Qur'anic ayaat with scientific phenomena and has stated that "the Qur'an did *not* contain a single statement that was assailable from a modern scientific point of view."

7 CONCLUSION

It thus appears that there is a present need for delineating a consistent methodology for the proper interpretation of ayaat containing references to science and technology, so as to avoid at least the most obvious inconsistencies on either side see, e.g., Qurashi[10] on coordination of Qur'an, science and Hadith. This is especially important today, as there are signs of a long period of convergence between the two streams of thought. An interacting group of ulama and Muslim scientists needs to be developed, as soon as possible.

ACKNOWLEDGEMENT

The author is grateful to Prof. Tufail Hashmi for his continued interest and to Dr. M. Mian Siddiqi for discussion of several points in this paper.

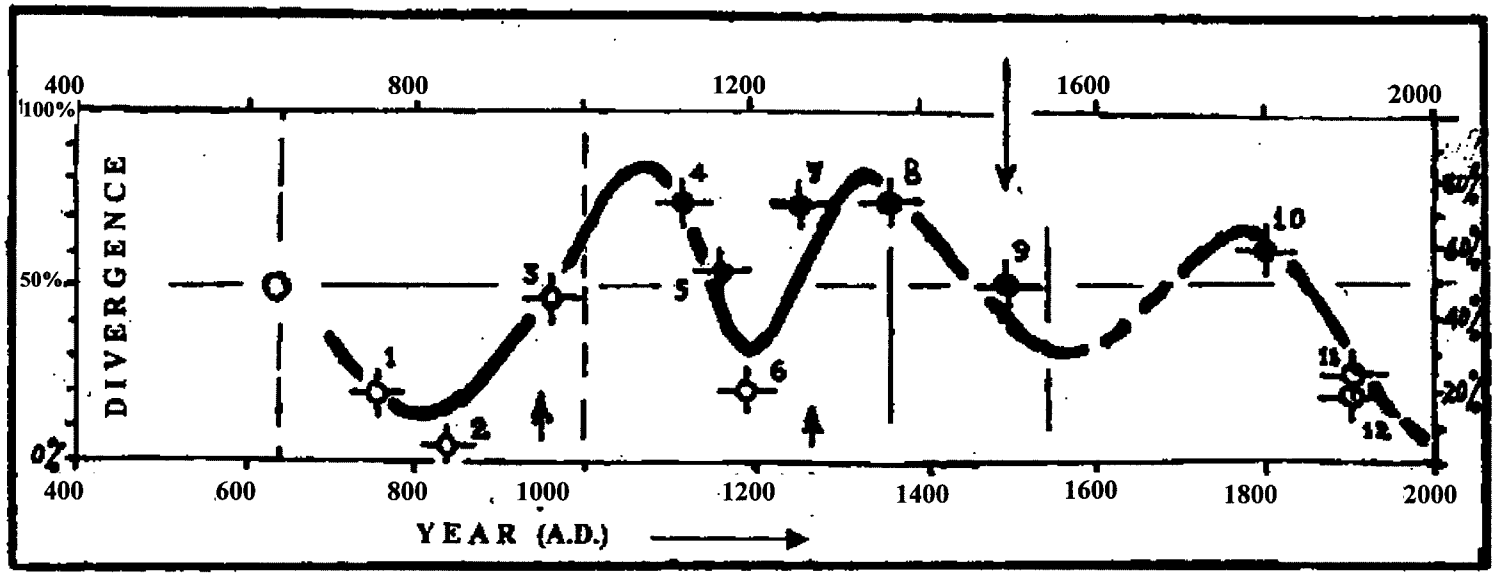


Figure 2. Plot, showing the estimated % of Divergence between Islamic Thought and Rational/Scientific Thought over fourteen centuries, using estimates for a dozen well-known Muslim personalities indicated as:1, Abu Hanifa; 2, the Mutazila; 3, Al Farabi; 4, Al Ghazali; 5, A. Q. Jilani; 6, Ibn Rushd; 7, Ibn al-Salah; 8, Ibn Taymiya; 9 Suyuti; 10, Shah Abdul Aziz; 11, M. Abduh; 12, Syed Ahmed Khan.

The smooth graph through the circles shows three clear cycles; the two short arrows correspond to the two main peaks of Muslim scientific activity, while the long arrow, pointing down, marks the end of Muslim rule in Spain and the discovery of America.

REFERENCES

1. A.K. Brohi, Preface to Quranic Ayaat containing References to Science and Technology (p.i.), by M. M. Qurashi, S.M. Bhutta & S. M. Jafar, S.S.W.C.T. and P.S.F., Islamabad, 1986.
2. (a) S.F. Mahmood, "A Short History of Islam", Oxford University Press, 1960, pp. 90-170.
   (b) Philip K. Hitti, "History of the Arabs," McMillan & Co. Ltd., 1951, pp. 240-278, 382-440, 745-757.
3. M. Khalid Masud, "Reasons for the Decline of Scientific Activity and Creativity in the Muslim World," (in International Conference on Science in Islamic Polity in the 21st Century, held at Islamabad, March 26-30, 1995) printed in Islamic Thought and Scientific Creativity, 6(3) April 1995, pp. 71-78.
4. Manazir H. Gilani, "Maqalaat-e-Ihsan," Said Brothers, Karachi (1991).
5. M. Moin Farooqui, "Science in Islamic Polity, Vision 2000," presented in International Conference on Science in Islamic Polity in the 21st Century, held at Islamabad, March 26-30, 1995, Conference papers, pp. 178-181.
6. Qaiser Mushtaq, "Decline of Scientific Activity in the Muslim World: Causes and Remedies" (presented in International Conference on Science in Islamic Polity in the 21st Century" held at Islamabad, March 26-30, (1995) and printed in Islamic Thought and Scientific Creativity, 6(2) June, 1995, pp. 69-79.
7. Ahmed Y. Ozmere, "Reasons for the Decline of Scientific Activity in the Ottoman Empire," in International Conference on Science in Islamic Polity in the 21st Century, held at Islamabad, March 26-30, 1995, Conference Papers, pp. 182-185.
8. A.D. Muztar, "Shah Waliullah: a Saint Scholar of Muslim India," National Commission on Historical and Cultural Research, Islamabad, 1979, pp. 104-111, 117-122.
9. Maurice Bucaille, "The Bible, the Quran and Science," translated from the French, D. Pannell and the Author, reprinted in 1979 by Anjum Ittahad-ul-Muslamin, Karachi, pp. viii and 152-154, etc.
10. M.M. Qurashi, Pak. J. Hist. & Phil. Sci., 2(1), 1996, 19-28.

