Center for Advanced Biotechnology and Medicine
http://cabm.umdnj.edu

2006 Report
An Advanced Technology Center
of the
New Jersey Commission on Science and Technology
Jointly Administered
By
Rutgers, The State University of New Jersey
and by the
University of Medicine and Dentistry of New Jersey
CONTENTS

Director’s Overview 3
Research Programs and Laboratories 5

Cell & Developmental Biology 6
  Molecular Oncology & Development  Cory Abate-Shen  7
  Molecular Chronobiology  Isaac Edery  11
  Tumor Virology  Céline Gélinas  13
  Growth & Differentiation Control  Fang Liu  17
  Protein Targeting  Peter Lobel  19
  Developmental Neurogenetics  James Millonig  22
  Mammalian Embryogenesis  Michael Shen  26
  Viral Transformation  Eileen White  28
  Sleep Genetics  Julie Williams  31
  Molecular Neurodevelopment  Mengqing Xiang  32

Structural Biology 34
  Biomolecular Crystallography  Eddy Arnold  35
  Structural Bioinformatics  Gaetano Montelione  39
  Protein Design and Evolution  Vikas Nanda  42
  Protein Crystallography  Ann Stock  44

Molecular Genetics 47
  Protein Engineering  Stephen Anderson  48
  Viral Pathogenesis  Arnold Rabson  50
  Molecular Virology  Aaron Shatkin  53

Education, Training and Technology Transfer 55

Lectures and Seminars 56
  CABM Lecture Series  57
  Annual Retreat  58
  20th Annual Symposium  60

Undergraduate & Graduate Students, Postdoctoral Fellows 63
  and Research Faculty (Non-tenure Track)
Administrative, Support and Technical Staff 68
  Patents 69

Scientific Advisory Board 71
  Current Members  72
  Past Members  73

Fiscal Information 75

Grants and Contracts 76
**Director’s Overview**

This was another productive, event-filled year at CABM and particularly noteworthy as it marked the Center's twentieth anniversary. The dedication of CABM faculty, students and staff remains stronger than ever in our mission to advance basic knowledge in the life sciences to improve human health. CABM researchers produced important original findings on the structure/function of key molecules and the interactions that control cell growth and early development. Elucidation of these processes has advanced our understanding of causative events underlying autism, cancer, neurodegenerative disorders, HIV/AIDS and other infectious diseases. In an effort to discover more effective therapies, CABM scientists are also fostering "bench to bedside" translation of their findings by partnering with colleagues at the Cancer Institute of NJ as well as other academic institutions and pharmaceutical companies.

CABM discoveries have opened new approaches to many challenging diseases. For example, CABM researchers have developed a mouse model that mimics human prostate cancer and are using it to optimize patient treatment and prevention. Another group is defining the molecular mechanisms of programmed cell death and is devising creative ways to restore this process in tumor cells as a way to eliminate them. Others are determining the role of inflammation and factors that regulate cell signaling and gene expression in cancer. Autism, neural development and neurodegenerative diseases of children are the focus of several CABM laboratories. An important breakthrough in understanding the genetic basis of autism was the recent discovery that a form of the Engrailed 2 gene is an autism susceptibility gene. Control of the formation of sight and hearing during early development has been defined at the level of specific transcription factors. The genetic bases of Batten disease and Niemann-Pick C2 disease, both fatal in childhood, were solved by a proteomics approach, making genetic counseling possible. Groundbreaking structural work, including molecular modeling, has given insights into protein design and evolution and helped to produce several drugs that are in clinical trials for HIV/AIDS. Structural approaches have also uncovered promising targets for developing new antibiotics and anti-virals to treat bacterial, influenza and hepatitis C virus infections. Biological clocks are important for maintaining homeostasis, and CABM investigators have deciphered molecular determinants of accurate timekeeping in a model system, results that impact on sleep disorders and suggest a genetic linkage between sleep and immunity.

Research progress depends on committed people, excellent facilities and long-term, dependable funding. We appreciate the continuing support of Rutgers University and the University of Medicine and Dentistry of NJ, our two sponsors that jointly administer CABM. In 2006 CABM investigators received $22 million in research grants and contracts, with a twenty-year total of more than $240 million. We are extremely grateful for this generous and essential funding provided by many public and private sources. They include the National Institutes of Health (NIH), Department of Defense, NJ Commission on Science & Technology, NJ Commission on Cancer Research, NJ Governor's Council on Autism, NJ Commission on Spinal Cord Research, the Howard Hughes Medical Institute, Johnson & Johnson, Merck Research Laboratories, Schering-Plough, Sanofi Aventis, the Ara Parseghian Medical Research Foundation, Batten Disease Support and Research Association, National Niemann-Pick Disease Foundation, The Cancer Institute of NJ, Foundation of UMDNJ, National Alliance for Autism Research, Autism Speaks and the American Psychological Association.
CABM tenured and tenure-track faculty now total eighteen with the appointment of Assistant Professor Joseph Marcotrigiano in the Rutgers Department of Chemistry and Chemical Biology. Dr. Marcotrigiano, a NJ native and Rutgers graduate, established an outstanding record of accomplishments as a graduate student and postdoctoral fellow at the Rockefeller University. Renewal of ideas and people is important to success in all human endeavors, and Dr. Marcotrigiano adds new strength to CABM research and teaching expertise in virology and protein X-ray crystallography.

CABM faculty are actively engaged in teaching graduate, medical and postgraduate students as well as undergraduates in both the laboratory and the lecture hall. The undergraduate students working in CABM laboratories presented their research results at the CABM Annual Retreat held in June at Cook College. Some of these students were selected to participate in the J&J-sponsored CABM Summer Scholars Research Program. In 2006 more than 125 CABM trainees worked and learned at the scientific frontiers in preparation for leadership roles in science and medicine. CABM faculty take special pride in the many hundreds of trainees who have gone on to independent positions in academia, biomedicine, and the pharmaceutical and biotechnology industry. These former students are well trained, highly competitive and represent one of CABM’s most important contributions to the future of science and economic growth.

The CABM twentieth anniversary was celebrated by the more than 200 participants attending the 20th Annual CABM Symposium held October 17th and 18th on the topic "Emerging Strategies in Molecular Medicine". The opening lecture was by MIT Professor and Nobel Laureate Phillip Sharp, and the meeting closed with a presentation by Dr. P. Roy Vagelos, retired Chairman of Merck & Co. and the first Chairman of the CABM Scientific Advisory Board. As part of the festivities, Dr. James Wells, Hind Professor in the Pharmaceutical Sciences at the University of California–San Francisco, received the 8th Paul Janssen Prize in Advanced Biotechnology and Medicine for his contributions in protein engineering. In another outreach program the CABM Lecture Series, sponsored again this year by Sanofi Aventis, brought to our scientific community leading investigators who described their latest findings and provided overviews of recent advances in their fields.

Despite great scientific progress, CABM, like all research and teaching institutions, is facing significant challenges precipitated by the limited funding available from NIH and other governmental sources. With the combined resources generated from patents and licensing, gifts, grants and contracts and the generous support of our corporate partners, we remain optimistic about the future of science and determined to find new paths of excellence in the pursuit of the CABM mission.

Aaron J. Shatkin, Professor and Director

Shatkin@cabm.rutgers.edu
http://cabm.umdnj.edu

Center for Advanced Biotechnology and Medicine
Research Programs and Laboratories
## Cell & Developmental Biology

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Faculty Director</th>
<th>Room</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Oncology &amp; Development</td>
<td>Cory Abate-Shen</td>
<td>7</td>
</tr>
<tr>
<td>Molecular Chronobiology</td>
<td>Isaac Edery</td>
<td>11</td>
</tr>
<tr>
<td>Tumor Virology</td>
<td>Céline Gélinas</td>
<td>13</td>
</tr>
<tr>
<td>Growth &amp; Differentiation Control</td>
<td>Fang Liu</td>
<td>17</td>
</tr>
<tr>
<td>Protein Targeting</td>
<td>Peter Lobel</td>
<td>19</td>
</tr>
<tr>
<td>Developmental Neurogenetics</td>
<td>James Millonig</td>
<td>22</td>
</tr>
<tr>
<td>Mammalian Embryogenesis</td>
<td>Michael Shen</td>
<td>26</td>
</tr>
<tr>
<td>Viral Transformation</td>
<td>Eileen White</td>
<td>28</td>
</tr>
<tr>
<td>Sleep Genetics</td>
<td>Julie Williams</td>
<td>31</td>
</tr>
<tr>
<td>Molecular Neurodevelopment</td>
<td>Mengqing Xiang</td>
<td>32</td>
</tr>
</tbody>
</table>
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.

Overview

I have had a long-standing interest in understanding the relationship between the processes that control normal development and those that lead to cancer. When I established my laboratory 15 years ago, I initiated a research program on vertebrate homeobox genes, which encode transcriptional regulatory proteins that are essential developmental regulators and are frequently deregulated in cancer. Over the years, my laboratory has taken a multi-disciplinary approach to investigate how homeobox genes regulate transcription during development, as well as how their aberrant expression contributes to tumorigenesis. This work has led to the generation of mouse models of human prostate cancer, which have provided new insights regarding prostate tumorigenesis, as well as pre-clinical models for chemoprevention and chemotherapy. In parallel, our investigations of the functions of homeobox genes in development have provided new insights regarding the fundamental problem of how homeoprotein transcription factors achieve target recognition in vivo during murine embryogenesis. Below, I discuss the research in my laboratory focused on these two general areas.

Modeling cancer in mice

Our entry into the generation of mouse models of cancer stemmed from our identification of the Nkx3.1 homeobox gene as a key regulator of prostate development and prostate epithelial differentiation. In collaboration with Dr. Michael Shen (Center for Advanced Biotechnology and Medicine, UMDNJ-Robert Wood Johnson Medical School), we found that Nkx3.1 is the earliest known marker of prostate epithelial differentiation and that its loss of function in mice leads to defects in prostate development and predisposes to prostate cancer. NKK3.1 is located on a region of human chromosome 8p21 that is frequently deleted in prostate cancer and, although NKK3.1 is not inactivated by mutation, its loss of protein expression is a hallmark of cancer progression in human prostate cancer as well as in mouse models of the disease. Our investigations of Nkx3.1 have provided a model for investigating the relationship between prostate epithelial differentiation and cancer initiation. For example, we found that defects in prostate epithelial differentiation in the Nkx3.1 mutant mice lead to increased susceptibility to oxidative damage coincident with the formation of pre-neoplastic lesions.
Although not sufficient for overt tumorigenesis in mice, *Nkx3.1* inactivation cooperates with loss of function of other tumor suppressor genes, particularly the *Pten* tumor suppressor gene, to promote prostate cancer. We have developed a series of mouse models based on combinatorial loss of function of *Nkx3.1* and *Pten*, which recapitulate the various stages of human prostate cancer, including prostate intraepithelial neoplasia (PIN), adenocarcinoma, and androgen-independent disease. These models have provided novel insights regarding mechanisms of prostate tumorigenesis and have enabled us to investigate pathways of prostate cancer using gene expression profiling. For example, we have found the AP1 transcriptional regulatory proteins, Fos and Jun, are up-regulated during prostate cancer progression in the *Nkx3.1; Pten* mutant mice and, in collaboration with Dr. William Gerald (Memorial Sloan-Kettering Cancer Center), that Fos and Jun are excellent predictors of survival in human prostate cancer. Our findings provide a means of stratifying patients at high risk for aggressive prostate cancer, and exemplify the value of cross-species comparisons for identifying novel biomarkers for human cancer.

