I-1 Seminal Research Contributions by T. D. C. Grace and S. Gao. K. MARAMOROSCH. Department of Entomology, Rutgers University, New Brunswick, NJ 08901. E-mail: maramor@rci.rutgers.edu

I had the good fortune to have Dr. Thomas D. C. Grace from CSIRO, Canberra, Australia as my associate at Rockefeller Institute from 1957 to 1959. I have also been privileged to meet personally Prof. Shangyin Gao who invited me to come to Wuhan, China in 1982. The two pioneers have never met and they were unaware of each others research. Their work led to the breakthrough that influenced all subsequent studies dealing with invertebrate cell cultures. Grace started experiments in insect cell culture as an Honors Year Project in 1955 and his work culminated in his establishment of the first four cell lines of Antheraea pernyi. His breakthrough was announced in October 1962 at the First International Conference on Invertebrate Tissue Culture in Montpellier, France and published in Nature (London) 195:788, 2. In 1959, Grace established the first mosquito cell lines. His achievements were greeted with understandable enthusiasm because they proved the feasibility of growing invertebrate cells in continuous culture. In 1959, three years before Grace’s successful cultivation of insect cells, Prof. Gao, Chair of Microbiology at Wuhan University, published his successful cultivation of Bombyx mori cells in continuous culture. Although the announcement appeared in English in Acta Virologica, it was overlooked by most invertebrate cell culture workers outside China. When I met Gao, I found out that after he obtained his Ph.D. degree at Yale University, he became a postdoctoral fellow in W. M. Stanley’s laboratory at the Rockefeller Institute in Princeton, NJ. Inspired by Ross G. Harrison at Yale, R. W. Glaser and William Trager at Princeton, and trained as a virologist, he decided to try the cultivation of grasserius virus in B. mori cells after returning to China. By 1959 Gao had carried his cell line of B. mori epithelial gonad cells through 22 passages, using cell monolayers for his virus studies. Although Gao was indisputably the first to grow insect cells continuously, China’s isolation during the Cultural Revolution prevented the wide dissemination of his work and did not influence invertebrate tissue culturists the way Grace’s success did. Following the solid and everlasting foundation provided by the work of Grace and Gao, invertebrate cell culture became widely used in biotechnology, the production of recombinant proteins, viral insecticides and vaccines, as well as in basic research in genetics, molecular biology, endocrinology, biochemistry, physiology, and virology.

I-2 Invertebrate Cell Culture Biology and Novel Cell Lines. ROBERT R. GRANADOS. Boyce Thompson Institute, Cornell University, Ithaca, NY 14853. E-mail: rg28@cornell.edu

The development in the 1980’s of the application of recombinant DNA technology to express cloned genes in insect host cells led to the remarkable growth and application of cultured insect cells in molecular cell biology research. The emergence of the baculovirus-insect-cell system as a versatile gene expression tool resulted from extensive and elegant studies on the molecular biology of baculoviruses and the establishment of new insect cell culture systems that supported high levels of protein expression. This expression system is now accepted as an important technology for the commercialization of products for use in agriculture and human health. Initially, many established cell lines from embryonic, ovarian, hemocytic, or larval tissues were readily available for use in expression systems. The highly popular Spodoptera frugiperda cell lines (SF21 and its clonal isolate, SF9) established in the 1970’s proved to be very good cells for gene expression. However, new insect cell lines with special characteristics needed to be developed to optimize protein expression. This led to the establishment of new cell lines such as the Trichoplusia ni cell line known as High Five Cells that is known for its very high levels of protein expression. Newly developed cell lines from the Monarch butterfly were shown to have native potential for complex glycosylation. More recently molecular cell engineering was used to stably engineer insect cell lines that expressed higher levels of complex glycans or were more resistant to nutritional or culture stress. Future directions in novel cell line design and development will be in molecular cell engineering and isolation of embryonic stem cell lines with unique properties.

I-3 Invertebrate Cell Culture Applications in China. Z. HU and T. Xie. Key Laboratory of Molecular Virology and Joint-Laboratory of Invertebrate Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, Peoples Republic of China. E-mail: hazh@pentium.whiow.ac.cn

Ever since Prof. Shangyin Gao started his early research of insect cell culture in 1958, the invertebrate cell culture and insect virology in China has developed rapidly. In this presentation, Prof. Gao’s activities and his contribution to the related researches in China will be introduced. Many invertebrate cell lines had been developed in China in the history, these cell lines as well as the researches related to the cell lines will be reviewed. Just like everywhere else in the world, using baculovirus-insect cells system for producing interested proteins for basic research or for therapeutic applied researches have been very popular since last decade. The status of using invertebrate cell culture to produce proteins in China will be presented. Several labs in China have a research program in insect cell culture either for basic baculovirus research or for expressing therapeutic proteins. The main labs will be introduced and their recent researches in virology of baculoviruses will be summarized.

