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Director’s Overview

CABM—25 and counting.
CABM experienced a momentous 2011. It was remarkable as our 25th anniversary year. Exciting events to celebrate this milestone included a festive dinner attended by nearly 200 former students and postdoctoral fellows who returned to CABM from around the country, Europe and Asia. This enjoyable reunion was held on October 20th. Announced at this event was the establishment of the Aaron J. Shatkin Endowed Lectureship which will start in the spring of 2012 and the naming of the CABM south atrium as the Shatkin Atrium – two overwhelming and highly appreciated honors. The CABM Annual Symposium was held on the next day. The meeting attracted more than 450 participants and featured lectures by current and former CABM Scientific Advisory Board members Bruce Alberts, Wayne Hendrickson, Robert Roeder and Phillip Sharp. In addition, several CABM alumni from academia and industry gave talks, and many others described recent their recent work in poster presentations.

Continuing the CABM Mission
The year was also noteworthy for the continued commitment to excellence of CABM faculty and students. Their research success made possible the vigorous pursuit of our mission--the advancement of basic knowledge to improve human health. CABM research programs were supported by NIH and several additional federal and state granting agencies as well as other public and private sources. The resulting research advanced our understanding of cancer, infectious diseases and hereditary and developmental disorders. As described in the individual research summaries, CABM researchers are collaborating with clinical investigators at The Cancer Institute of New Jersey to elicit new targets and to develop therapies for non-Hodgkins lymphomas and metastatic breast cancers as well as to design novel proteins for therapeutic use in cancer and other devastating diseases. Several CABM groups have gained new insights and found clever approaches to answer the health challenges of infections by antibiotic resistant bacteria, HIV/AIDS, hepatitis and influenza viruses. Other groups have used model systems to define a temperature dependent molecular mechanism for regulating biological clocks, uncover an autism susceptibility gene and decipher the differentiation cascade of retinal cells essential for vision. Another CABM laboratory has discovered the molecular basis of Batten Disease, a lethal childhood hereditary disorder that may become reversible and even curable based on this finding and developing technology licensed to a biotechnology company.

Fostering the Growth of Structural Biology
Another stellar event in 2011 has been the completion by Rutgers University of an impressive Center for Integrative Proteomics Research. The new building is physically connected to CABM and will accommodate computational research and training activities including the Biomaps Institute, high field NMR groups, the CABM Mass Spectrometry core facility, a cryoEM laboratory and the international Protein Data Bank (PDB). CABM had the pleasure of hosting the PDB group this year while the proteomics building was under construction. This proved to be an enjoyable arrangement that led to productive interactions and mutually beneficial collaborations, notably between PDB and the Northeast Structural Genomics Consortium, a multi-institutional center led by a CABM group and sponsored by NIH as part of the Protein Structural Initiative (PSI).
Teaching in the Classroom and Laboratory
All CABM faculty participate actively in the teaching of students and postdoctoral fellows both in the lecture hall and at the laboratory bench. Trainees include undergraduates, graduate students from many different MS and PhD programs and medical students. The NIH Biotechnology Program has been an excellent source of training and support for PhD students from various disciplines for more than 20 years. In recognition of its success, the training program was recently renewed for another five years. Outreach education programs also introduce scientific research to highly motivated high school students at local schools through SMART Team and Biolinks programs. In addition, CABM hosts a Summer Undergraduate Research Experience (SURE) that is now in its 8th year. In 2011 SURE students from Rutgers and several other leading universities participated in lectures and lab projects mentored by CABM graduate students and postdoctoral fellows—providing opportunities for both budding and committed scientists to interact. Other outreach programs include a seminar series that brings visiting scientists to talk about their work and to meet with trainees and the annual CABM Symposium each year on a topic of special interest. The next Symposium, our 26th, will be held in October, 2012.

Awards and Service
CABM faculty have received significant national honors for their scientific contributions and service. These include elected fellowship in AAAS, the American Academy of Microbiology, the American Academy of Arts & Sciences and the US National Academy of Sciences. Drs. Stock and Arnold are recipients of MERIT awards from NIH, and Drs. Gelinas, Millonig and Rabson are NIH study section members. Dr. Stock recently completed service as Chair of the Board of Scientific Counselors for NIH-NIDCR and is completing a long-held position as HHMI investigator. After completing service on the American Society of Biochemistry & Molecular Biology Council, she was appointed this year to the Society’s Education & Professional Development and Finance Committees. Dr. Montelione is advisor to the NSF Committee of Visitors. Many faculty serve on other national committees and in journal editorial positions. At the local level, Dr. Millonig recently joined Drs. Gelinas and Stock as Master Educators. In addition, Dr. Gelinas serves as Associate Dean for Research, and Dr. Millonig leads the RWJMS/Rutgers/Princeton joint MD/PhD program. This past year Dr. Rabson was appointed director of the Child Health Institute of New Jersey.

Financial Support
Advancing science through research requires strong support from many sources, administrative as well as financial. CABM is jointly administered by the University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School and Rutgers, the State University of New Jersey. We thank the Universities, in particular for providing a wonderfully functional building for doing science. CABM also benefits from the advice, wisdom and guidance freely given by a Scientific Advisory Board composed of leaders from academia and industry. We acknowledge with gratitude their help and support on many issues. Despite difficult economic times, CABM faculty were successful again in obtaining grants and contracts—nearly $18 million in 2011. Since the founding of CABM, a total of ~$300 million has been awarded, mostly for research but also for education and service functions that have had positive impacts on the two universities and stimulated the NJ economy. We are indebted to the numerous public agencies, private foundations, companies and individuals for their generous and essential financial assistance in our pursuit of excellence at CABM for the past 25 years. The sources of federal funding notably include the National Institutes
of Health, National Science Foundation, the Department of Defense and, at the state level, The NJ Commission on Spinal Cord Research, NJ Commission on Cancer Research, and the Governor’s Council for Medical Research and Treatment of Autism. The Howard Hughes Medical Institute has been supportive of CABM for nearly 20 years, and the Ara Parseghian Medical Research Foundation and Batten Disease Support and Research Association provided important help. CABM is very grateful to numerous companies including Johnson & Johnson, Hoffmann-LaRoche, Sanofi-Aventis, Pfizer, Takara Bio Inc., Cisco Inc., Nexomics Biosciences Inc., BioMarin Pharmaceuticals, Amicus Therapeutics, LifeGas, CIL, GenScript, Macrogen, New England BioLabs, Taxis Pharmaceuticals Inc., Rigaku and Genewiz. Without this combined financial help, CABM could not have maintained its commitment to excellence in advancing basic knowledge in the life sciences to improve human health. We are most grateful.

Aaron J. Shatkin, Professor and Director

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Research Programs and Laboratories
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The main focus of the Edery lab is to understand the biochemical and cellular bases underlying circadian (~24 hr) rhythms, using *Drosophila* as a model system. Our strength lies in applying biochemical strategies that are integrated with genetic, ecological and evolutionary perspectives to understand how circadian clocks function and regulate animal physiology and behavior. Circadian rhythms are driven by cellular clocks and enable organisms to anticipate daily and seasonal changes in environmental conditions, ensuring activities occur at optimal times in the day and appropriate seasonal responses are elicited. Understanding how clocks function is highly relevant for human health and well-being. Indeed, clock malfunctions in humans have been implicated in many diseases, including manic-depression, cancer, sleep problems and metabolic syndromes. Our lab is investigating the core clock mechanism, identifying novel clock proteins and how they interact to build a biological time-keeping device that is synchronized to local time. In a second major effort, we are determining the role of clocks in seasonal adaptation, which has led to novel ecological and evolutionary implications. These studies have also broadened our research scope by revealing mechanisms that regulate sleep and arousal. In addition, we are investigating how environmental stimuli, most notably temperature, controls global gene expression. Below is a brief summary of some of our ongoing research and future directions

**Mechanisms underlying circadian clocks:** The central clock mechanism is a dynamically changing multi-subunit biochemical oscillator based on a small set of interacting core “clock” proteins and auxiliary factors that as a unit generates a self-perpetuating daily transcriptional feedback loop that also drives global cyclical gene expression, which underlies many of the daily rhythms manifested by organisms. In addition, to the transcriptional architecture, numerous post-translational regulatory pathways, most notably reversible phosphorylation, regulate various aspects of clock
protein metabolism/activity, such that the biochemical oscillator has an endogenous period of ~24 hr. A conserved feature of animal clocks is that the key clock protein termed PERIOD (PER) undergoes daily rhythms in phosphorylation that are central to normal clock progression in animals. We showed that phosphorylated PER is recognized by the F-box protein, SLIMB (homolog of β-TrCP) and targeted to the 26S proteasome for rapid degradation (Ko et al., 2002, Nature), a key event in stimulating the next round of daily gene expression. We used mass spectrometry and phospho-specific antibodies to identify the critical phosphorylation events that promote the binding of SLIMB to PER (Chiu et al., 2008, Genes & Development). Converting key phospho-sites to Ala or Asp (phospho-mimetic) led to decreases or increases in the pace of the clock, respectively (Chiu et al., 2008, Genes & Development). In more recent work we identified a novel clock kinase, NEMO, and showed that different phospho-clusters have distinct functions (Chiu et al., 2011, Cell). Ongoing work is aimed at identifying the roles of different phospho-clusters on PER and the relevant kinases and phosphatases. We are also applying these techniques to other clock proteins, and investigating the effects of other post-translational modifications in clock function, such as SUMOylation and acetylation. Moreover, PER proteins not only function as the principle biochemical timer but also link to gene expression by acting as transcriptional repressors, whereby they “deliver” factors such as chromatin remodeling factors to central clock transcription factors, yielding cyclical gene expression and hence rhythmic physiology and behavior. We are undertaking proteomic strategies to identify native clock protein complexes and how they change throughout a daily cycle. In related work we are investigating the roles of micro RNAs (miRNAs) in clock function.

