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Overview

Celebratory Events and Honors for CABM Founding Director

The 25th anniversary of CABM was celebrated in the fall of 2011 with a special 25th Anniversary CABM Symposium on October 21 that included a CABM Alumni and Friends Dinner on the preceding evening. The Symposium featured keynote presentations by former and current CABM Scientific Advisory Board members Bruce Alberts, Wayne Hendrickson, Robert Roeder and Phillip Sharp as well as six oral presentations and approximately 30 poster presentations by CABM alumni. Over 450 researchers participated in this celebratory event with CABM alumni – former students, postdoctoral fellows and staff – returning from around the world. CABM faculty and friends took this opportunity to acknowledge the many contributions of Aaron J. Shatkin, founding Director of CABM, with two special awards. The CABM South Atrium, the hub of scientific and social gatherings at CABM, was named the Aaron J. Shatkin Atrium, with lettering affixed to the central kiosk and a plaque and portrait mounted on the west wall. Also announced at the 25th Anniversary celebration was the endowed annual Aaron J. Shatkin Lectureship that was established with >$100,000 contributed by over a hundred friends and donors. The first Shatkin Lecture was delivered by Dr. Harold Varmus, Director of the National Cancer Institute, on April 16, 2012. The presentation, entitled “Good Questions Drive Good Science” focused on new initiatives at NCI to address some of the most intriguing unanswered questions in the field of cancer research. Dr. Shatkin attended the lecture that filled the RWJMS Main Auditorium to capacity. The CABM gratefully acknowledges RWJMS for sponsoring the inaugural Aaron J. Shatkin Lecture and reception.

Biomedical Research Advances

CABM continued to vigorously pursue its mission to make fundamental discoveries in biomedical research, develop new technologies, and translate these to improve human health. CABM researchers conducted programs in the broad areas of cancer, infectious diseases and neurodevelopment and neurodegeneration with funding from NIH, other federal and state agencies, and public and private support. Programs in the field of cancer focused on mechanisms of invasion and metastasis in breast cancer, combination therapies for lymphoma and structural characterization of novel therapeutic targets identified in networks of proteins associated with cancer. Infectious disease programs made advances in development of new drugs to combat drug-resistance in HIV, new targets for hepatitis C drugs and identification of novel targets for new antibiotics effective against drug-resistant bacteria. Programs in neurodevelopment and neurodegeneration focused on a newly discovered autism susceptibility gene, an enzyme replacement treatment for the fatal hereditary childhood neurodegenerative disorder Batten disease, identification of key steps in retinal development and mechanisms of regulation of circadian clocks. Broad based structural biology and protein engineering projects produced approximately 200 protein structures providing clues to new functions, developed technologies for generating antibodies for all human transcription factors as key research tools for all areas of biomedical research, and designed new proteins relevant to engineering artificial tissues, producing peptide therapeutics and combatting food allergies. Descriptions of these advances are provided in the research summaries included in this report and documented in over 140 publications in highly ranked journals.
New Facilities for Proteomics Research

The newly completed Center for Integrative Proteomics Research (CIPR) at Rutgers University was dedicated in December 2011. The 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) led by Dr. Helen Berman. In the fall of 2011, the PDB group relocated from CABM where it had been based during construction of the CIPR building. The hosting of the PDB for the past two years was a productive and enjoyable experience that continued the tradition of CABM's role in fostering development of new institutes at UMDNJ and Rutgers. The arrangement strengthened interactions among structural biology researchers and facilitated collaborations, most notably the NIH-sponsored PSI-Nature Structural Biology KnowledgeBase that emerged from the Protein Structure Initiative (PSI) as a joint initiative of the PDB and the Northeast Structural Genomics (NESG) Consortium. The CABM looks forward to continuing collaborative interactions and joint activities with the CIPR.