While individuals diagnosed at early stages of prostate cancer now have an excellent chance of survival, most patients that progress to androgen-independent (hormone-refractory) stages eventually succumb to the disease. We have found that *Nkx3.1; Pten* mutant mice model salient features of androgen-independence in humans and, thus, enable investigations of androgen-independence within the context of the prostate microenvironment. In particular, our analyses of the emergence of androgen-independence in these mutant mice revealed that prostate cells can acquire the ability to survive in the absence of androgens long before they develop overt cancer phenotypes, which has significant implications for the treatment of prostate cancer using anti-androgen therapies. Furthermore, we demonstrated that the Akt/PI3 kinase and Raf/MEK/Erk MAP kinase signaling pathways act additively in culture, but synergistically in vivo to promote androgen-independence, which highlights the importance of investigating androgen-independence in the context of the prostate microenvironment. Notably, our findings prompted us to ask whether simultaneous inhibition of Akt/PI3 kinase and Raf/MEK/Erk MAP kinase pathways coincident with androgen ablation can prevent the emergence of androgen-independent tumors, and we now have promising data from pre-clinical studies in the *Nkx3.1; Pten* mutant mice to support this idea. We are working with Drs. Robert DiPaola and Eric Rubin (Cancer Institute of New Jersey) to translate these pre-clinical studies to clinical trials for patients with advanced prostate cancer.

In parallel with our analyses of these well-characterized *Nkx3.1; Pten* mutant mice, we are refining our mouse models to more closely recapitulate key features of human prostate cancer. In this regard, we have benefited from our collaboration with Dr. Michael Shen, whose group has developed conditional and inducible Cre alleles, which enable the spatial and temporal regulation of gene recombination in the prostatic epithelium. We are using these Cre alleles to investigate the consequences of sporadic deletion in the prostatic epithelium of *Nkx3.1* and *Pten* in combination with activatable K-Ras or B-Raf alleles (the latter in collaboration with Dr. Martin McMahon, UCSF Cancer Center). We envision that these new models will improve the “predictive utility” of our pre-clinical studies in mice for the design of clinical trials in humans. Undoubtedly, the best way to eradicate cancer is to delay or prevent its initial occurrence, and I believe that a particularly valuable, though surprisingly under-utilized, benefit of autochthonous
mouse models of cancer is their potential to provide mechanistic insights and in vivo models for cancer prevention. As proof of principle, we investigated the efficacy of Vitamin D₃ for chemoprevention of prostate cancer in the Nkx3.1; Pten mutant mice, in collaboration with Dr. Robert DiPaola, and found that the timing of its delivery relative to cancer progression is instrumental in predicting its efficacy for cancer prevention. Notably, while epidemiological studies have strongly implicated Vitamin D as a chemopreventive agent for prostate cancer prevention, most clinical trials have been performed on patients with advanced prostate cancer and have not been very promising. Our findings have prompted Dr. Robert DiPaola and his colleagues to initiate new clinical trials to investigate the efficacy of Vitamin D in patients with early-stage prostate cancer.

Although still a major health problem, improved methods for early detection and more effective treatment options have led to a decline in mortality from prostate cancer. Unfortunately, this is not the case for bladder cancer, for which the mortality rate has consistently increased in the past 10 years. My laboratory has recently developed a strategy using an adenovirus-expressing Cre to achieve sporadic recombination specifically in the bladder urothelium, the tissue layer that gives rise to both the invasive and non-invasive forms of the disease. Using this approach, we have found that deletion of both p53 and Pten result in invasive bladder cancer by 4 months of age with 100% penetrance. We are now collaborating with Dr. Carlos Cordon-Cardo (Memorial Sloan-Kettering Cancer Center) to investigate the relevance of p53 and Pten inactivation for invasive bladder cancer in humans. This new mouse model will provide a valuable resource for investigating the molecular mechanisms underlying invasive bladder cancer, as well as an excellent model for chemoprevention and chemotherapeutic preclinical studies.

Transcriptional regulation in development

Although a major emphasis of my research is now focused on modeling cancer in mice, I have a long-standing interest in transcriptional regulation during differentiation, and have been particularly interested in understanding how transcription factors are able to selectively interact with target genes in vivo. Our studies have focused on the Msx1 homeoprotein, which is expressed in undifferentiated progenitor cells during development and functions as a transcriptional repressor to negatively regulate differentiation. We found that Msx1 selectively regulates MyoD during muscle cell differentiation through a mechanism that requires its interaction with the linker histone, H1 as well as with the E3 SUMO ligase PIAS1. These proteins guide Msx1 to the nuclear periphery where the MyoD gene is located in undifferentiated muscle progenitor cells. Our studies uncovered a hitherto unappreciated role of sub-nuclear “address” for regulating the specific interactions of homeoproteins with target genes in vivo, which may be of broader relevance for regulating target gene specificity of other families of transcription factors.

We have found that in muscle cells Msx1 exists as a large multi-protein complex that includes histone H1 and PIAS1 as well as several other proteins. To investigate the functional relevance of this complex, we have generated a series of mutated Msx1 proteins that selectively disrupt individual protein interactions and/or functions of Msx1. We are constructing a molecular toolbox of Msx1 knock-in alleles that express these various mutant proteins in restricted domains during mouse development. This Msx1 allelic series will enable us to dissect the consequences of these individual interactions or activities for regulating differentiation and for repression of...
target genes in vivo. In particular, we are using these mice to identify genomic binding sites for Msx1 in vivo and to investigate the sub-nuclear location of these target sequences relative to that of Msx1, an approach we have called “FISH and ChIPs”. I believe that this general approach of dissecting biochemical activities in the context of the whole organism is essential to understand the complexity of homeoprotein transcriptional control in development and cancer, and will provide a paradigm for similar investigations of other transcriptional regulators.

Future directions

In summary, my research has encompassed basic mechanisms of vertebrate development and cancer biology, as well as translational studies that impact treatment options for patients with human cancer. By investing in a multi-disciplinary approach, we have made significant contributions in each of these areas and are now well-poised to tackle fundamental issues, including: (1) Exploiting mouse models to gain insights about cancer mechanisms, particularly using cross-species comparisons at the genomic, RNA and protein levels. (2) Developing mouse models for pre-clinical studies, with the goal of enhancing their predictive utility for the design of better chemotherapeutic and chemoprevention approaches for humans. (3) Generating more accurate mouse models that more closely recapitulate the evolution of human cancer. (4) Investigating transcriptional regulation in the context of the whole organism with the ultimate goal of understand how differentiation is controlled in normal cells and how these mechanisms go awry in cancer.

Publications:


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 circadian 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 synchronized to local time 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 1ε (CK1ε). 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 our studies will have broad significance for the understanding of PER function and clock mechanisms in humans. Also, it is anticipated that our work will yield 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 day length.

Based on 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. Indeed, recent work shows that mutations in the phosphorylation of human PER2 underlie certain familial sleep disorders.

Publications:


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 research is funded by the NIH and the New Jersey Commission on Cancer Research. Dr. Gélinas served for several years as a member of the NIH Experimental Virology Study Section, and she is currently a member of the NIH Virology B Study Section. In 2006 Dr. Gélinas was elected to the Stuart D. Cook Master Educators Guild.

The laboratory focuses on the function of the Rel/NF-κB transcription factors in cancer. NF-κB is renowned for its central role in normal immune and inflammatory responses and in the control of cell proliferation and cell death. Constitutive activation of NF-κB is a hallmark of many human cancers including leukemia, lymphomas, breast and lung carcinomas, and the viral NF-κB oncogene v-Rel causes aggressive and fatal leukemia/lymphomas in animal models. NF-κB is key to tumor cell survival, proliferation and chemoresistance and is an important target for therapy in immune and inflammatory disorders as well as in cancer. Our efforts focus on understanding how changes in the normal transcriptional activity and regulation of the NF-κB proteins and their targets contribute to oncogenesis and to tumor cell resistance to anti-cancer therapy.

This year there was significant progress in understanding the unique contribution of Rel/NF-κB’s transcriptional activity to oncogenesis. Following up on gene expression analyses suggesting that transforming Rel proteins can lead to gene-specific transcriptional repression, our studies revealed that this novel mode of action may be as important for Rel/NF-κB’s biological activity as is its well-documented transactivation function. We showed that expression of the B cell receptor (BCR) signaling mediators BCAP and BLNK is detrimental to v-Rel’s oncogenic activity, consistent with their transcriptional repression in human primary mediastinal B cell lymphomas (PMBCL) and Hodgkin’s lymphomas that depend on c-Rel for survival. Chromatin immunoprecipitation assays suggest that Rel proteins may directly repress the BCAP promoter. BCAP can lead to activation of the JNK signaling pathway that promotes cell death, whereas a high incidence of spontaneous pre-B cell lymphomas is seen in BLNK-deficient mice due to impaired B cell differentiation. Since NF-κB fails to protect cells from apoptosis when JNK activation is sustained, we hypothesize that suppression of BCAP and BLNK may be necessary to block BCR signaling and prevent B cell differentiation and cell death in Rel-dependent tumors. Studies are underway to test this hypothesis. Overall, these studies underscored an important role for transcriptional repression in NF-κB’s function and suggest that restoring
signaling downstream of BCAP and/or BLNK in Rel-dependent tumors could perhaps be therapeutically beneficial.

Céline Gélinas

During the past year our studies of Rel/NF-κB regulation uncovered that the peptidyl-prolyl isomerase Pin1 positively regulates Rel’s oncogenic activity. Pin1 is frequently upregulated in breast cancer and in mouse mammary tumors in which NF-κB is constitutively activated. However, evidence that Pin1 contributes to malignant cell transformation by NF-κB is still lacking. In collaboration with the groups of Drs. Kun Ping Lu (Harvard Med. School) and Fang Liu (CABM, Rutgers Univ.), we showed that Pin1 markedly enhances the transforming activity of the human c-Rel protein in primary lymphocytes, consistent with its ability to associate with c-Rel and v-Rel. This coincided with increased nuclear Rel protein accumulation in Pin1-expressing cells. Conversely, neutralization of Pin1’s function with the inhibitor juglone prompted the cytoplasmic relocalization of c-Rel in human lymphoma-derived tumor cell lines. These findings raise the possibility that approaches to suppress Pin1, or its association with Rel, may be helpful to antagonize Rel/NF-κB activity in some tumors.

