Center for Biotechnology

National Taiwan University has seven colleges that have research and teaching activities related to molecular biology and biotechnology. These colleges include science, medicine, engineering, agriculture, public health, electrical engineering and computer science, and law, totaling about 200 faculty members and 1000 graduate students.

In order to capitalize on teaching and research resources in these colleges, some faculty members have long advocated for forming a center of biotechnology. In March 1998, the Executive Council approved to form “Preparatory Office” for the Center for Biotechnology, and President Chen designated Professor Chia-Yin Tsai as the Director of the Office.

During the preparatory stage, a biotechnology teaching program was designed, approved and implemented starting September 1998. Finally, the Center for Biotechnology was established in June 1999. Professor Yih-ming Chen was appointed as the Director.

Major Tasks in the Teaching Program

I. Biotechnology Program

One of the most popular teaching programs on the campus since its installation in the fall semester of 1998, the Biotechnology Program has received about 274 applications from undergraduate and graduate students in the colleges of Science, Medicine, Engineering, Agriculture, Management, and Public Health. In order to improve teaching efficiency, the curriculum was designed to identify and differentiate core from specialized courses so that students may receive training in an orderly manner.

II. Biotechnology Core Techniques

Biotechnology Core Techniques is a core lab course in the Biotechnology Program. The purpose of this lab course is to familiarize students with all key techniques implicated in studies of the central dogma, where students are required to construct a recombinant DNA molecule involving the GUS gene and an expression vector, pQE-31.

Future Prospects

One of the major responsibilities of the Center is to educate and train students in the area of biotechnology so that they could meet the need and challenge of biotech industry. While working closely with all concerned departments and colleges, efforts will also be made to reach out in seeking financial supports and in establishing viable relationships with industrial and other academic organizations.

Current Research in National Taiwan University

(1) Protein chips Research Activities at NTU

Chii-Wann Lin, Ph.D.

The current biomedical detection paradigm is to use biochips that are multi-functional, are miniature in size, and possess parallel processing. Investigating the performance of worldwide leading biotech companies, bio-molecular detection, chip design, and biochemistry analysis are the three major branches of this core technology.

At NTU, a BioMEMS research team is organized and focuses on the integration of multidisciplinary resources for biochip developments, with committed researchers from various institutes supported by Taiwan’s National Project through the National Health Research Institute (NHRI). The ultimate goal has been to develop
a full bio-optical system that has the potential of meeting the demands of high throughput, ultra sensitivity, and precision biomedical measurements.

(2) cDNA Microarray Core Lab in National Taiwan University Hospital

Pan-Chyr Yang, M.D., Ph.D.

DNA microarray core lab

cDNA microarray is a powerful tool for genome-wide identification of differentially expressed genes, generating extensive applications in biomedical sciences. The cDNA microarray can be used to dissect functions of an unknown gene, to understand pathogenic mechanisms of a disease, to facilitate drug discovery, to diagnose disease, to predict disease prognosis and improve treatment outcome.

National Taiwan University Hospital started to form a microarray core lab since Feb. 2000, with Dr. Jeremy JW Chen in charge. The core lab can provide in-house multi-purpose array membranes, such as angiogenesis array, oncogene array or cytokine array.

Genome wide profiling of metastasis related gene

In an example of using cDNA microarray to perform a global analysis of metastasis related genes, a panel of novel metastasis related genes is identified, and further characterization indicated that some of these genes play a critical role in cancer metastasis. Metastasis is a multiple step process and begins with tumor cells leaving primary site to becoming lodged in a remote organ. The process involves interactions between cancer cells and their surrounding microenvironments. These model cell lines are used to identify invasion-associated genes using cDNA microarray with colorimetric detection.

A more invasive subline, CL1-5-F4, derived from metastatic lung tumor of SCID mice inoculated with CL1-5 cells, was combined with CL1-0, CL1-1, and CL1-5 in cDNA microarray screening. cDNA microarray membranes each containing 9600 PCR products of non-redundant expressed sequence tag (EST) clones were used to identify differentially expressed genes in these cell lines.

