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THE STATE UNIVERSITY OF NEW JERSEY
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ROBERT WOOD JOHNSON MEDICAL SCHOOL
University of Medicine & Dentistry of New Jersey

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Director's Overview

As our twentieth anniversary approaches, CABM scientists remain committed to the CABM mission of advancing basic knowledge in the life sciences to improve human health. Original findings discovered in CABM laboratories and published in leading journals are uncovering how molecules interact to regulate growth in organisms from bacteria to humans. These studies are providing new insights and a better understanding of the functional changes that lead to cancer, hereditary disorders, AIDS and other infectious diseases—opening new approaches to prevent and treat devastating illnesses.

One important focus of CABM research is cancer biology. Programmed cell death or apoptosis, a process essential for normal development, fails in tumor cells. CABM investigators are defining the molecular interactions that activate apoptosis in mammalian cells and developing novel ways to restore apoptosis and selectively kill cancer cells. Another CABM group has pioneered a mouse model of human cancer to elucidate the role of specific genes in suppressing prostate cancer progression. Many CABM researchers are also active members of the Cancer Institute of New Jersey, including the Deputy Director and Associate Director for Basic Science. These interactions are facilitating the translation of basic discoveries from the laboratory to the clinic via collaborations with physician-scientists.

Structural biology has been a driving force at CABM since its earliest days. Expert applications of an array of advanced technologies including X-ray crystallography, NMR spectroscopy, mass spectroscopy and the CABM-based Keck molecular modeling facility are providing detailed views of molecular machines that drive key biological processes such as cell signaling and gene expression. The CABM-based, NIH-sponsored Northeast Structural Genomics Consortium (NESG) is using robotic high-throughput techniques to translate human gene sequences into protein function and in the process eliciting potential new drug targets. More effective and less expensive HIV-1 inhibitors, designed and developed through CABM collaborations with pharmaceutical researchers, currently are in clinical trials, and AIDS vaccines based on engineered virus immunogens are showing promise in the laboratory.

Developmental biology is another main thrust at CABM. Stem cell self-renewal and differentiation, pattern formation in early embryogenesis and transcriptional control of nascent sight and hearing systems are being pursued in mouse and tissue models. Molecular mechanisms underlying biological clocks and their possible relationship to sleep are the focus of work by two CABM groups. The recent discovery in a CABM laboratory of a genetic link between a specific human gene and autism in children is stimulating new approaches to this increasing and overwhelming childhood disorder. CABM scientists have deciphered the genetic basis of Batten disease, a rare but fatal neurodegenerative illness in children. This work has provided a firm rationale for disease prevention and the potential for a cure by gene therapy and/or enzyme replacement.

Dedication, outstanding facilities and dependable financial support are all essential to progress in research. CABM is jointly administered by our two sponsoring universities, Rutgers and the University of Medicine and Dentistry of NJ, and we are grateful for their continuing and essential support. In 2005

investigators at CABM were awarded grants and contracts totaling more than $21 million. We thank the many private and public sources for providing this generous support. They include the National Institutes of Health, the Department of Defense, NJ Commission on Cancer Research, NJ Governor's Council on Autism, NJ Commission on Spinal Cord Research, the Howard Hughes Medical Institute, the Ara Parseghian Medical Research Foundation, Batten Disease Support and Research Association, The Cancer Institute of NJ, Foundation of UMDNJ, National Alliance for Autism Research, National Niemann-Pick Disease Foundation and the American Psychological Association. Industrial supporting partners included Bristol-Myers Squibb, Fisher Scientific, Hoffmann-LaRoche, Johnson & Johnson, Merck Research Laboratories, New England Biolabs, Ribapharm Inc., Sanofi-Aventis, Schering-Plough Research Institute and Wyeth Research.

CABM tenure track faculty this year total seventeen with the recent arrival of Assistant Professor of Biochemistry Vikas Nanda from the University of Pennsylvania. In addition to leading their research programs, they are fully engaged in teaching at all levels. More than 130 undergraduate, graduate, medical and postdoctoral students participated this year in CABM laboratories, gaining hands-on training at the scientific frontiers in preparation for positions of leadership in academia and industry. We point with great satisfaction and pride to the many accomplishments of our outstanding graduates as well as our current students. The Annual CABM Retreat in June provides one of many opportunities for them to present their results. Outstanding undergraduates, including many participants in the Johnson & Johnson-sponsored CABM summer research scholars program, are recognized for their scholarly contributions at this event. Another exciting event, the 19th Annual CABM Symposium in October attracted more than 200 participants who listened to talks by leading scientists on the topic, "Stem Cells in Development and Disease". Outstanding speakers also visited to give research updates in our monthly CABM Lecture Series.

This has been an exciting and challenging year. A notable highlight was the selection of CABM by NIH as one of only four sites across the nation for five-year, $50+m funding of a multi-member, large-scale structural proteomics center. This represents a strong vote of confidence in the leadership of the highly successful NESG. Two senior faculty members will be moving their laboratories to New Brunswick to nucleate research in developmental biology at the Child Health Institute of New Jersey, and CABM will continue to recruit jointly with the life sciences departments. The close of our first twenty years will likely be marked by new discoveries in 2006 that will celebrate and energize the start of the third decade of CABM science.

Aaron J. Shatkin, Professor and Director

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Research Programs and Laboratories

Cell & Developmental Biology

Cory Abate-Shen, Ph.D.

Resident Faculty Member, CABM; Professor, UMDNJ Robert Wood Johnson Medical School, Department of Neuroscience and Cell Biology and Department of Medicine; Program Leader, Prostate Program, Cancer Institute of NJ; Chief, Division of Developmental Medicine and Research-Department of Medicine

Molecular Oncology and Development Laboratory

Dr. Cory Abate-Shen joined CABM in August 1991 after spending three years as a postdoctoral and research fellow in Tom Curran's laboratory at the Roche Institute of Molecular Biology. Her work at Roche contributed to characterization of the DNA binding and transcriptional properties of the regulatory proteins Fos and Jun. Dr. Abate-Shen received her Ph.D. from Cornell University Medical College where she was awarded the Vincent du Vigneaud Award for Excellence in Graduate Research. She is a recipient of a Sinsheimer Scholar Award and an NSF Young Investigator Award and received a career recognition award from The American Society for Cell Biology.

Among the numerous genes that control patterning and organogenesis during development, homeobox genes are among the largest and arguably most important family. At least 500 members of this extensive superfamily are present in virtually all eukaryotes, and these regulate a plethora of developmental processes. Although discovered nearly 25 years ago, many fundamental aspects regarding how homeobox genes function during development are still largely unresolved. In particular, it remains a mystery as to how these proteins, which are notorious for their promiscuous DNA binding properties *in vitro*, display highly selective functions *in vivo*. Unraveling the mechanisms by which specific homeoproteins find their selective DNA regulatory elements *in vivo* has been a long-standing research interest of my laboratory. In addition to their functions during development, homeobox genes are often aberrantly expressed in cancer and as such exemplify the critical relationship between development and oncogenesis. Accordingly, research in my laboratory has also focused on understanding how de-regulated homeobox gene expression contributes to carcinogenesis.

How do homeobox genes function as transcriptional regulators during development?

To address this fundamental problem, we have employed as a model system the vertebrate *Msx* genes, which comprise a small, but important, family of homeobox genes because of their relevance for regulating cellular differentiation. Thus, although expressed in many diverse and unrelated tissues during development, a unifying feature of *Msx* gene expression is that in all spatial domains *Msx* expression is inversely correlated with differentiation. Moreover, *Msx1* functions as an inhibitor of differentiation through its ability to repress the expression of genes that control differentiation, such as *MyoD*, a critical regulator of muscle differentiation. Like other homeoproteins, these precise functions of Msx1 as a regulator of differentiation *in vivo* contrast with its rather promiscuous biochemical activities *in vitro*.

Based on the hypothesis that specificity *in vivo* requires interactions with other proteins, we employed several strategies to identify physiologically-relevant protein partners for *Msx1*. In our recently published study, we have found that Msx1 interacts with histone H1b and have shown that this interaction is necessary for Msx1 to regulate the expression of *MyoD* and to inhibit differentiation of myoblast cells. In particular, the interaction of Msx1 with this specific isoform

of histone H1 facilitates binding of this protein complex to a regulatory element of the *MyoD* enhancer, which is necessary for repressing *MyoD* expression in myoblasts cells.

Although an important component of specificity *in vivo*, the interaction of Msx1 with histone H1 cannot solely explain how Msx1 finds its specific target genes *in vivo*. Indeed, in our recent unpublished studies we have found that Msx1 exists in cells as a large multimeric protein complex of approximately 600 kDa. Moreover, we have found that association of Msx1 with this multimeric complex is necessary for its target gene selection *in vivo*. Our research now is focused on identifying the proteins that comprise this complex and deciphering their functions in determining the specificity of Msx1 target gene selection. We believe that our unique approach of employing biochemical approaches in relevant biological scenarios will not only be relevant for understanding homeobox gene function but also be more broadly applicable for other families of transcriptional regulators.

How does the altered expression of homeobox genes contribute to cancer?

To address this issue, we have been studying the vertebrate *Nkx3.1* gene, which is required for prostate differentiation, while its loss-of-function predisposes to prostate cancer. In humans, *NKX3.1* maps to 8p21, which undergoes allelic imbalance in precursor lesions for prostate cancer, called prostatic intraepithelial neoplasia (PIN). We have found that *Nkx3.1* mutant mice develop PIN, mimicking the presumed consequences of 8p21 loss in humans. Our analyses of these *Nkx3.1* mutant mice provide a unique model to understand how the altered expression of homeobox genes may contribute to cancer, as well as to elucidate the mechanisms involved in the earliest stages of prostate carcinogenesis. In our recent unpublished studies, we have found that *Nkx3.1* loss-of-function leads to defects in the expression of differentiation-associated genes, which in turn contributes to cancer predisposition. In particular, *Nkx3.1* mutant mice display altered expression of genes associated with protection against oxidative damage, which ultimately results in impairment of the integrity of the prostatic epithelium. Our findings have implications for understanding the molecular basis for the known sensitivity of the prostatic epithelium to oxidative damage, and also shed light on the specific cell types that give rise to prostate cancer.

Our analyses of *Nkx3.1* mutant mice have also provided the entry point for the generation of a distinctive series of mouse models of prostate cancer, which are based on loss of function of *Nkx3.1* and two other genes whose loss are known to be relevant for human prostate cancer, namely the *Pten* tumor suppressor and the $p27^{kip1}$ cell cycle regulator. Compound mutant mice lacking one or more alleles of *Nkx3.1, Pten,* and/or $p27^{kip1}$ recapitulate the various stages of prostate carcinogenesis, namely PIN, adenocarcinoma, androgen-independent disease and metastases. These compound mutant mice have provided a unique opportunity to survey the molecular mechanisms involved in prostate carcinogenesis. For example, in our recently published study, we uncovered a critical relationship between $p27^{kip1}$ gene dosage and prostate carcinogenesis, which is due to a requirement for elevated levels of *Cyclin D1* for prostate cancer. In addition to providing insights into the molecular pathways of prostate carcinogenesis, these mutant mice provide excellent pre-clinical models for chemoprevention and chemotherapy, which constitutes a major focus of our current studies.

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Isaac Edery, Ph.D.

Resident Faculty Member, CABM; Associate Professor, Rutgers University, Department of Molecular Biology and Biochemistry

Molecular Chronobiology Laboratory

Dr. Isaac Edery completed his doctoral studies in biochemistry as a Royal Canadian Cancer Research Fellow under Dr. Nahum Sonenberg at McGill University in Montreal. His Ph.D. research focused on the role of the eukaryotic mRNA cap structure during protein synthesis and precursor mRNA splicing. Subsequently, he was in the laboratory of Dr. Michael Rosbash at Brandeis University where he pursued postdoctoral studies aimed at understanding the time-keeping mechanism underlying biological clocks. He joined CABM in 1993, and his research is supported by NIH. Dr. Edery is a member of the editorial board of *Chronobiology International*.

A wide range of organisms from bacteria to humans exhibit daily or circadian rhythms in physiological and behavioral phenomena that are controlled by endogenous pacemakers or clocks. Circadian clocks are precisely synchronized to the 24-hr solar day by the daily light-dark and temperature cycles, enabling organisms to perform activities at advantageous times and manifest appropriate seasonal responses. We use the fruit fly *Drosophila* as our model system to understand the cellular and biochemical bases underlying clock function.

The isolation of "clock genes" has provided significant insights into the molecular underpinnings governing circadian rhythms. Tightly controlled oscillations in the levels of key clock proteins are essential for the normal progression of circadian clocks. Core features of many, if not all, circadian clocks are transcriptional feedback loops that generate daily cycles in the levels of clock mRNAs. Despite the centrality of transcriptional feedback loops and cycling mRNA levels in circadian clocks, it is clear that posttranslational regulatory schemes make significant contributions to the rhythm generation of clock protein abundance. Time-of-day specific differences in phosphorylation is emerging as an important regulatory scheme common to circadian clocks that can result in phase-specific changes in clock protein stability, subcellular localization, protein-protein interactions and activity. A strikingly conserved feature of animal clocks is that PERIOD (PER) proteins undergo daily rhythms in phosphorylation and levels that are regulated by casein kinase Iε (CKIε). We recently showed that phosphorylated PER is recognized by the F-box protein β-TrCP/Slimb and targeted for rapid degradation by the ubiquitin/proteasome pathway (UPP). Ongoing work is aimed at better understanding the intersection between clock protein dynamics and the UPP. Abnormal PER phosphorylation is associated with variant human sleep behavior. In addition, PER proteins have a role in cancer and apoptosis. Thus, it is likely that the proposed studies will have broad significance for the understanding of PER function and clock mechanisms in humans. Also, it is anticipated that the proposed studies will provide novel insights into the rules of engagement underlying substrate recognition by F-box proteins. This could be particularly rewarding in the case of β-TrCP/Slimb, which recognizes a variety of phospho-targets and has important roles in development, apoptosis, inflammatory responses and cancer.

We are also interested in understanding how circadian clocks influence seasonal physiological and behavioral responses. Recently, we showed that the splicing efficiency of an intron in the 3'

untranslated region of *per* is thermosensitive. At lower temperatures this intron is spliced more efficiently leading to higher levels of *per* mRNA and mainly daytime activity. By regulating the splicing efficiency of this intron flies are active during the warmer daytime hours during autumn/winter days typical of temperate zones and avoid the midday sun by manifesting mainly nocturnal activity during the warmer spring/summer seasons. Moreover, we identified a novel thermal response for a phospholipase C (PLC/NORPA) previously associated with photic signal transduction. We are continuing these studies to understand how a clock helps measure calendar time by adapting to seasonal changes in temperature and daylength.

Given the similarities in the circadian timing mechanisms operating in *Drosophila* and mammals, we anticipate that our findings will continue to provide important insights into disorders associated with clock malfunction in humans, including manic depression, seasonal affective disorders (SAD or winter depression), chronic sleep problems in the elderly and symptoms associated with trans-meridian flight ("jet-lag") and shift-work.

Publications:

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Céline Gélinas, Ph.D.

Resident Faculty Member, CABM; Professor, UMDNJ-Robert Wood Johnson Medical School, Department of Biochemistry

Tumor Virology Laboratory

Dr. Céline Gélinas came to CABM in September 1988 from the University of Wisconsin where she conducted postdoctoral studies with Nobel laureate Dr. Howard M. Temin on retroviruses and oncogenesis. She earned her Ph.D. at the Université de Sherbrooke in her native country, Canada, and received a number of honors including the Jean-Marie Beauregard Award for Academic and Research Excellence, the National Cancer Institute of Canada King George V Silver Jubilee Postdoctoral Fellowship, a Medical Research Council of Canada Postdoctoral Fellowship and a Basil O'Connor Starter Scholar Research Award. Her work is funded by the NIH. Dr. Gélinas has served as a member of the NIH Experimental Virology Study Section, and she is currently a member of the NIH Study Section on Virology B.

