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UNIDO

INTERNATIONAL CENTRE FOR GENETIC ENGINEERING AND BIOTECHNOLOGY

ACTIVITY REPORT - 1990

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. In the three years since it started operations at its provisional facilities in New Delhi and Trieste the Centre has produced a significant volume of work of admirable quality. The 1990 Report of ICGEB bears testimony to the foresight and commitment of those who contributed to its establishment.

Domingo Siazon Jr.,
Director-General, UNIDO

ICGEB is not intended to be just another molecular biology laboratory, but one that is dedicated to the needs of developing countries. In particular, it attempts to bring to them the benefits of modern biotechnology, through state-of-the-art training and research, relevant the their specific needs. ICGEB represents a progressive and comprehensive approach to international development. It aims at helping the developing nations to help themselves and holds out the prospect of alleviating some of the problems that have for so long hampered their socio-economic advancement.

The activities of ICGEB are geared to developing the basic research capabilities of its member countries, in addition to increasing awareness in biotechnology. The research activities of the ICGEB twin laboratories in New Delhi and Trieste, the complementary training schemes and the Centre's Collaborative Research Programme with its affiliated centres ensure that significant numbers of scientists are trained in the most recent theory and techniques, always in the context of the specific problems of their countries. The underlying idea is to circumvent the problems that impede the development and application of biotechnology in developing countries, by acting as a central resource of expertise and facilities of the highest quality.

The basic model for ICGEB was the International Centre for Theoretical Physics (ICTP), established in Trieste as a joint IAEA UNESCO effort in 1964 to provide training in physics for scientists from developing countries, to help the developing world to help itself through science, and to establish Trieste as a preferential forum for co-operation between industrialized countries and the Third World. ICGEB, being involved in a scientific activity of typically experimental nature, has obviously differentiated its training approach, but its philosophy is common to that of ICTP.

A final comment on the international character of the ICGEB: the staff and research fellows of the two Components come from 25 countries. The persons involved in the long-term training are exclusively from member countries, whereas the scientists are selected purely on the basis of their professional excellence (giving preference to member countries only when persons of comparable ability are identified).

The scientific activities in the two Components, after the definition of the research programme and the basic organization of the two laboratories, began only at the start of 1988. Three years later, significant progress has been made to warrant the publication of the 1990 ICGEB Activity Report.

Arturo Falaschi,
Director ICGEB

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ICGEB operates through the following instruments:

Research Programme: In New Delhi and in Trieste the laboratories have been organized both in terms of technical and administrative infrastructures as in terms of actual research activity. 6 Research Groups are operational in New Delhi, and 7 in Trieste. In Trieste the new Head of Component has taken his function in July 1990.

Short-term Training Programme: 12 activities (practical and theoretical courses and workshops) have been held in the two Components and in other laboratories of Member Countries, particularly in Affiliated Centres. 15 such activities are programmed for 1991.

Long-term Training Programme: A total of 29 new fellowships have been assigned in 1990 in the two Component laboratories, and in selected Italian laboratories. A new batch of applications is now under screening. It is proposed that this programme will be further enlarged, in parallel with the increased availability of laboratory space in the two Components, and will be extended to a pre-doctoral research programme.

Collaborative Research Programme: 18 Collaborative Research Projects have been approved for 1990, for research programmes in Affiliated Centres related to those of ICGEB. New proposals have been received for 1991 and are presently in the peer review process.

Scientific Services: A number of services have been activated for Affiliated Centres and other Member Countries: these concern the synthesis of oligopeptides, sequencing of proteins, synthesis of oligonucleotides, analysis of databases concerning sequences of biological macromolecules.

3/4/6

Introduction

The idea of creating an International Centre for Genetic Engineering and Biotechnology (ICGEB) was raised about ten years ago within the United Nations Industrial Development Organization (UNIDO), the UN agency established to promote the industrial development of third world countries. Several scientists advising UNIDO suggested that progress in genetic engineering techniques and advanced biotechnology could offer possible solutions to the most pressing problems (health, nutrition and economic development) of the developing world. Biotechnological products give high returns on investment in terms of energy, raw materials and capital expenditure. These features make such processes attractive for the economic development of third world countries. In 1982, at a conference held in Belgrade, the ministerial representatives of 35 UNIDO member countries agreed on the importance of creating "a centre of excellence for research and training in genetic engineering and biotechnology addressed to the needs of the developing world". The Centre was officially launched in 1983 with the signing of its Statutes by 26 countries. To date, the Statutes have been signed by 43 countries. It is stipulated in the Statutes that when 24 countries have ratified them, the ICGEB will become an Autonomous Intergovernmental Organization. In the interim, the Centre operates, provisionally, as a special project of UNIDO. It is governed by a Preparatory Committee consisting of representatives of its member countries, chaired by Ambassador Adolfo Taylhardat from Venezuela.

The member countries of ICGEB. There are 43 in all.

Component host countries	Affiliated Centres	Other member countries	
India	Algeria	Afghanistan	Morocco
Italy	Argentina	Bhutan	Pakistan
	Brazil	Bolivia	Panama
	Bulgaria	Colombia	Peru
	Chile	Congo	Poland
	China	Costa Rica	Senegal
	Cuba	Ecuador	Spain
	Egypt	Indonesia	Sudan
	Greece	Iran	Thailand
	Hungary	Iraq	Trinidad & Tobago
	Nigeria	Kuwait	Tunisia
	Venezuela	Mauritania	Turkey
	Yugoslavia	Mauritius	Vietnam
		Mexico	Zaire

A Panel of Scientific Advisers (PSA) comprising some of the most eminent researchers in genetic engineering and biotechnology has been set up to assist and advise the Preparatory Committee in its effort to establish and subsequently guide development of the Centre. ICGEB has been established as a twin centre having two component laboratories, one in Trieste, Italy and one in New Delhi, India with an additional network of affiliated centres in member countries. Direction of the Centre, as a whole, resides in Trieste.

Panel of Scientific Advisers

L.L. Cavalli-Sforza (Chairman)

A. Chakrabarty
P. Chambon
R. Haselkorn
A. Kornberg
J. Lederberg
S. Narang
W.J. Rutter
J. Salk
M.S. Swaminathan
C.C. Tan
R. Wu
F. Bolivar Zapata

In 1986 Prof. Irwin C. Gunsalus (Professor Emeritus of Biochemistry at the University of Illinois, IL, USA) was appointed as Director of ICGEB. Subsequently, Prof. Arturo Falaschi (formerly Director of the Institute of Genetics of the National Research Council in Pavia, Italy) and Prof. Krishna K. Tewari (Professor and Chairman of Molecular Biology and Biochemistry, University of California at Irvine, CA, USA) were selected respectively as the Heads of the two Trieste and New Delhi component laboratories.

After three years of dedicated service in the establishment of the basic activities of the Centre, Prof. Gunsalus returned to the USA, and Prof. Falaschi was appointed as Director. Prof. Francisco Baralle (formerly Senior Lecturer at the University of Oxford, UK) was appointed Head of the Trieste Component in September 1990.

In order to initiate the establishment of the Centre, the Preparatory Committee launched an Interim Programme that ran for a three-year period from 1986 to 1989. A Five-Year Programme with a budget of US$56 million was initiated in July 1989 to allow for continued development of the Centre's facilities, recruitment of scientific staff, etc., over the period 1989 to 1994.

To provide stability for its staff and unhindered continuation of the research activities of the Centre the Preparatory Committee decided to treat the new programme as a rolling five-year one, in that it will be annually reviewed and extended by an additional year. In line with this principle, the programme was extended in 1990 by a year to 30 June 1995 with additional resources provided by the Government of Italy.

Planned expenditures for the core activities of ICGEB
1 July 1990 to 30 June 1995

	Total	1990	1991	1992	1993	1994	1995
Personnel	23,393	1,813	4,288	4,675	4,825	5,174	2,618
CRP	5,000	500	1,000	1,000	1,000	1,000	500
Training	8,613	333	1,550	1,898	2,086	1,610	1,136
Equipment	14,355	1,245	3,004	2,980	3,231	2,430	1,465
Premises and Sundries	4,393	330	659	946	1,128	928	402
Totai	55,754	4,221	10,501	11,499	12,270	11,142	6,121

Figures for 1990 and 1995 are for six months only
Figures in thousands of U.S.$

Research Programme

The Trieste component

Facilities

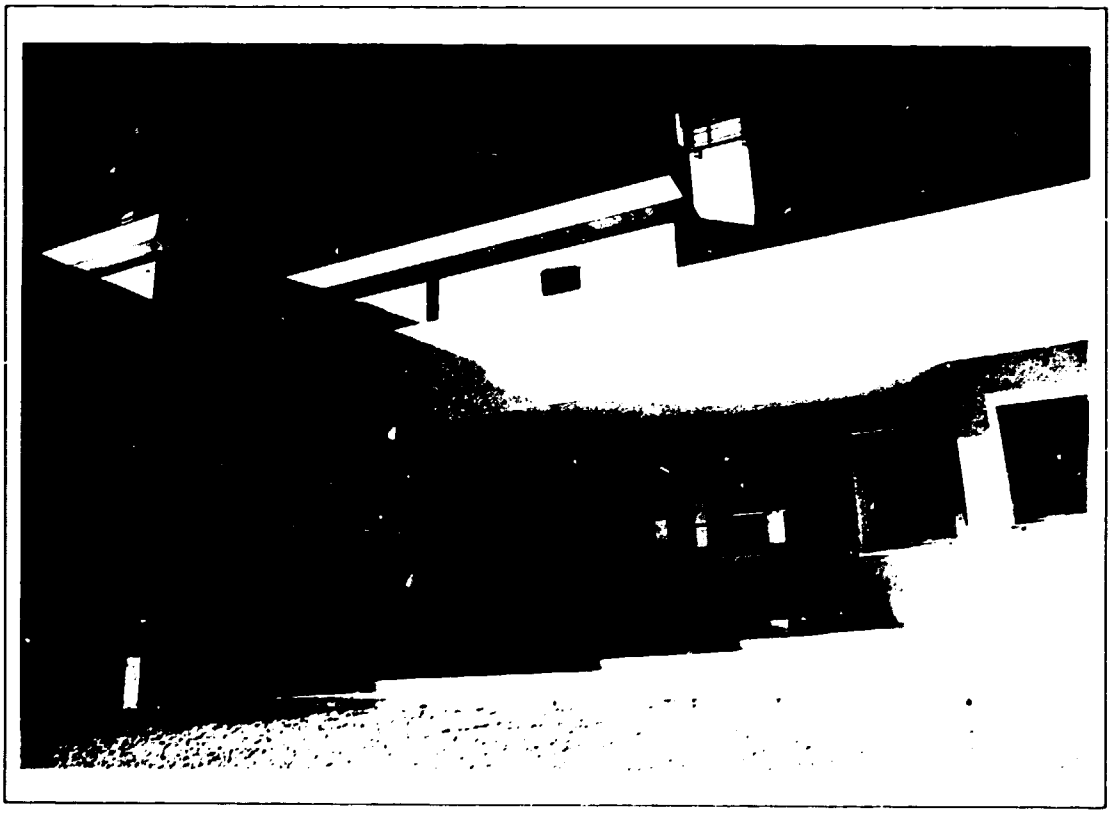
The Trieste Component and the direction of the ICGEB are located in the Trieste Research Area (AREA), a recently created science park in the outskirts of the city. Presently they are utilizing the F1 building (1024 sq.m.), the F2 building (930 sq. m.) and the F building (350 sq. m.), which are part of the original offer of the Italian Government.

The F1 building houses the administration for the Centre and for the Component, the library, plus the laboratories for the Cell - Molecular Biology Group and the Virology Group.

Building F2 houses: the stockroom, the dishwashing and sterilization facilities, the Head of Component's office, and the groups on Molecular Pathology, Immunology, Protein Structure and Function, and Microbiology.

The F building contains a technical shop for mechanical and electronic works, more storerooms and the chemical waste treatment facility.

The W building (5,400 sq.m.) has now started construction and is expected to be available by the middle of 1992. When the W building is available the ICGEB will return the F2 building to the Area.



Entrance and outside view of the F1 building, housing the direction of the Centre and part of the laboratories of the Trieste Component. These are located in the Trieste Research Area, a recently created science park in the outskirts of the city. The final premises will be completed in 1992 and will consist of approximately 7000 m^2 of laboratory and office space.

An auditorium (150 seats), a teaching lab., (24 places) and a cafeteria are available in the Area.

A guesthouse present in the Research Area is provisionally occupied by other Area initiatives (Synchrotron offices) and not available for the time being (possibly, at the end of 1991). The Area is offering, for this interim period, *ad hoc* agreements with nearby hotels, coupled with a shuttle service between them and ICGEB.

Personnel

Names in italic type correspond to ICGEB appointments

ICGEB Centre

Director	*Arturo Falaschi*
Personal Assistant to the Director	*Elena Stubel*
Programme Assistants for	*Susan Vincent Carli*
Collaborative Research and Training	*Diana Viti*
Senior Administrative Officer	*Mario Marchich**
Secretary to Senior Administrative Officer	*Loredana Ledovich**
Junior Associate Expert	Sanjay Pahwa
(Ministry of Foreign Affairs, Italy)	
Science Coordinator (Vienna Office)	*George Tzotzos*

Trieste Component

Head of Component:	*Francisco Baralle*
Personal Assistant to the Head of Component	*Ann Crum*
Secretary to the Head of Component	*Maria Santoro Airey*

General Administration Services

Accounting Clerk	*Giulia Bon Casseler*
Accounting Assistant	*Françoise Misiti Rusich*
Librarian	*Eleonora Millo*
Driver, Messenger	*Carlo Fratnik*
Receptionist, Switchboard, Typist	*Alessandra Borghesi*

Technical Services, Building, Safety

Responsible Officer	*Bodil Holle*
Stores Keeper	*Giorgio Pattavina*
Precision Mechanic	*Walter Schmitt*
Technician	*Fabiana Leuz*
Technician	*Mirella Dell'Oste*
Technician	*Carlo Gregori*

* Also servicing Trieste Component

One of the laboratories of the Trieste Component.

Procurement Unit

Responsible Officer	*Francisco Baralle*
Purchasing Assistant	*Ann Crum*
Typist, Assistant	*Grazia Denardo Spina*

Tissue Culture Services

Responsible Officer	*Georgine Faulkner Valle*
Technician	*Maria Elena Lopez*
Technician	*Sulena Polez*

Computer Services

Responsible Officer	*Sandor Pongor*
Associate Research Scientist	*Mark Vandevar*

Research Groups

Molecular and Cellular Biology

Senior Scientist (Director)	*Arturo Falaschi*
Associate Research Scientist	*Eva Csordas Toth*
Junior Scientist	*Narendra Intera*
Junior Scientist	*Mauro Giacca*
Junior Associate Expert (Ministry of Foreign Affairs, Italy)	Lorena Zentilin
Junior Associate Expert (Ministry of Foreign Affairs, Italy)	Giovanna Contreas
Trainee	*Li Ye Kang*
Trainee	*Dimiter Demirov*
Trainee	*Khalilur Rahman*
Graduate Student (International School for Advanced Studies - ISAS)	Francesca Demarchi
Graduate Student (ISAS)	Lidija Marusic
Graduate Student (ISAS)	Paolo Norio
Graduate Student (ISAS)	Gabriele Grassi
Fellow (Area di Ricerca, Trieste)	Giovanni Maria Severini
Fellow (Area di Ricerca, Trieste)	Silvia Diviacco
Technician	*Alexander Ochem*
Technician	*Maria Ines Gutierrez*

Genome Studies

Senior Scientist (Consultant)	*Claudio Schneider*
Junior Associate Expert (Ministry of Foreign Affairs, Italy)	Giannino Del Sal
Fellow (University of Trieste)	Guidalberto Manfioletti
Fellow (University of Trieste)	Elisabetta Ruaro Manfioletti
Fellow (Area di Ricerca)	Claudio Brancolini
Fellow (EMBO)	Catrine Albretsen Kvam
Graduate Student (ISAS)	Stefano Gustincich
Graduate Student (ISAS)	Rene Ufrera
Technician	*Michela Logar*
Technician	*Stefano Bottega*
Technician	*Federico Volpatti*

Virology	Senior Scientist	*Lawrence Banks*
	Associate Research Scientist	*Georgine Faulkner Valle*
	Junior Scientist	*Veronique Bouvard*
	Junior Scientist	*Alan Storey*
	Junior Scientist	*David Pim*
	Trainee	*Shang-Zhong Xi*
	Technician	*Paola Massimi*
Microbiology	Senior Scientist	*Carlo Bruschi*
	Junior Scientist	*Jacques Oberto*
	Trainee	*Prasert Suntinanalert*
	Trainee	*Maria Szakacs-Dobozi*
	Trainee	*Sarita Nazareth*
	Trainee	*Shoukat Parvez*
	Trainee	*Luis Rodriguez - Menocal*
	Technician	*Simone Ugolini*
	Technician	*Dale Ludwig*
	Technician	*Maristella Coglievina*
Protein Structure and Function	Senior Scientist	*Sandor Pongor*
	Trainee	*Alessandro Tossi*
	Trainee	*Gyorgy Simon*
	Trainee	*Jianwen Zhang*
	Technician	*Catherine Ludlow*
	Technician	*Rudolph Paladini*
Molecular pathology	Senior Scientist (Head of Component)	*Francisco Baralle*
	Junior Scientist	*Sergio Tisminetzky*
	Junior Scientist	*Renu Tuteja*
	Junior Scientist	*Rodolfo Garcia*
	Junior Scientist	*Eduardo Scodeller*
	Trainee	*Carlos Melo*
	Technician	*Giulia Devescovi*
	Technician	Bianca Rosa Guerra
Molecular immunology	Senior Scientist	*Oscar Burrone*
	Trainee	*Ileana Gonzalez-Rodriguez*
	Technician	*Sabrina Mancardi*

Research Staff: 50
Laboratory Technicians: 20
Administrative Staff: 16

Total: 86

Cell and Molecular Biology

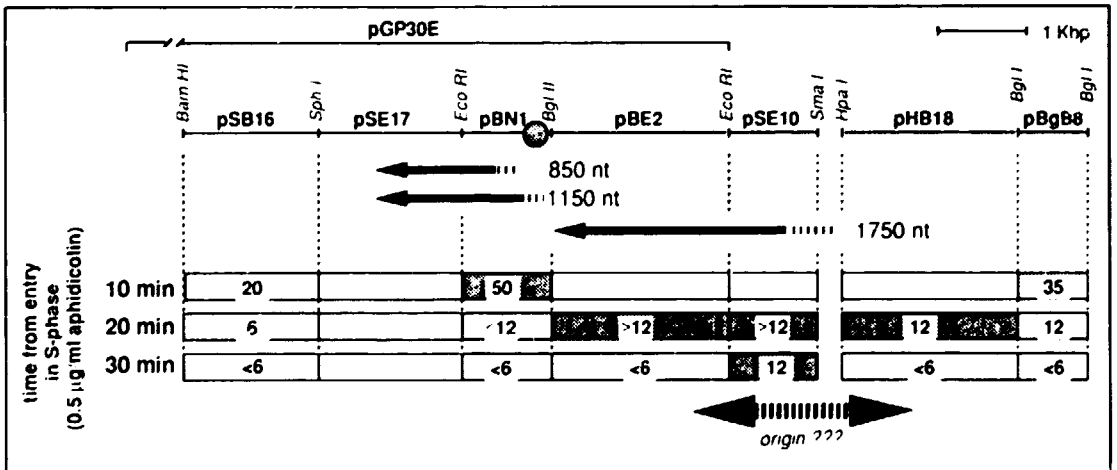
A. Falaschi, E.C. Toth, N. Tuteja, M. Giacca, L. Zentilin, G. Contreas, L.Y. Kang, D. Demirov, K. Rahman, F. Demarchi, L. Marusic, P. Norio, G. Grassi, G.M. Severini, S. Diviacco A. Ochem, M.I. Gutierrez

This programme addresses the study of the molecular aspects of the regulation of DNA replication in human cells. An understanding of this phenomenon may bring important light on the way by which higher organisms develop normally from the product of fertilization to the adult individual; furthermore it may bring light on the breakdown of regulation which causes the uncontrolled cell proliferation typical of tumoral growth. Finally, the availability of functional replication origins may allow the construction of new physiological vectors for cloning in mammalian cells.

Identification of replication origins

A functional human DNA replication origin has been located within 2 Kb of a 13.7 Kb genomic region from human chromosome 19, which had been originally cloned for its property of being replicated within the first two minutes after entry in S phase of human HL60 cells synchronized with aphidicolin. Most of the 13.7 Kb sequence was determined.

The movement of the replicative fork inside this large DNA region will be followed in order to identify with the highest possible resolution the actual site where the replication machinery is assembled. The exact initiation site will be determined by physically mapping the elongation of density labelled DNA by hybridization and quantitative PCR experiments, using, as probes, subcloned fragments of the region and specific oligonucleotide primers, respectively.



Localization of an origin of DNA replication of human cells. The DNA synthesized at the very onset of the S-phase in synchronized HL60 cells was isolated and hybridized to different DNA probes encompassing a putative replication origin. The intensity of the shading and the numbers indicate the extent of hybridization with the probes whose relative positions in the chromosome are indicated in the upper portion of the figure. This portion of the chromosome is heavily transcribed and the three main transcripts are indicated by the black arrows. The replication appears to start within the DNA region corresponding to probe pSE10.

Regulation of gene expression around an origin

The region around the origin is heavily transcribed, giving rise to several transcripts, some of which are differentially expressed in various cell lines and tissues. The upstream region of the two major mRNAs was characterized in detail. It contains a 600 bp long CpG-rich area with the properties of an HTF island. At one extreme of this region and very close to the presumed origin, a 17 bp sequence was located, which specifically binds to nuclear proteins. This sequence contains the core dyad symmetry element CACPuTG, a binding motif which constitutes a general cis-regulating element conserved throughout evolution as the target of a number of nuclear factors that share the same DNA binding specificity albeit in the context of different functions. In particular, it is analogous to the upstream element of the Major Late Promoter of Adenovirus 2, and is found present in the Long Terminal Repeat (LTR) of the Human Immunodeficiency Virus type 1 (HIV-1). South-Western type experiments revealed that at least three nuclear proteins are able to bind specifically to this sequence, of approximately 44, 70 and 110 kDa. The purification of these sequence-specific DNA binding proteins from HeLa cells is well advanced by using conventional ion-exchange chromatography, fast flow protein liquid chromatography and affinity chromatography on synthetic oligonucleotide-Sepharose matrix, containing several copies of the binding site. One of these specific proteins (the 44 kDa one) most probably corresponds to the Major Late Transcription Factor (MLTF USF) which was already proven to be involved in transcription initiation, both *in vivo* and *in vitro*. The structural and functional characterization of the three dominant sequence-specific DNA binding proteins is in progress.

