



Center for Virus Research
University of California, Irvine

FIVE-YEAR REVIEW

**CENTER FOR VIRUS RESEARCH
FIVE-YEAR REVIEW**

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I. MISSION OF THE CENTER

Purpose

The Center for Virus Research (CVR) seeks both to foster interdisciplinary scholarship, training and research among University of California, Irvine (UCI) faculty and to create scientific resources that use molecular virology as a foundation. This is accomplished by stimulating scientific communication amongst CVR members, as well as between CVR members and non-members from the UCI research community. Further promotion of these interactions can lead to the development of pilot studies and, if successful, into formal grant-supported interdisciplinary research. The CVR also promotes the creation of common resources to be shared by the membership and the UCI research community, which has led to the development of applied laboratories for BSL3 research, the Viral/Vector laboratory for the construction of recombinant virus, the applied proteome laboratory, and more recently, the applied immunology laboratory. The creation of these applied laboratories has also allowed the CVR to participate as a subcontractor with industry-based researchers and to support the University-Industry collaborations, especially in the form of (Small Business Innovative Research) SBIR grants. To further the establishment of the applied laboratories as well as their links to industry, the CVR has created several Project Scientist positions, each of which oversees a specific applied laboratory and is responsible for generating grant funding to support the applied lab operation. This recently implemented model allows the implementation of applied laboratories with relatively little resource requirement (aside from space) from UCI. Finally, the existence of our seminar and symposia series, as well as the existence of our applied laboratories, provides an excellent environment for advanced post-graduate training. Two training grants (currently involving 23 trainees) are overseen by the CVR. Since graduate training in virology encompasses six departments, in three schools, the CVR has also become the focus and administrative point for the organization of graduate virology courses and the virology track of the Combined Graduate Program in Molecular Biology Genetics and Biochemistry.

Prior, present, future goals and focus

The overall goal of this Organized Research Unit (ORU) is to use viruses to develop interdisciplinary collaborations and studies. The primary purpose of establishing the CVR is to significantly stimulate the interaction of UCI virologists with many other UCI researchers. Research on viruses has often provided a biological and technological foundation from which much has been discovered concerning the basic molecular processes of organisms. Indeed, this technology has had enormous impact on other areas. The very foundations of molecular biology owe much to virus research. Virology continues to teach us much about normal and disease processes (including cancer) of living systems, not only at the molecular and cellular level, but at the level of whole organisms and their populations as well. This is especially true for the animal viruses and their hosts. Viruses have long provided some of the most useful experimental models for disease, cancer, immunity, and genetic systems of gene control. In addition, viral-based technology is being vigorously pursued and developed in the context of gene therapy and is teaching us much about the control of cellular processes. With the growing worldwide

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threat of emerging viral diseases, interest in virus research at all levels has intensified and taken on a new global perspective; thus, there is a need at the international level to become more knowledgeable about viruses and disease. As a consequence, previously separate disciplines such as molecular biology, pathogenesis, evolutionary biology, neurology, and radiological sciences can now be readily linked by virus research. Such research pathways provide a highly interdisciplinary character to the Center for Virus Research at UCI. The ORU provides both the administrative structure as well as training and common facilities needed for this interdisciplinary research. Funding agencies, such as NIH, ACS, CDC, NSF, and MDA are all interested in supporting research on the mechanisms of disease. In addition, NIH (NIGMS/NIAID) has recently decided to support international training on disease emergence. These same agencies are currently supporting the research of participating ORU members.

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II. ORGANIZATION

The CVR is under the Directorship of Luis Villarreal, PhD, Professor in the Department of Molecular Biology and Biochemistry. An external advisory committee, composed of three senior virologists from other UC Campuses, advises the Director. In addition, an internal oversight committee chaired by Dr. Bert Semler works with the Director to develop policy. The CVR reports to the Executive Vice Chancellor for Research at UCI.

A brief history of the CVR

The CVR has a long evolutionary history at UC-Irvine. Originally, the Center was developed directly from an Irvine Research Unit (IRU) on animal virology, established by Dr. Edward Wagner in 1986. As founding IRU Director, he developed the focus of the IRU on the study of viral gene control. Following 14 years of strong success as an IRU, Dr. Villarreal proposed the establishment of an ORU on virology named the Center for Virus Research. Submitted in 1998 and approved by a systems-wide review in 1999, The Center represented the only ORU in the University of California system focused on virology. The CVR was to be administered by the Director (Dr. Luis Villarreal) and the Co-Director (Dr. Bert Semler). An external review committee (composed of senior virologists from UC campuses and from California viral-based private industry companies) met with the Co-Director to provide suggestions and critical feedback to the CVR.

For its continuation, the mission of the CVR will remain essentially unchanged, that is: the development of interdisciplinary research and training based on the study of viruses. We will conserve the interdisciplinary faculty membership, seminar series, symposia, and pilot research elements. The administrative structure will also remain essentially unchanged. As the CVR has been highly successful in promoting interdisciplinary research at UCI, we feel well justified to continue with this role.

Elements of the CVR

Seminar series

The CVR sponsors a regular seminar series every other Friday during the academic year in which leading worldwide researchers are invited to present their work in different areas of virology, gene regulation, cell transformation, and a variety of topics in molecular biology and molecular genetics. These seminars provide a stimulating forum for exchanges of scientific ideas and information and for a critical analysis of data generated by the postdoctoral fellows and graduate students making the oral presentations. Please refer to Appendix B for a complete listing of CVR sponsored seminars and symposia.

Summer support for incoming graduate student

The CVR has established a tradition of supporting an early start for incoming graduate students wishing to enter the labs of CVR members. This program covers the salary cost for new graduate students to start research the summer before the Fall quarter. This component has given CVR members an edge at attracting motivated graduate students, thus we feel it both aids in supporting

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CVR members and also aids in creating graduate student identification with our program. Typically a vote is taken of the CVR membership and support is awarded to as many students as the budget allows. We feel this has been a successful program that should be continued.

International trainees

In the past the CVR has also supported a limited number of international trainees. Some of these trainees have subsequently chosen to continue their research at UCI. (Itzel Calleja-Macias and Ester Magdelana Marques-Lona). Although this program pleases us, we have had some difficulty covering the cost of these international fellows beyond a very limited stay. And, although we would like to continue to support international fellows, we feel budgetary constraints will limit such support for the immediate future. However, we wish to maintain some capacity along these lines in that we hope to promote the applications for fellowship funding for such awards (such as UCMEXUS).

Pilot projects

One particularly fruitful and relatively inexpensive program that we have previously supported was a pilot project program. These are small budget science projects (typically less than \$2,000) that seek seed or reagent money to quickly evaluate new and promising ideas or interactions by CVR members. They are approved administratively at the discretion of the Director, and can thus go forward very quickly, sometimes starting within one day. Three examples of these pilot projects include: "The Rapid Total Synthesis of the Smallpox Gene for DNA Polymerase" (Villarreal-Hatfield), "The Synthetic Production of SARS Proteins" (Felgner) very early during the SARS outbreak, and more recently, the "Production of West Nile Virus Proteins" for the California State Department of Health. In several cases, these pilot projects have led to significant scientific interactions, including new grants. We feel this is a very effective program that makes the CVR highly adaptive to any situation that might arise and will thus seek to continue it. Given the seemingly spontaneous or rapid nature of emerging infectious disease (especially emerging viruses), maintaining a rapid-response ability gives CVR members a big advantage to adapt to new research opportunities.

Inter-institutional Collaborations

International

The CVR has been a principle participant in fostering international interaction between UCI members and colleagues in other countries. The CVR members have interacted with virologists of the Institute of Molecular Biology in Canto Blanco, near Madrid, Spain. Several UCI students were supported by the MIRT NIH funded program (Luis P. Villarreal, PI) to conduct summer research on HIV molecular genetic diversity. Other UCI students have worked with Dr. F. Almendral on the study of the molecular basis of parvovirus pathogenesis in mice. MIRT students were also involved in our most recent international Internet based symposia with colleagues from Universidad Nacional Automena de Mexico.

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Working with Dr. Alejandro Carranca, we have used the UCOP facility in Mexico, Casa California.

In addition to the student exchange programs, the CVR has also mediated the establishment of international research collaborations that have subsequently received federal funding. These international collaborations are mainly associated with the recruitment of Dr. Philip Felgner and the establishment of the Applied Laboratory for Proteomics for Biodefense. A most fruitful collaboration was started with Rick Titball in Porton Down, UK, at the Defense Science and Technology Laboratory (Dstl), and resulted in the awarding of two large NIH grants, one concerning the study of *F. tularensis* (Scanning the *F. tularensis* Proteome for Vaccine Agents) and the other examines *Burkholderia* (Scanning the *B. pseudomallei* proteome for vaccine antigens).