As part of our efforts to better understand the transcriptional and biological activities of Rel/NF-κB, we completed a functional analysis of defined phospho-acceptor sites conserved between the vertebrate Rel/NF-κB factors and the Drosophila morphogen Dorsal in collaboration with Dr. Ruth Steward (Waksman Institute, Rutgers Univ.). This work showed that a conserved serine phospho-acceptor site regulates the biological activities of the vertebrate Rel and fly Dorsal proteins through different mechanisms. While mutation of serine 317 in Drosophila Dorsal compromises its biological activity by antagonizing its nuclear localization, mutation of the corresponding serine 280 in the v-Rel oncogene significantly decreased its transforming activity by impairing its ability to transactivate specific genes, without compromising v-Rel’s nuclear localization or its DNA-binding activity. This is consistent with studies with RelA and suggests that site-specific phosphorylation in the Rel homology domain of vertebrate Rel/NF-κB proteins can modulate their transcriptional activity on particular gene subsets, perhaps via preferential recruitment of specific coactivators like CAPERα that we have recently characterized. Planned studies in collaboration with Drs. Arnold Rabson (CABM & Cancer Institute of New Jersey) and Roger Strair (Cancer Institute of New Jersey) will characterize the contribution of naturally occurring Rel gene mutations in human tumors.

Finally, we pursued our analysis of the function and regulation of the NF-κB-induced cell death inhibitor Bfl-1/A1, both independently and in collaboration with Dr. Eileen White (CABM and Rutgers Univ.). Bfl-1/A1 is overexpressed in several human cancers and is critical for tumor cell survival. Bfl-1 has recently emerged as the most discriminating gene associated with chemoresistance in microarray studies comparing human B cell chronic lymphocytic leukemia (B-CLL) refractory vs. responsive to chemotherapeutic treatment. We showed that Bfl-1/A1 functions similarly to cell death inhibitor Mcl-1, as a selective antagonist of the pro-apoptotic tBid and Bak proteins. This provided new insights into the mechanisms by which Bfl-1 counteracts the extrinsic and intrinsic death signaling cascades. This suggests that approaches to
block Bfl-1 interaction with tBid and/or Bak, or to promote its turnover, might improve the response of Bfl-1-expressing tumor cells to anti-cancer treatment. This may be important, since inhibition of Mcl-1 is necessary to sensitize certain tumors to the small molecule BH3 mimetic ABT-737. If Bfl-1 is an NF-κB-induced substitute for Mcl-1, antagonizing its function may be required for cancer therapy, and is perhaps one of the means by which NF-κB inhibitors provoke cancer cell apoptosis. In this regard, preliminary data suggest that certain kinases can decrease Bfl-1’s stability while others can promote it. While Bfl-1 mutants resistant to ubiquitin-mediated degradation could promote tumorigenesis in a mouse lymphoma model, microarray analysis of these tumors uncovered upregulation of tyrosine kinase Lck, that is incidentally a hallmark of human B-CLLs in which Bfl-1 is correlated with chemoresistance. However, little is known of the extent to which alterations in Bfl-1’s ubiquitination is associated with human tumors and of its impact on the chemotherapeutic response. Experiments are underway to address these issues and to further define the regulation of Bfl-1’s ubiquitination.

Overall, our analyses of the Rel/NF-κB proteins, the cellular factors with which they interact and the target genes that they regulate, including apoptotic inhibitor Bfl-1/A1, has contributed important insights into the mechanisms involved in oncogenesis associated with NF-κB. Since Rel/NF-κB is implicated in many disease conditions, these analyses may be helpful to design new approaches for therapy.

Publications:


---

Céline Gélinas


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.

We study TGF-ß signal transduction, transcriptional regulation, cell cycle control and their roles in tumorigenesis. TGF-ß potently inhibits cell cycle progression at the G1 phase. Smad proteins play an important role in mediating the TGF-ß growth-inhibitory responses by regulating the expression of several cell cycle components. For example, Smad proteins upregulate the expression of CDK inhibitors p15 and p21 and downregulate the expression of the c-myc protooncogene. We have discovered that Smad3, a key Smad protein for TGF-ß growth-inhibitory responses, is a major physiological substrate for G1 cyclin-dependent kinases CDK4 and CDK2. Except for the Rb family, Smad3 is the only CDK4 substrate demonstrated so far. We have mapped the CDK4 and CDK2 phosphorylation sites in Smad3 both in vivo and in vitro. Mutation of the CDK phosphorylation sites in Smad3 increases its transcriptional activity, resulting in a higher activity to stimulate p15 expression, and also a higher activity to downregulate c-myc expression. Using Smad3−/− primary mouse embryonic fibroblasts, we further showed that Smad3 inhibits cell proliferation from the G1 to S phase, and mutation of CDK phosphorylation sites augments this inhibitory function, accompanied by increased p15 expression and reduced c-myc expression at endogenous levels. Similar results were obtained in Mv1Lu mink lung epithelial cells that contain relatively low levels of Smad3. These observations strongly suggest that CDK phosphorylation of Smad3 inhibits its antiproliferative function. The vast majority of cancers, including breast cancers, are refractory to the TGF-ß growth inhibitory effects. Since CDK activity is often high in breast cancers, we hypothesize that inhibition of the activities of Smad3 and presumably the homologous Smad2 by CDK phosphorylation may allow breast cancers to be resistant to the antiproliferative effects of TGF-ß. We are examining this hypothesis.

We mapped the CDK phosphorylation sites to the Thr 8 in the N-terminal domain and the Thr 178 and S212 in the linker region of Smad3. Smad3 is also phosphorylated by ERK MAP kinase in response to EGF treatment. We mapped the ERK phosphorylation sites to Ser 207, Ser 203, and Thr 178 in Smad3, and showed that mutation of ERK phosphorylation sites increases its activity. In collaboration with Dr. Xin-Hua Feng’s laboratory at Baylor College of Medicine,
using the phosphopeptide antibodies we generated against each of the five phosphorylation sites, we identified small C-terminal domain phosphatases as specific phosphatases that dephosphorylate the Thr 8, Ser212, Ser 207 and Ser 203 in Smad3 and the analogous positions in Smad2. Interestingly, this phosphatase does not dephosphorylate Thr 178 in Smad3 or the analogous position in Smad2. Further studies indicate that the phosphatases enhance TGF-ß signaling, supporting our previous findings.

Runx2 is a Runt domain transcription factor involved in the activation of genes encoding osteoblast and chondrocyte-specific proteins. Runx2 activity is regulated by transcriptional and post-transcriptional mechanisms. The functional significance of the post-translational modification of Runx2 was not clear. In collaboration with Dr. Di Chen at the University of Rochester School of Medicine, we showed that cyclin D1-CDK4 induced Runx2 degradation in an ubiquitination- and proteasome-dependent manner. Mutagenesis of Runx2 serine-472, a consensus CDK site, to alanine increases the half-life of Runx2 and causes loss of sensitivity to cyclin D1-induced Runx2 degradation. The targeted Runx2 degradation by cyclin D1 identifies a novel mechanism through which Runx2 activity is regulated coordinately with the cell cycle machinery in bone cells.

Publications:


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. He was a Searle Scholar and received a Basil O’Connor Scholar Award.

Our laboratory has developed new methods for disease discovery that evolved from 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-Sachs 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 electrophoresis 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. We continue to study basic enzymological properties of the protein to learn more about its biological function and to develop improved diagnostic assays.

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. Finally, we have developed a LINCL mouse model and 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 proteins encoded by the human genome. We are in the final stages of completing a large scale project entailing purification and identification of mammalian mannose 6-phosphorylated proteins. We are in the process of developing high-throughput methods to validate the lysosomal localization of these and other proteins using classical subcellular fractionation and modern quantitative mass spectrometric methods.

One successful application of this proteomic research led to the determination of the molecular basis for Niemann Pick type C2 (NPC2) disease, a fatal cholesterol storage disorder. In collaboration with the laboratories of Drs. Ann Stock and Judith Storch, we are working to understand the biochemical and biophysical properties of the NPC2 protein. We have also created a NPC2 of mouse model and are using it to investigate the biological function of NPC2.

Publications:


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 understand the biological basis of human disorders such as autism, neural tube defects (NTDs), cataracts, and congenital heart defects (CHDs).

**Autism Spectrum Disorder (ASD)**

Individuals diagnosed with ASD exhibit deficiencies in communication and reciprocal social interactions that are accompanied by rigid or repetitive interests and behaviors. ASD is considered to be a neurodevelopmental disorder that has a polygenic basis. Increased risk for the disorder is believed to be due to multiple genes interacting with each other as well as environmental factors.

The cerebellum is a brain structure that has been shown to be consistently anatomically abnormal in individuals with autism. 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. These reported cerebellar anatomical defects could then contribute to some of the behavioral phenotypes associated with ASD.

My laboratory decided to test candidate genes for their involvement in ASD, by selecting genes that caused similar cerebellar anatomical defects in the mouse. One of the genes that we analyzed was the homeobox transcription factor gene, *ENGRAILED 2 (EN2)*. *En2* mouse mutants had been reported previously to display an autistic-like cerebellar anatomical phenotype, and human *EN2* maps to distal chromosome 7 where some evidence of linkage had been reported.

In collaboration with Dr. Linda Brzustowicz’s group at Rutgers University (Department of Genetics), we have demonstrated that two intronic SNPs (*rs1861972* and *rs1861973*) are consistently and significantly associated with ASD in 3 separate datasets (Gharani et al., 2004; Benayed et al., 2005). *EN2* is encoded by two exons and a single intron that spans 8.1 kb of genomic DNA. Our initial studies using 167 pedigrees demonstrated significant association for the A allele of *rs1861972* and the C allele of *rs1861973*, both individually and as a haplotype (Gharani et al., 2004). We have now replicated the association of these *rs1861972* and *rs1861973* alleles in two other datasets. Additional evidence of association was obtained when
the data were combined and analyzed 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 EN2 risk allele contributes to as much as 40% of ASD cases in the general population (Benayed et al, 2005). These genetic data are consistent with EN2 encoding an ASD susceptibility gene ASD [MIM 608636]; EN2 [MIM 131310]).

To identify candidates for the risk allele responsible for EN2 association, re-sequencing and LD mapping analysis were performed. These experiments identified the A-C rs1861972-rs1861973 haplotype as candidate disease alleles (Benayed et al., 2005). This possibility has been further supported by publicly available Hapmap data. Rs18161973 was typed as part of the Hapmap project and analysis of the Hapmap LD data demonstrated that rs1861973 is not in strong LD ($r^2>0.40$) with any other typed polymorphism within 2Mb surrounding EN2. These genetic data are consistent with the associated rs1861972-rs1861973 A-C haplotype being the best candidate for the EN2 disease allele.

