

## INTRODUCTION

Ever since the creation of living beings on this planet, Man has struggled to understand "life". Despite these efforts, the phenomenon of life has remained shrouded in mystery. Philosophers in the times of Aristotle and Omar Khayyam discussed "life" in abstract terms. However, recent breakthrough discoveries in biology such as unraveling the structure of DNA (1953), isolation of DNA polymerase (1958), reverse transcriptase (1969) and restriction enzymes (1970), practical uses of these enzymes in DNA mapping & gene cloning (1971),; plant transformation (1982) and sequencing of human genome (2000) have enabled biologists to penetrate deep into the intricate web of life. As a consequence, a scientific concept of life has emerged: that biological activity is the expression of structural and functional information stored in a defined sequence of four nitrogenous bases in DNA. The study of biologically active DNA molecules is called "Molecular Biology."

Biology has been harnessed since antiquity to fulfill humanity's most fundamental needs from increasing food supplies to improving health care. The availability of new and novel methodologies has greatly expanded the scope of applications of Molecular Biology, limited only by the imagination of the people using it. The vast panorama of applications of molecular biology is just unfolding. According to various international Resource Development research firms, the economic impact of applications of molecular biology during the next decade will be equal to, if not more than, that of plastics in the 1940's, transistors in the 1950's, computers in the 1960's, electronics in the 1970's and microelectronics in the 1980's and 1990's.

In view of such glowing hopes for making large profits, it is not surprising that private DNA companies have proliferated in Europe and USA. Most of these have focused attention on the areas in which there are obviously high profits to be made. Such developments will certainly result in substantial reductions in the overall demand for primary products from developing countries with serious economic consequences for their exports of raw materials which generate most of their foreign exchange earnings. In a background of such developments taking place at the international scene, it is imperative for a country like Pakistan to build indigenous capability in this new and emerging field of science.

## ESTABLISHMENT OF THE CENTRE

In order to build National capability in the new bioscience, University of the Punjab established a nucleus Centre for Advanced studies in Molecular Biology. In 1986, the Ministry of Education upgraded the University Centre into a National Centre of Excellence in Molecular Biology. In April 1987, the Federal Ministry of Science & Technology (MOST) approved the establishment of a Centre for Applied Molecular Biology (CAMB), located back to back with the laboratory block of the Centre of Excellence in Molecular Biology (CEMB).

The twin component Molecular Biology Laboratory Complex is spread over 60 acres of land, with a covered area of 7000 square meters, including a Laboratory Block, a Teaching Block, two Hostels for Research Scholars. The Laboratory Block is divided into four separate research units comprising a total of 30 Research Labs and four Conference Rooms; one Production Unit and one Support Facilities Unit comprising a Lab-aid Section (for washing, autoclaving and media preparation), an Animal House, an Insectary, six large Plant Growth Rooms, and storage space for research materials. The Teaching Block consists of a well equipped Library, Bio-informatic lab, Seminar Hall, Photography, Computer Rooms, a

Conference Hall, Director's Office, Administration and Accounts section. There is a self-service canteen.

### **FUNCTIONAL SET-UP**

Both components work in a symbiotic relationship to complement each other's efforts and together represent a unique, and most economical mode to optimally utilize the extremely limited resources of personnel and materials. A unique functional set-up is designed for the most expedient functioning at all levels. Administrative, financial and research work authority are delegated to the various functionaries of the Centre in line with the requirements of their technical and administrative responsibilities. As a result, most of the matters pertaining to the day to day functioning of the Centre are decided without bureaucratic hurdles. This has greatly helped to achieve high efficiency.

The overall control of the Centre is vested in a Board of Governors (BOG) which acts as the supreme body. The BOG is headed by the Vice-Chancellor, University of the Punjab, Lahore. For the day-to-day functioning, the Centre is headed by the Director who is incharge of all scientific and administrative matters. The Director is advised by an Executive Council (EC) and a Foreign Advisory Council (FAC) in developing the Centre's teaching and research programmes, in setting priorities for specific areas of research and in the appointment of senior faculty. The FAC reviews Ph.D. theses and also acts as a peer-review board. The appointments of faculty members and research staff are made by the Board of Governors on the recommendation of the Centre's Selection Committee and the Foreign Advisory Council. The Board of Studies prepares the teaching programme and policies for student admission and recommends M.Phil/Ph.D. synopses for approval by the Advanced Studies and Research Board of University of the Punjab. An Academic Review Committee, constituted by the BOG, meets once every year in December to review the annual progress of all individual research projects being carried out in the Centre as well as the academic programme of the Centre. The Review Committee submits its report to the BOG.

The administrative sections, viz. administration, establishment, procurement and stores etc., work under the control of an administrator. An accounts section works under the control of an accounts officer to maintain accounts of the Centre's funds. All disbursements are pre-audited by an auditor on deputation to the University from the Provincial Audit Department.

The research and training programme of the Centre is financed mainly by the Federal Government. However, contracted research brings in supplemental financial support for research projects from national and international funding agencies.

### **ACTIVITIES OF THE CENTRE**

The Centre has addressed itself to the following functions:

Teaching and training to generate a cadre of manpower specifically trained in molecular biology and recombinant DNA technology.

To undertake goal oriented molecular biological research on specific problems, related to economic needs of the country, in agriculture, health & medicine, industrial, energy and environmental sectors.

To create a repository of DNA modifying enzymes, DNA cloning vectors, novel bacterial strains and other such molecular tools for ready availability and use by various research groups at this Centre and other DNA research laboratories in Pakistan.

To organize national and international seminars and conferences for in-depth discussions on scientific and technological developments which will lead to new ideas and innovative applications of knowledge in gene cloning and recombinant DNA technology.

### **TEACHING AND TRAINING**

Every year, CEMB organizes 1-2 short specialized training courses for post-M.Sc. researchers/university teachers from Pakistan as well as neighboring countries. Collaborating scientists from institutions in Europe and USA help in the conduct of these programmes which are laboratory intensive and compare favourably with courses organized by Cold Spring Harbor Labs or European Molecular Biology Organization. In addition, young scientists from Pakistan as well as from other countries (Bangladesh, Egypt, India, Jordan, Nepal, Sri Lanka, Turkey, Philippines, Indonesia) come to learn specific techniques/methodologies and carry out experiments that cannot be carried out in their own laboratories due to lack of infrastructure and expertise.