Since the CACPuTG binding motif is also present in the HIV-1 LTRs, the question of its role in the viral transcriptional control was also addressed. Reporter gene expression experiments suggested that the HIV-1 binding site is a negative regulator of transcription, and that most of the negative function exerted by the Negative Regulatory Element of the LTR can be attributed to its activity.

Construction of novel linear vectors for human cells

One of the objectives of the present programme concerns the possibility of constructing linear vectors suitable for the introduction of foreign genes into higher eukaryotic cells. Such vectors should contain ends that behave as telomers, eukaryotic origins of replication, and should allow a cloning capacity of several kilobases of foreign DNA. These requirements can be satisfied by a vector containing autonomous parvovirus termini at the extremities - which should behave as telomers - and a chromosomal origin of replication in the middle. Several constructs based on the linear genome of the Minute Virus of Mice (MVM) and containing the SV40 or EBV origins of replication have been tested in permissive cells. Preliminary observations indicate that an inhibitory effect exists between functions encoded by the parvovirus genome and the functionality of these viral origins. This effect acts exclusively on sequences present *cis* with respect to the parvovirus genome, as concerns SV40 ori, while is present also *trans* in cotransfection experiments with EBV oriP. The molecular mechanisms responsible for these effects are currently under investigation.

Purification of DNA helicases from human cells

In parallel, we have started the purification and characterization of DNA helicases from human cells. These enzymes are known to be involved in the activation of origins and in the advancement of the growing fork (as well as in other aspects of nucleic acid metabolism). We have identified the

presence of four different DNA helicases in HeLa cells. One of them, named human DNA helicase I (HDH I), has already been purified and characterized. The direction of unwinding is 3' to 5' with respect to the single-stranded DNA to which the enzyme is bound. Among the other three DNA helicases that we have identified so far, HDH II and HDH III appear to have the same polarity as HDH I. We have also purified another species, namely HDH IV, which moves in the 5' to 3' direction along the DNA strand to which it binds. The purification of other species (HDH II and III) is in progress.

Genome Studies

C. Schneider, G. Del Sal, G. Manfioletti, E.R. Manfioletti,
C. Brancolini, C.A. Kvam, S. Gustincich, R. Utrera, M. Fogar,
S. Bottega, F. Volpatti

This group is studying a problem closely related to the one mentioned in the previous paragraph, namely the identification of the molecules which control the progress of the cells into the phase assuring replication of DNA. The analysis of a group of genes that are specifically expressed at growth arrest (gas) is shedding new light on the negative regulators of the 'in cycle' entry process. Furthermore we have identified, at the biological response level, an mRNA fraction derived from cells in active S phase that is able to induce recipient cells into the S phase. In order to experimentally address the above points, this group has developed and currently utilizes the first prototype of a highly sophisticated technique for automatic microinjection of macromolecules into the cell nucleus.

The group has also developed a highly innovative technology for the easy purification of cloned DNA fragments and genomic DNA of various origin.

NIH 3T3 fibroblast, viewed by laser scanning microscope. An automatic microinjection apparatus, available at ICGEB-Trieste, is utilized to study the function of genes involved in the regulation of progression in the cell cycle of mammalian cells.

which is an essential step towards permitting rapid and automated sequencing of large genomes, like the human one. They are furthermore progressing into the automation of the subsequent steps of DNA sequencing.

Virology

L. Banks, G. Faulkner, V. Bouvard, A. Storey, D. Pim, S. Xi, P. Massimi

The virology laboratory focuses its attention on the Human Papillomaviruses (HPVs). These viruses have been implicated as causative agents in a variety of human cancers, particularly uterine cervical cancers. Cervical cancer is particularly devastating in developing countries, with this being the second major cause of cancer related death in women after breast cancer. The work of the group is primarily aimed at elucidating the mechanisms by which HPV brings about cell transformation. The results of these studies will provide the means for designing strategies aimed at the prevention and treatment of HPV associated disease.

Biochemical studies on the HPV E7 proteins

The major tranforming gene of HPV-16 in rodent cells is encoded by the E7 open reading frame (ORF). Mutational analysis of this gene has shown that a major component of this activity is the ability of the protein product to bind the product of the retinoblastoma (rb) tumour suppressor gene. In addition we have shown that the region of the E7 protein responsible for this activity can also stimulate cellular DNA synthesis in serum starved NIH3T3 cells.

Current studies are now directed towards determining whether the E7 genes from the non oncogenic HPV types 6 and 11 can also stimulate cellular DNA synthesis. Our initial results indicate that these E7 genes have a markedly reduced ability to induce cellular DNA synthesis. Studies are also in progress to investigate the ability of the HPV-16 E7 gene to induce cellular DNA synthesis in cells deficient in the rb gene. This will tell us whether binding rb is essential for this activity of the E7 protein.

Loss of E7 expression correlates with increased c-myc expression

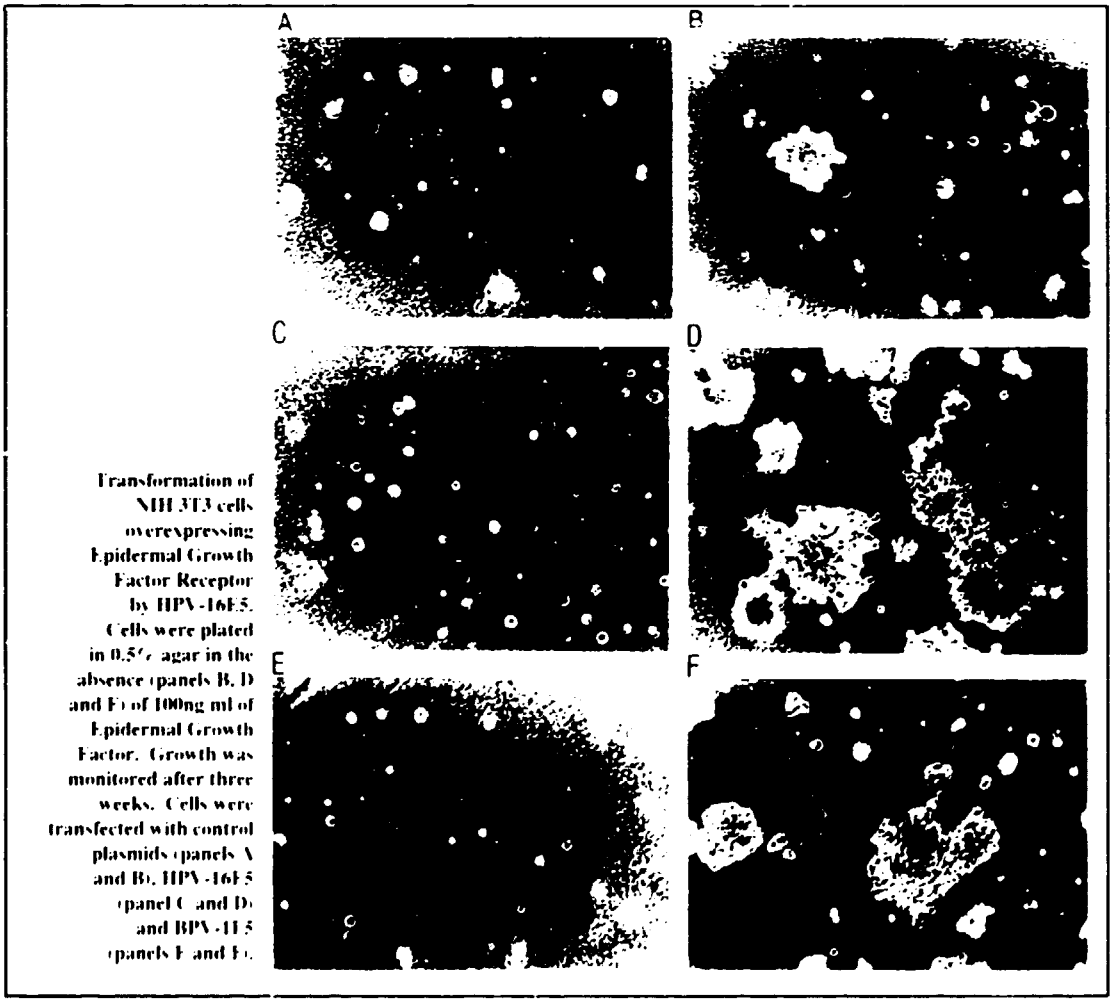
A rodent cell line transformed by an inducible HPV-16 E7 gene and constitutive EJ-ras was developed. This enabled us to demonstrate that continued E7 gene expression is required for maintenance of the transformed phenotype of these cells. Removal of E7 gene expression resulted in a cessation of cell growth. Studies were then initiated to isolate mutant cell lines which lost the requirement for continued E7 expression, with the intention of identifying cellular genes which could replace E7 activity. Several such lines were obtained and further analysis revealed that all such lines overexpressed the cellular c-myc gene. This indicates that there are certain similarities between the activities of E7 and c-myc in this system. Interestingly, amplifications of c-myc have been reported in a large number of cervical cancers and it remains to be seen whether this is of significance in the development of the tumour.

Activated macrophages specifically recognise E7 containing cells

It has been previously reported that HPV-16 containing NIH3T3 cells were specifically killed by mouse activated macrophages. To identify if any individual HPV gene products were responsible for this activity cell lines were generated containing each of the HPV-16 ORFs. Only lines containing the E7 gene were killed by the macrophages. Mutational analysis revealed that E7 mutated in the rb binding domain failed to stimulate macrophage killing. Other transformation defective E7 mutants could stimulate the macrophages. Studies are now in progress to identify which cellular genes are activated by E7 and may be responsible for the macrophage recognition.

Identification of the transforming activity of the HPV E5 gene

Most of the emphasis in transformation studies to date has been placed on the E6 and E7 genes with their well documented involvement in cell transformation. Following a recent report on Bovine Papillomavirus showing that the E5 gene could modulate the activity of the Epidermal Growth Factor Receptor (EGFR), we decided to further investigate the function of the HPV E5 gene product. Studies were performed on cells expressing high levels of the EGFR. Under normal circumstances these cells give weak anchorage independent growth (a measure of transformation) in the presence of Epidermal Growth Factor (EGF). We have shown however in cells containing the HPV E5 gene that this activity is greatly increased. In addition



Transformation of NIH 3T3 cells overexpressing Epidermal Growth Factor Receptor by HPV-16E5. Cells were plated in 0.5% agar in the absence (panels B, D and F) of 100ng ml of Epidermal Growth Factor. Growth was monitored after three weeks. Cells were transfected with control plasmids (panels A and B), HPV-16E5 (panel C and D) and BPV-1E5 (panels E and F).

these cells respond to much lower concentrations of EGF than cells lacking E5 sequences. This phenomenon appears to be true for all the E5 genes so far investigated, both from the oncogenic and benign HPVs. This suggests that HPVs may interact with the EGFR to stimulate cell growth.

Interestingly many cervical tumours have amplified EGFR levels of expression. The E5 gene is often deleted in later stage tumours and it is possible therefore that E5 may initially interact with the EGFR to stimulate cell growth, but later stage amplification of the EGFR renders the continued presence of E5 unecessary. Studies are now in progress to define the mechanism by which E5 interacts with the EGFR and to determine if other Growth Factor Receptors can be similarly affected.

Identification of transfomation defective HPV-16 from a normal cervix

Present epidemiology indicates that HPV is present in over 95% of human cervical tumours and in 10% of the normal population. Clearly not all this 10% will go on to develop cervical cancer and additional factors must be involved. HPV-16 was cloned from a biopsy taken from a histologically normal cervix. Transformation studies with this virus in rodent cells indicated that it was fully active. However transformation assays in cervical keratinocytes indicated that this virus failed to bring about immortalisation. Sequence analysis revealed normal E6 and E7 ORFs but the presence of a chain termination mutation in the E2 ORF. This study indicates that the inability of E2 to transactivate the expression of the E6 and E7 genes and their resultant lower levels of expression is responsible for the inability of this virus to bring about the transformation of human keratinocyte cells. Studies are now in progress to further characterise this naturally occurring mutant and to attempt to identify any additional mutant HPV-16 in normal cervix. Clearly this result has considerable significance for the epidemiology linking HPV-16 with cervical cancer.

Modulation of HPV-16 gene expression by E6

Transfection of the HPV-16 E6 gene into a cell line derived from a cervical carcinoma(SiHa) containing HPV-16 DNA has produced a number of interesting observations. Three different E6 constructs were transfected into these cells and selected for G418 resistance. The number of colonies able to grow was markedly affected by the particular E6 construct: two produced considerably more colonies above background whereas one decreased the number of colonies. Further analysis of the effect of the E6 constructs on CAT activity of the HPV-16 promoter or the adenovirus E2 promoter gives comparable results. Thus the E6 that stimulate colony formation activate gene expression whereas the E6 that inhibits colony formation downregulates gene expression. Studies are now in progress to identify the E6 responsive element in the HPV-16 regulatory region and to determine the mechanism of E6 action in controlling gene expression.

Characterisation of the E6-p53 protein interaction

Recent reports have indicated that a possible mechanism by which the HPV-16 E6 gene can bring about transformation is through an interaction with the cellular p53 tumour suppressor gene. Studies done in collaboration with C. Schneider and E. Ruaro of the Genome Studies laboratory indicate that p53 can inhibit cellular DNA synthesis. Work is now in progress to determine what effect the presence of the HPV E6 gene has upon this activity. Initial results indicate that E6 can inhibit the effect of the p53 gene and the mechanism of this activity is currently being investigated.

Effects of anti-sense phosphorothioate oligonucleotides on

Anti-sense oligonucleotides have been shown to modulate the expression of several viruses, including human immunodeficiency, and herpes viruses, and certain activated cellular oncogenes. We have investigated the use of these compounds as anti-HPV agents. A range of nuclease resistant phosphorothioate oligonucleotides complementary to the HPV16 E6, E7 and regulatory region were synthesised and their effects on cells containing HPV16 were examined. Oligos which overlap the initiation codons of either the E6 or E7 genes inhibit cell proliferation, determined by thymidine uptake. However, there was only slight decrease in the rates of synthesis and steady state levels of the E6 and E7 proteins. The results suggested that the observed inhibitory effects were mostly due to the binding of the oligonucleotides to cellular DNA sequences, and casts doubt over their use as modulators of HPV gene function or as potential therapeutic agents.

Transformation of human cervical keratinocytes

Most studies on the transforming activities of HPVs have so far been performed in rodent cell systems. Clearly if we are to make valid statements as to the role of HPV in human cancer these studies need to be performed in the cells which HPV normally infects *in vivo*. Thus we have developed systems for the propagation of human cervical keratinocytes in the laboratory. Initial studies now indicate that we can achieve immortalisation of these cells by HPV-16. We are now in a position to investigate the effects of HPV-16 upon the differentiation of these cells which will in turn provide valuable information on the control processes involved in regulating viral DNA replication.

Baculovirus expression of HPV-16 capsid proteins

Since no culture system is available for the propagation of HPV in the laboratory any studies on the viral late proteins have to be done using a variety of eukaryotic expression systems. We were interested in performing detailed characterisation of the HPV-16 capsid proteins with a view to using these as a possible means of intervention into HPV infection. The viral L1 and L2 proteins have now been expressed in a Baculovirus expression system. Both proteins appear to be phoshorylated and glycosylated. Expression of both proteins within the same cell results in a complex formation between the two viral capsid proteins. Studies are now in progress to determine whether this protein complex represents partial capsid assembly within these cells. In addition the Baculovirus produced L1 and L2 proteins are being used to investigate the human immune response to these proteins in both patients with obvious HPV lesions and in those who are asymptomatic.

Microbiology

C. Bruschi, J. Oberto, P. Suntinanalert, M. Szakacs-Dobozoi, S. Nazareth, S. Parvez, L. Rodriguez-Menocal, S. Ugolini, D. Ludwig, M. Coglievina

Research activity began around June 1990, following the establishment of the Microbiology Programme which started in January 1990. During the last six months activity has focused upon two of the three areas of research which have been proposed initially and, in particular: (i) microbial biodegradation of lignin; (ii) development of multi-host shuttle-vector systems for

the molecular cloning of biotechnologically relevant genes>

New facilities have been assigned to the Microbiology Programme consisting of the second floor of the F2 building of the Research AREA of Trieste, which includes office and laboratory space. These facilities allow the establishment of two physical laboratories, which is still underway. Almost all the equipment necessary for the implementation of the research activity has been installed. The Microbiology Programmeis now fully operational.

Microbial degradation of lignin

Four projects are currently in progress on this subject:

(1) In collaboration with the group of Prof. Piero Susmel, of the Institute for Animal Production of the University of Udine at Pagnacco, we have started to analyze the bovine's rumen microorganisms for their ability to degrade anaerobically lignin model compounds such as: *cinnamic acid*, *coumaric acid* and *ferulic acid* which are characteristic of soft woods, and *syringic acid*, *vanillic acid* and *vanillin*, which are characteristic of hard woods. The results show that the mixture of microorganisms from the rumen is able to degrade up 100% of the soft wood lignin model compounds in less than a week, while the hard wood compounds are degraded by only 20%. We then proceeded to isolate single microorganisms individually capable of the degradation. Several individual bacteria and one fungus were isolated and their identification is in progress. To this end the installation of the new anaerobic culture chamber facility will allow us from now on to continue the experiments at ICGEB instead of Pagnacco.

(2) A second project to clone and characterize the p-hydroxycinnamic acid decarboxylase from *Fusarium solani* has started. This enzyme is responsible, among others, for the decarboxylation of ferulic acid to 4-vinylguaiacol, a first important step in the oxidative degradation of lignin. We have constructed a colorimetric assay to detect mutants of *F. solani* unable to carry out the decarboxylation of ferulic acid. Using this visual screening, together with a nystatin-enrichment selection, we are searching for decarboxylase deficient mutants induced by chemical (MMS) and physical (UV) agents. At the same time we are also screening directly for ferulic acid resistant mutants.

(3) A third project is aimed at the study of the molecular mechanism of ligninase induction by veratryl alcohol in *Phanerochaete chrysosporium*. Veratryl alcohol is a catabolic product of lignin biodegradation and its mechanism of action seems to be rather complex. In addition to exerting an inducing effect on ligninase gene expression, it seems also able to inhibit peroxidase enzymes that inactivate the ligninases. We have begun to screen several lignin-degrading microorganisms, prokaryotic and eukaryotic, to determine whether the inducibility of the ligninase enzymes is a conserved characteristic between the two phyla. Ligninase activity of the various microorganisms will be measured by established colorimetric assays.

(4) A new, fourth project deals with the expression of known ligninase genes in the yeast *Saccharomyces cerevisiae*, using our high-expression vector pBLITZ, the first produced at ICGEB. The cloned ligninase gene Lib-Hb will be inserted into an inducible expression cassette and transformed into a recipient yeast strain which is deficient for endopeptidase processing of native proteins. The expression of a functional ligninase enzyme will be assayed with the same systems utilized for the third project.

Multi-host shuttle vector systems

Five projects are presently in progress on this subject:

(1) The construction of a pYAC (Yeast Artificial Chromosome) shuttle-vector able to re-circularize *in vivo* upon induction, called pYACHT, is almost completed. We have employed the yeast natural 2u DNA plasmid's site-specific recombination system to provide the substrate for re-circularization by the FLP recombinase. As soon as the construction will be finished we will address: (a) the re-circularization *in vivo* of a tester pYACHT carrying a 100 kilo-base human DNA fragment and, (b) the ability of this plasmid to replicate in *E. coli* when carrying very large amounts of cloned DNA, up to one magabase. Other bacterial origins of replication, such as the one from the F' plasmid and the ones from large R-plasmids will be tested.

(2) A second project deals with the cloning and sequencing of a yeast DNA fragment which we identified in the United States a few years ago, which seems to be able to stabilize *in cis* plasmids carrying a chromosomal centromere region. Several DNA subclones are under investigation to map the genetic determinant(s) responsible for this characteristic. Once the DNA region will be determined, sequencing and DNA-binding assay will be performed on it to further characterize the properties of this element.

(3) A third new large project has begun, in collaboration with the Molecular Biology Programme of Prof. Arturo Falaschi to construct a Human Artificial Chromosome, HAC. To insert the various structural elements of a human chromosome we will utilize, as origin of replication, the Parvovirus MVM (Minute Virus of Mice), a single-stranded 5.1 kilo-base DNA virus that replicates with a complex mechanism similar to the one of telomere replication. To this end we are first attempting to express the virus NS1 protein which is essential for DNA synthesis in mammalian cells and also controls expression of the non-structural proteins of the virus, VP1 and VP2. Once it has been demonstrated that this protein in non lethal for the cell, we will proceed to verify the complete replication of the vector in

Phenotypic expression of recombinant plasmid stability in the yeast *Saccharomyces cerevisiae* during clonal growth of a single colony. The sectorial pigmentation of the colony is due to plasmid loss during mitotic cell division.

mammalian cells by the maintenance of a selectable marker and CHEF pulse-field electrophoresis.

(4) The construction of an inducible, nonselectable high expression vector, based on the *in vivo* release of the natural 2u DNA plasmid by FLP site-specific recombination has been completed. The results of the tests of the stability and copy number of this plasmid have been reported in a paper which is now in press. We are now proceeding to analyze quantitatively the expression of various heterologous genes among which: (i) the F and HN surface antigenic glycoproteins from New Castle Disease Virus (NDV), in collaboration with the Department of Microbiology and Immunology of East Carolina University of Greenville, North Carolina, USA, and (ii) the mammalian tumor necrosis factor (TNF) in collaboration with the Boris Kidric Institute of Chemistry of Ljubljana, Yugoslavia and the penicillin acylase from *E. coli*, in collaboration with the Institute of Molecular Genetics and Genetic Engineering of Belgrade, Yugoslavia.

(5) A new project on the cloning of the telomerase of *Borrelia burgdorferi*, the etiological agent of Lyme disease, which is endemic in the Carso area between North-Eastern Italy and Yugoslavia, is in its initial stage of development. This spirochaeta is the only known eukaryotic microorganism which has a linear genome (chromosome and plasmids). The research, which has been established in collaboration with the Institute of Microbiology of the School of Medicine of the University of Trieste, should lead to the possibility to obtain a specific therapeutic system to inhibit the early replication of the bacteria after infection, by the interference with the regulation and or the activity of the telomerase thus preventing DNA synthesis.

Regulation of gene expression

Two projects on this subject are at their initial stage of development:
(i) the continuation of the study involving the regulation of the expression of the cloned yeast cell-division-cycle gene *CDC6*;
(ii) the cloning and sequencing of the yeast *RLC4 gene*, which is essential to regulate the proper dynamics of DNA recombination, in collaboration with the group of Dr. Michael Esposito, at the Cell & Molecular Biology Division of Lawrence Berkeley Laboratory, University of California, Berkeley, USA.

