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Biotechnology is a technology of great promise. It offers us an infinite number of ways of combating hunger, securing health and conserving our environment: concerns of crucial importance to the developing countries. UNIDO shares those concerns and is determined to harness the best resources to pave the way to sustainable development. Internationalization is a critical factor in that process. Centres, such as the International Centre for Genetic Engineering and Biotechnology, a major UNIDO project, provide an all-essential forum for effective international co-operation. They constitute an enabling scientific and educational environment for research and development into the pressing needs of developing countries. The Centre not only ensures access to state-of-the-art research and equipment, but it also disposes of a critical mass of scientific staff and can offer advanced training. By co-operating through the Centre with their counterparts in developed countries and engendering a sense of true partnership and dialogue, the scientists in developing countries not only remain at the cutting edge of a new technology, but they also help to keep it sharp and effective. The sense of community in the field of innovative research is heightened by the application of modern electronic communications, such as the Centre’s computer information resource, ICGEBnet, and more recently its newsletter HELIX. A welcome addition to the services provided by the Centre, the newsletter aims at enhancing the dialogue among its large membership.

Domingo L. Siazon Jr.
Director-General of UNIDO
Editorial

Biosafety is an emotionally charged term giving rise to fierce debate amongst scientists, policy makers and industry. In the West, safeguards demanded by environmentalists are often seen by industry as discrimination against biotechnology products, by subjecting them to extra levels of testing and regulation. In developing countries lack of safeguards or indeed of any regulatory framework is viewed with concern. It is often felt that deregulation will turn these countries into testing grounds for releases of transgenic organisms which would not be given test permits in the developed world. The testing of a rabies vaccine in Argentina in 1986 is often quoted as an example. On the other hand in the case of most developing countries, adoption of a regulatory framework, although legitimizing environmental releases, does not guarantee adequate monitoring of field tests due to lack of resources and expertise. In this sense, it is feared that regulation per se could be a more serious cause of relocation of potentially hazardous experiments to developing countries than lack of regulation itself. Some analysts argue that even if relocation is not on the cards, adoption of rigid regulations ridden with bureaucratic procedures is likely to be a serious impediment to technology transfer, limiting the availability of much needed products and processes to developing countries.

A number of initiatives is underway (see News), all trying to allay possible apprehensions over biotechnology applications, facilitate technology transfer and harmonize risk assessment methodologies.

But even if one denudes biosafety of all elements that tend to tint it politically, there remain questions to be answered before one takes a more relaxed attitude towards biological safety. True, a decade ago, a lot of the fears were originally generated by relative ignorance. Could genetic tampering through recombinant technologies lead to unpredicted genomic alterations? Is recombinant gene transfer through species barriers any different from that occurring in nature randomly through transduction and transformation? Most of these fears have been dispelled since. In fact the precision and contend...
ICGEB: scientists and programmes

Francisco E. Baralle, born in Buenos Aires on 26 October 1943, graduated in medicine and chemistry at the University of Buenos Aires, Department of Organic Chemistry. After gaining his Ph.D. he transferred to the Instituto de Investigaciones Bioquímicas Fundación Campoman directed by Prof. Luis F. Leloir. In 1974, he moved to the MRC Laboratory of Molecular Biology, Cambridge, UK, where he worked in the Division directed by Prof. Frederick Sanger. From 1980 to 1990 he was Professor of Pathology at Oxford University and Fellow of Magdalen College. He is a member of the European Molecular Biology Organization. He was appointed Head of the Trieste Component of ICGEB in September 1990.

Prof. Baralle was involved in the determination of the primary structure of eukaryotic mRNA. In 1977 he was the first to complete the sequence of the messenger of human beta-globin and, in 1979, to clone the gene of the beta-globin, a component of the human embryonic haemoglobin. He carried out research on thalassemia and is currently studying the genetic factors involved in the susceptibility to atherosclerosis. In his laboratory he is developing projects on the study of the genes of apolipoprotein AI and AI components of the High Density Lipoproteins (HDL) involved in the reverse cholesterol transport. High plasma levels of HDL seem to be associated with a low incidence of coronary heart disease. The knowledge of the genetic mechanisms which regulate these levels can be not only of great scientific interest but also of great value in clinical practice. Another project focuses on the different coding genes for the extracellular matrix proteins such as fibronectin and tenascin, and the RNA alternative splicing mechanisms involved in the generation of isoforms of these proteins. In the field of hypertension, Prof. Baralle is interested in adducin, a cytoskeletal protein whose properties could influence arterial tension.