The overall objective of this laboratory is to understand how the Rel/NF-κB transcription factors contribute to the onset and progression of hematopoietic and solid tumors. Rel/NF-κB proteins play fundamental roles in immune and inflammatory responses and are implicated in the control of cell proliferation, apoptosis and oncogenesis. Constitutive Rel/NF-κB activity is a hallmark of many human cancers. This is consistent with the acute oncogenicity of the viral Rel/NF-κB oncoprotein v-Rel that causes fatal leukemia/lymphoma in animal models. Aberrant Rel/NF-κB activity is characteristic of many human leukemia, lymphomas, myelomas and Hodgkin's disease. Chromosomal amplification, rearrangement, overexpression and/or persistent activation of the *rel* and *nf-κB* genes are also observed in breast, colon, lung, ovarian and prostate cancer. The ability of the viral and cellular Rel proteins to transform primary lymphoid cells offers a powerful assay to uncover the mechanisms involved in cancers associated with constitutive Rel/NF-κB activity. Our research focuses on the transcriptional activity and regulation of the Rel/NF-κB factors, on their role in cell growth, apoptosis and oncogenesis, and on the cellular genes that they regulate.

Our prior studies revealed that the transactivation domains (TADs) of Rel/NF-κB factors are critical determinants of their different oncogenic potentials. During the past year, a model has emerged from our analysis of Rel and RelA TAD mutants, highlighting that the direct relationship between the transactivation and transforming potentials of weakly transforming Rel factors evolves into an indirect relationship for Rel and RelA proteins whose transactivation potency exceeds a certain threshold. RT-PCR and microarray analyses identified two gene subsets differentially regulated by proteins harboring Rel vs. RelA TADs, suggesting that substitution or mutations affecting the Rel/NF-κB TADs may influence the repertoire of genes that they activate and the magnitude of their expression. These changes were correlated with their respective capacity to transform primary lymphoid cells. An intriguing observation is that transcriptional repression of a defined gene subset by Rel proteins may be a contributing factor to their transforming activity that is as important as transcription activation. We postulate that the impaired transforming activity associated with strong NF-κB TADs may result from the activation and/or the failure to repress genes whose activity is incompatible with lymphoid cell transformation. Functional studies are ongoing to address this model. Our findings raise the

possibility that defined Rel/NF-κB TAD mutations may promote manifestation of their oncogenic potential. The significance of this model is highlighted by the recent identification of two TAD point mutants that decrease the transcriptional activity of the human c-Rel protein in primary human lymphomas.

Complementary analyses focus on the protein interactions in which Rel/NF-κB TADs engage. Studies identified and characterized transcriptional coactivator CAPERα as a Rel TAD-interacting factor that potently enhances Rel-mediated gene activation, including that of cell death inhibitors Bcl-xL and Bfl-1/A1. The preferential interaction of CAPERα with Rel TADs vs. RelA TADs suggests that it may participate in differential Rel/NF-κB-dependent gene activation and perhaps contribute to Rel's oncogenic activity. Studies to probe this hypothesis are in progress. Since NF-κB complexes containing c-Rel or RelA are a hallmark of many human tumors, analysis of the cellular factors that modulate their transcriptional activity may help to clarify the mechanisms whereby the specificity and potency of Rel/NF-κB's transcriptional function contributes to oncogenesis associated with its persistent activation.

Another facet of our studies involves functional characterization of the Rel/NF-κB-dependent *bfl-1/a1* gene, which encodes an apoptosis inhibitor in the Bcl-2 family. Our studies of the past year revealed that Bfl-1 interacts with the endogenous pro-apoptotic factor Bak and that this interaction may be important for its protective activity. These findings add to the parallel that is emerging between the properties of Bfl-1 and those of another Bcl-2-related cell death inhibitor, Mcl-1. In this regard, we previously uncovered Bfl-1's regulation by the ubiquitin-proteasome pathway and found that a C-terminal deletion mutant of Bfl-1 induced leukemia/lymphoma in nude mice when co-expressed with mutant p53. Ongoing experiments are addressing the extent to which increased resistance to ubiquitin/proteasome-mediate turnover contributes to manifestation of Bfl-1's oncogenic potential.

Collectively, our continued analyses of the Rel/NF-κB TADs, the cellular factors with which they interact and the target genes that they regulate has provided important insights into the mechanisms involved in lymphomagenesis associated with aberrant Rel/NF-κB expression and activity. Since Rel/NF-κB activity is also implicated in many other disease conditions, systematic analysis of the factors that influence Rel/NF-κB activity and the relevant target genes that it controls may be helpful to design novel approaches for therapeutic intervention.

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Fang Liu, Ph.D.

Resident Faculty Member, CABM; Associate Professor, Rutgers University, Susan Lehman Cullman Laboratory for Cancer Research, Department of Chemical Biology, Ernest Mario School of Pharmacy

Growth and Differentiation Control Laboratory

Dr. Fang Liu obtained her B.S. in biochemistry from Beijing University and Ph.D. in biochemistry from Harvard University working with Dr. Michael R. Green. She conducted postdoctoral research with Dr. Joan Massagué at Memorial Sloan-Kettering Cancer Center and joined CABM in 1998. Dr. Liu has received awards from the American Association for Cancer Research-National Foundation for Cancer Research, the Pharmaceutical Research and Manufacturers of America Foundation, the Burroughs Wellcome Fund, and the Sidney Kimmel Foundation for Cancer Research. She also obtained fellowships from the K.C. Wong Education Foundation and the Jane Coffin Childs Memorial Fund for Medical Research.

1. Inhibition of Smad Transcriptional Activity and Antiproliferative Function by CDK Phosphorylation

TGF-ß is the most relevant physiological inhibitor of cell proliferation of a wide variety of cell types. During the earliest stage of tumorigenesis, the ability of TGF-ß to inhibit cell growth enables it to act as a potent tumor suppressor. TGF-ß inhibits cell proliferation by causing cell cycle arrest at the G1 phase. SMAD proteins are candidate tumor suppressors and can mediate the TGF-ß growth-inhibitory effects by regulating the expression of several cell cycle-related genes. We have discovered that Smad3 and Smad2 are phosphorylated by CDK4 and CDK2. We have carried out extensive studies on CDK phosphorylation of Smad3, which plays a critical role in the antiproliferative effects of TGF-ß. We demonstrate that Smad3 is a physiological substrate for CDK4 and CDK2. Except for the Rb family members, Smad3 is the only demonstrated substrate for CDK4. We have mapped both CDK4 and CDK2 phosphorylation sites *in vivo* and *in vitro*. Mutation of the CDK phosphorylation sites in Smad3 results in a higher transcriptional activity in the activation of p15 and also a higher activity to downregulate c-myc. Using Smad3$^{-/-}$ primary mouse embryonic fibroblasts and other epithelial cell lines, we further show that Smad3 inhibits cell proliferation from the G1 to S phase, and mutation of CDK phosphorylation sites augments this inhibitory function. These observations highly suggest that CDK phosphorylation of Smads inhibits their transcriptional activities and antiproliferative functions. Our findings have important implications in the understanding of TGF-ß resistance in cancers. Resistance to the TGF-ß growth-inhibitory effects is a necessary event in malignant transformation. Accordingly, over 90% of cancers have lost TGF-ß growth-inhibitory responses. Genetic inactivation of the TGF-ß receptor or Smad genes occurs at a relatively low frequency (~10%) and therefore cannot explain the broad loss of TGF-ß sensitivity in cancer cells. Tumor cells often contain high levels of CDK4 and CDK2 activities due to frequent amplification, translocation or overexpression of the cyclin D1 gene or inactivation of the tumor suppressor p16. In addition, overexpression of cyclin E and reduction of CDK inhibitor p27 levels also occur in cancer cells. Since Smad proteins are very good substrates for G1 CDKs, inactivation of Smad proteins by CDK phosphorylation may provide an important mechanism for TGF-ß resistance in cancers.

2. Identification and characterization of ERK MAP kinase phosphorylation sites in Smad3
The Smad3 linker region contains a total of four proline-directed kinase phosphorylation sites (T178, S203, S207 and S212). As described above, we have mapped the CDK4 and CDK2 phosphorylation sites to the T8, T178 and S212 in Smad3. Previous studies showed that Smad3 can also be phosphorylated by ERK MAP kinase. It was shown that mutation of the four proline-directed kinase phosphorylation sites in the Smd3 linker greatly reduced phosphorylation by ERK both *in vivo* and *in vitro*. The exact phosphorylation sites, however, were not mapped. We have identified and characterized ERK phosphorylation sites in Smad3. We show that EGF treatment induces a rapid phosphorylation of S207, S203 and T178 in Smad3. These sites can also be phosphorylated by ERK2 *in vitro*. Among the ERK phosphorylation sites in Smad3, the T178 site is also phosphorylated by CDK4 and CDK2 both *in vivo* and *in vitro*. The S207 is the best site for ERK in Smad3. Its phosphorylation is greatly induced by EGF treatment, and it is the most sensitive site in Smad3, responding to low doses of EGF. Mutation of the ERK phosphorylation sites to non-phosphorylatable residues does not appear to affect Smad3 subcellular localization. In contrast, mutation of the ERK phosphorylation sites increases Smad3 activity to stimulate a TGF-ß/Smad target gene, suggesting that ERK phosphorylation inhibits Smad3 activity.

3. The Smad3 linker region contains a transcriptional activation domain
Smad3 plays a key role in mediating the antiproliferative function of TGF-ß. We have shown that both CDK4 and CDK2 phosphorylate Smad3 and inhibit its transcriptional activity and antiproliferative function. We have mapped the CDK phosphorylation sites to Thr 8 (T8), Thr 178 (T178), and Ser 212 (S212). Two of the three CDK phosphorylation sites (T178 and S212) are located in the proline-rich linker region of Smad3. To provide insights into how CDK phosphorylation of Smad3 inhibits its transcriptional activity and antiproliferative function, we analyzed whether the Smad3 linker region contains a transcriptional activation domain. We found that indeed the linker region contains a transcriptional activation domain. When the linker region is fused to a heterologous DNA binding domain, the GAL4 DNA binding domain, it activates transcription from a reporter gene driven by the GAL4 binding sites. The transcriptional activity of the Smad3 linker region is constitutive, independent of TGF-ß, similar to the Smad activation domain (SAD) that is present in the proline-rich linker region of Smad4. We further show that the Smad3 linker region can physically interact with p300. The transcriptional activity of the Smad3 linker region is inhibited by E1a, which associates with p300. Overexpression of E1a can significantly but not completely rescue E1a-mediated repression. In contrast, a mutant E1a, which cannot interact with p300, has little effect on the transcriptional activity of the linker region. Taken together, these results indicate that p300 is important for the transcriptional activity of the Smad3 linker region, and that additional components that are yet to be identified may also contribute to the Smad3 linker transcriptional activity. We further show that the Smad3 linker region is also necessary to support TGF-ß-mediated transcriptional activation. A native Smad3 lacking the linker region can be activated by the TGF-ß receptor and has an increased capacity to interact with Smad4. However, it cannot support TGF-ß-mediated transcriptional activation responses. Thus, the Smad3 linker region has an important function in Smads-mediated transcriptional control.

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Peter Lobel, Ph.D.

Resident Faculty Member, CABM; Professor, UMDNJ-Robert Wood Johnson Medical School, Department of Pharmacology

Protein Targeting Laboratory

Dr. Peter Lobel trained at Columbia University and Washington University in St. Louis and joined CABM in 1989. He is currently conducting research on lysosomes and associated human hereditary metabolic diseases. This work resulted in identification of three disease genes that cause fatal neurodegenerative disorders. At CABM he was the first faculty member of UMDNJ to be named a Searle Scholar, a prestigious award given to newly appointed faculty members who show outstanding promise in biomedical research.

Our laboratory has developed new methods for disease discovery and identified the molecular bases for three fatal neurodegenerative disorders based on our research on lysosomal enzyme targeting. Lysosomes are membrane-bound, acidic organelles that are found in all eukaryotic cells. They contain a variety of different proteases, glycosidases, lipases, phosphatases, nucleases and other hydrolytic enzymes, most of which are delivered to the lysosome by the mannose 6-phosphate targeting system. In this pathway, lysosomal enzymes are recognized as different from other glycoproteins and are selectively phosphorylated on mannose residues. The mannose 6-phosphate serves as a recognition marker that allows the enzymes to bind mannose 6-phosphate receptor which ferries the lysosomal enzyme to the lysosome. In the lysosome, the enzymes function in concert to break down complex biological macromolecules into simple components. The importance of these enzymes is underscored by the identification of over thirty lysosomal storage disorders (e.g., Tay Sach's disease) where loss of a single lysosomal enzyme leads to severe health problems including neurodegeneration, progressive mental retardation and early death. Our approach to identify the molecular basis for unsolved lysosomal storage disorders is based on our ability to use mannose 6-phosphate receptor derivatives to visualize and purify mannose 6-phosphate containing lysosomal enzymes. For instance, we can fractionate proteins in normal and disease specimens by 2-dimensional gel eletecrophoresis and then, in a manner analogous to Western blotting, use a radiolabeled mannose 6-phosphate receptor derivative to selectively visualize phosphorylated lysosomal enzymes. This allows us to compare the spectrum of lysosomal enzymes present in normal and disease specimens. If the disease specimen lacks a given lysosomal protein, this may be responsible for disease. To investigate this, we purify and sequence the normal protein, clone the corresponding gene, and examine patients for mutations associated with disease. In this manner, we found that a fatal childhood neurodegenerative disease called LINCL (late infantile neuronal ceroid lipofuscinosis) is caused by mutations in a gene encoding a previously undiscovered lysosomal protease.

After we identified the gene and determined the function of the corresponding protein, we developed rapid biochemical and DNA-based assays for definitive pre-and postnatal diagnosis and carrier screening. This allows for genetic counseling to prevent further occurrence of the disease.

However, in the absence of universal carrier testing, new cases will continue to arise so it is important to develop effective therapies that can halt and reverse disease progression. To this

end, we have produced recombinant enzyme in a form that can be taken up by affected cells in culture to correct the primary defect. We have also developed a LINCL mouse model and, in collaboration with the Genzyme Corporation, are using it to study disease pathophysiology and to evaluate potential therapeutics strategies.

Another research program in the laboratory is to identify the spectrum of lysosomal enzymes encoded by the human genome. This research is particularly timely given the current effort towards determining the complete sequence of the human genome. Our approach is to purify mannose 6-phosphorylated proteins and then analyze each protein by peptide mapping, mass spectrometry and chemical sequencing. This information is used to search sequence databases to determine if a given protein corresponds to a known lysosomal enzyme or if it represents a previously unidentified species. We are in the process of completing our characterization of the mannose 6-phosphate glycoproteome from human brain and plasma and are extending this research to characterize new proteins and additional proteomes.

One recent application of our proteomic research was to determine the molecular basis for Niemann Pick type C2 (NPC2) disease, a fatal cholesterol storage disorder. In collaboration with Dr. Ann Stock's laboratory, we are working to understand the structural basis for cholesterol binding of the NPC2 protein. We have also created a NPC2 mouse model and are using it to investigate the biological function of NPC2 in lysosomal cholesterol transport.

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James H. Millonig, Ph.D.