CEMB also organizes a regular M.Phil/Ph.D programme. At present 54 M.Phil. and 46 Ph.D. students are enrolled in CEMB's M.Phil. and Ph.D. degree programmes in Molecular Biology. During the period between 1987 and 2004, 146 students completed the M.Phil degree programme in Molecular Biology and 33 students received the Ph.D. degree in Molecular Biology. In addition to this, 40 scientists from outside and 55 Pakistani researchers from various universities and institutes passed through the laboratory corridors of CEMB to either learn advanced gene cloning techniques or undertake specific experimentation.

#### **Research Programmes**

CEMB is working on the frontiers of molecular biological research to give birth to new ideas and to provide solutions to problems of national importance. Research problems are selected on the basis of scientific feasibility and overall impact on the understanding of mechanisms and processes which will lead to applications in health and medical, agricultural, industrial, energy and environmental sectors. All research is directed towards some specific goals and every effort is made to maintain "excellence" with "relevance" to the economical needs of the country. The following major research projects are underway:

## **MEDICAL MOLECULAR BIOLOGY**

### **FORENSIC DNA TYPING:**

The overall objective of the CEMB human DNA typing project is to introduce and help in establishment of forensic DNA typing facilities in the Pakistani criminal justice system viz. the Police and the Courts. CEMB is only one laboratory in Pakistan which is providing services to the police and Courts for DNA testing in criminal cases as well as to resolve paternity disputes. Whenever a person commits a violent crime such as sexual assault, Robbery or murder, there is a good chance that one will leave behind some amount of biological material in the form of skin cells, blood, saliva, sweat, semen, hair, nails etc. at the scene of the crime or on the body or clothes of the victims or carry away some of the victim's biological material. The modern forensic scientist can extract the tiny amount of human DNA present in such biological clues (crime stains) left behind by the criminal. This trace amount of DNA is amplified in quantity and analyzed to find out the genotype/DNA profile of the crime stain. The DNA profile obtained from crime scene biological material is compared with the DNA profile of the provided suspects.

It can lead to the eventual identification of the real criminal from a group of suspects and the acquittal of wrongfully accused persons. The specific goals of the project are a) Development, validation and application of newly emerging techniques in order to strengthen the DNA typing techniques for criminal investigation purposes b) conduct a genetic survey of the most common ethnic clans using Y-chromosome genetic markers and single nucleotide polymorphic markers. Besides, these studies may also generate informations about human chromosomal abnormalities i.e. trisomy, tetrasomy, deletion etc.

In order to reduce the crime rate, the DNA profile of criminals may be kept in a database. This will help to trace the crime repeaters. The DNA profile of all convicted offenders will be typed and stored in the database. The project uses state of the art polymerase chain reaction (PCR) and automated DNA sequencer for genotyping the crime scene or population DNA samples.

## MOLECULAR ANALYSIS OF HEARING IMPAIRMENT

Hereditary Hearing impairment, the inability to hear, is the most common neurosensory disorder affecting about 1 in 1000 children worldwide. According to an estimate, the prevalence of profound bilateral hearing loss is 1.6 per 1000 in Pakistan. To date, 135 non-syndromic deafness loci have been mapped including 72 autosomal recessive, 54 autosomal dominant and 8 X-linked. So far, 26 genes have been cloned for autosomal recessive deafness loci. Pendred Syndrome and Usher syndrome are the two common syndromes associated with recessive deafness. Some of the nonsyndromic loci are allelic variants of syndromes causing genes like *DFNB4/PDS* Syndrome, *DFNB2/USH1B*, *DFNB12/USH1D*, *DFNB18/USH1C* and *DFNB23/USH1D*. Deafness represents extreme genetic heterogeneity with an estimate of more than 300 genes to be associated with hearing.

CEMB team has identified 14 loci and 13 genes implicated in hearing impairment in Pakistani population. In addition, our Lab has also identified families segregating deafness phenotype linked to reported loci and novel mutations of known genes. The dominant modifier *DFNM1* suppresses *DFNB26* deafness phenotype. This is first example of suppressor of human deafness and it completely rescues deafness phenotype. This modifier is expected to yield valuable information about mechanisms of suppressors that may help in the prevention, treatment or better management of hearing impairment

Recessively inherited disorders such as deafness are more prevalent in endogamous populations; like Pakistan. As an outcome of the unique socio-cultural practices in Pakistan; approximately 60% of marriages are consanguineous. Genetic analysis of such large inbred populations manifesting recessive hereditary hearing loss is a powerful resource to map and identify new genes. Therefore these studies involve extensive fieldwork to identify and enroll large consanguineous families segregating deafness in multiple individuals, DNA extraction from blood samples of affected individuals and their normal family members, linkage analysis to exclude segregation of deafness in these families to reported loci, sequencing of genes to find deafness causing mutations and ultimately Genome-wide scan to map new loci and genes.

## MOLECULAR ANALYSIS OF VISION IMPAIRMENT

Vision impairment is a major problem causing loss of quality of life to the individual, family and society. CEMB has initiated a programme to study the genetics and molecular basis of Retinitis Pigmentosa, Cataract and Glaucoma. Vision impairment by Retinitis Pigmentosa and cataract affect with an estimated prevalence of 1 in 4000. Retinitis Pigmentosa is a set of hereditary retinal diseases and is characterized by the development of night blindness owing to the progressive death of the rod and cone photoreceptor cells. Congenital cataract is mainly the disorder of the lens of an eye. Lens of the eye is made of mostly water and proteins that are arranged to allow light to pass through and focus on the retina. Sometimes, some of the proteins clump together and start to cloud the lens, blocking light from reaching the retina, interfering with vision. Glaucoma is the diagnosis given to a group of ocular conditions that contribute high intraocular fluid pressure, damaged optic disk and loss of vision due to partial to complete damage of optic nerve that carries images from the retina of the eye to the brain.