Biotechnology and Health

MOSTEFA KHIATI*
Institute of Medicine
Hopital S. Zmerili
Algiers, Algeria

1 INTRODUCTION

Decoding the human genome is creating as much interest and passion as nuclear fission and conquering outer space did, if not more. Modern biotechnology, which is the direct application of a better understanding and a greater control of genetics is arousing a considerable degree of interest in the general public. This interest is linked with both the tremendous possibilities offered by these new techniques, particularly in their application in the areas of human health and nutrition, and also with the fears of risks involved in the use of these new techniques. In addition to these questions, there are also ethical debates, which are increasing in number, due to the constant new developments in the field of biotechnology. Finally, the fear also exists that these technical advances, which, at present, concern only developed countries, will, in fact, widen the gap between the industrialized countries and developing ones.

2 BIOTECHNOLOGY: A RANGE OF DEFINITIONS

Biotechnology is a very loose term that encompasses various molecular engineering techniques:

- It consists, firstly, of genetic engineering;
- It also applies to the use of micro-organisms in the production of food (yoghurts, cheese etc.) or pharmaceutical products (antibiotics);
- It includes the cultivation of plants (transgenic plants or genetically modified organisms, GMOs) and raising animals; and
- It also extends to techniques that use microorganisms and plants to improve production processes and clean up chemical waste.

* Professor of Medicine; and Fellow of the Islamic Academy of Sciences.

3 APPLICATIONS TO HUMAN HEALTH

3.1 General

Applications of biotechnology are not new. In the early 1980's, human insulin and a human growth hormone were obtained from genetically modified bacteria and then commercialised. What is relatively new, however, is the growing body of knowledge concerning genes and their widening application to ever increasing and varied fields: treatment and prevention of disease, nutrition etc.

3.2 Health care

In health care, while human cell cultures are widely used today in diagnosis or treatment (pregnancy tests, AIDS, leukaemia), the use of genes and DNA is now beginning to expand in its turn.

3.3 Cell banks and gene therapy

Cell banks exist in a number of developed countries. They receive various types of cells from human organs or different animals, examine their properties and, where necessary, decontaminate them. Some of these stem cells are isolated and then cloned to constitute a reference stock. These are evaluated at tens of thousands of specimens. This reserve, part of which remains untouched in order to "preserve a copy identical to the original," is placed in a nourishing environment in order to be able to reproduce. The cell bank will then conserve and distribute them to provide a supply for research workers or laboratories that wish to obtain them in return for payment for this service.

Great hope for the treatment of disease has been placed in a variety of stem cells, called embryonic stem cells, or ES cells, and there is already talk of therapeutic cloning or cell therapy. The cells used come from human embryos. They are removed at a very early stage of development, (that of the blastocyst), in the internal mass. At this stage they are quite undifferentiated and have the potential to become any kind of cell and go on multiplying indefinitely. Research at present underway is attempting to make it possible to repair damaged organs (brain, heart, or muscle) by regenerating them through cell transplants. Rejection is to be avoided by implanting the nucleus of a cell from the diseased tissue into the transplanted oocyte. These tests were interrupted by the international memorandum on bio-ethics in 1994, but have again been authorized in the United States as of this year, because of the tremendous therapeutic advances that are at stake.

In March 1999, children with severe combined immunodeficiency disease linked with an anomaly of the X chromosome, which takes the form of an absence of T and natural killer lymphocytes, were cured by gene therapy. They were given the stem cells (from their bone marrow) by intravenous injection. These cells had previously been purified and then placed in optimum conditions for reproduction. They were then infected with a retrovirus containing the Yc

gene, missing from their X chromosome.

The Bordatella pertussis bacterium which causes whooping cough is being brought under control by biotechnology. The coding gene for the pertussis toxin (PTX), which is responsible for most of the toxic effects, is removed from the germ. The bacterium thus obtained is less harmful and, in particular, capable of protecting mice from whooping cough and schistosomiasis by inserting the vaccination antigen into the genome of the coding gene bacterium. Scientists are soon hoping to obtain a recombining vaccine, which will be given via the nose. In the laboratory, tests on infected mice show that they produce a specific immune response by creating antibodies and lowering the number of parasites and eggs.

Genetically modified viruses or recombined viruses, in which the harmful element has been replaced by a therapeutic gene, are also undergoing tests, particularly in the treatment of AIDS. The virus is used as a vector to introduce fragments of DNA into the human cell in order to replace the deficient DNA.

3.4 Tissue engineering

Cell cultures, in particular, of keratinocytes or fibroblasts, form the basis of a new technique called tissue engineering, which consists of the preparation of living tissue and implantable organs. Tissue engineering makes it possible to carry out transplants of their own skin on large areas of the body in the case of sufferers from burns. Cell cultures of their skin or keratinocytes enable the surface removed to be multiplied a hundred fold and thus save those suffering from extensive burns to the body (victims of third-degree burns).

The same tissue engineering technique is used for repairing lesions to the cartilage, particularly when it has been worn away, as in the case of osteoarthritis, a disease caused by ageing of the cartilage and one which medication has been unable to prevent, cure or halt. The technique consists of removing cells from an area of healthy cartilage in the patient. These cells are reproduced in vitro and then re-injected into the unhealthy cartilage. The injected cells are held in place in the correct position by a "repair patch" made of a fragment of periosteum. Scientists are directing their research towards the culture of cells in biocompatible and biodegradable materials, for example, in the form of a gel, so that they may be more easily re-injected by means of an arthroscopy.

The technique of tissue engineering could revolutionize present treatments used in orthopaedics and traumatology by making it easier to use bone transplants obtained according to the same principles. The bio-materials, or bionic bones, thus obtained have the advantage of no longer creating rejection problems. They are either implanted by means of an operation or injected by micro-surgery.

3.5 Genetic engineering and preventive medicine

In the area of prevention, a molecule from genetically modified plants has been used as a vaccine against dental caries. Genetically modified tobacco plants can produce a vaccine against dental caries. A single adult plant is sufficient to provide the equivalent of one dose of the vaccine. The teeth are coated with the liquid vaccine and several applications will provide protection for at least four months. The vaccine contains antibodies that coat the germ responsible for the caries (streptococcus mutans) and prevent it from attaching itself to the tooth. This enables the tooth to be more easily colonized by the saprophyte bacteria, which attach to the teeth. The microbes neutralized by the antibodies can then be removed by brushing the teeth.