**Seasonal and thermal adaptation:** Many animals exhibit a bimodal distribution of activity, with ‘morning’ and ‘evening’ bouts of activity that are separated by a midday dip in activity or ‘siesta’. Ambient temperature is a key environmental modality regulating the daily distribution of activity in animals. In *D. melanogaster*, as temperatures rise there is less midday activity and the two bouts of activity are increasingly shifted into the cooler nighttime hours, almost certainly an adaptive response that minimizes the detrimental effects of the hot midday sun (Majercak et al., 1999, Neuron). We showed that the temperature-dependent splicing of the 3′-terminal intron (termed dmpi8) from the *D. melanogaster per* RNA is a major ‘thermosensor’ that adjusts the distribution of daily wake-sleep cycles, eliciting seasonably appropriate responses. In more recent work we showed that this mechanism does not operate in several *Drosophila* species with more restricted and ancestral locations in equatorial Africa wherein temperature and daylength do not show large seasonal variations (Low et al., 2008, Neuron). We investigated the molecular basis for the species-specific splicing phenotypes and found that multiple suboptimal splicing signals on dmpi8 underlie the thermosensitivity (Low et al., 2008, Neuron). Presumably, higher temperatures progressively destabilize interactions between the non-consensus 5′ splice site (ss) and the U1 snRNP, the initial step in the splicing reaction. Ongoing work is aimed at understanding how temperature regulates global gene expression. In addition, these studies are revealing new mechanisms that regulate sleep and arousal.
Publications (2010-2011):


Our laboratory focuses on the Rel/NF-kB signaling pathway and its role in cell survival and cancer. The Rel/NF-kB transcription factors are key to normal immune and inflammatory responses and play critical roles in tumor cell survival, pathogenesis and chemoresistance. They are thus important targets for therapy. Rel/NF-kB is constitutively activated in many human cancers, and the Rel proteins are implicated in leukemia/lymphomagenesis and in breast cancer. However, the detailed mechanism is not fully understood. Our studies aim at understanding how changes in NF-kB’s transcriptional activity and regulation contribute to carcinogenesis and tumor cell resistance to anti-cancer treatment.

We published a study demonstrating that defective ubiquitin-proteasome mediated turnover of the NF-kB-regulated apoptosis inhibitor Bfl-1 (Bcl2A1, A1) can predispose to lymphoma (Fan et al. 2010). Ubiquitination-resistant mutants of Bfl-1 showed decreased turnover and greatly accelerated tumor formation in a mouse model of leukemia/lymphoma. We also showed that these tumors also displayed upregulation and activation of tyrosine kinase Lck along with activation of the IKK, Akt and Erk signaling pathways, which are key mediators in cancer. Coexpression of Bfl-1 and constitutively active Lck promoted tumor formation, whereas Lck knockdown in tumor-derived cells suppressed leukemia/lymphomagenesis. Since Bfl-1 is overexpressed in many therapy-resistant leukemia and lymphomas and is necessary for tumor cell maintenance and chemoresistance, these data suggest that ubiquitination is an important tumor suppression mechanism to regulate Bfl-1 function. This also raises the possibility that mutations in bfl-1 or in the signaling pathways that control its ubiquitination may predispose to cancer. This work was conducted together with our colleagues, Drs. Eileen White (CINJ and Rutgers Univ.), Yacov Ron (RWJMS) and David Weissmann (RWJMS, RWJUH).
The findings above suggested that accelerating Bfl-1 turnover could perhaps be used to improve tumor cell response to anti-cancer therapy. We pursued our investigation of kinase-specific inhibitors that can accelerate Bfl-1 turnover and showed that some of them could significantly sensitize drug-resistant human leukemia and lymphoma cell lines to anti-cancer agents. Ongoing studies are evaluating the effectiveness of combination treatment in vivo in mouse xenograft models and in patient-derived specimens ex vivo together with our colleagues Drs. Roger Strair, Daniel Medina, Lauri Goodell and Susan Goodin (CINJ).

Our efforts looking into the mechanisms that regulate Bfl-1 turnover and function revealed that while Bfl-1 is regulated by ubiquitination, it is not a substrate for sumoylation. This indicated that ubiquitination is the primary mode of post-translational regulation to control its half-life. We identified specific mutations in putative Bfl-1 phosphorylation sites that markedly alter its turnover and have been investigating if these are substrates for phosphorylation. We also identified a naturally occurring gene mutation in human lymphoma cells that alters turnover of apoptosis inhibitor Bfl-1 and chemoresistance, and have optimized conditions to purify Bfl-1 binding partners with the ultimate goal to identify factors that control its turnover.

Collaborative studies with the group of Dr. Gaetano Montelione (CABM) elucidated the structure of Bfl-1ΔC (residues 1-151) bound to a Noxa BH3 peptide, refined to 2.25 Å resolution. This provided additional key insights into the structure of the helix-binding cleft of Bfl-1 and helped to understand how Bfl-1 selectively associates with some proapoptotic Bcl-2 family members and what dictates its affinity and specificity for Noxa compared to other Bcl-2 family members. A manuscript is in preparation (Guan et al. In preparation).

We also pursued our studies on the role of CAPER in breast cancer and its functional interaction with estrogen receptor and NF-kB. Constitutive Rel/NF-κB activity is inversely correlated with expression of estrogen receptor alpha (ERα) and is key to breast cancer cell survival, invasion and resistance to chemotherapy. Our studies uncovered a new role for CAPER in breast cancer cell invasion. In vivo studies using a mouse model of breast cancer metastasis are ongoing to elucidate its role in breast cancer progression and metastasis. Since CAPER was previously implicated both in transcriptional regulation as well as in alternative splicing, we used Affymetrix exon arrays to probe for possible effects on global gene expression in order to better understand the mechanisms by which it affects invasion. Preliminary results suggest interesting effects of CAPER at both levels. This is consistent with CAPER mutation data and with immunofluorescence data showing co-localization of CAPER with splicing factor SC35. These experiments are being conducted in collaboration with the group of Dr. Guna Rajagopal (CINJ).

Collaborative work emanating from the laboratories of Drs. Daniel Medina and Roger Strair (CINJ), together with Drs. John Glod (CINJ), Arnold Rabson
(CHINJ, CINJ and CABM) and Lauri Goodell (RWJUH) characterized the effects of mesenchymal stromal cell (MSC) on the survival and drug resistance of primary mantle cell lymphoma (MCL) cells, an aggressive subtype of non-Hodgkin’s lymphoma. Studies revealed an important role for the canonical and non-canonical NF-kB pathways and expression of cytokine BAFF by MSC in the long term survival of MSC. A manuscript was submitted (Medina et al. Submitted).

Finally, we pursued our collaborative work with Dr. Eileen White (CINJ) on the role of autophagy in tumorigenesis and the role of NF-kB in this context. These studies uncovered that activated Ras, commonly found in human tumors, requires autophagy to maintain oxidative metabolism and tumorigenesis. This suggests that therapeutic strategies to inhibit autophagy could perhaps be useful for the treatment of cancers in which Ras is activated. This work was published (Guo et al. 2011). Ongoing studies focus on understanding how p62 modulates autophagy and tumorigenesis and the role of NF-kB in this context.

**CAPER co-localizes with splicing factor SC35 in breast cancer cells, as seen by immunofluorescence and confocal microscopy.**
Publications:


Toxin-antitoxin (TA) systems; their roles in bacterial physiology and the development of novel antibiotics

Almost all bacteria including human pathogens contain suicide or toxin genes which are induced under stress conditions leading to cell growth arrest and eventual cell death in a way similar to apoptosis or programmed cell death in higher systems. *Escherichia coli* contains at least 35 TA systems and their toxins are highly diverse, targeting DNA, mRNA, protein and cell wall synthesis. The discovery of a sequence (ACA)-specific endoribonuclease from the *E. coli* TA systems led his laboratory to develop a unique protein production system converting *E. coli* cells to a single-protein production (SPP) bioreactor. This system allows one to label only a protein of interest in the cells with a specific isotope(s), an amino acid(s) or a toxic non-natural amino acid analogue(s) in a high yield. His laboratory also does research on the mechanism of ribosome stalling during protein synthesis. In May 2011, he received the Edward J. Ill Excellence in Medicine Award for Outstanding Medical Research Scientist. Dr. Inouye is a member of the American Academy of Arts & Sciences.

**Bacterial Physiology and Protein Engineering Laboratory**

Dr. Masayori Inouye joined CABM in October 2009. He is well-known for his discovery of antisense-RNA in 1984, the function of the signal peptide for secretion, biogenesis of outer membrane proteins, propeptide-mediated protein folding and molecular biology of histidine kinases. Currently he is working on the characterization of the toxin-antitoxin (TA) systems from *E. coli* and human pathogens such as *Mycobacterium tuberculosis* and *Staphylococcus aureus*. The discovery of a sequence (ACA)-specific endoribonuclease from the *E. coli* TA systems led his laboratory to develop a unique protein production system converting *E. coli* cells to a single-protein production (SPP) bioreactor. This system allows one to label only a protein of interest in the cells with a specific isotope(s), an amino acid(s) or a toxic non-natural amino acid analogue(s) in a high yield. His laboratory also does research on the mechanism of ribosome stalling during protein synthesis. In May 2011, he received the Edward J. Ill Excellence in Medicine Award for Outstanding Medical Research Scientist. Dr. Inouye is a member of the American Academy of Arts & Sciences.

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**Bacterial Toxin-Antitoxin (Apoptosis) System**
mRNA interferases

mRNA interferases are encoded by one of the TA systems. MazF from *E. coli* is the first mRNA interferase discovered in Dr. Inouye’s laboratory and functions as a sequence-specific (ACA) endoribonuclease. Its induction in *E. coli* cells results in growth arrest and eventual cell death. The Inouye group also observed that MazF induction in mammalian cells effectively causes Bak (a pro-apoptotic protein)-dependent programmed cell death. The application of bacterial mRNA interferases for mammalian cell growth regulation is currently being explored to develop an effective, novel method for cancer treatment and HIV eradication. In addition to *E. coli* MazF, the laboratory has identified a large number of mRNA interferases having different RNA cleavage specificity, including one from a highly halophilic archaeon that cleaves a specific seven base sequence. mRNA interference using highly sequence-specific mRNA interferases instead of antisense RNA or RNAi may be a novel and exciting way to regulate gene expression.

Single protein production in living cells

Expression of MazF, an ACA-specific mRNA interferase, results in nearly complete degradation of cellular mRNAs, leading to almost complete inhibition of protein synthesis. Intriguingly, MazF-induced cells are still fully capable of producing a protein at a high level if the mRNA for that protein is engineered to have no ACA sequences without altering its amino acid sequence. Therefore, it is possible to convert *E. coli* cells into a bioreactor producing a single protein of interest. This “single-protein production” (SPP) system allows NMR structural studies of proteins without purification, which makes this system especially useful for structural studies of membrane proteins. In addition, the SPP system provides a unique opportunity to produce proteins in which all the residues of a specific amino acid in a protein are replaced with toxic non-natural amino acid analogues to create proteins of novel structures and functions.