To begin testing whether the EN2 intron has biological activity, we are performing transient transfection analysis in P6 mouse cerebellar granule cells, a cell type in which En2 is expressed. These experiments have demonstrated that the EN2 intron acts as a transcriptional repressor ($P=0.0000055$, Student’s T-test). Ongoing experiments have also determined that there is a functional difference between the associated A-C and non-associated G-T haplotype. These data provide additional evidence that rs1861972-rs1861973 A-C haplotype is the risk allele responsible for EN2 association with ASD.

The next step in our analysis is to generate a series of mouse transgenics to determine the developmental ages (when) and cell types (where) in which the A-C haplotype is functional. These data will help develop hypotheses of how the A-C haplotype affects CNS development and will be important in order to translate our genetic findings into better therapies for ASD.

**Neural Tube Detects (NTDs)**

To understand the genetic and developmental basis of human NTDs, the lab has been studying the spontaneous mouse mutant called *vacuolated lens* (*vl*). NTDs affect neurulation, which is the closing and fusion of the neural plate to generate the neural tube along the entire neuraxis. NTDs are the second most common congenital birth defect in humans and are considered to have a multi-factorial basis with both genetics and environment contributing to increased risk.

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 Bev Paigen PhD 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), Gpr161, which results in a frame shift and early termination of the protein. *In situ* hybridizations by us have demonstrated that the orphan GPCR is expressed in the developing spinal cord and eye. These studes
have identified one of the first GPCRs to be implicated in neurulation and eye development (Desai et al submitted).

Most GPCRs have a similar secondary protein structure with an extracellular N terminus, seven trans-membrane domains and a cytoplasmic C terminal tail. The vl mutation is an eight base pair deletion that causes a frameshift and premature stop codon, truncating the C terminal tail by 143 amino acids. The C terminal tail of GPCRs functions as an important regulator of receptor activity. We have demonstrated that the mutation has multiple effects on Gpr161 protein, including decreased receptor-mediated endocytosis, which is a common mechanism to attenuate GPCR signaling.

Interestingly, the vl NTD phenotypes are also multi-genic. On the C3H background, 100% of vl/vl homozygotes display a NTD phenotype (50% spina bifida, 50% a closed NTD phenotype). In addition, 50% of the mutants die, which is likely to be due to the spina bifida phenotype. However, when the vl mutation was crossed onto different genetic backgrounds (C57BL6/J, Muscastaneus (CAST/Ei) and Musmolossinus (MOLF/Ei)) to map the genetic position of the locus, ~50% of the vl/vl homozygotes displayed no phenotype and lethality was not observed. This result indicates that unlinked genetic modifiers on these backgrounds can rescue the associated vl mutant phenotypes. We have mapped 5 different vl modifier loci on these different genetic backgrounds (Modifiers of vacuolated lens, Modvl 1-5, LOD 3.7-5.0)(Korstandjie et al, submitted).

To investigate whether the MOLF/Ei modifier, Modvl5 (chr 18 LOD=5.0), is sufficient to rescue the vl phenotypes, we have generated a congenic line where the entire genome is C3H except for the region around Modvl5 on chromosome 18 which retained a MOLF/Ei haplotype. By crossing the Modvl5 modifier to vl mice, we have now demonstrated that Modvl5 is sufficient to rescue vl associated lethality. Ongoing experiments are examining vl/vl embryos to determine whether Modvl5 rescues the spina bifida phenotype. Our future goals are to clone the gene responsible for Modvl5 and to determine whether the other modifiers are also sufficient to rescue the vl associated phenotypes.

Cataract

Cataract is defined as an opacity of the lens. Age related cataract is common and estimated to affect ~20.5 million Americans over the age of 40 or 17.2% of the population. Like NTDs, it is considered to be multi-factorial with both genetics and environment playing a role in susceptibility. Congenital cataract is less common in developed countries, occurring in ~30:100,000 births, but is still a leading cause of childhood blindness worldwide. Mutations in genes that affect lens development in mice have been shown to cause congenital cataracts in humans. Because lens fiber cells continue to be generated in the adult, genes that cause congenital cataracts may also contribute to age related cataracts.

We have also mapped a vl MOLF/Ei cataract-specific modifier to chromosome 4, Modvl3 (LOD=4.2). Ongoing congenic analysis is investigating the sufficiency of Modvl3. However,
bioinformatic analysis has identified Foxe3, a winged helix-forkhead transcription factor, as a possible candidate gene for Modvl3. Mutations in Foxe3 cause cataracts in both mice and humans and a non-synonymous SNP exists between C3H and MOLF/Ei. We are in the process of performing molecular analysis to determine whether this SNP is functional.

In summary, because the different vl disease phenotypes (NTDs, cataracts) are due to multiple genetic loci and the vl locus encodes Gpr161 which binds to an unidentified endogenous ligand, our analysis has established an important mouse model to study the multi-factorial basis of both NTDs and cataracts. Future experiments are directed at determining whether Gpr161 and Modvl modifiers contribute to the human disease as we have determined for EN2 with ASD and to identify the endogenous ligand for Gpr161.

**Congenital Heart Defects (CHDs)**

CHDs is the most common human birth defect occurring at ~1:50 live births. Like NTDs and cataracts it is considered to have a multi-genic basis. The Splotch mouse mutation displays both NTDs and CHDs in 100% of Sp/Sp embryos on a C57BL6/J background. To determine whether these disease phenotypes could be rescued by crossing the mutation onto different backgrounds, we performed crosses to DBA/2, MOLF/Ei and NZW. In all 3 crosses, we observed complete rescue of the CHD but not the NTD phenotype. These data indicate that the CHD Splotch phenotype is multi-genic and we are in the process of mapping these modifiers.

**Publications:**


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 was 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.

In recent studies, we have focused on the formation of the anterior visceral endoderm (AVE), which represents a critical event in patterning of the anterior-posterior axis in the pre-gastrulation mouse embryo. We have shown that the TGFβ family member GDF3 (Growth-Differentiation Factor-3), a close relative of Xenopus Vg1, resembles the TGFβ ligand Nodal in both its signaling activity and its role in AVE formation in vivo. Thus, in cell culture, GDF3 signaling requires the EGF-CFC co-receptor Cripto and can be inhibited by Lefty antagonists. In Xenopus
embryos, GDF3 misexpression results in secondary axis formation, and induces morphogenetic elongation and mesendoderm formation in animal caps. In mouse embryos, Gdf3 is expressed in the inner cell mass and epiblast, and null mutants frequently exhibit abnormal formation or positioning of the AVE. This phenotype correlates with defects in mesoderm and definitive endoderm formation, as well as abnormal Nodal expression levels. These findings indicate that GDF3 acts in a Nodal-like signaling pathway in pre-gastrulation development and provide evidence for the functional conservation of Vg1 activity in mice.

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. Our recent studies have focused on the emergence of androgen-independence in these compound mutant mice. This process is significant since androgen-deprivation therapy is a widely-used treatment for patients with advanced prostate cancer but ultimately results in the emergence of a hormone-refractory disease that is invariably fatal and lacks effective clinical treatments.

Publications:


Eileen White, Ph.D.
Resident Faculty Member, CABM; Professor, Rutgers University, Department of Molecular Biology and Biochemistry; Associate Director for Basic Science, Cancer Institute of New Jersey; Adjunct Professor, UMDNJ-Robert Wood Johnson Medical School

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.

Regulation of Apoptosis by Viral Oncogenes
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 adenovirus 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.
Hypoxia and Defective Apoptosis Drive Genetic Instability and Tumorigenesis
Genomic instability is a hallmark of cancer development and progression, and characterizing the stresses that create, and the mechanisms by which cells respond to genomic perturbations is essential. We discovered that antiapoptotic BCL-2 family proteins promote tumor formation of transformed baby mouse kidney (BMK) epithelial cells by antagonizing BAX- and BAK-dependent apoptosis. Cell death in vivo correlated with hypoxia and induction of PUMA (p53 up-regulated modulator of apoptosis). Strikingly, carcinomas formed by transformed BMK cells in which apoptosis was blocked by aberrant BCL-2 family protein function displayed prevalent, highly polyploid, tumor giant cells. Examination of the transformed BMK cells in vivo revealed aberrant metaphases and ploidy changes in tumors as early as 9 days after implantation, which progressed in magnitude during the tumorigenic process. An in vitro ischemia system mimicked the tumor microenvironment, and gain of BCL-2 or loss of BAX and BAK was sufficient to confer resistance to apoptosis and to allow for accumulation of polyploid cells in vitro. These data suggest that in vivo, even in cells in which p53 function is compromised, apoptosis is an essential response to hypoxia and ischemia in the tumor microenvironment and that abrogation of this response allows the survival of cells with abnormal genomes and promotes tumorigenesis.

Autophagy Promotes Tumor Cell Survival and Restricts Necrosis, Inflammation, and Tumorigenesis
Defective apoptosis renders immortalized epithelial cells highly tumorigenic, but how this is impacted by other common tumor mutations is not known. In apoptosis-deficient cells, inhibition of autophagy by AKT activation or by allelic disruption of beclin1 confers sensitivity to metabolic stress by inhibiting an autophagy-dependent survival pathway. While autophagy acts to buffer metabolic stress, the combined impairment of apoptosis and autophagy promotes necrotic cell death in vitro and in vivo. Thus inhibiting autophagy under conditions of nutrient limitation can restore cell death to apoptosis-refractory tumors, but this necrosis is associated with inflammation and accelerated tumor growth. Thus, autophagy may function in tumor suppression by mitigating metabolic stress, and in concert with apoptosis, by preventing death by necrosis.

Caspase-Dependent Processing Activates the Pro-Apoptotic Activity of Deleted in Breast Cancer-1 During Tumor Necrosis Factor-α-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 and 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.

**Publications:**


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.

**Publication:**

Mengqing Xiang, Ph.D.
Resident Faculty Member, CABM; 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. In 2003 he received the Award in Auditory Science from the National Organization for Hearing Research Foundation. 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 a variety of molecular genetic approaches to identify and study transcription and other regulatory factors that are required for programming development of the retina, inner ear, spinal cord, and other CNS areas. A major focus of our work is to develop animal models to study the roles of these regulatory genes during normal sensorineural development, as well as to elucidate how mutations in these genes cause sensorineural disorders such as blindness and deafness.