National

In addition to these international collaborations, the CVR has promoted interactions with other researchers within USA. Working with Rafi Ahmed, PhD at the Emory Vaccine Center, we have collaborated to conduct a study of the human immunological memory to smallpox vaccine (see Appendix A). This collaboration also involved colleagues at Center for Disease Control and Prevention in Atlanta and was the basis for a successful NIH grant application by them. We have used this same study as part of a large Program Project proposal investigating vaccinia that is currently under review by NIH. It should be noted that that Program Project involves many other UCI researchers. As a consequence of the CVR-Atlanta collaboration, we also established collaboration and submitted grant applications with Shane Crotty, who moved from Atlanta and is now in San Diego. Other prominent collaborations have also been recently established via the CVR. These include collaborations and grants submitted with the Oregon Health Science Primate Center. More recently, and of significance to our interest to develop in the area of Biodefense, we have established a collaboration with Jay Hooper, head of virology at United States Army Medical Research Institute for Disease (USAMRID). Dr. Hooper oversees a BLS4 level experiments involving Monkeypox virus in primates and he is collaborating with us to evaluate effective immunity against smallpox using our proteome technology. We have also established a recent collaboration with Dr. Dennis Taub, Chief of the Immunology Division, NIH/IRP in Bethesda. Dr. Taub has a unique set of human reagents, including archived sera (80 years old) from patients that have recovered from an actual Smallpox infection. He has sent us some of these human sera so that we might use our whole proteome technology to evaluate human immunity to smallpox infection. We feel these preliminary studies are likely to lead to additional grant funding opportunities for the CVR.

CVR Symposia

Historically, the CVR has sponsored various international symposia. Most recently, the CVR sponsored back-to-back symposia at two institutions in Mexico, at the Department of Biochemistry, School of Medicine in Monterrey

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and in the Institute of Genomic Technology in Reynosa. The majority of the CVR members visited Mexico for these two symposia. These symposia were supported by UCMEXUS of the University of California. The CVR's international links to Mexico have continued. On Aug 30th 2004, the CVR, in collaboration with UNAM, hosted the very first internet-based meeting at the Casa California, the University of California facility in Mexico City. This new facility was developed to promote interactions between UC and UNAM. The topic of this meeting was the application of CVR based proteome technology to the development of new anti-cancer vaccines for the treatment of cervical cancer in Latinas. We hope to further develop this international cancer vaccine study during the next five years of CVR support.

Please see Appendix B for meeting agenda.

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III. RESEARCH ACTIVITIES

Biodefense

The research scope of the ORU has evolved noticeably during this initial five years. The CVR has significantly added to its structure. The CVR has acquired a new emphasis to better reflect major changes in the priority of federal research funding and national defense and health priorities, especially in the area of biodefense. In the context of virology, Smallpox represents the greatest potential biological threat to our country and to the world as a whole. Accordingly, the CVR has sought to develop and use poxvirology as a platform for the creation of new programs and expand them into other areas of virology and biotechnology. Since neither UCIrvine nor any other UC campus has had significant strength in pox virology, the CVR has sought to obtain an FTE for the recruitment of a senior professor in this field. In addition, the CVR sought to use this FTE to develop expertise in both proteome and protein-protein interaction technology. Along with the support of the Dean of Biological Sciences, the Chair of the Department of Molecular Biology and Biochemistry, as well as the Executive Vice Chancellor for Research, our effort to generate this FTE, its corresponding startup funding, space and instrumentation have all been successful, resulting in the recruitment of Dr. Paul Gershon to UCI from the University of Texas, Houston. Along with his recruitment and the assistance of the CVR, Dr. Gershon was also successful in obtaining a new TOF-TOF mass spectrometer from UC Irvine central administration which will be developed as a UCI-wide resource for the study of proteins, especially protein-protein interactions. This will bring UCI expertise in both poxvirology and protein technology. Thus, we have leveraged this appointment to address several important needs of UCI.

The initial CVR focus for proteomics and biodefense was to be on poxviruses, aka, vaccinia virus, as vaccinia virus is a manageable and important genome. This would provide the platform for the development of proteome technology and we feel we have been a highly successful in this strategic decision. Early on, we realized that the traditional methods for the expression of all the proteins from any particular genome would be problematic. Problems of protein solubility and cellular toxicity were common. The CVR then sought to develop new, in vitro based, proteome technologies that could circumvent these issues by recruiting new researchers and establishing new laboratories to conduct work in this area. The CVR was quickly able to express the entire genome of vaccinia virus. We then sought to leverage this vaccinia-based technology and apply it to the development of the whole proteomes, made in vitro, of non-viral pathogens, in the context of biodefense as well as emerging disease. The CVR now promotes the expression of numerous bacterial proteomes, and has successfully expanded its laboratory services to the study of non-viral pathogenic genomes. The CVR has now established a Proteomics Laboratory for Biodefense under the supervision of CVR Project Scientist, Dr. Phil Felgner. The emphasis of this laboratory is on the rapid in vitro expression of proteomes as well as on rapid vaccine development. This laboratory will also participate in a proposed new Regional Center on Biodefense that is to be submitted for NIH review later this year.

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In addition to the Proteome Laboratory, the CVR has also recently developed an Applied Immunology Laboratory under the supervision of CVR Associate Project Scientist Dr. Huw Davies. The space for both of these applied laboratories was negotiated with the Dean of the School of Biological Sciences, Sue Bryant. Both of these newly developed laboratories have further allowed the CVR to stimulate linkages with industry in the form of subcontracts and SBIR grants.

Such successes have also stimulated the CVR to foster other SBIR contracts and create another laboratory service. This is the library display laboratory of CVR member Dr. Gregory Weiss in the Department of Chemistry, which is supporting an ImmPort Therapeutics SBIR funded research as a subcontract. As NIH recently awarded this grant, the Phage Display Library is only now being established by Dr. Weiss.

Cancer Research

An early interest of the UCI virologists (when we were still an IRU) was to promote the study of cancer and the development of the Comprehensive Cancer Center. As one of our most prominent CVR members, Dr. Hung Fan is interested in the study of retrovirus mediated tumor development and is the Director of the Cancer Research Institute (CRI). Dr. Fan has been highly productive, successful, and well recognized for his studies of retrovirus mediated lung cancer in bovine systems. There has long been a close working relationship between the virologists of the CVR and the CRI. The CRI and the Comprehensive Cancer Center are also very tightly coordinated, as the Comprehensive Cancer Center is composed of 'groups' or programs with common scientific interests. The CVR has generally provided the scientific network for the Virology Program of the Cancer Center. The CVR Director, Luis Villarreal, PhD, then working with human polyomavirus, was the initial leader of the Cancer Center Virology Program and participated directly in both the initial application and the NIH site visit of that center grant. In keeping with the recommendations of the study panel of the site visit, the building of scientific strength in cancer virology at UCI was recommended, especially in the area of small and large DNA cancer viruses. Accordingly, the CVR worked closely with the CRI and the Comprehensive Cancer Center to identify and recruit a basic scientist working in these areas of virology. In so doing, we were one of the first interdisciplinary efforts to be awarded a senior position from the UCI central administration for the promotion of a Center. As CVR director, it was Dr. Villarreal's duty to identify a possible home department for that recruitment, which ended up being his home department, Molecular Biology and Biochemistry. He then recruited Dr. Hans-Ulrich Bernard to UCI and was successful in promoting a departmental appointment for him. The Cancer Center was also highly successful in generating the resources needed for this position, and provided set-up funds. Dr. Bernard is now here at UCI and our efforts in the area of cervical cancer biology, caused by human papillomavirus, are now well underway. Since Dr. Bernard has a compelling and long-term interest in cancer virology, he has succeeded in obtaining NIH funding for his research on HPV. His recruitment will also help us develop human studies with colleagues in Mexico in the area of cervical cancer.

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Following the successful recruitment of Dr. Bernard, and to further advance cancer research at UCI and continue our support for the CRI and the Cancer Center, the CVR also spearheaded an effort to obtain an FTE for a virologist that would work on a large DNA virus that was a cancer virus. Continuing with our close work with the CRI and the Cancer Center, we were able to obtain an FTE for such a position and were also successful in proposing to the Department of Molecular Biology and Biochemistry that they provide an academic home for this appointment. Subsequently, we successfully recruited Dr. Ingrid Ruf to join UCI, who works on Epstein Bar Virus (EBV). With her recruitment, we feel that CVR members now add considerable strength to the major areas of cancer virology.

Team Based Science

Although the CVR still promotes specific member-to-member scientific interactions these recent CVR-based initiatives reflect a strategic decision to develop and promote team-based scientific research at UCI. This change is also reflected by changes in federal grant funding, which have also developed new programs that emphasize team based scientific research. Center grants are especially designed to promote the development of team based research. Such teams can take advantage of the products and infrastructure of the associated laboratory resources: whole proteome technology, applied immunology, library display and mass spectrometer based study of protein-protein interactions are generally rapid and high throughput and well developed to serve the expanded needs of research teams. The CVR has subsequently spearheaded the development of several Program Project and Center grants, composed of teams and core lab facilities (applied labs), which have been submitted to NIH.

Member Research and Collaborative Research Interactions

1. Luis P. Villarreal, PhD, Department of Molecular Biology and Biochemistry & Center for Virus Research, Director

My laboratory was initially interested in the link between small DNA virus replication to host cell differentiation with both polyomavirus and papillomavirus. Our interest is both basic (mouse polyomavirus studies of viral persistence), applied (human papillomavirus and cervical cancer) as well as theoretical (virus and host evolution). This theoretical interest has stimulated the development of integrating approaches and has also led us to become involved in the integration of large-scale projects, such as biodefense and emerging viral disease.