Publications:


Vaiphei, S.T., Tang, Y., Montelione, G.T., Inouye, M. The Use of the Condensed Single Protein Production System for Isotope-Labeled Outer Membrane Proteins, OmpA


We study TGF-β/Smad signal transduction, transcriptional regulation, cell cycle control and their roles in cancer. TGF-β regulates a wide variety of biological activities. It induces potent growth inhibitory responses in normal cells, but promotes migration and invasion of cancer cells. Smads mediate the TGF-β responses. TGF-β binding to the cell surface receptors leads to the phosphorylation of Smad2/3 in their carboxyl-terminus as well as in the proline-rich linker region. The serine/threonine phosphorylation sites in the linker region are followed by the proline residue. Pin1, a peptidyl-prolyl cis/trans isomerase, recognizes phosphorylated serine/threonine-proline (pS/T-P) motifs. We show that Smad2/3 interacts with Pin1 in a TGF-β-dependent manner. We further show that the phosphorylated threonine 179-proline motif in the Smad3 linker region is the major binding site for Pin1. Although EGF also induces phosphorylation of Smad3 in the carboxyl-terminus as well as in the proline-rich linker region the same as TGF-β, Pin1 is unable to bind to the EGF-stimulated Smad3. Further analysis suggests that phosphorylation of Smad3 in the carboxyl-terminus is necessary for the interaction with Pin1. Depletion of Pin1 by shRNA does not affect significantly TGF-β-induced growth inhibitory responses and a number of TGF-β/Smad target genes analyzed. In contrast, knockdown of Pin1 in human PC3 prostate cancer cells strongly inhibited TGF-β-mediated migration and invasion. Accordingly, TGF-β induction of N-cadherin, which plays an important role in migration and invasion, is markedly reduced when Pin1 is depleted in PC3 cells. The catalytic activity of Pin1 is essential for TGF-β-induced migration and invasion (see Figure). Since Pin1 is overexpressed in many cancers, our findings highlight the importance of Pin1 in TGF-β-induced migration and invasion of cancer cells.
Smad3 plays an important role in inhibiting cell proliferation and promoting apoptosis. Human Smad3 is localized near a hot spot mutation area for breast cancer and for several other types of cancers. We have found that Smad3 levels are reduced in human primary breast cancer samples and in human breast cancer cell lines. Importantly, Smad3 expression is high in normal breast stem cells but low in breast cancer stem cells. In addition, our preliminary studies indicate that 7,12-dimethylbenz-[a]-anthracene (DMBA), a chemical carcinogen, induces mammary tumor formation at a much higher frequency in Smad3-/- or Smad3+/- mice than in Smad3+/+ mice. Thus, we hypothesize that Smad3 has important tumor suppressor function for breast cancer.

Breast cancer stem cells are CD44+CD24-/low. Although CD44 lacks its own signaling domain, it associates with and co-stimulates signaling by a number of growth factor receptors, such as Her2 and EGF receptor 1. CD44 is overexpressed in the vast majority of breast cancers. CD44 is not just a marker for breast cancer stem cells. It is necessary for breast tumor initiation, tumor growth, and metastasis. Knockdown of CD44 greatly reduces tumor-initiating frequency, tumor weight, and colony formation in soft agar assay. Disruption of tumor cell surface CD44 function induces apoptosis in metastatic mammary carcinoma cells in vivo. A predominant function of CD44 is to promote growth and to inhibit apoptosis. The growth-inhibitory and tumor-suppressive functions of p53 depend on its repression of CD44 expression. Our preliminary studies have discovered that Smad3 potently represses CD44 transcription in mammary epithelial cells. Thus, we further hypothesize that Smad3 repression of CD44 expression is essential for its tumor suppressor activity, and that Smad3 and p53 cooperate to inhibit breast tumorigenesis. Our research is directed towards testing these hypotheses. Our findings will be highly significant for preventing and treating breast cancer.
Publications:


Dhar-Mascareno, M., Belishov, I., Liu, F., and Mascareno, E. J. (2011) Hexim-1 modulates androgen receptor and the TGFß signaling during the progression of prostate cancer. The Prostate in press
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 Sach's disease) where loss of a single lysosomal enzyme leads to severe health problems including neurodegeneration, progressive mental retardation and early death.

Our approach to identify the molecular basis for unsolved lysosomal storage disorders is based on our ability to use mannose 6-phosphate receptor derivatives to visualize and purify mannose 6-phosphate containing lysosomal enzymes. Once we discover the cause of a given disease, we conduct further research to understand the underlying system and potentially to develop therapeutics. We also investigate the basic mechanism for targeting of lysosomal proteins and the composition of the lysosome.
In the prior year, we have made considerable progress investigating late infantile neuronal ceroid lipofuscinosis (LINCL), a recessive neurodegenerative disease of childhood that is due to deficiencies in the lysosomal protease tripeptidyl-peptidase 1 (TPP1). In one study, we performed mass spectrometric analyses on storage material and lysosomal fractions from an LINCL mouse model. This revealed several candidates for proteins stored in brain. In depth analysis of the most prominent of these, glial fibrillary acidic protein, revealed that this protein represented a contaminant that adventitiously associated with storage material and lysosomes during the isolation procedure. We also used the mouse model to explore potential therapeutic approaches and found that intrathecal administration of recombinant human TPP1 resulted in significant delivery of enzyme to the brain. Importantly, this treatment prolonged lifespan and improved decreased disease symptoms. Finally, in collaboration with scientists at the University of Missouri and BioMarin Therapeutics, as a step towards the clinic we conducted pilot enzyme replacement studies using an LINCL dog model.

We are also continuing our investigation of the lysosomal proteome. Following the principles of de Duve and colleagues that lead to the discovery of the lysosome, we analyzed rat liver subcellular fractions using mass spectrometry to identify and determine the distribution of different proteins. This led to the identification of numerous candidate lysosomal proteins and provided further evidence that ABCB6 is lysosomal and that superoxide dismutase 1 has a mixed cytoplasmic-lysosomal localization.

Acute intrathecal administration of recombinant human TPP1 extends lifespan of a LINCL mouse model. Animals were administered the indicated dose of TPP1 at the age of 28-30 days and then followed for survival.
Publications:


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, Department of Defense, National Alliance for Research on Schizophrenia and Depression, Autism Speaks, National Alliance for Autism Research, N.J. Governor’s Council on Autism and New Jersey Commission on Spinal Cord Research. He is director of the RU/Princeton/RWJMS M.D./Ph.D. program.

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.

Risk for ASD is likely due to both genetic and non-genetic environmental factors, with epigenetic regulation of genes providing a possible interface between genetic and environmental factors. Our previous research has focused on the homeobox transcription factor, ENGRAILED 2 (EN2). The common alleles (underlined) of two intronic EN2 SNPs, rs1861972 (A/G) and rs1861973 (C/T), are significantly associated with ASD (518 families, P=.00000035). Subsequent association, LD mapping, and re-sequencing identified the associated rs1861972-rs1861973 as the most suitable candidate to test for function.

Function was then tested in primary cultures of cerebellar granule cells since endogenous En2 is expressed at high levels in these cells. Luciferase assays demonstrated that the A-C haplotype results in an ~250% increase in expression. Additional analysis determined that both associated alleles are sufficient and necessary for this activator function. EMSAs revealed that nuclear factors specifically bind the A-C haplotype. An unbiased proteomic screen identified two transcription factors, Cux1 and NfiB, that bind significantly better to the A-C haplotype. Knock-down, over-expression, ChIP and EMSA supershift analysis demonstrated that both transcription factors bind the A-C haplotype at the same time and are required for activator function. These studies determined that the ASD-associated A-C haplotype functions as a transcriptional activator.
We then investigated if EN2 levels are increased in individuals with autism. Ninety cerebellar samples were acquired and Taqman QRT PCR measured normalized EN2 mRNA levels. A significant increase was observed in affected individuals with an A-C haplotype compared to controls (74% increase, P=0.0005). Thus the A-C haplotype is also correlated with increased EN2 levels in individuals with ASD.

Because ASD is a neurodevelopmental disorder, we generated transgenic mice. EN2 was replaced with a DsRed reporter and 25kb of evolutionarily conserved sequence was used to drive expression of the transgene. 6 A-C and 8 G-T lines were generated. QRT PCR measured Ds-Red levels for all the lines at 5 developmental time points (E9.5, E12.5, E17.5, P6 and adult). At all stages the A-C haplotype results in increased expression (P<.001). ISH was then used to determine if this increased expression was due to elevated levels in the same cell types or expanded expression domains. Broader expression domains are at E9.5, E17.5 and adult for the A-C haplotype. For instance, in the adult the A-C haplotype is expressed at high levels in the raphe nuclei and the locus ceruleus while little or no expression is detected for the G-T haplotype. Together these data demonstrate that the ASD associated A-C haplotype is functional and increased EN2 levels is correlated with ASD risk.

**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. We have positionally cloned the vl locus and determined that a mutation in the orphan G protein coupled receptor (GPCR), Gpr161, results in the vl disease phenotypes. In situ hybridizations demonstrated that Gpr161 is expressed in the lateral neural folds of the neural plate, the developing lens, retina, limb and CNS. Characterization of the vl mutation indicates that C terminal tail of Gpr161 is truncated, leading to multiple effects on the protein including reduced receptor-mediated endocytosis (Matteson et al., 2008).

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, Mus castaneus (CAST/Ei) and Mus molossinus (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)(Matteson et al., 2008; Korstanje et al., 2008). To identify the genes in the \( Modvl \) 95% CIs that may contribute to these modifying effects, transcripts co-expressed with \( Gpr161 \) during development were identified by bioinformatic analysis. Ingenuity Pathway Analysis indicates the Wnt and EMT pathways are over-represented (P<.001).

\( Modvl5^{MOLF} \) congenics have been generated to test whether this locus is sufficient to rescue the \( vl \) phenotypes. Mating analysis determined that \( vl \)-associated lethality occurs between E8.5-E9.5 and \( Modvl5^{C/M} \) is sufficient to rescue the lethality. Morphological analysis indicates \( Modvl5^{C/M} \) partially suppresses the \( vl \)-associated NTDs. Bioinformatics determined the transcription factor, Cdx1, is the only gene within the \( Modvl5 \) 95% CI co-expressed with \( Gpr161 \) in the neural folds. Re-sequencing Cdx1 identified a poly-glutamine polymorphism (5Q-C3H, 7Q-MOLF) predicted to affect secondary and tertiary protein structure. Genetic and \textit{in vitro} functional analyses demonstrate the poly-Q polymorphism is functional. Additional \textit{in vitro} and \textit{in vivo} analyses indicate that Cdx1 modulates Wnt and retinoic acid signaling during neural fold development, which may be responsible for the phenotypic rescue. These results establish \( vl \) as one of the first multigenic mouse models of NTDs and support that Cdx1 is a modifier for \( Modvl5 \).

**Schizophrenia:**
Schizophrenia is a common neuropsychiatric disorder that afflicts \( \sim 1:100 \) individuals. Our collaborator, Linda Brzustowicz, M.D. (Rutgers University), has previously reported positive linkage and association results for the Nitric Oxide Synthase 1 Adaptor Protein (NOS1AP) gene (Brzustowicz et al., 2004; Brzustowicz et al., 2000). Molecular genetic experiments (luciferase assays, EMSAs) using human neural cell lines have demonstrated that all three schizophrenia-associated alleles are functional by increasing gene expression. An unbiased proteomic screen is being used to identify the proteins that mediate this activity.
Figure 1. A) Luc assays demonstrate 50% increase in A-C haplotype after 1 day in vitro (left), and ~250% increase after 3 days in vitro in P6 mouse granule cells (right). B) Luc assays demonstrate that a 200bp fragment encompassing both SNPs is sufficient for activator function (left), while analysis of the rare haplotypes (A-T and G-C) determined that both associated alleles are necessary (right) for function. C) EMSAs with P6 mouse granule cell nuclear extract demonstrate nuclear factors (arrow) bind better to A-C than G-T haplotype. D) Knockdown of both Cux1 and NfIB demonstrate that both factors are required for endogenous EN2 expression in HEK-293T cells (n=3).