In similar fashion, experiments in which the gene which produces the Escherichia coli (the bacterium which causes gastro-enteritis) is introduced into potatoes have shown that volunteers who have eaten the vegetable are able to produce antibodies, which react against the E. coli germ. This research could lead to the development of a vaccine that would prevent diarrhoea caused by the E. coli bacteria.

Genetic engineering can also be used to eliminate allergenic proteins found in plants, vegetables and fruit, when these proteins do not play an essential role in the plant. In fact, in most cases, allergenic proteins are proteins, which serve to stock food for the germinating seeds. For example, researchers at Nagoya University in Japan managed to reduce the amount of the main allergenic protein in rice by 70 - 80% by incorporating a gene called antisense, which blocked the production of this protein in the plant. Studies are underway to set up a database concerning the principal allergenic proteins. They will be followed by the identification and isolation of the genetic code specific to each of these proteins.

3.6 Biotechnology and food production

Viruses which contaminate plants constitute a major problem for agriculture in countries all over the world, since they are responsible for the destruction of what is sometimes a huge percentage of crops. Genetic engineering has made it possible to make these crops resistant to insects and herbicides.

Biotechnology can also improve the quality of farm-produce, for instance, by increasing the number of vitamins present in crops, or the amount of vitamin C and H in certain fruits and vegetables. More spectacularly, by selecting varieties of potatoes, bananas or tomatoes which are richer in vitamin A, it could be possible to combat the vitamin A deficiency which is the cause of blindness in certain regions of the globe. Through biotechnology, decisive progress will be made in the feeding of premature babies. The preparations available at present contain a palmitic acid (the fatty acid present in greatest quantities in breast milk), which is very difficult for babies to digest and which also causes a drop in calcium levels. In the new preparation for premature babies, alpha lipase, an enzyme produced by genetically modified Aspergillus,

is used to produce a natural form of palmitic acid.

Biotechnology can also contribute to creating a better nutritional balance in people by lowering their cholesterol level through their eating certain fermented dairy products and particular kinds of margarine and butter substitutes.

4 ETHICAL PROBLEMS

The application of biotechnology to health and the farm-produce industry today raises a whole series of ethical problems that are difficult to solve. Following are some examples:

- Is it acceptable to modify human cells genetically?
- Is manipulating the fertilized oocyte, that is to say, the first cell in what will become a human being any less legal?
- How far will biotechnology go in this field?
- With regard to nutrition, can one accept the sale of certain wines which are rich in flavoids obtained by genetic engineering, on the grounds that they can help avoid coronary diseases?

5 MISUNDERSTANDINGS OR REAL RISKS

The interest in biotechnology shown by the general public is considerable, as current events concerning GMOs show. It is generally admitted that there is no zero risk as far as biotechnology is concerned. Some useful insects suffer because of GMOs. However, it must be noted that pesticides in use destroy 60% of useful insects today, that is, three times more than by biotechnology. Should this, nevertheless, be considered an acceptable risk? The advantages of biotechnology seem to allow one to be optimistic: it makes food production safe by rigorous testing at each stage and also enables equally rigorous legislation for its marketing; it plays a positive role in the environment and simplifies the management of water resources by reducing the use of pesticides. All in all, it is a technique that is both economical and ecological. Is all the media hype surrounding biotechnology merely due to a misunderstanding? Is it due to a lack of information about the nature and function of genes? There is certainly a great deal of confusion, and adequate communication is needed in order to clear up ambiguities and clarify the data available.

6 THE PROBLEM OF DEVELOPING COUNTRIES

Most of the research carried out throughout the world is concentrated in the industrialized countries. Developing countries seem to be following this technological progress from afar. In fact, none of the new crops that have been

created has benefited the developing countries, which are in such need of them. There are many examples of this. For instance, rice is deficient in lysine, an essential amino-acid. This deficiency is the cause of blindness in children in countries such as China, where rice is the principal food crop. The selection and production of rice that is richer in lysine would make it possible to reduce this type of blindness. The Feathery Mottle Virus contaminates the sweet potato, which is one of Africa’s main crops. Its production there is 57% lower than in China. The selection of a potato that is more resistant to FMV would increase its production in many of Africa's poorest countries.

7 CONCLUSION

Biotechnology offers enormous, even unfathomed possibilities for health care and nutrition. Its extension to developing countries will constitute a strong factor in making lasting progress. In order to achieve this, it is essential to lift the ethical restrictions in place today as well as those that could arise in the future. Better communication, with wider information of the public, should make it easier for new biotechnological products to be accepted.

It is up to the leaders of our countries to speed up, broaden and encourage the introduction of biotechnologies that are already formidable arms in winning the battle for development.

REFERENCES

1. Food Safety Evaluation, Organisation pour la Coopération et le Développement Economique (OCDE), Paris, 1996.
2. The Safety Assessment of Novel Foods, Food and Chemical Toxicology, 34 (10) 931-940, 1996.
3. Biotechnology and Food Safety, FAO Food and Nutrition, Paper n°61, FAO, Rome, 1996.
4. Allergenicity of foods produced by genetic modification, Food Science and Nutrition, 36 Supplément,1996.
5. Centre for plant breeding and agricultural research (CRPO-DLO), Droevendaalsesteeg 1, P.O. Box 16, NL-6700 AA Wageningen, Pays-Bas. (http://www.bib.wau.nl/crpo/" www.bib.wau.nl/crpo/).
6. Johnston D.J., Ehlermann-Cache N., la biotechnologie: quelques questions essentielles, OCDE Observateur, 6 Octobre 1999.

Conference Committees

Advisory Science Committee

Prof. Mehmet Ergin FIAS
Prof. Adnan Badran FIAS
Prof. Abdelhafid Lahlaidi FIAS
Prof. Ahmed Marrakchi FIAS
Prof. Anwar Nasim FIAS
Prof. S Q Mehdi FIAS
Prof. A A Azad FIAS
Eng. Moneef R Zou'bi DG-IAS

IAS Organising Committee

Eng. Moneef R Zou'bi DG-IAS.
Ms Lina Jalal, IAS.
Ms Taghreed Saqer, IAS.

Local Committee

Prof. A Jirari, Prof. Abdelhafid Lahlaidi FIAS (Chairman), Prof. Abddul-Latif Berbich, Prof. Hamza Kettani FIAS, Prof. M. Bensouda, Prof. M. Jiddane, Prof. Naima Alami-Ouahabi, Prof. M. Lahrichi, Prof. S. El Ayoubi.