Teaching, Training and Outreach

CABM faculty members participate actively in the teaching of students and postdoctoral fellows both in the lecture hall and at the laboratory bench. Research faculty in CABM laboratories, Drs. Sangita Phadtare and David Sleat also participate in medical school courses as course directors and instructors. Trainees at CABM include undergraduates, graduate students from many different MS and PhD programs and medical students. Many CABM faculty members have been recognized for their excellence in teaching. In 2012, Dr. Nanda was inducted into the UMDNJ Master Educators Guild, joining Drs. Millonig, Gélinas and Stock as Master Educators. The NIH Biotechnology Program, co-directed by Drs. Martin Yarmush and Aaron Shatkin has been an excellent source of training and support for Ph.D. students from various disciplines for more than 20 years. In recognition of its success, the training program was recently renewed and this year, the program began its fifth five-year cycle, achieving distinction as the longest running NIH training grant at Rutgers.

CABM continues to be active in research oriented outreach activities, hosting visiting faculty scholars from around the world, this year from Spain and China. CABM also hosted the annual meeting of the Northeast Structural Genomics Consortium which attracted over 100 scientists from the US and Canada. Other CABM-sponsored programs that enhance the research environment on the university campus include a seminar series that brings visiting scientists to talk about their work and to meet with trainees, the newly inaugurated Aaron J. Shatkin Lecture and the annual CABM Symposium held each year on a topic of special interest.

Outreach education programs such as SMART Team and Biolinks programs introduce scientific research to highly motivated high school students at local schools. In addition, CABM hosts a Summer Undergraduate Research Experience (SURE) that is now in its ninth year. In 2012 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. A new initiative seeks to build bridges between US and Chinese academic programs. Dr. Montelione has been awarded a grant from the Chinese government to develop a joint Rutgers research program at Jiangnan University. This program began in the summer of 2012 with a six-week course in Proteomics and Functional Genomics at CABM for 14 visiting Chinese students. The program has already generated a joint publication and a joint patent application.
Awards and Service

CABM faculty members 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, Dr. Nanda is recipient of an NIH New Innovator Award, Drs. Gélinas, Millonig, Rabson, Stock and Xiang served on NIH study sections and Dr. Montelione is a member of the National Science Foundation BIO Advisory Panel. Dr. Montelione served as an assessor at the international CASP10 protein structure prediction meeting and competition in Italy. Dr. Stock serves on the American Society of Biochemistry & Molecular Biology's (ASBMB) Education & Professional Development and Finance Committees and represents (ASBMB) on the FASEB Committee on Science Policy's Subcommittee on Training. Dr. Montelione is advisor to the NSF Committee of Visitors. Many faculty serve on other national committees, as members of grant review panels and in journal editorial positions. At the local level, Dr. Gélinas serves as Associate Dean for Research, Dr. Millonig leads the RWJMS/Rutgers/Princeton joint MD/PhD program, Dr. Rabson serves as Director of the Child Health Institute of New Jersey and Dr. Lobel serves as Director of the RWJMS-Rutgers Biological Mass Spectrometry Core Facility. In 2012 Dr. Nanda was recognized with the UMDNJ Foundation Excellence in Research Award. Postdoctoral fellows Drs. Hisako Masuda, Angela McKoy, Yu Meng, Kamana Misra and Jim Stapleton were awarded competitive fellowships and graduate student Fuguo Jiang received a prestigious award from the Chinese government.

Initiatives with Biotech and Pharma

Interactions with industry are a central component of the CABM mission to translate research discoveries and new technologies to improve human health. CABM has spawned two biotechnology companies, both of which marked major advances this year. Prodaptics Pharmaceuticals, Inc., co-founded by Dr. Arnold, was established in Fall 2011. Based on expertise the Arnold laboratory acquired during development of anti-AIDS drugs in collaboration with Janssen Pharmaceutica, Prodaptics is pursuing therapeutics targeting flu virus. Nexomics Biosciences, established in 2008 by Dr. Montelione, uses licensed technologies developed at CABM for protein production and structure determination to enable drug discovery and development by client biotechnology and pharmaceutical companies. In February 2012, Nexomics entered a new growth phase with official opening of a new headquarters in state-of-the-art laboratory space at the Commercialization Center for Innovative Technologies (CCIT) in North Brunswick, a component of the New Jersey Commission on Science and Technology (NJCST) Incubator program.