Role of Barhl1 in the maintenance of superior collicular neurons. The mammalian superior colliculus of the midbrain is a brainstem center that integrates sensorimotor signals involved in the control of orienting behaviors. Its structure is characterized by seven well-organized cellular and fibrous layers associated with distinct physiological properties. For instance, the superficial layers, which consist of the zonal, superficial gray and optic layers, are innervated by axons from the retina and visual cortex, thus receiving inputs almost exclusively related to vision. To date, little is known about the molecular bases governing the lamination, differentiation and survival of superior collicular neurons. Barhl1 is a homeodomain transcription factor that has been demonstrated to play an essential role in maintaining inner ear hair cells, cerebellar granule cells and precerebellar neurons. We have shown that Barhl1 exhibits a select expression pattern in the superior colliculus with positive neurons largely restricted to the zonal layer, as visualized by the β-galactosidase activity expressed from the lacZ reporter knocked in the Barhl1 locus. Targeted disruption of Barhl1 results in the loss of a large population of neurons from the zonal layer of the superior colliculus, as indicated by reduced β-galactosidase staining and marker gene expression as well as by increased apoptotic cell death. Taken together, these data suggest that Barhl1 is crucially required for the survival but not for the specification of zonal layer neurons in the superior colliculus. Therefore, we have thus far identified Barhl1 as a key transcriptional regulator essential for the maintenance of inner ear hair cells, cerebellar granule cells, as well as precerebellar and superior collicular neurons.
Role of Ptf1a in specifying horizontal and amacrine cell fates during retinal development.

The vertebrate neural retina comprises six classes of neurons and one class of glial cells, all derived from a population of multipotent progenitors. There is little information on the molecular mechanisms governing the specification of cell type identity from multipotent progenitors in the developing retina. In a collaborative study with Dr. Christopher Wright’s laboratory, we have found that Ptf1a, a basic-helix-loop-helix (bHLH) transcription factor, is transiently expressed by post-mitotic precursors in the developing mouse retina. Recombination-based lineage tracing analysis in vivo revealed that Ptf1a expression marks retinal precursors with competence to exclusively produce horizontal and amacrine neurons. Inactivation of Ptf1a leads to a fate-switch in these precursors that causes them to adopt a ganglion cell fate. This mis-specification of neurons results in a complete loss of horizontal cells, profound decrease of amacrine cells and an increase in ganglion cells. Furthermore, we identify Ptf1a as a primary downstream target for Foxn4, a forkhead transcription factor involved in the genesis of horizontal and amacrine neurons. These data together with the previous findings on Foxn4 provide a model in which the Foxn4-Ptf1a pathway plays a central role in directing the differentiation of retinal progenitors towards horizontal and amacrine cell fates.

Publications:


Structural Biology

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Faculty Director</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomolecular Crystallography</td>
<td>Edward Arnold</td>
<td>35</td>
</tr>
<tr>
<td>Structural Bioinformatics</td>
<td>Gaetano Montelione</td>
<td>39</td>
</tr>
<tr>
<td>Protein Design and Evolution</td>
<td>Vikas Nanda</td>
<td>42</td>
</tr>
<tr>
<td>Protein Crystallography</td>
<td>Ann Stock</td>
<td>44</td>
</tr>
</tbody>
</table>
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. Eddy 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 and a Fellow of the American Academy of Microbiology in 2006. 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. Drs. 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 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 and Phase III 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 Dr. 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 Dr. Richard Ebright at Rutgers University); 2) the human mRNA capping enzyme and its associated factors (with Dr. Aaron Shatkin at CABM), and 3) influenza virus proteins with Dr. Gaetano Montelione at CABM and Dr. Robert Krug (University of Texas-Austin).

**Publications:**


Gaetano Montelione, Ph.D.
Resident Faculty Member, CABM; Professor, Rutgers, Department of Molecular Biology and Biochemistry; Adjunct Professor, UMDNJ-RWJMS, Department of Biochemistry; Director, Northeast Structural Genomics Consortium

Structural Bioinformatics 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. Kurt 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-$\alpha$ 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. He was recently elected as a Fellow of the American Association for the Advancement of Science.

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 certain 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 and bioinformatics.

Publications:


Baran, M.; Moseley, H.N.B.; Aramini, J.M.; Bayro, M.J.; Monléon, D.; Lau, J.; Montelione,
Gaetano Montelione


Vikas Nanda, Ph.D.
Resident Faculty Member, CABM; Assistant Professor, UMDNJ Robert Wood Johnson Medical School, Department of Biochemistry

Protein Design and Evolution Laboratory
Dr. Vikas Nanda 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 among 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 viral genomes for estimating the extent of possible mutations in order to abet drug design efforts.

Publications:


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. Stock has received National Science Foundation Predoctoral and Damon Runyon-Walter Winchell Postdoctoral Fellowships, a Lucille P. Markey Scholar Award in Biomedical Science, an NSF Young Investigator Award, a Sinsheimer Scholar Award and an NIH MERIT Award in 2002. 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 at UMDNJ. In 2004 Stock was selected as an Outstanding Scientist by the NJ Association for Biomedical Research and she was elected Fellow of the American Association for the Advancement of Science in 2006. Stock is an investigator of the Howard Hughes Medical Institute. She serves on the Board of Scientific Counselors for the NIH-National Institute of Dental and Craniofacial Research and on the Editorial Board of the Journal of Bacteriology.

Our laboratory focuses on structure/function studies of signal transduction proteins. Effort is concentrated on response regulator proteins that are the core of two-component systems, the most prevalent 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, containing >1500 members and accounting for approximately one third of all response regulators. Following our recent structural characterization of several representative proteins of this family (Escherichia coli ArcA, KdpE, TorR, PhoB, PhoP, Thermotoga maritima DrrB, and DrrD, and Mycobacterium tuberculosis MtrA) we have focused our efforts on understanding how different intramolecular and/or intermolecular domain arrangements in the inactive states of these proteins provide different modes of regulation that appear to be optimized for the specific needs of the signal transduction pathways in which each protein functions. The observation that in the phosphorylated active state, all of these proteins adopt a similar dimeric structure mediated by the regulatory domains and involving a set of highly conserved residues along the α4-β5-α5 face has raised questions about the specificity of dimer formation. We postulated that heterodimer formation may be possible among different subfamily members within a single cell, potentially providing for integration of signaling pathways. We have demonstrated the formation of heterodimers in vitro and have been developing methods to assess the existence and physiological significance of heterodimer formation in vivo.

We have determined the X-ray crystal structure of the DNA-binding domain of AgrA, a response regulator transcription factor that regulates the expression of virulence genes in Staphylococcus aureus. This provides the first structure for a member of the family of LytTR domains, the fourth largest class of response regulator transcription factors and ones that are associated with virulence factor expression in many pathogenic bacteria. The structure of AgrA establishes the fold for this very important family and defines both a novel fold, and a novel DNA-binding domain with a unique mode of DNA recognition.
Additional advances have been made in characterizing molecular mechanisms in bacterial chemotaxis, a model sensory system and arguably the most extensively characterized signaling system. We have extended our previous work on the reversible posttranslational modification of transmembrane chemoreceptors. Our studies have demonstrated that, unlike the extensively characterized methylation systems of enteric bacteria, methylation in *Thermotoga maritima* does not involve a tethering interaction between receptors and the methyltransferase. We have identified determinants in the methyltrasferase that allows categorization of the enzymes into tethering-dependent and tethering-independent classes. The majority of bacterial chemotaxis systems appear to function via a tethering-independent mode. We have also characterized the interaction between the chemotaxis response regulator CheY and its phosphatase CheZ. Numerous crystal structures and complementary biochemical assays have established two distinct binding modes for the CheY-CheZ interaction, with the two modes corresponding to the inactive and active conformations of CheY. The study provides a structural explanation for the previously observed acceleration of CheY phosphorylation upon binding to a peptide of CheZ. Additionally, the study provides direct evidence for an intermediate state of CheY, indicating that the widely accepted "two-state" model for response regulator activation is an oversimplification.

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. A ligand-binding assay was developed and was used to characterize the binding specificity of NPC2 for a variety of sterol ligands. Together with our recently determined crystal structure of NPC2 bound to the cholesterol analog, cholesteryl sulfate, our data suggest that the sterol binding pocket of NPC2 is malleable, capable of accommodating and molding itself around a variety of different sterol ligands. We have also continued our attempts to identify weakly associating cellular partners of NPC2 by affinity chromatography and photo-activated cross-linking.

**Publications:**


# Molecular Genetics

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Faculty Director</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein Engineering</td>
<td>Stephen Anderson 48</td>
</tr>
<tr>
<td>Viral Pathogenesis</td>
<td>Arnold Rabson 50</td>
</tr>
<tr>
<td>Molecular Virology</td>
<td>Aaron Shatkin 53</td>
</tr>
</tbody>
</table>
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 NESG, (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.

With regard to the above throughput challenge, during the past year we have found a host-vector combination that is promising: so far, after testing a small sample of targets, we have been able to achieve fairly high levels of expression (30 – 50 mg/L from initial transformants) for approximately 50% of the genes tested. Work is proceeding with a larger sample to get a better estimate of the success rate. One of the proteins expressed at a high level using this method is
mouse MERP (mammalian ependymin-related protein), and this is currently being scaled up for structure determination (Chiang et al, in preparation). In collaboration with Professor Shin-Geon Choi (Kangwon National University, South Korea) we are continuing to evaluate the utility of high-yield episomal P. pastoris expression vectors (Hong et al, 2006). And we have also shown that mutagenesis followed by high-density screening using immunohistological methods can be used to isolate clones secreting significantly greater amounts of recombinant heterologous protein (Nallaseth et al, 2006).

Working with Codon Devices (Cambridge, MA), we are also exploring the use of total gene synthesis as a cost-effective substitute for molecular cloning in the preparation of expression constructs; this approach enables us to convert sequences from databases directly into expressible genes without having to first acquire a genomic DNA- or cDNA-based physical intermediate. Such genes have been made and tested for expression in P. pastoris and shown to work. A particularly exciting application of this approach involves our 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 aiming 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 will provide a source of novel Plasmodium antigens to use as the bases for improved malaria vaccines. Since Plasmodium genes are notoriously difficult to express in normal host-vector systems due to their high AT content, the total gene synthesis route, with codons optimized for expression in standard eukaryotic hosts, will be a crucial enabling strategy.

Publications:

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 is currently Chair, NIH Oncologic Sciences IRG Cancer Molecular Pathology Study Section.

Dr. Rabson’s laboratory studies the molecular basis of cancer and human retroviral infections. His research activities are focused in two major areas: 1) viral and cellular mechanisms regulating the expression of human retroviruses that cause cancer and AIDS; and 2) the roles of cellular transcription factors, particularly those of the NF-κB/Rel family, in the development and progression of human cancer.