2. Bert L. Semler, PhD, Department of Microbiology and Molecular Genetics & Center for Virus Research, Co-Director

Our research focuses on how RNA viruses specify complex RNA-protein and protein-protein interactions to carry out their replication mechanisms in infected mammalian cells. The viruses under investigation are picornaviruses, which include poliovirus, human rhinovirus, coxsackievirus, hepatitis A virus, and others. We are investigating the mechanism of picornavirus translation initiation as directed by an internal ribosome entry site (IRES) in the 5' noncoding region of viral genomic RNAs. We found that the translation initiation signals encoded in the 5' noncoding region of picornavirus RNAs are

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comprised of specific RNA-RNA and RNA-protein interactions. In particular, the RNA-protein interactions form signals important in the mechanism of cap-independent translation initiation of picornavirus mRNAs, signals that may have counterparts in eukaryotic cellular translation of specific messenger RNAs that harbor IRES elements. Using cell-free translation assays in extracts made from human cells, we are investigating the role of a cellular RNA binding protein (known as PCBP2) in translation initiation functions required by poliovirus and other picornaviruses (e.g., human rhinovirus and coxsackievirus) in an attempt to identify the precise step(s) in translation initiation in which PCBP2 functions. Another focus of our research is elucidation of the mechanisms involved in replicating picornavirus genomic RNAs during an infection of human cells. Such mechanisms are of interest because picornaviruses employ covalent linkages between viral proteins and newly-synthesized viral RNAs to effect the efficient replication of their genetic information. Similar to IRES-dependent translation initiation, the initiation of viral RNA replication also utilizes RNA-protein interactions. These interactions occur between sequences present in the termini of viral RNAs and both viral and cellular polypeptides. Using defined viral mutants and inter-species chimeric templates, we are investigating the sequence determinants of such interactions and how they confer template specificity to the viral replication apparatus that allows synthesis of progeny RNAs from defined templates present among a myriad of cellular mRNAs in the cytoplasm of infected cells. Part of this specificity is imparted by viral polypeptide called 3CD. This protein is both a viral-specific proteinase and a sequence-specific RNA binding protein. As such, 3CD is a key player in the picornavirus life cycle. Protein 3CD also interacts with cellular proteins to augment its functions in protein processing and viral RNA replication. We are utilizing cell culture assays and in vitro RNA replication reactions to identify the specific domains in polypeptide 3CD responsible for its RNA binding/replication properties for human rhinovirus, poliovirus, and coxsackievirus. In addition to revealing the nature of specific macromolecular interactions that regulate viral and cellular gene expression, results from our studies should identify molecular targets for antiviral chemotherapy.

Semler/Robinson/Lane Collaboration

Recently, Ed Robinson, Tom Lane, and Bert Semler initiated a collaborative study to identify novel therapeutic agents against pathogenic, positive-strand RNA viruses. They plan to submit a grant application for NIH support of this project in the near future. The potential of positive-strand RNA viruses for bioterrorism, while currently theoretical, is real and could prove devastating. The cost in human lives, while perhaps only small, would be far less than the devastation to public markets and the economy that fear alone would instill. Moreover, drugs with activity against such agents are either non-existent or are of fairly limited efficacy. For these reasons, we propose an interdisciplinary approach to improve high through-put screening, drug discovery, and sensitive measures of viral replication for positive-strand RNA viruses that replicate within the cytoplasm of infected cells in association with intracellular membranes. The specific viruses initially targeted will be members of the *Flaviviridae* specifically, dengue and yellow fever viruses, and *Coronaviridae*, mouse hepatitis virus. Ultimately we will translate findings on these viruses into studies on West Nile virus (a flavivirus) and SARS-CoV, a coronavirus. To accomplish our goals, we propose the following specific aims: (1) Develop high through-

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put cytolytic assays for screening viral replication inhibitors. (2) Screen natural product libraries and generate small combinatorial libraries for inhibitors of RNA virus replication based upon leads identified in the natural products. (3) Develop sensitive and quantitative measures of viral RNA replication within infected cell cultures. (4) Determine the effect of compounds with antiviral properties on *in vitro* RNA replication. (5) Synthesize drug-like molecules based on lead compounds directed at bona fide RNA replication targets. The successful completion of these specific aims will allow the identification of lead molecules and perhaps drug candidates for the treatment of viral infection in the event of a terrorist event or natural outbreak. In addition, our proposed approaches should result in the development of sensitive measures of viral RNA replication that can be used for studies evaluating viral pathogenesis, drug development, and potentially vaccine development. Ultimately, our studies will determine the mechanisms of action for lead molecules with antiviral activity, mechanisms that should also reveal novel insights into specific steps of viral RNA template recognition and assembly of multi-component replication complexes that are essential features of the intracellular life cycles of positive strand RNA viruses.

3. Edward K. Wagner, PhD, Department of Molecular Biology and Biochemistry

Herpes simplex virus is a highly adapted human pathogen with an extremely rapid lytic replication cycle, and yet with the ability to invade sensory neurons where highly restricted gene expression occurs with the absence of cytopathology. Such latent infections are subject to reactivation whereby infectious virus can be recovered in peripheral tissue enervated by the latently infected neurons following a specific physiological stress. A major factor in these "switches" from lytic to latent infection and back involves the interaction between viral promoters, the viral genome, and cellular transcriptional machinery. Basic properties of viral promoters controlling the expression of specific classes of viral genes are being investigated by the introduction of specific modifications to critical promoters which lead to altered patterns of viral gene expression, and ultimately replication and pathogenesis. Also of particular interest are those features of the HSV genome and its interaction with the neuronal cell that lead to latency and reactivation.

Wagner/Sandri-Goldin Collaboration Project in which we are doing a global analysis of the effects of defined alterations in the structure of the HSV-1 immediate early protein ICP27 both on overall transcript abundance and on transport of individual mRNA species from nucleus to cytoplasm.

4. Hung Fan, PhD, Department of Molecular Biology and Biochemistry

Our lab is studying the molecular biology and pathogenesis of retroviruses. These viruses have been important models for studying gene expression and cancer. HIV-1, the causative agent of AIDS, is also a retrovirus. Historically, we have worked on Moloney murine leukemia virus (M-MuLV). Current M-MuLV projects include using enhancer variants of M-MuLV to study the complex (and multi-step) process of leukemogenesis in mice, and using M-MuLV as a system to study cellular chromatin and chromatin-associated proteins. A second project involves study of jaagsiekte sheep retrovirus, the etiologic agent of a transmissible lung cancer in sheep. We were the first to obtain an

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infectious and oncogenic molecular clone of JSRV, and we have been studying the virus from the viewpoints of tissue-specific expression and oncogenesis. It is particularly interesting that the JSRV Envelope protein functions as an oncogene. We are currently studying the mechanism of oncogenic transformation by the Env protein, and developing small animal models for the JSRV-induced disease. A third project involves the generation of simian immunodeficiency virus (SIV)-based vectors. These vectors are being used to study aspects of SIV cell tropism, and (in collaboration with Dr. Chris Miller, UC Davis) to study the cellular pathways of SIV infection in vivo in rhesus macaques.

Fan/Robinson Collaboration

Analysis of JSRV integrase. The Robinson lab has expertise in expressing and purifying retroviral integrases, while the Fan lab isolated a molecular clone of JSRV. The two labs are collaborating in cloning and expressing JSRV integrase for structure-function studies. The two laboratories have also collaborated in studies on SIV. The Fan lab has identified a post-entry block for T-tropic SIV in primary macaque macrophages that is dictated by the tropism of the viral envelope protein. To locate the step at which the post-entry block occurs, the Robinson lab is providing assistance in real-time PCR measurements of viral DNA intermediates -- 2-LTR circles in particular (a marker for nuclear import).

Fan/Ruf Collaboration

The Ruf lab is interested in EBV-encoded EBER RNAs. These RNAs are known to bind the LA protein in infected cells. In studies on glyco-Gag protein of M-MuLV, the Fan lab has found that this protein interacts with LA protein in a yeast 2-hybrid assay. This raises the possibility that LA protein may be involved in M-MuLV particle production through its interaction with glyco-Gag. Dr. Ruf's lab generated NIH3T3 cells stably expressing different levels of EBER RNAs, and they have been infected with wild-type and glyco-Gag mutant M-MuLV. Effects on M-MuLV production will be studied in the EBER-expressing cells.

5. Tom Lane, PhD, Department of Molecular Biology and Biochemistry

Work in this laboratory at the University of California, Irvine has focused on understanding the molecular and cellular mechanisms involved in regulating inflammation following microbial infection. Specifically, Dr. Lane is interested in evaluating the functional contributions of chemokines (chemotactic chemokines) and their receptors in the initiation and maintenance of inflammation following infection. Current work is divided into three major research areas: i) utilization of a mouse model of viral-induced encephalomyelitis and immune-mediated demyelination to study the functional contributions of chemokine and chemokine receptor expression in regulating neuroinflammation, host defense, and disease development; ii) evaluation of the mechanisms by which chemokine and chemokine receptors participate in linking innate and adaptive immune responses following viral infection, and iii) utilization of a model of remyelination developed in my laboratory to identify unique gene(s) involved in this complex process.