Publications:


It is estimated that over 20 million people are infected by HTLV-1, the first identified human retrovirus, and 2-5% of them are likely to develop a serious HTLV-1-associated disease. Dr. Rabson is studying the differential mechanisms by which HTLV-1 causes an aggressive and fatal T cell leukemia/lymphoma (Adult T-Cell Leukemia, ATL) in some infected individuals and a series of immunological disorders including a neurological disease of the spinal cord (HTLV-associated Myelopathy, HAM/TSP) in other infected people. These disorders occur in only a minority of patients, years after initial infection. This suggests that there are important interactions between the virus and the host that determine its pathogenicity. Furthermore, a strong host immune response against HTLV-1 gene products favors the establishment of latent infection in vivo. Nonetheless, expression of HTLV-1 gene products, particularly the Tax transactivator, is required for disease development. The Rabson laboratory has identified and characterized the mechanisms by which the expression of HTLV-1 can be activated in infected human T-lymphocytes leading ultimately to disease pathogenesis. They have shown that stimulation through the T-cell receptor can potently induce HTLV-1 gene expression, including the expression of Tax, leading to T cell immortalization. They have recently confirmed this in a mouse model of HTLV-1 latency and gene expression. T cell receptor stimulation of CD4+ cells in a Tax transgenic mouse leads to increased T cell proliferation and long-lived survival (>13 months). Thus, activation of latently infected T cells in HTLV-1-infected individuals may induce expression of Tax, ultimately leading to proliferation of subsets of cells, based on specific T cell receptor-ligand interactions. This could explain the progressive polyclonal to oligoclonal proliferation to ultimately monoclonal proliferation of infected T-cells that characterizes HTLV-1-associated diseases.
Another important feature of HTLV-1-infected T cells expressing the Tax transactivator is the production of multiple cytokines. The Rabson laboratory has shown that Tax-expressing CD4+ T cells fail to properly “bias”, i.e. fail to differentiate into specific T helper subtypes, such as Th1, Th2, Th17 and Treg cells. Instead, the Tax-expressing cells express cytokines characteristic of all of these different cells, suggesting a “confused” phenotype that may in turn lead to immune dysregulation with the potential for both opportunistic infections and autoimmune-like 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 Drs. Ruth Steward (Waksman Institute), Dale Schaar (CINJ), and Hatem Sabaawy (CINJ), the Rabson lab is investigating the functions of PDCD2, a highly conserved nuclear and cytoplasmic protein, the mutation of which disrupts hematopoiesis in Drosophila (Drs. Steward and Minakhina). The Rabson lab has shown high levels of expression of this enigmatic protein in very early developing embryos and is currently performing mouse genetic experiments and transcriptional studies to determine its function. Dr. Rabson has also continued collaborations with Dr. Roger Strair at CINJ on the potential utility of NF-κB inhibition in leukemia therapy. A clinical trial, examining the effects of NF-κB inhibition, as part of induction chemotherapy, on the molecular phenotype of Acute Myeloid Leukemia is in the late stages of development and is a follow-up to a published trial showing the feasibility of inhibiting NF-κB in patients.
Publications:


One of the earliest steps in the cascade of events necessary for mRNA formation and function is the addition of a 5'-terminal \( m^7GpppN \) "cap" to nascent RNA Polymerase II transcripts. This structural hallmark is present on most eukaryotic cellular as well as viral mRNAs and is essential for viability. The presence of a cap enhances several downstream events in cellular gene expression including RNA stability, splicing of pre-mRNAs in the nucleus and initiation of protein synthesis in the cytoplasm. These important effects have fostered many studies that defined the enzymatic mechanisms of capping. We have cloned and sequenced the mouse and human capping enzymes (CEs, ~98% identical) and mapped the human protein to 6q16, a region implicated in tumor suppression. The 597-amino acid, 68kD mammalian polypeptides consist of two functional domains --N-terminal RNA 5’ triphosphatase (RTase) and C-terminal guanylyltransferase (GTase). Mutational, biochemical and genetic analyses demonstrated that the GTase active site is a lysine in the sequence 294 Lys-X-Asp-Gly 297, one of several highly conserved motifs characteristic of a nucleotidyltransferase superfamily of proteins that includes other cellular and viral CEs. A haploid deletion strain of \( S. \ cer evisiae \) missing the guanylyltransferase enzyme was complemented for growth by the mouse wild type cDNA clone despite only ~25% sequence identity. However, a mouse clone containing alanine in place of lysine in the KXDG motif did not complement. The results demonstrated the functional conservation of CEs from yeast to mammals.

We found that mammalian capping enzyme binds via its GTase region to the hyperphosphorylated C-terminal domain (CTD) of the largest subunit of RNA
polymerase II, facilitating the selective capping of pre-mRNAs. Similarly, the full length and C-terminal domain of CE were localized to the nucleus in transfected cells and also bound poly (U) in vitro, suggesting that the C-terminal domain of CE can bind nascent transcript 5' termini for capping directly. The CE N-terminal RNA 5'-triphosphatase (amino acids 1-237) contains the sequence VHCTHGFNRTG which corresponds to the conserved active-site motif in protein tyrosine phosphatases (PTPs). Mutational analyses identified the Cys and Arg residues in this motif and an upstream aspartate as required for triphosphatase activity. These and other results indicate that removal of phosphate from RNA 5' ends and from modified tyrosine residues in proteins occurs by a similar mechanism.

We also cloned and characterized the third essential enzyme for mRNA 5'-capping, human mRNA (guanine-7-) methyltransferase (MTase). It mapped to 18p11.22-p11.23, a region encoding brain transcripts that have been suggested as positional candidates for susceptibility to bipolar disorder. Sequence alignment of the 476-amino acid MTase protein within the corresponding yeast, C. elegans and Drosophila enzymes demonstrated several required, conserved motifs including one for binding S-adenosylmethionine. MTase bound to human CE and also formed ternary complexes with the elongating form of RNA polymerase II. To identify other proteins that interact with CEs, we used a yeast two-hybrid system to screen a human fetal brain cDNA library with full length human CE and isolated transcription elongation factor SPT5. It bound to CE and stimulated RNA guanylylation but not the triphosphatase step of capping. Purified, hyperphosphorylated CTD similarly stimulated RNA guanylylation in vitro, but the effects of P-CTD and SPT5 were not additive, suggesting a common or overlapping binding site on CE. By using two-hybrid, GST-pulldown and co-immunoprecipitation approaches, we also found that MTase interacts with the nuclear transporter, importin-α (Impα). MTase selectively bound and methylated RNA containing 5'-terminal GpppG, and both activities were stimulated several-fold by Impα. MTase/RNA/Impα complexes were dissociated by addition of Impβ which also blocked Impα stimulation of RNA cap methylation. RanGTP but not RanGDP prevented these effects of Impβ. The results suggested that, in addition to a linkage between capping and transcription, mRNA biogenesis and nucleocytoplasmic transport are functionally connected.

A general model of capping: RNA Polymerase II containing hypophosphorylated CTD initiates transcription, produces 20-25 nucleotide 5'-triphosphorylated transcripts and pauses with SPT5 bound as part of a large transcription complex. Serine 5 residues in the heptad repeats that comprise the CTD and SPT5 are phosphorylated by transcription factor TFIIH, changing the CTD conformation to allow CE binding. The 5' end of the ~25 nucleotide nascent transcript is capped as it becomes exposed at the surface of the polymerase complex, stimulated by SPT5 as well as by the hyperphosphorylated CTD (P-CTD). MTase binds to CE (mammals) or to P-CTD (yeast). Impα stimulates MTase binding and N7 guanine methylation. After phosphorylation of SPT5 and RNA Polymerase II on CTD
serine 2 residues by pTEF-b, polymerase bound complexes of factor DSIF and negative elongation factor (NELF) dissociate and processive elongation ensues. In an effort to decipher how the critically important human GTase works, we determined that the minimum enzymatically active domain resides in CE residues 229-567. The expressed and purified active fragment was crystallized and the structure determined by X-ray crystallography. Seven related conformational states were obtained in the crystal. Position differences of the oligonucleotide/oligosaccharide (OB) binding fold lid domain over the conserved GTP binding site in the seven structures provided snapshots of the opening and closing of the active site cleft via a swivel motion. While the GTP binding site is structurally and evolutionarily conserved, the overall GTase mechanism in mammalian and yeast systems differs somewhat. Experiments are underway to crystallize complexes of human GTase with CTD and RNA as well as GTP. Protein engineering is being applied in an effort to crystallize the full length human CE.

Structure of the GTase domain of human CE consisting of the base, hinge and flexible lid. The predicted single-stranded RNA binding track, marked by sulfate ions incorporated from the crystallization solution, lies in a positively charged cleft below the lid and close to modeled GMP with phosphoamide linkage to K294 of the conserved KXDG active site sequence near the center of the structure. Also shown is the predicted CTD binding site modeled based on the structure of a mouse homolog complex (Ghosh et al. 2011 Mol. Cell 43: 299-310). Blue to red correlates with increasing flexibility.

Publications:


Our laboratory investigates the molecular mechanisms that govern the determination and differentiation of the 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.