For statistical analysis, self-organizing map algorithm was performed to identify the expression patterns. In vivo tracheal graft assay confirmed that cell lines with greater invasiveness had greater metastasis potential. Positive correlation between gene expression levels and cell line invasiveness was found in 2.9 percent of the 9,600 putative genes. On the other hand, negative correlation was found in 3.3 percent of the genes. The data demonstrated that genes related to cell adhesion, motility, angiogenesis, signal transduction, and others may play significant roles in the metastasis process. Several novel candidate genes were identified and further characterized. Expression of these genes correlated with the cell line invasive ability as well as clinical metastasis in lung cancer patients.

Several genes were identified as novel invasion and metastasis suppressor genes. These results substantiate the model system with which one can identify invasion-associated genes by using cDNA microarray and cancer cell lines of different invasiveness. This technique may allow us to explore complex interactions between multiple genes in orchestrating the process of cancer metastasis.

CRMP-1 is a novel invasion and metastasis suppressor gene

Collapsin Response Mediator Protein CRMP-1 is a candidate gene we identified from cDNA microarray screening of the differentially expressed genes in lung cancer cells with high versus low invasive activity. Having identified one gene, collapsing response mediator-1 (CRMP-1), which can suppress cancer cell invasion, the CRMP-1 gene expression both in mRNA and protein levels were inversely associated with the invasive activity of cancer cells. When the CRMP-1 gene was transfected into highly invasive cells and then over expressed, the transfected cells revealed lower invasive activity than untransfected cells. It is concluded that CRMP-1 appears to be involved in cancer invasion and metastasis, and may be a novel invasion suppressor gene.

(3) Vibration Arthrometry

Ching-Chuan Jiang, M.D., Ph.D.

The vibration arthrometry laboratory's first objective was to develop a non-invasive diagnostic tool for detecting knee joint disorders. The vibration signals picked up from the accelerometer on patella with the knee under test passively moved at two different speed
protocols are measured. In a study of 37 patients with knee injuries, the accuracy, sensitivity and specificity of VAM in diagnosing meniscal tear was 81 percent, 75 percent and 100 percent respectively.

In another study of PPC recordings in 53 patients with knee osteoarthritis, the accuracy, sensitivity and specificity of the diagnosis of degenerative change of the patellofemoral joint by PPC was found to be 94.3 percent, 97.2 percent and 88.2 percent respectively. VAM was also found to be useful in accessing patella subluxation, intra-articular loose bodies, plica syndrome and prosthetic metal wear. The results also showed that vibration signals in rapid knee motion could be effectively used for detection of early stage prosthetic polyethylene wear.

**Application of VAM in sports medicine**

As the sensitivity and specificity of the diagnosis of meniscal tear by VAM is 75 percent and 100 percent respectively, meniscal signal would only appear in 3/4 of the cases with meniscal lesions and is definitely negative in the absence of any meniscal tear. The diagnostic value of VAM is complementary to that of MRI as the latter is known for its exceptionally high sensitivity but relatively low specificity for detecting meniscal lesion (Jiang 1994, 1995).

Results show that VAM is a valuable non-invasive tool for diagnosing different types of knee disorders including patellar subluxation, patellofemoral maltracking, joint mice or plica syndrome.

**VAM in the diagnosis of total knee prosthetic wear**

Clinically, total knee prosthetic wear is grouped into three types as follows: 1) metal wear; 2) polyethylene wear; 3) malpositioning of prosthetic components without wear.

The study showed that vibration signals of knee in rapid motion could be used to detect early prosthetic polyethylene wear. When metal wear occur, which is usually noted at a relative late stage of prosthetic wear, characteristic PPC signals could also be seen. VAM, therefore, provides a simple, effective and non-invasive method for not only detecting the presence or absence of prosthetic wear but also assessing the extent of the wear that has occurred.