Chairpersons of Conference Sessions

Session 1:	Prof. Abdul Salam Majali FIAS	Jordan.
Session 2:	Prof. Ishfaq Ahmad FIAS	Pakistan.
Session 3:	Prof. Sami Al-Mudhaffar FIAS	Iraq.
Session 4:	Prof. Ahmet Hikmet Ucisik FIAS	Turkey.
Session 5:	Prof. Abdelhafidh Lahlaidi FIAS	Morocco.
Session 6:	Prof. Mohamed B E Fayez FIAS	Egypt.
Session 7:	Prof. A M Sow FIAS	Senegal.
Session 8:	Prof. Mustafa Khiati FIAS	Algeria.
Session 9:	Prof. Hamza Kettani	Morocco.

List of Conference Participants

1. Mr Mohammed Adad, Head of Informatics Division, Ministry of Social Economy, Rabat, Morocco.

2. Dr Nazeer Ahmad, Director General, S&TC Division, Dr A Q Khan Laboratories, Kahuta, PO Box 502, Rawalpindi, Pakistan.

3. Prof. Ishfaq Ahmed (FIAS), Advisor to the President/Chief Executive of Pakistan (Federal Minister), Government of Pakistan, Chief Executive Secretariat, Islamabad, Pakistan.

4. Prof. Shamsher Ali (FIAS), Professor of Physics, Physics Department, Dhaka University, Dhaka 1000, Bangladesh.

5. Dr Imad El-Dien Aradeeb, Associate Professor, Faculty of Veterinary Science, University of Khartoum, PO Box 32, Khartoum, Sudan.

6. Prof. Wiranto Arismunandar (FIAS), Institute of Technology Bandung (ITB), GANESA 10, Bandung 40132, Indonesia.

7. Prof. Muhammad Asghar (FIAS), Institute of Sciences Nucleaires, 53-avenue des Martyrs, 38026 Grenoble Cedex, France.

8. Prof. Attia A. Ashour (FIAS), Professor Emirates, Mathematics Department, Faculty of Science, Cairo University, Giza, Egypt.

9. Mr Wasfi Ayad, Jordan Embassy, Rabat, Morocco.

10. Prof. Said El-Ayoubi, Faculty of Medicine and Pharmacy, Mohammed V University, BP 6203, Souissi, Rabat, Morocco.

11. Prof. Ahmed Abdullah Azad (FIAS), Director of Research, University of Cape Town, Faculty of Health Sciences, Anzio Road, Observatory, Cape Town 7925, South Africa.

12. Prof. Adnan Badran (FIAS), President, Philadelphia University, PO Box 1, Philadelphia University, 19392, Jordan.

13. Prof. Mohammed El-Bakoury, Faculty of Medicine and Pharmacy, Mohammed V University, BP 6203-Souissi, Rabat, Morocco.

14. Dr Elias Baydoun, American University of Beirut, Biology Department, Beirut, Lebanon.

15. Prof. Abdelmajid Belmahi, Faculty of Medicine and Pharmacy, Mohammed V University, BP 6203, Souissi, Rabat, Morocco.

16. Prof. Faouzia Benabdallah, Professor of Pediatric Surgery, Rabat Children Hospital, Rabat, Morocco.

17. Prof. Abdelkamel Benaissa, Professor of Medicine, Faculty of Medicine and Pharmacy, Mohammed V University, BP 6203, Souissi, Rabat, Morocco.

18. Dr. Bouziane Benali, Faculty of Science, Department of Physics Ibn Tofail University, Kenitra, Morocco.

19. Prof. Abdelaziz Benjouad, Director of Laboratories (Biochemistry and Immunology), Faculty of Science, Mohammed V University, Avenue Ibn Batouta, BP 1014, Rabat, Morocco.

20. Prof. Mohamed Bensouda, Faculty of Medicine and Pharmacy, Mohammed V University, BP 6203, Souissi, Rabat, Morocco.

21. Prof. Abddul-Latif Berbich, Secretary General, Academy of the Kingdom of Morocco, PO Box 5062, Rabat, Morocco.

22. Dr Faiq Billal, Director (Science), ISESCO, Hay Riyadh, PO Box 2275, Post Code 10104, Rabat, Morocco.

23. Dr Younis Bjijou, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat Morocco.

24. Prof. Naci Bor (FIAS), Editor-in-Chief, IAS Journal, Mithatpasa Caddesi, No. 66/5, Ankara, Turkey.

25. Prof. Abdeslam El Bouchikhi, Faculty of Medicine and Pharmacy, Mohammed V University, BP 6203, Souissi, Rabat, Morocco.

26. Prof. Mohamed Larbi El Bouchikhi, Faculty of Medicine and Pharmacy, Mohammed V University, BP 6203, Souissi, Rabat, Morocco.

27. Prof. Abdellatif Bour, Nutrition Laboratory, Department of Biology, Faculty of Science, Ibn Tofail University, BP 133, Kenitra 14000, Morocco.

28. Prof. Mohammad Ilyas Burney (FIAS), Scientist Emeritus, National Institute of Health, Islamabad, Pakistan.

29. Prof. Noor Mohammad Butt (FIAS), Scientist Emeritus, Pakistan Atomic Energy Commission, PINSTECH, PO Nilore, Islamabad, Pakistan.

30. Prof. Ahmed Chahlane, Mohammed V University, Rabat, Morocco.

31. Prof. A Cherkaoui, Ibn Tofail University, Faculty of Science, PO Box 133, Kenitra, Morocco.

32. Prof. Mustapha Chokairi, Mohammed V University, Department of Biology, Agdal, Rabat, Morocco.

33. Ms Suzy Touma Christian, Conference Interpreter, 21A ch. Des Cornillons, CH-1292 Chambesy-Geneva, Switzerland.

34. Dr. Abdelmajid Choukri, University Ibn Tofail, Faculty of Sciences, B. P 133, 14000, Morocco.

35. Prof. Ali A. Al-Daffa' (FIAS), Professor of Math, King Fahd University of Petroleum and Minerals, P O Box 324, Dhahran 31261, Saudi Arabia.

36. Ms Khadija Al-Debai, PO Box 8952, Rabat, Morocco.

37. Prof. Ibrahima Mar Diop (FIAS), IAS Vice-President, Honorary Dean, Faculty of Medicine, UCAD, Clinique Fann Hock, Rue 55 angle 70, BP 15504, Fann, Dakar, Senegal.

38. Prof. Mustafa Doruk (FIAS), Dept. of Metallurgical and Materials Engineering, Middle East Technical University, 06531, Ankara, Turkey.