Dr. Rabson’s laboratory studies the Human T-Cell Leukemia Virus type 1 (HTLV-1), the first identified human retrovirus. Over 20 million people are likely infected by HTLV-1 and 2-5% of them are likely to develop a serious HTLV-1-associated disease. Dr. Rabson is studying the mechanisms by which the human T-cell leukemia virus (HTLV-1) causes aggressive T cell leukemia/lymphoma (Adult T-Cell Leukemia, ATL) in some infected individuals and a neurological disease of the spinal cord (HTLV-associated Myelopathy, HAM/TSP) in other 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 the mechanisms by which the HTLV-1 virus may infect target human T-lymphocytes and yet remain in a latent, silent form from which virus may be induced for further infection and disease induction. They have shown that immune activation of HTLV-1-infected T-cells by stimulation through the T-cell receptor 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 worked to develop a mouse model of HTLV-1 pathogenesis using a transgenic mouse model in which the HTLV-1 LTR promoter region directs
the expression of the oncogenic Tax protein, which is responsible for transformation of infected T-cells. His laboratory has shown that T-cell activation in these animals will induce HTLV-1 LTR expression, resulting in induction of Tax oncogene expression. This leads to hyperproliferation and decreased death of these T-cells with sustained replication in culture of up to 15 weeks, as compared with a maximal survival of two weeks by non-transgenic T-cells. 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. Studies to examine the effects of immune activation on T-cell proliferation and survival in the transgenic mice are now in progress and offer hope of creating an authentic model of HTLV-1 induced leukemia/lymphoma that may be of utility in developing treatment and prevention strategies of HTLV-1-associated diseases.

The second major area of study in Dr. Rabson’s laboratory continues to be the roles of transcriptional regulation 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. He has been particularly interested in the “non-canonical” NF-κB pathway in human lymphomas. A number of years ago, his laboratory identified the frequent occurrence of molecular alterations in the gene encoding the NFκB2 transcription factor, in the malignant cells of patients with cutaneous T-cell lymphoma (CTCL). His laboratory has now identified a series of target genes through which mutated NFκB2 may act to induce increased T-cell proliferation, survival and oncogenesis and is now characterizing how these targets may function in development of lymphomas.

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. Recent studies have shown that combinations of TPA with other agents such as Taxol, can potently inhibit the growth of prostate cancer cells in mouse models, when used at clinically achievable doses. This provides a rationale for possible clinical trials in refractory prostate cancer.

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 (Rutgers’ Ernest Mario School of Pharmacy), 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:


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. Shatkin was the recipient of the 1977 National Academy of Sciences Molecular Biology Award, and in 2003 he received the Association of American Medical Colleges Award for Distinguished Research in the Biomedical Sciences.

One of the earliest events in eukaryotic gene expression is 5’terminal capping of pre-mRNAs. The m7GpppN cap structure is added to nascent RNA polymerase II transcripts selectively by the sequential catalytic action of RNA triphosphatase (RT), guanylyltransferase (GT) and methyltransferase (MT). The cap marks gene transcription start sites and has multiple downstream effects on mRNA maturation, stability, nuclear transport and translation initiation. These critical events involve recognition of the cap m7G by cap binding proteins. Uncapped transcripts are rapidly degraded in cells while RNAs that contain an unmethylated cap fail to direct protein synthesis.

Cap-forming enzymes of human, mouse and worm origin were cloned, sequenced and characterized. In contrast to the separate enzymes in yeast, metazoans contain a bifunctional capping enzyme (CE) consisting of N-terminal RT and C-terminal GT. We showed that CE selectively binds RNA polymerase II by interaction of the GT domain with the large subunit C-terminal heptad repeat sequences (CTD) after they are phosphorylated by general transcription factor TFIIH. The interaction of CE with P-CTD, or with factor DSIF, stimulated RNA capping. CE binding to DSIF also relieved transcription repression by negative elongation factor (NELF), suggesting that CE plays a critical role in elongation checkpoint control during promoter clearance. Protein-protein interactions between MT and nuclear transporter importin-α increased MT binding to unmethylated substrate RNA and also stimulated cap methylation of GpppN RNA 5’ends. The results point to a functional connection between capping, transcription and protein targeting, suggesting that capping is part of a network of interacting pathways essential for viability.

We used small interfering (si) RNAs to demonstrate that, as in yeast, capping is essential in C. elegans, mouse and human cells. Knockdown of CE was embryonic lethal in worms, and CE siRNA treatment of a variety of human and mouse cells resulted in apoptosis as measured by TUNEL.
assay and caspase-3 activation. Although death induction was p53 independent, a mouse embryo fibroblast cell line that was missing BAK and BAX failed to undergo apoptosis in response to siRNA CE knockdown. Instead, autophagy was induced with cleavage and relocalization of LC3 to punctate bodies in the cytoplasm. siRNA-mediated decreased MT also resulted in apoptosis in mammalian cells. Viability could be restored by transfection of a truncated MT that retained a nuclear localization sequence (NLS) and was capable of forming complexes with RNA polymerase II in the nucleus. By contrast, a truncated MT that was catalytically active, but missing a NLS and thus localized to the cytoplasm, 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 to maintain homeostasis in eukaryotic cells.

Publications:


Education, Training & Technology Transfer
Lectures and Seminars
January 18, 2006
Dorothee Kern, Brandeis University, HHMI
“On the Move in the NMR Tube, the Crystal and the Computer: Protein Dynamics during Catalysis and Signaling”

February 8, 2006
Vincenzo Pirrotta, Rutgers University
“Polycomb Complexes and Epigenetic Chromatin Silencing Mechanisms”

March 22, 2006
Mario R. Capecchi, University of Utah, HHMI
“Gene Targeting into the 21st Century: Modeling Human Disease in the Mouse, from Cancer to Neuropsychiatric Disorders”

April 19 2006
Daria Hazuda, Merck Research Laboratories
“HIV-1 Integrase Inhibitors: Basic Research to Clinical Development”

May 3, 2006
John Blenis, Harvard Medical School
“Regulation of Cell Growth through the Integration of Mitogenic and Nutrient Signaling Pathways”

November 1, 2006
Robert Benezra, Memorial Sloan-Kettering Cancer Center
“Modeling Tumor Angiogenesis in the Mouse”

November 15, 2006
Hiroaki Mitsuya, NIH
“Development of Therapeutics for HIV Infection: The Past, Present, and Future”

- The above lectures were supported in part by Sanofi Aventis and New England Biolabs -
Program

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

8:45 AM  Opening Remarks – Aaron J. Shatkin

9:00 AM  Session I – Harvesting Genomics Information at the Functional Level
Chair:  Xuesong Ouyang – Molecular Oncology and Development Lab (Cory Abate-Shen)

Vikas Nanda – Protein Design and Evolution Lab (Vikas Nanda)
“Why are Proteins Even-handed?”

Joseph Bauman – Biomolecular Crystallography Lab (Eddy Arnold)
“In Search of New Crystal Forms from Engineering of HIV-1 Reverse Transcriptase”

Janet Huang – Protein NMR Spectroscopy Lab (Guy Montelione) “Targeting Proteins from the Human Cancer Protein Interaction Network (HCPIN) for Structural Genomics”

Meiqian Qian – Protein Targeting Lab (Peter Lobel)
“Proteomic Investigation of Mannose 6-phosphate Receptor Function”

Ferez S. Nallaseth – Protein Engineering Lab (Stephen Anderson)
“Towards Generic Optimization of a P. pastoris Expression Strain: A Screen for Mutants Secreting Elevated Levels of a Reporter Protein”

Eduardo Perez – Protein Crystallography Lab (Ann Stock)
“Characterization of Thermotoga maritima Methylation System and the Identification of Methylation Sites in Chemotaxis Receptors”

10:30 AM  Coffee Break

11:00 AM  Session II – Systems Regulation in Flies and Mammals
Chair:  Pragnya Das – Molecular Neurodevelopment Lab (Menqing Xiang)

Chun Chu – Molecular Virology Lab (Aaron J. Shatkin)
“Knocking-down mRNA Capping Induces BAK and BAX-dependent Apoptosis”

Tzu-Hsin Kuo – Sleep Genetics Lab (Julie Williams)
“Molecular Analysis of the Interaction between Sleep and Immune Response in Drosophila”
Annual CABM Retreat

Wen-Feng Chen – Molecular Chronobiology Lab (Isaac Edery)
“Regulating Splicing of the Period Gene 3’-terminal Intron by Light, Circadian Clock Factors, and Phospholipase C”

Ramesh Chellappa – Molecular Neurodevelopment Lab (Menqing Xiang)
“Auto-regulation of Barhl1 Expression during Inner Ear and Cerebellar Development”

James H. Millonig – Developmental Neurogenetics Lab (James H. Millonig)
“Suppression of the Vacuolated Lens Embryonic Lethality Phenotype”

12:15 PM
Lunch and Break

3:00 PM
Posters

4:00 PM
Session III – New Insights in Cancer Biology
Chair: Vikas Nanda – Protein Design and Evolution Lab (Vikas Nanda)

Anna Puzio – Molecular Oncology and Development Lab (Cory Abate-Shen)
“New Mouse Models of Ovarian and Bladder Cancer”

Isao Matsuura – Growth & Differentiation Control Lab (Fang Liu)
“Analysis of Proline-Directed Phosphorylation Sites of Smad3”

Peter J. Simon – Viral Pathogenesis Lab (Arnold Rabson)
“HTLV-1 Exit from Latency Is Driven by a Positive Feedback Induction of Tax”

Robin Mathew – Viral Transformation Lab (Eileen White)
“Computerized Fluorescence Video Time-Lapse Microscopy for the Analysis of Dynamic Cellular Processes in Living Cells”

Jui Dutta – Tumor Virology Lab (Céline Gélinas)
“Role of Transcriptional Coactivator HCC1.4/CAPERα in Rel/NF-κB Mediated Transcription and Oncogenesis”

Xi Wang – Mammalian Embryogenesis Lab (Michael Shen)
“Analysis of Progenitor Cells in the Mouse Prostate”

5:30 PM
Close
20th Annual CABM Symposium

Emerging Strategies in Molecular Medicine

October 17-18, 2006

Sponsored by

The Center for Advanced Biotechnology & Medicine

and

Johnson & Johnson

Program

Tuesday, October 17, 2006

12:45 pm Registration

1:15 pm Welcoming Remarks
Aaron J. Shatkin, Professor & Director, CABM

Session I

Co-chairs: Michael Shen, Professor, Pediatrics, UMDNJ-Robert Wood Johnson Medical School; Associate Director, Child Health Institute of New Jersey and CABM Faculty Member; and Mengqing Xiang, Professor, Pediatrics, UMDNJ-Robert Wood Johnson Medical School and CABM Faculty Member

1:30 pm Phillip A. Sharp, Institute Professor, Center for Cancer Research, Massachusetts Institute of Technology
"The Biology of Small RNAs"