In collaboration with pharmaceutical companies he has initiated biotechnology projects aimed at producing polypeptides of pharmaceutical value such as erythropoietin, the coagulation factor VIII and some lymphokines.

In his laboratory at ICGEB, apart from carrying out basic research in the above fields, an applied project has been initiated for the development of diagnostic kits, innovative vaccines and the production of recombinant monoclonal antibodies for immunodiagnostic therapy. Particular attention is focused on Hepatitis B and C vaccines. They are being developed using a novel epitope presentation system based on the capsid of the Black Beetle Virus (BBV). This structure allows a considerable flexibility for the blending of foreign T and B cell epitopes in a strongly immunogenic context.
The virology group at ICGEB-New Delhi

Research activities, headed by Dr. Shahid Jameel, are currently directed towards studies at the molecular level of viral hepatitis: hepatitis B and enteric non-A non-B hepatitis.

The Hepatitis B Programme

Hepatitis B is a major world health problem with an estimated 200 million carriers of this disease worldwide. The predominant mode of transmission is parental, where chronic carriers constitute the reservoir for spread of infection to other susceptible individuals, either horizontally or vertically. Severe chronic hepatitis B frequently leads to premature death from liver failure. Chronic hepatitis B is also associated with the development of primary hepato cellular carcinoma (PHC) with risk of PHC development being about 300-fold that of age-matched non-carriers.

The aetiological agent for this disease, hepatitis B virus (HBV), is a small (42 nm), partially double-stranded DNA virus (Fig. 1). The host range of HBV is narrow, to date productive infections have been established only in human beings and higher primates. In permissive hosts, viral antigens and DNA are found primarily within liver cells, which harbour abundant quantities of replicative and assembly intermediates as well as mature virions. The genome of HBV is circular DNA of only ~2 kilobases in length which encodes at least four viral antigens: DNA polymerase (P), core (HBc), surface (HBsAg) and X (HBx) proteins.

The hepatitis B programme is centred on the following aspects:

1. Expression and characterization of functional domains of the X-protein (HBx).
2. Analysis of the enhancer element of HBV.
3. Novel approaches towards the design of a molecular vaccine for hepatitis B.
4. Lymphokine-derived immunostimulatory agents as potential adjuvants.

1. Hepatitis B virus X Protein

Of all the HBV-encoded proteins, X is the least understood. Recently, it has been shown to be capable of trans-activating a number of viral and cellular promoters or enhancers. Most significant in this respect is trans-activation of the long terminal repeat (LTR) of the human immuno deficiency virus (HIV) by X, as it is the first evidence of an interaction between HBV and HIV at the molecular level. This supports clinical observations that a majority of AIDS patients also test positive for hepatitis B. Does HBV infection in any way predispose towards HIV infection? With the HBV X protein trans-activating the HIV-1-LTR, it is possible that HIV infection may activate a latent form of HIV into full-blown AIDS.

Research is directed towards an understanding of the mechanism of this trans-activation. A simplified mechanism would involve binding of X to its target DNA sequences just like a number of known transcription factors. Preliminary evidence rules this out because X does not seem to bind DNA, and no consensus nucleotide sequence can be localized on target DNA. Trans-acting factors that do not bind DNA generally act by interacting with or modulating other DNA-binding proteins.

This problem is being approached by generating a number of site-directed mutants of the X protein. The trans-activating properties of these mutants will define the functional domains of this protein. Simultaneously, co-precipitation of X and other cellular proteins with anti-X antibodies is being pursued to define proteins capable of associating with X.

2. HBV Enhancer

Enhancers are cis-acting DNA elements that are able to potentiate transcription from RNA polymerase II (TBP) transcribed promoters independent of orientation and distance. They also confer tissue-specific gene expression and most interestingly, they are often found transposed to proto-oncogenes thereby inducing tumor formation.