**VAM in assessing P-F joint cartilage wear**

In the studies of Physiological Patellofemoral Crepitus (PPC), 36 patients with knee osteoarthritis were enrolled for VAM study. The PPC signals were analyzed with a mathematical model. By means of different parameters like R10Hz<fd<100Hz, R100Hz<fd<450Hz or intraclass distance, these signals could be grouped into three different types each with different clinical meaning. In type 1 signal, the P-F joint is intact. Type 2 signal indicates wear of the trochlear groove cartilage but the patellar cartilage is intact. Type 3 signal appears only when damage of patellar cartilage occurs. These results allow for the evaluation of the integrity of the P-F joint cartilage and the extent of cartilage wear by recording the PPC signals. VAM is therefore a valuable tool for evaluation of patients with anterior knee pain.

**Other clinical applications**

Audible or vibration signals are often noted from human joints. For example, patients with temporomandibular (TM) joint dislocation or TM joint arthritis may notice an audible click when they open their mouths. Other examples include the Ortolani’s click of degenerative dysplasia of hip, the McMurray’s sign of ruptured meniscus, snapping sound of the snapping hip or shoulder.

Although these signs have important and specific clinical implications, in the absence of VAM, their documentation often depends on the subjective description of either the patient or physician doing the physical examination, which is not objective enough for further analysis.

**Current Research in Canine Transmissible Venereal Tumor in NTU**

Dept. of Veterinary Medicine

Rea-Min Chu, Ph.D.

Canine transmissible venereal tumor (CTVT) is an excellent model for investigating the interaction between host immunity and tumor growth. CTVT is a naturally
occurring, poorly-differentiated, round-cell neoplasm, which can be experimentally transplanted into allogeneic dogs. This tumor spontaneously regresses (R phase) following a period of progressive growth (P phase). CTVT can be allo-transplanted across major histocompatibility complex barriers. Although CTVT is an allograft, the host's immune system is unable to destroy the tumor cells in the first few months, and the tumor grows progressively for almost 20 weeks (Chu et al., 2001).

After a short stable phase, the tumor undergoes regression. The mechanism(s) of these phenomena was the focus of our studies.

CTVT inoculation significantly reduces the proportion of B lymphocytes among all peripheral blood lymphocytes (PBL) during the P and R phases, but the proportion of B lymphocytes returns to normal after complete removal of CTVT. CTVT secretes soluble, heat- and proteinase K-sensitive cytotoxic substance(s) that destroys PBBL but spares other types of immune cells.

These novel anti-B cell factors may be eventually used to regulate specific B cell responses for clinical therapies of various diseases, because the factors also affect B cells of humans and other mammals (Liao et al., 2001).

While the mechanisms behind the transition from the P to R phases are not well understood, it is believed that during spontaneous regression of CTVT cells, slow tumor cell proliferation must contribute to the decrease in tumor size.

However, shortening of tumor cell telomeres is not directly involved in this process (Chu et al., 2001). Other factors, such as expression of MHC antigens on CTVT cells, humoral immunity, cytokines released by the inflammatory cells and, especially, tumor infiltrating lymphocytes may contribute to the decrease in tumor size.

The expression of MHC molecules is usually low in P phase and then greatly increases during R phase. The results indicate that TIL isolated from R phase CTVT secreted a heat-sensitive, soluble substance(s) that triggered over-expression of MHC I and II after 12 weeks PI. This caused the tumor to enter R phase and helped stop CTVT growth (Hsiao et al., 2001).

In a study to detect heat shock proteins (HSPs) 60, 70 and 90 in CTVT, only level of HSP 60 was significantly higher in CTVT cells than in other canine tumors and most of the normal canine tissues.

HSP 60 is especially higher in CTVT cells of regressing than those in progressing phases. These data suggest that canine HSP 60 is a potential marker for this tumor and appears to be involved in CTVT regression (Chu et al., 2001).

(5) From *Drosophila* Genetics to Human Functional Genomics

Dept. of Zoology

Tze-bin Chou, Ph.D.

After systematic analysis of human disease-associated gene sequences in *Drosophila melanogaster*, it is clearly indicated that 74 percent of disease genes searched matching unique *Drosophila* sequences, which can be categorized into various subclasses based on various clinical phenotypes (http://homophila.sdsc.edu).