2:15 pm Joseph Schlessinger, Professor, Pharmacology, Yale University School of Medicine
“Cell Signaling by Receptor Tyrosine Kinases: From Basic Concepts to Cancer Therapy”

3:00 pm Gaetano Montelione, Professor, Molecular Biology & Biochemistry, Rutgers University and CABM Faculty Member
“Structural Proteomics in Drug Target Discovery”
3:45 pm  Coffee Break and Posters

4:15 pm  Cory Abate-Shen, Professor, Medicine and Neuroscience, UMDNJ-Robert Wood Johnson Medical School and CABM Faculty Member
"Modeling Cancer in Mice—New Models, New Insights into Human Disease"

5:00 pm  Arnold J. Levine, Professor, School of Natural Sciences, Institute for Advanced Study and UMDNJ-Robert Wood Johnson Medical School
“Single Nucleotide Polymorphisms in the p53 Pathway that Influence Cancers in Humans”

5:45 pm  Reception – CABM, South Atrium

Wednesday, October 18, 2006

8:30 am  Registration & Continental Breakfast

Session II
Chairperson: Céline Gélinas, Professor, Biochemistry, UMDNJ-Robert Wood Johnson Medical School and CABM Faculty Member

9:00 am  Sean J. Morrison, Professor, Internal Medicine, HHMI, Center for Stem Cell Biology, University of Michigan
“Stem Cell Self-renewal, Cancer Cell Proliferation, and Aging”

9:45 am  James Millonig, Assistant Professor, Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School and CABM Faculty Member
"Support for the Homeobox Transcription Factor ENGRAILED 2 (EN2) as an Autism Susceptibility Gene"

10:30 am  Coffee Break and Posters

11:00 am  Michael Snyder, Professor and Chair, Molecular Cell and Developmental Biology, Yale University
“Regulatory Networks in Yeast and Humans”

11:45 am  Richard Allen Young, Professor, Whitehead Institute of Biomedical Research
"Regulatory Circuitry of Human Embryonic Stem Cells"

12:30 pm  Box Lunch (Ticket Required) – CABM, South Atrium
Session III  
Chairperson: Ann Stock, Professor, Biochemistry, HHMI, UMDNJ-Robert Wood Johnson Medical School and CABM Faculty Member

1:30 pm  Peter Lobel, Professor, Pharmacology, UMDNJ-Robert Wood Johnson Medical School and CABM Faculty Member  
“Proteomics, Lysosomes and Disease”

2:15 pm  James A. Wells, The Harry W. and Diana Hind Distinguished Professor in Pharmaceutical Sciences, UCSF  
"Site-directed Chemical Biology for Allosteric Sites"

3:00 pm  2006 Paul Janssen Prize

Coffee Break and Posters

3:30 pm  Eddy Arnold, Professor, Chemistry, Rutgers University and CABM Faculty Member  
"Development of Novel HIV/AIDS Therapeutics: Strategies for Overcoming Drug Resistance"

4:15 pm  P. Roy Vagelos, Retired Chairman and Chief Executive Officer, Merck & Co., Inc.  
"The Changing Pharmaceutical Industry"

5:00 pm  Closing Remarks
UNDERGRADUATE AND GRADUATE STUDENTS, POSTDOCTORAL FELLOWS AND RESEARCH FACULTY (NON-TENURE TRACK) INCLUDING COMPETITIVE FELLOWSHIP AWARDS

Undergraduate Students
- Gregg Barcan, Biomolecular Crystallography
- Leo Barinov, Protein Crystallography
- Mukta Baweja, Biomolecular Crystallography
  J&J Research Scholar
- Rachel Bradley, Biomolecular Crystallography
- Kevin Bray, Viral Transformation
- Mariam Buker, Molecular Chronobiology
- Acacia Carreon, Biomolecular Crystallography
- Zhe Chen, Mammalian Embryogenesis
- Coleen Cissosanti, Protein NMR Spectroscopy
- Deep Desai, Molecular Oncology & Development
- Pooja Dharia, Protein NMR Spectroscopy
- Anna Dulencin, Protein Targeting
- Marwa Elshishiny, Protein Targeting
- Michael Fakhry, Protein NMR Spectroscopy
- Erin Folger, Mammalian Embryogenesis
  J&J Research Scholar
- Keith Hamilton, Protein NMR Spectroscopy
- Arun Handa, Sleep Genetics
  J&J Research Scholar
- James Hsu, Protein Crystallography
- Ashley Hutson, Biomolecular Crystallography
- Mehreen Iqbal, Molecular Virology
  J&J Research Scholar
- Arthee Jahangir, Mammalian Embryogenesis
- Sindhu Kadambi, Developmental Neurogenetics
- Neha Kaushik, Protein Crystallography
- Pushpa Keshav, Developmental Neurogenetics
  Rutgers Aresty Research Center Award
- Sameera Kongara, Viral Transformation
- Olga Kravchuk, Viral Transformation
  NJCCR Summer Fellowship
- Dong Yup Lee, Protein NMR Spectroscopy
- Hoi Ching Lee, Protein Targeting
Undergraduate Students
Graduate Students

- Joel Marimuthu, Molecular Oncology and Development
  J&J Research Scholar
- Patricia Moore, Protein Targeting
- Elton Muci, Molecular Oncology and Development
  J&J Research Scholar
- Aleksandra Nagorny, Biomolecular Crystallography
- Anna Natenzon, Biomolecular Crystallography
  J&J Research Scholar
- Ebony Ortiz, Lab Management
- Jenna Pacheco, Lab Management
- Dipikaben Patel, Molecular Virology
  J&J Research Scholar
- Neel Patel, Protein Targeting
- Shyam Patel, Viral Transformation
- Taral Patel, Protein NMR Spectroscopy
- Brian Radvansky, Protein NMR Spectroscopy
- Abraham Rashin, Biomolecular Crystallography
  J&J Research Scholar
- Rebecca Rice, Protein NMR Spectroscopy
- Jung Seo, Protein Engineering
  Henry Rutgers Scholars Program Award
- Mayank Shah, Developmental Neurogenetics
- Jamil Shaikh, Sleep Genetics
  J&J Research Scholar
- Norain Siddiqui, Protein NMR Spectroscopy
- Kate Stokes, Protein NMR Spectroscopy
- Xiaonan Sun, Developmental Neurogenetics
  Rutgers Aresty Research Center Award
- Tiffany Tsay, Biomolecular Crystallography
- James Tyminski, Biomolecular Crystallography
- Rushabh Vakharia, Protein NMR Spectroscopy
- Adarsh Yagnik, Molecular Oncology and Development
  J&J Research Scholar

Graduate Students

- Priti Bachhawat, Protein Crystallography
- Joseph Bauman, Biomolecular Crystallography
- 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
- Kevin Bray, Viral Transformation
- 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
- 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
- Hua Han, Protein Crystallography
- Chi Kent Ho, Protein NMR Spectroscopy
- Kangxin Jin, Molecular Neurodevelopment
- Silky Kamdar, Developmental Neurogenetics
- Kwi-Hye Kim, Protein Targeting
  Batten Disease Support & Research Association Fellowship
- 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
- Anna Puzio-Kuter, Molecular Oncology and Development
  NIH NRSA Predoctoral Fellowship
- Meiqian Qian, Protein Targeting
  NIH Interdisciplinary Research Workforce Training Grant
- Matthew Simmons, Tumor Virology
- David Snyder, Protein NMR Spectroscopy
- Victoria Swiss, Molecular Oncology and Development
  NIH Biotech Training Grant
  New Jersey Commission on Cancer Research Fellowship
- Yuan Tao, Protein Crystallography
  NIH NRSA Fellowship
- Xiongying Tu, Biomolecular Crystallography
Graduate Students
Postdoctoral Research Fellows

- 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
- Hailong Yu, Mammalian Embryogenesis

Postdoctoral Research Fellows
- Brian Benoff, Protein Crystallography
  - Howard Hughes Medical Institute
- Ramesh Chellappa, Molecular Neurodevelopment
- Joanna Chiu, Molecular Chronobiology
  - NIH NRSA Postdoctoral Fellowship
- Pragnya Das, Molecular Neurodevelopment
- Kyriakos Economides, Molecular Oncology and Development
  - NIH NRSA Postdoctoral Fellowship
- Yongjun Fan, Tumor Virology
- Mary Fitzgerald, Biomolecular Crystallography
- Hui Gao, Molecular Oncology and Development
- Rong Gao, Protein Crystallography
  - Howard Hughes Medical Institute
- Dehua Hang, Protein NMR Spectroscopy
- Daniel Himmel, Biomolecular Crystallography
  - NIH NRSA Postdoctoral Fellowship
- William Ho, Biomolecular Crystallography
- Marianna de Julio, Mammalian Embryogenesis
  - DOD Postdoctoral Fellowship
- Shih-hsin Kan, Mammalian Embryogenesis
  - DOD Postdoctoral Fellowship
- Vassiliki Karantza, Viral Transformation
- Marinela Cristina Karp, Viral Transformation
- Ning Lei, Mammalian Embryogenesis
- Shengguo Li, Molecular Neurodevelopment
- Edward Licitra, Viral Transformation
- Donglin Liu, Molecular Virology
  - New Jersey Commission on Cancer Research Fellowship
- Huijun Luo, Molecular Neurodevelopment
- Robin Mathew, Viral Transformation
Postdoctoral Research Fellows
Research Faculty (Non-Tenure Track) and Research Associates

-Paul Matteson, Developmental Neurogenetics
-Zeqian Mo, Molecular Neurodevelopment
-Feng Qiu, Molecular Neurodevelopment
-David Sidote, Protein Crystallography
    Howard Hughes Medical Institute
-Yuefeng Tang, Protein NMR Spectroscopy
-Jingqiang Wang, Molecular Oncology & Development
-Xi Wang, Mammalian Embryogenesis
-Yanhong Wang, Protein Targeting

Research Faculty (Non-Tenure Track)
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
Kurt Degenhardt-Assistant Research Professor, MBB, RU
Palchamy Elango-Research Associate, MBB, RU
Swapna Gurla-Assistant Research Professor, MBB, RU
Yuanpeng Huang-Assistant Research Professor, MBB, RU
Brian Hudson-Research Associate, Chemistry, RU
Scott Hughes-Research Associate, Chemistry, RU
Eun Young Kim-Research Associate, MBB, RU
Hansol Lee, Adjunct Instructor, Medicine, UMDNJ-RWJMS
Li Chung Ma-Associate Research, MBB, RU
Thomas Mariano-Assistant Research Professor, Chemistry, RU
Isao Matsuura-Research Associate, Chemical Biology, RU
Hunter Moseley-Assistant Research Professor, MBB, RU
Ferez Nallaseth-Research Associate, MBB, RU
Wilberto Nieves-Neira, M.D.-Research Assistant Professor, Ob., Gyn. & Reprod. Sci., UMDNJ-RWJMS
Deena Oren-Assistant Research Professor, Chemistry, RU
Xuesong Ouyang-Adjunct Instructor, Medicine, UMDNJ-RWJMS
Paolo Rossi-Assistant Research Professor, MBB, RU
Stefan Sarafianos-Associate Research Professor, Chemistry, RU
Seema Sharma-Research Associate, MBB, RU
David Sleat-Research Associate Professor, Pharmacology, UMDNJ-RWJMS
Lincong Wang-Research Associate, MBB, RU
# Administrative and Support Staff / Technical Staff