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One major area of ongoing work in the laboratory is to study how chemokines and chemokine receptors contribute to defense and disease following mouse hepatitis virus (MHV) infection of the central nervous system (CNS). Persistent MHV infection of the CNS results in an immune-mediated demyelinating disease that is similar clinically and histologically to the human demyelinating disease Multiple Sclerosis (MS). As such, the MHV system offers a relevant model to study the underlying molecular and cellular mechanisms contributing to MS. Importantly, chemokines and their receptors have been shown to be expressed within demyelinating lesions present in MS patients and have been proposed to be important in contributing to demyelination by attracting inflammatory cells into the CNS. Over the past four years, the lab has systematically determined that expression of chemokine and chemokine receptor genes is regulated within the CNS following MHV infection. Specifically, studies have determined that expression of CXCL10/IP-10 (interferon inducible protein 10 kDa) is important in host defense during acute disease by attracting T lymphocytes into the CNS that participate in host defense. Conversely, chronic expression of CXCL10/IP-10 is important in amplifying demyelination by attracting T lymphocytes into the CNS of persistently infected mice. Other chemokines such as CCL5/RANTES are also important in disease by attracting both T lymphocytes and macrophages into the CNS of MHV-infected mice. In addition, this laboratory has also determined that chemokine receptors including CCR2 and CCR5 also enhance leukocyte accumulation within the CNS following MHV infection. Collectively, these studies highlight the importance of chemokines and their receptors in enhancing inflammation within the CNS of virally-infected mice and indicate these molecules may be relevant targets for treatment of MS as well as other neuroinflammatory diseases.

Additional efforts are now focused on understanding how chemokines/chemokine receptors regulate innate immunity within the CNS following viral infection as well as how chemokine signaling contributes to the generation of anti-viral effector T cells.

6. Rozanne M. Sandri-Goldin, PhD, Department of Microbiology and Molecular Genetics

Regulatory functions of a post-transcriptionally acting herpesvirus protein Herpes simplex virus type 1 (HSV-1) immediate-early protein ICP27 is a 63 kilodalton nuclear phosphoprotein that is required for viral growth during lytic infection. ICP27 has a number of effects on gene expression including: a contribution to the shut off of host protein synthesis; the stimulation of HSV-1 early gene expression, and the induction of late viral gene products. Studies from my laboratory have demonstrated that ICP27 performs these functions predominantly at the post-transcriptional level. Specifically, ICP27 inhibits host cell splicing early in infection by mediating improper phosphorylation of splicing proteins, which results in stalled spliceosomal complexes (Sciabica, K.S., Q.J. Dai and R. M. Sandri-Goldin. 2003. ICP27 interacts with SRPK1 to mediate HSV splicing inhibition by altering SR protein phosphorylation. *EMBO J.* 22:1608-1619). This results in cellular pre-mRNAs being retained in the nucleus, which contributes to the shut off of host protein synthesis. Later in infection, ICP27 dissociates from spliceosomal complexes and moves to sites of HSV-1 transcription/replication, taking with it an important cellular nuclear export factor that binds to cellular mRNAs

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that are undergoing splicing. This factor is termed Aly/REF. ICP27 binds to HSV-1 intronless mRNAs and the ICP27-RNA-Aly/REF complex is exported to the cytoplasm via the cellular mRNA export receptor, TAP/NXF. Thus, ICP27 commandeers a cellular export pathway to favor viral RNA export and thus, stimulates expression of viral early and late gene products (Chen, I.B., K.S. Sciabica, and R.M. Sandri-Goldin. 2002. ICP27 interacts with the RNA export factor Aly/REF to direct herpes simplex virus type 1 intronless mRNAs to the TAP/NFX1 export pathway. *J. Virol.*76:12877-12889). Further, we have found that ICP27, like many cellular RNA processing factors interacts with the C-terminal domain of RNA polymerase II, and ICP27 appears to be one of the viral factors that is involved in recruiting RNAP II to sites of HSV-1 DNA in replication complexes., which also contributes to the stimulation of early and late gene expression. Current studies are directed toward unraveling the interaction with RNAP II and to a detailed structure-function analysis of ICP27. NMR spectroscopy is being performed on selected domains that are known to be involved in ICP27's RNA binding and protein interactions.

Sandri-Goldin/Wagner Collaboration

Drs. Sandri-Goldin and Wagner are using global analysis of HSV-1 transcripts to determine the stages of HSV-1 infection that are affected by ICP27 expression. Specifically, because ICP27 is an immediate early protein, we have asked what the consequences are to HSV gene expression if the expression of ICP27 is delayed. This was accomplished by generating recombinant viruses with late promoters substituted for the IE promoter of ICP27. Surprisingly, the results showed no significant effect on viral gene expression or replication (Sun, A., G.B. Devi-Rao, M.K. Rice, D.C. Bloom, R.M. Sandri-Goldin, P. Ghazal and E.K. Wagner. 2004. Immediate-early expression of the HSV-1 ICP27 transcript is not critical for efficient replication in vivo or in vitro. *J. Virology*. In press). In another collaborative study, Dr. Sandri-Goldin and Wagner are determining whether or not all viral transcripts require ICP27 for efficient export to the cytoplasm. Nuclear and cytoplasmic RNA fractions are being analyzed by microarrays from cells infected with WT HSV-1 and with a series of viral mutants in which regions of ICP27 involved in its export or RNA binding activity are deleted. Preliminary results demonstrate an effect on viral RNA export at late times after infection.

7. Suzanne B. Sandmeyer, PhD, Department of Biological Chemistry

Research in the Sandmeyer laboratory is focused on the *Saccharomyces cerevisiae* retrovirus-like element Ty3. Ty3 is a model system for the role of the host cell in retroviral replication and integration. Ty3 is unusual in that it integrates specifically in the vicinity of polymerase III transcription initiation sites. Information about what mechanism is responsible for this specificity may help in understanding the basis of more subtle discrimination in insertion site selection of retroviruses and may help in the design of gene therapy vectors which are safer by virtue of a more predictable pattern of insertion.

Work in the laboratory for the last several years has resulted in the basic characterization of the element. It is 5.4 kbp in length and occurs in one to five copies in typical laboratory strains of yeast. Its 5.2 kb transcript is directly analogous to the retroviral

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genome. It contains plus- and minus-strand primers for reverse transcription and encodes proteins containing domains similar to retroviral Gag and Pol in the first and second open reading frames, *GAG3* and *POL3*, respectively. The proteins encoded in *GAG3* are the structural proteins, capsid and nucleocapsid. Ribosomal readthrough from *GAG3* into *POL3* results in production of Gag3-Pol3 fusion proteins. The *POL3* reading frame encodes a protease required for processing the primary translation products of both frames into active species, a reverse transcriptase required for replication, and an integrase required for integration of the replicated Ty3 DNA.

Expression of Ty3 RNA results in production of intracellular virus-like particles (VLPs) composed primarily of Ty3 structural proteins and also catalytic proteins and Ty3 RNA and DNA. Reverse transcription of Ty3 RNA into DNA occurs within the VLP. *In vivo* transposition can be detected by a variety of genetic techniques and accompanies high level expression of the Ty3 element.

The first objective of research in the laboratory is the understanding of the basis of positional targeting of Ty3 insertions. Testing *in vivo* of the effects of mutations in tRNA gene promoter elements showed that intact promoter elements are essential for integration activity. An *in vitro* integration assay was developed which is dependent upon VLPs as the source of DNA and integrase activity, a target tRNA gene, and cell extracts with pol III transcription factor activity. Fractionation of cell extracts showed that TFIIB and TFIIC were essential for integration, but that polymerase III was not. Mutational analysis of the Ty3 element, particularly the integrase, is underway in order to identify protein domains which contribute to targeting. Highly-purified transcription factor proteins produced in *E. coli* were tested and Brf1 and TBP were shown to constitute the minimal *in vitro* target for integration into the U6 RNA gene which has an upstream TATA box. Information gained from these studies is being used to modify the insertional specificity of model retroviruses.

The second major area of research in the laboratory is identification of host genes that affect replication of the Ty3 element. A screen of a collection of random insertion mutants and a screen of the yeast knockout collection of mutants have identified over 120 mutants that are directly or indirectly affected in Ty3 transposition. These mutants are defective in genes involved in chromatin structure, nuclear transport, intracellular trafficking, and the cytoskeleton among other functions. Experiments are underway to identify the point in the lifecycle that is affected in the different mutants.

Sandmeyer/McPherson Collaboration

A collaborative effort between the McPherson laboratory, which is expert in structural biology of viruses, and the Sandmeyer laboratory, which is expert in Ty3 molecular biology, is ongoing to characterize the structure of the Ty3 viruslike particle. Because Ty3 does not have matrix or a membrane envelope, it has a relatively simple particle structure. In contrast, retroviruses have more complicated structures and tend to be heterogeneous. This limits the application of certain modeling approaches to determination of retrovirus structure. In addition, because retroviruses have to exit and enter cells to undergo the complete lifecycle, it is more complicated to characterize the

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different structures present at different stages. Currently the particles formed from wt and mutant Ty3 elements are being characterized in order to understand how the structure changes during the lifecycle. The primary approach to structure characterization at the current time is atomic force microscopy.