39. Prof. Radhouane Ellouz, Director, Biotechnology Centre, Route de la Soukra, Km 3.5, 3038 Sfax, Tunisia.

40. Prof. Mehmet Ergin (FIAS), Head of Chemistry Department, Fatih University, 34900 Buyukcekmece, Istanbul, Turkey.

41. Dr Achour Essaid, Mohammed V University, Faculty of Sciences, Department of Physics, Kenitra, Morocco.

42. Prof. Mustafha Fadili, Mohammed V University, Faculty of Medicine, BP 6203, Souissi, Rabat, Morocco.

43. Prof. Mohamed Baha-Eldin Fayez (FIAS), Emeritus Professor, National Research Centre, Tahrir Street, Dokki, Cairo, Egypt.

44. Prof. Abdelhak Garch, Faculty of Medicine, Casablanca, 19 Rue Tarik Ibn Zyad, Casablanca, Morocco.

45. Mr Ibrahim Ait Garrot, Faculty of Medicine, Mohammed V University, BP 6203, Souissi, Rabat, Morocco.

46. Ms. Saude Habash, Ministry of Higher Education, Rokn Al-Deen, Damascus, Syria.

47. Prof. Hashim Mohamed El-Hadi (FIAS), Vice-Chancellor, Sudan International University, PO Box 12769, Elmogran, Khartoum, Sudan.

48. Prof. Mohamoud Hafez (FIAS), Entomology Department, Faculty of Science, Cairo University, Cairo, Egypt.

49. Prof. Syed Z. Haider (FIAS), Professor of Chemistry, c/o Dept. of Chemistry, Dhaka University, Dhaka 1000, Bangladesh.

50. Prof. Nour Eddin El-Haloui, Professor, Faculty of Science, Ibn Tofail University, Kenitre, Morocco.

51. Prof. Mohammad Hamdan (FIAS), Rector of AOU, Arab Open University, PO Box 138 Jubeiha, Amman, Jordan.

52. Prof. Mohamed H. A. Hassan (FIAS), Executive Director, TWAS, c/o ICTP, Trieste, Italy.

53. Prof. Bambang Hidayat (FIAS), Bosscha Observatory (ITB), Lembang 40391, Java, Indonesia.

54. Prof. Mohamed Jiddan, Professor of Medicine, Faculty of Medicine, Mohammed V University, BP 6203, Souissi, Rabat, Morocco.

55. Prof. Najia Kacemi, Faculty of Medicine, Mohammed V University, BP 6203, Souissi, Rabat, Morocco.

56. Dr Ahmed Parveez Ghulam Kadir, Senior Research Officer, Malaysian Palm Oil Board, No 6, Persiaran Institusi, Bandar Baru Bangi, 4300 Kajang, Selangor, Malaysia.

57. Miss Najia El-Kaoui, Mohammed V University, Faculty of Medicine, Laboratory of Anatomy, Rabat, Morocco.

58. Prof. M'hammed Said El-Kebbaj, University Hassan II, Faculty of Science, BP 5366, Casablanca, Morocco.

59. Mr Hamza Ali Kettani, 11 Rue Ouled Bou-Tabet, Souissi, Rabat, Morocco

60. Prof. Hamza El-Kettani (FIAS), Bi'r Qasem Street, Souissi, Rabat, Morocco.

61. Mr. Hassan Ali Kettani, 11 Rue Ouled Bou-Tabet, Souissi, Rabat, Morocco.

62. Dr Najib Kettani, President, MEDIKA Group, 121 Avenue Oued Akrach, Souissi, Rabat, Morocco.

63. Prof. Abdel Quadeer Khan (FIAS), President, Pakistan Academy of Sciences, G-5/2, Constitution Avenue, Islamabad, Pakistan.

64. Prof. Mostefa Khiati (FIAS), Villa 41, Cite du 20 Aout, Oued Roumane, El Achour, Algiers, Algeria.

65. Prof. Abdelhafid Lahlaidi (FIAS), Faculty of Medicine and Pharmacy, Mohammed V University, BP 6203, Souissi, Rabat, Morocco.

66. Mr Karim Lahlaidi, c/o Faculty of Medicine and Pharmacy, Mohammed V University, BP 6203, Souissi, Rabat, Morocco.

67. Dr Nadia Lahlaidi, c/o Faculty of Medicine and Pharmacy, Mohammed V University, BP 6203, Souissi, Rabat, Morocco.

68. Prof. Mohamed Lahrichi, Faculty of Medicine and Pharmacy, Mohammed V University, BP 6203, Souissi, Rabat, Morocco.

69. Prof. Walali Laudyi, Hassan II Institute of Agronomy and Veterinary Medicine, Rabat, Morocco.

70. Major Mamdooh Maharma, Jordan Armed Forces, Amman, Jordan.

71. Dr Mohammed Ali Mahesar, Executive Secretary, COMSTECH, Constitution Avenue, Islamabad 44000, Pakistan.

72. Prof. Mohamed Kamel Mahmoud (FIAS), Emeritus Research Professor, National Research Centre, Tahrir Street, Dokki, Cairo, Egypt.

73. Prof. Abdel Salam Majali (FIAS), President, Islamic Academy of Sciences, PO Box 830036, Amman 11183, Jordan.

74. Prof. Iftikhar Ahmad Malik (FIAS), 129 Hali Road, Westridge – I, Rawalpindi, Pakistan.

75. Dr Kauser Abdulla Malik, Member (Biosciences), Pakistan Atomic Energy Commission, PO Box 1114, Islamabad, Pakistan.

76. Prof. Ahmed Marrakchi (FIAS), 2 Mohammed Bensaleh Street, Menzah 6, 1004 Tunis, Tunisia.

77. Prof. Ahmet Mazgarov (FIAS), Director, Doctor of Science, Professor, Tatarstan Academy of Sciences, N. Ershov str., 35-a, Kazan 420045, Tatarstan Republic, Russia.

78. Prof. S. Qasim Mehdi (FIAS), Director General, Biomedical and Genetic Engineering Division, Dr A Q Khan Research Laboratories, 24-25 Mauve Area, PO Box 2891, Islamabad, Pakistan.

79. rof. Amdulla Mehrabov (FIAS), Middle East Technical University, Department of Metallurgical and Materials Engineering, 06531 Ankara, Turkey.

80. Dr Saad Mrani, Rabat Children Hospital, Rabat, Morocco.

81. Prof. Sami Al-Mudhaffar (FIAS), Professor of Biochemistry, Academy of Sciences, Waziriya, PO Box 4023, Baghdad, Iraq.