**Generation of a transgenic line for studying V2 neuronal lineages and functions in the spinal cord.** During spinal neurogenesis, the p2 progenitor domain generates at least three subclasses of interneurons named V2a, V2b and V2c, which are crucial components of the locomotor central pattern generator. We have previously shown that the winged-helix/forkhead transcription factor Foxn4 is expressed in a subset of p2 progenitors and required for specifying V2b interneurons. To determine the cell lineages derived from Foxn4-expressing progenitors, we generated a Foxn4-Cre BAC transgenic mouse line that drives Cre recombinase expression, mimicking endogenous Foxn4 expression pattern in the developing spinal cord. We used this transgenic line to map neuronal lineages derived from Foxn4-expressing progenitors and found that they gave rise to all neurons of the V2a, V2b as well as the newly identified V2c lineages. These data suggest that Foxn4 may be transiently expressed by all p2 progenitors and that the Foxn4-Cre line may serve as a useful genetic tool not only for lineage analysis but also for functional studies of genes and neurons involved in locomotion.
**Diverse developmental roles of Foxn4.** In our previous studies, we have shown that Foxn4 is transiently expressed in a subset of progenitors during retinogenesis and spinal neurogenesis. Targeted inactivation and gain-of-function analyses have revealed an essential function of Foxn4 in the generation of amacrine and horizontal cells during retinal development as well as in specifying the V2b interneurons during spinal cord development. In collaboration with Drs. Stavros Malas (University of Cyprus) and William Richardson (University College London), we have now used genetic fate mapping and loss-of-function analysis to show that the transcription factor Sox1 is expressed in and required for a third type of p2-derived interneuron, which we named V2c. These are close relatives of V2b interneurons, and, in the absence of Sox1, they switch to the V2b fate. The absence of Foxn4 results in a complete loss of Sox1-expressing V2c neurons, indicating that Foxn4 is necessary for specifying not only V2b but also V2c interneuron subtypes. In another collaboration with Dr. Jeffrey Goldberg (University of Miami, Miller School of Medicine), we observed a developmental delay in the distribution of RGC (retinal ganglion cell) projections to the superior colliculus. Moreover, RGC axons failed to penetrate into the retinorecipient layers in Foxn4 mutant mice. Foxn4 was not expressed by RGCs and was undetectable in the superior colliculus itself. Thus, Foxn4 may be indirectly required for RGC distal axon patterning. Aside from its neural expression, we have also shown that during murine lung development Foxn4 is expressed in proximal airways by a subpopulation of postmitotic epithelial cells which are distinct from basal and ciliated cells. Foxn4 inactivation causes dilated alveoli, thinned alveolar walls and reduced septa in the distal lung but no overt gross alterations in proximal airways, suggesting that Foxn4 may have a non-cell-autonomous role critical for alveologenesis during lung development. Together, our data suggest that Foxn4 may play multiple cell-autonomous and non-cell-autonomous roles during epithelial cell development.

**Brn3a regulates dorsal root ganglion sensory neuron specification and axonal projection into the spinal cord.** The sensory neurons of the dorsal root ganglia (DRG) must project accurately to their central targets to convey proprioceptive, nociceptive and mechanoreceptive information to the spinal cord. How these different sensory modalities and central connectivities are specified and coordinated still remains unclear. Given the expression of the POU homeodomain transcription factors Brn3a and Brn3b in DRG and spinal cord sensory neurons, we analyzed subtype specification of DRG and spinal cord sensory neurons as well as DRG central projections in Brn3a and Brn3b single and double mutant mice. Inactivation of either or both genes causes no gross abnormalities in early spinal cord neurogenesis; however, in Brn3a single and Brn3a;Brn3b double mutant mice, sensory afferent axons from the DRG fail to form normal trajectories in the spinal cord. The TrkA+ cutaneous nociceptive afferents stay outside the dorsal horn and fail to extend into the spinal cord, while the projections of TrkC+ proprioceptive afferents into the ventral horn are also impaired. Moreover, Brn3a mutant DRGs are defective in sensory neuron specification, as marked by the excessive generation of TrkB+ and TrkC+ neurons as well as TrkA+/TrkB+ and TrkA+/TrkC+ double positive cells at early embryonic stages. At later stages in the mutant, TrkB+, TrkC+ and parvalbumin+...
neurons diminish while there is a significant increase of GRP+ and c-ret+ neurons. In addition, Brn3a mutant DRGs display a dramatic down-regulation of Runx1 expression, suggesting that the regulation of DRG sensory neuron specification by Brn3a is mediated in part by Runx1. Our data together demonstrate a critical role for Brn3a in generating DRG sensory neuron diversity and regulating sensory afferent projections to the central targets.

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Altered TrkA+ and TrkC+ central afferent projection patterns in Brn3a mutant spinal cords at E12.5. The bundles of TrkA+ and TrkC+ afferent fibers are detected by immunostaining from lateral to medial regions of the dorsal horn edge in Brn3a+/− spinal cords at E12.5. In Brn3a−/− spinal cords, TrkA+ and TrkC+ fibers are narrowly located in a much smaller region, and extensively overlap with each other.

Publications:


Li, S., Misra, K., and Xiang, M. A Cre transgenic line for studying V2 neuronal lineages and functions in the spinal cord. Genesis 2010 48:667−672.


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In 2011 the lab embarked on a three-year $2.8M NIH-funded project to express a representative set of antigens derived from human transcription factors. The collaborators and co-PIs on this project are Profs. Gaetano T. Montelione and Joseph Marcotrigiano, both of CABM and Rutgers, and Prof. Cheryl Arrowsmith of the Ontario Cancer Research Center and the University of Toronto. Late in 2011, NIH identified two “affinity capture reagent” consortia for our antigen production group to partner with: one, headed by Prof. Anthony Kossiakoff at the University of Chicago and including groups at UCSF and University of Toronto, that will be making Fabs and antibodies using high throughput phage display technology; and one based at Johns Hopkins University, headed by Prof. Jef Boike, that has invented a low-cost, high throughput method of isolating and screening traditional monoclonal antibodies.

NIH’s overarching goal is to eventually produce renewable cognate affinity capture reagents (including antibodies) directed against every human protein, an effort that has been unofficially dubbed the “Human Anti-Proteome Project”. NIH wants to make such reagents available to the research community as powerful tools for studying human biology. A description of this initiative is at http://commonfund.nih.gov/proteincapture/index.aspx. The effort that we are undertaking, namely producing human transcription factor antigens for affinity capture reagent production, is a pilot project designed to kick-start this overall effort. The first envisioned application for the anti-TF antibodies is to enable chromatin immunoprecipitation (ChIP) studies aimed at obtaining a complete map of potential transcription start sites in the human genome – much of this work is being carried out by the NIH-funded ENCODE Consortium.
Human transcription factors are typically complex, multidomain proteins, and in terms of numbers of unique genes represent roughly 10% of the entire human proteome. Our goal is to express native, folded, individual domains from these different transcription factors with the hope that optimal specificity and affinity may be obtained by raising affinity capture reagents against 3D epitopes. In the first year the Rutgers group has designed single-domain expression constructs using advanced “domain parsing” software developed by Dr. Janet Huang and Prof. Gaetano T. Montelione (see figure below). Together with Dr. Thomas Acton and Prof. Montelione, we have also developed a new series of high-efficiency *E. coli* vectors using “transcript optimized expression-enhancement technology” (“TOEET” – patent applied for). These vectors enable unprecedented levels of expression and solubility to be achieved. In 2011 the group cloned domains from nearly 1400 separate human transcription factors in >2700 distinct constructs and expression-tested approximately 80% of these at small scale. We are now moving on to scale-up and production of multi-milligram quantities of purified antigen for our affinity capture reagent partners. In December, 2011, we shipped our first batch of purified antigens.

**Fig. 1.** Example of a portion of the output of the DisMeta server and how it can be used to analyze transcription factor sequences for structural information. Shown schematically is a portion of the sequence of a human transcription factor, IF-16 (γ-interferon-inducible protein 16), analyzed by the DisMeta server. In this segment of the sequence the server predicts two regions that appear to have a complex, folded, tertiary structure separated by a region of polypeptide chain that is predicted to be relatively disordered. Three-dimensional structures for domains represented by the two ordered regions shown were determined by the NESG. The leftmost structure (PDB ID 2oq0) is a HIN–200/IF120x domain (InterPro ID IPR004021), and the rightmost structure (PDB ID 3b6y) is a pyrin domain (InterPro ID IPR004020) (Liao et al, unpublished). This example illustrates how the DisMeta server can be used to scan TF protein sequences to find the regions encoding domains that are likely to have 3D epitopes.
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 treatment, 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 his colleagues are working to understand molecular mechanisms of drug resistance and apply structure-based drug design for the treatment of serious human diseases. In pursuit of these goals, the laboratory uses research tools from diverse fields, including X-ray crystallography, molecular biology, virology, protein biochemistry, and macromolecular engineering. Eddy’s team of very experienced and gifted coworkers, many of whom have been in the lab 10 years or longer, is the driving force behind the continuing progress.

Since its establishment at CABM in 1987, our laboratory has studied the structure and function of reverse transcriptase (RT), an essential component of the AIDS virus and the target of most widely used anti-AIDS drugs. Using the powerful techniques of X-ray crystallography, we have 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 inhibition and resistance, and structure-based design of RT inhibitors. The team has solved a variety of crystal structures representing multiple functional states of HIV-1 RT. These structures include HIV-1 RT in complex with a double-stranded DNA template-primer, HIV-1 RT complexes with RNA:DNA template-primers, structures of RT with AZTMP-terminated primer representing pre-translocation and post-translocation complexes, and ternary complexes of wild-type and drug-resistant RT with DNA, and AZT-triphosphate/tenofovir-diphosphate. We have also determined the structures of numerous non-nucleoside inhibitors with wild-type and drug resistant HIV-1 RT, and the structural information was used in the design of two recently approved non-nucleoside drugs. Also, we have obtained structures of RT:RNase H inhibitor and RT:DNA:AZTppppA (an ATP-mediated AZT excision product) complexes (Tu et al., 2010).

Drug development against 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 long-term collaborations with the groups of Stephen Hughes (NIH NCI-Frederick), Roger Jones (Rutgers), Michael Parniak (U. of Pittsburgh), and Ronald Levy (Rutgers). 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 since its inception.

Through collaboration with the late Dr. Paul Janssen we participated in a structure-based drug design effort that resulted in the discovery and development of non-nucleoside inhibitors (diarylpyrimidine, or DAPY analogs) with high potency against all known drug-resistant variants of HIV-1 RT. Crystallographic work from the Arnold and Hughes laboratories allowed precise visualization of how potential anti-HIV drug candidates latch onto RT, their molecular target. Janssen and colleagues at the Center for Molecular Design successfully used this structural information to guide the design and synthesis of new molecules with improved potency against wild-type and drug-resistant HIV-1 strains. Scientists at Tibotec, a subsidiary of Johnson & Johnson, tested the compounds for antiviral activity against wild-type and resistant HIV-1, and have led the clinical trials.

The DAPY compounds are simple, inexpensive to make and have near ideal pharmacological properties. Etravirine (TMC125/Intelence) was approved for treatment of HIV infection by the FDA in 2008, and rilpivirine (TMC278) was approved as Edurant in May 2011. In what may be unprecedented for a new best-in-class drug, Johnson & Johnson is permitting rilpivirine to be available in generic form immediately in developing nations; this will make the drug available to millions of people. The prototypical DAPY compound, TMC120/dapivirine, is now being developed as a microbicide for blocking sexual transmission of HIV-1. A broader outcome of this study is a drug design concept for overcoming drug resistance; the strategic flexibility that permits the DAPY compounds to “wiggle” and “jiggle” in a binding pocket to accommodate mutations apparently accounts for their potency against a wide range of drug-resistant variants. Through a systematic protein engineering effort we obtained high-resolution crystals of HIV-1 RT and demonstrated that strategic flexibility of rilpivirine was responsible for its resilience against drug-resistant RT variants. Recent efforts include using the high-
resolution HIV-1 RT crystals for drug-like fragment screening. A number of novel allosteric sites for inhibition of both polymerase and RNase H activity have been identified from the fragment screening effort.