The study is based mainly on three objectives. Firstly to search affected families of RP, cataract and glaucoma from all over Pakistan. Secondly genotyping for reported loci/genes and sequence analysis to identify new mutations in our population. Finally search for new RP, cataract and glaucoma loci by performing genome wide scans on selected unlinked families.

CEMB team has identified four new genes and three new loci responsible for RP and cataract. RP1, GRK1, RP32 and PROM1 are the genes/loci that have a causative role in autosomal recessive Retinitis Pigmentosa.  $\beta$ B3 gene and locus on chromosome 19 & chromosome 1 were found to be responsible for congenital cataract. Our major goal is to identify novel mutations in known genes and new loci / genes responsible for vision impairment in Pakistani population.

The present study will have long term benefits for visually impaired Pakistani population. Pakistani consanguineous families are a useful genetic resource to elucidate the molecular and genetic basis of recessively inherited vision impairment. Genetic testing will enable presymptomatic and prenatal diagnosis of members of such families. Moreover, carrier diagnosis can be offered for individuals whose family members are known to be associated with a particular locus. This research will ultimately pave the way for the development of new specific therapies.

## **LABORATORY OF INFECTIOUS DISEASES & MOLECULAR DIAGNOSIS**

During the last six years our laboratory has been engaged in studies on infectious diseases. We have focused attention on tuberculosis and hepatitis, being the two major and most devastating diseases in the region. In regard to studies on hepatitis our efforts have focused on the diagnosis of all types of hepatitis viruses, sequence variability study in the genomes of hepatitis B, C and G. Efforts are continuing to raise antibodies against viral antigens. Molecular (Pcr-Based) Diagnosis Of Infectious Diseases

Our main task is to run a diagnostic laboratory offering a broad range of laboratory tests in virology and bacteriology. These include the full range of serological assays as well as PCR-based assays to detect and quantify viral DNA or RNA in clinical specimens. Another area that we are interested in is the development of methods for antiviral susceptibility testing. Our research interests focus on the use of modern molecular biology tools like PCR and sequencing for purposes of molecular epidemiology and pathogenesis. In that way we can collect information on the prevalence of certain virus types or subtypes in different patient groups and identify prognostic markers or viral factors that influence the success rate of antiviral therapy in chronic viral infections (e. g. genotypes of hepatitis C virus).

## **MOLECULAR VIROLOGY**

Current focus of this laboratory is; to unravel the molecular pathways that are associated with hepatitis C virus (HCV) induced pathogenesis. It is estimated that ~250 million individuals are infected with HCV worldwide including 10-15 million in Pakistan. Chronic infection is strongly associated with liver cirrhosis, inflammation, insulin resistance, steatosis, fibrosis, and the hepatocellular carcinoma. HCV encodes at least 3 structural components, Core (C), Envelope-1 (E1) and Envelope-2 (E2) and 7 non-structural components, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B. The non-coding sequences present at the 5' and 3' termini are essential for translation and RNA-dependent RNA viral replication. In the western hemisphere, the current antiviral development include inhibitors of HCV replicative enzymes, such as protease, helicase, and polymerase, as well as several genetic approaches, such as ribozymes and antisense oligonucleotides against internal ribosome entry site (IRES). Currently developed HCV inhibitors display maximum efficacy for genotype 1, because genotype 1 is generally used for

screening. The hallmark of the disease is its propensity to evolve into chronicity, probably because of viral heterogeneity that allows the virus to escape immune-mediated neutralization. Major ongoing research projects of the laboratory are outlined below:

**(1) To develop in vitro culture models of surrogate viruses with the aim to arrest viral infection.**

Infectious pseudo type particles (HCVpp) harboring unmodified E1/E2 glycoproteins of HCV genotypes 3a/b and 4a/b (predominant in Pakistan) onto retroviral core particles will be generated. Due primarily to the identical physiological and immunological conditions between chimpanzee and humans, these pseudo type viral particles have good potential to be tested as protective vaccine in chimpanzees. Trimeric and SCID/uPA mice are difficult to use for drug screening since their livers are humanized by hepatocyte transplantation and is therefore susceptible to de novo HCV infection with serum viral RNA levels equivalent to those seen in human.

**(2) To delineate the molecular pathways associated with HCV-induced pathogenesis.**

In vitro cellular model of HCV 3a/b and 4a/b replication is developed with the aim to test antiviral compounds. The cloning of Pakistani HCV full-length genome, sub genome replicon and individual regulatory components will open up many avenues of research to address the complexity of hepatic cellular processes and immune responses associated with HCV infection. In depth analysis of the regulator regions of the key genes associated HCV pathogenesis is carried out using some powerful molecular and genetic tools. In particular the focus is on steatosis, insulin resistance and fibrosis and on purinergic (p2X and p2Y) signaling cascades.

**(3) Generation of HCV cellular replicon and mapping conserved functional domains.**

Full-length genomic cDNA and subgenomic replicon expressing Huh-7 cells are generated from the HCV 3a/b and 4a/b genotype isolated from patients and/or infected liver biopsies. DNA sequencing is performed to determine the genetic variation. The next generations of anti-HCV therapeutic agents will be analyzed in the cellular/animal model of HCV replication and/or gene expression. The generation of HCV 3A/3B sub genome replicon will be instrumental in the study of HCV replication and viral-host interactions. Viral and cellular factors required for HCV replication will be defined by cutting edge gene and micro-array, chromatin-immunoprecipitation (CHIPs), proteomics, protein-protein interactions methodologies.

**(4) To define liver specific gene expression patterns and signaling cascades, which are perturbed during viral infection?**

The inflammatory cytokines (interleukins, MIP, SAP1, Cox-2, and MCP-1) and genes for gluconeogenesis and insulin signaling via their transcriptional regulators such as p-CREB, p-AMPK, Pi3K, pAKT-2, HNF1/4, C/EBP beta are being investigated (recent focus of Dr. Qadri's research). The immune responses will be correlated during insulin resistance in chronic HCV infection. This information will help to design strategies to arrest stage specific viral replication and associated events. This project will utilize, FACS analysis, RT-PCR, proteomics, gene array, immunoblotting, immunostaining, confocal imaging and targeted mechanistic studies in the cellular and animal models using liver specific promoter region fused with a reporter LUC gene.