Resident Faculty Member, CABM; Assistant Professor, UMDNJ-Robert Wood Johnson Medical School, Department of Neuroscience and Cell Biology; Adjunct Assistant Professor, Rutgers University, Department of Genetics

Developmental Neurogenetics Laboratory

Dr. James H. Millonig came to CABM in September 1999 from the Rockefeller University where he was a postdoctoral fellow in the laboratory of Dr. Mary E. Hatten. His postdoctoral research combined neurobiology and mouse genetics to characterize and clone the *dreher* mouse locus. He did his doctoral research at Princeton University with Dr. Shirley M. Tilghman. Dr. Millonig is a recipient of the March of Dimes Basil O'Connor Starter Research Award and grants from the National Institutes of Health, National Alliance for Autism Research, N.J. Governor's Council on Autism and New Jersey Commission on Spinal Cord Research.

My laboratory uses human and mouse genetics to study the genetic and developmental basis of human disorders such as autism, congenital cataracts and neural tube defects (NTDs) like spina bifida.

***ENGRAILED 2* (*EN2*) is an Autism Spectrum Disorder (ASD) susceptibility locus.**
Individuals diagnosed with ASD exhibit deficiencies in communication and reciprocal social interactions that are accompanied by rigid or repetitive interests and behaviors. Post-mortem and neuroimaging studies are consistent with ASD being a neurodevelopmental disorder. Twin, family and disease modeling studies have demonstrated that ASD is a polygenic disorder that is likely to be a result of multiple genes interacting with each other and unknown environmental factors.

One of the CNS structures consistently affected in individuals with autism is the cerebellum. Twenty four out of 29 post-mortem studies have demonstrated a decrease in the number of Purkinje cells, a type of cerebellar neuron. This decrease in Purkinje cell number occurs mostly in the absence of any obvious signs of degeneration, suggesting that the phenotype is due to a developmental defect. Neuroimaging studies have also revealed cerebellar defects. Eighteen separate studies from eight different research groups have demonstrated that the cerebellum is often hypoplastic, making this abnormality another consistent morphological defect observed in autistic individuals. Although the cerebellum is classically thought to control only motor coordination, functional imaging studies have demonstrated that the cerebellum is also active during tasks that are defective in ASD including language and attention. Thus, the anatomical defects reported for the cerebellum could directly contribute to some of the behavioral phenotypes associated with ASD.

My laboratory, in collaboration with Linda Brzustowicz's group at Rutgers University, has demonstrated that the homeobox transcription factor, *ENGRAILED 2* (*EN2*), is associated with ASD using family-based linkage disequilibrium methods (Gharani et al., 2004; Benayed et al., 2005). Our initial studies using 167 nuclear pedigrees from the Autism Genetic Resource Exchange (AGRE I dataset) demonstrated significant association for two intronic SNPs (*rs1861972* and *rs1861973*) both individually and as a haplotype (*rs1861972- rs1861973* haplotype: narrow: P= 0.0009 broad: P=0.0024)(Gharani et al., 2004).

Significant replication for *rs1861972* and *rs1861973* has been observed in two additional datasets: another 222 AGRE families (*rs1861972- rs1861973* haplotype: *P*=0.00185) and a separate sample of 129 NIMH families (*rs1861972- rs1861973* haplotype: *P*=0.043). Additional evidence of association was obtained when the data were combined and analyzed from both AGRE datasets (389 families) (haplotype: *P*=0.0000043) or from all three datasets (518 families) (haplotype: *P*=0.000000427). Population Attributable Risk (PAR) calculations for the associated haplotype using the entire sample of 518 families determined that the risk allele contributes to as much as 40% of ASD cases in the general population (Benayed et al., 2005).
These data represent one of the more significant associations of any gene with ASD. Furthermore, *EN2* is the one of the few ASD associated gene in which statistical significant association was observed for the same genetic allele in the replication datasets. These genetic data are consistent with *EN2* encoding an ASD susceptibility locus (OMIM #131310).

To identify polymorphisms that are inherited in a manner consistent with them being the risk allele, 18 additional polymorphisms have been tested for association in the AGRE I dataset. None of these polymorphisms are consistently associated, indicating that they are not the risk allele. Linkage disequilibrium (LD) mapping studies have demonstrated that only the other intronic SNPs are in strong LD with the associated SNPs, *rs1861972* and *rs1861973*, suggesting that the risk allele maps to the intron. Further association and re-sequencing analysis have identified only *rs1861972* and *rs1861973* as being associated with ASD, identifying them as candidate risk alleles. Ongoing experiments are investigating whether these SNPs functionally alter the expression of *EN2* (Benayed et al., 2005).

Further supporting our *EN2* association results are mouse genetic experiments by collaborators at UMDNJ-RWJMS and Rutgers. To investigate whether misexpression of *En2* could cause a neuronal phenotype, we have misexpressed *En2* in primary cortical cultures in collaboration with Emanuel DiCicco-Bloom's group in the Department of Neuroscience and Cell Biology at UMDNJ-RWJMS. *En2* misexpression reduced the number of neurons displaying a differentiated phenotype as determined by morphology and marker expression, indicating that the misexpression of *EN2* during CNS development could have phenotypic consequences (Benayed et al., 2005).

Considering our human genetic results, we have investigated the *En2* mouse knockout for behavioral phenotypes in collaboration with George Wagner PhD (Rutgers University). Behavioral abnormalities have been observed including reduced play behavior, allogrooming and sniffing. The *En2* mutants also display deficiencies in spatial memory tasks. Neurochemical analysis of different brain structures has uncovered a consistent increase in serotonin levels specifically in the cerebellum. These neurochemical results are intriguing considering that previous physiological, pharmacological and genetic data have implicated the serotonin pathway in ASD (Cheh, Millonig et al., submitted).
Altogether these experiments are consistent with *EN2* acting as an ASD susceptibility locus. Future experiments are focused on identifying the disease allele and generating a mouse model of *EN2* ASD susceptibility so that developmental and behavioral analyses can be performed on a genetically relevant mouse model.

***Vacuolin*, an orphan GPCR, important for neural tube closure and lens development.**
To understand the genetic and developmental basis of human NTDs and congenital cataracts, the lab has been studying the spontaneous mouse mutant called *vacuolated lens* (*vl*). A single allele of the *vacuolated lens* mutation has arisen on the *C3H/HeSnJ* inbred background. *Vl* homozygotes display congenital cataracts or spina bifida.

In collaboration with Dr. Bev Paigen at The Jackson Laboratories, we have used genetic crosses to positionally clone the *vl* locus. An 8 base pair deletion has been identified in an orphan G protein coupled receptor (GPCR) that results in a frame shift and early termination of the protein. This 8 base pair deletion is not observed in 23 other inbred strains indicating that it is not a polymorphism. In addition, *in situ* hybridizations by us have demonstrated that the orphan GPCR is expressed in the developing spinal cord and eye, which is consistent with its mutant phenotypes. For these reasons, we conclude that this orphan GPCR is responsible for the *vl* mutation, and in accordance with mouse and human nomenclature, we are calling this orphan GPCR, *vacuolin*. We have also investigated the effect of the *vl* mutation on *vacuolin* expression, function and subcellular localization as well as the effect of the mutation on lens and spinal cord development. Future analysis will investigate whether human *VACUOLIN* is associated with NTDs.

Because the cloning of *vacuolin* has identified a novel receptor important for normal spinal cord and lens development, it is important to identify other genes in the pathway. When the *vl* mutation has been crossed onto different genetic backgrounds, including *C57BL6/J*, *Mus castaneus* (*CAST/Ei*) and *Mus. molossinus* (*MOLF/Ei*), approximately 50% of the *vl/vl* embryos display no phenotype. This result indicates that unlinked genetic modifiers on these backgrounds can suppress the associated *vl* mutant phenotypes, indicating that they are likely to function in the *vacuolin* pathway.

We have mapped 5 different *vl* modifier loci on these different genetic backgrounds. Our future goals will be to positionally clone these modifier loci as a means of further understanding the *vacuolin* pathway and identifying other genes that might contribute to human NTDs and congenital cataracts.

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Michael M. Shen, Ph.D.

Resident Faculty Member, CABM; Professor, UMDNJ-Robert Wood Johnson Medical School, Department of Pediatrics; Chief, Division of Developmental Biology-Department of Pediatrics

Mammalian Embryogenesis Laboratory

Dr. Michael Shen completed his doctoral work in genetics under Dr. Jonathan Hodgkin at the MRC laboratory of Molecular Biology in Cambridge, England. He then performed postdoctoral work at Harvard Medical School under Dr. Philip Leder before joining CABM in 1994. He is a former recipient of a Leukemia Society of America Special Fellowship, a Howard Hughes Medical Institute Postdoctoral Fellowship, a Jane Coffin Childs Postdoctoral Fellowship and a National Science Foundation Graduate Fellowship. Dr. Shen is a former member of the NIH *Development-2* Study Section and is currently an Instructor for the Cold Spring Harbor Laboratory course *Molecular Embryology of the Mouse*.

The Shen laboratory investigates the molecular mechanisms involved in pattern formation and organogenesis during vertebrate development. In particular, the lab is examining *(i)* pattern formation during pre-gastrulation and gastrulation stages of embryogenesis, *(ii)* regulation of transforming growth factor-beta (TGFβ) signaling in early mouse embryogenesis, and *(iii)* prostate development and cancer in mouse model systems. These studies primarily utilize experimental approaches involving genetically-engineered mice but also employ cell culture and biochemical approaches to investigate molecular mechanisms. Finally, the laboratory has initiated new projects on the molecular regulation of embryonic stem cell self-renewal and differentiation, as well as on the identification and molecular analysis of prostate epithelial stem cells.

To address the first two areas, we have been investigating the signaling pathway and *in vivo* functions of Nodal, a member of the Transforming Growth Factor-beta (TGFβ) family that is essential for multiple critical processes in early vertebrate development. Our studies of the molecular mechanisms of pattern formation during development have focused on understanding the regulation of Nodal signaling at the extracellular level. In particular, members of the *EGF-CFC* family encode glycosyl-phosphatidylinositol (GPI) linked proteins that are essential for activity of Nodal. Our earlier studies have demonstrated that the *EGF-CFC* gene *Cripto* is required for correct orientation of the anterior-posterior (A-P) axis in the pre-gastrulation mouse embryo, whereas *Cryptic* is required for left-right specification. Furthermore, using cell culture and biochemical approaches, we have shown the ability of EGF-CFC proteins to act as co-receptors for Nodal, as well as the activity of Lefty proteins as soluble Nodal inhibitors.

While genetic and biochemical studies have indicated that EGF-CFC proteins function as cell-autonomous co-receptors for Nodal, our cell culture data have also suggested that the mammalian EGF-CFC protein Cripto can act as a secreted signaling factor. We have recently shown that Cripto acts non-cell-autonomously during axial mesendoderm formation in the mouse embryo and may possess intercellular signaling activity *in vivo*. Phenotypic analysis of hypomorphic mutants demonstrates that *Cripto* is essential for formation of the notochordal plate, prechordal mesoderm, and foregut endoderm during gastrulation. Remarkably, *Cripto* null mutant cells readily contribute to these tissues in chimeras, indicating non-cell-autonomy.

Consistent with these loss-of-function analyses, gain-of-function experiments in chick embryos show that exposure of node/head process mesoderm to soluble Cripto protein results in alterations in cell fates towards anterior mesendoderm in a manner that is dependent on Nodal signaling. These findings support a model in which Cripto can function *in trans* as an intercellular mediator of Nodal signaling activity.

In a third major area of interest, we are investigating mouse models of prostate development and cancer (in collaboration with Dr. Cory Abate-Shen's lab). Our mouse models have been primarily based on the combined loss-of-function of the homeobox gene *Nkx3.1* and the tumor suppressor gene *Pten*, both of which are known to play central roles in human prostate cancer. In previous studies, we had shown that our *Nkx3.1; Pten* mouse models could develop a spectrum of pre-cancerous lesions that resemble human prostatic intraepithelial neoplasia (PIN). We have now investigated the malignant potential of the high-grade PIN lesions that form in *Nkx3.1; Pten* compound mutant mice and have demonstrated their neoplastic progression in a serial transplantation/tissue recombination assay. Furthermore, we have found that a majority of *Nkx3.1; Pten* mice greater than one year of age develop invasive adenocarcinoma, which is frequently accompanied by metastases to lymph nodes. Finally, we have observed androgen-independence of high-grade PIN lesions following androgen ablation of *Nkx3.1; Pten* mice. These results indicate that *Nkx3.1; Pten* mice recapitulate key features of advanced prostate cancer and represent a useful model for investigating associated molecular mechanisms and for evaluating therapeutic approaches.

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Eileen White, Ph.D.

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Viral Transformation Laboratory

Dr. Eileen White transferred her research program to CABM in July 1990 from Cold Spring Harbor Laboratory where she was a staff investigator. She conducted postdoctoral research at the Cold Spring Harbor laboratory as a Damon Runyon–Walter Winchell Fellow, working with Dr. Bruce Stillman. Her work is currently funded by a MERIT Award from NIH and Howard Hughes Medical Institute. Dr. White is a member of the National Cancer Institute Board of Scientific Counselors.

Primary epithelial cells become transformed as a result of the combined action of the deregulation of cell growth control and inhibition of programmed cell death (apoptosis). Expression of the human adenovirus E1A oncogene releases normal restrictions on cell cycle progression through interactions with the retinoblastoma tumor suppressor protein and its relatives, and with the p300 and CBP transcriptional co-activators. The cellular response to this deregulation of cell growth control by E1A is the stabilization of the p53 tumor suppressor protein and the induction of p53-dependent apoptosis of transformed cells and p53-independent apoptosis in virus infected cells. The activation of this apoptotic program prevents transformation and limits virus replication. The adenonvirus E1B oncogene encodes E1B 19K, an apoptosis inhibitor which thereby sustains transformation and productive infection. E1B 19K is a viral homologue of cellular BCL-2, and its expression blocks apoptosis induced by E1A and death receptor signaling during infection, and by p53 during transformation. Furthermore, inhibition of p53-independent apoptosis by E1B 19K or BCL-2 facilitates tumorigenesis in vivo, and defects in apoptosis confer resistance to chemotherapy. Therefore, determining the mechanisms of apoptosis regulation is essential for understanding tumorigenesis and for developing successful treatments.

E1B 19K functions as a general apoptosis inhibitor by binding to and inhibiting pro-apoptotic BCL-2 family members BAX and BAK that propagate cell death signaling through mitochondria. The E1B 19K protein blocks apoptosis by inhibiting BAK and a specific form of BAX in mitochondria, thereby preventing the release of pro-apoptotic mitochondrial proteins, caspase activation and apoptosis. Since either BAX or BAK is essential for apoptosis, the interaction between E1B 19K and both BAX and BAK is required to inhibit their oligomerization and the release of proteins from mitochondria which promote caspase activation and cell death. Inhibition of BAX and BAK oligomerization by E1B 19K bears striking similarity to the means by which bacterial immunity proteins block pore formation by bacterial toxins, which have structural homology to BAX and BAK. The long-term focus of the White laboratory has been to determine the mechanism by which oncogenes and tumor suppressor genes regulate apoptosis, and the role of apoptosis in infection and oncogenesis. As deregulation of apoptosis is a common feature of many disease states, knowledge gained by this pursuit should provide new opportunities for the development of novel therapies, particularly for cancer treatment.