Protein Structure and Function

S. Pongor, A. Tossi, G. Simon, J. Zhang, C. Ludlow, R. Paladini

The fundamental problem of protein engineering is the understanding of structural principles that underly specific functions of proteins and their genes. The knowledge gained from a better understanding of this problem will play a key role in designing new proteins for practical uses, such as vaccines and other therapeutic agents. Our interest is centered around the study of structural domains that convey a specific function to a variety of otherwise unrelated proteins. Such domains are required for binding proteins to receptors, to nucleic acids and to biological membranes and the interactions involved play important roles in many pathological processes.

We are using three approaches:

1) biocomputing methods are developed in order to extract structural principles from databases;
2) techniques of molecular modelling and molecular mechanics are used to design and test 3D models of the peptides;
3) the peptides are produced by methods of protein chemistry and molecular biology. The experimental approaches are as follows:

Biocomputing

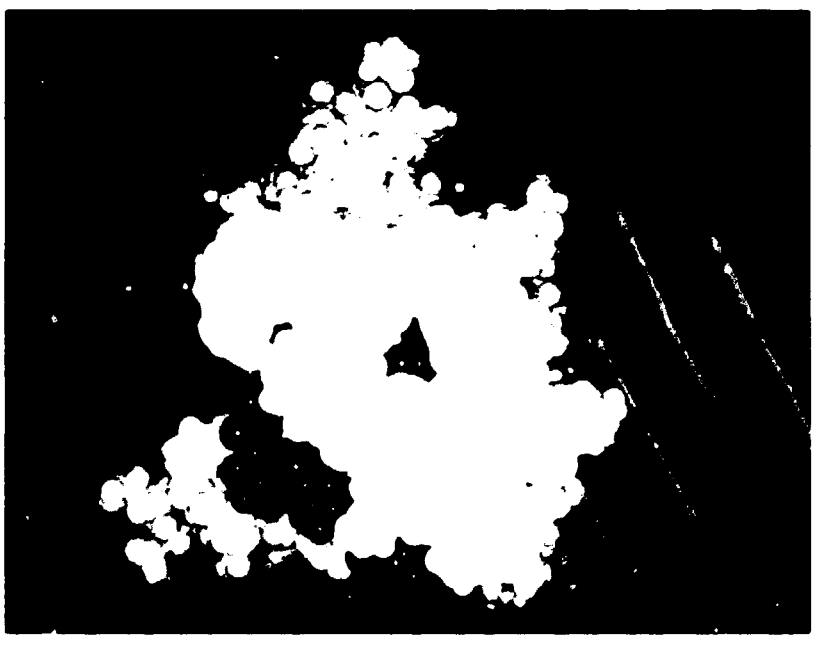
The long term goal is the application of computational methods to the rational design of proteins and their genes. Our efforts centre around methods for building rational consensus models from existing structures, this being a fundamental problem of molecular modelling.

We are continuing our efforts to develop specific pattern search techniques for macromolecular databases. The programmes are developed in C and run on a SUN 4 390 under UNIX BSD 4.2. The programming environment includes the sequence databases EMBL, Genbank, Swiss-prot and PIR, the Intelligenetics package for sequence analysis, as well as public domain softwares such as FastA and the Phylip programme package for phylogenies.

Part of this research is carried out in collaboration with the ABC Institute of Protein Research, Godollo, Hungary using their VAX VMS system that features the GCG analysis programs along with its programming library.

Molecular modelling

Techniques of molecular graphics and mechanics are used to model peptides and to study their interaction with other macromolecules. This programme is a collaborative effort with the International Centre for Pure and Applied Chemistry whose researchers use similar techniques for the study of organic molecules and polymers. A Silicon Graphics IRIS workstation 4D 210 GTX was purchased in 1990 and Insight and Discover of BIOSYM.



Interleukin-1 antagonist model structure generated with the molecular graphics programme Insight. Computer-aided design is used to improve the properties of anti-inflammatory agents.

powerful programme packages for building and modelling molecular structures were installed along with public domain softwares such as AMBER. Molecular dynamics studies of peptides in various interactions were initiated in collaboration with Dr. B. Schurmann (Max-Planck-Institute, Mainz).

Protein chemistry

This laboratory employs the techniques of peptide synthesis and N-terminal sequencing, and both methods are provided as a service to ICGEB researchers. Part of this work is carried out in collaboration with the ABC Institute of Protein Research, Gödöllö, Hungary. Solid phase peptide synthesis is carried out using the FMOC (Milligen 9050) and the tBOC (Applied BioSystem 430) methodologies. The peptides are purified by reverse phase HPLC, characterized by PICO-TAG amino acid analysis and N-terminal sequencing. In 1990, a total of 16 peptides were synthesized. Sequencing is carried out on an Applied BioSystems 477 sequencer.

The subjects under study are described in the following.

Detection of structural motifs in protein sequences

Current alignment algorithms are sufficiently fast to search large sequence databases for homologous sequences. Interpretation of the results becomes an increasing problem in the range of 15-25% identities, even though distant similarities (like those between domains that are well conserved among the members of a superfamily) are very important for biologists. We are developing a set of programmes that search for distant similarities defined in terms of pattern size and residue properties. Central to this approach is the generalized data model that represents molecular structure as a set of substructures and relationships with quantitative and qualitative properties assigned (Pongor, Nature, 323:24,1987). Our programmes were tested by detecting distant similarities among the members of the tnf-receptor family, between HIV coat protein and members of the Ig family as well as different actin-binding proteins. A collaborative effort is underway with Drs. V. Veljkovic and R. Metlas of the Boris Kidric Institute in Belgrade to test whether parametric representations could provide better tools for identifying known patterns in sequences.

Analysis of protein/nucleic acid interactions

Sequence-specific interactions of proteins with DNA plays a central role in gene regulation. 3D structures of protein-DNA complexes are now available for a number of repressors and it has been experimentally shown that short peptides derived from the 'leucine-zipper' domain bind to DNA in vitro. We will use the helix-turn-helix motif of the repressor protein of the rhizophage 16-3, cloned by Dr. L. Orosz's group as a model (Dallmann et al. Nature, 26:398-401, 1987) to design mutants and test their activities both *in vitro* and *in vivo*. In 1990, three synthetic peptides corresponding to fragments of various DNA-binding domains were synthesized.

Structure/function studies on bactenecins and interleukins

Bactenecins are a small family of bactericidal peptides present in the storage granules of bovine neutrophiles that were discovered by the group of Prof. D. Romeo at the University of Trieste. As bactenecins constitute a unique structural class of peptides we became interested in the structural principles underlying their biological activity in the hope of designing pharmaceutically useful bactenecin analogs. Several segments corresponding

to bactenecin fragments were synthesized and their structure checked by circular dichroism in various solvents. Preliminary results show that some of the peptides have an ordered structure in solution and several of them are quite resistant to proteolysis. The endogenous antagonist of interleukin-1 acting on the human IL-1 receptor provides a significant reduction in bacterial endotoxin-caused mortality in septic shock and it is also a potentially useful agent in treating chronic inflammatory diseases.

We are interested in designing analogs of this protein in the hope of obtaining a potent, therapeutically useful analog with long biological half-life and good bioavailability. We use structural predictions based on homologous sequences present in the Brookhaven database and are trying to identify segments from which acceptable models of recombinant proteins and or conformationally restricted fragment-analogues can be derived. Central to our approach is the prediction of surface-bound structural segments on the antagonist molecule, that we try to achieve by a combination of predictive methods.

Sequence specificity of protein phosphorylation

Protein kinases phosphorylate oncogene products, transcription and replication factors in a specific and regulated manner. In collaboration with the laboratory of Prof. J. Allende, we will design synthetic substrate analogues to the target sequences of casein kinase II, that was recently cloned from X. laevis oocytes by that laboratory. In 1990 a total of 4 peptides were synthesized that represent analogs to the sequences occurring *in vivo*. Two of these were found to inhibit casein kinase II *in vitro*.

Structural principles of proline repeats in proteins

Proline rich repeats are found in many non-globular proteins of interesting biological and physicochemical properties such as the immunoactive circumsporozoite protein of the malaria parasite or the adhesive proteins of various organisms. It was recently suggested that the proline repeats constitute a new class of secondary structure. We have found that proline repeats are indeed much more widespread than previously thought and started to model-build smaller fragments corresponding to various chain symmetries. We plan to study the dynamic behaviour of these models through molecular dynamics as well as energy minimization experiments.

Molecular Pathology

F. Baralle, S. Tisminetzky, R. Tuteja, R. Garcia, E. Scodeller, C. Melo, G. Devescovi, B.R. Guerra

Infectious diseases

(a) Vaccine Development Programme:

Peptide fragments used as antigens have recently raised the possibility of the development of new vaccines. This antigenic fragment can be obtained as chemically synthetized peptide or can be introduced by genetic manipulation in chimeric proteins.

Using this last approach it is also feasible to locate antigenic peptides on the surface of particulate viruses or virus like particles. Peptides presented to the immune system in this way induce a substantially higher response than

when presented free or fused to a carrier (chemically or genetically). Several particles have already been engineered for carrying foreign epitopes:
(i) empty capsids formed of HBsAg;
(ii) hepatitis B core particles;
(iii) virus like particles of yeast;
(iv) poliovirus particles.

Several viral epitopes have been tested as immunogens under this form; these include: Human Immunodeficiency Virus (HIV); Herpes Simplex Virus 1 (HSV -1); Papilloma Virus; Influenza Virus; Food and Mouth Disease Virus etc.

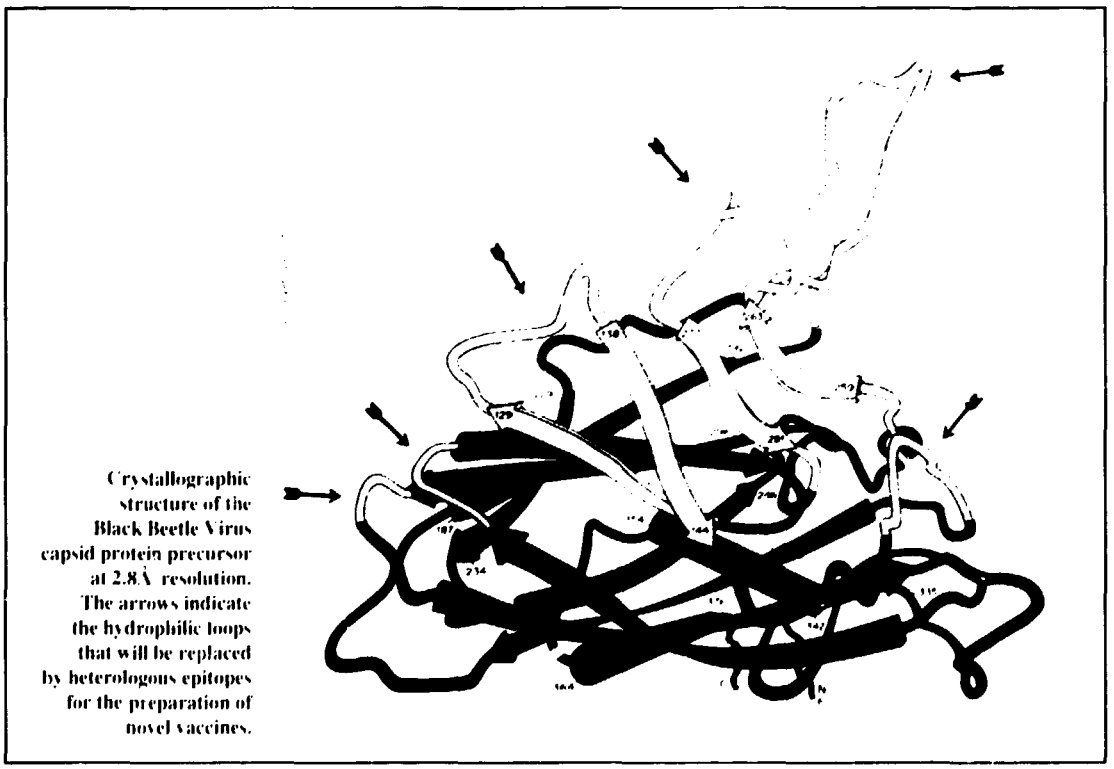
We are now developing a new vector for the production of an insect RNA virus carrying foreign epitopes.

The vector chosen is a small RNA virus, member of the Nodaviridae family, the Black Beetle Virus. Members of this family have some features that can be very advantageous:

(1) They grow vigorously in cultured cells, producing yields as high as 20% of the total protein after a replication cycle. The viruses also grow efficiently in several lepidopteran larvaes, which can mean a very simple way to produce virus on a large scale.

(2) Viruses of this family also have an extraordinary resistance to inactivation by heat, detergents and other denaturants. Such stability should be very advantageous during the process of purification and storage of any vaccine.

(3) These viruses should be self replicating carriers endowed with the known mutational rate of RNA replicases and from which a high number of mutated strains can be easily derived by routine procedures. This should facilitate the selection of putatives variants where the foreign epitope has a more favourable conformation.



Crystallographic structure of the Black Beetle Virus capsid protein precursor at 2.8Å resolution. The arrows indicate the hydrophilic loops that will be replaced by heterologous epitopes for the preparation of novel vaccines.

We are now looking for regions exposed at the capsid surface capable of accepting foreign aminoacid sequences without affecting capsid assembly or virus viability. This work is greatly facilitated by the fact that after X-ray crystallographics studies a detailed knowledge of its fine capsidal structure is available. We have found three major loops outward of the capsid that are expected not to contribute significantly to hold the protomers together. This area also shows the higher rate of aminoacid substitution between different strains.

These two features make these loops candidates for location of foreign sequences. The initial constructs will be carried out with epitopes derived from Hepatitis B virus, rotavirus, HIV, FMV and in a second phase after their identification epitopes from Hepatitis C virus.

(b) Parasite defences and inflammation:

Eosinophils play a crucial role in the defence against parasites and in the development of inflammatory reactions. Their response depends on their ability to generate oxygen metabolites and secrete basic proteins which are cytotoxic or degradative (ECP: eosinophil cationic protein, EPO: peroxidase, EPX: eosinophil protein X). The main aim of this project at the present stage was to separate different granule populations of the human eosinophil, where the cytotoxic and degradative activity is stored, and characterize them in terms of:

(a) their ability to degranulate upon cell stimulation;

(b) strength of binding of their proteins to intragranular matrices.

Human eosinophils were purified from normal peripheral blood on Percoll gradients and left untreated or treated with interleukin-5 (IL-5), which is the most efficient priming agent identified so far. Secretion from the cell granules was then triggered with opsonised Sephadex beads, mimicking physiological situations. Proteins secreted into the extracellular medium were measured by radioimmunoassay using specific antibodies to ECP, EPO and EPX. Cell granules were analyzed before and after priming and before and after stimulation of secretion by separating the different granule populations by isopycnic sucrose centrifugation. The interaction between proteins and matrix proteoglycans of the granules was quantified by consecutive extractions at acid pH and with a cationic detergent. This methodology will provide a way to determine the effect of interleukins and serum factors obtained from normals and from patients with inflammatory conditions on the secretory capacity of eosinophils, i.e the modulation of eosinophil responses.

(c) HIV protease inhibition:

The production of HIV protease in *E. coli* has been achieved as a hybrid βgal-protease polypeptide and as mature protease. The enzymatic activity was tested using as substrates synthetic oligopeptides. The results were reasonable and encouraged us to proceed to the preparation of sufficient amounts of protease to be used for the isolation of polyclonal and monoclonal antibodies and the screening for inhibitory peptides derived from the regions that will be isolated by direct specific DNA amplification of cDNA obtained from the spleen RNA of the HIV protease immunized mice.

Molecular biology of hyperlipidemia

An apolipoprotein A-I gene promoter polymorphism, due to an adenine (A) to guanine (G) transition 78 base pairs upstream from the transcription initiation site, was studied by amplification of the corresponding region of the apoA-I gene, DNA sequencing, and allele-specific oligonucleotide hybridization.

The frequency of the polymorphism was studied on female and male individuals classified into three groups according to the high density lipoprotein (HDL) cholesterol concentration. Statistical analysis showed a significant difference of allelic frequencies between females with high and low HDL cholesterol.

As the sequences surrounding the polymorphism are known to be involved in transcription modulation, it is possible that the A-G transition polymorphism may have an influence on apoA-I synthesis and, in consequence, on the HDL cholesterol levels in women. We are currently analyzing the behaviour *in vitro* of both promoter alleles and the influence of the neighbouring sequences involved in steroid regulation of gene expression.

Molecular biology of hypertension

Differences that are genetically associated with the development of hypertension in a strain of genetically hypertensive rat (MHS), were described in ion transport across erythrocyte membranes compared to normotensive control (MNS). Antibodies against the MNS ghost proteins were raised in the MHS, producing an immunoreaction against a 105 KDa protein later identified as adducin.

A clone coding for a portion of mouse adducin was isolated with these antibodies. Using this clone, overlapping cDNA clones coding for a 63 KDa adducin-like protein were isolated. A database search revealed an over-all similarity to filamentous proteins and local similarity to the actin-binding regions of alfa-actin.

A family of related mRNAs of about 3500, 3800, 4200 nt, was found to be present in spleen, kidney and heart tissues. Similar mRNAs and an additional tissue specific 8000 nt mRNA are present in brain. All mRNAs seem to be generated by alternative splicing from the transcript of a single gene.

In view of tryptic peptide fingerprint differences of MHS and MNS adducin, a structural study has been carried out to determine any eventual sequence variations between the MHS and MNS adducin gene and a single amino acid polymorphism has been detected that seems to be a condition necessary but not sufficient for hypertension in rats of the MHS strain.

We are currently studying the possible different functions of the alternative adducin forms, particularly the possibility that some of them do not form a complex with actin. We have also started to identify human families with hypertension clinically similar to the rat model. We plan to determine whether polymorphisms exist in the adducin genes in these families and whether they are associated with hypertension.

Molecular biology of the extracellular matrix.

(a) Fibronectin: The tissue specific patterns of fibronectin pre mRNA processing during development and aging in rat are studied. Fibronectin variation is generated by alternative splicing of a primary transcript derived from a single gene. At least three regions of the molecule are involved in rat: EIIIA, EIIIB and V.

We have investigated by ribonuclease protection analysis the splicing patterns of these regions during development and aging in rat. Between fetal and adult rat the extent of inclusion of the EIIIA and or EIIIB region in fibronectin pre-mRNA varies according to the tissue analyzed.

On the contrary the inclusion of the V region, and in particular the V25 alternative variant, is significantly higher in all fetal than adult tissues

studied. This data is consistent with a crucial role of the V25 variant, possibly related to the interaction with the a4b1 integrin receptor during development. These studies are being extended to EIIIA and EIIIB regions. Another line of research that is being followed is the identification of the *cis* and *trans*-acting elements that determine the differential RNA processing observed.

(b) Tenascin: Tenascin (TN) is a polymorphic high molecular weight extracellular matrix glycoprotein composed of six similar subunits joined together at their NH$_2$-terminal by disulfide bonds. By sequencing cDNA clones which cover the complete coding region of human TN, we have established its primary structure. This confirms that, as in the case of chicken, TN is mainly made up of three groups of sequences with a high homology to Epidermal Growth Factor (EGF) fibronectin (FN) type III repeat and fibrinogen. Furthermore, we have determined the amino-terminal sequence of the mature peptide directly on purified TN.

The main differences with respect to the chicken TN molecule are that in the human there are 14 and half EGF-like repeats compared to 13 and half in the chicken and that as previously reported (Gulcher, J.R., Nies, D.E., Marton, L.S. and Stefansson, K. (1989) Proc. Natl. Acad. Sci. USA., 86, 1588-1592), there are 15 FN-like repeats compared to 11 in the chicken.

By Polymerase Chain Reaction (PCR) amplification we have also studied the different splicing patterns of the TN pre-mRNA in cultured cells. The results show the presence of at least four different isoforms containing different numbers of FN-like type III repeats. Using purified human TN as immunogen, we have obtained numerous monoclonal antibodies (Mabs) to TN.

By screening a human melanoma cDNA library in the expression vector λgt11 with these Mabs and subsequently sequencing the insert of the positive clones, we have been able to localize, within the TN molecule, the epitopes recognized by two of these Mabs: BC-4, which recognizes an epitope within the EGF-like sequence and BC-2 which recognizes an epitope within the FN-like type III repeats whose expression is regulated by alternative splicing of the TN pre-mRNA.

Thus, while the Mab BC-4 may be useful in studies on TN distribution (since it recognizes all different TN isoforms) BC-2 may be useful in the study of the expression of particular TN isoforms generated by the alternative splicing of the TN primary transcript.

Molecular Immunology

O. Burrone, I. Gonzalez-Rodriguez, S. Mancardi

Recombinant monoclonal antibodies

Monoclonal antibodies obtained against specific human cancers are powerful tools to study and characterize relevant antigens on tumor cells, and could be of wide application in diagnosis and therapy. One limitation in their use *in vivo* is their murine origin. This project focuses on developing the technology to produce chimeric genes for both Ig light and heavy chains, consisting of the mouse antigen- specific V region and the human C region and to obtain their expresion in different cell lines.

Alternatively, chimeric molecules carrying the binding specificity of the antibody associated to toxins or enzymatic activities, will also be investigated. We have constructed appropriate expression vectors containing the C

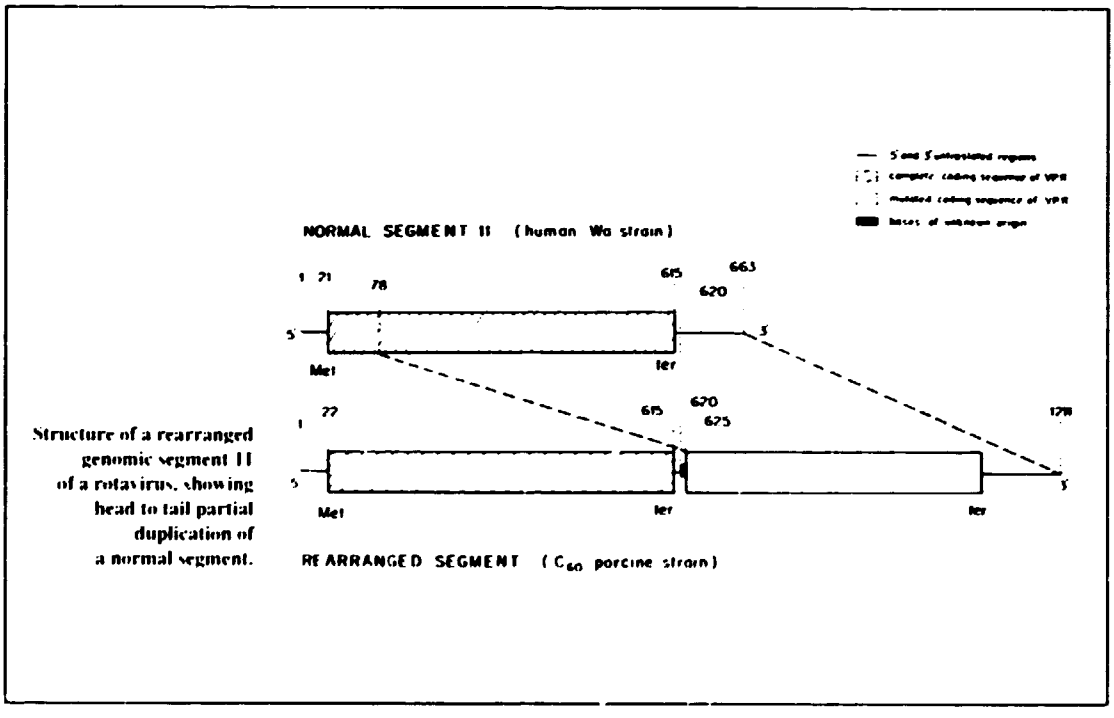
region of human IgG1 and K chains, using two different promotors: the LTR of the Rous Sarcoma Virus and the mouse Immunoglobulin heavy chain promoter associated to its tissue specific enhancer. A monoclonal antibody already developed and directed against a membrane surface protein present in most human breast cancer will be first humanized. Partial sequence of the light chain V region has already been obtained.