## HEPATITIS-B VIRUS

Hepatitis B is also a deadly liver disease caused by HBV causing 2 million deaths every year globally. It spreads 100 times faster than AIDS. In Pakistan about 8% people are carrying HBV. So, active & passive immunization of the whole nation is very urgent. Active vaccines are commercially available but their efficacy is very low due to variants therefore, passive vaccines are also important. Work is in progress for the development of monoclonal antibodies for passive immunity & diagnosis. Immunization will prevent vertical transmission of the virus from mother to baby. HBV isolates have been genotyped from different geographical regions of Pakistan. HBV genotypes B (41%) & C (40%) were the most predominant HBV genotypes. The response rate of HBV genotype C is very poor that is 15-20% to IFN and lamivudine.

## RESEARCH ON GB VIRUS C/HEPATITIS G VIRUS

HGV infection is a matter of intense debate especially in Pakistan. As it is controversial throughout the world regarding their site of replication & its co-infection with HCV, HBV and hepatitis non-A-E. Our laboratory has initiated a study to find out the site of replication & to know the co-infection of HG V with HCV, HBV and non A-E hepatitis. We have identified some new sites of replication of HGV such as bone marrow, spleen, testes, and brain along with liver. Multiple Drug Resistant Genes In Mycobacterium Tuberculosis

Tuberculosis (TB) is a chronic communicable disease caused by *Mycobacterium tuberculosis*, resulting in lung, brain, liver, urogenital tract and bone infection and sometimes causing a generalized infection ('miliary' tuberculosis) (Kumar, et al, 1997). Although certain other microbes referred to as atypical mycobacteria also cause TB, (eg., *M. avium-intracellulare* complex in AIDS patients), it is the organism *Mycobacterium tuberculosis* hominis, discovered by Robert Koch in 1882, that is responsible for the vast majority of cases of tuberculosis. Tuberculosis is a highly contagious disease that has caused more morbidity and mortality than any other bacterial infection. The disease is still alive, spreading wildly and killing more people worldwide than even malaria and HIV. Every year there are 8-10 million new cases of TB and 2-3 million deaths. In Asian countries, 60 to 80% of children below the age of 14 are infected. About 1/3<sup>rd</sup> of the world's population harbors *M. tuberculosis*, therefore, is at risk for developing the diseases. In Pakistan about 1.5 million people suffer from TB, and more than 21,000 new cases occur each year. This is an under estimate since only one in five new cases is diagnosed and despite progress in biological, biochemical and immunological techniques, a reliable identification is not easy. For the detection of tuberculosis, the most sensitive & specific technique is AFB culture. It takes about 4-8 weeks; smear examination is quick but it lacks sensitivity & specificity (less than 10%). PCR techniques offers rapid, specific, sensitive and accurate detection methodology but it is very costly.

In regard to our studies on tuberculosis, we have focused attention on the development of more sensitive and specific methodologies for the detection of tuberculosis and the mechanism of genetic and molecular basis of drug resistance in mycobacterium.

As a result of our input during the last three years into studies on tuberculosis, rapid and more specific methodologies for the early diagnosis of tuberculosis in clinical samples have been established.

Rapid and simple screening methods are being developed for the detection of mutations responsible for multiple drug resistance in tuberculosis (MDR-TB). For this purpose DNA

sequence analysis, dideoxy fingerprinting (ddF) and single strand conformation polymorphism (SSCP) is used to detect these mutations. Preliminary studies have been published.

## **FUTURE RESEARCH IN VIROLOGY**

We are particularly interested in basic research on virus-host interactions involved in transmission, pathogenesis, persistence, immune responses and antiviral resistance associated with hepatitis C virus (HCV) infections.

We are highly interested to study the interaction between HCV proteins and cellular proteins including signal transduction pathways that contribute to HCV pathogenesis, evasion of immune responses and antiviral resistance, and the combined effects of HCV. We are also interested in the study of HCV quasispecies with respect to resistance to antiviral therapy, HCV persistence and progression of liver disease. Regulation of HCV protein expression, high-throughput screen assays for measurement of IFN efficacy and the recently described HCV replicon system are important areas of research. All of these research experiments will be performed in cultured human liver cells that are the natural reservoir for HCV infection and replication in vivo.

## **BIOPHARMACEUTICAL LAB.**

### **CYTOKINE PROGRAMME**

Cytokine is a group of protein cell regulators, including lymphokines, monokines, interleukins and growth factors. They are produced by a variety of cells in the body and play an important role in the pathophysiology of range of diseases and have therapeutic potential. Such proteins also act as immunomodulators. The therapeutic impact of cytokines has been felt mainly in the field of cancer, infectious diseases, blood disorders, rheumatic and autoimmune diseases. Research about “cytokines in clinical medicine” has been revolutionized with the availability of recombinant cytokines and some of the corresponding monoclonal antibodies.

The cytokines therapy is the area where we can expect the greatest impact of molecular biology and recombinant DNA in clinical medicine in the near future. Cytokine group is working in many directions in which growth factors, colony stimulating factors, interleukins and interferon will be developed and engineered in appropriate hosts in the coming years. The group is also working on for the development of specific immunoglobulins for diagnostic as well as therapeutic use.

### **HBV VACCINE PROGRAMME**

HBV is one of the most threatening health problems causing hepatitis B infection. It is responsible for 80% cases of liver cancer. According to WHO Pakistan is amongst one of the most endemic countries and therefore, declared a high-risk zone. No satisfactory, specific and proven treatment against HBV is available. The prevention through vaccination is more economical and ensures high protection. A programme is being initiated to develop hepatitis B peptide vaccine by employing molecular and recombinant DNA technology.