Caspase-dependent Processing Activates the Pro-Apoptotic Activity of Deleted in Breast Cancer-1 During Tumor Necrosis Factor-alpha-Mediated Death Signaling

Deleted in breast cancer-1 (DBC-1) was initially cloned from a homozygously deleted region in breast and other cancers on human chromosome 8p21, although no function is known for the protein product it encodes. We identified the generation of amino-terminally truncated versions of DBC-1 during tumor necrosis factor (TNF)-α-mediated apoptosis. Full-length 150 kDa DBC-1 underwent caspase-dependent processing during TNF-α-mediated death signaling, to produce p120 DBC-1 and p66 DBC-1 carboxy-terminal fragments. Endogenous DBC-1 localized to the nucleus in healthy cells but localized to the cytoplasm during TNF-α-mediated apoptosis, consistent with the loss of the amino-terminus containing the nuclear localization signal. Overexpression of an amino-terminal truncated DBC-1, resembling p120 DBC-1, caused mitochondrial clustering, mitochondrial matrix condensation, and sensitized cells to TNF-α-mediated apoptosis. The carboxy-terminal coiled-coil domain of DBC-1 was responsible for the cytoplasmic and mitochondrial localization, and for the death promoting activity of DBC-1. Thus, caspase-dependent processing of DBC-1 may act as a feed-forward mechanism to promote apoptosis and possibly also tumor suppression. DBC-1, like its homolog cell cycle and apoptosis regulatory protein-1 (CARP-1), may function in the regulation of apoptosis.

Key Roles of BIM-driven Apoptosis in Epithelial Tumors and Rational Chemotherapy

Defective apoptosis not only promotes tumorigenesis but also can confound chemotherapeutic response. Here we demonstrate that the proapoptotic BH3-only protein BIM is a tumor suppressor in epithelial solid tumors and also is a determinant in paclitaxel sensitivity in vivo. Furthermore, the H-ras/mitogen-activated protein kinase (MAPK) pathway conferred resistance to paclitaxel that was dependent on functional inactivation of BIM. Whereas paclitaxel induced BIM accumulation and BIM-dependent apoptosis *in vitro* and in tumors *in vivo*, the H-ras/MAPK pathway suppressed this BIM induction by phosphorylating BIM and targeting BIM for degradation in proteasomes. The proteasome inhibitor Velcade (P-341, Bortezomib) restored BIM induction, abrogated H-ras-dependent protection against paclitaxel, and promoted BIM-dependent tumor regression, suggesting the potential benefits of combinatorial chemotherapy of Velcade and paclitaxel.

Tumorigenesis results in the acquisition of mutations that promote tumor growth and chemoresistance, and relating tumor genotype to prognostic indications and to effective treatment regimens is essential for successful therapeutic outcome. Determining the mechanism of apoptosis induction by the chemotherapeutic drug paclitaxel revealed that BIM suppressed tumorigenesis and was required for paclitaxel responsiveness. The targeting of BIM for degradation in proteasomes by the H-ras/MAPK pathway was the molecular basis for paclitaxel resistance in tumors with activating mutations in RAS, and paclitaxel responsiveness was restored by joint administration of the proteasome inhibitor Velcade. Thus rational combinatorial chemotherapy using proteasome inhibitors to enhance chemosensitivity to paclitaxel in tumors where the H-ras/MAPK pathway is activated may be therapeutically beneficial.

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Julie A. Williams, Ph.D.

Resident Faculty Member, CABM; Assistant Professor, UMDNJ-Robert Wood Johnson Medical School, Department of Pharmacology

Sleep Genetics Laboratory

Dr. Julie Williams came to CABM in October 2004 from the University of Pennsylvania where she conducted postdoctoral studies with Dr. Amita Sehgal. Her research was aimed at understanding circadian and homeostatic mechanisms of sleep in Drosophila. She completed her Ph.D. in Neuroscience at the University of British Columbia in Vancouver Canada where she received honors for her research focused on the brainstem mechanisms of sleep. Subsequently, she was awarded a Pickwick Fellowship from the National Sleep Foundation to pursue postdoctoral studies in circadian rhythms with Dr. Steven Reppert at Harvard University. Her current work is funded in part by a grant from the UMDNJ Foundation.

Sleep is controlled by two processes. One process is homeostatic, which determines *how much* sleep occurs depending on the length of time spent awake. The other process is circadian, which determines *when* or what time of day sleep occurs. While the molecular basis of circadian clocks is well understood, little is known about the molecular components of the sleep homeostatic system. Our approach to this challenging problem is to investigate the mechanisms of sleep in a model organism, *Drosophila melanogaster*. Many features of *Drosophila* cell biology are highly conserved and have historically provided valuable information that is directly relevant to humans. Some examples include advances in cancer biology and circadian rhythms. Our goal is to use this model organism to make similar advances in the field of sleep research.

To identify molecular candidates of sleep homeostasis, we performed a genome wide microarray analysis of changes in gene expression associated with sleep deprivation in flies. Genes involved in immune function constituted a major component of those affected by sleep deprivation. While some molecules, including those involved in inflammatory/immune responses, have been implicated in the control of sleep, their role in this process remains unclear. We further demonstrated that acute sleep deprivation augments the immune response in flies such that they become more resistant to bacterial infection. Immune-related mutants also show defects in baseline levels of sleep as well as in responses to sleep deprivation. We are currently investigating the mechanisms underlying these effects using behavioral, genetic, tissue culture, and *in vivo* monitoring strategies.

Sleep disturbances and fatigue are known to be associated with a wide range of illnesses in humans, including diseases such as cancer and diabetes. Delineating the underlying mechanisms that control the interaction between sleep and immunity may identify therapeutic targets for treating sleep disorders as well as disturbances or fatigue associated with cancer and other diseases.

Mengqing Xiang, Ph.D.

Resident Faculty Member, CABM; Associate Professor, UMDNJ-Robert Wood Johnson Medical School, Department of Pediatrics

Molecular Neurodevelopment Laboratory

Dr. Mengqing Xiang came to CABM in September 1996 from the Johns Hopkins University School of Medicine where he conducted postdoctoral studies with Dr. Jeremy Nathans. He earned his Ph.D. at the University of Texas M.D. Anderson Cancer Center and has received a number of honors including a China-U.S. Government Graduate Study Fellowship, a Howard Hughes Medical Institute Postdoctoral Fellowship, a Basil O'Connor Starter Scholar Research Award and a Sinsheimer Scholar Award. His work is currently supported by NIH and the New Jersey Commission on Spinal Cord Research.

Our laboratory investigates the molecular mechanisms that govern the determination and differentiation of highly specialized sensory cells and neurons. We employ molecular genetic approaches to identify and study transcription factors that are required for programming development of the retina, inner ear and somatosensory ganglia. A major focus of our work is to develop animal models to study roles of transcription factor genes in normal sensorineural development, as well as to elucidate how mutations in these genes cause sensorineural disorders such as blindness and deafness.

Role of Foxn4 in the specification of spinal sensory interneurons. Neuronal subtype diversification is essential for the establishment of functional neural circuits, and yet the molecular events underlying neuronal diversity remain largely to be defined. During spinal neurogenesis, the p2 progenitor domain, unlike others in the ventral spinal cord, gives rise to two intermingled but molecularly distinct subtypes of interneurons, termed V2a and V2b. In a collaborative study with Michael Matise's laboratory, we have demonstrated that the Foxn4 winged helix/forkhead transcription factor is co-expressed with the Mash1 basic helix-loop-helix transcription factor in a subset of p2 progenitor cells. Loss of *Foxn4* function causes a complete loss of V2b neurons, a concomitant fate-switch to V2a neurons, and a complete down-regulation of Mash1 expression. The absence of *Mash1* results in a similar but partial loss of V2b neurons associated also with an increase of V2a neurons. Overexpression of Foxn4 alone in spinal neural progenitors promotes the V2a fate at the expense of the V2b fate, whereas, Mash1 suppresses both the V2a and V2b fates. However, co-expression of both Foxn4 and Mash1 promotes the V2b fate while inhibiting the V2a fate. Thus, these data together suggest that Foxn4 cooperates with Mash1 to specify the identity of V2b neurons from bi-potential p2 progenitors.

Identification of Brn3b downstream genes by microarray analysis of gene expression. Brn3b is a POU domain transcription factor gene whose expression is restricted to ganglion cells during retinal development. We have shown previously by gene targeting and overexpression studies that Brn3b plays an essential role in regulating the differentiation, axon outgrowth and pathfinding, and survival of retinal ganglion cells. To identify candidate Brn3b downstream genes, we carried out microarray analyses using the GeneChip® Mouse Genome 430A arrays (Affymetrix). The obtained data were analyzed using the Microarray Suite and DNA-Chip Analyzer softwares to calculate fold changes of transcripts between the wild-type and mutant.

We found that 156 cDNA clones displayed a decrease or increase of 1.7-fold or higher in their expression levels in the *Brn3b* mutant retina. One hundred and six of them are downregulated and 50 are upregulated. Of these 156 genes, 143 are known genes and 13 are unknown. Our gene list includes only 20 genes that have previously been identified as Brn3b downstream genes by various other approaches. Thus, our gene expression profiling experiment has greatly expanded the repertoire of genes that are regulated by Brn3b and hence will provide significant insight into the molecular basis by which Brn3b controls the differentiation and survival of retinal ganglion cells. For instance, the Iroquois-related homeobox genes *Irx2, Irx3, Irx4* and *Irx5* and the early B-cell factor genes *Ebf1* and *Ebf3* are all found to be significantly downregulated in *Brn3b* null retinas. These affected genes may explain why loss of *Brn3b* function causes defects in axon outgrowth and guidance of ganglion cells since all these transcription factor genes have been implicated in the regulatory pathway of axon pathfinding. Similarly, Brn3b is found to regulate the expression of proapoptotic genes, providing a link between Brn3b and its role in ganglion cell survival.

Effect of *Brn3a* deficiency on sensory neuron development. We have been investigating the function of Brn3a during sensorineural development. The absence of *Brn3a* in mice causes developmental defects in the trigeminal, dorsal root, and inner ear sensory ganglia, as well as in the brainstem. In an ongoing collaborative study with Dr. Hiroyuki Ichikawa's laboratory, we have been analyzing in *Brn3a* null mice immunoreactivities of various neurochemical substances in sensorineural tissues and examining sizes of neurons expressing these substances. We found that in *Brn3a* null trigeminal ganglia, the proportion of small neurons immunoreactive for substance P markedly increased and that of medium- to large-sized immunoreactive ones correspondingly decreased compared to wildtype ganglia. Loss of *Brn3a* function also caused a decrease in the number of neurons immunoreactive for somatostatin receptor sst2A or for delta-opioid and an increase in the number of mu-opioid receptor-immunoreactive neurons. However, the number and size of vanilloid receptor subtype 1-immunoreactive neurons was barely affected by the deficiency. These data suggest that *Brn3a* deficiency affects the survival of trigeminal nociceptors and their expression of neurochemical substances. In the dorsal root ganglia, we analyzed the effect of *Brn3a* inactivation on dopaminergic neurons immunoreactive for tyrosine hydroxylase (TH). TH-immunoreactive neurons were detected in the dorsal root ganglia of wildtype and heterozygous mice, but their proportion was greatly increased by the loss of *Brn3a* function. TH-immunoreactive neurons were of various sizes in wildtype and heterozygous mice. In the knockout mice, however, these neurons were mostly small. Therefore, Brn3a may normally suppress TH expression in small neurons of the dorsal root ganglion but may activate its expression in large ones.

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Structural Biology

Eddy Arnold, Ph.D.

Resident Faculty Member, CABM; Professor, Rutgers, Department of Chemistry and Chemical Biology, Adjunct Professor, UMDNJ-RWJMS, Department of Molecular Genetics, Microbiology and Immunology

Biomolecular Crystallography Laboratory

Dr. Arnold obtained his Ph.D. in organic chemistry with Professor Jon Clardy at Cornell University in 1982. From 1982 to 1987, he was a postdoctoral researcher with Professor Michael G. Rossmann at Purdue University and was a central member of the team that solved the structure of a human common cold virus by X-ray crystallography. Among the awards and fellowships Arnold has received are a National Science Foundation Predoctoral Fellowship, Damon Runyon–Walter Winchell and National Institutes of Health Postdoctoral Fellowships, an Alfred P. Sloan Research Fellowship, a Johnson and Johnson Focused Giving Award, and a Board of Trustees Award for Excellence in Research at Rutgers. Dr. Arnold is the director of a multi-center NIH Program Project. He received an NIH MERIT Award in 1999 and was elected a Fellow of the American Association for the Advancement of Science in 2001. His laboratory is supported by grants from NIH and industrial collaborators, and by research fellowships.

Many of the underlying biological and chemical processes of life are being detailed at the molecular level, providing unprecedented opportunities for the development of novel approaches to the cure and prevention of human disease. A broad base of advances in chemistry, biology and medicine has led to an exciting era in which knowledge of the intricate structure of life's machinery can help to accelerate the development of new small molecule drugs and biomaterials such as engineered viral vaccines. Eddy Arnold and Gail Ferstandig Arnold and their colleagues are working to develop and apply structure-based drug and vaccine designs for the treatment and prevention of serious human diseases. In pursuit of these goals, their laboratory uses research tools from diverse fields, including X-ray crystallography, molecular biology, virology, protein biochemistry and macromolecular engineering.

The approaches being developed in the Arnold laboratory are applicable to a wide array of human health problems, ranging from infectious diseases to cancer and diseases caused by hereditary genetic defects. Much of the lab's research effort to date has focused on the development of drugs and vaccines for the treatment and prevention of AIDS. Examples of the results of these studies include: 1) collaborative development of drugs for the treatment of AIDS, some of which appear to be more effective than treatments in current use; and 2) production of AIDS vaccine candidates that have elicited protective immune responses against HIV.

Eddy Arnold and coworkers study the structure and function of reverse transcriptase (RT), an essential component of the AIDS virus and the target of many of the most widely used anti-AIDS drugs. Using the powerful techniques of X-ray crystallography, his team has solved the three-dimensional structures of HIV-1 RT in complex with a variety of antiviral drugs and model segments of the HIV genome. These studies have revealed the workings of an intricate and fascinating biological machine in atomic detail and have yielded numerous novel insights into polymerase structure-function relationships, detailed mechanisms of drug resistance and structure-based design of RT inhibitors. Synthesis of this information has led to the development of a number of inhibitors that show great promise as potential treatments for AIDS. The group is also pursuing structural studies of HIV-1 RT with inhibitors of the RNase H functionality of this

enzyme. Although RNase H activity is essential for HIV replication, no drugs targeting this activity are currently available. Detailed knowledge of how RNase H inhibitors bind to HIV-1 RT will establish a platform for structure-based design and development of RNase H inhibitors as anti-AIDS drugs.

Drug development and structural studies of a molecule as complex as HIV RT require immense and highly coordinated resources. The Arnold group has been fortunate to have highly successful collaborations with the groups of Stephen Hughes (NIH NCI, Frederick, MD), of Paul Lewi and the late Paul Janssen (Center for Molecular Design, Janssen Research Foundation, Belgium), of Craig Gibbs and Michael Miller (Gilead Sciences), and of Roger Jones (Rutgers Chemistry and Chemical Biology) and Michael Parniak (U. of Pittsburgh). The group also benefits from generous access to synchrotron X-radiation sources (CHESS, APS, and BNLS). Hughes and his coworkers have contributed expertise in protein engineering, production, and biochemistry at every stage of the RT project. Lewi, Janssen and their coworkers have synthesized hundreds of molecules from a number of chemical families in search of optimal drug candidates, eventually leading to the discovery of agents effective against isolates of HIV containing drug-resistance mutations that can cause the currently available drugs to fail. Crystallographic work from the Arnold and Hughes laboratories has allowed precise visualization of how potential anti-HIV drug candidates latch onto RT, their molecular target. Lewi, Janssen, and colleagues at the Center for Molecular Design have used this structural information to guide the design and synthesis of new molecules with improved properties. Following further evaluation against drug-resistant variants, scientists at Tibotec-Virco (Mechelen, Belgium) have coordinated testing of two of the molecules with highly promising results in Phase II clinical trials. Some of these anti-HIV compounds are inexpensive to make, potently and broadly effective, non-toxic and simple to administer. These molecules have the potential to be widely accessible for treating AIDS even in underdeveloped nations.