A major HBV enhancer has been mapped to a region between the surface and X genes. This enhancer can transcriptionally regulate at least three HBV promoters -- the X and core promoters are located downstream while the surface antigen promoter is located upstream. It also binds several cellular proteins and exhibits liver specificity. A second liver-specific enhancer has recently been identified adjacent to the core promoter of HBV. It may be developmentally regulated as excessive core gene expression is observed in advanced hepatocellular carcinoma when HBV replication is virtually absent.

The research programme includes a detailed mutational analysis of certain repeated sequence elements present within the major enhancer region of HBV. Cloning of enhancer and/or factors from liver DNA libraries using the "South Western" technique is also underway. These studies are likely to help in understanding
mechanisms of gene expression in cells infected with the virus, its role in pathogenesis of hepatitis and hepatocellular carcinoma and tracing the evolutionary origin of hepatitis viruses.

3. A Molecular Vaccine for Hepatitis B

The synthetic peptide methodology has proved immensely useful in mapping important domains within surface antigen proteins of a variety of pathogens. However, keeping in mind the problem of MHC restriction and antigenic variation, it appears unlikely to us that a synthetic peptide vaccine will prove effective in a genetically outbred human population. A novel approach is being followed to overcome these problems. Proteins will be designed that code exclusively for a variety of selected immunologically and functionally relevant determinants of the hepatitis B surface antigen. The synthesis, assembly and expression of the gene for one such construct is currently underway. It is anticipated that studies of this nature will also help elucidate some basic principles applicable towards the design of molecular vaccines in general and in addition provide an excellent model system to study the mechanism of antigen processing and presentation.

As a corollary to these above described studies, synthetic peptides are used extensively to identify important and potentially useful regions of HBsAg. Recent efforts have also been focussed at reconstituting conformation-dependent antigenic determinants of HBsAg with synthetic peptides.

The development of a recombinant HBV vaccine is also planned. This will include sequences from the pre-S regions to improve the immunogenicity as compared to the vacines currently available. Certain novel approaches aimed at maximizing HBsAg expression in cultured cells are currently being pursued to provide a cost-effective alternative to the presently available recombinant HBV vaccines.

4. Lymphokine-derived Immunostimulatory Agents

Broadly speaking, the approach here involves the use of synthetic peptides from lymphokine sequences that are capable of potentiating the immune response against a given immunogen. Our initial efforts are focussed on human interleukin-1β (IL-1β) since a nonapeptide derived from this protein with immunostimulatory activity has already been described. We have shown that co-administration of this nonapeptide sequence to a given peptide immunogen can confer in-built adjuvanticity at least in the murine system. We are currently examining the potency of other IL-1β derived sequences and, in particular, peptides that combine various functional regions of the native IL-1 protein. It is expected that such ‘bionas’ versions of IL-1 will find application as adjuvants in a variety of vaccine preparations, including that for hepatitis B.

By enhancing immunogenicity of a given vaccine in such a manner, it should be possible to reduce the number of immunizations required to confer total protection. This would render it not only more appealing but also a useful immunization both by reducing cost and increasing efficacy in terms of lower dropout rates and thereby be of particular benefit to the developing world.

The Enteric Non-A Non-B Hepatitis Programme

The development of diagnostic tests for viral hepatitis A and B has led to the realization that another form of viral hepatitis exists. The diagnosis of this Non-A, Non-B (NANB) hepatitis is currently one of exclusion. Two major forms of NANB hepatitis are recognized worldwide.

Of these enteric NANB hepatitis follows a fecal-oral transmission, with contaminated drinking water as the major source of infection. This form of hepatitis is endemic to the Indian subcontinent and a number of major epidemics have been reported in the last few years. A seroepidemiologically similar disease has also been reported from Southeast and Central Asia, parts of Africa, the Middle East, the Soviet Union, as well as parts of North and Central America. Current estimates project that with the availability of definitive diagnostic protocols, this form of hepatitis may exceed hepatitis B as the world’s most common form of hepatitis.

The candidate aetiological agent has been identified as a 27-31 nm viral particle in the stools of patients (Fig. 1). Initial efforts con...
Concerning enteric NANC hepatitis are aimed at cloning the genome of this virus. To this end, an animal model system has been set up involving transmission of the disease from human patients to rhesus monkeys by inoculation of infectious stool suspensions. Subtractive cDNA libraries from animal materials in bacteriophage lambda are currently in preparation. These libraries will be screened with convalescent sera to identify specific clones. Regions of the viral genome once cloned will provide the basis for developing definitive diagnostic agents, and later on, a recombinant vaccine. Based on minimal nucleotide sequence information available, polymerase chain reaction (PCR) based strategies are also being pursued for developing a confirmatory diagnostic test for enteric NANC hepatitis.