## MEDICAL MOLECULAR BIOLOGY

### STEM CELL RESEARCH GROUP

*Stem cells* are an undifferentiated population of cells present in the body that can differentiate into specialized cell type upon receiving a suitable signal from the environment. This means that *stem cells* from one organ can form cells of a completely different organ. This property of *stem cells* can be utilized for the treatment of different diseases that are incurable by the existing therapeutic means. Stem cells are present in almost all the organs of the body and are involved in the repair process. Keeping this in view, CEMB in 2003 initiated the Stem Cell Research program with the aim to investigate the potential of *stem cell* based repair of different damaged organs.

The main focus of the group is to repair damaged areas in the heart, liver, kidney, pancreas and eye. The potential of *stem cells* in the repair of damaged myocardium has been extensively studied and the results obtained are extremely promising. Heart tissue once damaged cannot be repaired by the currently available therapeutic means. However, in recent experiments we have demonstrated that *stem cells* can repair the damaged heart muscle and improve the cardiac function in a myocardial infarction mice model.

Similarly, a strategy has been developed for the restoration of eye sight in patients with damaged corneal surface. For this purpose, patients' own *stem cells* are being grown and transplanted in the affected eye. Initial results are extremely promising and further experiments are in progress. Other projects have been initiated to determine whether *stem cells* can help in restoring the normal functioning of a damaged kidney. In order to assess the ability of *stem cells* in the repair of pancreas we have made a diabetic rat model and future experiments are underway to determine the role of *stem cells* in the treatment of diabetes. Liver fibrosis is an extremely serious medical condition with a large number of people suffering from hepatitis and other associated disorders in our country. We have initiated work on the project aimed to highlight the role of *stem cells* for the repair of liver fibrosis with promising results.

Preliminary studies have showed a lot of potential for the repair of damaged organs with stem cells. Our present studies will have long term benefits in improving the existing medical therapies for the treatment of various disorders. Furthermore, it will provide basis for the development of a cell based therapy with the potential to treat even the most complicated medical problems.

### MOLECULAR IMMUNOLOGY/ENZYMOLGY

Immunology lab was established to provide service for the production and characterization of polyclonal and monospecific antibodies to researchers. The facility has been carrying out all the necessary procedures for the immunization of animal (rabbits) and the production of polyclonal antibodies. These polyclonal antibodies are purified by using affinity chromatography. The resulting pure antibody is supplied to researchers after western blotting and immuno-dotblot.

Another objective of Immunology lab is easy detection of GMOs (Genetically modified organisms), which include protein detection methods such as immunoassays or Enzyme Linked

ImmunoSorbent Assays (ELISA), dipsticks. These methods are based on the properties of antibodies to detect expression of cry genes in seed extracts of transgenic crops (rice, cotton).

In enzymology, Production of molecular tools like Taq DNA polymerase, restriction endonucleases (EcoR1, HindIII, Pst1) Lambda DNA and Lambda-HindIII marker are included. Maintenance of these molecular tools and their distribution to all labs of the institute, according to the needs of researchers.

## **PLANT MOLECULAR BIOLOGY**

### **Plant Tissue Culture and Transformation**

The Centre is involved in advanced research and training aimed at improving crop plants. A multidisciplinary programme provides molecular, physiological and entomological information essential to the development of insect resistant and physiologically improved crop plants. Tissue culture technique leading to the transformation of plants is the main step towards achieving the goal. Plants under study include chickpea, cotton and rice. *Agrobacterium*-mediated and biolistic method are being used for the transformation of these plants. *Agrobacterium* induce a tumor in plants which is a natural form of genetic engineering. The piece of DNA which *Agrobacterium* injects in plants for tumor formation is being used as a tool for genetic modification of chickpea and cotton. Different genes have been successfully transformed into different crop plants by using this method. Transgenic plants of chickpea and cotton has been obtained which contain a modified CryIA(b) gene of *Bacillus thuringiensis*. In biolistic methods, metal particles coated with DNA are fired in to the plant tissues. Plant cell incorporates the DNA into its chromosomes and then divides and regenerates into full plants. Insect resistant rice plants have been obtained by this methods. A synthetic CryIA(c) gene in combination with Cry II A gene with Marker gene has been transformed into Cotton and Basmati rice. These plants are being grown in greenhouse conditions to study the inheritance of foreign genes.

### **Biosafety Studies**

Before GM crops are being released and commercialized careful field trials should be conducted in order to study and evaluate different aspects of these plants while keeping in view, biology of the crop, introduced trait, the receiving environment and interaction between these. Transgenic cotton and rice transformed with single and multiple genes have been produced in the centre against lepidopteran insects pests . This group is actively involved in studying the horizontal and vertical flow of the genes and to assess the risk/evaluation of transgenic plants to the ecological environment in Pakistan. Different experiments regarding the effect of transgenic rice and cotton on soil organisms and other animals, fate of Bt protein in soil and horizontal and vertical gene flow have been conducted and many others are in process.

### **GMO Testing**

The cultivation of genetically modified crops is becoming increasingly important; more traits are emerging and more acres than ever before are being planted with GM varieties. The release of GM crops and products in the markets worldwide has increased the regulatory need to monitor and verify the presence and the amount of GM varieties in crops and products. Labeling legislation and trade requirements differ from one country to another, leading to the necessity for the development of reliable and sensitive analytical methods for detection, identification and quantification of GM varieties in crops and their products. GM crops and their products can be

identified by detecting either the inserted genetic material at DNA level, the resulting protein or phenotype. Several analytical methods such as methods based on the PCR, for detecting the inserted DNA, immunological assays for detecting the resulting protein, or using bioassays to detect the resultant phenotype have been developed. A lateral-flow immunoassay (LFT) was developed by immunology Lab (first time in Pakistan) and used to detect Bt-GM crops for the expression of insecticidal crystal protein (ICP) of *Bt*. One-step lateral flow test, immunochromatographic strips (ICS) or dipsticks, have been a popular platform for qualitative, rapid, visual, portable and easy to use test. The immunological (ELISA, Dot blots, Dipsticks) and insect biotoxicity assays are being used as the tools for the screening and detection of Cry 1A ICP in several Bt. cotton plants for insect resistance trait.