In addition to working to study HIV-1 RT and to develop 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 in collaboration 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 including the polymerase from 2009 H1N1 pandemic strain.
Figure from Das et al., 2011 showing how the nonnucleoside inhibitor TSAO has a shape that resembles a dragon when bound to HIV-1 RT.

**Publications:**


Structural Biology and Bioinformatics

Dr. Berman's research area is structural biology and bioinformatics, with a special focus on protein-nucleic acid interactions. She is the founder of the Nucleic Acid Database, a repository of information about the structures of nucleic acid-containing molecules; and is the co-founder and Director of the Protein Data Bank, the international repository of the structures of biological macromolecules. Professor Berman also leads the Protein Structure Initiative Structural Biology Knowledgebase. She is a Fellow of the American Association for the Advancement of Science and of the Biophysical Society, from which she received the Distinguished Service Award in 2000. A past president of the American Crystallographic Association, she is a recipient of the Buerger Award (2006) and a member of the inaugural class of ACA Fellows (2011). Dr. Berman received her A.B. in 1964 from Barnard College and a Ph.D. in 1967 from the University of Pittsburgh. She received the Department of Chemistry Alumni Award from the University of Pittsburgh in 2010.

The Protein Data Bank

The Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) supports scientific research and education worldwide by providing an essential resource of information about biomolecular structures.

The PDB archive is the single repository of information about the 3D structures of large biological molecules, primarily proteins and nucleic acids. Scientists from around the world submit their data about their experiments to the PDB, which is then processed and made freely available in the PDB archive. The RCSB PDB, which is based at Rutgers and the University of California, San Diego, manages the PDB archive as a member of the Worldwide PDB (wwPDB).

The wwPDB organization (www.wwpdb.org) was formed to ensure that the PDB archive is and will be freely and publicly available to the global community. The wwPDB members (RCSB PDB, Protein Data Bank in Europe (PDBe), Protein Data Bank Japan (PDBj), and the BioMagResBank (BMRB)) host deposition, processing, and distribution centers for PDB data and collaborate on a variety of projects and outreach efforts. As 'archive keeper' for the wwPDB, the RCSB PDB maintains the central repository of the PDB archive.

The central activity of the wwPDB is the collection, validation, archiving and distribution of high quality structural data to the scientific community on a timely basis. The systems developed by the RCSB PDB are reliable and stable to meet the challenges of high data rates and complex structures. As of November 8, 2011, the PDB archive contains more than 77,000 entries.
In 2011, the wwPDB commemorated the PDB’s 40th anniversary with a special symposium. The meeting was attended by 19 distinguished speakers, approximately 250 participants, and 93 poster presentations. 34 travel awards for early career scientists were made using funds raised by the wwPDB. More information about the meeting is available at http://meetings.cshl.edu/meetings/pdb40.shtml.

The RCSB PDB’s website at www.pdb.org provides access to the PDB FTP archive as well as tools that help users search, analyze and visualize PDB structural data. In 2010, the RCSB PDB website had more than 5.6 million visits by users in 218 countries. On average, the website has more than 196,000 unique visitors each month. Additionally, 160,713,233 data files were downloaded from the FTP server in 2010.

The website provides access to a relational database that integrates PDB data with related information from external sources (such as journal abstracts, functional descriptors, sequence annotations, structure annotations, and taxonomy). Users can search for structures using simple searches (PDB ID, keyword, sequence, or author) and complex queries.

PDB-101 is a unique view of the RCSB PDB that packages together the resources of interest to teachers, students, and the general public. Major topics include Molecule of the Month columns, a top-down exploration from high-level functional categories to PDB structures called Structural View of Biology, and related Educational Resources and materials.

The RCSB PDB group at Rutgers is involved with undergraduate and graduate education, and supports local K12 education with programs such as the protein modeling event at the New Jersey Science Olympiad, and K12 education throughout the world with online resources.

**Structural Genomics and the PSI Structural Biology Knowledgebase**

With the overarching goal of creating new knowledge about the interrelationships of sequence, structure, and function, the Protein Structure Initiative Structural Biology Knowledgebase (PSI SBKB, sbkb.org) is a free online resource that captures and highlights the structural and technical advances made by the PSI structural genomics efforts for use by the broader biological community. This information is integrated with publicly available biological information so that it can enable a better understanding of living systems and disease. Created in collaboration with the Nature Publishing Group, the Structural Biology Knowledgebase also provides a research library, highlights focused on new research advances and technical tips, the latest science news, and an events calendar to present a broader view of structural genomics and structural biology.

Searches of the SBKB by sequence or PDB ID will yield a list of related protein structures from the Protein Data Bank, theoretical 3D models, associated
descriptions (annotations) from biological databases, as well as structural genomics protein target information, experimental protocols, and the ability to order available DNA clones. Text searches will find technology reports and publications that were created by the PSI’s high-throughput research efforts, as well as the monthly features and highlights from the website. Web tools that aid in bench top research, such as protein construct design, are also available. This information can enable scientists by providing everything that is known about a protein sequence, including any experimental successes or failures, thus streamlining their own research efforts.

The SBKB group also developed two entirely new resources for the Structural Genomics community. First, a new experimental data tracking resource, TargetTrack (sbkb.org/tt/), was created that merged two existing (and mostly redundant) and expanding to support new types of high-throughput and biological research. Second, a “Metrics” resource was created to track the progress of the whole PSI program.

The Unified Data Resource for Cryo Electron Microscopy

The PDB archives large biological assemblies determined by cryo-electron microscopy (cryoEM), a maturing methodology in structural biology that bridges the gap between cell biology and the experimental techniques of X-ray crystallography and NMR. In addition to 3D density maps, cryoEM experiments often yield fitted coordinate models. Through an NIH/NIGMS-funded collaboration, a joint EM map and model deposition tool has been developed. The unified resource for deposition and retrieval network for cryoEM map, model, and associated metadata is available at EMDatabank.org. This work has been carried out in collaboration with the RCSB PDB at Rutgers, PDBe, and the National Center for Macromolecular Imaging at Baylor College of Medicine.

Publications:


Hepatitis C virus (HCV) continues to be a major public health problem. In most cases, HCV infection becomes chronic and can persist for decades, leading to cirrhosis, end-stage liver disease and hepatocellular carcinoma. Currently, 2% of the human population – approximately 123 million people – is infected with HCV. In fact, there are 3-4 times more individuals infected with HCV than HIV, making virus transmission a major public health concern. In the United States, HCV infection is the most common cause of liver transplantation and results in 10,000 to 20,000 deaths a year. There is no vaccine, and current HCV therapy, pegylated interferon-alpha in combination with ribavirin, leads to a sustained response in only 50% of genotype 1-infected patients, the prevalent genotype in the United States. The current HCV treatment stimulates the patient’s immune system to clear the virus, but numerous side effects cause many patients to prematurely stop treatment. Given the high prevalence of infection and poor response rate, inhibitors that specifically target HCV proteins with fewer side effects are desperately needed. In addition, an effective vaccine would greatly reduce the spread of the virus.

Our laboratory studies how HCV enters a host cell and avoids the cellular innate immune response to infection. To elucidate these processes we employ a variety of structural, biophysical, biochemical and virological techniques. HCV is a member of the family *Flaviviridae*, which also includes Pestiviruses and Flaviviruses.
The HCV virion consists of an enveloped nucleocapsid containing the viral genome, a single-stranded, positive sense RNA that encodes a single open reading frame. Once the virus penetrates a permissive cell, the HCV genome is released into the cytosol where the viral RNA is translated in a cap-independent manner by an internal ribosome entry site (IRES) located within the 5’ nontranslated region (NTR). Translation generates a viral polyprotein that is proteolytically processed by cellular and viral encoded proteases into ten proteins. HCV is an enveloped virus with two glycoproteins (E1 and E2) that form the outermost shell of the virion. These two proteins are thought to be involved in cell receptor binding, entry, membrane fusion and immune evasion. Both E1 and E2 are type I transmembrane proteins with an amino-terminal ectodomain and a carboxy-terminal membrane-associating region. The transmembrane regions are involved in ER retention and the formation of noncovalent E1/E2 heterodimers. Since HCV is thought to bud into the ER, retention of the glycoproteins at the ER membrane ensures their placement on the virion particles. The E1 and E2 ectodomains are heavily glycosylated and contain several intramolecular disulfide bonds. E1 and E2 contain 4 and 11 predicted N-linked glycosylation sites, respectively, and many highly conserved cysteines.

Intracellular double-stranded RNA (dsRNA) is an important signal of virus replication. The host has developed mechanisms to detect viral dsRNA and initiate antiviral responses. Pathogen Recognition Receptors (PRR) are cellular proteins that sense the presence of pathogen associated molecular patterns (PAMPs) to induce an antiviral state. Retinoic acid inducible gene I (RIG-I) is one member of a family of PRR that resides within the cytoplasm of a cell and senses the presence of 5’ triphosphorylated dsRNA, an intermediate of virus replication. RIG-I encodes two caspase recruitment domains (CARD), a DExD/H box RNA helicase, and a repressor domain (RD). The RD blocks signaling by the CARD domains under normal growth conditions. To investigate the contributions of the individual domains of RIG-I to RNA binding, we determined the equilibrium dissociation constants (Kd) of the protein–RNA complexes. The tightest RNA affinity was observed with helicase-RD, whereas the full-length RIG-I, helicase domain and RD bind dsRNA with a 24-fold, 8,600-fold and 50-fold weaker affinity, respectively. Crystals of RIG-I helicase-RD in complex with ADP-BeF3 and 14 base-pair palindromic dsRNA were obtained and the structure was determined to 2.9Å resolution. RIG-I helicase-RD organizes into a ring around dsRNA, capping one end, while contacting both strands using previously uncharacterized motifs to recognize dsRNA. Small-angle X-ray scattering, limited proteolysis and differential scanning fluorimetry indicate that RIG-I is in an extended and flexible conformation that compacts upon binding RNA. These results provide a detailed view of the role of helicase in dsRNA recognition, the synergy between the RD and the helicase for RNA binding and the organization of full-length RIG-I bound to dsRNA. The results provide evidence of a conformational change upon RNA binding.
The long-term goals of our studies are to provide a structural and mechanistic understanding for how the HCV glycoproteins (E1 and E2) interact with each other and with cellular receptors. Also we are focused on how HCV evades the host antiviral response and how RIG-I discriminates viral from cellular RNAs.

Publications:


As director of the NIH-funded Northeast Structural Genomics Consortium of the NIGMS Protein Structure Initiative, Dr. Montelione leads an inter-institutional pilot project in large-scale structural proteomics and bioinformatics. 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 collaborations with academic laboratories and 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 several hundred 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 is yielding three-dimensional structures and new functions for some 200 proteins per year, and can thus have tremendous scientific impact. Research areas include networks of proteins associated with human cancer biology, protein complexes involved in influenza virus infection, innate immune response, and ubiquitination pathways.
Publications:


Our group is interested in constructing new proteins for applications in biomedical research, nanotechnology and as tools for understanding how proteins fold and evolve. Significant progress has been made in the last decade using sophisticated computer programs to design proteins with novel folds and functions. We maintain and develop the software package, protCAD (protein computer aided molecular design), which has been applied to protein design, structure prediction and docking of protein ligand complexes.