The Virology group at ICGEB has undertaken to identify sequence or structural peptide and oligonucleotide synthetizers and an animal model for hepatitis.

Profile of research staff

**Vidhu Jain** (Laboratory Technician, M.Sc., Jawaharlal Nehru University, 1989). Purification of peptides by h.p.l.c. Purification of oligonucleotides and assembling synthetic genes.

**Shahid Jameel** (Assistant Scientist, Ph.D., Washington State University, Pullman, USA, 1984). Expression of HBV genes in heterologous systems. Mammalian cloning and PCR analysis of the ET-NANC genome.

**Ravinder Kumar** (Laboratory Technical Assistant, B.Sc., University of Delhi, 1984). Maintenance of eukaryotic cell lines, preparation of media and care of P3 lab. Serodiagnosis of hepatitis markers in patient sera.

**Vijay Kumar** (Research Scientist, Ph.D., All-India Institute of Medical Sciences, New Delhi, 1984). Characterization of the enhancer element of HBV. Structure-function analysis of HBX and gene expression.

**Venkatasamy Manivel** (Senior Research Fellow, Ph.D., Indian Institute of Science, Bangalore, 1983). Characterization of immunomodulators and peptide antigens derived from HBsAg.

**Kanury V. S. Rao** (Research Scientist, Ph.D., M.S. University of Baroda, 1983). Designing a molecular vaccine against HBV by recombinant DNA and synthetic peptide techniques. Characterization of immunomodulators and immunologically relevant determinants of HBsAg.

**Girish C. Shukla** (Laboratory Technical Assistant, B.Sc., University of Delhi, 1983). Routine DNA cloning, plasmid isolation, gene expression and preparation of laboratory reagents.

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**Published work from the Virology group at ICGEB — New Delhi**


ICGEB: affiliated centres

BIOTECHNOLOGY OF MINING — BACTERIAL LEACHING OF MINERALS IN CHILE

by Jorge I. Allende President of the
National Committee on Biotechnology
of Chile

The Mexican National Committee on
Biotechnology is also affiliated to CONICYT

for Development's National Council on Research Science and Technology. The National Committee on Biotechnology coordinates the activities of ICGB affiliated laboratories in Chile.

Chile, like many other developing countries, is highly dependent on mining. Copper exports account for nearly 50% of the foreign currency the country receives annually. It is not surprising, therefore, that one of the priority areas of the National Committee for Biotechnology of Chile is the bacterial leaching of minerals.

As the grade of the existing copper ores becomes lower and lower, high grade sulfide copper ores below 3% are extracted through the classical pyrometallurgical process, which is faster and more economical but which has serious problems related to atmospheric contamination. Oxide ores of copper can be chemically leached with concentrated sulfuric acid treatment of the minerals in heaps or piles. The most abundant copper ores, however, are chalcopyrites which are mixed cuprous and ferrous sulfides. These sulfide ores are quite recalcitrant to chemical leaching and can only be leached effectively through the action of bacteria. Bacterial leaching employs microorganisms that require a very acid environment (below pH 4) to grow and derive their energy from ion oxidations. There are a number of microorganisms that can participate in the leaching process, including bacteria from the genus floated, Leptothrix, and sulfur. Some of these bacteria are shown in figure 1. However, the best known and most abundantly found is Thiobacillus thiooxidans. This gram-negative bacterium can fix atmospheric CO2 and N2 to obtain carbon and nitrogen compounds for its metabolism and uses atmospheric O2 to oxidize ferrous to ferric ions and sulfides to sulfate. These oxidations are its sources of energy.

Figure 2 shows that floaters can attack chalcopyrites directly to solubilize ferrous and sulfates ions. The ferric ion can in turn oxidize the cuprous ion of the chalcopyrite to cupric ions that go into solution.

Empirically, the action of these bacteria can be observed in the situation when a heap of ore containing copper oxides and chalcopyrites is irrigated with dilute sulfuric acid as shown in the experimental heap presented in Fig. 3.