8. W. Edward Robinson, PhD, Departments of Pathology, Microbiology and Molecular Genetics, and Medicine

The acquired immune deficiency syndrome (AIDS) is of worldwide importance. In some areas of the world, specifically in central Africa, the human immunodeficiency virus (HIV) is predicted to cause negative population growth. The research in the laboratory has concentrated on anti-HIV drug development. Working with organic chemists, we are developing new anti-HIV compounds that target a specific viral protein called integrase. Integrase is absolutely required for HIV replication and has no mammalian homolog. Furthermore, integrase is the third of three viral enzymes and the only enzyme for which no inhibitors are used to treat patients. To date, we have identified over 100 compounds that inhibit HIV integrase; some of these are the most potent small molecule inhibitors of HIV integrase yet described. We are using these compounds as tools to better understand the molecular mechanisms involved in HIV integrase function and retroviral integration. In particular we are studying the effects of drug resistance mutations on integrase function and on HIV replication. We are also attempting to identify the amino acids that comprise the inhibitor-binding pocket on the integrase protein. Continued synthesis of anti-HIV compounds is ongoing, especially compounds that inhibit primary clinical isolates of HIV and both North American and African isolates. Techniques that are in use in the laboratory include molecular biological techniques, the polymerase chain reaction (PCR), quantitative real-time PCR, and HIV replication studies utilizing both clinical isolates of HIV and tissue-culture adapted strains of HIV.

9. Walter Fitch, PhD, Department of Ecology and Evolutionary Biology

Molecular evolutionary studies can shed much light on a vast array of interesting biological problems. My research is eclectic, and tries to answer any and all of these problems to the extent that the sequence information in proteins and/or nucleic acids is available and the methods are suitable.

To perform such studies one must detect significant similarity among sequences, align them homologously, and infer ancestral relationships and sequences. I spend a considerable amount of time inventing new and improving old ways of accomplishing such tasks. I am especially interested currently in ways of assigning weights to different nucleotide positions so that one may have greater confidence in the resulting phylogeny (evolutionary tree). An associated problem is how to allow for the fact that some amino acids may not be allowed to vary among insects but are variable among vertebrates and vice versa. The best part of research is when a new improvement permits one to see new things. New things we've seen recently include the following:

1. An analysis of 350 isoaccepting tRNA's for eight amino acids from all the kingdoms showed that the various isoacceptors evolved by gene duplications that occurred prior to the most recent common ancestor of everything that is alive today. It also showed that the pattern of gene duplications follows the pattern of the genetic code as if the earliest

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duplications separated recognition of purines from pyrimidines and later duplications separated recognition of A from G and C from U. This is a progression that suggests the genetic code was originally ambiguous and that the gene duplications arose so as to permit an increasingly refined specification of the amino acid to be encoded. Thus we appear to be recognizing evolutionary processes that occurred 3.5 billion years ago.

2. An analysis of the genes of the influenza virus isolated from 1933 to the present has permitted us to study the evolution of flu where the "fossils" are accurately dated. The rate of evolution for the hemagglutinin gene turns out to be as fast as for any known gene, 10⁻² substitutions/site/year with a rate so constant that I can determine the year of the virus' isolation if given the sequence. Moreover, we were able to prove that this enormous rate is associated with positive Darwinian selection where the coat of the virus is changing rapidly, the benefit being the change in the viral antigenic sites. This confers a temporary escape of the virus from immune surveillance. Thus we appear to be recognizing evolutionary processes that occurred in the last few decades.

3. With the flu hemagglutinin for both A and B types we can identify the positions at which there is positive selection for change of amino acids. Rates of evolution in these selected positions reduce the effectiveness of the host antibodies. Using them as referents, we can predict from which sequence group will be the source of future generations of flu virus with an accuracy exceeding 80%.

10. Gregory A. Weiss, PhD, Department of Chemistry and Molecular Biology & Biochemistry

The Weiss laboratory uses vast collections of proteins ("libraries") displayed on the surface of filamentous bacteriophage to identify the rare molecule that binds tightly to a target molecule or catalyzes an interesting chemical reaction. Our experiments investigate forces responsible for non-covalent interactions, which govern essentially all processes in biology. We seek to uncover general rules for how these forces work, and then apply principles elucidated to the development of novel receptors, biosensors and catalysts. For example, we have recently used phage-displayed shotgun scanning to uncover the role of second sphere residues in protein recognition of DNA sequences. Our libraries are also being used for proteomics projects in collaboration with other members of the Center for Viral Research, as described below.

Weiss/Felgner/Gershon/Villarreal Collaboration

This close collaboration focuses on developing nimble technology platforms to counter bioterrorism threats. For example, with funding from the NIH, we are identifying reagents from phage-displayed libraries with high affinity and specificity for vaccinia virus proteins. The ultimate goal is to identify a specific, high affinity binding partner, or "Artificial Antibody" (ArtAb), for each protein in the vaccinia virus (smallpox vaccine) proteome. Our approach applies vast libraries of novel protein variants fused to filamentous phage particles ("phage display"). Libraries include ankyrin and leucine-rich repeat scaffolds with vast diversity (>10¹¹ different molecules). Various phage-display techniques are being used to hone binding affinity and specificity of vaccinia protein binding partners.

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11. David Camerini, PhD, Department of Molecular Biology & Biochemistry

During the last few years we have focused our efforts on understanding the pathogenesis of CCR5 tropic strains of HIV-1 (R5 HIV-1). We are studying the selection for, and maintenance of R5 HIV-1 following viral transmission, and during the first few years of infection as well as the development of greater pathogenic potential by late stage R5 HIV-1. These topics are of crucial importance to an understanding of HIV-1 pathogenesis since R5 HIV-1 is predominant in infected individuals. Our data suggest that HIV-1 mediated signaling through CCR5 allows infection of resting memory CD4+ T cells, which are crucial targets of HIV-1 infection. In the early stages of infection this may provide a selective pressure for R5 HIV-1 since few cells are activated in a healthy individual. In contrast, during AIDS many cells may be activated by HIV-1 and opportunistic pathogens, allowing the replication of X4 HIV-1. We use normal human lymphocytes, tonsil and lymph node organ cultures to realistically study HIV-1 infection of the mature immune system. We also study HIV-1 infection of the thymus, an important aspect of AIDS, using SCID mice bearing human thymic grafts (SCID-hu mice), and fetal thymic organ culture. These systems, which maintain the complexity of lymphoid tissue, are ideal for our studies of HIV-1 pathogenesis since they accurately mimic the *in vivo* situation. Other interests in the lab include the mechanisms of action of natural antiviral proteins including defensins. Defensins block HIV-1 replication by unknown mechanisms. Understanding these mechanisms may shed light on viral pathogenesis and lead to new treatments for AIDS. Finally, we have data suggesting that HIV-1 and other retroviruses replicate via an RNA lariat intermediate like yeast retroelements. We plan to study the interaction of cellular and viral factors that lead to the formation and resolution of the HIV-1 RNA lariat intermediate. This work may also open new avenues for the development of antiviral agents to combat AIDS.

Camerini/Robinson/Weiss Collaboration on HIV Integrase

Our lab is part of a collaborative effort with the Robinson and Weiss labs plus Richard Chamberlin's group from the Chemistry Department and Robert Hamatake's group of ICN Pharmaceuticals to develop inhibitors of the HIV-1 integrase (IN). We are currently testing lead compounds in organ culture and will soon resubmit an NIH Program Project Grant application to support this work

12. Ingrid Ruf, PhD, Department of Molecular Biology and Biochemistry

The main focus of research in the Ruf lab relates to the basic molecular biology and pathogenesis of a ubiquitous herpesvirus, Epstein-Barr virus (EBV). As is characteristic of all herpesvirus, once an individual is infected with EBV the infection is maintained for the life of that individual. While most individuals encounter no lasting difficulties as a result of this infection, several malignancies, including Burkitt Lymphoma (BL) and Nasopharyngeal carcinoma, have been linked to EBV infection and occur years to decades after the initial infection with the virus. The lab is interested in determining how the virus contributes to the development of these malignancies, with an interest in both the role of viral gene products and the effects these gene products have on the host. Current projects in the lab are focused on the specific contribution of two small non-coding viral RNA molecules in the establishment and maintenance of tumorigenicity in BL. Additionally, the lab is interested in the role of the virus in promoting cell survival (or inhibiting cell death) and the potential mechanisms used by the virus to accomplish this enhanced cell survival. The long term goals of this work are to enhance our understanding of viral latency in healthy hosts and virus-mediated oncogenesis.

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Ultimately, we hope to contribute to the development of treatments or methods of prevention for those most at risk for the development of EBV-dependent tumors.