I-4 Scale-up and Optimizing the In Vitro Growth of Insect Cells for Production of Recombinant Proteins and Viral Pesticides. S. N. AGATHOS. Unit of Bioengineering, University of Louvain, Louvain-la-Neuve, B-1348 Belgium. E-mail: agathos@gebi.ucl.ac.be

In vitro cultivated insect cells have been used extensively in the last decades for the production of viral pesticides and, more recently, also for the production of recombinant proteins, thanks to the Baculovirus Expression Vector System (BEVS). Lepidopteran insect cells infected with a lytic insect virus (baculovirus) can be used to produce recombinant viral particles for use as biopesticides. Alternatively, in the BEVS a gene coding for the protein of interest is inserted in the baculovirus under the control of a strong promoter. Upon infection of cultured insect cells, large amounts of recombinant proteins are transiently expressed. This system is extremely popular as the easiest means to produce diagnostic, veterinary vaccine- or research-grade proteins, since a lower level of post-translational authenticity is required compared to human injectable therapeutics. However, advances in vector construction and the realization that for many therapeutic applications structural authenticity is less important than actual biological function now make the BEVS a mature technology platform contender for the production of therapeutics. This trend is supported by the efficiency, low cost and robustness of the technology compared to mammalian cell culture systems. The commercial production of both bioprocesses and proteins requires efficient scale-up of insect cell culture, based on fast-growing infection-prone cell lines, low-cost culture media (preferably, serum-free and protein-free) and maintenance of good specific protein (or viral particle) productivity at high-density infections. Better knowledge of insect cell metabolism allows more rational design of low-cost culture media and better proteolysis control. A more complete understanding of cell infection by the wild-type or recombinant baculovirus vector leads to a less empirical choice of parameters such as multiplicity (MOI) and time of infection (TOI). Engineering issues related to high-cell density cultivation (new bioreactor types or successful retrofitting of established designs, improved gas exchange, nutrient feeding strategies involving fed-batch or continuous perfusion, etc.) are progressively leading to a panoply of solutions for successful large-scale production of recombinant proteins and viral pesticides.
I-5
Apoptosis Regulation in Cultured Insect Cells: Facing Death on a Hair Trigger. R. J. CLEM. Molecular, Cellular, and Developmental Biology Program, Division of Biology, Kansas State University, Manhattan, KS 66506. E-mail: rclem@ksu.edu

Apoptosis is a common response of cells to various stress stimuli. In mammalian cells, intrinsic death signals induce apoptosis by triggering the release of pro-apoptotic factors from mitochondria, including cytochrome c. Once in the cytoplasm, cytochrome c binds to an adaptor protein called Apaf-1 and allows for the formation of a large complex called the apoptosome, which results in the activation of an apical caspase, caspase-9, and subsequent activation of effector caspases such as caspase-3. In contrast, the induction of apoptosis in insect cells appears to be regulated somewhat differently. There does not appear to be a requirement for cytochrome c or other mitochondrial factors in Drosophila S2 cells. Instead, an apopTosome-like complex is constantly active in S2 cells, generating an active form of the apical caspase Dronc via its autotaxalytic activity. This continuous generation of active Dronc requires the presence of the Apaf-1-homologous protein Dark. The levels and/or activity of this active form of Dronc are normally kept in check by the protein DIAP1. However, DIAP1 protein has a half life of only around 40 minutes, and interruption of DIAP1 synthesis, either by RNAi or as a result of cellular stress, results in rapid spontaneous apoptosis due to the accumulation of active Dronc and subsequent cleavage and activation of the effector caspase Drice by Dronc. Once activated, Drice in turn cuts uncleaved Dronc at a different site, possibly amplifying the apoptotic signal. Knockdown of the SF-IAP protein in the lepidopteran Sf21 cell line also results in spontaneous apoptosis. Thus many insect cells are continuously poised to undergo apoptosis, which has been suggested to be an ancient defense response against virus infection.