Vaccines have proven to be the most effective tools for worldwide control of infectious diseases. Our laboratory's vaccine development project, co-directed by Gail and Eddy Arnold, involves engineering a human common cold virus, rhinovirus (HRV), to display immunogenic segments from more dangerous pathogens for the purpose of developing vaccines against these pathogens. This work involves generating "combinatorial libraries" of chimeric human rhinoviruses using a technique called random systematic mutagenesis. Foreign sequences are linked to the HRV sequences via adapters of randomized sequences and lengths, leading to a constellation of presentations. Large sets of such viruses presenting the foreign sequences in many conformations are generated and then selected with appropriate antibodies aimed at the target pathogen, allowing for the isolation of vaccine candidates with the most effectively reconstructed foreign segments. Our combinatorial approach to the vaccine problem is akin to buying many tickets for a lottery: the chances of winning the jackpot are increased by having more tickets.

Chimeric rhinovirus constructs have been made that elicit antibodies (in guinea pigs) capable of

potently neutralizing the AIDS virus in cell culture. Virus libraries incorporating immunogens from the HIV gp120 and gp41 envelope glycoproteins have been constructed. We are also working collaboratively with Professor John Taylor of the Rutgers Chemistry and Chemical Biology Department to probe the immunogenic determinants of an epitope from gp41 using synthetic peptides. In addition to looking for chimeric viruses and peptides capable of eliciting the most potent and broad immune responses possible, we are also interested in elucidating the molecular determinants of immunogenicity. Knowledge of the relationship between structure and function (i.e., neutralization) would give us the opportunity to develop better vaccine candidates. The laboratory team is also using X-ray crystallography and computational chemistry to analyze the structures of some of the engineered viruses (and soon peptides), alone and in complex with anti-HIV antibodies. Ultimately we hope to identify three-dimensional correlates of immunogenicity and use this information to develop a structural basis for design of more effective human vaccines. There is every reason to expect that a structure-based approach to vaccine development will become as important to vaccinology as has structure-based drug design to drug discovery and development.

In addition to working to develop novel vaccines and chemotherapeutic agents, the laboratory aims to gain greater insights into the basic molecular processes of living systems. Other projects currently being pursued in the lab include structural studies of: 1) bacterial RNA polymerase holoenzyme complexes with inhibitors and substrates (with Richard Ebright and Helen Berman at Rutgers and Sergei Borukhov at SUNY-Brooklyn); 2) proteins from the SARS coronavirus (with Susan Weiss and Paul Bates at U Penn); and 3) the human mRNA capping enzyme and its associated factors (with Dr. Aaron Shatkin at CABM). The structural group also collaborates with Gaetano Montelione (CABM) on crystallography of proteins targeted by the Northeast Structural Genomics Consortium.

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Gaetano Montelione, Ph.D.

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Protein NMR Spectroscopy Laboratory

Dr. Gaetano Montelione did graduate studies in protein physical chemistry with Professor Harold Scheraga at Cornell University. He learned nuclear magnetic resonance spectroscopy at the Swiss Federal Technical Institute in Zürich where he worked with Nobel laureate Dr. Kürt Wüthrich, the first researcher to solve a protein structure with this technique in 1985. Two years later Dr. Montelione solved the structure of epidermal growth factor. He has developed new NMR techniques for refining 3D structures of proteins and triple resonance experiments for making ^{1}H, ^{13}C, and ^{15}N resonance assignments in intermediate-sized proteins. His laboratory has determined 3D solution structures for epidermal growth factor, type-α transforming growth factor, RNA-binding proteins involved in cold-shock response, and immunoglobulin-binding proteins. He has served as a member of the NSF Molecular Biophysics Study Section. Dr. Montelione has received the Searle Scholar Award, the Dreyfus Teacher-Scholar Award, a Johnson and Johnson Research Discovery Award, the American Cyanamid Award in Physical Chemistry, the NSF Young Investigator Award, and the Michael and Kate Bárány Award of the Biophysical Society.

Goals of our work involve developing high-throughput technologies suitable for determining many new protein structures from the human genome project using nuclear magnetic resonance spectroscopy (NMR) and X-ray crystallography. These structures provide important insights into the functions of novel gene products identified by genomic and/or bioinformatic analysis. The resulting knowledge of structure and biochemical function provides the basis for collaboration with pharmaceutical companies to develop drugs useful in treating human diseases that are targeted to these newly discovered functions. The approach we are taking is opportunistic in the sense that only proteins which express well in bacterial expression systems are screened for their abilities to provide high quality NMR spectra or well-diffracting protein crystals. Those that provide good NMR or X-ray diffraction data are subjected to automated analysis methods for structure determination. The success of our approach relies on our abilities to identify, clone, express and analyze hundreds of biologically-interesting proteins per year; only a fraction of the initial sequences chosen for cloning and analysis result in high-resolution 3D structures. However, this "funnel" process can yield new functions for tens of new structures per year and can thus have tremendous scientific impact. Prof. Montelione is the director of the NIH-funded Northeast Structural Genomics Consortium, an inter-institutional pilot project in large-scale structural proteomics.

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Protein Design and Evolution Laboratory

Dr. Vikas Nanda has recently joined CABM in September of 2005 after studying as an NIH National Research Service Award postdoctoral fellow in the laboratory of Dr. William DeGrado at the Department of Biochemistry and Biophysics at the University of Pennsylvania School of Medicine. His research focused on studying the molecular basis of transmembrane interactions amongst integrins involved in regulation of platelet hemostasis. Prior to that, Dr. Nanda received his doctorate in Biochemistry from Johns Hopkins University.

The sequence of a natural protein is just one of a large subset of allowed sequences that result in a functional molecule. While a sequence has been optimized for function, it also must be an evolvable sequence – one that can easily mutate in response to evolutionary pressures without radical perturbation of structure or function. An optimal protein is one that not only is functional but also is well connected to neighboring sequences, giving mutational pathways for adaptation to traverse. Our lab will investigate the extent of these limitations using novel computational protein design algorithms, bioinformatic tools and protein library screening methods. Large scale mutagenesis studies of proteins have demonstrated a remarkable malleability of a protein sequence to change without disruption of structure.

The goal of our research is to understand the molecular underpinnings of mutational tolerance and apply them to problems in protein and drug design. De novo designed proteins with significant sequence plasticity are optimal starting points for engineering functional active sites. Additionally, understanding how mutations accumulate helps predict how pathogens evolve drug-resistance, giving us the opportunity to anticipate viral evolution. We will study sequence malleability in three ways – (1) develop de novo design methods that computationally search the accessible sequences of a given fold for those that are optimally tolerant to mutations, (2) map the mutational tolerance of the small protein signaling domains through high throughput screening of large libraries of mutations and (3) explore the existing sequence variability of HIV protease and other targets for estimating the extent of possible mutations in order to abet drug design efforts.

Publications:

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Ann Stock, Ph.D.

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Protein Crystallography Laboratory

Dr. Ann Stock performed graduate work on the biochemistry of signal transduction proteins with Professor Daniel E. Koshland, Jr. at the University of California at Berkeley. From 1987 to 1991 she pursued structural analysis of these proteins while a postdoctoral fellow with Professor Clarence Schutt at Princeton University and Professor Gregory Petsko at the Structural Biology Laboratory in the Rosenstiel Center at Brandeis University. Her CABM laboratory has solved structures of several signal transduction proteins, including receptor modification enzymes and members of the two-components family of signal transduction proteins. Stock has received National Science Foundation Predoctoral and Damon Runyon-Walter Winchell Postdoctoral Fellowships, a Lucille P. Markey Scholar Award in Biomedical Science, the NSF Young Investigator Award, a Sinsheimer Scholar Award and an NIH MERIT Award. She has received the Foundation of UMDNJ Excellence in Teaching Award, the Molecular Biosciences Graduate Association Educator of the Year Award and the designation of Master Educator. In 2004 Stock was selected as an Outstanding Scientist by the NJ Association for Biomedical Research. Stock is an investigator of the Howard Hughes Medical Institute.

Our laboratory focuses on structure/function studies of signal transduction proteins. Effort is concentrated on bacterial histidine protein kinases and response regulator proteins that are the core of two-component systems, the predominant signal transduction pathways in bacteria. These systems are important for virulence in pathogenic organisms and are targets for development of new antibiotics. We have concentrated our research efforts on understanding how phosphorylation regulates the activities of response regulator switch domains. Investigations have been directed at the OmpR/PhoB subfamily of response regulators. This subfamily of response regulator transcription factors, characterized by a novel winged-helix domain, is the largest response regulator subfamily with over 1000 members and accounting for >30% of all response regulators. Substantial progress has been made in establishing the mechanism of phosphorylation-mediated activation of response regulators of the OmpR/PhoB subfamily. Structures have been determined for the activated regulatory domains of seven response regulators of the OmpR/PhoB family (*Escherichia coli* ArcA, KdpE, TorR, PhoB, PhoP, *Thermotoga maritima* DrrB, and DrrD). We have also determined structures for four regulatory domains in their inactive states (*E. coli* PhoB, *T. maritima* DrrB and DrrD, and *Mycobacterium tuberculosis* MtrA), three of these within the context of full-length multi-domain proteins. The structures have led us to propose a model for regulation in this family in which the response regulator proteins have distinct inactive states but adopt a common active state upon phosphorylation. The different inactive states involve different intra- and/or intermolecular domain arrangements that provide for molecular interactions with a variety of regulatory consequences. Thus the distinct inactive states allow for different modes of regulation that have been optimized for the specific needs of the signal transduction pathways in which each protein functions. In the active phosphorylated state, all proteins adopt a similar dimeric structure mediated by the regulatory domains and involving a set of highly conserved residues along the $\alpha4$-$\beta5$-$\alpha5$ face. The rotationally symmetric regulatory domain dimer connects to the DNA-binding domains via flexible linkers, allowing the DNA-binding domains to associate with the head-to-tail symmetry that is required for interaction with their tandemly arranged direct-repeat DNA recognition half-sites. Current *in vitro* and *in vivo* studies are aimed at correlating the different inactive state structures with physiologically relevant regulatory strategies.

Additional advances in our laboratory include the identification of the sites of reversible post-translational modification in the six transmembrane chemoreceptor proteins of *T. maritima*. This work, performed in collaboration with the Mass Spectrometry Facility directed by Dr. Peter Lobel (CABM), establishes a unique consensus sequence for methylation in *T. maritima* and abolishes the previously and widely held assumption that consensus sequences for post-translational modifications determined for one organism can be used to predict modification sites in proteins of another organism. We are close to completing the NMR solution structure of a *Staphylococcal* DNA-binding domain, something that has eluded crystallographic approaches in many other laboratories. This will represent the first structure of a member of the family of LytR domains and will establish the fold for this very important family of transcription factors that regulate the expression of virulence genes in pathogenic bacteria.

In collaboration with the laboratory of Dr. Peter Lobel (CABM) we have continued structure/function investigations of NPC2, a protein deficient in Niemann-Pick type C2, a disease characterized by accumulation of cholesterol in lysosomes. Our previous determination of the X-ray crystal structure of NPC2 allowed us to hypothesize about the nature of the cholesterol-binding pocket in NPC2. This year we solved the structure of bovine NPC2 bound to the cholesterol analog, cholesteryl sulfate. The structure confirmed our hypothesized binding site and provides details about ligand binding relevant to understanding the sterol specificity of this protein. We have also attempted to identify weakly associating cellular partners of NPC2 by affinity chromatography and photo-activated cross-linking. We have observed several proteins that appear to associate specifically with NPC2. Purification and identification of these proteins is in progress.

Publications:

Lubetsky, J.B. and **Stock, A.M**. (2005) Two-component signal transduction and chemotaxis. In *Structural Biology of Bacterial Pathogens*. G. Waksman, M. Caparon and S. Hultgren, eds., American Society for Microbiology Press, Washington, D.C. 17-36.

Toro-Roman A., Mack, T.R. and **Stock, A.M.** (2005) Structural analysis and solution studies of the activated regulatory domain of the response regulator ArcA: a symmetric dimer mediated by the a4-b5-a5 face. *J. Mol. Biol.,* 349:11-26.

Bachhawat, P. Swapna, G.V.T., Montelione, G.T. and **Stock, A.M.** (2005) Mechanism of activation for transcription factor PhoB suggested by different modes of dimerization in the inactive and active states. *Structure,* 13:1353-1363.

Toro-Roman, A. Wu, T., and **Stock, A.M.** (2005) A common dimerization interface in bacterial response regulators KdpE and TorR. *Protein Sci.,* 14:3077-3088.

Molecular Genetics

Stephen Anderson, Ph.D.

Resident Faculty Member, CABM; Associate Professor, Rutgers University, Department of Molecular Biology and Biochemistry

Protein Engineering Laboratory

Dr. Stephen Anderson conducted his postdoctoral research under Nobel laureate Dr. Frederick Sanger at the MRC Laboratory of Molecular Biology in Cambridge, England. That project involved the sequencing of human and bovine mitochondrial genomes. He went on to a position at the California-based biotechnology start-up company Genentech. Anderson has held research or teaching positions at Harvard University, the MRC Laboratory of Molecular Biology, Genentech, Inc., University of California, San Francisco, and Rutgers University. While at Genentech he was responsible for all specialty chemical projects and second generation tissue plasminogen activator research.

We are primarily focused on recombinant protein expression projects as part of our participation in the Northeast Structural Genomics Consortium (www.nesg.org), one of the four large U.S. structural genomics centers funded by the NIH Protein Structure Initiative. In particular, the lab's goal is to establish the methylotrophic yeast *Pichia pastoris* as a moderate throughput and relatively high-yield expression host for protein sample production. The attempted expression of many gene products, especially those of eukaryotic origin, often fails to yield soluble native material when *E. coli* is used as the heterologous production host. Expression systems based on simple microbial eukaryotes such as yeast could, in theory, be a cost-effective alternative to *E. coli*.

P. pastoris is a familiar and well-established expression system for recombinant proteins. It is capable of producing proteins in extremely high yields and has been used to produce samples for 3D structural characterization by X-ray crystallography and NMR spectroscopy. However, to achieve high yields of a given protein it is usually necessary to empirically try different expression plasmids and host strains, select for integrants of the foreign expression construct in the host strain's chromosomal DNA, and study multiple clonal isolates for each vector-host combination to find ones that produce relatively high levels of protein. This trial-and-error approach is rather cumbersome and thus not suitable for a high-throughput protein expression effort. Some laboratories have attempted to adapt the *P. pastoris* system to a high-throughput environment, but these efforts have typically reported relatively modest yields. We are trying to eliminate this yield vs. throughput trade-off and engineer new *P. pastoris* expression systems that will be more suitable for routine use by structural biologists and other proteomics researchers.

In the area of technology development, work during the past year has included the development of a prototype high-yield episomal *P. pastoris* expression vector (Choi et al, manuscript in preparation). This vector promises to streamline protein production for proteomics applications. Also initiated is a screening program for novel host strains that are able to secrete arbitrary heterologous recombinant proteins at relatively high levels.

In the area of current applications we have begun a careful curation of novel NESG targets taken from a list of human extracellular proteins. Special attention has been paid to the issues of distinguishing full-length from fragmentary cDNAs in sequence databases and of correctly predicting the boundaries of domains ("domain parsing") in multidomain proteins. We are also exploring the feasibility of using total gene synthesis as a cost-effective substitute for molecular cloning in the preparation of expression constructs: this approach should enable us to convert sequences from databases directly into expressable genes without having to first acquire a genomic DNA- or cDNA-based physical intermediate.