Analysis of the effluents emerging from such a heap reveals that there is aminal yield of solubilized copper due to the action of copper oxides present in that ore. After a variable period of several weeks, during which the heap has been intermittently treated with the acid solution, the effluent again contains solubilized copper in water.
Cable amounts and also shows the presence of leaching bacteria in numbers that normally range from 10 to 10 bacteria/ml. The extraction of copper by this method is very slow since it may take several months to achieve a commercially significant percent of the copper-sulfide in the ore. Very often, however, the low grade ore that can be treated in this way has already been withdrawn from the mine together with richer ores and of the atmospheric gases required by the bacteria. For this reason, a biotechnology project that deals with improving the bacterial leaching of copper ores requires a large trans-disciplinary team of researchers that ranges from basic bacteriologists and molecular biologists to mining engineers, geologists and electrochemists.

In 1985, a group belonging to the Chilean National Committee for direct budget of approximately US$2 million and addition much larger resources, when in-kind contributions are considered. This major project has been divided into three sub-projects dealing with the biological, the engineering, and the mining aspects. Table 1 shows some of the research topics that have been investigated by these three different groups.

Biotechnology, won the approval of the Chilean government, the United Nations Development Programme (UNDP) and the United Nations Industrial Development Organization (UNIDO) for a project to carry out research in the bacterial leaching of Chilean copper ores. This project had a first stage covering 1985-1987 and second stage 1988-1990. It has involved six institutions—the University of Valparaiso, the Technological Institute of Chile, the Center for Mining and Metallurgy and the Chilean Copper Corporation (CODELCO). CODELCO is one of the largest copper mining companies in the world which controls all the state-owned mines of Chile. There are more than 50 researchers who have actively participated in this project which has had a

Attentive year-time, considerable progress has been achieved in understanding the biochemistry and physiology of the bacteria, in determining some of the key parameters that limit leaching in heaps and piles, in designing the bio-leaching operations and in monitoring the progress of a leaching process from a level of practically zero knowledge about the process. Chilean researchers now constitute a group with expertise that is recognized internationally. A large number of publications and two patent applications have resulted from the work carried out. More important, however, a large number of young researchers have been trained in advanced biological techniques and have been exposed to the philosophy of working on top
Although the UNDP-sponsored project came to an end in December 1990, the researchers involved in this project will continue to work in this area with funds from the Chilean National Fund for Science and Technology and from international sources. They are now working on collaborative Research Project No. 10685.

Studies of the stress response in biominging microorganisms, possible implications in improvement of bioleaching processes which have been carried out in collaboration with the laboratory of Dr. Hector Terres of Argentina, Mining biotechnology is still in its infancy and certain much work has yet to be done to achieve large breakthroughs in increased productivity. However, this is clearly an area in which the developing countries must keep alert and which can be used to establish research teams and to link scientists to the problems of their societies.

**TABLE 1**

**Scientific Research Activities of the Three Subprojects of the Bacterial Leaching of Minerals**

<table>
<thead>
<tr>
<th>Biological Subproject</th>
<th>Engineering Subproject</th>
<th>Mining Operations Subproject</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Biochemistry and genetics of CAO, iron in these bacteria.</td>
<td>2. Ideal conditions for the leaching of concentrates in shaker tanks.</td>
<td>2. Monitoring of various parameters including flow of metal-reducing organisms in actual mining operation that involve leaching.</td>
</tr>
<tr>
<td>3. Chemotaxis of bacterial attachment to minerals.</td>
<td>3. Monitoring of CO₂, O₂, pH and other parameters under experimental conditions.</td>
<td>3. Design of piles and dump for biohydrometallurgy.</td>
</tr>
<tr>
<td>4. Strain identification by DNA probes and specific antibodies.</td>
<td>4. Fluid dynamics of different kinds of piles and heaps.</td>
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</tbody>
</table>
ICGEB coming of age

The Proceedings of the Colloquium on "Life Sciences and Radiation" held at ICGEB Trieste in June 1991 will be published by ICGEB as a special publication.

The network plans to identify laboratories and develop new productive activities and services for commercial exploitation.

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tuated by the end of year at the same science centre where the Trieste Coment of ICGB is situated, a new laboratory will be equipped in gene mapping, the development of automated systems for DNA sequencing and will also act in interface between the Italian universities and ICGB.