ClassI-HLA genes

HLA class I proteins are essential components of the cell surface. Their main function is to act as receptors for binding viral peptides that are then presented as target molecules for recognition by virus-specific citotoxic T cells through their TCR (T cell receptor) molecules. We have previously studied the control of expression of the alfa chain of class I genes by Interferon and established a set of regulatory mutant cell lines. From one of these cells we have recently isolated and sequenced a new member of a non-classical class I gene, belonging to the AR H locus. Members of this locus are characterized by having a specific one base deletion in the alfa-3 domain, thus introducing a premature termination codon. Since the expression of this gene seems to be down regulated, we will investigate the nature of the regulatory sequences involved with the aim of understanding different mechanisms of regulation of class I gene expression.

Molecular biology of rotavirus.

This projects focuses on the study of the biology of rotavirus replication. Among the infectious agents that produce diarrohea, rotavirus represents the most extended viral infection. Rotaviruses are widely spread and are able to infect a number of species of mammals and birds. They are non-enveloped viruses that belong to the reoviridiae family and as such they have a segmented genome, composed of 11 segments of double stranded RNA.



Structure of a rearranged genomic segment 11 of a rotavirus, showing head to tail partial duplication of a normal segment.

Some of the viral non structural proteins, found only in virus infected cells are not yet fully characterized and their functions still unknown. One such protein of interest is VP11, the protein coded for in the genomic segment 11. We will be looking into the role of VP11 in viral genome replication and virion assembly.

We have recently found by sequencing genomic segment 11, that this is a highly conserved protein, very rich in serines and threonines. We have also demonstrated that VP11 in its mature form, is found in the cytoplasm of virus infected cells, post-translationally modified by the addition of monomeric O-glucosamine residues. At present, we are also analyzing different natural occuring variants of both humans and animal rotavirus showing rearrengements in the structure of genome segment 11.

The two main components of rotavirus outer shell, VP7 and VP4, are the proteins against which neutralizing antibodies can be raised. Since human serotype specific epitopes have been mapped, we will look into the possibility of designing recombinant vaccines using VP4 and VP7 epitopes either alone or in combination with the aim of inducing both antibody and cytotoxic immune responses.

The New Delhi component

Facilities

The existing interim facility of ICGEB New Delhi is housed at the National Institute of Immunology (NII) building and the Life Sciences building of Jawahrlal Nehru University (JNU). The NII facility consists of 1,000 sq.m. devoted to laboratories and an additional 200 sq.m. for administration. The laboratories are distribated on four floors; the ground, first and second floors are devoted to the Mammalian Biology, Plant Biology and Structural Biology Groups respectively. These three floors have been modified and are fully functional with all the equipment necessary for the research. The third floor of about 100 sq.m. has been modified to suit the requirements of a P-3 facility, which requires a contained atmosphere with negative pressure and no mixing of circulating air to other laboratories. Animal tissue culture equipment has been installed which is essential for the hepatitis and malaria research.

Provisional premises of ICGEB-New Delhi. These correspond to a wing of the National Institute of Immunology, located in the campus of the Jawaharlal Nehru University. The permanent premises, of a total of 10,000 m² of laboratory and office space, are in construction in a nearby area and will be completed in 1992.

The laboratory at JNU required major modification. With frequent disruption of power, it was decided to completely change the power lines and the electrical connections; the laboratory is now functional. Since this laboratory is devoted to plant tissue culture, a large growth chamber has been procured and installed. Cooling facilities, emergency generator, -70 freezers and an incubator have been provided.

The construction of the 10,000 sq.m. for the permanent facility is in progress; the contractor is a semi-governmental organization called U.P. Rajkiya Nirman Nigam (UPRN). The stone-metal boundary wall, the foundation and the first floor of the building have been constructed, the provisional roads have been laid out and the second floor is in progress. The permanent facilities are expected to be ready by the middle of 1992.

The Government of India is providing a newly built guest house for the ICGEB New Delhi. The guest house, sited approximately 2 km. from NII, consists of 10 single and 10 double rooms that are utilized for training courses and speakers. It is located on the JNU campus and is available for ICGEB use until the permanent facilities are built, when it will become the property of JNU.

Personnel

Names in italic correspond to international appointments.

Head of Component	*Krishna Kumar Tewari*
Executive Secretary	Gita Srinivasan

Administration:

Administrative Officer	*Gurudas Chatterjee*
Research Management Officer	Yashpal Roy Saxena
Purchase Officer	Vikas Dwivedi
Secretary to Administrative Officer	Tina Dias
Computer Analyst	Dinkar Chaturvedi
Receptionist Typist	Ramachandra Radha
Driver	Madan Lal Yadav
Procurement	Ashok Kumar
Plumber	Umaid Singh Rana
Electrician	Bhagwan Das
Messenger	Ramakant Pal

Research Groups

Mammalian biology

a) Hepatitis Virus:

Assistant Scientist	*Shahid Jameel*
Research Scientist	Vijay Kumar
Assistant Scientist	*KaluryVenkatta Subba Rao*
Research Fellow	Narayana Jaysuryan
Senior Research Fellow	Vankatasamy Manivel
Lab Technician	Vidhu Bansal
Lab Technician Assistant	Girish Shukla
Lab Technician Assistant	Ravinder Kumar

b) Malaria

Assistant Scientist	*Fred Alexander S. Kironde*
Trainee	*Jordan Kriakov*
Research Scientist	Pawan Sharma
Research Fellow	Amiya Ranjan Nayak
Research Fellow	Anil Kumar
Trainee	*Fakhreldin Omer*
Lab Technician	Balwan Singh
Lab Technician	Sangeeta Shah
Lab Technician Assistant	Naresh Sahoo

Plant biology

a) Stress Resistance:

Senior Scientist	*John Bennett*
Research Scientist	Raj Kamal Bhatnagar
Research Fellow	Angamuthu Selvapandiyan
Trainee	*Myron Williams*
Trainee	*Rossitsa Bachvarova*
Senior Research Fellow	Madan Mohan
Research Fellow	Suresh Nair
Trainee	*Farkad A. Fattah*
Liasion Officer	Piyasooli Bennett
Lab Technician	Geetha Vasudevan
Lab Assistant	Naresh Arora
Lab Attendant	Prakash Singh Patwal

b) Plant Transformation: Gene Structure, Replication & Transcription

Senior Scientist	*Krishna Kumar Tewari*
Assistant Scientist	*Sunil Mukherjee*
Research Scientist	Navin Khanna
Senior Research Fellow	Chalivendra Chenchu Subbaiah
Research Fellow	Malireddy Kondadarami Reddy
Research Fellow	Sujata Lakhani
Research Fellow	Vanga Siva Reddy
Lab Technician	Dhirendra Kumar
Trainee	*Edmundo Barrios*
Lab Attendant	Kedar Singh

Structural biology

a) Protein Chemistry

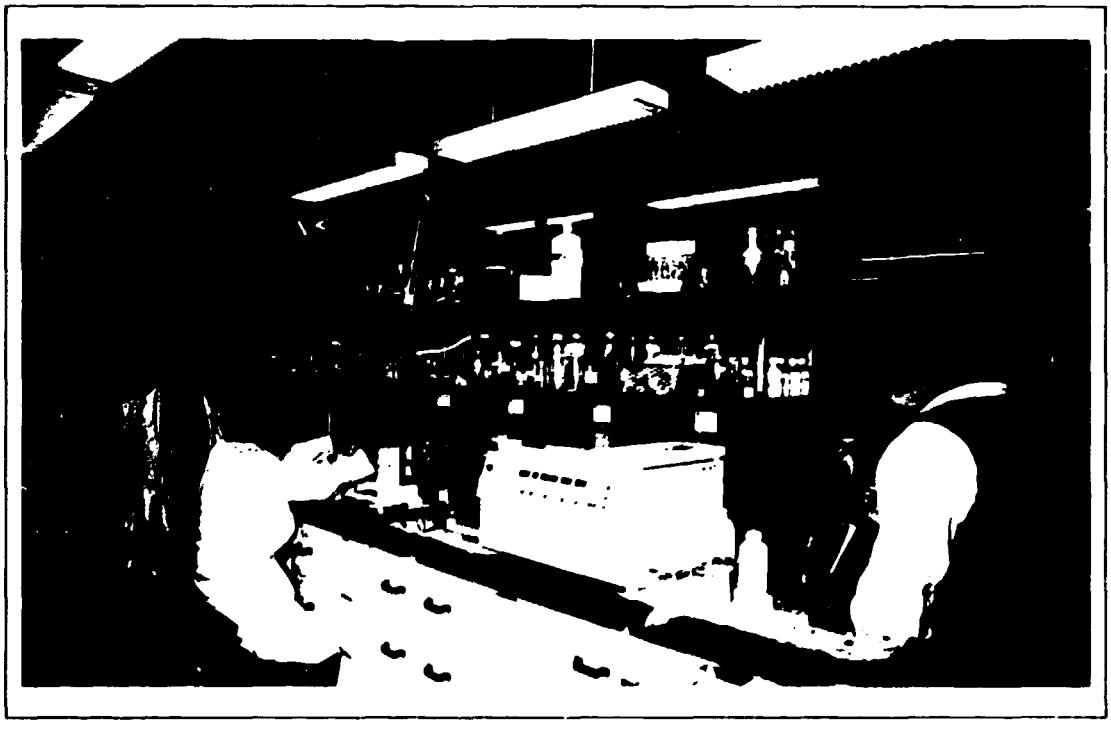
Assistant Scientist	*Virander Singh Chauhan*
Research Fellow	Paramjeet Kaur
Lab Technician	Akash Mathur
Lab Technician	Ashima Bhardwaj
Lab Technician	Shyama Chatterjee
Lab Technician Assistant	Shyama Nagpal
Lab Technician	Rakesh Arora
Lab Attendant	Narinder Singh

b) Mammalian Gene Products

Senior Scientist	*Qi Song Wang*
Research Associate	Yong Jie Min
Senior Research Fellow	Kumud Majumder
Lab Technician	Alok Chatterjee
Lab Technician	Manisha Malhotra

Research Scientists: 31
Laboratory Technicians: 19
Administration Staff: 12

Total: 62



One of the laboratories of the New Delhi Component.

Mammalian Biology: a) Hepatitis

S. Jameel, V. Kumar, K.V. Rao, N. Jayaryan, V. Manivel, V. Jain, G. Shukla, R. Kumar

Hepatitis B virus (HBV)

The aim is to understand in detail the basic molecular biology of HBV and to design a molecular vaccine against HBV infection.

Of the four HBV-encoded proteins, the X-protein is least understood. It is a kinase and is capable of activating transcription from a wide variety of *cis*-elements, both viral and cellular. However, the protein does not directly bind to DNA. To understand the mechanistic details of X action, the protein has been expressed to high levels in *E. coli*, purified to homogeneity and shown to be biologically active. A variety of mutant X proteins have been made and are being evaluated for activity to gain an insight into functionally relevant domains. Monoclonal antibodies are being raised against purified X to be able to study its interactions with cellular proteins. Such protein-protein interactions could provide important clues to the role of X in viral transactivation and transformation.

HBV contains a hepatotropic enhancer sequence which is crucial to its replicative life-cycle. To understand the transcriptional regulation due to this element, a number of repeat regions within it have been changed by site-directed mutagenesis. These mutants are being characterized in terms of (a) transcriptional activity in reporter gene (CAT) assays and (b) DNA-protein interactions *in vitro*. Future work would involve the cloning out of enhancer-binding protein sequences from liver cDNA libraries using the "Southwestern" technique. The network of HBV X protein and enhancer and their interactions with cellular proteins is likely to be important in understanding the pathogenesis of hepatitis B infections and the factors that lead to HBV-related hepatocarcinomas.

Studies on hepatocyte receptors for HBV are in progress using synthetic peptides derived from the pre-S1 and pre-S2 regions of HBV surface antigen (HBsAg). In a comparative study it was found that the pre-S1 region represents the dominant participant in the attachment of HBV to human hepatocytes as opposed to the pre-S2 region. From binding studies with the pre-S1 derived peptide it was found that there are between 5 to 10×10^5 sites for this region of HBV on HepG2 cells (a human hepatoma cell line). Cross-linking studies with radiolabelled peptide identified the putative receptor as a 30-34 kDa protein which appears to be glycosylated. Attempts are underway to purify this protein to near homogeneity using a combination of lectin and peptide-affinity chromatography. Subsequent to its purification, the amino-terminus of this protein will be sequenced and compared with that of known proteins.

Synthetic peptides are being used to delineate immunologically relevant domains of HBsAg. Here the major focus is on reconstructing the conformational 'a' determinant of HBsAg. We have recently shown that a peptide representing the sequence 124 to 147 of the major protein spontaneously oligomerizes in a disulfide-dependent manner to reconstruct a conformational, disulfide-dependent 'a' determinant on the native protein. Furthermore, this epitope represents the dominant constituent of the repertoire

recognized by the human immune system when exposed to HBsAg. This oligomeric peptide is immunogenic in mice and rabbits and elicits 'a'-specific antibodies.

Using a panel of peptide-analogues that represent either deletion, substitution or chemical modification of single residues we have identified the 'a' epitope as a hitherto undescribed one that includes Met 133 and Lys 141. It was also found that this peptide aggregates in aqueous solution to reconstitute spherical and tubular particles visible by Transmission Electron Microscopy. These aggregates can also be resolved by sucrose density-gradient centrifugation and this is being used to examine the kinetics and effect of single amino acid changes on the aggregation capacity. These studies are being conducted with the aim of eventually being able to design such self-aggregating sequences from other systems as a general route to the construction of determinants dependent on the quarternary structure of native proteins. The potential of this particular peptide as a candidate vaccine for hepatitis B is also being currently explored using murine, rabbit and primate models. Finally, an alternative approach is also being tested wherein this particular self-aggregating sequence has been incorporated as a component of a multiple epitope type 'designer' vaccine.

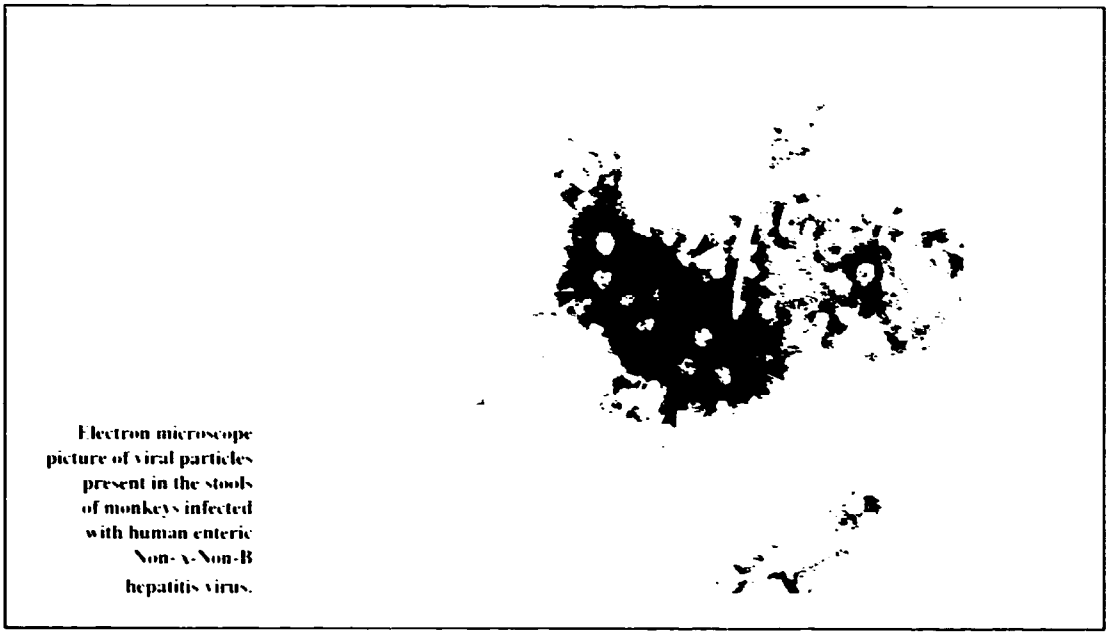
In conjunction with the studies on vaccine design, we have an ongoing parallel study on the use of synthetic IL-1B derived peptides as adjuvants. We had earlier demonstrated that coupling of the immunostimulatory nonapeptide sequence from human IL-1B to a peptide immunogen results in enhanced immunogenicity. To arrive at an adjuvant of general applicability we have focussed on circumventing the need for coupling the IL-1 derived sequence to immunogens. Towards this end an IL-1B derived peptide that includes the nonapeptide immunostimulatory sequence and the IL-1 receptor binding domain was used. This construct was found to be several-fold more active than the nonapeptide alone in its immunostimulatory capacity. When mixed with a commercially available hepatitis B vaccine preparation, the IL-1 derived peptide was capable of enhancing anti-HBsAg response in both high and low responder strains of mice. Increased anti-HBsAg response was also accompanied by an increased anti 'a' response. This peptide may thus prove useful as a general co-adjuvant in vaccine formulations.

Enteric Non-A, Non-B virus

The aim is to isolate the aetiogical agent for enteric non-A, non-B (ET-NANB) hepatitis, molecular cloning of its genome and the development of recombinant diagnostics and vaccines.

The poorly understood ET-NANB hepatitis is endemic in Asia, Africa and Central America, thus affecting a majority of the world's population. It is a disease transmitted through contaminated water and food and appears to have a high mortality rate among pregnant women. Still, the diagnosis has remained one of exclusion (of hepatitis A and B) in the absence of definitive diagnostic tests. The need in developing countries, therefore, is the availability of such tests.

The ICGEB group has covered hepatitis epidemics in India and after careful serology, chosen cases of ET-NANB hepatitis. The disease has been successfully transmitted to rhesus monkeys and present efforts are aimed at using animal materials (bile, liver and stools) to clone the viral genome. This is being done by making cDNA libraries which will be screened with sera from the infected animals as well as WHO reference standard sera for ET-NANB hepatitis. Such an approach may be feasible since cross-reactivity



Electron microscope picture of viral particles present in the stools of monkeys infected with human enteric Non-A-Non-B hepatitis virus.

between sera from different geographical locations is high.

Recently, very limited nucleic acid sequence information for a candidate virus, HEV was reported. Using this information, we are attempting to devise a nucleic acid hybridization based diagnostic protocol. With the advent of polymerase chain reaction (PCR) amplification methodology, the detection of very low levels of HEV or related sequences during ET-NANB hepatitis episodes may be possible. A PCR-based strategy is also being followed for the cloning work.

The ET-NANB hepatitis program at ICGEB is a long-term program with initial thrust at diagnosis and vaccines. The molecular biology of the virus itself could be very interesting and would be an area of interest in future.

Mammalian Biology: b) Malaria

F. Kironde, J. Kriakov, P. Sharma, A.R. Nayak, A. Kumar, F. Omer, B. Sing, S. Shah, N. Sahoo

Human malaria is caused by four species of the genus *Plasmodium: P. falciparum, P. vivax, P. malariae* and *P. ovale*. With the spreading emergence of multidrug-resistant isolates of malaria parasites and the decline in efficacy of mosquito control, improved approaches to developing an antimalaria vaccine are a priority. Malaria is the cause of death of several million people each year. However, several difficulties frustrate the efforts towards an antimalarial vaccine. They include diversity of parasite antigens, variable human immune response and other mechanisms of parasite evasion of host immunity.

Our research is aimed at identifying and characterizing protective peptides or epitopes from described antigens and identifying new protective antigens of *Plasmodium*. We are also engaged in devising hybrid or composite antigenic peptides of potential for incorporation in anti-malarial subunit vaccines. Over the past year, the studies described were undertaken.

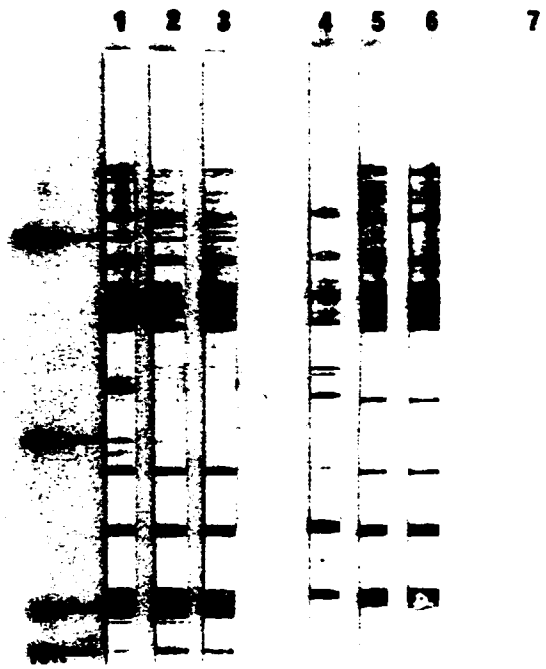
Plasmodium yoelii antigens

Isoelectrofocusing separation and immunogenicity of antigens of the malaria parasite *Plasmodium yoelii*. Knowledge of the relative humoral and cellular host immunity against separated antigens of malaria parasites will help in selection of the protective antigens suitable for incorporation in an anti-malarial vaccine. Therefore, we separated Triton X-100 extract antigens of *P. yoelii* (a malaria parasite of rodents) by isoelectric focusing. We then prepared two types of mouse anti-*P. yoelii* sera: one by immunizing BALB c mice with the extracted *P. yoelii* antigen emulsified in Freund's adjuvant (immunized mice), and the other by thrice infecting BALB c mice with *P. yoelii* and curing the infection with anti-malarial drugs (convalescent mice).