82. Prof. Zaghloul Raghib El-Naggar (FIAS), 14 Road 262-A, New Ma'adi, Cairo, Egypt

83. Prof. Anwar Nasim (FIAS), Adviser Science, COMSTECH Secretariat, 3 Constitution Ave, G5/2, Islamabad, Pakistan.

84. Prof. Souleymane Niang (FIAS), Presidential Advisor, Former Rector, University Cheikh Anta Diop of Dakar, PO Box 5000, Dakar, Senegal.

85. Prof. Naima Alami Ouahabi, Professor of Molecular Biology, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco.

86. Prof. Korkut Ozal (FIAS), Akoz Wakf, Kisikli Caddesi, 18 Blok B, Altunizade, Uskudar, Istanbul, Turkey.

87. Prof. Mehmet Nimet Ozdas (FIAS), Professor Emeritus, Technical University of Istanbul, Makina Fakultesi, Gumussuyu 80191, Istanbul, Turkey.

88. Prof. Munir Ozturk, Professor of Ecology, Chairman Biology Department, Fatih University, Istanbul, Turkey.

89. Dr Mohammed Al-Qahtani, Assistance Professor, King Abdulaziz University, Jeddah, Saudi Arabia.

90. Prof. Mazhar M. Qurashi (FIAS), Pakistan Academy of Sciences, Constitution Avenue, G-5, Islamabad, Pakistan.

91. Prof. Kamal Raddaoui, Professor of Psychiatry, Faculty of Medicine, Ar-Razi Hopital, BP 34, Sale, 11005 Rabat, Morocco.

92. Prof. Atta-ur-Rahman (FIAS), Minister of Science and Technology, Coordinator General, COMSTECH, COMSTECH Secretariat, 3 Constitution Avenue, G-5/2, Islamabad 44000, Pakistan.

93. Prof. Najih M. Khalil El-Rawi (FIAS), President, Iraqi Academy of Sciences, P O Box 423 Aadhamea, Baghdad, Iraq.

94. Prof. Mohammed Salouhi, Al-Mohammedia School of Engineers, Ave Ibn Sina, BP 765, Agdal, Rabat, Morocco.

95. Mr. Haitham Samaan, Al-Baath University, Homs, Syria.

96. Prof. Samaun Samadikun (FIAS), Senior Researcher, Inter University Research, Center on Microelectronics Technology, Bandung Institute of Technology, GANESA 10, Bandung 40132, Indonesia.

97. Ms Taghreed Saqer, Secretary, Islamic Academy of Sciences, PO Box 830036, Amman 11183 Jordan.

98. Dr Mohamad Mokhtar Sellami, Nahj Mahmoud Al-Matery, Tunis 1002, Tunisia.

99. Prof. Misbah-Ud-Din Shami (FIAS), Secretary General, Pakistan Academy of Sciences, G-5/2, Constitution Avenue, Islamabad, Pakistan.

100. Prof. Ahmad Shamsul Islam (FIAS), University of Texas, Department of Botany, Austin, TX 78713, USA.

101. Dr Said Skali, 132 Rue Mostafa El Maani, Casablanca, Morocco.

102. Prof. Ahmedou Moustafaha Sow (FIAS), Professor of Internal Medicine, Polyclinique Cheihk El-Hadj Malick, Rue B angle Allees Ababacar SY, Dakar, Senegal.

103. Prof. Mohammed Taquiollah, Mohammed V University, PO Box 250 Rabat, Morocco.

104. Prof. M'hammed Tijane, Head of Department of Biology, Faculty of Sciences, Morocco.

105. Prof. Abdelaziz Touijri, Director General, ISESCO, Riyadh Area, PO Box 2275, Rabat 10104, Morocco.

106. Prof. Ahmet Hikmet Ucisik (FIAS), Bogazici University, Biomedical Engineering Institute, 80815 Bebek, Istanbul, Turkey.

107. Prof. Muhammad Anwar Waqar (FIAS), Professor of Biochemistry, The Aga Khan University, Department of Biological and Biomedical Sciences, PO Box 3500, Stadium Road, Karachi, Pakistan.

108. Prof. Ibrahima Wone (FIAS), Villa No 9059, Sacre Coeur III, Dakar, Senegal.

109. Dr Thami El-Zahraoui, Directorate of Higher Education (Research and Development), PO Box 6598 Rabat, Morocco.

110. Eng. Moneef R Zou'bi, Director General, Islamic Academy of Sciences, PO Box 830036, Amman 11183, Jordan.

Council Members of the Islamic Academy of Sciences (1999-2003)

President:	**Dr Abdel Salam Majali**	Jordan.
Vice-President:	**Dr Iba Mar Diop**	Senegal.
Vice-President:	**Dr Ali Kettani***	Morocco.
Vice-President:	**Dr Misbahuddin Shami**	Pakistan.
Treasurer:	**Dr Adnan Badran**	Jordan.
Secretary General:	**Dr Mehmet Ergin**	Turkey.
Member:	**Dr Naci Bor**	Turkey.
Member:	**Dr Sayed Zahir Haider**	Bangladesh.
Member:	**Dr Mohamed H A Hassan**	Sudan.
Member:	**Dr Naeem Ahmad Khan**	Pakistan.
Member:	**Dr Zaghloul El-Naggar**	Egypt.

Council of the Islamic Academy of Sciences (2003-2007)

President:	**Dr Abdul Salam Majali**	Jordan.
Vice-President:	**Dr J Younis Katili**	Indonesia.
Vice-President:	**Dr Ahmed Marrakchi**	Tunisia.
Vice-President:	**Dr Misbahuddin Shami**	Pakistan.
Treasurer:	**Dr Adnan Badran**	Jordan.
Secretary General:	**Dr Mehmet Ergin**	Turkey.
Member:	**Dr M Shamsher Ali**	Bangladesh.
Member:	**Dr Naci Bor**	Turkey.
Member:	**Dr Mohamed H A Hassan**	Sudan.
Member:	**Dr Anwar Nasim**	Pakistan.
Member:	**Dr Muthana Shanshal**	Iraq.

IAS Executive Staff

Eng. Moneef R Zou'bi	*Director General.*
Ms Lina Jalal	*Programme Officer.*
Ms Najwa Daghestani	*Programme Officer.*
Ms Taghreed Saqer	*Secretary.*

* Passed away, 2001, Prof. Hamza Kettani elected, November 2001.

.