### **Plant Genomics**

There are tremendous changes in Plant Biological studies during the last decade as the concept of “Genomics” has been introduced. Plant Genomics Group is working on identification and characterization of drought tolerant genes in cotton (*Gossypium arboreum*). The effect of abiotic and biotic stresses was studied on the expression of genes using techniques such as microarray, differential display, gene homology and making wax mutants. These studies will provide the number and nature of all genes that are implicated in stress tolerance and their response to alternation of stresses.

Seven drought responsive transcripts and six full length genes has been identified and characterized by using differential display, RACE and Gene Homology techniques. Real time PCR indicate that these genes are mainly expressed in drought stressed leaves as compared to control and other tissues. *Hsp26* and *USp* were cloned in plant expression vector and transformed to a local cotton variety (*Gossypium hirsutum*) through agrobacterium mediated transformation. Three promoters of stress-related genes have been identified by genome walking and cloned. To get minimal promoter sequence deletion constructs have been made and one promoter has been confirmed by agro-infiltration in tobacco. On the basis of comparative studies of morphological and physiological characteristics of six varieties of *Gossypium arboreum* the relatively most drought tolerant variety was selected. cDNA libraries were constructed from the mRNA isolated from leaf samples under drought stress condition. Clones were PCR amplified for spotting on microarray slides and hybridized with probe from control and water stressed plant samples. The data obtained by scanning of hybridized slides is being analyzed. For identification of wax genes, wax mutants were developed by physical and chemical mutagens and confirmed by SEM and GCMS. cDNA libraries were constructed from the mRNA isolated from leaf samples. The clones were further PCR amplified for spotting on microarray slides.

### **Quality Seed Production/ Floriculture**

#### **Biotechnological Improvement of Gladiolus:**

Tissue culture conditions for the four reported cultivars of gladiolus susceptible to *Fusarium oxysporum* have been developed. Friable, nodular and embryogenic callus have been obtained from the four cultivars. Cell suspension cultures of gladiolus cultivars were developed.

Fusarium resistant cell lines of gladiolus have been regenerated using fusaric acid (toxin). The resistant plantlets were multiplied and shifted to the field. The toxin tolerant plants were sprayed with the conidia of the fungus and found highly resistant against the *Fusarium*.

RAPD analysis of genome from ten selected lines with control using five primers, gave polymorphic bands compared to the control. Identification and sequencing of Fusarium resistant gene is in progress.

### **Biotechnological Improvement of Potato:**

Commercial production of disease free potato seed is in progress. The annual production of potato disease free seed has reached upto 0.13 million mini-tubers.

Another aspect of this project is to develop a reliable virus detection protocol for variant strains of PVY, PVX and PLRV. Reverse Transcriptase Polymerase chain reaction (RT-PCR) based detection conditions were optimized. Capsid protein gene of Potato virus Y was cloned into cloning vector pGEM-T, and Capsid Protein of PVY was sequenced. Using RT-PCR cDNA was synthesized. cDNA was sub cloned into cloning vector, later on cloned gene of PVY was sequenced in automated sequencer. Homology of the sequenced gene of PVY with reported genes in Gene Data Bank was observed with in range of 91 % to 99%. Out of total gene size approximately 50% of the gene size was sequenced.

### **Synthetic Seed Production:**

Synthetic seed production protocol has been developed by encapsulating the somatic embryos which is being exploited for the production of synthetic F1 hybrid seeds of economically important vegetables

Meristematic cells of four tomato, three cucumber cultivars and four Chilli cultivars were isolated, to make disease free seed stock under aseptic conditions and were developed into friable and embryogenic callus. These cells were further developed into cell suspension cultures for single cell production and for the production of somatic embryos to be encapsulated. Genetic relatedness among regenerants produced via synthetic seed and their respective parents was determined through RAPD and AFLP genotyping.

### **Biotechnological Improvement of Sugarcane**

Tissue culture conditions for the seven cultivars of sugar cane (*Saccharum officinarum*) have been established. In-vitro multiplication of disease free stock of sugar cane has been obtained, starting with meristem tip culture. Disease Free plantlets were acclimatized and shifted in field. Plantlet regeneration from friable, nodular and embryogenic callus have been obtained from all the seven cultivars.

### **Disease Resistance Through siRNA Gene Silencing Technique**

Posttranscriptional gene silencing (PTGS) is an epigenetic form of mRNA degradation important in the defense of plants against virus infection and widely used as a tool for inactivating gene expression. Discovered in plants, PTGS have been demonstrated to have a role in a diverse range of functions including regulation of gene expression, development, chromatin structure, and defense responses to viruses and transposons. Small non coding RNA sequences of about 20–25 nucleotides in length can serve important targeting functions in eukaryotic cells through a process called RNA silencing, or RNA interference (RNAi). Remarkably, the silent state in transgenic plants can spread from cell to cell and even systemically throughout the plant via a mobile silencing signal that can cross a graft junction.

Currently, we are employing siRNA technology in order to silence the expression of Potato virus Y (PVY) and Sugarcane Mosaic Virus (SCMV).

### APPLIED AND FUNCTIONAL GENOMICS LAB

Applied Genomics to drugs and diagnostics is fostering novel approaches to analyze the genomes of the target organisms responsible for these diseases. This provides an unprecedented opportunity to use whole genome based methodologies, computational biology, and functional genomics to identify new drug/targets and diagnostic reagents. Functional genomics technologies are utilization of genome data to infer gene functions, protein and transcript profiling and pharmacologic analysis. We are currently utilizing **expression profiling via microarray**, which offers a non-biased approach to identifying specific genes, gene networks and mechanisms of gene regulation operant in various diseases such as Hepatitis C disease. Expression analysis of HCV infected patient's blood and biopsy will help to find the genes that are expressed in circulating white blood cells as well as in the liver of patients with varying degrees of liver damage (fibrosis). We anticipate that results from differential gene expression analysis will allow us to make predictions about likelihood of disease progression and/or response to the treatment. Additionally, these studies may provide serum marker for indicative of various stages of HCV infection that will eliminate the need for biopsy.