We found that upon being challenge-infected with 10^8 *P. yoelii* parasites, the convalescent mice showed no parasites at all in their blood and survived the infection, whereas with similar challenge infection, the immunized mice were only partially protected and died in about twelve days. After equivalent challenge infection, normal unprotected mice typically die within eight days. These results suggested that sera of the fully-protected convalescent mice may recognize protective *P. yoelii* antigens that are not recognized by the partially-protected immunized BALB c. On testing this possibility by the immunoblot assay, we observed that indeed a number of antigens (M_r 16, 19, 40 and 150 kDa) were recognized by the sera of the fully-protected but not by those of the partially-protected mice. We feel that the antigens uniquely recognized by the convalescent sera may be protective against infection, a notion which we are currently testing. However, the results described here strongly justify the suggestion that similar studies should be undertaken to specifically identify protective antigens of *P. falciparum* and *P. vivax* using experiments in non-human primates. This is more urgently appropriate because the malaria endemic-area sera currently used in identification of protective human-malaria parasite antigens recognize both protective and nonprotective antigens. Consequently, most antigens identified so far by such sera appear nonprotective. The approach suggested by our results provides a simpler and potentially more specific method for identifying protective parasite antigens (from recombinant clones of *Plasmodium* DNA expression libraries, for example). In follow-up work to these studies, the *P. yoelii* antigens 16, 19, 40 and 150 kDa and specific antisera to them will be prepared and tested for protection against infection. In addition, using sera of fully-immune and partially-immune mice, we have started immunoscreening an expression library of *P. yoelii* which we recently constructed in lambda ZAP vector. We have found a number of recombinant clones uniquely recognized by the sera of fully-protected convalescent mice and we will characterize them this year.

Plasmodium falciparum T-cell epitope

CS.T_3 the promiscuous T-cell epitope of *Plasmodium falciparum* circumsporozoite protein contains a B-cell epitope. In a collaborative study with Dr. Kanury Rao of the Virology Group (ICGEB New Delhi), the peptides CST_3 (sequence: IEKKIAKMEK ASSVFNNV), R_3 (sequence:



Improved identification of protective malaria parasite antigens. Antigens of 16kDa, 19kDa, 40kDa and 150kDa were recognized by fully protected convalescent-mice sera (lanes 1, 2 and 3) but neither by partially protected hyperimmunized-mice sera (lanes 4, 5 and 6) nor by normal-mice sera (lane 7).

(QGPGAP)$_3$), and CSTR$_3$ (sequence: CST$_3$-gly-gly-R$_3$), a conjugate of CST$_3$ and R$_3$, were synthesized and immunized in groups of mice of differing genetic background, namely, BALB c(H-2^d), C3H(H-2^k), C57BL 6 (H-2^b). CST$_3$ had previously been found by Dr. Francesco Sinigaglia (Switzerland) to be a widely recognized T cell helper epitope, inducing formation of anti-NANP antibodies (antibody against lysine-alanine-lysine -proline) in otherwise nonresponder H-2^b mice. Also, CST$_3$ binds to a variety of major histocompatibility complex (MHC HLA) molecules of humans and, *in vivo*, probably acts similarly as in mice. R$_3$ is the central repeat sequence of the *P. yoelii* circumsporozoite protein (CSP), whereas NANP is the equivalent central repeat in *P. falciparum* CSP, a vaccine-candidate molecule.

We sought to analyze the immunogenicity of CSTR$_3$ in order to determine whether CST$_3$ could be used to provide T-cell help for immune response to antigens from *Plasmodium* species other than *P. falciparum*. This aspect of anti-malarial vaccines is important as it has become increasingly clear that an effective anti-malarial subunit vaccine will have to be a multiple-antigen epitope composite or hybrid construct.

We found that neither CST$_3$ nor R$_3$ alone induced any antibody whereas CSTR$_3$ induced both anti-CST$_3$ and anti R$_3$ antibodies. This suggested that in the CSTR$_3$ construct, CST$_3$ and R$_3$ mutually provide T-cell help for antibody formation. However, to resolve whether perhaps a new intervening sequence had been created by adjoining CST$_3$ to R$_3$, we conducted competition experiments between CSTR$_3$ against the individual constituent peptides and against a mixtures of CST$_3$ and R$_3$ (namely, CST$_3$ plus R$_3$). The mixture competed effectively against CSTR$_3$ whereas neither CST$_3$ nor R$_3$

did so individually, suggesting that no immunogenic intervening sequences had been created and that, therefore, CST_3 and R_3 each contain a B-cell epitope.

Thus, CST_3 can act as a T-cell helper epitope for antigens other than those from *P. falciparum*. However, in view of possible immunosuppression due to competition of formed anti-CST_3 antibody against memory T-cell and MHC HLA molecules in binding to CST_3, the epitope, before being incoporated into subunit vaccines, should be carefully evaluated to rule out B-cell stimulation. Such competition would reduce the efficacy of a vaccine.

Enhanced immunogenicity by foreign T-cell epitopes

Enhancing immunogenicity of a malarial (*P. falciparum*) peptide: use of foreign T cell epitopes. Among individuals of different genetic backgrounds, the immune responsiveness to synthetic peptides is variable (genetically restricted) due to availability or lack of appropriate T cell epitopes in the immunized peptide. We have evaluated the role of two T cell epitopes in enhancing the immunogenicity of a 20 residue synthetic peptide P8 (sequence: LDNIK GNVGK MEDTI KKNNK) representing a portion of the precursor of a major merozoite surface antigen (PMMSA), a vaccine candidate antigen of *P. falciparum*.

The peptide P8 represents a sequence in a highly conserved N-terminal region of PMMSA. Two independent studies by others had indicated the presence of a B-cell epitope as well as a T-cell epitope in the P8 sequence region. We found high levels of anti-P8 antibodies in sera of 32 out of 35 blood slide-positive malaria cases, suggesting that P8 is a target of immune response during natural infection.

One of two rabbits immunized with P8, without carrier protein, made high titer (10^{-6}) anti-P8 antibodies. However, immunization of mice of seven different strains stimulated anti-P8 antibody in only one strain, SJL ($H\text{-}2^s$), suggesting genetic restriction in production of anti-P8 antibody. Indeed, when P8 was conjugated to tetanus toxoid (TT), a commonly used carrier protein, high titer anti-P8 antisera were produced by all seven mouse strains immunized with the P8-TT (whole) conjugate.

However, use of tetanus toxoid as a carrier protein has the disadvantage of epitope-specific suppression of antibody response in individuals previously primed with tetanus toxoid. To circumvent carrier-specific suppression, the whole TT molecule was replaced by a 15-mer synthetic peptide representing residues 830 to 844 of tetanus toxoid (tt830-844, sequence: QYIKA NSKFI GITEL). This sequence has been shown by other workers to be immunogenic in a majority of genetic (HLA MHC) backgrounds. When we primed inbred mice of different genetic (H-2) strains with tt 830-844 and boosted with P8-TT (whole), the mice made a boostable antibody response against P8, indicating that tt 830-844 could efficiently provide T-cell help to P8.

In another set of experiments, similar inbred mice of differing H-2 backgrounds were immunized and boosted with the P8-tt830-844 construct. Boostable high titer antibody was produced in all mouse strains used. Thus, tt830-844, a small synthetic peptide representing only a portion of the whole TT molecule, was successfully used to overcome genetic restriction of immune response as well as to circumvent the problem of carrier specific suppression of immune response.

This work was further extended to the T epitope peptide CST_3 of *P. falciparum* CSP. Seven strains of mice, immunized with P8-CST_3 and boosted twice, induced high levels of anti-P8 antibodies. Comparison of anti-P8

antibody levels elicited with the help provided by tt830-844 and CS1.3 indicated that CS1.3 was a more efficient T cell epitope.

The peptides used in this collaborative study were synthesized by Dr. V. S. Chauhan and colleagues from the Structural Biology Group.

Thrombospondin related peptide

Immunonogenicity of thrombospondin-related anonymous peptide (TRAP). A peptide P6 (sequence: WSPCSVTCG) from the TRAP protein of *P. falciparum* was further investigated to confirm our previous findings that P6 inhibited *P. falciparum* invasion of erythrocytes. At 0.08 and 1.2 mg/ml, purified anti-P6 immunoglobulin caused 20% and 98% inhibition of parasite growth, respectively, strongly suggesting that TRAP is a target of protective antibody immunity. Studies designed to localize TRAP on the parasite body are underway and will be continued this year.

Plant Biology: a) Gene structure, replication and transcription

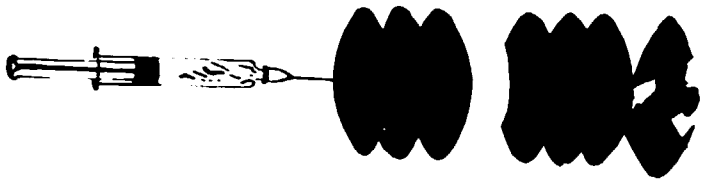
K.K. Tewari, S. Mukherjee, N. Khanna, C.C. Subbaiah, M.K. Reddy, S. Lakhani, V.S. Reddy, D. Kumar, E. Barrios, K. Singh

We are studying the molecular aspects and mechanisms of DNA replication and transcription within the chloroplast. We hope to combine knowledge derived from these studies to construct vectors which would propagate autonomously and express efficiently within the organelle and thus permit chloroplast transformation.

Replication of chloroplast DNA

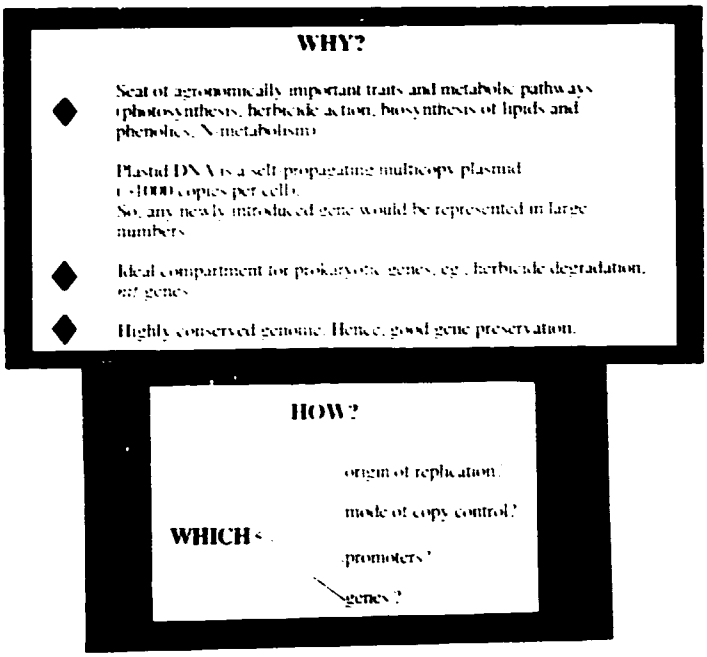
We have analysed the static structure of the replication origin (ori) sequences of pea chloroplast DNA which had been used previously to construct a successful vector for organellar transformation and transient expression. The ori regions are very A-T rich and contain repeated sequences, two characteristics of other ori stretches of both prokaryotic and eukaryotic sources. The sequences are sensitive to S1 nuclease digestion indicating that these regions are molten or unwound. Electron microscopy confirms that a small portion of the regions indeed remains in a denatured condition under the influence of superhelicity. We have been able to set up an *in vitro* replication system using a semi-crude lysate of pea chloroplasts. This system can faithfully replicate plasmids containing pea chloroplast ori sequences. DNA synthesis is linear with time and within 30 seconds of incubation, the chain length of the product synthesized is as big as 9 kb.

In this crude extract, we have detected topoisomerase I, helicase and primase activities, in addition to DNA polymerase. These and some other unidentified proteins probably form a tight complex. A replication proficient fraction (fraction C) has been derived from this extract by density-banding in a glycerol gradient. The fraction can replicate a single-stranded DNA template containing ori sequences and contains helicase activity as revealed



REDESIGNING PLASTIDS

Plastids are cellular organelles present in all plants and occurring in various forms corresponding to different functions. Chloroplasts are green and are devoted to photosynthesis; chromoplasts are coloured (other than green) and help in pollination and seed dispersal; leucoplasts store starch, etc... They are semi-autonomous and have a prokaryotic type of genome. The programme aims at altering the genome of the plastids by transformation.



by strand displacement assays. In the initial semi crude-lysate, the helicase factor is associated with a nuclease which is removed by density sedimentation through the glycerol gradient. Silver staining after SDS-PAGE reveals that fraction C yields 20 polypeptides, but in a non-denaturing 10% acrylamide gel, only one band is visible. When the gel containing this single band is excised and examined by reducing SDS-PAGE, about 12 prominent polypeptides are revealed. Currently, we are examining this complex in activity gels to assess the activity profile of the complex.

We have also identified a crude fraction of the lysate which increases replication five-fold compared with either the semi-crude extract or fraction C. This polymerase accessory fraction yields higher synthesis of "templated-sized" products, suggesting that increased synthesis is not due to simple nucleolytic degradation of the template by the proteins of the accessory fraction. When the normal replicated products are visualised by E. M. we find σ-shaped structures suggesting that nicking might play a role in the initiation of DNA replication. When the products are linearised at different ends, branched molecules are observed. Measuring the lengths of branches of variously cut molecules, we have formed a fair idea of the directionality and start-points of DNA synthesis. Replication is unidirectional in the probed plasmids and startpoint maps at or near the D-loop where replication had

been reported to initiate in the intact chloroplast *in vivo*.

The crude lysate of pea chloroplasts also contains three different single-stranded DNA-binding (SSB) proteins. The amino-terminal sequence analysis shows that these SSBs are novel and are related to many nucleic acid binding proteins of other organisms. Southwestern blotting further demonstrates that these proteins bind strongly to single-stranded DNA and preferentially to ct-DNA derived probes. The minimum DNA-binding domain is also mapped to a 12 kD papain fragment of the 30 kD polypeptide by Southwestern analyses.

Polyclonal antisera have been raised against the 33 and 30 kD polypeptides. Immunoblot analysis shows that the SSBs are antigenically distinct polypeptides. Peptide mapping further demonstrates that these are different gene products. Earlier experiments using translational inhibitors show that the SSB genes are located in the nucleus. These studies are further confirmed by *in vitro* translation of leaf tissue mRNAS. Extracts of cyanobacterial and algal cells as well as higher plant chloroplasts show the presence of similar polypeptides in immunoblots, indicating the evolutionary conservation of SSB genes. Attempts are being made to understand the role of these proteins in plastid DNA replication or transcription.

We have obtained a pUC19-based library of rice chloroplast DNA. Some of these clones replicate very well *in vitro* using the semi-crude rice chloroplast extract. These clones also replicate *in vitro* when incubated with heterologous extract of pea chloroplasts. These clones presumably contain rice chloroplast DNA ori sequences. Currently we are trying to delimit the ori sequences. When the various proteins of the semi-crude rice chloroplast extract are electrophoretically separated in an activity gel, we have detected DNA polymerase activity as a single 80 kD polypeptide.

Transcription of chloroplast DNA

We are investigating the mechanism of transcriptional regulation of plastid genes using homologous *in vitro* chloroplast transcription systems. This involves the purification and characterization of chloroplast RNA polymerase and its accessory proteins, and the cloning of functional promoters and terminator sequences of the chloroplast DNA. We have developed an *in vitro* "run-off" assay system using the plastid RNA polymerase and recombinant plasmids containing the putative promoters of the 16S rRNA gene and the *psbA* gene (which codes for a 32 kDa polypeptide of Photosystem II). Using conventional methods of protein purification, we have isolated two transcriptional activities from pea chloroplasts which are distinguished by their metal ion requirements and template preference for ribosomal and messenger genes *in vitro*. However, it is not known whether the two distinct transcriptional activities can be attributed to the existence of more than one functional form of the RNA polymerase. Three monoclonal antibodies that specifically recognize the 27, 75, 90 and 95 kD polypeptides of the ribosomal-specific enzyme preparation have been used to address the question of common polypeptides between the two transcriptional activities. Both activities have been found to contain all four polypeptides by Western blots. These antibodies inhibit transcription of both 16S rRNA and *psbA* genes by their interaction with the RNA polymerase. Our experiments have shown that although two highly specific ribosomal and messenger transcriptional activities can be separated by protein fractionation, they contain at least four common functional polypeptides suggesing that the specificity of transcription is probably conferred by as yet unidentified factor(s).

The technique of photoaffinity labelling is also being used to identify the template, transcript and nucleotide binding subunits of chloroplast RNA polymerase. We have found that two polypeptides with mol. wts of 56 and 60 kD bind to the nascent RNA during transcript elongation in an *in vitro* ribosomal transcriptional system. Similar studies are being extended to transcriptional systems using mRNA promoters. Future studies will aim to identify and characterize the other functional polypeptides of the chloroplast RNA polymerase.

Transformation of rice genomic DNA

Attempts have also been made to develop and standardise techniques for chromosomal modification of rice. Following the PEG mediated direct DNA uptake method, we have been able to transform rice protoplasts with two genes (neomycin phosphotransferase which confers resistance to kanamycin, and glucuronidase (GUS) gene, a very sensitive reporter gene). Transformation of foreign DNA and its integration into genomic DNA have been confirmed through Southern hybridization of genomic DNA of rice using probes of *neo* and GUS gene coding regions. The expression of *neo* gene has been confirmed by the ability of callus to grow on kanamycin containing medium. The GUS gene expression also has been confirmed by a histochemical method where the GUS gene expressing cells turn blue.

Plant Biology: b) Plant Stress Resistance

J. Bennett, R. Bhatnagar, A. Selvapandiyan, M. Williams, R. Bachvarova, M. Mohan, S. Nair, F.A. Fattahh, P. Bennet, G. Vasudevan, N. Arora, P.S. Patwal

Insect and fungal diseases of rice

Insect and fungal diseases of rice account for global yield losses of 20-40% annually. Our research programme aims to use molecular and cellular techniques (transformation, gene mapping, tissue culture) to enhance disease resistance in this important cereal. Four insect pests and one fungal disease have been targetted in the initial programme: gall midge (*Orseolia oryzae*), leaffolder (*Cnaphalocrocis medinalis*), yellow stem borer (*Scirpophaga incertulas*), brown planthopper (*Nilaparvata lugens*), and the narrow brown leaf spot fungus (*Cercospora oryzae*). Transformation of rice protoplasts with genes encoding insecticidal proteins offers a potentially powerful approach to enhancing insect resistance in rice. Such proteins include amylase inhibitors, proteinase inhibitors and *Bacillus thuringiensis* d-endotoxins, all of which are targetted against the digestive system of the insect. This approach is likely to be particularly effective against larval pests of rice.

We have targetted yellow stem borer, leaffolder and the eight Asian biotypes of gall midge for the transgenic approach. Our initial programme involves classifying the digestive proteinases of these pests and assessing their sensitivity to protein inhibitors. Most progress has been made with the trypsin of yellow stem borer, which is potently inhibited by soybean trypsin inhibitor (Kunitz-type). The published gene sequence for SBTI was used to

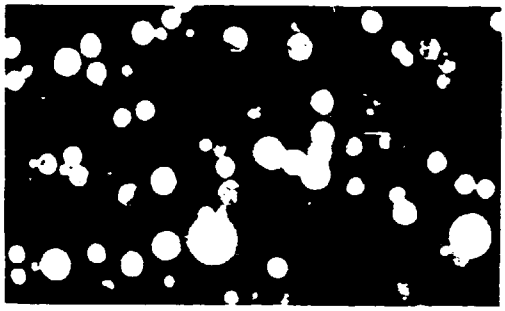
design primers for amplification of the gene from soybean DNA by the polymerase chain reaction (PCR). The same primers also allowed for direct insertion of the gene into a T7-based expression vector for transformation of *Escherichia coli*. SBTI was expressed in *E. coli* to the extent of 10-50% of total cellular protein. The inhibitor was recovered from sonicated cells in inclusion bodies and could be released in active form by treatment with heat, urea or sodium dodecyl sulphate.

Large quantities of recombinant SBTI have been prepared for inclusion in artificial diets on which the larvae of stem borer, leaffolder and other insects can feed. Such tests will indicate whether SBTI is as toxic to insect digestion *in vivo* as it is to insect trypsin *in vitro*. It is possible that SBTI will prove toxic to many important phytophagous pests. Accordingly, we have begun a survey to identify additional insect targets among both dipterans and lepidopterans. In addition, the gene encoding SBTI will be transferred to plasmids suitable for transformation of rice and other plants. Antibodies against SBTI will be used to monitor the spatial and temporal expression of SBTI in transgenic plants and to assess the influence of subcellular targetting on the levels of SBTI accumulation. As plants are usually regarded as devoid of trypsin-like proteinases, it is possible that SBTI will not be toxic to plant cells.

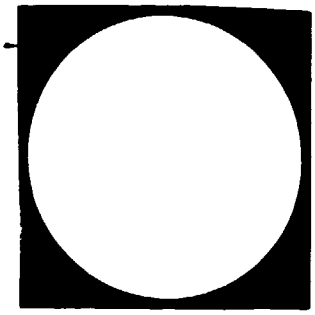
SBTI is potentially an anti-nutritional factor in human and animal diets. We are therefore keen to isolate mutant forms of the SBTI gene which have reduced toxicity to mammals but retain insect toxicity. Site-specific and random mutations will be generated and expressed in *E. coli*. We are currently developing a method for the rapid screening of bacterial colonies for the presence of recombinant SBTI with differential toxicity to insect and mammalian trypsins. The fact that the crystal structure of the complex between porcine trypsin and the soybean trypsin inhibitor has been published will aid our mutation strategy.

Very little is known about the structure, activation and gene expression of proteinase zymogens in insects. This is a serious deficiency, given the likely importance of insect proteinases as targets for inhibition in transgenic plants. There are indications that the presence of trypsin inhibitors in the diet might trigger trypsin secretion in some insects. We shall use PCR to amplify the trypsin gene of *Drosophila melanogaster* for use as a probe for trypsin gene expression in insects and for expression in *E. coli*. Antibodies raised against the recombinant zymogen will be used in assays of trypsin localization and abundance. SBTI is toxic to the trypsin of *Drosophila melanogaster*: this indicates the feasibility of a laboratory study on the ability of insects to evolve resistance to protein inhibitors.

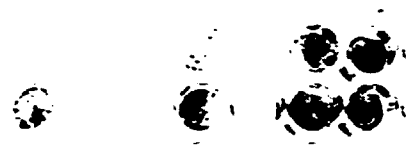
The gene mapping programme involves the use of restriction fragment length polymorphism (RFLP) analysis. Rice contains 12 haploid chromosomes, for which a map of over 350 DNA markers has been prepared by Steve Tanksley's group at Cornell University. The clones mapped by the Cornell group have been made available to us for mapping of genes which confer resistance to brown planthopper (Bph), gall midge (Gm) and *C. oryzae*. These phenotypes are highly suitable for RFLP mapping because they are known to be controlled by major genes. The mapping population for Bph and Gm resistance is a set of 47 recombinant inbred lines (F_{6-7}) derived from a cross between the high yielding cultivar Phalguna (which has resistance to Gm biotype 1) and the Assam landrace ARC6650 (which carries resistance to the Indian biotype of Bph). The recombinant inbred lines have already been screened for Bph and Gm resistance by our collaborators at the



RICE PROTOPLASTS



KANAMYCIN SELECTION FOR TRANSFORMED CALLI



GUS ASSAY: CALLI SHOWING β-GLUCURONIDASE EXPRESSION (BLUE)

Procedure utilized at ICGEB New Delhi for the tranformation of rice. Rice protoplasts (90% viable, as shown by fluorescent staining) are incubated with plasmid DNA in the presence of polyethylene glycol. The top right figure shows calli growing on selection medium containing kanamycin. The bottom right figure shows the expression of the β-glucuronidase gene introduced with the plasmid conferring a blue colour to the transformed cells. The bottom left part of the figure shows transformed rice plants regenerated from the calli.