I-7
Transgenic Insect Cell Lines That Support Production of Humanized Glycoproteins by Baculovirus Expression Vectors. D. L. Jarvis, J. H. Hollister, and J. J. Aumiller. Department of Molecular Biology, University of Wyoming, Laramie, WY 82071. E-mail: dljarvis@uwyo.edu

One widely-recognized advantage of the baculovirus-insect cell system is its ability to produce recombinant proteins with eucaryotic modifications, such as glycosylation. However, the protein glycosylation pathways of insect cells appear to be less extensive than those of mammalian cells and, as a result, insect cells rarely produce sialylated glycoproteins. The inability of the baculovirus-insect cell system to produce authentic recombinant glycoproteins, with carbohydrate side chains identical to those found on native human glycoproteins, can be a serious problem because these side chains can influence glycoprotein behavior in various ways. For the past several years, our group has been creating transgenic lepidopteran insect cell lines with extended protein glycosylation pathways to address this problem. Sf9 and Tr-5B1-4 cells were transformed with expression plasmids that encode various mammalian enzymes involved in protein glycosylation. Expression of these genes was controlled using baculovirus enhancer and promoter elements because they provide constitutive foreign gene expression in the absence of baculoviral infection. Clones containing stably integrated copies of these genes were isolated and extensively characterized. Generally, the resulting insect cell lines constituted express the enzymatic activities encoded by the mammalian transgenes. These cells typically had normal morphologies, growth properties, and could support baculovirus infection and recombinant protein production. Furthermore, these transgenic insect cell lines could routinely produce sialylated recombinant glycoproteins under appropriate conditions. Additional details regarding the isolation and characterization of these cell lines will be discussed in this presentation, which will show that we have created transgenic insect cell lines that can support humanized recombinant glycoprotein production by baculovirus expression vectors.

I-9
Molecular Biology and Genomics of Shrimp Viruses and Their In Vitro Culture. J. M. Vlah. Laboratory of Virology, Wageningen University, 6709 PD Wageningen, The Netherlands. E-mail: just.vlak@wur.nl

White spot syndrome virus (WSSV, Nimaviridae). Taura syndrome virus (TSV, Dicistroviridae) and Yellow head virus (YHV, Roniviridae) are the most important viral pathogens affecting cultured shrimp. These viruses emerged in the last decade and quickly spread around the world. WSSV is a notorious scourge as it not only affects shrimp, but also other crustaceans such as crabs and crayfish. Intervention strategies are being sought to control virus diseases in shrimp, in particular against WSSV, and vaccination—although in its infancy—shows promise. The genomes of TSV, YHV and WSSV have been completely sequenced and their genes are awaiting functional analysis. But molecular and cell-biological studies of these viruses are hampered by the fact, that no convenient cell culture system from shrimp is available. Primary shrimp cultures have been made, but their generation is often cumbersome and time consuming. They usually originate from lymphoid organs or ovaries and show outgrowth of fibroblast-like epitheloid cells. They are susceptible to virus (WSSV) infection. However, stable cell lines are not available and key features for the rapid and robust establishment of shrimp cell cultures have not been identified. The basic media (M-199, L-15) are supplemented with foetal calf serum and salts, with an osmolality equal to shrimp hemolymph. Cultured shrimp cells appeared very sensitive to tissue-dissociating enzymes. Survival after 50 days has been reported. By necessity and convenience, most if not all of the experiments involving shrimp viruses are carried out using live shrimp or other crustaceans. However, such an in vivo approach does not reveal the intricacies of viral replication and virus-host interactions at the cell level. This contribution will concentrate on the state of the art in shrimp cell culture technology and its potential applications in shrimp virus research.
I-12
Molecular Engineering and Biology of Invertebrate Cell Cultures: Summary and Conclusions. DWIGHT E. LYNN. Insect Biocontrol Laboratory, USDA/ARS, Beltsville Agricultural Research Center, Beltsville, MD 20705. E-mail: lynnd@ba.ars.usda.gov

This session was in honor of Dr. Thomas D. C. Grace and Prof. Shangyin Gao and has shown how far we have come since their historic papers on the long term maintenance and establishment of cell lines from insects. Dr. Grace focused on ovarian tissue to create his continuous lines and while Prof. Shangyin initiated primary cultures from a variety of tissues, the longest surviving/subcultivable cells also were from reproductive tissue. In the four and a half decades since their early successes, well over 500 continuous insect cell lines have been developed from a variety of insect species and tissues. As more material has become available, the diversity of uses has also grown. Since the earliest days, insect cells have been used to study insect viruses which, in addition to providing a means to study and produce these biocontrol agents, also led to the development of the baculovirus expression vector. The virus research, in turn, has driven studies on cell biology and pathobiology so that we now know much more about the events regulating cell death and pathogenesis. We’ve also learned how to manipulate the cells so that the products, such as proteins from expression vectors, have a greater biological activity or so we can create long-term stably transformed cultures. Additionally, we have branched out to other invertebrates and expanded our scope for discovery of new uses for the technology originated by Dr. Grace and Prof. Shangyin.