**Generation of sub genomic replicon (S) of HCV 3a genome in liver cells:** The most prevalent HCV genotype in Pakistan is 3a and to date, there is no replicon system available for 3a genotype. *In vitro* cellular model of HCV 3a replication will be developed with the aim to test antiviral compounds and pathological pathways. This *in vitro* replicating model will open many avenues of research to address the complexity of hepatic cellular processes and immune responses associated with HCV infections.

In **therapy and technology development**, we are studying gene expression analysis of natural antiviral herbal compounds against HCV. **Mechanisms of action of Ayurvedic Medicines for Hepatitis C:** There are limited therapeutic options for patients with chronic hepatitis C, especially for those who fail to respond to interferon (IFN) therapy; identifying new and improved therapeutic drugs against HCV is urgently needed. Our lab has identified certain herbal compounds, which showed potential antiviral activity against HCV. We intend to screen out more herbal compounds and also determine their antiviral activity in 3a sub-genomic replicon. Several of the natural herbs have complementary and overlapping mechanisms of action, including antiviral effects by either inhibiting the formation of viral DNA or RNA or inhibiting the activity of viral reproduction. The mechanism of action of the potential herbal compounds after their toxicological and antiviral screening will be analyzed using DNA microarray. DNA microarray can provide novel insights into the mechanism(s) of disease processes and drug action by identifying patterns of gene expression correlated with the biologic process of compounds.

**siRNA as a potential therapeutic target:** Gene functions can be revealed by loss of function assays. The recently introduced RNA interference (RNAi) is a particularly effective mechanism for selective inhibition of gene expression, which in a very short time has become the preferred method for inhibiting expression of targeted genes. To prove the **siRNA as a potential therapeutic target** we are currently using siRNA technology against various genotypes of HCV.

**Liver cDNA library printing:** DNA microarray technology has now developed to a stage where the state of expression of complete genomes can be recorded with high accuracy on a single chip. These cDNA libraries which contain the total genomic data of the liver diseases can be printed on a single chip with DNA microarray technology and will provide an opportunity to survey transcription modulation in context of liver infectious diseases. We are generating human liver cDNA chip and study gene expression analysis of liver related diseases. cDNA

microarray technology is useful for making estimates of the abundance of particular messages relative to a designated source of mRNA that serves as a reference point. Commercial support of this technology has recently reached a level where it is reasonable for departments or large laboratories to consider setting up their own cDNA array facility.

## **INDUSTRIAL AND ENVIRONMENTAL BIOTECHNOLOGY**

This programme has different aspects in order to augment environmental quality.

There are different ways to ameliorate the environmental health bioremediate the toxic waste replace the synthetic products with biological products regulate the use of anthropogenic products. This group make effort to generate a cadre of biological products which may reduce the use of synthetic items if not replaced.

An environmental synthetic contaminant like plastics can be replaced with biodegradable plastic materials. Certain microbes produce polymers which possess properties very much similar to synthetic plastic. The difference between synthetic and biodegradable plastics is that plastic materials stay in the environment for decades and are non-degradable. Therefore, environmental aesthetics can be improved by replacing synthetic plastic with biodegradable plastic. Biodegradable plastic materials are produced by different microorganisms including *pseudomonas* spp., and *bacillus* spp. These biological plastics can be degraded by microorganisms during dumping in sewage, soil or in water. The project aims at the development of efficient microbial strains, cheaper media and process development for polymer production. With the introduction of biodegradable plastic in the environment, the problems related to plastic waste may be reduced if not eliminated completely.

In textile industry, fabrics are treated with synthetic detergents during different treatments before and after printing and dyeing. The synthetic detergents may be replaced by enzymes comparable to detergents. Alpha-amylase, Alkaline protease and cellulase are potent Candidates and produced by different microbial and fungal strains. The objective of this project is to manipulate microbial strains for efficient production of textile related enzymes.

These efforts will certainly help to augment environmental condition by reducing the use of anthropogenic chemicals and materials.

## **PRODUCTION ACTIVITIES**

In addition to carrying out original research, the laboratories of CEMB are engaged in the " production of research materials that are used as molecular tools in DNA research. At present, eleven restriction enzymes are being routinely prepared to satisfy 70-80% of the need for DNA modifying enzymes at the Centre. In due course, it is planned to raise the stock to twenty enzymes and market them to other R&D institutes and laboratories in the country. It is anticipated that easy availability of routinely needed DNA enzymes will promote research and teaching in Molecular Biology in the country. In addition to restriction enzymes, the Centre produces its own deoxynucleotide oligomers, plasmids and bacteriophage DNAs. The production group is also working to develop the most promising Bt isolates into pesticidal formulations as economical, efficacious and environmentally friendly bioinsecticides that can be used in conjunction with chemical insecticides to lessen the environmental burden of chemical insecticides.

The production group also maintains a microbial culture collection section where approximately 1000 bacterial and fungal strains are maintained and are available at minimal costs to research laboratories in the country.

### **EXPERT GROUP MEETINGS**

One of the main difficulties of working in a scientifically developing country like Pakistan is the lack of opportunities for scientific discussions and cross-fertilization of research ideas. There are only a handful of laboratories working at the forefront of biological research and even those have less than the required critical mass of trained manpower. To provide some relief from these inherent difficulties, the Centre regularly organizes expert group meetings, symposia and seminars to bring a galaxy of eminent scientists from Europe and North America to Pakistan. The meetings provide a comprehensive coverage of the latest developments in selected areas in regard to unique and specific problems of economic importance. The International symposia are usually preceded by introductory lecture programmes for a selected group of young university teachers to acquaint them with concepts that form the theoretical basis of the subjects to be covered in the symposia. Such International symposia gather a wealth of valuable scientific research information in the relevant areas; expose Pakistani scientists and science policy-makers to the frontiers in genetic engineering and recombinant DNA technology; create general awareness about the unlimited potential of molecular biology; and provide opportunities for exploring possibilities of collaborative research endeavours. Proceedings of such meetings are published to provide a compendium of information valuable to the scientific community as well as science planners in Pakistan. Contacts emanating through such meetings have led to an enhanced relationship between Pakistani and foreign scientists resulting in major initiatives by the Pakistani scientists.