Finally, in the area of future applications we have begun planning a project to use the know-how and infrastructure in protein expression developed for the NESG structural genomics effort to address the problem of new vaccine development for malaria. Specifically, we are proposing to express a consensus set of the predicted *Plasmodium* sporozoite secreted or surface-bound proteins and test these for protective immunogenicity in animals. Recombinant proteins emerging from this screen may provide a source of novel *Plasmodium* antigens to use as the basis for improved malaria vaccines.

Publications:

Shin-Geon Choi, In-Pyo Hong, and Stephen Anderson (2006) "Development of a high-throughput screening expression vector for structural genomics in *Pichia pastoris*", in preparation.

Arnold B. Rabson, M.D.

Resident Faculty Member, CABM; Deputy Director, CINJ; Professor, UMDNJ-Robert Wood Johnson Medical School, Department of Molecular Genetics, Microbiology and Immunology; Adjunct Professor, UMDNJ-RWJMS, Department of Pathology and Laboratory Medicine

Viral Pathogenesis Laboratory

Dr. Arnold Rabson joined CABM in the summer of 1990. Previously, he performed his residency in pathology at Brigham and Women's Hospital in Boston. He was a medical staff fellow and a senior staff fellow in Malcolm Martin's laboratory at the National Institute of Allergy and Infectious Diseases from 1981 to 1990. Dr. Rabson has served on a number of federal grant review boards, including an NIH Special Review Committee for AIDS. He is a past member of the NIH Pathology B and CAMP study sections and has served as Chairman of several Special Emphasis Panels of the NIH Center for Scientific Review. He is a Senior Editor of the journal *Clinical Cancer Research*. He completed a 12-year term on the editorial board of the *Journal of Virology* as well as *Cancer Research*. Dr. Rabson is also the Deputy Director of the Cancer Institute of New Jersey (CINJ), Chief of the Division of Cancer Genomics and Molecular Oncology at CINJ and directs its Transcriptional Regulation and Oncogenesis Program.

Dr. Rabson's laboratory studies the molecular basis of cancer and human retroviral infections. One aspect of his research program focuses on the viral and cellular mechanisms regulating the expression of human retroviruses that cause cancer and AIDS, the human T-cell leukemia virus type 1 (HTLV-1) and the human immunodeficiency virus (HIV). A second aspect of his laboratory studies the roles of cellular transcription factors, particularly those of the NF-κB/Rel family, in the development and progression of human malignancies, including leukemia, lymphomas and prostate cancer.

HTLV-1 causes an aggressive T cell leukemia/lymphoma (Adult T-Cell Leukemia, ATL) in some infected individuals and causes a demyelinating neurological disease (HTLV-associated Myelopathy, HAM/TSP) in other infected patients. These disorders occur in only a minority of infected patients, years after initial infection. This suggests that there are important interactions between the virus and the host that determine its pathogenicity. The Rabson laboratory has identified and characterized models of latent HTLV-1 infection in which T-cells can be chronically infected with HTLV-1, yet not express its genes, and therefore evade immune detection. Furthermore, they have shown that activation of the T-cells through the T-cell receptor (i.e. immune activation) can potently induce HTLV-1 gene expression. This suggests that stimulation of particular T-cell clones through their T-cell receptor could lead to enhanced HTLV-1 gene expression, resulting in increased T-cell proliferation. This could explain the polyclonal to oligoclonal proliferation of infected T-cells that characterizes HTLV-1-associated diseases. Over the last year, Dr. Rabson's laboratory has further elucidated the mechanisms responsible for activation of latent HTLV-1 proviruses. They have shown critical roles for the LCK tyrosine kinase, downstream of T-cell receptor activation and for activation of the Ras/MAP kinase pathways leading to induction of HTLV-1 gene expression. Importantly, they have demonstrated that the activation of latent HTLV-1 proviruses is the result of a potent feedback loop in which T-cell receptor activation leads to a low level of LTR activation, resulting in expression of the HTLV-1 Tax transactivator leading to a further marked activation of the HTLV-1 long terminal repeat (LTR) promoter. Using a transgenic mouse model of

HTLV-1 LTR-Tax transactivator expression, the laboratory has shown that T-cell activation in these animals will also induce HTLV-1 LTR expression, resulting in induction of Tax expression. This leads to hyperproliferation and decreased death of these T-cells with sustained replication in culture of up to 4-6 weeks, as compared with a maximal survival of two weeks of non-transgenic T-cells. Thus, these mice model activation of Tax in human T-cells leading to sustained proliferation which serves as the ground for additional mutations leading to ultimate malignant transformation.

The second major area of study in Dr. Rabson's laboratory continues to be the roles of different transcriptional regulators in the pathogenesis of human cancer. In collaboration with Dr. Gélinas at CABM, his laboratory has continued to study the roles of the NF-κB in cancer. Studies over the past year have focused on the "non-canonical" NF-κB pathway in human lymphomas. A number of years ago, his laboratory identified the frequent occurrence of molecular alterations in another NF-κB factor, the gene encoding the *NFKB2* transcription factor, in the malignant cells of patients with cutaneous T-cell lymphoma (CTCL). He has shown that the mutations in this gene in CTCL and other lymphomas are associated with a loss of the ability of this protein to inhibit the classical NF-κB pathway. His laboratory has been identifying cellular target genes that may mediate the oncogenic activity of these tumor-associated mutations. Further studies are on-going as are studies of the Bcl-3 protooncogene, a coactivator for NF-κB dimers that is overexpressed in certain human lymphomas. The laboratory has shown that Bcl-3 interacts with Lck, a tyrosine kinase also implicated in T-cell lymphomas and that this interaction increases expression of subsets of NF-κB target genes. Possible targets of Bcl-3 activation have also been identified.

Dr. Rabson's laboratory has continued a number of important collaborations over the last year. He has collaborated with Dr. Strair at the Cancer Institute of New Jersey to study the cytotoxic effects of attenuated adenoviruses for human mantle cell lymphoma cells. These studies have shown that highly debilitated mutant adenoviruses can still specifically kill mantle cell lymphoma cells, presumably due to complementation of the adenovirus mutation by mutations (such as cyclin D1 overexpression) occurring in the lymphoma cells. Collaborations are continuing with Dr. A. Conney (Rutgers University) to test the effects of the phorbol ester, TPA, in human cancer. These studies have demonstrated the efficacy of TPA (at clinically achievable concentrations) in inducing differentiation and death of myeloid leukemia cells, and the possible effects of combinations of TPA with other agents in overcoming resistance to TPA induced differentiation in leukemic cells. They provide the rationale for further clinical trials. They have also demonstrated the potential efficacy of TPA in combination with other agents for the therapy of pancreatic cancer, as demonstrated in pre-clinical animal models.

Finally, over the last year, Dr. Rabson has continued to collaborate with several laboratories in studies of inhibitors of HIV replication. In collaboration with Drs. Sinko (R.U. School of Pharmcy), Leibowitz and Stein, his laboratory has shown that conjugated forms of peptides

derived from the HIV Tat transactivator have the ability to inhibit HIV replication. Dr. Sinko is pursuing further studies to demonstrate the potential clinical applicability of such an approach.

Publications:

Lin, H.-C., Hickey, M., Hsu, L., Medina, D., and **Rabson, A.B**. (2005) Activation of Human T Cell Leukemia Virus Type 1 LTR and Cellular Promoters by T-Cell Receptor Signaling and HTLV-1 Tax Expression. *Virology*, 339:1-11.

Avila, G.E., Zheng, X., Cui, X.X., Ryan,A., Hansson, A, Suh, J., **Rabson, A.B**., Chang,R.L., Shih, W.J., Lin, Y., Crowell, P., Lu, YP., Lou,Y.R. and Conney, A.H. (2005) Inhibitory effects of 12-O-tetradecanoylphorbol-13-acetate (TPA) alone or in combination with all-trans retinoic acid on the growth of cultured human pancreas cancer cells and pancreas tumor xenografts in immunodeficient mice. *J. Pharmacology and Experimental Therapeutics*. 315:170-187.

Zhao, Y., Ramakrishnan, A., Kim, K.-E., and **Rabson, A.B**. (2005) Regulation of Bcl-3 through interaction with the Lck tyrosine kinase. *Biochemical and Biophysical Research Communications,* 335:865-873.

Hansson, A., Marin Y.E., Suh, J., **Rabson, A.B**., Chen, S., Huberman, E., Chang, R.L., Conney, A.H., Zheng, X. (2005) Enhancement of TPA-induced growth inhibition and apoptosis in myeloid leukemia cells by BAY11-7082, an NF-kappaB inhibitor. *Int. J. Oncol*. 27L9410948.

Rabson, A.B. and Weissman, D. (2005) From microarray to bedside: targeting NF-κB for therapy of lymphomas. *Clinical Cancer Research*, 11:2-5.

Medina, D.J., Sheay, W., Osman, M., Goodell, L., **Rabson, A.B**., and Strair, R.K. (2005) Adenovirus infection and cytotoxicity of primary mantle cell lymphoma cells. *Experimental Hematology*, in press.

Aaron J. Shatkin, Ph.D.

Director, CABM; University Professor of Molecular Biology at Rutgers University and Professor, UMDNJ-Robert Wood Johnson Medical School, Department of Molecular Genetics, Microbiology and Immunology

Molecular Virology Laboratory

Dr. Aaron J. Shatkin, a member of the National Academy of Sciences, has held research positions at the National Institutes of Health, The Salk Institute, and the Roche Institute of Molecular Biology. He has taught at Georgetown University Medical School, Cold Spring Harbor Laboratory, The Rockefeller University, UMDNJ-Newark Medical School, University of Puerto Rico, Princeton University and other institutions. He was editor of the Journal of Virology from 1973-1977, founding editor-in-chief of Molecular and Cellular Biology from 1980-1990 and is currently editor of Advances in Virus Research. Shatkin serves on the advisory boards of a number of organizations. In 1989 he was recognized by New Jersey Monthly magazine with a New Jersey Pride Award for his contributions to the State's economic development. In 1991 the State of N.J. awarded Shatkin the Thomas Alva Edison Science Award. He was elected Fellow of the American Academy of Arts and Sciences in 1997 and the American Association for the Advancement of Science in 1999. In 2003 he received the Association of American Medical Colleges Award for Distinguished Research in the Biomedical Sciences.

An important feature of gene expression in a broad range of eukaryotes from yeast to humans is the addition of a m7GpppN cap to nascent mRNA 5´ends. The cap structure marks transcription start sites and regulates mRNA stability, splicing, nuclear transport and translation initiation facilitated by cap binding proteins. Caps are synthesized by three enzymatic steps catalyzed sequentially by RNA triphosphatase (RT), guanylyltransferase (GT) and methyltransferase (MT). All three activities have been shown to be required for viability in yeast. Transcripts that are uncapped are rapidly degraded while RNAs containing unmethylated caps fail to direct protein synthesis.

We cloned, sequenced and characterized the human, mouse and worm capping enzymes. In contrast to separate enzymes in unicellular eukaryotes, metazoans contain a bifunctional capping enzyme (CE) consisting of N-terminal RT and C-terminal GT. CE selectively bound RNA polymerase II via interaction of the GT domain with the C-terminal heptad repeat sequences in the polymerase largest subunit. This association stimulated capping. CE binding also relieved repression by negative elongation factor in transcription complexes, suggesting that it plays a critical role in elongation checkpoint control during promoter clearance. These and other results point to a functional connection between capping and transcription. Additional protein-protein interactions, for example between MT and the nuclear transporter importin-α, imply linkage also between capping and protein transport.

RNA interference (RNAi) knockdown of CE in *C. elegans* was embryonic lethal. We showed that capping is also essential for viability in mammalian cells. Knockdown of CE in human cells by small interfering RNA (siRNA) resulted in apoptosis as measured by TUNEL assay and caspase-3 activation. siRNA knockdown of the cap MT also induced programmed cell death. Viability could be restored by transfection of truncated MT that retained a nuclear localization sequence (NLS) and associated with transcription complexes in the nucleus. By contrast, a truncated MT that was missing a NLS and thus localized to the cytoplasm, although catalytically active, failed to reverse the siRNA-induced apoptotic effects of MT knockdown. The results demonstrate that mRNA capping is a key

component of the network of interacting pathways required for homeostasis in mammalian cells and eukaryotes generally.

Publications:

Shafer, B., Chu, C., and **Shatkin, A.J**. (2005) Human mRNA Cap Methyltransferase: Alternative Nuclear Localization Signal Motifs Ensure Nuclear Localization Required for Viability. *Mol. Cell. Biol.* 25:2644-49.

Chu, C., Shafer, B. and **Shatkin, A.J**. (2005) Apoptosis Is Induced in Mammalian Cells by siRNA Knock-down of mRNA Capping Enzymes. Cold Spring Harbor Meeting on Eukaryotic mRNA Processing.

Shatkin, A.J. (2005) Gene Regulation: mRNA Capping. Henry Stewart Talks, London (on line).

Education, Training & Technology Transfer

Lectures and Seminars

CABM Lecture Series

January 26, 2005
Gregory Hannon, Cold Spring Harbor Laboratories
"RNAi: Mechanism and Application"

February 23, 2005
Barry Honig, Columbia University
"Combining Biophysics and Bioinformatics to Predict Protein Structure and Function"

March 23, 2005
Co-sponsored by the Child Health Institute of New Jersey
Matthew P. Scott, Stanford University
"Hedgehog Signaling in Development and Disease"

April 6, 2005
Joan Brugge, Harvard Medical School
"Morphogenesis and Oncogenesis in 3D Breast Epithelial Cultures"

November 9, 2005
Mark Hochstrasser, Yale Medical School
"Ubiquitin-dependent Protein Degradation at the Nuclear Envelope"

November 16, 2005
Clifford J. Tabin, Harvard Medical School
"Patterning the Vertebrate Limb and Heart"

- The CABM Lecture Series is supported in part by Sanofi-Aventis -

ANNUAL CABM RETREAT, June 9, 2005
Forest Lodge, Warren, NJ

Program

8:15 AM **Registration, Poster Set-up and Continental Breakfast**

8:45 AM **Opening Remarks – Aaron J. Shatkin**

9:00 AM **Session I – Gene Expression Regulatory Mechanisms**
Chair: Julie Williams – Sleep Genetics Lab

Nupur Gupta – Tumor Virology Lab (Céline Gélinas)
"Transcriptional Activity of Rel/NF-kappaB Subunits: Insights Into Their Oncogenic Function"

Chun Chu – Molecular Virology Lab (Aaron J. Shatkin)
"siRNA Knock-down of Human mRNA Capping Enzymes Induces Apoptosis"

Brian Benoff – Protein Crystallography Lab (Ann Stock)
"Binding Specificity of OmpR, a Winged-Helix Transcription Factor"

Guanan Wang – Growth and Differentiation Control Lab (Fang Liu)
"Characterization of TGF-beta Induced Phosphorylation in the Smad3 Linker Region"

Eun Young Kim – Molecular Chronobiology Lab (Isaac Edery)
"Role for Doubletime in the Posttranscriptional Regulation of dCLOCK Protein"

Julie Williams – Sleep Genetics Lab (Julie Williams)
"Mechanisms of Sleep in the Immune Response"

10:30 AM **Coffee Break**

11:00 AM **Session II – Genomics/Proteomics Approaches to Elucidating Function**
Chair: James Aramini – Protein NMR Spectroscopy Lab (Guy Montelione)