Directorate of Rice Research, Hyderabad. Results from a colleague at Raipur suggest that Gm is located on chromosome 6, fairly close to the *waxy* gene. Accordingly, we are concentrating our efforts on determining the segregation patterns among the inbred lines of 10-15 DNA markers known to be located on chromosome 6. After the Gm gene has been mapped, we shall extend our analysis to all 12 chromosomes to locate the Bph gene. In this case about 80 probes will have to be used to place the Bph gene on one of the chromosomes and then another 10 will have to be used to locate the gene along the correct chromosome.

We are determined that RFLP map data will be put to use in plant breeding institutes in developing countries. To facilitiate transfer of this technology, we are adopting an approach based on the use of PCR for RFLP analysis. PCR lends itself to simple automation. We believe that for many purposes PCR will replace the more demanding technique of Southern hybridization. More than 30 mapped clones of the rice genome have been sequenced at each end to obtain information on which to base the synthesis of suitable primers for PCR amplification of the corresponding loci from rice varieties of interest. About one-third of these pairs of primers have been synthesized and

tested: in most cases they amplify from indica rice a single DNA species of the expected size.

We are currently investigating the utility of these PCR products for the detection of RFLPs by direct staining of agarose gels, without the need for blotting or hybridization. The size of PCR products amplified from a given locus appears to be reasonably constant among indica varieties but can be quite different among indica, japonica and other species of *Oryza*. We are testing the hypothesis that some of these size differences are due to the involvement of mobile elements. RFLP analysis is also part of our genetic and biochemical studies on the resistance of rice to the causal agent of narrow brown leaf spot disease, *C. oryzae*. This fungus is one of many species of the genus *Cercospora* which are known to produce the red toxin cercosporin, which is believed to play an important role in symptom development through its ability to generate free radicals when excited by light. It is almost universally toxic to living cells and has been proposed as an agent for photodynamic chemotherapy of cancer.

Several major and minor crops are quite adversely affected by *Cercospora* diseases, including maize, soybean, peanut, banana, beet and asparagus, especially in the humid tropical regions. Natural resistance to species of *Cercospora* is generally weak or non-existent, and all attempts to generate somaclonal variants with such resistance have failed. Rice, however, is exceptional in that resistance to many races of *C. oryzae* has been incorporated into popular US and Central American rice varieties since the 1940s. In particular, the common weed "red rice" is resistant to almost all of the approximately 47 known races of the fungus. The once-popular variety Lemont is resistant to about half of these races. However, many of the rice varieties currently popular in the Americas (such as Leah and Labelle) are susceptible to the newer races. The same is true of some popular Chinese varieties. Accordingly, we initiated a programme to determine whether resistance to *C. oryzae* was due to cellular resistance to cercosporin or to other reasons. We have purified cercosporin from an isolate of *C. oryzae* and have added it to solid medium supporting the growth of rice callus derived from seeds of red rice, Lemont, Leah and Labelle. We have measured the toxicity of cercosporin in terms of inhibition of callus growth and abolition of the capacity of calli to convert fluorescein diacetate to fluorescein. Both assays give the same result: red rice and Lemont are resistant to cercosporin at the cellular level but Leah and Labelle are sensitive. This is the first demonstration of cellular resistance to this toxin in plants. In addition, calli of red rice and Lemont remain yellow, while calli of the other varieties turn purple and die. This suggests that resistance to cercosporin involves either rapid degradation of the toxin or its exclusion from the cells. This discovery opens the door to a biochemical and genetic analysis of resistance mechanisms in rice, which should have relevance to the development of resistance in other crops.

Herbicide tolerance

The herbicide glyphosate (phosphonomethylglycine) is widely used against mono- and dicotyledonous weeds. Its target is enolpyruvylshikimate 5-phosphate (EPSP) synthase, an enzyme of aromatic amino acid biosynthesis. However, the inability of glyphosate to discriminate between crop plants and weeds restricts its use during the growth season.

Discrimination has recently been generated by incorporating into several crop plants an altered EPSP synthase gene which encodes a target with

reduced affinity for the herbicide. Another possible strategy for introducing glyphosate tolerance into plants is to confer upon them the ability to detoxify the herbicide. At ICGEB we are pursuing both strategies.

The gene for EPSP synthase from *Bacillus subtilis* has been cloned into pUC18 and transformed into *E. coli* AB2829, an EPSP synthase-deficient mutant. Transformants were selected for prototrophy.

The EPSP synthase gene was detected by Southern hybridization using as probe an oligonucleotide corresponding to a conserved segment of the protein. The transformant was resistant to glyphosate, as demonstrated by its ability to grow in the presence of 100 mM herbicide. Reasons for this high level of tolerance are being investigated but may lie in part in the 15-fold overexpression of the enzyme in the transformant compared with wild-type *E. coli*.

Glyphosate degradation has been studied at three levels:

(1) isolation of a Pseudomonad with a high capacity for growth on glyphosate as sole phosphorus source.
(2) detection of C-P lyase *in vitro*.
(3) cloning of the C-P lyase gene.

We are working with two Pseudomonads capable of growth on glyphosate as sole P source. They are designated PG2982 (from Dr. Shinabarger) and GLC 11 (an ICGEB isolate).

While both strains are able to grow on 10 mM glyphosate as P source, they differ markedly in their capacity for growth on the commercial formulation of glyphosate (the isopropylammonium salt known as Glycel). PG2982 can grow on no more than 5 mM Glycel, whereas GLC 11 can grow on 100 mM Glycel. The basis of this difference is unknown but it does not appear to be due to sensitivity of PG2982 to the counterion. Both strains can also grow on other phosphonates as sole P source (e.g., 2-aminomethylphosphonate and 2-aminoethylphosphonate).

The ability of strains PG2982 and GLC 11 to grow on phosphonates as P source strongly suggests that they possess C-P lyase activity. C-P lyase activity has been detected in extracts of both strains, through measurement of glyphosate-dependent release of phosphate.

Detection of the other product of the C-P lyase reaction, sarcosine, has been hampered by the presence of a sarcosine dehydrogenase activity in the extracts.

The highest specific activity has been recorded in preparations of proteins from the periplasmic space. It appears that C-P lyase is secreted into the periplasm, whereas sarcosine dehydrogenase is intracellular.

Cloning of the C-P lyase gene is being attempted from both PG2982 and GLC 11. Most progress has been made with the former. Chromosomal DNA was partially digested with Hind III or Pst I and 10-30 kb fragments were ligated into the broad host range cosmid pLA2917.

After packaging and transfection on *E. coli* strain S17-1, colonies were selected on plates for growth on appropriate antibiotics and then tested for growth on 1 mM glyphosate as sole P source. The medium was supplemented with phenylalanine, tyrosine and tryptophan to counteract the effects of glyphosate on EPSP synthase. Two clones have been obtained, each of which contains a 20 kb insert within pLA2917. These clones are being characterized further.

Structural Biology:
a) Protein Chemistry

V.S. Chauhan, P. Kaur, A. Mathur, A. Bhardwaj, S. Chatterjee, S. Nagpal, R. Arora, N. Singh

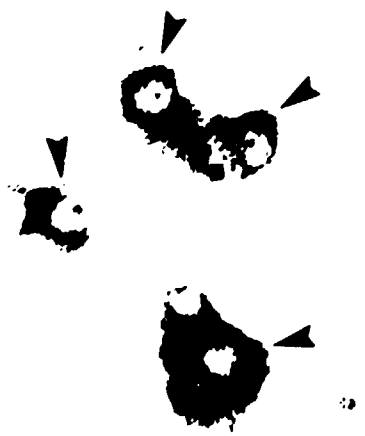
Design, synthesis and structural studies of conformationally restricted peptides

Conformationally constrained peptides are of tremendous potential in understanding structure-function, in developing highly active analogues of bioactive peptides, in designing enzyme inhibitors and drug design in general. Our objective is to design linear peptides to serve as models for secondary structural motifs and, using these, to synthesise highly active and selective bioactive peptides. Our studies with α, β-dehydroamino acids containing model peptides have shown them to be excellent models for introducing turns and helical motifs.

Solution conformational studies using high resolution nuclear magnetic resonance (NMR) have shown that dehydrophenylalanine (DPhe) and dehydroleucine (DLeu) show a strong tendency to induce β-turns in peptide chain. Crystal structure of these peptides, i.e., Boc-Phe-DLeu-Val-OMe, For-Met- DPhe-Phe-OMe have confirmed these findings.

Another such peptide has shown the presence of two consecutive β-turns. Solution conformational studies on synthetic peptides I, II and III containing two or more dehydrophenylalanines show these to be excellent models for helical structures. Crystal structure of peptide I has clearly indicated the presence of a 3_{10} helix.

(I) Boc-Gly-DPhe-Leu-DPhe-Ala-NHCH$_3$ (3_{10} helix)
(II) Boc-Phe-DPhe-Val-Phe-DPhe-Val-OCH$_3$ (3_{10} helix)
(III) Boc-Gly-DPhe-Ala-Phe-Leu-DPhe-Ala-NHCH$_3$ (α-helix)



A chemically synthesized peptide, derived from the hepatitis B surface antigen, oligomerizes spontaneously, giving rise to macromolecular aggregates in aqueous solution, indicated by the arrows in the electron microscope picture. The aggregates seems a likely candidate for a novel vaccine for hepatitis B.

Interestingly dehydroalanine (DAla) and dehydroaminobutyric acid (DAbu) peptides produce different constraints. NMR studies have shown that DAla introduces a γ-turn, whereas DAbu prefers an extended structure. It is clear that the size of β-substituent plays an important role in deciding the nature of the peptide backbone constraint in dehydro peptides. We are presently exploring if this theme can be extended to generate other secondary structures, such as a β-sheet, etc.

We now plan to use these principles in designing amphipathic helices, and also to extend these studies in synthesising highly active analogues of Luteinizing hormone-releasing hormone (LH-RH) and of Bombesin, a highly potent neuropeptide, and to study the structural requirements of the chemotactic receptor through constrained analogues of the chemotactic peptide (FMLP). These studies will be helpful in establishing ground rules for peptide design and for correlating structure with function.

Malaria antigens

All malaria antigens show an intriguing structural feature: they contain tandemly repeated amino acid sequences which are highly antigenic. These antigens also show unusual cross-reactivity, the reasons for which are not clearly defined. We synthesised peptides corresponding to these repeat units and have studied their immunological and structural characteristics. Circular dichroism studies have indicated that these repeat unit peptides from different antigens show noticeable structural similarities in solution. Antibodies raised against these peptides showed high levels of cross-reactivity indicating that the repeat units may indeed be responsible for immunological cross-reactivity at the antigen level and also for the parasite defence. Usefulness of these repeat unit peptides in serological studies is being tested in collaboration with Malaria Research Centre in Delhi.

A 195 kD protein, recursor to major merozoite surface antigen (PMMSA), has emerged as the main target for a blood stage vaccine. We have focussed our attention on a 83 kD protein, N-terminal fragment of PMMSA, which is believed to contain elements required for protective immunity. We have synthesised a number of peptides from the conserved region of 83 kD fragment, corresponding to the most hydrophilic portions, in order to locate the dominant B-cell epitopes. We have found that these peptides cross react with antibodies in the sera from all malaria patients that we have tested. A peptide corresponding to the non-conserved region showed poor response.

A major problem in designing a synthetic peptide vaccine is that it usually requires a carrier protein. It is now recognised that this may be circumvented by including T-cell epitopes in the designed peptide. We have synthesised composite peptides consisting of a B-cell epitope from 83 kD protein (residues 266- 279) and universally recognised T-cell epitopes from tetanus toxoid (peptide IV) and from a malaria antigen CSP (peptide V). We have found that inclusion of T-cell epitope enhances the immunogenecity significantly, and also helps overcome the genetic restriction in antigen presentation. These findings will be useful in peptide vaccine design. Based on the above

(IV) LDNIKGNVGKMEDYIKKNKKEEKLAKMEKASSVFNVV
(V) LDNIKGNVGKMEDYIKKNKKQYIKANSKFIGITEL

observations, we have synthesised two candidate peptides, 48 and 52 residues long, containing two B-cell and two T-cell epitopes each. We have

shown that these composite peptides also recognise antibodies in malaria infected human sera. We are currently checking T-cell response in mice and with human T-cells, with these composite peptides.

Two synthetic peptides from thrombospondin related anonymous protein (TRAP), a nonapeptide and a 18-residue peptide, were synthesised. These peptides have shown very encouraging results in both sporozoite and merozoite invasion inhibition (upto 60% inhibition). Structural studies on the smaller peptide are in progress. We now plan to concentrate on the development of a blood stage synthetic multiple epitope peptide. After obtaining a complete immunological profile on the above two polypeptides we propose to carry out toxicology experiments in at least two animal systems, before planning any clinical experiments. Based on our recent results with B- and T-cell epitope identification, we also propose to design more multiple epitope peptides corresponding to 83 kD protein, in order to develop a blood stage malaria peptide vaccine.

Studies on anticoagulant peptides

Hirudin, a 65 amino acid polypeptide from the leech, is a highly specific and potent inhibitor of thrombin. It has been shown that C-terminal fragments of hirudin can form the basis for developing a synthetic peptide inhibitor of thrombin. We have synthesised several analogues to further correlate structure-function relationships and have found that analogues designed to have preferences for helical structure showed poor or no antithrombin activity.

We found that the bioactivity is highly sequence specific and that residues 52-63 contain the minimal sequence with maximum inhibition activity. We have successfully designed and synthesised peptides containing the C-terminal binding domain joined through a non-peptide linker to a tripeptide inhibitor sequence (D-Phe-Pro-Arg-X) that is supposed to fit in the catalytic groove of thrombin. These bifunctional peptides are more potent than the C-terminal fragments alone. Now that the crystal structure of the thrombin-hirudin complex is known, it might be possible to design better analogues for thrombin inhibition.

Structural Biology: b)Mammalian Gene Products

Q.S. Wang, Y. G. Min, K. Majumder, A. Chatterjee, M. Malhotra

Recombinant bovine growth hormone

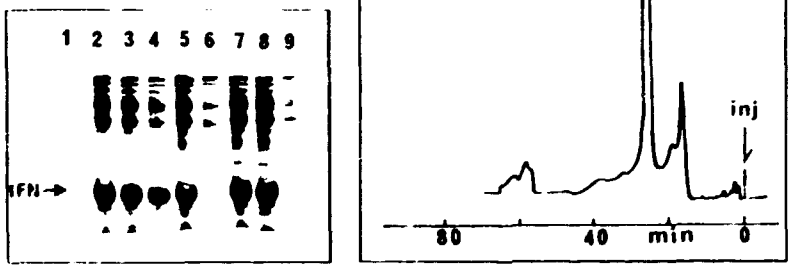
Earlier we had synthesised, cloned and sequenced the three fragments of the bGH gene. These fragments were assembled into a complete gene of correct sequence. We have inserted the bGH gene into a vector with the heat-inducible p_L promoter of phage λ. An expression level of 10-15% of total cellular protein was achieved in *E. coli*. This was shown by protein staining after SDS-PAGE and by immunoassay.

Attempts are being made to improve the level of expression by trying a combination of the following: different origins of *E. coli*, different vectors using various strong promoters, a two [illegible] system and variations in the conditions of gene induction.

Recombinant human γ-interferon

The γ-IFN gene has been expressed to about 60% of total protein in *E. coli*. Because of this high level of expression, the protein is obtained as an inclusion body which needs to be solubilized by denaturation-renaturation. To this end we hope to develop a simple and economical purification procedure for potential industrial use. We purified the inclusion bodies (IB) containing recombinant γ-IFN by sonication, removing the cell debris by centrifugation and then collecting the IB by further centrifugation at higher speed. The purity of recombinant IFN in the IB is more than 90% as assessed by SDS-PAGE. Further purification was carried out by gel filtration HPLC. The HPLC purified material showed positive results with ELISA carried out using anti-human γ-IFN antibodies raised in rabbits using commercially available recombinant human γ-IFN.

Initial efforts to set up a biological assay for γ-IFN using the WISH cell line and vesicular stomatits virus (VSV) were not very successful due to the slow growth rate of WISH cell line. We have now switched over to the VERO cell line and VSV. The preliminary results obtained from using this assay system and γ-IFN purified by controlled pore glass column chromatography and reversed phase HPLC are encouraging.



Recombinant γ-interferon expressedd in *E. coli* under the influence of the λ-PL promoter. The left panel shows the SDS-PAGE analysis. Lane 1: standard γ-INF; lanes 2-5 and 7-8: clones bearing γ-INF genes; lanes 6 and 9: control plasmid. The right panel shows the h.p.l.c analysis of the γ-INF obtained from purified inclusion bodies.

Recombinant hirudin.

Hirudin, an extremely potent anti-coagulant, has been widely proposed as a possible drug for thrombosis. We have chemically synthesized, ligated into vector pINIII0mpA3, cloned and expressed in *E. coli* a hirudin gene based on the known protein sequence of leech hirudin variant 2. Two clones (pHKME210 and pHKME219) were tested for reproducibility of expression as monitored by a clot-inhibition assay and ELISA. The estimated yield based on activity determinations is 0.5 mg l of culture. Optimization of yield is being carried out. The recombinant hirudin has a 6 amino acid long N-terminal extension which can be removed by CNBr treatment of the protein.

Removal of the extension leads to increased activity of recombinant hirudin. It is known that the presence of extra amino acids in the N-terminus of hirudin reduces its capacity to bind specifically to thrombin.

To investigate the importance of individual amino acid residues at the N-terminus of hirudin in the binding of the protein to the active center of thrombin, two mutations of hirudin (viz., tyrosine at position 3 to phenylalanine or alanine) have been generated using PCR. The phenylalanine mutant gene has been cloned and characterised by restriction mapping of positive clones. The DNA sequence was correct. Cloning and characterisation of the other mutant is in progress. Future work will involve:

(i) purification and sequencing of recombinant hirudin and the two mutant forms;

(ii) characterization of mutants with respect to binding to thrombin.

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Lagatolla, C., Dolzani, L., Gutierrez, M.L., Giacca, M., Monti-Bragadin, C.
A restriction polymorphism for the identification of *rpsL* gene Alleles in partial diploid strains of *Escherichia coli*: Biochem. Pharmacol., (in press)

Gustincich, S., Carninci, P., Manfioletti, G., Del Sal, G. and Schneider, C.
A fast method for high quality genomic DNA extraction from whole human blood: Biotechniques, (in press)

Doorbor, J., Parton, A., Harley, K.O., Banks, L., Crook, T., Stanley, M. and Crawford, L.
Detection of novel splicing patterns in a HPV-16 containing keratinocyte cell line: J. Virol. (in press)

Kaur, P., Sharma, P., Kumar, A., Chauhan, V.S.
Synthetic, immunological and structural studies on repeat unit peptides of *plasmodium falciparum* antigens: Int. J. Peptide Protein Res. (in press)

Ludwig, D.L., Bruschi, C.V., The 2μm Plasmid as a nonselectable, stable, high-copy number yeast vector: Plasmid. (in press)

Siri, A., Carnemolla, B., Saginati, M., Leprini, A., Casari, G., Baralle, F.E., Zardi, L.
Complete primary structure and analysis of different pre-mRNA splicing patterns of human tenascin. Characterization of specific epitopes recognized by monoclonal antibodies: Nucl. Acids Research (in press)

Dash, S., Rao, K.V.S., Joshi, B., Nayak, N.C., Panda, S.K.
Significance of Natural Polymerized Albumin and its Receptor in Hepatitis B infection of Hepatocytes. Hepatology (in press)

Manivel, V., Rao, K.V.S. An Interleukin-1 derived synthetic Peptide as an added co-adjuvant in vaccine formulations: Vaccine (in press)

Bennett, J.
Protein phosphorylation in green plant chloroplasts: Ann. Rev. Plant Physiol. Plant Mol. Biol. (in press)

Bagnarelli, P., Menzo, S., Manzin, A., Giacca, M., Varaldo, P.E., Clementi, M.
Sensitive detection of human immunodeficiency virus type 1 (HIV-1) genomic RNA in plasma samples by reverse-transcription polymerase chain reaction" J. Med. Virol., (in press)

Bagnarelli, P., Menzo, S., Manzin, A., Varaldo, P.E., Montroni, M., Giacca, M., Clementi, M.
Efficient and fast detection of human immunodeficiency virus type 1 transcripts in peripheral blood lymphocytes by the polymerase chain reaction: J. Virol. Methods, (in press)

Collaborative Research Programme, Training Activities and Scientific Services

Affiliated Centres and Collaborative Research Programme

The role of the Affiliated Centres

These are research institutes laboratories in member states, recognized by the PSA and the Preparatory Committee as having attained a demonstrably high standard to allow them to enter into a special relationship with ICGEB.

They form an effective network enabling the Centre strengthen research in developing countries and to reach a large number of scientists at home, through an extension of its services and training and research programmes.

At present ICGEB has 13 affiliated centres in member countries. Applications for affiliation of laboratories in Mexico and Tunisia have been evaluated by expert group missions and the Panel of Scientific Advisors. The recommendations of the Panel on the above affiliations will be considered by the Preparatory Committee in its 16th session (May 1991).

ALGERIA, ARGENTINA, BRAZIL, BULGARIA, CHILE, CHINA, CUBA, EGYPT, GREECE, HUNGARY, NIGERIA, VENEZUELA, YUGOSLAVIA

The Collaborative Research Programme (CRP)

The CRP was established to encourage joint research between ICGEB and its affiliate centres in areas of common interest and to stimulate research in applied projects, not directly related to the ICGEB programme, but of particular interest to the developing world.

Under the provisions of the current Five-Year Programme, each affiliated centre can submit up to five proposal per year, all of which are subject to peer reviewing by ICGEB staff and external referees.

Grants do not exceed US$ 35,000 per annum and are extended for a maximum period of three years. They are strictly meant to cover expenditures related to expendable supplies, small items of equipment, educational travel and research visits.

The collaborative research programme of ICGEB continued in 1990 for a second year. During the first year of the collaborative research programme, 1989, 14 collaborative projects received grants amounting to US$860,000. In 1990, from a total number of 44 research proposals submitted, 18 received ICGEB grants amounting to US$1,119,000.00 (Fig. 1). Listings of the projects approved in the first two years of the operation of this programme are given in Tables 1 and 2.