Laboratories at CABM continue to collaborate with industrial partners to advance research programs and novel therapeutic strategies. Dr. Lobel received a focused giving award from Johnson & Johnson and is working with BioMarin Pharmaceuticals to develop a drug using recombinant TPP1 for an enzyme replacement therapy to treat late infantile neuronal ceroid lipofuscinosis, a hereditary neurodegenerative disease of childhood that is due to a deficiency in the lysosomal protease tripeptidyl peptidase I (TPP1). Dr. Marcotrigiano collaborates with researchers at Avatar Biotechnologies LLC, Gilead Sciences, GlaxoSmithKline and Novartis to develop vaccines and therapeutics against hepatitis C virus. Dr. Inouye collaborates on new technologies with Dainippon Sumitomo Pharma. Additional advances with potential commercial value led to 10 provisional and awarded patents for protein expression technologies and strategies relevant to treatment of lysosomal storage diseases.
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 for many contributions and 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 members were successful again in obtaining grants and contracts – more than $15 million in fiscal year 2012. Notably, two large-scale NIH projects are based at CABM, the Protein Structure Initiative's NESG structural genomics program and the Human Anti-Proteome Project's production of antigens for human transcription factors.

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 was 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 appreciative of support from numerous companies including Johnson & Johnson, Hoffmann-LaRoche, Sanofi-Aventis, Pfizer, Takara Bio Inc., Cisco Inc., Nexomics Biosciences Inc., BioMarin Pharmaceuticals, Amicus Therapeutics, Cambridge Isotopes Laboratory, GenScript, Macrogen, New England BioLabs, Taxis Pharmaceuticals Inc., Rigaku America 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.

Transitions

With great sadness CABM experienced the passing of its founding director, Dr. Aaron J. Shatkin, on June 4, 2012 at the age of 77. The happier celebratory events of the past year, the 25th Anniversary CABM Symposium, the dedication of the Aaron J. Shatkin Atrium and the inaugural Aaron J. Shatkin Lecture provided an opportunity for us to share with Dr. Shatkin reflections on the history of CABM, acknowledgement of our achievements, and our overwhelming gratitude for his leadership. A book of essays from faculty and friends was presented in draft form to Dr. Shatkin in May, with publication scheduled for Fall 2012. A Memorial Program in conjunction with the 26th Anniversary Symposium “Advances in Biology and Medicine: A Tribute to Aaron J. Shakin” will be held in October 2012. Colleagues from around the world composed retrospectives for publication in Science, Proceedings of the National Academy of Sciences, and Advances in Virus Research recapping Dr. Shatkin’s seminal scientific achievements and poignantly capturing his enthusiasm for science, his remarkable mentorship and his humble, caring nature – descriptions that resonate deeply with all who had the privilege of working with Dr. Shatkin.

Dr. Shatkin was recruited as Director of CABM in 1985 when the institute was little more than a vision.
He oversaw design and construction of the beautiful CABM building, recruited exceptional faculty and established and nurtured a culture of scientific excellence and collaborative spirit. For almost three decades, until his death, Dr. Shatkin guided CABM with an inimitable style of leadership that will be greatly missed. The CABM that exists today reflects Dr. Shatkin’s passion for scientific discovery and his dedication to its pursuit. CABM is his legacy and we are resolute that it thrive and grow as he envisioned.

*Ann M. Stock, Professor and Associate Director*

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Molecular Chronobiology Laboratory

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

The main focus of Dr. Isaac Edery’s 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 mid-day 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 seasonally 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:


Low, K.H., Chen, W.F., Yildirim, E. and Edery, I. Natural variation in the *Drosophila melanogaster* clock gene *period* modulates splicing of its 3’-terminal intron and mid-day siesta. *PLOS One* 2012 7:e49536.
Dr. Céline Gélinas came to CABM in September 1988 from the University of Wisconsin, where she conducted postdoctoral studies with Nobel laureate Dr. Howard M. Temin. She earned her Ph.D. at the Université de Sherbrooke in her native country, Canada, and received a number of honors including the Jean-Marie Beauregard Award for Academic and Research Excellence, the National Cancer Institute of Canada King George V Silver Jubilee Postdoctoral Fellowship, a Medical Research Council of Canada Postdoctoral Fellowship and a Basil O’Connor Starter Scholar Research Award. Her research is funded by the NIH, the Leukemia and Lymphoma Society and the Department of Defense Breast Cancer Research Program. Dr. Gélinas served for several years as a member of the NIH Experimental Virology Study Section and the Virology B Study Section. In 2006, she was elected to the UMDNJ Stuart D. Cook Master Educators Guild. Dr. Gélinas was appointed Associate Dean for Research for RWJMS in 2008. She was elected to Fellowship in the American Academy of Microbiology in 2010.