This creates a picture of disease genes that are amenable to study using *Drosophila* as the model organism. Nevertheless, more than 60 percent of these fly unique genes do not have mutant alleles. Dr. Tze-Bin Chou has recently established the P-transposase insensitive cFRT2L2R chromosome that allows direct recombination analysis after P-transposon-induced mutagenesis. This new technique provides at least 10-fold higher efficiency than the original tool and will facilitate the exploration of *Drosophila* functional genomics and, thereafter, of human functional genomics.

(6) Engineering Crops with Insect-resistance Genes

Dept. of Botany

Prof. Yeh Kai-wen and Yih-ming Chen

To overcome the agricultural problem of insect damage, engineering crops with insect-resistant genes is becoming an important and urgent item in Taiwan agricultural biotechnology. A research team in National Taiwan University has discovered the trypsin inhibitor genes from local sweet potato cultivar, and delivered the genes in several brassica vegetable plants by means of Agrobacterium-mediated transformation method.

(7) Department of Agronomy

Huu-Sheng Lur, Ph.D.

1. Molecular marker aiding crop breeding
2. Molecular fingerprinting and profiling of crop genotypes
3. Transformation of valuable genes in rice
4. Functional genomics in stress resistance
5. Bioinformatics and consulting

(8) Marine Molecular Biology and Biotechnology (MMBB) Lab

HJ Tsai, Ph.D.

MMBB lab is headed by Prof. Huai-Jen Tsai, Institute of Fisheries Science, National Taiwan University. The primary research areas are molecular biology of aquatic animals and marine biotechnology. The following projects are currently underway: (1) the study of the molecular structure, regulation and expression of Myf-5 (a muscle regulatory factor), troponin (a muscle structural protein) and rhodopsin (a retinal photoreceptor) genes; (2) the study of human disease by using transgenic fish as a model organism; (3) the development of useful transfer systems for aquacultural fish, shellfish and invertebrates; and (4) the creation of colorful varieties of ornamental fish.

This is the first group to identify unique cis-regulatory elements and binding nuclear proteins controlling the specific expression of zebrafish myf-5 and carp rhodopsin genes by using transgenic approach. Many useful transgenic lines of medaka and zebrafish are generated, carrying GFP cDNA fused with the upstream regions of myf-5, rhodopsin, troponin and actin genes. Interestingly, the research generated a germ-line transmitting transgenic zebrafish having fluorescent heart.

A transgenic line of glowing medaka expressing green fluorescence strong enough to be viewed under bright light was developed as well. These transgenic lines are potentially useful in both academic research and applied science. Furthermore, the lab also generates fast-growing varieties of transgenic loach and abalone, which are transferred DNA fragments containing the exogenous fish growth hormone cDNA via sperm-mediated gene transfer system.

Hsinchu Biomedical Science Park
Integration of Knowledge-based Economy and Biotechnology — Managing Taiwan's Future

T he development of knowledge-based economy involves not only the creation, accumulation, and imparting of knowledge, but also the conversion of knowledge paradigm to enhanced production effectiveness and doubled increase in the per capita income. Biotechnology is internationally regarded as the best performer of the future, and with the industrially advanced countries having pinpointed the sector as the strategic industry, the industry will be developed at full speed and with all available resources.

Building a strong base of biomedical science and industry will play a critical role in upgrading Taiwan’s competitiveness considerably and prompting further economic development. In order to make use of the limited national resources effectively, the development of the nation’s biomedical industry should begin by integrating the strengths of the government, academic institutions, and private enterprises, and start from the segments where there are pressing needs for further development and where the effects will be the most enormous.

Key Factors to the Successful Development of Biomedical Science Park

In 1997 various government agencies invested NT$1.4 to NT$1.5 billion (US$1:NT$28 in March 1997) as R&D funds for the development of biotechnology industry. However, due to a lack of the “cooperation between the academic institutions and private industry” and limited investment in the biotechnology sector subsequently, no concrete results were reported.