## Administrative and Support Staff

<table>
<thead>
<tr>
<th>Butler, Lynnette</th>
<th>Khan, Rana</th>
<th>Rossi, Kristin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drudy, John</td>
<td>Kornhaber, Jane</td>
<td>Sharkey, Thomasina</td>
</tr>
<tr>
<td>Dugenio, Ramon</td>
<td>LaFliandra, Alba</td>
<td>Shaver, Barbara</td>
</tr>
<tr>
<td>Esteves, Teresa</td>
<td>Lafond, Aileen</td>
<td>Sinha, Minoti</td>
</tr>
<tr>
<td>Frances, Madeline</td>
<td>Marshall, Sally</td>
<td>Sowa, Angela</td>
</tr>
<tr>
<td>Graham, Calreather</td>
<td>Miller, Loretta</td>
<td>Sutterlin, Diane</td>
</tr>
<tr>
<td>Grossman, Amy (HHMI)</td>
<td>Molina, Julia</td>
<td>Vaughn, Camille</td>
</tr>
<tr>
<td>Hebenstreit, Donna</td>
<td>Mortos, Flordelisa</td>
<td>Venner, Laura</td>
</tr>
<tr>
<td>Hedlhi, Mahdi</td>
<td>Patel, Shakuntala</td>
<td>Waltz, Shelley</td>
</tr>
<tr>
<td>Holowczak, Mary Ann</td>
<td>Pulz, Sharon</td>
<td>Williams, Keith</td>
</tr>
</tbody>
</table>

## Technical Staff

<table>
<thead>
<tr>
<th>Baran, Michael</th>
<th>Kadambi, Sindhuja</th>
<th>Tong, Saichu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beaudoin, Brian</td>
<td>Kinkade, Caroline</td>
<td>Walker, David</td>
</tr>
<tr>
<td>Chen, Chen Xiao</td>
<td>Lackland, Henry</td>
<td>Wang, Dongyan</td>
</tr>
<tr>
<td>Chen, Guanghua</td>
<td>Lazar, Gloria</td>
<td>Wang, Huang</td>
</tr>
<tr>
<td>Chiang, Yi-Wen</td>
<td>Lim, Cecilia</td>
<td>Wu, Ti (HHMI)</td>
</tr>
<tr>
<td>Chu, Jennifer</td>
<td>Locke, Jessica</td>
<td>Xiao, Rong</td>
</tr>
<tr>
<td>Clark, Art</td>
<td>Mukherjee, Chandreyee</td>
<td>Zhao, Caifeng</td>
</tr>
<tr>
<td>Conover, Kenith</td>
<td>Nader, Kamyar</td>
<td>Zhao, Li</td>
</tr>
<tr>
<td>Cunningham, Kellie</td>
<td>Nwosu, Jenifer</td>
<td>Zheng, Haiyan</td>
</tr>
<tr>
<td>Dharia, Chhaya</td>
<td>Okubo, Yasushi</td>
<td></td>
</tr>
<tr>
<td>El-Banna, Mahmoud</td>
<td>Owens, Leah</td>
<td></td>
</tr>
<tr>
<td>El-Banna, Mukarram</td>
<td>Patel, Jay</td>
<td></td>
</tr>
<tr>
<td>Fang, Yingyi</td>
<td>Petrosky, Whitney</td>
<td></td>
</tr>
<tr>
<td>Gao, Jizong (HHMI)</td>
<td>Pike, Douglas</td>
<td></td>
</tr>
<tr>
<td>Halili, Vivienne</td>
<td>Price, Sandy</td>
<td></td>
</tr>
<tr>
<td>He, Dongming</td>
<td>Rahman, Taslima</td>
<td></td>
</tr>
<tr>
<td>Hu, Yaping</td>
<td>Shetty, Karishma</td>
<td></td>
</tr>
<tr>
<td>Janjua, Haleema</td>
<td>Sohar, Istvan</td>
<td></td>
</tr>
<tr>
<td>Jiang, Mei</td>
<td>Sun, Yvonne</td>
<td></td>
</tr>
</tbody>
</table>
**PATENTS**


Anderson, S., and Ryan, R. "Inhibitors of urokinase plasminogen activator" U. S. Patent No. 5,550,213


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., 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


Lobel, P and Sleat, D. , "Human lysosomal protein and methods of its use." US Patent No. 6,302,685 B1

Millonig, James H., Brzustowicz, Linda M., Gharani, Neda. “Genes as diagnostic tools for autism”, Provisional application submitted 7/1/03


White, E., Chiou, S.-K. and Lin, H.-J. Cells having oncogene-suppressed p53-mediated apoptosis and methods of use to identify anti-oncogenic compounds. US Patent No. 5,604,113

White, E., Thomas, A., Kasof, G., and Goyal, L. Recombinant cell line and screening method for identifying agents which regulate apoptosis and tumor suppression. US Patent No. 6,890,716 B1


Degenhardt, K., Nelson, D., Cuconati, A. and White, E. Transformed epithelial baby mouse kidney cell lines (BMK) with targeted gene disruptions in Bax, Bak and Bax plus Bak (pending). Patent Application 60/323,392


CABM Scientific Advisory Board

Outside Academic Members:

Dr. Wayne A. Hendrickson  
Investigator, Howard Hughes Medical Institute  
Professor  
Department of Biochemistry & Molecular Biophysics  
Columbia University

Dr. Robert G. Roeder  
Arnold and Mabel Beckman Professor  
Head, Laboratory of Biochemistry and Molecular Biology  
The Rockefeller University

David D. Sabatini, M.D., Ph.D.  
Frederick L. Ehrman Professor and Chairman  
Department of Cell Biology  
NYU School of Medicine

Industry Representatives:

Dr. Lee E. Babiss  
Vice President  
Pre-Clinical Research & Development  
Hoffmann-La Roche, Inc.

Dr. Kenneth S. Koblan  
Vice President  
Neuroscience Research  
Merck Research Laboratories  
Merck & Co., Inc.

Dr. C. Elliott Sigal, M.D., Ph.D.  
Chief Scientific Officer, President, PRI  
Bristol-Myers Squibb Company

Dr. Catherine Strader  
Executive Vice President and  
Chief Scientific Officer  
Schering-Plough Research Institute
Dr. Theodore John Torphy (Chair)
Corporate Vice President and Chief Scientific Officer
Corporate Office of Science and Technology
Johnson & Johnson

Dr. Frank S. Walsh
Senior Vice President and Head,
Discovery Research
Wyeth Research

**UMDNJ Members:**

William N. Hait, M.D., Ph.D.
Director, The Cancer Institute of New Jersey
Associate Dean, Oncology Program
Professor of Medicine & Pharmacology

Dr. Masayori Inouye
Professor and Chairman
Department of Biochemistry

Dr. Leroy F. Liu
Professor and Chairman
Department of Pharmacology

**Rutgers University Members:**

Dr. Kenneth J. Breslauer
Linus C. Pauling Professor of Chemistry
Associate Dean and Director
Division of Life Sciences
Wright/Rieman Chemistry Laboratory

Dr. Allan H. Conney
Director, Laboratory for Cancer Research
State of New Jersey Professor of Pharmacology
William M. and Myrle W. Garbe Professor of Cancer and Leukemia Research

Dr. Joachim W. Messing,
University Professor and Director
Waksman Institute
FORMER CABM SCIENTIFIC ADVISORY BOARD MEMBERS

Alberts, Bruce, Prof., UCSF (Pres. National Academy of Sciences), '86
* Baltimore, David, Director, Whitehead Inst.,'86-'90
Black, Ira, Chairman, Dept. Neuroscience and Cell Biology, RWJ/UMDNJ, (deceased), '97-'06
Blumenthal, David, Exec. Dir., Ctr. for Health Policy & Mgmt. @ Harvard, '86-'88
Bolen, Joseph, Vice President, Hoechst Marlin Roussel, '98
Burke, James, Chairman of the Board, J & J, (retired), '86-'89
Denhardt, David, Prof. & Chairman, Rutgers, Dept. Biol. Sci., '88-'91
Drews, Jürgen, Pres., Intl. R & D, Hoffmann-La Roche Inc., (retired), '93-'96
Gage, L. Patrick, Pres., Wyeth-Ayerst, (retired), '01
Gussin, Robert, Corporate V. P., Johnson and Johnson, (retired), '01
Haber, Edgar, Pres., Bristol-Myers Squibb Pharma. Res. Inst., (deceased), '90-'91
Harris, Edward, Jr., Prof. & Chairman, Dept. Med., RWJ/UMDNJ '86-'88
Lerner, Irwin, Pres. & CEO, Hoffmann-La Roche Inc., (retired), '86-'93
Loh, Dennis, Vice President Preclinical Res. & Dev. Hoffmann-La Roche Inc. '98
Luck, David, Prof., Rockefeller Univ., (deceased), '94-'97
* Merrifield, Robert, Prof., Rockefeller Univ., (deceased), '91-'93
Moliteus, Magnus, Pres., Pharmacia, '86-'88
Morris, N. Ronald, Associate Dean & Professor, Dept. of Pharma, RWJ/UMDNJ, (retired), '86-'92
* Nathans, Daniel, Sr. Invest., HHMI, Johns Hopkins Univ., (deceased), '93-'96
Reynolds, Richard C., Executive Vice President, RWJ Foundation, (retired), '88-'91
Rosenberg, Leon, Pres., Bristol-Myers Squibb, (retired), '91-'97
Ruddon, Raymond W., Corp. V.P. Johnson & Johnson, (retired), '01
Olson, Wilma, Prof., Dept. of Chemistry, Rutgers, '86-'92
Palmer, James, CSO and Pres., Bristol-Myers Squibb Company, (deceased), '00-'04
Pickett, Cecil, Executive V.P., Schering-Plough Research Inst., (retired), '00-'06
Pramer, David, Dir., Waksman Inst. '86-'88
Ringrose, Peter, Pres., Bristol-Myers Squibb, (retired), 2000
Sanders, Charles, Vice Chairman, E.R. Squibb & Sons, (retired), '88-'91
Scolnick, Edward M., M.D. President, Merck & Co. Inc., (retired), '95 - '97
Shapiro, Bennett, Executive V.P., Merck & Company, (retired), '01
* 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