Our research focuses on the mechanisms involved in cancer. Our studies aim at understanding how changes in specific signaling pathways and in the regulation of gene expression contribute to cancer development, progression, and tumor cell resistance to anti-cancer therapy.

We pursued our studies of the NF-κB-regulated apoptosis inhibitor Bfl-1 (Bcl2A1, A1), which contributes significantly to chemoresistance and is overexpressed in many therapy-resistant leukemia/lymphoma and in metastatic melanoma. Building on our previous findings that Bfl-1 turnover is regulated by the ubiquitin-proteasome and that this can significantly impact its role in cancer, we pursued our investigation of approaches to accelerate Bfl-1 turnover as a novel avenue to sensitize drug-resistant tumor cells to anti-cancer treatment. During the past year, we followed-up on kinase-specific inhibitors that can accelerate turnover of both Bfl-1 and its homologue Mcl-1, and quantitated their combinatorial effects with anti-cancer agents. This demonstrated their synergistic action in restoring apoptosis in drug-resistant human lymphoma and melanoma cell lines in vitro. We also evaluated their effectiveness in combination treatment in vivo, using a mouse NOD/SCID/gamma (NSG) lymphoma xenograft model. Combination treatment significantly reduced tumor growth and induced tumor regression compared to single treatment. Additionally, tumor rebound was significantly slower in animals that received combination treatment compared to either drug alone. These data suggest that compounds that can accelerate turnover of Bfl-1 and/or Mcl-1 could lead to novel combination treatments to overcome drug-resistance in leukemia/lymphoma and metastatic melanoma. These experiments were carried out as part of an ongoing collaboration with Drs. Roger Strair, Daniel Medina, Lauri Goodell and Susan Goodin (CINJ). A manuscript is in preparation (Yang et al. In preparation).
Since deubiquitinating (DUB) enzymes can rescue their protein substrates from degradation by the proteasome and represent potential anticancer targets, we hypothesized that identifying DUBs important for the ubiquitin-mediated turnover of Bfl-1 could perhaps provide alternative targets to sensitize drug-resistant tumor cells in which Bfl-1 is elevated. We found that cell treatment with a partly selective small molecule DUB inhibitor could significantly decrease Bfl-1 protein levels, and that this effect was rescued by treatment with proteasome inhibitor. We conducted a focused shRNA library screen to suppress the expression of known or putative human DUBs, and identified two DUBs whose inhibition altered the steady state levels of Bfl-1. Ongoing studies aim at further validating these candidates, at determining their mode of action and their effects on chemoresistance. Overall, these preliminary results suggest that specific DUBs could potentially constitute novel targets in drug-resistant tumor cells in which Bfl-1 is upregulated.

Collaborative studies with the group of Dr. Gaetano Montelione (CABM), who elucidated the structure of Bfl-1\(\Delta C\) (residues 1-151) in complex with human Bak, Noxa and Bim BH3 peptides, are ongoing. These provided key insights into the structure of the helix-binding cleft of Bfl-1 and have helped to better understand how Bfl-1 selectively associates with some proapoptotic Bcl-2 family members and what dictates its affinity and specificity. A manuscript is in preparation (Guan et al. In preparation).

There was significant progress in our studies looking at the role of CAPER in breast cancer. We followed up on our findings that CAPER promotes breast cancer cell invasion in vitro. In vivo studies, using a mouse xenograft model, uncovered significant effects on breast cancer cell engraftment and metastasis. Consistent with the implication of CAPER in alternative splicing as well as in transcription, validation of our Affymetrix exon array data suggested important roles for both activities. A manuscript is in preparation (Molli et al. In preparation). These studies are part of ongoing collaborations with Whitney Petrosky and Drs. Michael Reiss, Shridar Ganesan and Guna Rajagopal (CINJ). Collaborative studies are also ongoing with the group of Dr. Gaetano Montelione (CABM), who elucidated the structure of one of the CAPER RNA Recognition Motifs (RRM), whose mutation we showed significantly attenuates breast cancer cell invasion. A manuscript is in preparation (Rossi et al. In preparation).