Fig.1 **ICGEB COLLABORATIVE RESEARCH PROGRAMME**

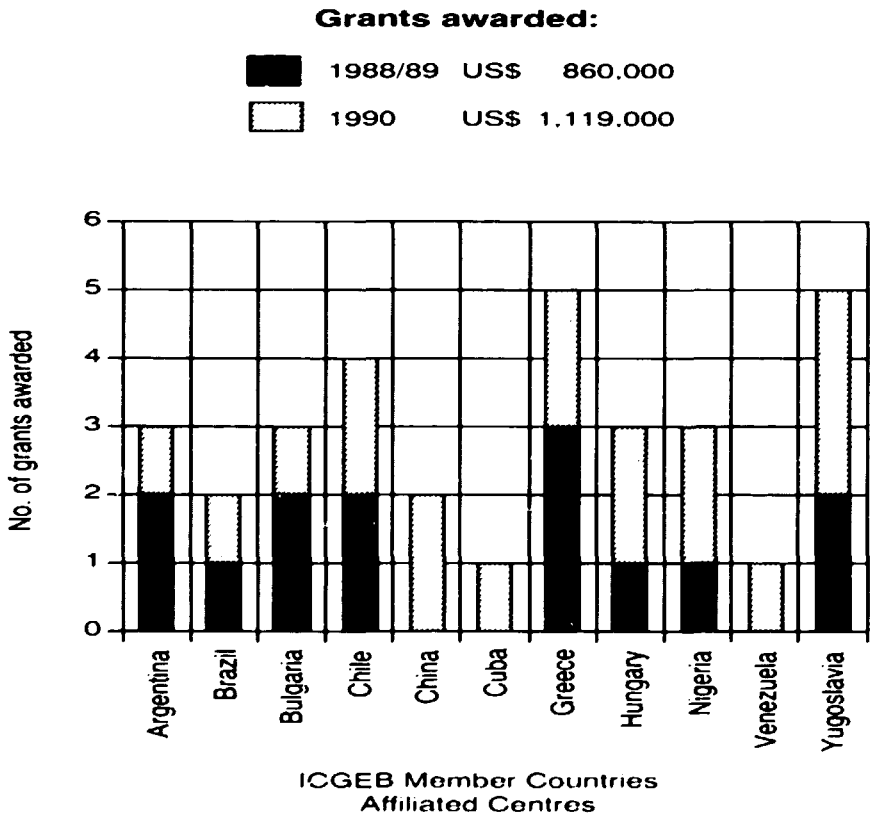

Table 1
UNIDO/ICGEB COLLABORATIVE RESEARCH PROGRAMME 1988/89
Total Number of Projects Submitted = 77
Total Number of Grants awarded = 14 (US$860,000)

Country	Title of Project	Principal Investigator	Institute and Address
ARGENTINA	Genetic and Antigenic relationships between human and animal rotaviruses. Production of antigens on high density cultures of animal cells	Jose L. LA TORRE	Centro de Virologia Animal Serrano 665 1414 Buenos Aires ARGENTINA
ARGENTINA	Regulation of gene expression during developmental processes in higher plants	Nestor CARRILLO	Universidad Nacional de Rosario Facultad de Ciencias Bioquimicas y Farmaceuticas 2000 Rosario - Santa Fe ARGENTINA
BRAZIL	Nutritional improvement of root crops by engineering	Mauro CARNEIRO	S.A.I.N. Parque Rural 70.770 Brasilia-DF BRAZIL
BULGARIA	Tomato Spotted Wilt Virus (TSWV): Genome structure & development of resistant tobacco plants	Atanas ATANASSOV	Central Laboratory of Genetic Engineering 2232 Kostinbrod - 2 BULGARIA
BULGARIA	Development of new vectors for gene expression in mammalian cells	Kalin P. DUDOV	Central Laboratory of Genetic Engineering 2232 Kostinbrod - 2 BULGARIA
CHILE	Microbial degradation of lignin studies on the basidiomycete ganoderma applanatum and bacteria metabolizing lignin-related compounds	Rafael VICUNA	Universidad Catolica de Chile Departamento de Biologia Celular Casilla 114-D Santiago CHILE
CHILE	Development of improved diagnostic and prevention systems for typhoid fever	Arturo YUDELEVICH	Microbiology & Molecular Genetics Unit Dept. of Cell Biology Faculty of Biological Sciences Catholic University of Chile Casilla 114-D Santiago CHILE
GREECE	Structural and genetic analysis on the four alph-helix motif in proteins	Michael KOKKINIDIS	Institute of Molecular Biology & Biotechnology (IMBB) P.O. Box 1527 711 10 Heraklion Crete GREECE
GREECE	Structure and pathogenicity of the acetylcholine receptor	Socrates TZARTOS	Hellenic Pasteur Institute 127 Vas. Sofias Ave. 11521 Athens GREECE
GREECE	Analysis of the organization and expression of the Plum Pox Virus (PPV) genome	Efthimia Mina TSAGRIS	Institute of Molecular Biology & Biotechnology (IMBB) P.O. Box 1527 711 10 Heraklion Crete GREECE

Country	Title of Project	Principal Investigator	Institute and Address
HUNGARY	Study of the mechanism of transactivation in HTLV and BLV	BOROS	Institute of Biochemistry Biological Research Center P.O. Box 521 6701 Szeged HUNGARY
NIGERIA	Mass propagation of trees for wood pulp and paper industry in Nigeria	S.N.C. OKONKWO	Dept. of Botany University of Nigeria Nsukka NIGERIA
YUGOSLAVIA	Study of the new gene (NCU) that affects resistance to gyrase inhibitors	Dragutin SAVIC	Genetic Engineering Centre Vojvode Stepe 283 P.O. Box 794 11001 Belgrade YUGOSLAVIA
YUGOSLAVIA	Development of vectors for gene expression in lactobacilli	Ljubisa TOPISIROVIC	Genetic Engineering Centre Vojvode Stepe 283 P.O. Box 794 11001 Belgrade YUGOSLAVIA

Table 2
UNIDO/ICGEB COLLABORATIVE RESEARCH PROGRAMME 1990
Total Number of Proposals Submitted = 44
Total Number of Grants Awarded = 18 (US$ 1,119,000.00)

Country	Title of Project	Principal Investigator	Institute and Address
ARGENTINA	Ionic channels in plant cells: Molecular basis for plant improvement in semi-arid regions	F.J. BARRANTES	Instituto de Investigaciones Bioquimicas Universidad Nacional del Sur Consejo Nacional de Investigaciones Cientificas y Tecnicas C.C. 857, Camino La Carrindanga Km. 7 8000 Bahia Blanca ARGENTINA
BRAZIL	Oncogenes and anti-oncogenes in cell proliferation control	Mari C.S. ARMELIN	Instituto de Quimica Departamento de Bioquimica Universidade de Sao Paulo C.P. 20780 Sao Paulo 01498 Sao Paulo BRAZIL
BULGARIA	Molecular basis of cystic fibrosis in Bulgaria	Luborodna KALAYDJIEVA	Laboratory of Molecular Pathology Institute of Obstetrics 2 Zdrave St 1431 Sofia BULGARIA
CHILE	Studies of the stress response in biomining microorganisms. Possible implications in the improvement of the bioleaching process	Carlos A. JEREZ	Dept. Bioquimica Facultad de Medicina Universidad de Chile Casilla 70086 Santiago CHILE
CHILE	Saccarification of straw: use of enzymes from native fungi	Jaime EYZAGUIRRE	Department of Cell and Molecular Biology Universidad Catolica de Chile Casilla 114-D Santiago CHILE
CHINA	A novel, efficient and powerful method for site-specific mutagenesis	Qi Song WANG	Institute of Genetics Fudan University Shanghai 200433 PEOPLE'S REPUBLIC OF CHINA
CHINA	Studies on structural mechanism of prolonged-acting and highly potent human insulin	Da Cheng WANG	Institute of Biophysics Chinese Academy of Sciences Zhong Guan Cun Beijing 100080 PEOPLE'S REPUBLIC OF CHINA
CUBA	Transformation of sweet potato (Ipomonea Batata L.) for increasing its nutritional value as food and animal feed	Sergio PEREZ TALAVERA	Centro de Ingenieria Genetica y Biotecnologia Ave 31 entre 190 y 158 Cubanacan Cuidad Habana CUBA

Country	Title of Project	Principal Investigator	Institute and Address
GREECE	Photosynthetic water cleavage and inhibitory effect of herbicides	Demitrios GHANOTAKIS	Department of Chemistry University of Crete P.O. Box 1470 GR-71110 Iraklion Crete GREECE
GREECE	Structural and functional analysis of human glutamate dehydrogenase	Nicholas MOSCHONAS	Institute of Molecular Biology & Biotechnology Foundation for Research & Technology P.O. Box 1527 71110 Heraklion, Crete GREECE
HUNGARY	Characterization of DNA binding proteins involved in the regulated expression of a wheat chlorophyll a b binding protein	Ferenc NAGY	Institute of Plant Physiology Biological Research Center (BRC) Hungarian Academy of Sciences P.O. Box 521 H-6701 Szeged HUNGARY
HUNGARY	Structural studies on sequence specific DNA-protein interactions	Laszlo OROSZ	Agricultural Biotechnology Center P.O. Box 170 2101 Godollo HUNGARY
NIGERIA	The biology of bananas, plantains and of sigatoka in the breeding for resistance to the sigatoka leaf spots	Tunde FATUNLA	Department of Plant Science Obafemi Awolowo University ILE-IFE NIGERIA
NIGERIA	Screening of the antimutagenic and genotoxic activities of extracts of several edible vegetables plants and mushrooms commonly consumed in Nigeria	Emmanuel OBASEIKI-EBOR	Department of Pharmaceutical Microbiology College of Medical Sciences University of Benin Benin City NIGERIA
VENEZUELA	A pilot project of the application of nucleic acid probes to malaria diagnosis in Venezuela	Hilda A. PEREZ	Instituto Venezolano de Investigaciones Cientificas (IVIC) Centro de Microbiologia y Biologia Celular Lab. Immunoparasitologia Apdo 21827 Caracas 1010A VENEZUELA
YUGOSLAVIA	Molecular Diagnostics of Genetic and Infectious Diseases	Ana SAVIC	Institute for Molecular Genetics and Genetic Engineering P.O. Box 794 11001 Belgrade YUGOSLAVIA
YUGOSLAVIA	Sequencing by hybridization: Method development on gamma vectors	Radomir CRKVENJAKOV	Institute for Molecular Genetics and Genetic Engineering P.O. Box 794 11001 Belgrade YUGOSLAVIA
YUGOSLAVIA	Genetic and protein engineering of penicillin acylase	Vladimir GLISIN	Institute of Molecular Genetics and Genetic Engineering P.O. Box 794 11001 Belgrade YUGOSLAVIA

For 1991, 38 collaborative research proposals were submitted for consideration. The number of proposals per Affiliated Centre is given below:

Algeria	2	Greece	4
Argentina	4	Hungary	7
Brazil	2	Mexico	5
Bulgaria	1	Nigeria	1
Chile	3	Tunisia	3
Cuba	5	Yugoslavia	1

Peer-reviewing of these proposals has commenced and a final evaluation will be made in the third quarter of 1991.

The collaborative research programme includes also a training component. Provisions are made within each project for least one long-term training fellowship. The trainee fellows supported under this scheme are selected by ICGEB, in consultation with the principal investigator of the project, and must originate from a member country other than that of the host laboratory. Financial support for these fellowships is in addition to the collaborative research grant and may not exceed US$ 15,000.

Training Programme

A two-component programme of short and long-term training activities has been initiated to allow the Centre to reach one of its most foundamental targets, namely to disseminate state-of-the-art biotechnology to scientists from developing countris.

Long-term training

In so far as long-term training is concerned, ICGEB awards post-doctoral fellowships to member-country scientists (ten fellowships a year per Component) for one year with the possibility of renewal for a second year. Another ten fellowships are tenable for work in selected Italian laboratories on programmes related to those of the Centre.

ICGEB received 99 and 202 applications for fellowships in 1989 and 1990 respectively. Of these, 22 candidates were selected in 1989 and 26 in 1990. They have been assigned to research teams in the New Delhi and Trieste Components of ICGEB as well as in selected Italian Institutes. The fellowships are distributed to candidates from 21 Member Countries.Fig. 2 gives the collective data related to this programme component, whereas Tables 3 and 4 provide a comprehensive list of placements.

ICCEB fellowships range between US$ 15.000-20.000.

Fig. 2

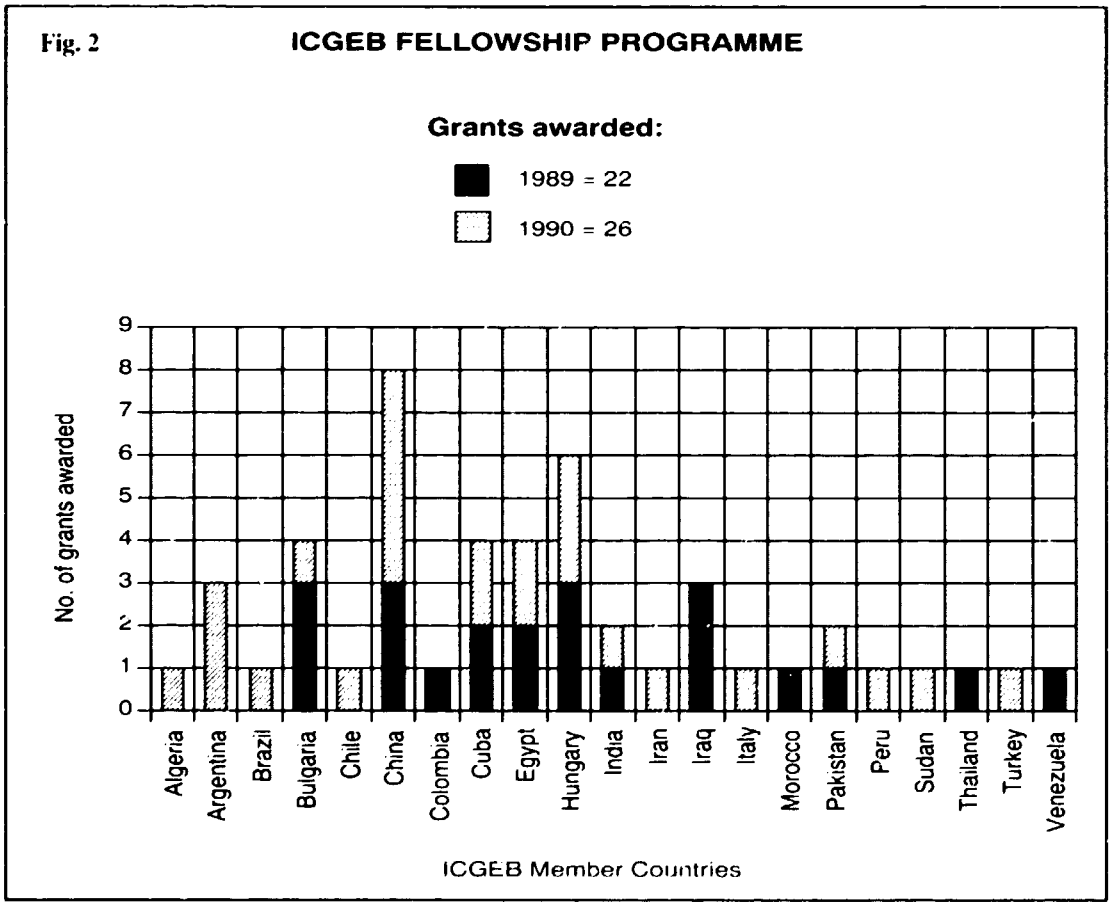

Table 3

ICGEB FELLOWSHIP PROGRAMME 1989

Number of grants awarded = 22

Name	Country	Host Laboratory
Dimiter DEMIROV	Bulgaria	ICGEB, Trieste, Italy
Jordan KRLAKOV	Bulgaria	ICGEB, New Delhi, India
Daniella DIMITROVA	Bulgaria	ICGEB, Trieste, Italy
Shang-Zhong XI	China	ICGEB, Trieste, Italy
Jianguo LIU	China	ENEA, Rome, Italy
Li Ya KANG	China	ICGEB, Trieste, Italy
Jesus Abelardo GRANJA	Colombia	IBC CNR, Rome, Italy
Maribel GUERRA	Cuba	IGBE CNR, Pavia, Italy
Luis RODRIGUEZ MENOCAL	Cuba	ICGEB, Trieste, Italy
Mohammed S. A-SALAM MAHMOUD	Egypt	ICGEB, New Delhi, India
Magdy S. RADY	Egypt	IGBE CNR, Pavia, Italy
Maria SZAKACS-DOBOZI	Hungary	ICGEB, Trieste, Italy
Peter NAGY	Hungary	CSVVCM CNR, Bari, Italy
Attila MOLNAR	Hungary	UNIV, Florence, Italy
Sarita Winifred NAZARETH	India	ICGEB, Trieste, Italy
Akeel Abed YASSEEN	Iraq	CSAN CNR, Rome, Italy
Esmail K. SHUBBER	Iraq	IGBE CNR Pavia, Italy
Farkad FATLAH	Iraq	ICGEB, New Delhi, India
Mohamed ETTAYEBI	Morocco	IBC CNR, Rome, Italy
Shoukat PARVEZ	Pakistan	ICGEB, Trieste, Italy
Prasert SUNTINANALERT	Thailand	ICGEB, Trieste, Italy
Edmundo BARRIOS	Venezuela	ICGEB, New Delhi, India

Table 4

ICGEB FELLOWSHIP PROGRAMME 1990

Number of grants awarded = 26

Name	Country	Host Laboratory
Chikh BENGRA	Algeria	ICGEB. Trieste. Italy
Carlos Alberto MELO	Argentina	ICGEB. Trieste. Italy
Alejandro Jose VILA	Argentina	UNIV. Florence LCIB. Italy
Susana GIAMBIAGI	Argentina	ICGEB. Trieste. Italy
Elizabeth Kinuyo GIMBO	Brazil	IGBE CNR Pavia. Italy
Svetlana TOMOVA	Bulgaria	CBM CNR. Rome. Italy
Luisa Angelica ARANEDA	Chile	UNIV. Rome. Italy
Xian Jin ZHOU	China	ISC Bergamo. Italy
Jianwen ZHANG	China	ICGEB. Trieste. Italy
Zhiping WENG	China	ICGEB. Trieste. Italy
Zibai QI	China	UNIV.Rome Ist.Vir.. Italy
Han Ying YUAN	China	ICGEB. New Delhi. India
Rene DELGADO HERNANDEZ	Cuba	IREMN Milan. Italy
Ileana A. GONZALEZ RODRIGUEZ	Cuba	ICGEB. Trieste. Italy
Mohamed Helmy EL-BORAI	Egypt	CSII CNR. Turin. Italy
Ahmed Abdel Salam SETTIN	Egypt	IBC CNR Rome. Italy
Gyorgy SIMON	Hungary	ICGEB. Trieste. Italy
Bela SZABO	Hungary	ISS Rome. Italy
Miklos CSERZO	Hungary	ICGEB. Trieste. Italy
S. BALAJEE	India	ICGEB. Trieste. Italy
Bita NAKHAI	Iran	ICGEB. Trieste. Italy
Alessandro TOSSI	Italy	ICGEB. Trieste. Italy
Abdul Razaque MEMON	Pakistan	ICGEB. New Delhi. India
Carlos Alberto BARRON	Peru	UNIV. Milan. Italy
Fakhreldin OMER	Sudan	ICGEB. New Delhi. India
Huseyin BAGCI	Turkey	ICGEB. Trieste. Italy

Table 5

ICGEB SHORT-TERM TRAINING PROGRAMME
LIST OF TEACHERS

Theoretical and Practical Courses

John Abelson	California Institute of Technology, Pasadena	USA
Paolo Amati	University of Rome, Rome	ITALY
Jorge Allende	University of Chile, Santiago	CHILE
Amos Barroch	Centre Medicale Universitaire, Geneve	SWITZERLAND
Hugo Barrera-Saldena	Universidad de Nuevo Leon, Monterray	MEXICO
Lucio Benedetti	Institut Jacques Monod, Paris	FRANCE
Bruno Betschart	Swiss Tropical Institute, Basel	FRANCE
Mervyn J. Bibb	John Innes Institute, Norwich	UK
Martin Bishop	Medical Research Council, Cambridge	UK
Imre Boros	Institute of Biochemistry, Szeged	HUNGARY
John Brady	National Cancer Institute, NIH, Bethesda	USA
Roger Brent	Massachusetts General Hospital, Boston	USA
Douglas Brutlag	Stanford University, Stanford	USA
Celia J. Bruton	John Innes Institute, Norwich	UK
Gabriella Campadelli-Fiume	University of Bologna, Bologna	ITALY
Pilar Carvallo	University of Chile, Santiago	CHILE
Luca L. Cavalli-Sforza	Stanford University School of Medicine, Stanford	USA
Rahajit Chakraborty	University of Texas, Houston	USA
Keith F. Chater	John Innes Institute, Norwich	UK
Lawrence Cohen	Applied BioTechnology Inc., Cambridge	USA
John Collins	University of Edinburgh, Edinburgh	UK
Jan Conn	University of Florida, Gainsville	USA
Jacinto Convit	Universidad Central de Venezuela, Caracas	VENEZUELA
Henry Daniell	University of Idaho, Moscow	USA
F. Deak	Institute of Biochemistry, Szeged	HUNGARY
Marcella Devoto	Gaslini Institute, Genoa	ITALY
Heidi Diggelman	ISREC, Epalinges	SWITZERLAND
Erno Duda	Institute of Biochemistry, Szeged	HUNGARY
Fulvio Esposito	University of Camerino, Camerino	ITALY
H. Fan	University of California, Irvine	USA
Laura Frontali	University of Rome, Rome	ITALY
Roberta Gambella	University of Camerino, Camerino	ITALY
A. Gnanam	University of Madras, Chepauk	INDIA
Colin Goding	Marie Curie Research Institute, Surrey	UK
Christine Guthrie	University of California, San Francisco	USA
Ulf Gyllenstein	Biomedical Centre, Uppsala	SWEDEN
Rebecca Hackett	National Institutes of Health, Bethesda	USA
G. Hadlacky	Institute of Biochemistry, Szeged	HUNGARY
Benjamin Hall	University of Washington, Seattle	USA
A. Hernandez	Universidad Central de Venezuela, Caracas	VENEZUELA
David Allan Hopwood	John Innes Institute, Norwich	UK
Eric Hunter	University of Alabama, Birmingham	USA
Keith Iams	ILRAD, Nairobi	KENYA
Y.W. Kan	Howard Hughes Medical Institute, San Francisco	USA
David Kemp	Walter & Eliza Hall Institute of Medical Research, Melbourne	AUSTRALIA
P.S. Khandekhar	National Institute of Immunology, New Delhi	INDIA
Tobias Kieser	John Innes Institute, Norwich	UK
Helen M. Kieser	John Innes Institute, Norwich	UK
I. Kiss	Institute of Biochemistry, Szeged	HUNGARY