Expert Group Meeting on the Commercialization of Biotechnology

Application of biotechnology has already resulted in products of high commercial value in industrialized countries. The increasing recognition of the obvious opportunities, development countries are keen to engage in applications of relevant priority sectors. UNIDO will assist these programmes promote "Biotechnology Development", has scheduled a meeting at its head-quarter in Vienna on October 1 to November 1, on "Commercialization of Biotechnology". The meeting will focus on biotechnology applications in health care and food processing and is expected to review mechanisms opted by developed nations for the industrialization of biotechnology products; related policies, programmes and constraints of developing nations; and later proposals for promo-
tion of commercialization of biotechnology through cooperation. The role of ICGEB in this regard will be considered.

**Biosafety Code of Conduct**

UNIDO is developing an international voluntary Code of Conduct (CoC) for the release of GMOs to the environment. The Code attempts to strike a balance between the need to protect public interest and the desire to attract investment in biotechnology applications. A first draft of the CoC was prepared by the UNIDO Secretariat based on the inputs of some 15 experts in the field, as well as the work of other international organizations. It includes a section on the operational modalities of an international mechanism which, upon request, could provide transparent and scientifically sound advice to national authorities on how to implement the Code. UNIDO is working on the CoC in close cooperation with the other members of the UNIDO/UNEP/WHO/FAO informal working group on biosafety. It is expected to finalize the draft in meeting convened by UNIDO 8-10 July 1991 in Trieste. The conference facilities will be provided by ICGEB.

**Information Resource for the Release of Organisms into the Environment**

The United Nations Environment Programme and the Microbial Strain Data Network (MSDN) organized a workshop that took place at UNIDO Headquarters in Vienna, 11-15 March 1991, with a view to consider the needs of establishing such an information resource as well as its operative modalities. The workshop was sponsored by UNIDO and was attended by ICGEB staff. Its proceedings are expected later in the year as a UNEP publication.

In the meantime, the Environment Directorate of OECD has developed an information pointer system (BIOTRACK) on field releases of modified organisms. BIOTRACK, restricted at present, contains some 300 entries. It is planned to be made available to a large number of users within 1991.

ICGEB keeps a close watch on regulatory issues in its member countries and is building a database of information based on the responses to a questionnaire, distributed earlier on this year, requesting information on biosafety regulations adopted in its member countries. Mechanisms of facilitating information exchange between ICGEB and the different database hosts and or accessing other information resources such as BIOTRACK are being examined. It is anticipated to provide the ICGEB user community with on-line access to such databases resources through ICGEBnet.

**Assessing Biotechnology Risks**

An ad hoc Workshop of Senior Experts on International Procedures for Assessing Biotechnology Risks is to be held in London, 17-19 June 1991. The Workshop is organized by the United Nations Conference on Environment and Development (UNCED) and is being hosted by the Government of the United Kingdom. The major purpose of the Workshop will be to consider a draft document on international procedures for assessing biotechnology risks, for subsequent presentation to the Third Session of the UNCED Preparatory Committee.
Calendar
June - December
1991

International Symposium
PSEUDOMONAS BIOLOGY AND BIOTECHNOLOGY
ICGEB-Trieste, 10—20 June

Theoretical Course
GENETICALLY MODIFIED ORGANISMS: SAFETY IN
THE LABORATORY AND THE ENVIRONMENT
ICGEB-Trieste, 1—3 July

Conference
GENETICALLY MODIFIED ORGANISMS
FOR THE 1990s
ICGEB-Trieste, 3—5 July

Practical Course
COMPUTER APPLICATIONS IN
MOLECULAR BIOLOGY
ICGEB-Trieste, 22 July—2 August

Practical Course
PLANT TRANSFORMATION
ICGEB-N. Delhi, 15 July—3 August

Practical Course
NUCLEIC ACID SYNTHESIS AND GENE ASSEMBLY
ICGEB-N. Delhi, 2—21 September

Practical Course
TECHNIQUES IN GENOME RESEARCH
ICGEB-Trieste, 22—27 September

Theoretical Course
MARINE MICROBIOLOGY AND BIOCHEMISTRY
ICGEB-Trieste, 16—20 December

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