Patrons of the Islamic Academy of Sciences

His Excellency the President of the Islamic Republic of Pakistan.
His Royal Highness Prince Al Hassan Ibn Talal of the Hashemite Kingdom of Jordan, Founding Patron.

Honorary Fellows of the Islamic Academy of Sciences

1. His Excellency Rauf Denktash, President of the Turkish Republic of Northern Cyprus.
2. Datuk Patinggi Tan Sri Haji Dr Abdul Taib Mahmud, Chief Minister, State of Sarawak, Malaysia.
3. His Excellency Dato' Seri Dr Mahathir Mohamad, Prime Minister of Malaysia.
4. Prof. Ferid Murad, 1998 Nobel Laureate (Medicine), USA.
5. His Excellency Nursultan Abishevich Nazarbayev, President of the Republic of Kazakhstan.
6. Dr Enver Oren, President and Chairman, Ihlas Gazetecilik Holding, Turkey.
7. Sheikh Hamad Al-Zamil, Chairman, Al-Zamil Group, Saudi Arabia.
8. Prof. Ahmed Zewail, 1999 Nobel Laureate (Chemistry), Egypt/USA.
9. *The late* Dr Hakim Mohammed Said, President, Hamdard Foundation, Pakistan.

Fellows of the Islamic Academy of Sciences (May 2004)

1.	Prof. Omar **Abdul Rahman**	Malaysia	Veterinary Medicine
2.	Prof. Naim **Afgan**	Bosnia-Herzegovina	Mechanical Engineering
3.	Prof. Ishfaq **Ahmad**	Pakistan	Physics
4.	Prof. Askar **Akayev**	Kyrghyzistan	Computer Engineering
5.	Prof. Sajjad **Alam**	Bangladesh/USA	Physics
6.	Prof. M Shamsher **Ali**	Bangladesh	Physics
7.	Prof. Qurashi Mohammed **Ali**	Sudan	Medicine/Anatomy
8.	Prof. Huda Saleh Mehdi **Ammash**	Iraq	Biology
9.	Prof. Wiranto **Arismunandar**	Indonesia	Mechanical Engineering
10.	Prof. Muhammad **Asghar**	France	Physics
11.	Prof. Attia A **Ashour**	Egypt	Mathematics
12.	Prof. Saleh A **Al-Athel**	Saudi Arabia	Mechanical Engineering
13.	Prof. Ahmad Nawawi **Ayob**	Malaysia	Botany
14.	Prof. Ahmad Abdullah **Azad**	Bangladesh/Australia	Biochemistry
15.	Prof. Agadjan **Babaev**	Turkmenistan	Geography
16.	Prof. Adnan **Badran**	Jordan	Biology
17.	Prof. Ibrahim Gamil **Badran**	Egypt	Medicine
18.	Prof. Farouk **El-Baz**	USA	Geology
19.	Prof. Kazem **Behbehani**	Kuwait	Immunology
20.	Prof. Azret Yusupovich **Bekkiev**	Balkar/Russia	Physics
21.	Prof. Naci **Bor**	Turkey	Medicine
22.	Prof. Rafik **Boukhris**	Tunisia	Medicine
23.	Prof. Mohammad Ilyas **Burney**	Pakistan	Medicine
24.	Prof. Noor Mohammad **Butt**	Pakistan	Physics
25.	Prof. Muhammad Iqbal **Choudhary**	Pakistan	Organic Chemistry
26.	Prof. Ali A **Daffa'**	Saudi Arabia	Mathematics
27.	Prof. Mamadou **Daffe**	Mali/France	Biochemistry
28.	Prof. Fakhruddin **Daghestani**	Jordan	Mechanical Engineering
29.	Prof. Ramazan **Demir**	Turkey	Biology
30.	Prof. Oussaynou Fall **Dia**	Senegal	Geology
31.	Prof. Ugur **Dilmen**	Turkey	Medicine
32.	Prof. Ibrahim Mar **Diop**	Senegal	Medicine
33.	Prof. Mustafa **Doruk**	Turkey	Metallurgical Engineering
34.	Prof. Mehmet **Ergin**	Turkey	Chemical Engineering
35.	Prof. Mohamed B E **Fayez**	Egypt	Chemistry
36.	Prof. Mehdi **Golshani**	Iran	Physics
37.	Prof. Kadyr G **Gulamov**	Uzbekistan	Physics
38.	Prof. Hashim M **El-Hadi**	Sudan	Veterinary Medicine

39. Prof. Mahmoud **Hafez**	Egypt	Entomology
40. Prof. Syed Zahir **Haider**	Bangladesh	Chemistry
41. Prof. Mohammad **Hamdan**	Jordan	Mathematics
42. Prof. Mohamed H A **Hassan**	Sudan	Mathematics
43. Prof. Ali Ali **Hebeish**	Egypt	Chemistry
44. Prof. Bambang **Hidayat**	Indonesia	Astronomy
45. Prof. Abdul Latif **Ibrahim**	Malaysia	Microbiology
46. Prof. Mohammad Shamim **Jairajpuri**	India	Zoology
47. Prof. J (Younis) Ario **Katili**	Indonesia	Geology
48. Prof. Hamza **El-Kettani**	Morocco	Physics and Chemistry
49. Prof. Pulat K **Khabibullaev**	Uzbekistan	Physics
50. Prof. Salambek **Khadjiev**	Chechnya	Chemistry
51. Prof. Idriss **Khalil**	Morocco	Mathematics
52. Prof. Abdul Qadeer **Khan**	Pakistan	Metallurgical Engineering
53. Prof. Hameed Ahmed **Khan**	Pakistan	Physics
54. Prof. Naeem Ahmad **Khan**	Pakistan	Physics
55. Prof. Mostefa **Khiati**	Algeria	Medicine
56. Prof. Abdelhafid **Lahlaidi**	Morocco	Medicine
57. Prof. Zohra Ben **Lakhdar**	Tunisia	Physics
58. Prof. Abdel Salam **Majali**	Jordan	Medicine
59. Prof. Iftikhar Ahmad **Malik**	Pakistan	Medicine/Pathology
60. Prof. Ahmed **Marrakchi**	Tunisia	Electronic Engineering
61. Prof. Akhmet **Mazgarov**	Tatarstan/Russia	Petrochemistry
62. Prof. Syed Qasim **Mehdi**	Pakistan	Molecular Biology
63. Prof. Amdoulla **Mehrabov**	Azerbaijan	Materials Science
64. Prof. Sami **Al- Mudhaffar**	Iraq	Biochemistry
65. Prof. Badri **Muhammad**	Malaysia	Chemistry
66. Prof. Zaghloul **El-Naggar**	Egypt	Geology
67. Prof. Ibrahim Saleh **Al- Naimi**	Qatar	Chemistry
68. Prof. Anwar **Nasim**	Pakistan	Genetics
69. Prof. Jamal **Nazrul-Islam**	Bangladesh/UK	Mathematics
70. Prof. Souleymane **Niang**	Senegal	Mathematics
71. Prof. Gulsen **Oner**	Turkey	Medicine
72. Prof. Ramdane **Ouahes**	Algeria	Chemistry
73. Prof. Korkut **Ozal**	Turkey	Civil Engineering
74. Prof. Mehmet Nimet **Ozdas**	Turkey	Mechanical Engineering
75. Prof. Sinasi **Ozsoylu**	Turkey	Medicine
76. Prof. Munir **Ozturk**	Turkey	Biology
77. Prof. Iqbal **Parker**	South Africa	Biochemistry
78. Prof. Syed Muhammad **Qaim**	Germany	Nuclear Chemistry
79. Prof. Subhi **Qasem**	Jordan	Agriculture
80. Prof. Mazhar M **Qurashi**	Pakistan	Physics
81. Prof. Atta-ur-**Rahman**	Pakistan	Chemistry
82. Prof. Najih Khalil **El-Rawi**	Iraq	Civil Engineering
83. Prof. **Riazuddin**	Pakistan	Physics
84. Prof. Makhmud **Salakhitdinov**	Uzbekistan	Mathematics
85. Prof. Hussein Samir **Salama**	Egypt	Entomology