Michael Baran – Protein NMR Spectroscopy Lab (Guy Montelione)
"Bionet-based Target Selection in Structural Genomics"

Yi-Wen Chiang – Protein Engineering (Stephen Anderson)
"Development of *Pichia Pastoris* as an Expression Host for Structural Genomics"

Feng Qiu – Molecular Neurodevelopment Lab (Mengqing Xiang)
"Gene Expression Profiling Analysis of Retinal Development"

Xuesong Ouyang – Molecular Oncology and Development Lab (Cory Abate-Shen)
"Discovery and Functional Analyses of Genes Involved in Prostate Carcinogenesis: A Microarray Approach"

12:00 PM **Lunch and Break**

3:00 PM **Posters**

4:00 PM **Session III – Pathways of Development and Disease**
Chair: Marianna de Julio – Mammalian Embryogenesis Lab (Michael Shen)

Jianhua Chu – Mammalian Embryogenesis Lab (Michael Shen)
"Non-cell-autonomous Role for Cripto in Axial Midline Formation During Vertebrate Embryogenesis"

Rym Benayed – Developmental Neurogenetics Lab (Jim Millonig)
"Support for the Homeobox Transcription Factor ENGRAILED 2 as an Autism Susceptibility Gene"

Kwi-Hye Kim – Protein Targeting Lab (Peter Lobel)
"Mouse Models to Evaluate Potential Therapeutics for Late Infantile Neuronal Ceroid Lipofuscinosis"

Yulia Frenkel – Biomolecular Crystallography Lab (Eddy Arnold)
"Hydrophobic Drug Aggregation: Drug Bioavailability and Aggregate Properties"
Alison Tuske – Viral Pathogenesis Lab (Arnold Rabson)
"Phenotypic Characterization of Lymphoid Cells Derived from HTLV-1 LTR-tax Transgenic Mice"

Brian Beaudoin – Viral Transformation Lab (Eileen White)
"Functional Hierarchy Among Death Programs: Defective Apoptosis Reveals Autophagy and Necrosis"

5:30 PM **Close**

19th Annual CABM Symposium

Stem Cells in Development and Disease

October 18, 2005

Sponsored by
The Center for Advanced Biotechnology & Medicine
and
The Child Health Institute of New Jersey
With support from
Bristol-Myers Squibb
Fisher Scientific International Inc.
Hoffmann-LaRoche Inc.
Merck Research Laboratories
Schering-Plough Research Institute
Wyeth Research

Program

8:15 am **Registration & Continental Breakfast** - CABM South Atrium

9:00 am **Welcoming Remarks**
Aaron J. Shatkin, Director, CABM

Session I

Chairperson: ***Francesco Ramirez,*** Director, Child Health Institute of New Jersey, UMDNJ-Robert Wood Johnson Medical School

9:15 am ***Allan Spradling,*** Carnegie Institution of Washington and HHMI
"*Drosophila stem cells as models of vertebrate stem cell function and dysfunction"*

10:00 am ***Linheng Li,*** Stowers Institute for Medical Research
"Cellular and molecular regulation of adult stem cells"

10:45 am **Coffee Break**

11:15 am ***Elaine Fuchs,*** Rockefeller University and HHMI
"Stem cells and their lineages in the skin"

12:00 noon ***Ihor Lemischka,*** Princeton University
"Molecular regulation of stem cell fates"

12:45 pm **Lunch (Ticket required)** - CABM Room 010
South Atrium

Session II

1:45 pm **Chairperson: Ira Black,** Acting Director, Stem Cell Institute of New Jersey; Chair, Neuroscience and Cell Biology, UMDNJ- Robert Wood Johnson Medical School

2:00 pm ***Michael Clarke***, University of Michigan
"Solid tumor stem cells"

2:45 pm ***George Daley,*** Harvard Medical School
"Stem cells in regenerative medicine and reproductive biology"

3:30 pm **Break**

3:45 pm ***Connie Cepko***, Harvard Medical School and HHMI
"Retinal progenitor and stem cell regulation"

4:30 pm ***Alejandro Sanchez-Alvarado,*** University of Utah School of Medicine and HHMI
"Stem cell and regeneration regulators in the planarian Schmidtea mediterranea*"*

5:15 pm ***Philip Beachy,*** The Johns Hopkins University School of Medicine and HHMI
"Hedgehog signaling in tissue repair, stem cell renewal, and neoplasia"

6:00 pm **Reception** - CABM South Atrium

UNDERGRADUATE AND GRADUATE STUDENTS, POSTDOCTORAL FELLOWS AND RESEARCH FACULTY (NON-TENURE TRACK) INCLUDING COMPETITIVE FELLOWSHIP AWARDS

CABM faculty members also participate in UMDNJ and Rutgers University graduate student rotation programs that place students in CABM labs each year in order to give them education and experience in a range of research methods and subjects.

Undergraduate Students

-Matthew Alter, Protein NMR Spectroscopy
-Gregg Barcan, Biomolecular Crystallography
-Mukta Baweja, Biomolecular Crystallography
-Sapna Bolikal, Molecular Chronobiology
-Kevin Bray, Viral Transformation
-Chen Xiao Chen, Protein NMR Spectroscopy
-Zhe Chen, Mammalian Embryogenesis
NJCCR Summer Fellowship
-Oliver Choo, Developmental Neurogenetics
-Usman Choudhry, Molecular Oncology and Development
-Melissa Ciano, Protein NMR Spectroscopy
-Coleen Cissosanti, Protein NMR Spectroscopy
-Drew Clark, Biomolecular Crystallography
-Arti Dabholkar, Lab Management
-Kathy Dao, Protein Targeting
-Fahima Dewan, Protein NMR Spectroscopy
-Pooja Dharia, Protein NMR Spectroscopy
-Nadishani Dissanayaka, Protein Targeting
-William Domm, Molecular Chronobiology
-Anna Dulencin, Protein Targeting
-Michael Fakhry, Protein NMR Spectroscopy
-Yvonne Farnacio, Molecular Oncology and Development
-Erin Folger, Mammalian Embryogenesis
-Arun Handa, Sleep Genetics
-Eriona Hysolli, Lab Management
-Sindhu Kadambi, Developmental Neurogenetics
-Hina Kapadia, Lab Management
-Neha Kaushik, Protein Crystallography
-Pushpa Keshav, Developmental Neurogenetics
-Tanuja Ko, Developmental Neurogenetics
-Kheng-Jim Lim, Molecular Oncology and Development
-Dan Marchalik, Molecular Virology
-Joel Marimuthu, Molecular Oncology and Development
-Elton Muci, Molecular Oncology and Development
-Aleksandra Nagorny, Biomolecular Crystallography
-Ebony Ortiz, Lab Management

-Jenna Pacheco, Lab Management
-Shruti Parikh, Lab Management
-Dipikaben Patel, Molecular Virology
-Shyam Patel, Viral Tansformation
  NJCCR Summer Fellowship
-Brian Radvansky, Protein NMR Spectroscopy
-Ramya Rao, Biomolecular Crystallography
-Abraham Rashin, Biomolecular Crystallography
-Boris Rozenfeld, Developmental Neurogenetics
-Mayank Shah, Developmental Neurogenetics
-Pavithra Sivakumar, Protein NMR Spectroscopy
-Xiaonan Sun, Developmental Neurogenetics
-Nora Taha, Developmental Neurogenetics
-Laura Tavarez, Administration
-Jessica Tippett, Molecular Oncology and Development
-Sam Tischfield, Developmental Neurogenetics
-James Tyminski, Biomolecular Crystallography
-Adarsh Yagnik, Molecular Oncology and Development
-Ayesha Zahiruddin, Lab Management

Graduate Students
-Diana Anderson, Viral Transformation
-Priti Bachhawat, Protein Crystallography
-Michael Baran, Protein NMR Spectroscopy
  GAANN Fellowship
-Joseph Bauman, Biomolecular Crystallography
  NIH Virus Host Training Grant
-Rym Benayed, Developmental Neurogenetics
  American Psychological Association Fellowship
-Aneerban Bhattacharya, Protein NMR Spectroscopy
-Tanya Borsuk, Developmental Neurogenetics
  New Jersey Commission on Spinal Cord Research Graduate Fellowship
-Wen-Feng Chen, Molecular Chronobiology
-Chun Chu, Molecular Virology
  NIH BioMaps Training Grant
-Jianhua Chu, Mammalian Embryogenesis
-Cecilia Della Valle, Protein Targeting
  NIH Supplement for Minorities
-Jigar Desai, Developmental Neurogenetics
-Sayali Dixit, Protein Crystallography
-Jui Dutta, Tumor Virology

-John Everett, Protein NMR Spectroscopy
  NIH Training Grant
-Gaofeng Fan, Tumor Virology
-Yulia Frenkel, Biomolecular Crystallography
  NIH Interdisciplinary Research Workforce Training Grant
-Jayita Guhaniyogi, Protein Crystallography
-Nupur Gupta, Tumor Virology
-Kangxin Jin, Molecular Neurodevelopment
-Silky Kamdar, Developmental Neurogenetics
-Kwi-Hye Kim, Protein Targeting
  Batten Disease Support & Research Association Fellowship
-Gregory Kornhaber, Protein NMR Spectroscopy
-Tzu-Hsing Kuo, Sleep Genetics
-Jung Eun Lee, Molecular Chronobiology
-Kwang Huei Low, Molecular Chronobiology
-Timothy Mack, Protein Crystallography
  NIH Biochemistry Training Grant
-Eduardo Perez, Protein Crystallography
  NIH Supplement for Minorities
-Anna Puzio, Molecular Oncology and Development
  NIH NRSA Predoctoral Fellowship
-Meiqian Qian, Protein Targeting
-Jayashree Rao, Molecular Oncology and Development
-Matthew Simmons, Tumor Virology
-David Snyder, Protein NMR Spectroscopy
  NIH Interdisciplinary Research Workforce Training Grant
-Victoria Swiss, Molecular Oncology and Development
  NIH Biochemistry Training Grant
-Tingting Tan, Viral Transformation
-Alejandro Toro, Protein Crystallography
  NIH NRSA Fellowship
-Xiongying Tu, Biomolecular Crystallography
-Thomas Vorrius, Mammalian Embryogenesis
-Nancy Vranich, Developmental Neurogenetics
  NJ Commission Spinal Cord Research Predoctoral Fellowship
-Guannan Wang, Growth and Differentiation Control
-Xi Wang, Mammalian Embryogenesis
-Evrim Yildirim, Molecular Chronobiology
-Cuifeng Yin, Protein NMR Spectroscopy
-Hailong Yu, Mammalian Embryogenesis

Postdoctoral Research Fellows

-Dr. Brian Benoff, Protein Crystallography
Howard Hughes Medical Institute
-Dr. Ramesh Chellappa, Molecular Neurodevelopment
-Dr. Canhe Chen, Mammalian Embryogenesis
-Dr. Yun Chen, Molecular Oncology and Development
-Dr. Joanna Chiu, Molecular Chronobiology
NIH NRSA Postdoctoral Fellowship
-Dr. Pragnya Das, Molecular Neurodevelopment
-Dr. Natalia Denissova, Protein NMR Spectroscopy
-Dr. Kyriakos Economides, Molecular Oncology and Development
NIH NRSA Postdoctoral Fellowship
-Dr. Yongjun Fan, Tumor Virology
-Dr. Hui Gao, Molecular Oncology and Development
-Dr. Daniel Himmel, Biomolecular Crystallography
NIH NRSA Postdoctoral Fellowship
-Dr. Marianna de Julio, Mammalian Embryogenesis
DOD Postdoctoral Fellowship
-Dr. Shih-hsin Kan, Mammalian Embryogenesis
DOD Postdoctoral Fellowship
-Dr. Vassiliki Karantza, Viral Transformation
-Dr. Marinela Cristina Karp, Viral Transformation
-Dr. Hyuk Wan Ko, Molecular Chronobiology
-Dr. Ning Lei, Mammalian Embryogenesis
-Dr. Shengguo Li, Molecular Neurodevelopment
-Dr. Edward Licitra, Viral Transformation
-Dr. Heng-Ling Liou, Protein Targeting
-Dr. Donglin Liu, Molecular Virology
New Jersey Commission on Cancer Research Fellowship
-Dr. Huijun Lo, Molecular Neurodevelopment
-Dr. Robin Mathew, Viral Transformation
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-Dr. Zeqian Mo, Molecular Neurodevelopment
-Dr. Devi Mukherjee, Viral Transformation
Howard Hughes Medical Institute
-Dr. Feng Qiu, Molecular Neurodevelopment
-Dr. David Sidote, Protein Crystallography
Howard Hughes Medical Institute
-Dr. Yu Tian, Protein Targeting
Batten Disease Support & Research Association Postdoctoral Fellowship
-Dr. Steven Tuske, Biomolecular Crystallography
-Dr. Xi Wang, Mammalian Embryogenesis
-Dr. Yanhong Wang, Protein Targeting
-Dr. Sujuan Xu, Protein Crystallography

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Thomas Acton-Assistant Research Professor, Molecular Biology & Biochemistry (MBB), RU
James Aramini-Assistant Research Professor, MBB, RU
Gail Arnold-Research Professor, Chemistry, RU
Kalyan Das-Associate Research Professor, Chemistry, RU
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Isao Matsuura-Research Associate, Chemical Biology, RU
Hunter Moseley-Assistant Research Professor, MBB, RU
Ferez Nallaseth-Research Associate, MBB, RU
Deirdre Nelson-Research Associate, MBB, RU; HHMI
Wilberto Nieves-Neira, M.D.-Assistant Professor, Viral Transformation, RU
Deena Oren-Assistant Research Professor, Chemistry, RU
Xuesong Ouyang-Adjunct Instructor, Medicine, UMDNJ-RWJMS
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Stefan Sarafianos-Associate Research Professor, Chemistry, RU
David Sleat-Research Associate Professor, Pharmacology, UMDNJ-RWJMS
Li Zhao, Research Assistant, MBB, RU

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PATENTS

Anderson, S., Lazarus, R. A., Hurle, M., Anderson, S., and Powers, D. B. "Enzymes for the production of 2-keto-L-gulonic acid" U. S. Patent No. 5,376,544

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Anderson, S. "Methods for identifying useful t-PA mutant derivatives for treatment of vascular hemorrhaging" U. S. Patent No. 6,136,548

Anderson, S., and Banta, S. "Design and production of mutant 2,5-diketo-D-gluconic acid reductase enzymes with altered cofactor dependency" U. S. Patent No. 6,423,518

Anderson, S. "Methods for the prevention or treatment of Alzheimer's disease" U. S. Patent No. 6,471,960

Anderson, S. "Methods for identifying useful t-PA mutant derivatives for treatment of vascular hemorrhaging" U. S. Patent No. 6,136,548

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White E., Strair, R., Rabson, A.B. and D. Medina. "Cytotoxic agents for the selective killing of lymphoma and leukemia cells and methods of use thereof" (pending)

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Scolnick, Edward M., M.D. President, Merck & Co. Inc. (retired), '95 - '97
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* Sharp, Phillip, Prof. & Dir., Ctr. for Cancer Res., MIT, '90-'93
Tilghman, Shirley M., Prof. , Princeton University (Pres. Princeton U.), '01
Turner Mervyn J., Senior V.P. Merck & Co., '01-'05
Vagelos, P. Roy, Chairman & CEO, Merck & Co., (retired), '86-'94
Wilson, Robert, Vice Chairman, Board of Directors, J & J, (retired), '91-'95