### **LABORATORY FACILITIES**

The laboratories of the Centre are well-equipped with advanced facilities and sophisticated equipment such as a automated DNA synthesizer and a DNA sequencer, an amino acid analyzer, three Beckman Ultracentrifuges (model L8), six medium speed J2-21 centrifuges, one Packard Scintillation Counter, two Microferm laboratory bench fermenters, two high pressure liquid chromatographers, one Pulsaphore electrophoresis system for Pulsed Field Gel Electrophoresis, two spectrophotometers.

One Fast Performance Liquid Chromatography system, one PHASTTM system for automated polyacrylamide gel electrophoresis and staining, six Thermal Cyclers for PCR, two complete sets of DNA sequencing apparatuses and several IBM-compatible computers. This is in addition to routinely needed equipment such as microscopes, electrophoresis apparatus, gel dryers, fraction collectors, incubators, refrigerators, ovens, microwave ovens, and autoclaves.

### **THE CEMB LIBRARY**

The Centre houses a moderate-sized library that receives many of the relevant journals in molecular biology, molecular genetics and recombinant DNA research. The CEMB library currently subscribes to 19 major research journals and 8 review journals. In addition, there is an excellent collection of recent editions of important scientific texts on various aspects of

molecular biology and biotechnology some of which are not available anywhere else in the country.

### **BIO-INFORMATICS/COMPUTER SECTION**

In order to retrieve, store and carry out analysis of stored information, powerful computing facilities have been provided at the Centre. The labs of the CEMB are connected via Local Area Network of tool for comparing a DNA or protein sequence to other sequences in various databases, is available on the local area. Also, Entrez search and retrieval system is made easily accessible on high speed computers via INTERNET for real-time ON-LINE links with foreign databases like GENE BANK, EMBL, SWISS-PROT, MEDLINE, Genome Data Base, etc. to obtain newly sequenced DNA/Protein sequences as well as three dimensional structure of Proteins etc.

The Informatics Section of the CEMB library provides E-mail and Web-browsing facilities to the researchers of the Centre through which they can connect and communicate with foreign labs and search international databases to retrieve the required information.

### **LINKAGE WITH INTERNATIONAL UNIVERSITY/ORGANIZATION**

#### **S. # NAME OF THE FOREIGN INSTITUTION**

1. John Hopkins University Baltimore,
  2. Cold Spring Harber Labs, New York, U.S.A.
  3. University of Washington, Seattle, U.S.A.
  4. Rockefeller Foundation, US NSF, U.S.A.
  5. Department of Biochemistry, Ohio State University 484 W, 12th Avenue, Columbus, U.S.A.
  6. Department of Entomology, North California State University U.S.A.
  7. John Innes Centre, Norwich, Research Park, Colney Lane Norwich, NR47UH U.K.
  8. National Institute of Bioscience and Human Technology, Tsukuba, Japan.
  9. Department of Biochemistry University of Ottawa, Ontario, Canada.
  10. International Rice Research Institute, Philippines.
  11. National Institute of Biotechnology, (GBF) Germany.
  12. Plant Genetic Systems, Belgium.
  13. Institute of experimental medicine, CNR, ROME, Italy.
  14. Institute of Clinical and Biological Research, Microcitemie Hospital, University of Cagliari, Sardinia, Italy.
- National Institute of Deafness & other communication disorder, National Institute of health, Laboratory of Molecular genetics 5-Research court, Rockville, MD.

### **LINKAGE WITH NATIONAL UNIVERSITY/ORGANIZATION**

#### **S. # NAME OF THE INSTITUTION**

1. Pakistan Agriculture Research Council.
2. Centre for Applied Molecular Biology, Ministry of Science & Technology.
3. Quaid-e-Azam University Islamabad.
4. Pakistan Atomic Energy Commission Islamabad.
5. Centre for Molecular Genetics, University of Karachi, Karachi.
6. Centre Cotton Research Institute, Multan.

## **EXTRA CURRICULUM ACTIVITIES AND REKNOWN ALUMNI**

Following extra curriculum activities are being undertaken in the Centre.

1. The Journal Club of the Centre arranges study/excursion tours to the various areas of the Country.
2. The Journal Club also arranges Cricket matches within the members of the Centre from time to time.
3. A table tennis court is available in the Centre to play table tennis by the members of the Centre.
4. A badminton court is also available in the Centre for playing badminton by the members/research scholars of the Centre.

## **DEGREES PROGRAMME AND ADMISSION REQUIREMENTS DURATION, SCHEME OF STUDY (SUBJECT ETC) AND NUMBER OF SEATS.**

The Centre offer's M.Phil/Ph.D degree programme in Molecular Biology.

### **1. Admission Requirements**

Following are the admission requirements in the M.Phil/Ph.D programme of the Centre.

- a) Having 1<sup>st</sup> Class or CGPA-03.00 in Master Degree in Agriculture, Bio-Chemistry, Bio-Technology, Botany, B-Pharmacy, Chemistry, DVM, Environmental Sciences, MBBS, Medical technology, Microbiology, Microbiology & Molecular genetics, Molecular Biology and Zoology or equivalent qualification.
- b) Desire to pursue future teaching/research career in Molecular Biology and recombinant DNA technology.
- c) Not above the age of 30 year.
- d) No 3<sup>rd</sup> division in academic career.

### **2. Duration**

Ph.D. (four to five year)

M.Phil (two years)

### **3. Scheme of Study:- Research plus Theoretical Work.**

Number of Seats:- Number of seats are not fixed it depends upon on going research programmes in the Centre.

### **DUES:-**

Admission Fee	Rs 1000/=	
Bench Fee	Rs 5000/=	
General Club Fee	Rs 450/=	
Registration Fee (Per Student)	Rs. 1700/=	(Students other than Punjab University)

**For Foreign Students US\$750 Per Semester**