I-13
Comparative Approaches to Understanding Mechanisms of Toxicity: The Comparative Toxicogenomics Database (CTD). C. J. MATTINGLEY1, G. T. Colby1, M. C. Rosenstein1, J. N. Forrest, Jr.2, and J. L. Boyer2. 1Mount Desert Island Biological Laboratory, Salisbury Cove, ME 04672 and 2Department of Medicine, Yale University School of Medicine, New Haven, CT 06520. E-mail: cmattin@mdibl.org

The molecular mechanisms underlying the toxic action of many environmental chemicals are not well understood. Scientists have long exploited diverse experimental models to understand the complexity of gene-environment interactions. The value of these models is further enhanced through comparative studies, which are proving to be essential for elucidating biological systems and annotating accumulating genomic data. To facilitate understanding about toxicologically significant genes, the Comparative Toxicogenomics Database (CTD) is being developed at the Mount Desert Island Biological Laboratory (MDIBL; http://www.mdibl.org/) with support from the NIEHS and in collaboration with investigators at other NIEHS Marine and Freshwater Biomedical Science (MFBS) Centers. CTD will be the first publicly available resource to provide the following collection of features: 1) curated and integrated sequence, reference and toxicant data for toxicologically important genes and proteins; 2) results from comparative studies of these genes and proteins across evolutionarily diverse organisms, with special attention to aquatic organisms; and 3) integrated data from existing molecular and toxicology resources. Data curatiation and integration in CTD will resolve many of the limitations to accessing molecular toxicology data in existing resources and provide users with a novel, comparative perspective on genes of toxicological significance. Major goals of this project are to provide unique insights into molecular evolution, the functional significance of conserved sequences and the genetic basis of variable toxicity.

I-14
“Fish & Chips” Using DNA Arrays to Study Environmental Stress in Non-model Organisms. A. Y. GRACEY, A. R. Cossins, and G. N. Somero. Hopkins Marine Station of Stanford University, Oceanview Blvd. Pacific Grove, CA 93950. E-mail: agracey@stanford.edu

A key factor in the survival and continued success of organisms in climatically variable environments is their ability to display a suite of adaptive responses that promote both the constancy of their constituent physiological processes and increase tolerance to damaging or lethal conditions. Relatively little is known about the mechanisms underpinning these responses in aquatic organisms. Deploying a high-throughput microarray-based approach, we have investigated the transcriptional component of the teleost fishes, common carp (Cyprinus carpio), and the long-jaw mud-sucker (Gillichthys mirabilis), to a variety of environmental challenges. A microarray for each species was constructed using >10,000 mostly non-redundant cDNA amplicons isolated from a collection of normalized and serially-subtracted cDNA libraries. A time-course analysis of the transcriptional response to environmental conditions and hypoxia will be discussed in detail. Discrete and overlapping sets of genes were regulated in different tissues in response to stress, suggesting that the differentiated tissues possess distinct functional roles in adaptation. The integration of the expression data with other indices of physiological condition, in particular metabolite levels, will be presented. These studies provide a unique broad overview of the complex process of adaptive regulation in vertebrate animals following exposure to environmentally significant stress.

I-15
The Effects of Arsenic on the Function of CFTR Cl Channels in Killifish, a Euryhaline Teleost. BRUCE A. STANTON1, Alexander Lankowski1, Joseph R. Shaw2, Katherine Karlson1, Caitlin R. Stanton3, Renee Thibodeau4, Maria V. Strelcovitch4, and A. STANTON1. 1Mount Desert Island Biological Laboratory, Salisbury, ME 04672; 2Brown University, Providence, RI 02912; 3Whitman College, Walla Walla, WA 99362; and 4Wellesley College, Wellesley, MA 02481. E-mail: bas@Dartmouth.edu