13. Hans-Ulrich Bernard, PhD, Department of Molecular Biology and Biochemistry

Our lab investigates the molecular biology of human papillomaviruses (HPVs) that are involved in cervical and anal carcinogenesis and laryngeal papillomatosis. Our research has three objectives, namely studies (i) of gene expression and (ii) of genomic diversity of HPVs and (iii) molecular biological support to preclinical and clinical vaccine developments. Our long-term investigation of HPV gene expression has moved from the identification of transcriptional activators and repressors, the role of the nuclear matrix and viral chromatin to the present focus of epigenetic regulation induced by methylation of HPV DNA. We have completed projects that document the correlation of HPV DNA methylation with neoplastic progression and identified targets of the HPV genomes that are either hotspots of or protected from DNA methylation. Presently, we are following projects based on the hypothesis that DNA methylation is a major regulator of latency and progression of HPV infections. Our study of the genomic diversity of HPVs has just led to a final and official classification (taxonomy) of all HPV types. Continuing projects investigate the worldwide diversity of variants of each HPV type and its potential impact on the increased risk of the populations of some countries to develop HPV induced cancers. As to support of vaccine development, our lab defines and constructs fusion proteins that are used in animal models in the labs of Dr. L. Villarreal and Dr. P. Felgner to develop therapeutic anti-HPV vaccines, and we determine the genomic properties of HPV genomes in an ongoing clinical phase II study at the UCI and UCLA medical centers under leadership of Dr. F. Meysken

14. Philip Felgner, PhD, Center for Virus Research

Despite the ever-increasing availability of genome sequences from many human pathogens the production of complete proteomes remains a bottleneck. To address this, a high throughput PCR recombination cloning and expression platform has been developed by Felgner and colleagues at the University of California Irvine (UCI). The method relies on high throughput amplification of each predicted open reading frame using gene specific primers, followed by in vivo homologous recombination into a T7 expression vector. The proteins are then expressed in an E. coli-based cell-free in vitro transcription/translation system and the proteins are printed directly onto microarrays without further purification. The chips can then be used to screen sera from individuals naturally or experimentally exposed to the organisms of interest. With support from 3 NIH/NIAID biodefense grants, the laboratory has completed the proteomes for vaccinia virus (194 ORFs) and for F. tularensis (1933 ORFs), and has initiated synthesis of the B. pseudomallei proteome (~5600 ORFs). This high throughput technology platform for vector generation, protein expression, chip printing and serological analysis will be made available to the CVR. Serology from vaccinated or infected, animals and humans using the protein microarrays is being applied by this laboratory to the problems of vaccine and diagnostic antigen discovery. Protein subunit vaccines and DNA vaccines are being investigated and four potent but non-toxic immunological adjuvants have been acquired by the lab. These adjuvants and vaccine formulations are also available to the CVR.

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Felgner/Villarreal Collaboration

Protein microarrays are being investigated as a tool for diagnostic antigen discovery against emerging infectious diseases. Protein microarrays containing the West Nile virus and SARS proteomes have been synthesized, and the utility of these arrays for diagnostic purposes is being investigated.

Felgner/Bernard Collaboration

The laboratories have a significant collaborative research and development activity underway to develop a therapeutic vaccine to clear early stage HPV cervical infections. The vaccine will be a cocktail of five antigens delivered as a DNA prime followed by a protein boost. The antigens used will be the five early genes, E1, E2, E4, E6 & E7. The genes for the DNA vaccine prime are codon optimized for optimal expression in mammalian tissue, and the proteins for boosting will be formulated into one of four adjuvants. The preferred adjuvant will be either PROVAX, Tomatine, Perham Scaffold or Zhibin Guan Nanoparticles selected based on performance. The optimal vaccine formulation will be prepared for preclinical and clinical evaluation at the UCI Medical Center.

15. Huw Davies, PhD, Center for Virus Research

Dr. Davies is a cellular immunologist with expertise in vaccine design. His main experience is in the investigation of novel protein and nucleic acid based vaccines for the prevention and treatment of viral infections, particularly human papillomavirus, orthopoxviruses, West Nile virus. His current interests are the application of a high throughput gene cloning and expression platform developed in the proteomics facility for the rapid discovery of T and B cell antigens and rational vaccine design.

Davies/Felgner Collaboration

In collaboration with Dr. Felgner he has developed a technique for screening sera from vaccinated and infected humans and other animals against proteome microarrays. Several candidate vaccines have been identified in the vaccinia proteome and he is currently evaluating these antigens for protection in a mouse model using both protein and DNA delivery. He is also involved in the production and screening of protein chips of the proteomes of Plasmodium falciparum, Francisella tularensis, and Burkholderia pseudomallei.

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IV. TEACHING ACTIVITIES

The CVR has a long tradition of being very supportive of the university's teaching mission. Although ORU's do not offer graduate courses and such courses are offered through individual departments, these graduate virology-based courses are all team-taught by faculty who come from various departments, many of who are members of the CVR. The CVR has always functioned as the de facto organization that coordinates this teaching. The CVR proposes to continue in this coordination role. Most members of the CVR are also involved in the combined graduate program Molecular Biology Genetics and Biochemistry (MBGB), as well as participants in the MBGB Virology track program, headed by CVR member Dr. Ed Robinson, Pathology. The CVR has been a supporting unit of this graduate track providing logistical support to the School of Biological Sciences. Accordingly, an NIH funded training grant in Virology (Bert Semler, PI), is administered by the CVR and its relationship to the MBGB combined program is coordinated through the CVR. The Virology trainees are required to participate in graduate virology classes as well as the CVR seminar series and symposia. Thus, the CVR is very closely involved in graduate teaching but not directly responsible for it as this is an unapproved activity of an ORU. Please see Appendix H for a copy of the Virology training grant entitled, "Molecular Biology of Eukaryotic Viruses".

CVR Relationship to Virology Track of Combined Graduate Program

The Virology Track of the Combined Graduate Program in Molecular Biology, Genetics and Biochemistry in the School of Biological Sciences is comprised of faculty, students, postdoctoral fellows, and laboratory staff who have common research and teaching interests in virology and related disciplines. It shares a common core curriculum with all other tracks in the MBG&B graduate program. The research programs of faculty participants include the study of genome replication, viral specific transcription, viral RNA processing, viral translation, viral protein processing, and assembly and transport of viral structural proteins. There are also research efforts aimed at understanding virus-host interactions that include studies of how virus gene products alter and program host functions, alteration of host regulatory molecules, growth control, cell cycle regulation, differentiation control, the role of the innate immune response, the integration specificity of viral genomes, and the subversion of host functions for virus gene expression. The viruses/viral systems being studied include murine leukemia virus, human immunodeficiency virus (HIV), retrotransposons in yeast, poliovirus and human rhinovirus, coronaviruses, papillomavirus, herpes simplex virus, polyomavirus, and adenovirus.

The graduate training program for the Virology Track, which is formally administered by Combined Graduate Program in Molecular Biology, Genetics and Biochemistry in the School of Biological Sciences, includes core elective courses in viral gene expression, molecular pathogenesis of viral infections, and immunopathogenic mechanisms of disease. Students in the School of Biological Sciences Graduate Virology Track also participate in the seminar series sponsored by the Center for Virus Research (described in further detail in the Public Service Section).

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The current offerings of graduate courses in the Virology Track of the Combined Graduate Program in Molecular Biology, Genetics and Biochemistry that the CVR assisted in developing and currently assists in coordinating are as follows:

Virology Track: Course Offerings and Training Activities

(1) Mol Bio 205: Topics in Viral Gene Expression (required course)

The course is taught by Bert Semler, Roz Sandri-Goldin, Luis Villarreal, Suzanne Sandmeyer, and Ed Robinson. It includes lectures by faculty as well as student presentations and discussions of topics involving replication, control of gene expression, and interactions with the host following infections with selected DNA and RNA viruses. These include (among others) small DNA-containing viruses, herpesviruses, picornaviruses, influenza virus, and retroviruses (including HIV).

(2) M&MG 222: Molecular Pathogenesis of Viral Infections (elective course)

Lecturers for the course include: W. Edward Robinson, Rozanne Sandri-Goldin, Bert Semler, Edward Wagner, and Hung Fan

In addition to lectures by faculty, students give presentations and evaluate papers on selected topics.

(3) M&MG 224: Immunopathogenic Mechanisms of Disease (elective course)

Lecturers include Ed Robinson, Jerry Manning, Andrea Tenner, Christopher Hughes, and Michael Selsted

The course is divided between didactic teaching of general principles using predominantly an “experimental design” approach and discussion of timely or especially intriguing works. The latter will require the students to be more active in the course. Grading in the course includes the participation of the students in these interactive sessions. In addition, each student will be required to write a short NIH style proposal on some topic of direct relevance to immunopathogenesis. Grading of that proposal will be performed by two students and one or two faculty acting as a “study section.” The study section will consist of oral discussion/critique of the grant applications.

Workshops

These workshops are offered as a component of the training program in Gene Therapy of Cancer. The workshops are a hands-on demonstration of the use and application of virus-based technology. This training is conducted in the viral vector BSL3 lab in McGaugh Hall and by the staff of the Vector Laboratory for a period of several weeks. These workshops are offered to graduate students, MD/Ph.D. students, and Medical Residents-Fellows here at UCI. In addition, as part of our international training program, these workshops will also be offered in the summer to international visiting scholars and to some of our highly qualified undergraduate students as a component of their participation in international training.

CVR Relationship to Masters Biotechnology Program

In addition to its role in these Ph. D. trainee courses above, the CVR has also been active in supporting the Master’s Biotechnology Program, formally titled the Master’s of

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Science degree in Biological Sciences. The CVR also assisted in the development of this program, which is formally administered through the Department of Molecular Biology and Biochemistry in the School of Biological Sciences. It was a member of the CVR that initially proposed the development of two high level laboratories in the area of virology and immunology that were to be used for the creation of the Master of Science in Biotechnology program. Dr. Villarreal developed the MB&B 124 - Virus Engineering course, which is required for Masters students. CVR member Dr. Tom Lane of MB&B coordinated the development of immunology lab. Recently, Dr. Lane was appointed as the Director of the Master's Biotechnology Program. These two Master's program labs, along with the graduate core classes, make up a basic course requirement for the Masters Biotechnology Program. It should also be noted that both of these labs are also offered to advanced undergraduates and apply the MB&B undergraduate major.