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Janet Kurjan	Columbia University, New York	USA
Corinna La Rosa	University of Camerino, Camerino	ITALY
Michael Levitt	Stanford University, Stanford	USA
Giovanna Lucchini	University of Milan, Milan	ITALY
Lucio Luzzatto	Royal Postgraduate Medical School, London	UK
Giovanni Magni	University of Milan, Milan	ITALY
Kamini N. Mendis	Department of Parasitology, Colombo	SRI LANKA
Gabriella Milanesi	CNR, Pavia	ITALY
Sharon Mitchell	US Department of Agriculture, Gainsville	USA
Tony Monaco	Imperial Cancer Research Fund, London	UK
L. Nagy	Institute of Biochemistry, Szeged	HUNGARY
Maine Nicolas	Universite de Paris-Sud, IMG, Cedex	FRANCE
Juan Olate	University of Chile, Santiago	CHILE
R.W. Old	University of Warwick, Coventry	UK
Nduka Okafor	Nigerian Society for Microbiology, Enugus	NIGERIA
Omar Orellana	University of Chile, Santiago	CHILE
Cristian Ortego	University of California, Berkeley	USA
Michael Parkhouse	National Institute of Medical Research, London	UK
John S. Parkinson	The University of Utah, Salt Lake City	USA
Eugina Pates	Baylor College of Medicine, Houston	USA
Hilda Perez	IVIC, Caracas	VENEZUELA
Raul Perez-Berkott	University of Rome "La Sapienza", Rome	ITALY
J.B. Perrone	Beckon Dickinson Tropical Disease Diagnostics, NJ	USA
Thomas Peters	The University of North Carolina, Chapel Hill	USA
Mario Pirastu	University of Cagliari, Sardinia	ITALY
Robert Possee	NERC Institute of Virology, Oxford	UK
John Pulitzer	International Institute of Genetics and Biophysics, Naples	ITALY
Talvio Ramalho-Ortigao	University of Ulm, Ulm	GERMANY
Jose Luis Ramirez	Universidad Central de Venezuela, Caracas	VENEZUELA
Victor Romanowski	Universidad Nacional, La Plata	ARGENTINA
Giovanni Romeo	Gaslini Institute, Genoa	ITALY
Poly Roy	Institute of Virology & Environmental Microbiology, Oxford	UK
Florian Ruker	Institute of Applied Microbiology, Vienna	AUSTRIA
Jesus Sainz	Lawrence Berkeley Laboratory, Berkeley	USA
Chris Sander	European Molecular Biology Laboratory, Heidelberg	GERMANY
Paolo Sassone-Corsi	CNRS, Strasbourg	FRANCE
Jose Vincente Scorza	Universidad de Los Andes, Trujillo	VENEZUELA
Heinz Hartmut	SchigerUniversitat Ulm - Ulm	GERMANY
Duncan Shaw	Institute of Medical Genetics, Cardiff	UK
T.F. Slienk	Princeton University, Princeton	USA
Rolando Stiontes	Ministerio de Sanidad y Asistencia Social, Aragua	VENEZUELA
Thomas J. Silhavy	Princeton University, Princeton	USA
Cassandra Smith	Lawrence Berkeley Laboratory, Berkeley	USA
Edwin M. Southern	Oxford University, Oxford	UK
Jack Stevens	University of California, Los Angeles	USA
Felice Tapia	Universidad Central de Venezuela, Caracas.	VENEZUELA
John W. Taylor	The Rockefeller University, New York	USA
Ronald K. Taylor	The University of Tennessee, Memphis	USA
Glauco Tocchini-Valentini	Institute of Cell Biology, CNR, Rome	ITALY
Christopher K. Tuggle	Norris Cancer Hospital and Research Institute, Los Angeles	USA
V. Udvardy	Institute of Biochemistry, Szeged	HUNGARY
Meena Upadhyaya	Institute of Medical Genetics, Cardiff	UK
I. Verma	Salk Institute, San Diego	USA
Edward Wagner	University of California, California	USA
Moises Wassermann	Instituto Nacional de Salud, Bogota	COLOMBIA
George M. Wormstock	The University of Texas Medical School, Houston	USA
Thomas J. White	Cetus Corporation, Emeryville	USA
Prapon Wilairat	University of Mahidol, Bangkok	THAILAND
T. Zakany	Institute of Biochemistry, Szeged	HUNGARY
Robert Zimmerman	Officina Sanitaria Panamericana, Maracay	VENEZUELA

In addition, a pre-doctoral training programme is currently being organized. This is envisaged as a compensatory mechanism in aid to those developing countries that have not instituted post-graduated programmes in their educational systems. To achieve this, ICGEB will enter into an agreement with University institutions of an international character in Trieste and New Delhi willing to enroll ICGEB member-country students (with at least a BSc degree in a scientific subject) into a three- to five-year PhD programme. The programme will require that each student obtains credits in advanced subjects of the biological and physical sciences for at least two semester courses per year, for three years. The graduate students will be working at the scientific programmes of ICGEB and will eventually defend a thesis based on the research performed under the supervision of the Centre's staff.

It is planned that pre-doctoral training will commence in 1991. Some 18 pre-doctoral fellows are expected to be resident at each of the two Component laboratories of ICGEB by 1995.

Short-term training

The short-term component of the training programme includes a number of practical, theoretical courses and workshops held at ICGEB and Affiliated Centres. These focus on specialized subjects or techniques and are of 2-6 weeks' duration. They are conducted by ICGEB staff and external instructors who have gained an international reputation in their field (see Table 5). A comprehensive listing of courses held between 1988 and 1990 is given in Table 6, whereas those scheduled to take place in 1991 are shown in Table 7.

The number of trainees and the countries of their origin is illustrated in Fig. 3. Participation in the training programme is given to member-country scientists preferentially, but not exclusively. In particular, non-member country scientists are considered for those activities which are sponsored in collaboration with other international agencies whose membership is more extended than that of ICGEB.

The current Five-Year Programme provides for up to 24 weeks of courses workshops annually at each of the two ICGEB components. The costs are borne exclusively by the Centre. At the same time, up to five such courses per annum are programmed to take place at affiliated centres, with ICGEB covering up to 80% of the costs.

In the first three years of its operation the training programme of the Centre has been expanded significantly and its high quality is attracting increasing attention from Member States and international oreganizations. World Health Organization (WHO), United Nations Environment Programme (UNEP) and the International Scientific Committee for Biotechnology (COBIOTECH), are already collaborating with ICGEB or have expressed a keen interest in organizing joint training activities.

In addition, ICGEB holds annually a number of research symposia, conferences and colloquia aimed at enhancing information exchange on latest research related to the overall objectives of the Centre's programme. These are held at ICGEB and or its affiliated centres. Table 8 provides an account of meetings held in the period 1988-1990.

Table 6
ICGEB SHORT-TERM TRAINING PROGRAMME
Theoretical and Practical Courses

Type of programme	Title	Location	Dates
1988			
Practical Course	Eukaryotic Expression Vectors	La Plata, Argentina	10 07 88-30 07 88
Practical Course	Genetic Engineering	Enugu, Nigeria	01 09 88-10 09 88
1989			
Theoretical Course	Molecular Genetics of Yeast	Trieste, Italy	29 03 89-31 03 89
Practical Course	Genetic Manipulation of Streptomyces	Wuhan, China	09 04 89-24 04 89
Practical Course	Computer Applications in Molecular Biology	Trieste, Italy	03 07 89-13 07 89
Practical Course	Methods in Eukaryotic Gene Expression	Szeged, Hungary	01 10 89-14 10 89
Theoretical Course	Genetic Pathologies and the Human Genome	Trieste, Italy	06 10 89-10 10 89
Practical Course	Molecular Virology	New Delhi, India	01 11 89-10 12 89
Practical Course	Molecular Biology of Chloroplasts	New Delhi, India	03 07 89-10 08 89
1990			
Theoretical Course	Bacterial Genetics	Trieste, Italy	23 03 90-29 03 90
Theoretical Course	Modern Techniques in Nucleic Acid and Protein Synthesis and Analysis	New Delhi, India	26 03 90-14 04 90
Theoretical Course	Molecular Genetics of Yeast	Trieste, Italy	09 04 90-13 04 90
Practical Course	Techniques in Human Genome Research	Santiago, Chile	18 06 90-06 07 90
Practical Course	Computer Applications in Molecular Biology	Trieste, Italy	16 07 90-27 07 90
Theoretical Course	Molecular Virology	Trieste, Italy	04 11 90-09 11 90
Practical Course	Molecular Basis of Protozoan Parasitism	New Delhi, India	05 11 90-30 11 90
Theoretical and Practical Course	New Tools for the Study and Diagnosis of Parasitic Diseases	Caracas, Venezuela	26 11 90-14 12 90

Table 7
ICGEB Courses - 1991

Practical Course: P. Amati, Rome **MOLECULAR BIOLOGY AND DIAGNOSIS OF HUMAN PAPILLOMA VIRUS**	1-23 February Havana, Cuba
Practical Course: J. Bennett, ICGEB **RFLP's IN PLANT BREEDING**	4-22 February New Delhi, India
Practical Course: J. Santome O. Cascone, Buenos Aires **PROTEIN AND PEPTIDE PURIFICATION, MICROSEQUENCING, BIOTECHNOLOGICAL APPLICATIONS**	11-27 March Buenos Aires, Argentina
Theoretical Course: T.J. Silhavy, Princeton **BACTERIAL GENETICS**	18-22 March Trieste, Italy
Theoretical Course: G. Romeo, Genova **HUMAN GENETICS**	21-27 April Trieste, Italy
Theoretical Course: G. Tocchini-Valentini, Rome **YEAST MOLECULAR GENETICS**	8-12 May Trieste, Italy
Practical Course: C.V. Bruschi, ICGEB **YEAST MOLECULAR GENETICS**	13-24 May Trieste, Italy
International Symposium: E. Galli C.V. Bruschi, Milano ICGEB **PSEUDOMONAS BIOLOGY AND BIOTECHNOLOGY**	16-20 June Trieste, Italy
Theoretical Course: T.G.B. Howe, Bristol **GENETICALLY MANIPULATED ORGANISMS: SAFETY IN THE LABORATORY AND THE ENVIRONMENT**	1-3 July Trieste, Italy
Conference: J. Beringer G. Tzotzos, Bristol ICGEB **GENETICALLY MANIPULATED ORGANISMS: RECENT DEVELOPMENTS IN RESEARCH AND RELEASE**	3-5 July Trieste, Italy

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Practical Course: 22 July - 2 August
D. Brutlag, Stanford
COMPUTER APPLICATIONS IN MOLECULAR BIOLOGY Trieste, Italy

Practical Course: 17 July - 3 August
K.K. Tewari, ICGEB
PLANT TRANSFORMATION New Delhi, India

Practical Course: 22-27 September
L.L. Cavalli-Sforza F.L. Baralle, Stanford ICGEB
TECHNIQUES IN GENOME RESEARCH Trieste, Italy

Practical Course: 4-22 November
Q. Wang, ICGEB
NUCLEIC ACID SYNTHESIS AND GENE ASSEMBLY New Delhi, India

Theoretical Course: 16-20 December
M.L. Sinnott S. Paoletti, Chicago Trieste
(joint with UNEP)
MARINE MICROBIOLOGY AND BIOCHEMISTRY Trieste, Italy

Information: Ms Daisy Vatt, ICGEB, Padriciano 99, I-34012 Trieste, Italy - Tel. (39-40) 37573[illegible] Telex 460392 ICGEB I Fax (39-40) 226555

Fig. 3 **ICGEB SHORT-TERM TRAINING PROGRAMME**

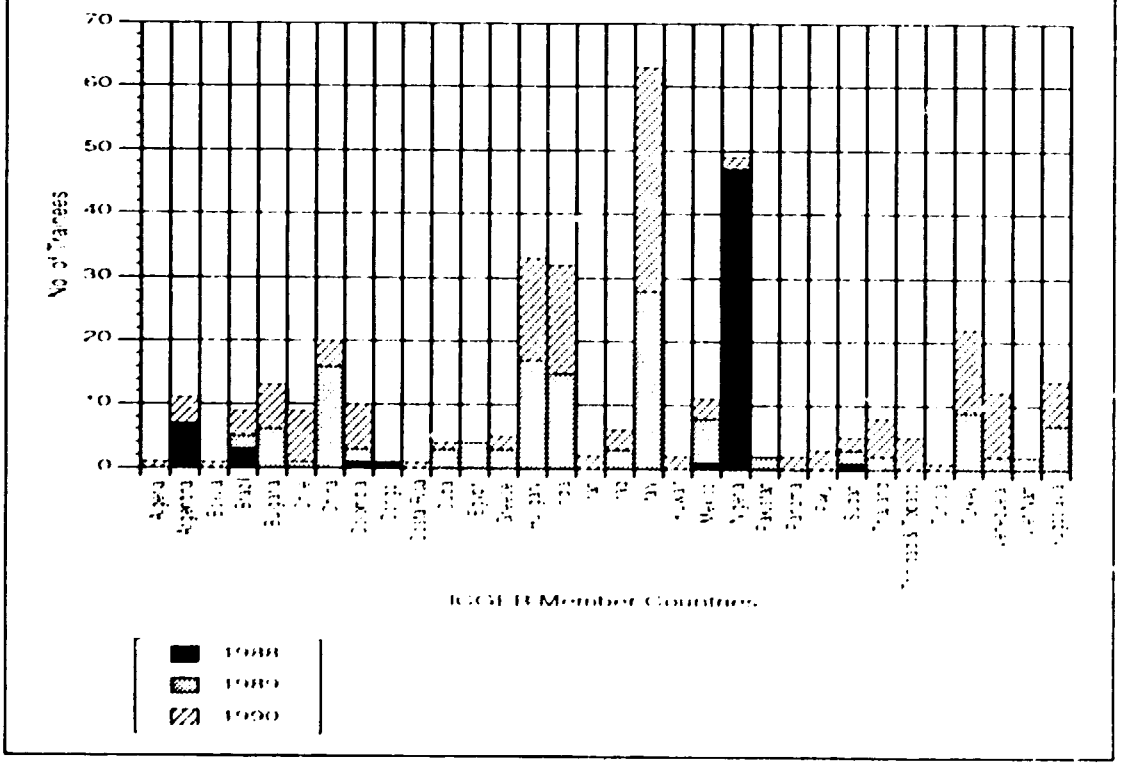


Table 8
ICGEB MEETINGS - 1988/1989/1990

1988

Workshop Symposium

FROM PROTEIN STRUCTURE TO PROTEIN ENGINEERING	I.C. Gunsalus, ICGEB	21-25 March Trieste, Italy

1990

Symposium

MOLECULAR AND GENETIC APPROACHES TO PLANT STRESS	John Bennett ICGEB	14-17 February N. Delhi, India

Colloquium

EUKARYOTIC GENE REGULATION AND EXPRESSION	Joseph Papamatheakis Greece	22-24 May Heraklion, Greece

Colloquium

LIGNIN: STRUCTURE, BIODEGRADATION AND PRACTICAL UTILIZATION	E. Katchalski-Katsir Israel	26-28 June Trieste, Italy

1991

International Symposium

PSEUDOMONAS BIOLOGY AND BIOTECHNOLOGY	E. Galli, Italy C.V. Bruschi, ICGEB	16-20 June Trieste, Italy

Conference

GENETICALLY MODIFIED ORGANISMS FOR THE 1990s.	J. Beringer, UK G. Tzotzos, ICGEB	3-5 July Trieste, Italy

Scientific Services

Apart from the collaborative research and training programmes, ICGEB provides a wide range of services to its member states.

Chemical Services

Facilities for protein sequencing, oligopeptide and oligonucleotide synthesis are available on request by scientists from Affiliated Centres and member countries.

Consultation Services

ICGEB personnel and scientists appointed by ICGEB, upon request of member countries, carry out site visits and provide advise on matters relating to bioscience policy, consultation on specific research programmes and or for planning and setting up new research laboratories.

ICGEB net: the ICGEB computer resource for molecular biology

The computer information resource service (ICGEBnet) is already operational at the Trieste Component of ICGEB. The service is intended to provide the means of combining dispersed researchers, resources and information into a single integrated computer and communications environment, thus strengthening Member Countries' capability in biotechnology.

Access to the ICGEBnet resource is available to all ICGEB Member Country scientists. User support and training is provided and a detailed manual describing the system's services and software has been distributed to Member Country scientists and Affiliated Centres. In addition, on-line help will be available for the major programmes on the system. In-house and regional training sessions will be arranged as the system develops.

The ICGEB computer resource for molecular biology was created in 1990 with the purpose of disseminating the best of currently available computational technology in molecular biology to the ICGEB research community. The major objectives of the ICGEBnet resource are:

(a) The creation of an international computer network linking ICGEB New Delhi, the Affiliated Centres, and Member Country laboratories to a central computer resource located at ICGEB Trieste.
(b) The provision of computational assistance to ICGEB molecular biologists in planning experiments, and analyzing protein and nucleic acid sequences. ICGEBnet provides on-line access to the major sequence data banks, including: GenBank, EMBL, PIR, and SWISS-PROT
(c) To serve as a focal point for the development and sharing of new software developed by ICGEB scientists.
(d) To promote the rapid sharing of information and collaboration among the ICGEB Member Country scientists through the means of electronic bulletin boards and electronic mail.

The ICGEBnet system is fully operational and access is available to all the ICGEB, Trieste scientists through a local area network connecting all

the laboratories and offices. The principal mechanism for remote access to the ICGEBnet resource is via the X.25 Public Data Networks (PDN). ICGEBnet is connected to the ITAPAC X.25 PDN via a leased data communication line allowing for 16 simultaneous incoming connections. Access to the ICGEBnet resource is available to all ICGEB Member Country scientists, with preference given to those scientists whose research is directly related to the research goals of ICGEB. We are currently in the process of assigning user accounts to Member Country scientists in addition to assisting them with setting the appropriate tools to communicate with the ICGEBnet resource. A detailed Users Manual has been prepared and will be distributed to all users of the system. The ICGEBnet system also serves as a training resource and was used extensively during the 1990 ICGEB Practical Course "Computer Applications in Molecular Biology".

The following is a summary of the facilities and services available to users of ICGEBnet.

On-line Service Hardware:

(a) Interactive, time sharing computers including a SUN 4/390, Silicon Graphics 4D/210 and associated peripheral equipment (tape drives, disks).
(b) Telecommunication access to the computer through the use of the Public Data Network (X.25) services. A direct dial telephone line is also available for direct connection to the computer.
(c) Access to the Internet for worldwide electronic mail, remote login, and file transfer capabilities.

On-line Service Software:

(a) IntelliGenetics Suite which contains comprehensive programs for representing and manipulating nucleic acid and protein sequence data.
(b) INSIGHT and DISCOVER packages for the analysis of protein tertiary structures.
(c) FASTA and TFASTA for sequence similarity searching.
(d) IRX for the rapid retrieval of sequences from the databases.
(e) Clustal for the alignment of multiple sequences.
(f) Phylip for the construction of phylogenies.
(g) GM for the automated analysis of eukaryotic sequences.
(h) MM for electronic mail and for bulletin board access.

On-line Service Database Library:

The IntelliGenetics formatted versions of the GenBank and European Molecular Biology Laboratory (EMBL) databases of nucleic acid sequences, the Protein Identification Resource (PIR) and SWISS-PROT libraries of protein sequences, the VectorBank database of cloning vectors, the KeyBank databank of consensus sequence and other patterns, and the REBASE restriction enzyme database from Dr. Richard Roberts of Cold Spring Harbor.

Support:

User consultation by telephone and electronic mail in addition to on-line and hardcopy documentation of the major programmes and training through the ICGEB course "Computer Applications in Molecular Biology".

Software library for personal computers:

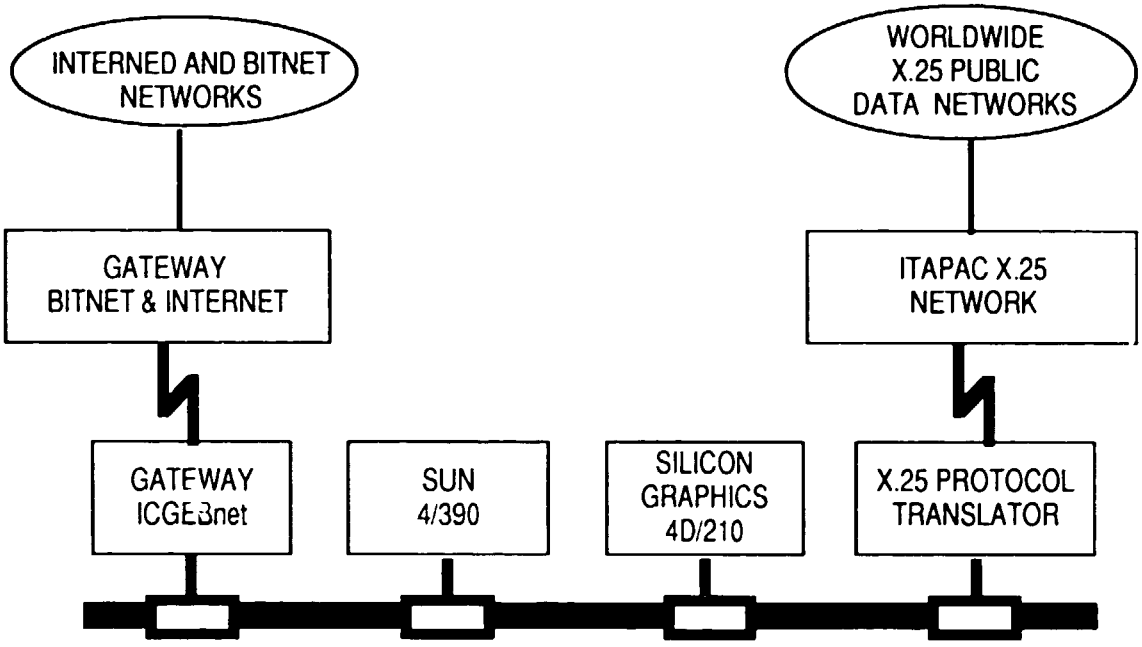
Over 50 public domain scientific programmes from IBM personal computers in addition to another 35 programmes for the Apple Macintosh are available for copying by ICGEBnet users.

Other Database Functions:

ICGEB also acts as a source of data, in the broadest sense of the term, by:

- gathering and collating information on research activities in member countries;
- acting as a hub of an information network to promote awareness of commercial products produced by member country institutes, such as restriction enzymes, diagnostic reagents and vaccines;
- monitoring matters related to the release of genetically modified micro-organisms into the environment and general safety procedures in biotechnology.

The ICGEBnet Data Communication Network



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