Computational Design of an Extracellular Matrix

The extracellular matrix (ECM) is a complex network of collagens, laminins, fibronectins and proteoglycans that provides a surface upon which cells can adhere, differentiate and proliferate. Defects in the ECM are the underlying cause of a wide spectrum of diseases. The ECM mediates endothelial cell polarity and under normal conditions can suppress pre-oncogenic transitions to a neoplastic state. We are constructing artificial, de novo collagen-based matrices using a hierarchic computational approach. These matrices will be physically characterized in the laboratory and used to probe the role of chemical and spatial organization in the ECM on the tumor forming potential of adhered cells. Successfully designed matrices will be applied to engineering safer artificial tissues.

Rational Design of Enhanced Peptide Therapeutics

Peptides are an emergent and important class of therapeutics with over forty compounds on the market and nearly 700 more in clinical or pre-clinical trials. During the development of peptide drugs, D-enantiomers of amino acids are frequently incorporated to improve pharmacokinetic and pharmacodynamics.
properties by lowering susceptibility to proteolysis. Typically, such modifications are introduced in lead compounds by trial-and-error or combinatorial approaches.

Our laboratory is developing components in protCAD to simulate the impact of non-natural amino acids on structure and stability. Using fundamental principles of protein design, we will pursue the computational, structure-based development of peptides with variable chirality, broadly extending our capacity to create safe and potent therapeutics.

**Biochemical Basis of Food Allergies**

A crucial and unanswered question in the field of food allergy research is why certain proteins elicit an IgE mediated immune response, while others are tolerated. One compelling model is that non-allergens are more digestible, resulting in sufficient protein degradation in the stomach and intestine to render the remaining fragments immunologically inert. In this project, we develop a highly defined system for exploring the relationship between digestibility and allergenicity using engineered variants of protein allergens from peanut and shrimp.

Natural collagens in the connective tissue or the basement membrane assemble in a hierarchic fashion. Our peptides proceed from monomer to trimer to fiber, and finally to a hydrogel. Hydrogels are versatile materials with applications in drug delivery, tissue scaffolds and enzyme immobilization. Insight gained from this study will improve molecular design of biomimetic materials. TOP PICTURE: Phase diagram of peptides CPB and CPC combinations showing the diversity of self-assembling structures formed. BOTTOM PICTURE: Electron Microscopy images of negatively stained CPB:CPC samples at various mixing ratios.
Publications:


The research of the Stock 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.

The Stock laboratory has continued to focus efforts on characterizing representative members of the OmpR/PhoB transcription factor subfamily of response regulator proteins, but now from a systems biology perspective. Studies have shifted from in vitro analyses to in vivo analyses with the goal of being able to describe the parameters of protein levels, phosphorylation states, and gene expression activity during induction of the phosphate assimilation pathway, a model two-component system in *E. coli*. Tools have been developed and data are being accumulated that will hopefully allow the mathematical modeling of the mechanism of regulation in a representative two-component system.

An additional research focus is characterization of two-component systems of *Staphylococcus aureus*, a re-emerging pathogen that is currently associated with more deaths annually in the United States than HIV1 (AIDS). New classes of drugs are needed to combat the economic and health burden of drug-resistant *S. aureus* strains (i.e. methicillin-resistant, MRSA). Several response regulators in *Staphylococcus aureus* play important roles in virulence and are potential drug targets. The Stock laboratory has focused efforts on two *S. aureus* transcription factors, AgrA and VraR. In 2008, the Stock laboratory reported the structure of the...
DNA-binding domain of AgrA, establishing a novel fold for the LytTR domain that regulates virulence factor expression in many pathogenic bacteria. Recent studies have focused on purification and characterization of full-length AgrA with an emphasis on understanding the role of the extreme C terminus that is altered by a phase-variation mechanism, inactivating AgrA at late stages of infection. WATERGATE LOGSY NMR experiments were used to identify drug-like fragments that bind to AgrA. All six ligands identified bind to the same site, overlapping the surface required for binding. Three of the fragments have been shown to inhibit DNA binding and are being pursued for inhibitor development. The Stock laboratory has determined the structures of both inactive and activated full-length VraR, a vancomycin-resistance-associated regulator that plays a central role in maintaining the integrity of the cell wall peptidoglycan and in coordinating responses to cell wall damage. These structures represent the first inactive and active pair of full-length response regulator transcription factors. The structures establish the mechanism of activation of VraR via dimerization and reveal an unusual deep binding pocket with great potential for inhibitor development. Virtual docking analyses using the Zinc database of available compounds have identified many compounds predicted to bind to this pocket. In collaboration with Ed LaVoie (School of Pharmacy, Rutgers University) and John Kerrigan (CINJ), these compounds have been ranked and several have been selected as a starting point for inhibitor development.

**Dimer interface of active VraR receiver domain.** The protruding Methionine 13 from one monomer fits into a deep binding cleft of the other monomer, a site that is being targeted for development of inhibitors as leads for development of novel antibiotics.
Publications:


Dixit, S.S., Jadot, M., Sohar, I., Sleat, D.E., Stock, A.M. and Lobel, P. Biochemical alterations resulting from the loss of either NPC1 or NPC2 suggest that these proteins underlie Niemann-Pick C disease function in a common lysosomal pathway. PLOS One 2011 in Press.
Education, Training and Technology Transfer

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Lectures and Seminars
Lecture Series 2010-2011

Lectures will be held in CABM Seminar Room 010 unless otherwise noted

October 19, 2010 – Waksman Auditorium
(Pre-Registration Required)
24th Annual CABM Symposium
“Cancer – Progress & Prospects”

Wednesday, November 17, 12:00
Robert Lamb, HHMI, Northwestern University
“Influenza Virus Budding: Who Needs an ESCRT When There Is M2 Protein”

Wednesday, December 1, 12:00
Alanna Schepartz, Yale University
“Exploring Biology with Molecules that Nature Chose not to Synthesize”

Wednesday, January 19, 12:00
Evgeny Nudler, New York University, School of Medicine
“Trafficking and Cooperation in Gene Regulation and Genome Instability”

Wednesday, February 2, 12:00
Smita Patel, UMDNJ-Robert Wood Johnson Medical School
“Mechanistic studies of a ring-shaped helicase that coordinates DNA replication”

Wednesday, March 30, 12:00
Charles Rice, The Rockefeller University
“Hepatitis C virus: New insights into replication and control”

Wednesday, April 13, 12:00
Nahum Sonenberg, McGill University
“Translational Control of Cancer”

Supported by Sanofi Aventis
CABM RETREAT, June 30, 2011
Cook Campus Center, New Brunswick, NJ

Program

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

8:45 AM  Opening Remarks – Aaron J. Shatkin

9:00 AM  Session I – Gene Expression – From Structure to Behavior and Steps In Between
Chair: Janet Huang - Structural Bioinformatics and Proteomics Lab (Guy Montelione)

Shuchismita Dutta – Protein Data Bank Lab (Helen Berman)
“Molecular Anatomy Project: Promoting a Structural View of Biology”

Lili Mao – Bacterial Physiology and Protein Engineering Lab (Masayori Inouye)
“The Single Protein Production (SPP) system in E. coli”

Hua Han – Protein Crystallography Lab (Ann Stock)
“The Kinetics of phospho regulation by PhoBR two-component system in E. coli”

Evrim Yildirim – Molecular Chronobiology Lab (Isaac Edery)
“Phosphorylation of S826/828 on PER sets the pace of circadian clock”

Weihua Qiu – Molecular Virology Lab (Aaron Shatkin)
“Structure of the Guanylyltransferase Domain of Human mRNA Capping Enzyme”

Stephen Anderson – Protein Engineering Lab (Stephen Anderson)
“Production of human transcription factor immunogens”

10:30 AM  Coffee Break and Posters

11:00 AM  Session II – Model Systems to Study Growth and Development
Chair: Su Xu – Protein Targeting Lab (Peter Lobel)

Kenneth McGuinness – Protein Design and Evolution Lab (Vikas Nanda)
“Designing Collagen Self-Assembly using Hydrophobic Interactions”

Céline Granier – Viral Pathogenesis Lab (Arnold Rabson)
“PDCD2, a Novel Nuclear Body-associated Protein in Embryonic Stem Cells and Mouse Development”

Bo Li – Developmental Neurogenetics Lab (Jim Millonig)
“Vacuolated lens (vl): a multigenic mouse mutant model of Neural Tube Defects (NTDs)”
Kamana Misra – Molecular Neurodevelopment Lab (Meng Xiang)
“To be or not to be” A neuron's perspective

12:30 PM  Lunch and Break

2:15 PM  Posters and Coffee

Session III – Understanding Disease at the Molecular Level as a Basis for Developing Novel Therapies
Chair: Kamana Misra – Molecular Neurodevelopment Lab (Meng Xiang)

Mary Fitzgerald Ho – Biomolecular Crystallography Lab (Eddy Arnold)
“GE23077: A novel bacterial RNA polymerase inhibitor”

Joseph Marcotrigiano – Structural Virology Lab (Joe Marcotrigiano)
“The Structure of a Precursor from the Alphavirus Replication Complex sheds light on pathogenesis and mechanisms of polyprotein processing”

Rongjin Guan – Structural Bioinformatics and Proteomics Lab (Guy Montelione)
“Structural Basis for the Sequence-Specific Recognition of Human ISG15 by the NS1 Protein of Influenza B Virus”

Yu Meng – Protein Targeting Lab (Peter Lobel)
“Intravenous enzyme replacement therapy for late-infantile neuronal ceroid lipofuscinosis (LINCL)”

Fang Liu – Growth and Differential Lab (Fang Liu)
“Pin1 promotes TGF-beta-induced migration and invasion”

Na Yang – Tumor Virology Lab (Céline Gélinas)
“Understanding and targeting Bfl-1 turnover to overcome chemoresistance”

4:30 PM  Close
The 25th Anniversary CABM Symposium

BRUCE ALBERTS
WAYNE HENDRICKSON
ROBERT ROEDER
PHILLIP SHARP

CABM Alumni Speakers:
SCOTT BANTA  JIANPING DING
SNEZANA DJORDJEVIC  JOSEPH MARCOTRIGIANO
HUNTER MOSELEY  KEN VALENZANO  ZHENYU YUE
and Poster Session

CENTER FOR ADVANCED BIOTECHNOLOGY AND MEDICINE
CELEBRATING 25 YEARS OF EXCELLENCE

October 21, 2011
Sponsored by:
Johnson & Johnson
Roche  Rutgers Office of VP for Health Sciences
UMDNJ–Robert Wood Johnson Medical School
Pfizer
Amicus Therapeutics  LifeGas
Cambridge Isotope Labs  GenScript Corporation  MacrogenUSA
New England Biolabs  Rigaku Americas Corporation