Chronic arsenic exposure is associated with increased risks of cancers, diabetes, vascular and other disease. A potential mechanism(s) for these outcomes involves the effects on stress-induced pathways (e.g., gluocorticoid-receptor, GR, gene activation). The killifish, Fundulus heteroclitus, provides an excellent model to study the effects of environmental toxins on GR-mediated and the molecular biology of cystic fibrosis transmembrane regulator (CFTR) Cl channels. As euryhaline teleosts, killifish can inhabit a wide range of salinities (salinity range 0-1200 mOsm). They are able to transition from freshwater (FW) to seawater (SW) and maintain NaCl balance because the opercula epithelium secretes more Cl in SW than in FW. This is accomplished by increasing the number of CFTR Cl channels in the plasma membrane of the opercula. Cortisol, which rises during migration into SW, increases CFTR expression by activating GR. Thus, studies were conducted to test the hypothesis that in killifish arsenic reduces Cl secretion and blocks acclimation to SW by: 1) acute (i.e. non-transcriptional) inhibition of CFTR function and 2) inhibition of cortisol/GR-mediated CFTR expression. Opercula membranes from FW adapted killifish were isolated and mounted in Ussing chambers for measurements of CFTR-mediated Cl secretion. Arsenic was independently applied to apical and basolateral membranes (2.5 to 30 μM) and the effects on Cl secretion were evaluated. In a second set of experiments, FW and SW acclimated fish were injected (IP) with arsenic, and the effects on Cl secretion and CFTR gene expression were measured. In a third set of experiments designed to mimic the rise in plasma cortisol levels during SW acclimation, FW fish were injected with arsenic (5 μmol/kg fish, IP; cortisol (40 μmol/L), or co-injected with each. Studies were also initiated to relate IP injections to waterborne arsenic exposure. Acute (96-h) toxicity tests were conducted on fish acclimated to FW, SW, and abruptly transferred from FW to SW. Arsenic applied directly to the opercula rapidly inhibited Cl secretion (i.e., 30 minutes, LC50 4.1μM) when applied to the apical (sea water) but not the basolateral solution (blood side). Arsenic (5 μmol/kg fish) injected IP also inhibited Cl secretion in FW and SW fish, and reduced CFTR gene expression as determined by Q-RT-PCR. In addition, 24 hrs of arsenic exposure also decreased CFTR gene expression in a dose-dependent fashion over the range of 1.25-10 μmol As/kg fish. However, at 5 μmol As/kg fish, arsenic increased Cl increased in fish sampled within the effects range (160-320 μmol As/kg fish). Significant differences were observed between FW and SW fish (214 ± 31 μmol As/kg fish, FW vs. SW, P < 0.05). This supports our hypothesis that arsenic acutely inhibits Cl secretion and blocks acclimation to SW by: 1) acute (i.e. non-transcriptional) inhibition of CFTR function and 2) inhibition of cortisol/GR-mediated CFTR expression. Opercula membranes from FW adapted killifish were isolated and mounted in Ussing chambers for measurements of CFTR-mediated Cl secretion. Arsenic was independently applied to apical and basolateral membranes (2.5 to 30 μM) and the effects on Cl secretion were evaluated. In a second set of experiments, FW and SW acclimated fish were injected (IP) with arsenic, and the effects on Cl secretion and CFTR gene expression were measured. In a third set of experiments designed to mimic the rise in plasma cortisol levels during SW acclimation, FW fish were injected with arsenic (5 μmol/kg fish, IP; cortisol (40 μmol/L), or co-injected with each. Studies were also initiated to relate IP injections to waterborne arsenic exposure. Acute (96-h) toxicity tests were conducted on fish acclimated to FW, SW, and abruptly transferred from FW to SW. Arsenic applied directly to the opercula rapidly inhibited Cl secretion (i.e., 30 minutes, LC50 4.1μM) when applied to the apical (sea water) but not the basolateral solution (blood side). Arsenic (5 μmol/kg fish) injected IP also inhibited Cl secretion in FW and SW fish, and reduced CFTR gene expression as determined by Q-RT-PCR. In addition, 24 hrs of arsenic exposure also decreased CFTR gene expression in a dose-dependent fashion over the range of 1.25-10 μmol As/kg fish. However, at lower concentrations of arsenic (0.625 μmol As/kg fish) CFTR gene expression increased. Low concentrations of arsenic have been shown to be more acutely (e.g., chick embryo, cell culture). Cortisol stimulated CFTR gene expression in FW fish, and the increase was blocked by arsenic. Median lethal (LC50) and no observable effect concentrations calculated for waterborne arsenic were similar for FW and SW fish (214 ± 31 μmol As/kg fish, respectively). Toxicity increased when exposure to arsenic in FW was followed by SW challenge (LC50 150-175 μmol As/kg fish). Plasma Cl increased in fish sampled within the effects range (160-320 μmol As/kg fish), consistent with inhibition of CFTR Cl secretion by the opercula. Collectively, these studies support the hypothesis that arsenic in SW has an acute, inhibitory effect on Cl secretion by the opercula, and that arsenic blocks acclimation to SW by inhibiting cortisol-induced and GR-mediated CFTR gene expression and Cl secretion.