* Nobel Laureate

Fiscal Information

CENTER FOR ADVANCED BIOTECHNOLOGY AND MEDICINE
GRANTS AND CONTRACTS SUMMARY
FY 2005: JULY 1, 2004 - JUNE 30, 2005

	DIRECT COSTS	INDIRECT COSTS	TOTAL COSTS
FEDERAL	14,573,440	3,619,158	18,192,598
FOUNDATION	2,027,741	513,522	2,541,263
INDUSTRY	311,333	106,100	417,433
STATE	395,184	26,546	421,730
UNIVERSITY	13,261	0	13,261
TOTAL	17,320,959	4,265,326	21,586,285

Date: January 20, 2006

CABM Grants and Contracts
FY 2005: 7/01/2004 - 6/30/2005

Prepared:1/18/06

		Total Award	Start - End	FY 2005 Direct Costs	FY 2005 Indirect Costs	FY 2005 Total Costs
Abate-Shen UMDNJ						
Federal	6	$8,033,226		$1,191,736	$437,760	$1,629,496
Department of Defense						
Isolation of Target Genes for Nkx3.1 in Prostate Carcinogenesis (Postdoctoral Fellowship Award for Dr. Xuesong Ouyang)		$98,000	3/1/03 3/30/05	$21,007	$11,659	$32,666
The Role of Hoxb13 and Nkx3.1 in Prostate Development and Carcinogenesis (Postdoctoral Fellowship Award for Dr. Kyriakos Economides)		$125,000	10/1/04 9/30/06	$30,145	$16,730	$46,875
NIH						
Mouse Models of Ovarian Cancer (NRSA Predoctoral Fellowship Award for Anna Puzio)		$88,070	9/1/04 8/31/07	$24,565	$0	$24,565
Roles of Nkx3.1 in Prostate Development and Cancer		$1,644,415	7/1/03 6/30/08	$211,500	$117,383	$328,883
A Mouse Model for Prostate Cancer (Mouse Models of Human Cancers Consortium Project)		$4,485,615	4/1/04 3/31/09	$679,519	$175,618	$855,137
Roles for MSX1 in Vertebrate Embryogenesis		$1,592,126	4/1/03 11/30/07	$225,000	$116,370	$341,370
Foundation	2	$20,000		$6,666	$0	$6,666
Foundation of UMDNJ						
Postdoctoral Stipend - Xuesong Ouyang		$10,000	3/1/03 2/28/05	$3,333	$0	$3,333
Postdoctoral Stipend - Kyriakos Economides		$10,000	11/1/04 10/31/06	$3,333	$0	$3,333
Total - Abate-Shen	**8**	**$8,053,226**		**$1,198,402**	**$437,760**	**$1,636,162**

		Total Award	Start - End		FY 2005 Direct Costs	FY 2005 Indirect Costs	FY 2005 Total Costs
Arnold **Rutgers**							
Federal	5	$10,337,663			$1,455,034	$586,665	$2,041,699
NIH							
Bisphosphonate Inhibitors of NRTI Excision		$765,034	2/1/04	1/31/09	$94,950	$52,697	$147,647
NRSA Postdoctoral Fellowship - Steven Tuske		$118,000	8/6/01	8/5/04	$3,868	$0	$3,868
Structures of HIV Reverse Transcriptase with Substrates (MERIT Award)		$4,258,110	2/15/04	1/31/09	$527,331	$290,241	$817,572
Solvent Modeling for HIV RT Drug Design (NRSA Postdoctoral Fellowship Award for Dr. Daniel Himmel)		$153,968	3/8/04	3/7/07	$49,801	$0	$49,801
Structure-Based Design of Drugs and Vaccines for Targeting AIDS (Program Project)		$5,042,551	8/16/02	7/31/07	$779,084	$243,727	$1,022,811
Foundation	1	$1,890,000			$100,000	$0	$100,000
Janssen Foundation							
HIV Reverse Transcriptase Interactions with TIBO Inhibitors		$1,890,000	7/1/91	12/31/04	$100,000	$0	$100,000
Industrial	3	$633,700			$237,333	$106,100	$343,433
Merck							
HIV-1 Reverse Transcriptase Structures		$155,500	12/1/04	11/30/05	$58,333	$32,375	$90,708
Evaluating Chimeric Human Rhinovirus (HRV)		$373,200	9/15/04	2/14/07	$95,000	$52,725	$147,725

			Total Award	Start - End		FY 2005 Direct Costs	FY 2005 Indirect Costs	FY 2005 Total Costs
Ribapharm Inc.								
HIV-1 Reverse Transcriptase Structures with Bound Non-Nucleoside Inhibitors			$105,000	7/1/04	6/30/05	$84,000	$21,000	$105,000
Total - Arnold		**9**	**$12,861,363**			**$1,792,367**	**$692,765**	**$2,485,132**
Edery	**Rutgers**							
Federal		3	$3,808,268			$483,333	$243,669	$727,002
NIH								
Clock Mechanism Underlying Drosophila Rhythmic Behavior			$1,400,600	1/15/04	12/31/07	$231,250	$119,180	$350,430
Seasonal Adaptation of a Circadian Clock			$1,356,318	3/1/05	2/28/09	$77,083	$37,067	$114,150
Seasonal Adaptation of a Circadian Clock			$1,051,350	7/15/01	2/28/05	$175,000	$87,422	$262,422
Total - Edery		**3**	**$3,808,268**			**$483,333**	**$243,669**	**$727,002**
Gélinas	**UMDNJ**							
Federal		2	$2,560,411			$330,000	$177,428	$507,428
NIH								
Functional Analysis of Bfl-1/A1 in the NF-kB Signaling Pathway, Apoptosis and Oncogenesis			$1,232,725	7/1/00	6/30/05	$157,500	$84,545	$242,045
Trans-acting Function of the v- and c-Rel Oncoproteins			$1,327,686	7/1/01	6/30/06	$172,500	$92,883	$265,383
Total - Gélinas		**2**	**$2,560,411**			**$330,000**	**$177,428**	**$507,428**
Liu	**Rutgers**							

		Total Award	Start - End		FY 2005 Direct Costs	FY 2005 Indirect Costs	FY 2005 Total Costs
Federal	2	$1,768,298			$266,250	$144,811	$411,061
Department of Defense							
TGE-beta Resistance on Breast Cancer		$466,500	7/1/03	6/30/06	$100,000	$55,500	$155,500
NIH							
Role of Smad Proteins in Cell Growth Regulation		$1,301,798	12/14/01	11/30/06	$166,250	$89,311	$255,561
Total - Liu	**2**	**$1,768,298**			**$266,250**	**$144,811**	**$411,061**
Lobel UMDNJ							
Federal	3	$3,884,360			$1,001,686	$271,482	$1,273,168
NIH							
Maldi TOF TOF Mass Spectrometer		$500,000	5/1/05	4/30/06	$500,000	$0	$500,000
Novel Lysosomal Enzymes and Associated Human Genetic Diseases		$1,507,795	5/1/03	3/31/07	$244,893	$128,962	$373,855
Novel Lysosomal Enzyme Deficient in Batten Disease		$1,876,565	4/1/03	3/31/08	$256,793	$142,520	$399,313
Foundation	6	$895,500			$208,094	$12,500	$220,594
Ara Parseghian Medical Research Foundation							
Molecular Characterization Niemann-Pick C2 Disease		$625,000	7/1/01	6/30/06	$112,500	$12,500	$125,000
Batten Disease Support & Research Assoc.							
Substrate Specificity of Tripeptidyl Peptidase I (Postdoctoral Fellowship Award for Dr. Yu Tian)		$120,000	8/20/03	8/19/06	$40,000	$0	$40,000

		Total Award	Start - End	FY 2005 Direct Costs	FY 2005 Indirect Costs	FY 2005 Total Costs
Evaluation of potential therapeutic targets for classic late infantile neuronal ceroid lipofuscinosis (Predoctoral Fellowship Award for Kwi-Hye Kim)		$45,000	9/1/04 8/31/06	$18,750	$0	$18,750
Foundation of UMDNJ						
Postdoctoral Stipend: Heng-Ling Liou		$10,000	3/1/03 2/28/05	$3,333	$0	$3,333
Postdoctoral Stipend - Yu Tian		$15,000	9/1/03 8/30/06	$5,000	$0	$5,000
National Niemann-Pick Disease Foundation						
The Role of NPC2 Protein in Lysosomal Cholesterol Trafficking (Postdoctoral Fellowship Award for Dr. Heng-Ling Liou)		$80,500	3/15/03 3/14/05	$28,511	$0	$28,511
Total - Lobel	**9**	**$4,779,860**		**$1,209,780**	**$283,982**	**$1,493,762**
Millonig UMDNJ						
Federal	1	$25,000		$25,000	$0	$25,000
NIH						
Environmental influences of autism spectrum disorder (NIEHS - EOHSI Pilot Project)		$25,000	4/1/04 3/31/05	$25,000	$0	$25,000
Foundation	3	$415,000		$124,494	$8,006	$132,500
National Alliance for Autism Research						
Studying Mouse Cerebellar Development as a Means of Identifying Autism Susceptibility Genes		$120,000	7/1/03 6/30/05	$54,545	$5,455	$60,000
Genetic analysis of the homeobox transcription factor, ENGRAILED 2, in autism spectrum disorders		$70,000	7/1/04 6/30/06	$35,000	$0	$35,000

		Total Award	Start - End		FY 2005 Direct Costs	FY 2005 Indirect Costs	FY 2005 Total Costs
Whitehall Foundation, Inc.							
A Transcription Factor Code for Dorsal CNS Development		$225,000	1/1/02	12/31/04	$34,949	$2,551	$37,500
State	4	$696,151			$269,275	$17,455	$286,730
NJ Commission on Spinal Cord Research							
The development of spinal cord therapies through a genetic analysis of mouse spinal cord development		$399,967	7/1/04	6/30/06	$182,545	$17,455	$200,000
Spinal cord proliferation & differentiation: the role of neural tube closure (Predoctoral Fellowship Award for Nancy Vranich)		$60,000	7/1/04	6/30/06	$30,000	$0	$30,000
NJ Governor's Council on Autism							
Functional Analysis of the ENGRAILED2 Pathway in Autistic Individuals		$48,928	7/1/04	6/30/05	$48,928	$0	$48,928
Association Analysis of Genes in Engrailed 2 Pathway		$187,256	6/1/05	5/31/07	$7,802	$0	$7,802
University	1	$1,500			$1,500	$0	$1,500
Rutgers University							
Undergraduate Research Fellows Program		$1,500	7/1/04	6/30/05	$1,500	$0	$1,500
Total - Millonig	**9**	**$1,137,651**			**$420,269**	**$25,461**	**$445,730**
Montelione Rutgers							
Federal	3	$34,209,284			$8,235,752	$989,577	$9,225,329
NIH							
Acquisition of 800 MHz NMR System with Cryogenic Probe		$760,000	7/1/04	6/30/05	$760,000	$0	$760,000

		Total Award	Start - End	FY 2005 Direct Costs	FY 2005 Indirect Costs	FY 2005 Total Costs
Structural Genomics of Eukaryotic Model Organisms (Northeast Structural Genomics Consortium Project)		$33,060,534	9/30/00 8/31/05	$7,288,252	$885,515	$8,173,767
Structural Genomics of Eukaryotic Model Organisms - Conference Supplement		$388,750	9/1/02 8/31/05	$187,500	$104,062	$291,562
Total - Montelione	**3**	**$34,209,284**		**$8,235,752**	**$989,577**	**$9,225,329**
Shatkin UMDNJ						
Industrial	1	$74,000		$74,000	$0	$74,000
Pharmaceutical Companies						
CABM Symposium & Lecture Series		$74,000	7/1/04 6/30/05	$74,000	$0	$74,000
University	1	$11,761		$11,761	$0	$11,761
Rutgers University						
Computer-Based Enhancement of CABM Teaching Capabilities		$11,761	7/1/04 6/30/05	$11,761	$0	$11,761
Total - Shatkin	**2**	**$85,761**		**$85,761**	**$0**	**$85,761**
Shen UMDNJ						
Federal	3	$4,697,219		$601,875	$331,958	$933,833
NIH						
EGF-CFC Genes in Mammalian Left-Right Axis Determination		$1,561,092	4/1/00 3/31/05	$151,875	$83,193	$235,068
Functional Analysis of Cripto in Mouse Embryogenesis		$1,744,380	4/1/02 3/31/07	$225,000	$124,875	$349,875
Growth Factor Signaling in Prostate Development and Cancer		$1,391,747	9/30/01 6/30/05	$225,000	$123,890	$348,890

		Total Award	Start - End		FY 2005 Direct Costs	FY 2005 Indirect Costs	FY 2005 Total Costs
Total - Shen	**3**	**$4,697,219**			**$601,875**	**$331,958**	**$933,833**
Stock **UMDNJ**							
Federal	2	$1,735,476			$277,484	$115,052	$392,536
NIH							
Structure and Function of Response Regulator Proteins (MERIT Award)		$1,642,788	7/1/03	6/30/08	$246,454	$115,052	$361,506
Mechanisms of Activation of Response Regulator Proteins (NRSA Predoctoral Fellowship Award for Alejandro Toro)		$92,688	9/1/03	8/31/06	$31,030	$0	$31,030
Foundation	2	$8,452,872			$802,701	$239,455	$1,042,156
Ara Parseghian Medical Research Foundation							
Structural Analysis of NPC2		$280,556	1/1/03	6/30/05	$101,136	$10,670	$111,806
Howard Hughes Medical Institute							
Howard Hughes Medical Institute Investigatorship		$8,172,316	8/1/94	8/31/05	$701,565	$228,785	$930,350
Total - Stock	**4**	**$10,188,348**			**$1,080,185**	**$354,507**	**$1,434,692**
White **Rutgers**							
Federal	1	$1,879,354			$305,144	$103,419	$408,563
NIH							
Function of the Adenovirus E1B Oncogene		$1,879,354	7/1/01	6/30/06	$305,144	$103,419	$408,563
Foundation	1	$7,221,996			$785,786	$253,561	$1,039,347

		Total Award	Start - End	FY 2005 Direct Costs	FY 2005 Indirect Costs	FY 2005 Total Costs
Howard Hughes Medical Institute						
Howard Hughes Medical Institute Investigatorship		$7,221,996	5/18/98 8/31/05	$785,786	$253,561	$1,039,347
State	1	$35,000		$35,000	$0	$35,000
NJ Commission on Cancer Research						
Mitotic Checkpoint Function and Cancer Sensitivity (Postdoctoral Fellowship Award for Dr. Robin Mathew)		$35,000	6/1/04 5/31/05	$35,000	$0	$35,000
Total - White	**3**	**$9,136,350**		**$1,125,930**	**$356,980**	**$1,482,910**
Xiang UMDNJ						
Federal	3	$3,571,146		$400,146	$217,337	$617,483
NIH						
Role of the Foxn4 Gene during Retinogenesis		$801,872	5/1/05 4/30/08	$29,167	$15,413	$44,580
Transcriptional Regulation of Retinal Development		$1,723,335	9/30/02 7/31/07	$225,000	$119,667	$344,667
Roles of Homeodomain Factors in Neural Development		$1,045,939	8/1/01 5/31/05	$145,979	$82,257	$228,236
State	1	$400,000		$90,909	$9,091	$100,000
NJ Commission on Spinal Cord Research						
Role of Foxn4 in Spinal Cord Development and Regeneration		$400,000	12/15/04 12/14/06	$90,909	$9,091	$100,000
Total - Xiang	**4**	**$3,971,146**		**$491,055**	**$226,428**	**$717,483**

	Total Award	Start - End	FY 2005 Direct Costs	FY 2005 Indirect Costs	FY 2005 Total Costs
CABM GRAND TOTALS	**$97,257,185**		**$17,320,959**	**$4,265,326**	**$21,586,285**