86. Prof. Eldar Yunisoglu **Salayev**	Azerbaijan	Physics /Mathematics
87. Prof. Samaun **Samadikun**	Indonesia	Electronic Engineering
88. Prof. Lorenzo **Savioli**	Italy	Medicine
89. Prof. Misbah-Ud-Din **Shami**	Pakistan	Chemistry
90. Prof. Ali **Al-Shamlan**	Kuwait	Geology
91. Prof. Ahmad **Shamsul-Islam**	Bangladesh	Botany
92. Prof. Muthana **Shanshal**	Iraq	Chemistry
93. Prof. Ahmedou M **Sow**	Senegal	Medicine
94. Prof. Mahmoud **Tebyani**	Iran	Electronic Engineering
95. Prof. Ahmet Hikmet **Ucisik**	Turkey	Materials Science
96. Prof. Mohammed A **Waqar**	Pakistan	Biochemistry
97. Prof. Ibrahima **Wone**	Senegal	Medicine
98. Prof. Bekhzad **Yuldashev**	Uzbekistan	Physics/ Mathematics
99. Prof. Mikhael **Zalikhanov**	Balkar/Russia	Glaciology/Biology

Deceased Fellows of the Islamic Academy of Sciences

Prof. Mohammad Ibrahim	(1911-1988)	Bangladesh.
Prof. Djibril Fall	(1930-1992)	Senegal.
Prof. Salimuzzaman Siddiqui	(1897-1994)	Pakistan.
Prof. Abdus Salam Mia	(1925-1995)	Bangladesh/USA.
Prof. Suleiman Gabir Hamad	(1937-1996)	Sudan.
Prof. Mohammad R Siddiqi	(1908-1998)	Pakistan.
Prof. Abdullah M Sharafuddin	(1930-1998)	Bangladesh.
Prof. Achmad Baiquni	(1923-1998)	Indonesia.
Prof. Mumtaz Ali Kazi	(1928-1999)	Pakistan.
Prof. Faramaz G Maksudov	(1930-2000)	Azerbaijan.
Prof. Ali Kettani	(1941-2001)	Morocco.
Prof. Mohamed Kamel Mahmoud	(1926-2003)	Egypt.

Laureate(s) of the IAS Ibrahim Memorial Award

Prof. Ugur Dilmen	1996	Turkey.

IAS Supporters

The Hashemite Kingdom of Jordan
The Islamic Republic of Pakistan
The State of Kuwait
The Republic of Turkey
Malaysia
The Republic of Senegal
The Republic of Sudan
The Islamic Republic of Iran
The State of Qatar
The Republic of Tunisia
The Kingdom of Morocco
The State of Sarawak/Malaysia
The Republic of Indonesia

The OIC Standing Committee on Scientific and Technological Co-operation (COMSTECH).
The Islamic Development Bank (IDB).
The OPEC Fund for International Development, Vienna, Austria.
Arab Potash Company, Jordan.
United Nations Educational Scientific and Cultural Organisation (UNESCO).
Islamic Educational Scientific and Cultural Organisation (ISESCO).
The World Bank.
The United Nations Environment Programme (UNEP).
Kuwait Foundation for the Advancement of Sciences (KFAS).
Turkish Scientific and Technical Research Council (TUBITAK).
The Royal Scientific Society (RSS), Jordan.
Pakistan Ministry of Science and Technology.
Ministry of Science, Technology and the Environment, Malaysia.
University Cheikh Anta Diop, Dakar, Senegal.
Ministry of Higher Education and Scientific Research, Sudan.
National Centre for Research, Sudan.
Ministry of Culture and Higher Education, Iran.
Iranian Research Organisation for Science and Technology (IROST).
The Academy of Sciences, Tehran, Iran.
The Academy of Medical Sciences, Tehran, Iran.
Saudi Arabian Oil Company, Saudi Arabia (ARAMCO).
Ihlas Holding, Turkey.

Arab Bank, Jordan.
Jordan Kuwait Bank, Jordan.
Rafia Industrial Company, Jordan.
Secretariat of State for Scientific Research and Technology, Tunisia.
Academy of the Kingdom of Morocco.
Petra Private University, Jordan.
Higher Council of Science and Technology (HCST), Jordan.
Pakistan Academy of Sciences.
Majlis Islam Sarawak, Malaysia.
Sasakawa Peace Foundation, Tokyo, Japan.

IAS Waqf

Islamic Academy of Sciences
Jordan Islamic Bank
Shemeisani Branch
Account No: 809/$91
Telephone: 5677107
Facsimile: 5691700
PO Box 925997
Amman 11110
Jordan.

IAS Endowment Fund

Islamic Academy of Sciences
Arab Bank
Fifth Circle Branch
Account No: 0134/034765-5/71
Telephone: 5526870
Facsimile: 5526874
PO Box 141107
Amman
Jordan.

IAS on the Internet

http://www.iasworld.org