- Advanced Immunology Lab, Molecular Biology 221L, 4 units
- Advanced Virology Lab, Molecular Biology 224, 4 units

CVR Tutorial for Advancement to Candidacy

The CVR has also developed a tutorial program whose purpose is to help CVR members prepare their trainees for advancement to candidacy. This program is a self-paced tutorial that schedules student development and prepares them for their advancement to candidacy exam. The CVR membership realized that the scientific writing and presentation skills that are so essential for Ph. D. student development, and were not being formally developed within the context of the MBGB combined graduate program. Thus the CVR created a self paced tutorial for its members, to be administered prior to the exam, in which the students would be coached for developing their written and oral proposal. The virology track students are the only track that provides such assistance. We feel this model has been relatively successful and will explore incorporating as a formal component into the proposed Ph. D. program in Biotechnology.

Postgraduate Minority Science Training

The CVR also oversees an NIH post-baccalaureate training grant aimed at enhancing minority trainee participation in graduate school. This program, funded in 2000 by NIH entitled, "Post-Baccalaureate Research and Education Program" PREP, supports an average of 10 full time technicians who have finished their baccalaureate degree but have not matriculated to graduate school. These trainees are employees of the CVR and are placed into various participating labs to work as technicians. During this training period, PREP participants are asked to enroll in various courses as UCI employees (mainly graduate courses) as well as participate in an advanced graduate tutorial offered by the CVR. The PREP program makes extensive use of CVR personnel and infrastructure to provide the necessary advanced training. Both the CVR project scientists applied laboratories and member laboratories are utilized (and supported) to provide this highly effective environment for the advanced technical training of the PREP participants. CVR members Dr. Toai Nguyen, Dr. Huw Davies, Dr. David Camerini, Dr. Rozanne Sandri-Goldin, Dr. Paul Gershon, Dr. Hans Ulrich Bernard and Dr. Phil Felgner as well as non-member faculty participate in this training. We feel this has been a highly effective program since most of our trainees do indeed attain the goal of the program by subsequently enrolling in graduate school in the biomedical research field. In summary,

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the CVR has been highly active in post-graduate training, both in course and program development. We propose to continue and to possibly expand this successful training activity. A copy of the Post-Baccalaureate Research and Education Program PREP proposal is provided in Appendix I.

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V. PUBLIC SERVICE

The CVR has a well-established tradition in public service. This has especially been apparent in the context of public communication. The CVR has often been turned to by regional, national and international news agencies with respect to ongoing developments of viral disease and modern molecular technology.

The CVR also participates in various public service ventures with associations such as: UCI Today, Orange County Register, KPRN, KMEX, in order to increase public awareness and educate laypersons regarding virology related topics of emerging infectious diseases.

Seminar Series

As previously mentioned, an extremely successful and highly promoted public service component of the CVR is its prominent seminar series. These presentations are open to the public and exceptionally well attended. Occasionally, the CVR also co-sponsors seminars with other departments and programs (MB&B, MGB, MD-PhD program, MSP). Over the years, the CVR has hosted numerous internationally recognized scientists in this series and has significantly assisted in the promotion and wider recognition of the entire UCI campus. The reputation of the UCI virology based research community has also been promoted by these seminars. A complete listing of the past seminar speakers with corresponding university affiliation and seminar titles are included in Appendix B.

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VI. RESOURCES

Administration

The Director and Administrator have separate offices of approximately 100 sq ft., both of which are negotiated space by the CVR with the Department of Molecular Biology and Biochemistry. Faculty members and staff have available to them either PC or Mac computers, dedicated fax, and phone lines. The CVR Administrator works closely with bookkeeper and personnel analyst in the Department of Molecular Biology and Biochemistry for the personnel and financial maintenance of the ORU administration.

Major Equipment and Research Facilities

The CVR members individual research laboratories range in size from approx. 1,000 to 2,500 sq. ft., and are located in various buildings across campus, including: McGaugh Hall, Medical Sciences I, Steinhaus Hall, Med Surge I and II, Sprague Hall, Hewitt Hall and Natural Sciences I. These labs, which are not formally under the CVR administration, are suitable for research in virology, biochemistry, protein expression and purification, molecular genetics, crystallography, and protein structure computational research. The laboratories are well-equipped for the proposed studies and include liquid scintillation counters, recording spectrophotometers, high speed centrifuges, gel electrophoresis equipment and power supplies, ultra-low freezers, lyophilizers, fraction collectors, UV monitors, shaking and cabinet incubators, microcentrifuges, tissue culture hoods, inverted microscopes, CO₂ incubators, and thermal cycling apparatuses for polymerase chain reactions. The investigators in these laboratories have access to cold rooms, preparative ultracentrifuges and rotors, photographic equipment and dark rooms, and a comprehensive array of chromatographic equipment and reagents. In addition, the UCI campus has core facilities in protein analysis, confocal microscopy and FACS analysis, DNA sequencing and DNA microarrays and mass spectroscopy. Major computer facilities are available on campus with access to individual laboratories via fiber optics, and now wireless, network.

Self-Sustaining Facilities that the CVR Administers and Promotes

CVR currently oversees the following: Viral/Vector facility (a recharge unit), the School of Biological Science BSL3 laboratory, the proteome laboratory for biodefense, and the laboratory of applied immunology. We should note that the space for all of these labs, as well as the space for the administration of the CVR, has come so far only from the School of Biological Science, due to the strong support of the Sue Bryant, the Dean, as well as support from the past and current Chair of the Department of Molecular Biology and Biochemistry. We should also note that future growth of CVR activities is desired and likely, although space for such growth is problematic, especially since CVR activities are not limited to interactions with faculty members in the School of Biological Sciences. For example, the CVR is proposing to assume leadership of a proposed nanomedicine center, which would involve a faculty mainly in the Department of Engineering and the Department of Physical Sciences. If these activities also require resources or applied laboratory development, it would not represent a space that has high priority for the School of Biological Sciences. This issue identifies one of the two major problems of an ORU: that is there is not clear way for an ORU to generate needed space. The other

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major problem discussed below is that lack of administrative support for large grant oversight.

For a detailed, technical/scientific description of these facilities, please refer to Appendix G.

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VII. FUTURE DIRECTIONS

In the near future, the CVR will continue to foster the development of viral proteomics and viral-host proteomics – applied labs, biodefense and emerging diseases. To this aim, the CVR will thus propose and seek interdisciplinary, team-based Center and Program Project grants. Program projects in virology and biodefense as well as in vaccine development, are already being proposed and some have been submitted. The CVR will also seek to become involved, as a core facility, in the recently proposed Regional Center for Excellence in Biodefense and Emerging Infectious Diseases (via PI Alan Barbour, Phil Felgner, core lab director). The same applied laboratories that can function as core facilities for Center grants will also be used as core facilities for this regional center. As a major goal for the next 5 years, the CVR will seek to maximize its successes in whole genome proteomics into the newly developing area of protein-protein interactions on the scale of whole proteomes.

The availability of whole proteome naturally leads us to the next major challenge: understanding how these proteins interact with each other with their host proteins. Such a focus is of interest to the entire CVR membership for it affects numerous issues central to the control of almost all biological processes. This new theme will foster a new set of research interactions within the CVR membership, as well as between CVR members and other researchers both within UCI and other Universities, government and industrial labs. It will also be reflected in the focus of the seminar series and symposia.

Team-Based Research

In addition to our previously stated goals, the CVR's newly identified goals will include greater support for interdisciplinary team-based research and the further development and utilization of applied laboratories. A specific goal for the last two years was the development of Biodefense related, whole proteome based research. This has been successful. Over the next five years and beyond, we propose to develop and focus our collaborations on the study of protein-protein interactions. As our interest is to promote team-based research, in the form of large center grants and contracts, the CVR will need to develop its administrative infrastructure to administer and coordinate these larger, more complex grants. In the first year of continued funding, the CVR will attempt to obtain an NIH funded program project grant in the area of virology and biodefense. The recently developed applied laboratories, as core facilities for the participating teams, will support these center grants. Consistent with this aim, the CVR will also seek to participate in a proposed Regional Center of Excellence in Biodefense and Emerging Disease that will be submitted by Dr. Alan Barbour by providing a core proteome facility.

Cancer Virology

In the last five years, the CVR, working with the Cancer Center, successfully recruited two new faculty members that study cancer virology. We feel we now have good strength in this area and propose to further stimulate the development in that direction. The CVR will continue to promote interactions with CRI and the Comprehensive Cancer Center. Along these lines we will also continue to promote the use of viruses to study Cancer. Our new faculty (Dr. Bernard and Dr. Ruf) will be encouraged to invite seminar

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speakers and develop symposia in which the virology of cancer is explored. We hope to stimulate new interactions that could result in either new research projects or new studies that affect human health. Dr. Ruf is a beginning assistant professor and is still in the stage of establishing her own lab. The CVR will seek to promote her success by stimulating interactions with other UCI researchers and invited seminar speakers. For example, the CVR proteome lab could provide her with valuable resources for the study of EBV. Dr. Bernard is an internationally recognized expert in the study of human papillomavirus and cervical cancer. He is now the leader of the virology program of the Cancer Center. The CVR will seek to further stimulate the international study of cervical cancer. Because cervical cancer is the number one cause of cancer death of woman in Mexico, the CVR hopes to stimulate interactions between virus based cancer research at UCI and cancer research at UNAM, Mexico.