Pre-registration is required.
Please register online by October 7, 2011 at cabm.rutgers.edu/symposium

Location:
UMDNJ  Robert Wood Johnson
Medical School Main Lecture Hall
(adjacent to the CABM building)

For further information:
CABM Symposium
679 Hoes Lane  Piscataway, NJ 08854
Email: symposium@cabm.rutgers.edu
25th Anniversary CABM Symposium
Friday, October 21, 2011

Program

8:15 am  Registration & Continental Breakfast
          RWJMS Great Hall

9:00 am  Welcoming Remarks
          Aaron J. Shatkin, PhD
          Professor and Director, CABM
          Richard L. McCormick, PhD
          President, Rutgers, The State University of New Jersey

Session I: Chairperson: Joanna Chiu, PhD
            Assistant Professor, UC Davis

9:10 am  Phillip Sharp, PhD
          Institute Professor, MIT
          “The Cell Biology of Small Noncoding RNAs”

9:50 am  Ken Valenzano, PhD
          Vice President, Amicus Therapeutics
          “Pharmacological Chaperones Offer a Two-pronged Approach to Treat Lysosomal Storage
          Disorders: Fabry Disease as a Case Study”

10:10 am  Zhenyu Yue, PhD
           Associate Professor, Mt. Sinai Medical School
           “From LRRK2 Kinase to the Hope of Understanding and Treating Parkinson’s Disease”

10:30 am  Coffee Break

Session II: Chairperson: Wei-xing Zong, PhD
           Assistant Professor, SUNY Stony Brook

11:00 am  Robert Roeder, PhD
           Arnold and Mabel Beckman Professor, Rockefeller University
           “Transcriptional Regulatory Mechanisms in Animal Cells”

12:00 pm  Scott Banta, PhD
           Associate Professor, Columbia University
           “Development of the Beta Roll Motif as a Novel Biomolecular Recognition Scaffold”
12:20 pm  

**Lunch Break**  
RWJMS Great Hall

1:00 pm  

**Poster Session**  
RWJMS Great Hall

**Session III:** Chairperson: Jun Zhu, PhD  
Director, DNA Sequencing and Computational Biology Core, NIH-NHLBI

2:00 pm  

**Wayne Hendrickson, PhD**  
University Professor, Columbia University; Investigator, HHMI  
“Structural Analysis of SLAC1-family Anion Channel Activity”

2:40 pm  

**Snezana Djordjevic, PhD**  
Senior Lecture, University College London, UK  
“Small Molecule Inhibitors of the VEGF-Neuropilin Interaction”

3:00 pm  

**Hunter Moseley, PhD**  
Assistant Professor, University of Louisville  
“Metabolic Modeling of Converging Metabolic Pathways: Analysis of Non-Steady State Stable Isotope-Resolved Metabolomics Data of UDP-GlcNAc and UDP-GalNAc”

3:20 pm  

**Coffee Break**

**Session IV:** Chairperson: Minjung Kim, PhD  
Assistant Professor, Moffitt Cancer Center, Tampa, FL

3:50 pm  

**Joseph Marcotrigiano, PhD**  
CABM Resident Faculty Member; Assistant Professor, Rutgers University  
“The Innate Immune Response to Viral RNA”

4:20 pm  

**Bruce Alberts, PhD**  
Editor, Science; Professor Emeritus, UCSF  
“Stimulating Innovation in Scientific Research”

5:00 pm  

**Closing**

5:10 pm  

**Reception**  
RWJMS Great Hall
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Stein, S. and Montelione, G.T. “Site specific 13C-enriched reagents for magnetic resonance imaging”. U.S. Patent No. 6210655


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Dean of Biological Sciences
Vice President of Health Science Partnerships

Dr. Allan H. Conney
William M. And Myrle W. Garber
Professor of Cancer and Leukemia Research

Dr. Joachim W. Messing
University of Professor and Director
Waksman Institute

Martin L. Yarmush, M.D.
Paul and Mary Monroe Professor of Science and Engineering
Director, Center for Innovative Ventures of Emerging Technologies, Rutgers
Director, Center for Engineering in Medicine, Massachusetts General Hospital
Former CABM Scientific Advisory Board Members

Alberts, Bruce, Prof., UCSF (Pres. National Academy of Sciences), '86
Babiss, Lee, Vice President Pre-Clinical Res. & Dev. Hoffmann-La Roche Inc.'99-06
Bayne, Marvin L., VP Discovery Technologies Merck, '07-10
Bolen, Joseph, Vice President, Hoechst Marin Roussel, '98
Black, Ira, M.D., Prof. & Chairman, Dept. of Neurosci. & Cell Biol., RWJ/UMDNJ '91-06, (deceased)
Blumenthal, David, M.D., Exec. Dir., Ctr. for Health Policy & Mgmt. @ Harvard, '86-88
Burke, James, Chairman of the Board, J & J, '86-89
Denhardt, David, Prof. & Chairman, Rutgers, Dept. Biol. Sci., '88-91
Drews, Jürgen, M.D., Pres., Intl. R & D, Hoffmann-La Roche Inc., '93-96
Gage, L. Patrick, Pres., Wyeth-Ayerst, '01
Gill, Davinder, Vice President and Head Global Biotherapeutic Technologies, Pfizer, Inc. '10-11
Gussin, Robert, Corporate V. P., Johnson and Johnson, '01
Haber, Edgar, M.D., Pres., Bristol-Myers Squibb Pharma. Res. Inst., '90-91(deceased)
Hait, William, M.D., Ph.D., Prof., Associate Dean & Director, CINJ, '06-07
Harris, Edward, Jr., M.D., Prof. & Chairman, Dept. Med., RWJ/UMDNJ '86-88 (deceased)
Inouye, Masayori, Ph.D., Prof. of Biochemistry, UMDNJ-RWJMS 1986-2010
Koblan, Kenneth V.P. and Site Head for Basic Research, Merck, '06-10
Lerner, Irwin, Pres. & CEO, Hoffmann-La Roche Inc., '86-93
Loh, Dennis, Vice President Preclinical Res. & Dev. Hoffmann-La Roche Inc. '98
Lowry, Stephen F., M.D., Prof and Chair Dept. of Surgery, UMDNJ-RWJMS '07-11 (deceased)
Luck, David, M.D., Ph.D., Prof., Rockefeller Univ., '94-97 (deceased)
Mansour, Tareq S., C.S. Interim Head Wyeth Research, '09-10
* Merrifield, Robert, Prof., Rockefeller Univ., '91-93 (deceased)
Moliteus, Magnus, Pres., Pharmacia, '86-88
Morris, N. Ronald, M.D., Associate Dean & Professor, Dept. of Pharma, RWJ/UMDNJ, '86-92
* Nathans, Daniel, M.D., Sr. Invest., HHMI, Johns Hopkins Univ., (Pres., Johns Hopkins, deceased), '93-96
Olson, Wilma, Prof., Dept. of Chemistry, Rutgers,'86-92
Palmer, James, CSO and Pres., Bristol-Myers Squibb Company, '00-04 (deceased)
Pickett, Cecil, Executive V.P., Schering-Plough, '00-06
Pramer, David, Dir., Waksman Inst. '86-88
Reynolds, Richard C., M.D., Executive Vice President, Robert Wood Johnson Foundation,'88-91
Rosenberg, Leon, M.D., Pres., Bristol-Myers Squibb, '91-97
Ruddon, Raymond W., M.D., Ph.D., Corp. V.P. Johnson & Johnson, '01
Ringrose, Peter, Pres., Bristol-Myers Squibb, 2000
Sabatini, David, M.D., Ph.D., Prof. & Chair, Dept. Cell Biol. NYU School of Med., '93-08
Sanders, Charles, M.D., Vice Chairman, E.R. Squibb & Sons, '88-91
Scolnick, Edward M., M.D., President, Merck & Co. Inc., '95 - '97
Shapiro, Bennett, M.D., Executive V.P., Merck & Company, '01
* Sharp, Phillip, Prof. & Dir., Ctr. for Cancer Res., MIT, '90-'93
Sigal, Elliott, M.D., Ph.D., President and CSO, Bristol Myers Squibb, '05-'08
Strader, Catherine, Exec. V.P. and CSO, Schering-Plough Research Institute, '06
Tilghman, Shirley M., Prof., Princeton University (Pres. Princeton U.), '01
Torphy, Theodore John, Corp. V.P. and CSO, Corp. Office of Science and Technology J&J, '03-'07
Turner, Mervyn J., Senior V.P. Merck & Co., '01-'05
Vagelos, P. Roy, M.D., Chairman & CEO, Merck & Co., '86-'94
Walsh, Frank S., Ph.D., Senior V.P. and Head Wyeth Research, '02-'08
Wilson, Robert, Vice Chairman, Board of Directors, J & J, '91-'95

* Nobel Laureate
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<td>Orphan GPCR, Gpr161, and Epithelial-Mesenchymal Transition</td>
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<td>NJ Governor’s Council for Medical Research and Treatment of Autism</td>
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<td>$75,000</td>
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<td>Tumor suppressor role of CAPER alpha in ER alpha negative &amp; Rel NF-kB positive breast cancer (Postdoctoral Fellowship)</td>
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<td>Targeting the NS1 Protein for the Development of Influenza Virus Antivirals (University of Texas subcontract)</td>
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| Structural Genomics of Eukaryotic Domain Families | $37,810,498 | 9/1/10 - 6/30/15 | $6,066,300 | $1,442,501 | $7,508,801 |

| Membrane Protein Production using the Yeast SPP System (UMDNJ subcontract) | $121,266 | 9/30/10 - 7/31/14 | $34,766 | $18,774 | $53,540 |

| Industry | 1 | $80,228 | $17,821 | $9,713 | $27,534 |
| Nexomics Biosciences, Inc. | Protein Production and NMR Data Collection Services | $80,228 | 7/1/08 - 6/30/11 | $17,821 | $9,713 | $27,534 |

| Total - Montelione, Gaetano | 4 | $39,463,271 | $6,322,131 | $1,580,816 | $7,902,947 |

<p>| Nanda, Vikas | UMDNJ |  |  |  |</p>
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<tr>
<th>Project Description</th>
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<th>FY 2011 Indirect Costs</th>
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<td>National Science Foundation</td>
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<td>Design of Programmable, Self-Assembling Collagen Biomaterials</td>
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**Phadtare, Sangita**  
UMDNJ

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<td>6/1/09 - 5/31/11</td>
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<td>Transcription Antitermination by OB-fold Family Proteins (ARRA Award)</td>
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**Shatkin, Aaron**  
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<td>Cisco, Inc.</td>
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<td>NIH</td>
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<td>Transcriptional Regulation of Retinal Development</td>
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<td>Dll4 Gene Regulation and Function during Retinogenesis</td>
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