HIV and AIDS

HIV and AIDS represents one of the major health challenges for the entire planet. It has been targeted by the Gates Foundation as a priority disease for which a new vaccine is needed. The most recent addition to the CVR is Dr. Don Forthal, from the Department of Medicine and Chief of the Division of Infectious Disease at the College of Medicine, whose current area of scientific research is in the study of immunity to human immune response to HIV. With this new appointment, we believe that the CVR now has a critical mass of retrovirologists with interest in AIDS (Drs. Robinson, Fan, Forthal, Camerini). In addition, the newly recruited CVR Project Scientist, Dr. Huw Davies (a viral-immunologist), and Dr. Phil Felgner also bring expertise and technical capacity (proteome and applied immunology labs) that could significantly assist a project dedicated to the study of HIV. During the next funding cycle, the CVR will seek to stimulate the research interactions amongst the CVR retrovirologists. We have already hosted a meeting of CVR members in which new concepts for vaccines against HIV were discussed and evaluated. Soon, we will seek to support pilot projects that will attempt to generate reagents or studies that could provide ‘proof of concept’ results. The CVR will seek to stimulate interactions that could lead to new ideas for possible vaccine therapies against HIV.

Herpesvirus

A significant strength of the UCI virologist has always been in the area of herpesvirus research, due to the presence of Edward Wagner and Roz Sandri-Goldin. With the recent recruitment of Dr. Ingrid Ruf, who works on Epstein Bar Virus, a member of the herpes virus family, the CVR has continued to build basic science strength in the field of herpes virology. However, there is now an opportunity to expand the link of the CVR to a clinical department in the School of Medicine. Since human herpesvirus is also a leading cause of serious eye disease (conjunctival dermatitis), the Department of Ophthalmology has recently recruited several new faculty members that study herpesvirus mediated disease. The CVR has established a programmatic linkage with these new faculty members in the College of Medicine and is currently developing therapeutic vaccines for the treatment of HSV (herpes simplex virus) -1 and -2. The high throughput PCR cloning platform has been used to amplify all the ORFS from the HSV-1 and HSV-2 proteomes and clone them into CMV and T7 vectors. Proteome microarrays are used to monitor the

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antibody profiles of individuals without known HSV infection, with recrudescent infections, and individuals with single or low numbers of recurrent infections. The latter are thought to be mounting protective T cell responses. For T cell assay, autologous antigen presenting cells from human donors are transfected with CMV plasmids expressing individual HSV genes and used to restimulate T cells. Responses by the T cells are monitored by gamma-interferon ELISPOTs. These experiments, which are being conducted in collaboration with Dr. Don Forthal (UCI, College of Medicine) and Lbachir Ben Mohamed (Department of Ophthalmology & Center for Immunology) and Dr. David Bloom (University of Florida College of Medicine) will help define an antigen set recognized by HSV-specific T cells appropriate for inclusion in a therapeutic subunit vaccine.

Viral and Pathogen Proteomics

The above plans reflect the continuation or growth of new research programs that have, for the most part, already been initiated at UCI under the CVR leadership. However, the main change for the next five years will be to add an entirely new focus, or emphasis, to the interdisciplinary study of viruses. This focus will now be in the general area of proteomics. We wish to develop interactions based on the study of entire proteomes of viruses and other pathogens. Initially, we will seek to consolidate our gains in this area and implement the several recently awarded research grants in Biodefense. However, since the science and technology of biodefense research is essentially the same as that needed for the study of emerging pathogens, this biodefense focus will also serve to support research in emerging disease (such as SARS, Avian influenza, West Nile Virus). Since we have already established an applied laboratory in proteomics, this facility and technology will be available to stimulate any additional proteome studies. For example, the production of the entire Herpesvirus proteome can now readily be done, and depending on the developing interactions of the herpesvirologists, could serve as a basis of a group research project. A similar proteome-based approach could apply to HIV and papillomavirus based research. In fact, we have already begun making many human papillomavirus proteins for the purposes of evaluating various vaccine strategies. (See below for further details).

Agile Vaccines

Another area that will be developed by the CVR as a consequence of our proteome research is in the area of agile vaccines. Agile vaccines are produced from our rapid proteome technology, and also evaluated very rapidly in the CVR applied immunology laboratory. We have developed and used whole proteome chip technology for this purpose that has been shown to be capable of globally measuring the humoral response to proteins. In addition, the applied immunology laboratory is also evaluating various new technologies that will aid in the effectiveness of vaccine development (adjuvant technology). This capacity to identify potential antigens, produce them and evaluate them for biological outcome has many potential applications to various CVR researchers. Furthermore, agile vaccine research opens another door for CVR interactions. Biotechnology companies are often interested to identify and evaluate potential vaccines. By developing CVR based capacity in vaccine research, we will be well positioned to stimulate research interactions and contracts with industrial labs. The CVR will seek to

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stimulate such interactions with SBIR grants. For example, several SBIR grant submissions to evaluate influenza and smallpox vaccines have recently been submitted with several small companies.

Protein-Protein Interactions

At a recent meeting of the CVR membership, possible scientific themes for the next cycle of the CVR funding were considered and evaluated. We sought to identify new group serving opportunities and to develop issues of common interest to the all the CVR members, as well as the development of new technical capacity that could serve the needs of members. As a consequence of this meeting, the members reached a consensus that the theme of ‘protein-protein’ interactions in the context of proteomics was the most appropriate theme to develop. The interaction of proteins with other proteins constitutes a basic process that is involved in the control of most biological phenomena and relates to the interest of the entire CVR membership. However, there currently exist no coherent way to study or understand how all the proteins of a virus (or cell) interact with all the other viral or cellular proteins. Yet we now know such interactions are crucial control points for the virus and the host. This issue not only relates to the study of all viral systems, it also relates to understanding all host systems. The study of protein-protein interactions also requires the origination of new technology, which should serve as a useful platform for the creation of new resources. Given our recent activity and success in whole proteome technology, we will seek to take advantage of our newly developed whole proteome technical capacity and exploit our own resources. Our initial focus to develop in this area is already clear. With the successful recruitment of Dr. Paul Gershon and the corresponding establishment of the UCI shared resource on protein mass spectroscopy, we now have a major new instrument at UCI (a TOF-TOF mass spec.) that can be developed as a UCI-wide resource for the advanced study of protein-protein interactions. Dr. Gershon’s area of scientific interest is exactly in the study of protein-protein interactions (in the context of vaccinia proteins). When he gets his lab operating, he also want to interface this mass. spec. based technology with a Biacore system that uses light refraction to measure protein-protein interactions. If this Biacore and mass. spec. technology can be effectively linked, we will have established a powerful new technology to study protein-protein interactions in a much higher throughput way. Since essentially all the CVR membership have interest in evaluating protein-protein interactions, as soon as technical issues can be dealt with, a mini-symposium will then be held to develop interactions amongst the CVR membership.

Nuclear Magnetic Resonance (NMR)

It should also be stressed that there are alternative technical approaches that will also be explored. The CVR has worked with Dr. Melanie Cocco of the Department of Molecular Biology and Biochemistry to evaluate the use of high field strength NMR for the study of protein-protein interactions. Although there are some clear limitations to this technology in terms of sizes of proteins, it is a very powerful technology that needs further evaluation. The CVR will be supporting pilot projects in which the proteome based technology derived by Dr. Felgner will be linked to the NMR technology of Dr. Cocco. Another alternative also exists for the study of protein-protein interactions, in the area of nanotechnology, wherein nanodevices could be developed for this purpose. This would

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also include the possible application of microfluidic devices. Along these lines, the CVR has already initiated discussion with D. G. P. Lee, PhD of Engineering to explore the interface of such technology to the study of protein-protein interactions. Working with Dr. Greg Weiss, several prototypical devices have already been made. In addition, working with Dr. James Nowick of the Department of Chemistry, the CVR has submitted a 'white paper' or initial proposal to NIH in which we propose to develop a nanotechnology center for medicine at UCI. The CVR proposes to promote these and other interactions during the next five years.

The CVR has developed a good working relationship with the Institute for Information and Genomic Biotechnology (IGB) application of information technology to modern biological science is now an ingrained and essential feature. In most of our efforts, the CVR membership needs the support of information technology. Many of the recent biodefense grants we have received, including all of our whole proteome work, have formal elements in which a relationship with IGB is supported. Inherent in all of the above proposals will be the need to continue the close working relationship with IGB.

Overview

The CVR has expanded during its initial review to include new applied laboratories and to recruit their directors (senior project scientists). The center has also generated grants and contracts with other research institutions (Department of Defense, Navy Medical Research Institute, United States Army Medical Research Institute for Disease (USAMRID)) as well as private, industrial labs. We feel that this new applied ORU lab situation, with their emphasis on producing deliverables, may help define a new model by which ORU's can generally stimulate the development of intellectual property and via SBIR contracts, promote both the development of and initial funding for new spin-off biotech companies. As indicated above, our initial focus will be to continue our efforts to promote the interdisciplinary study of viruses and the technology associated with them as well as training. Essentially all of the elements and activities of the ORU described herein (seminars, symposia, training, applied labs development, etc.) will be continued. Some of these ongoing activities (Cancer virology, HIV-AIDS study, Herpes research), however, are scheduled for immediate expansion. Other activities will be developed as new initiatives (e.g. viral and pathogen proteomics, protein-protein interaction studies). For the next five years, we propose a new expanded pattern of interaction: CVR interactions with other disciplines such as Engineering and Physical Science, Information and Computer Science and Environmental Science. These collaborations are in the initial, conceptual stages and will be further developed, as interactions are currently ongoing.