Finally a manuscript was published, emanating from collaborative work with 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) (Medina et al. 2012). This work demonstrated that the canonical and non-canonical NF-\(\kappa B\) signaling pathways and expression of cytokine BAFF by mesenchymal stromal cells plays an important role in protecting mantle cell lymphoma cells from spontaneous- and drug-induced apoptosis. This highlights a key role for these factors in this aggressive subtype of non-Hodgkin’s lymphoma.

**Publications:**

Cell Death Regulation 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. His laboratory found that the TA systems regulate cell growth and death in reminiscence of apoptotic cell death in the eukaryotes, targeting highly diverse cellular functions including DNA replication, mRNA stability, ribosome function, cytoskeleton assembly, fatty acid biosynthesis and cell wall biogenesis. The earlier discovery of a sequence (ACA)-specific endoribonuclease or mRNA interferase 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, which provides a major impact on NMR structural study of large proteins. The research on the various TA systems likely yields a novel means to induced cell death in bacteria. Furthermore, since many of the bacterial toxins are also able to cause apoptotic cell death in human cells, his laboratory explores the application of bacterial toxins as therapeutic tolls for human diseases such as cancer and AIDS. Dr. Inouye is a member of the American Academy of Arts & Sciences.

Regulation of cell growth and death: the roles of the toxin-antitoxin (TA) systems in bacterial growth and death and their application for human diseases

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 importance of these TA systems in medical science has not been fully appreciated until recently. The Inouye laboratory works to decipher the cellular targets of these toxins and the molecular mechanisms of toxin functions.

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 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 (for example, Canavanine for Arginine) to create proteins of novel structures and functions, which cannot be achieved by any other means using living cells.

**Publications:**


Ishida, Y., Park, J-H., Mao, L., Yamaguchi, Y. and Inouye, M. Replacement of all arginine residues in MazF-bs mRNA interferase with canavanine; specificity alteration of a five-base to a si recognition for RNA cleavage. *J. Biol. Chem.* In Press.
Growth and Differentiation Control Laboratory

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

Cancer stem cells are thought as the origin of cancer. Breast cancer is the most common cancer in women. Human breast cancer stem cells are often 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 not just a marker for breast cancer stem cells. It is necessary for breast tumor initiation, tumor growth, and metastasis.

Mammospheres, which grow in nonadherent surfaces in liquid culture, are enriched in cells with functional characteristics of stem/progenitor cells. Knockdown of CD44 in breast cancer cells greatly reduced its ability to form mammosphere (see the figure). Similarly, knockdown of CD44 significantly inhibited colony formation on soft agar. Furthermore, knockdown of CD44 markedly inhibited the tumorigenicity of the breast cancer cells. The xenograft assay was carried out in collaboration with Dr. Nanjoo Suh. Knockdown of CD44 also significantly reduced basal and ligand-induced migration and invasion.

The tumor suppressor p53 is mutated or functionally inactivated in 70% cancer. In breast cancer, p53 is mutated in ~20% cases, and ~50% breast cancers contain MDM2 or MDMX overexpression. p53 causes cell cycle arrest, cellular senescence, or induces apoptosis in response to stress. It has been shown that the tumor suppressive activity of p53 is dependent on its repression of CD44 expression.

We analyzed p53 repression of CD44 expression in the MCF10A human breast cancer progression model. We show that p53 repression of CD44 expression is progressively reduced along breast cancer progression in the MCF10A model. Our preliminary studies have discovered that Smad3 also potently represses CD44 expression. Furthermore, only Smad3 of the Smad family can repress CD44, and the repression is also progressively reduced along breast cancer progression in the MCF10A model. Our ongoing studies are elucidating the mechanisms by which Smad3 and p53 repress CD44 expression, and to answer the question how repression of CD44 expression is deregulated during breast cancer progression.
TGF-β has a distinct role in renal fibrosis associated with epithelial-mesenchymal transition (EMT) of the renal tubules and synthesis of extracellular matrix. Smad3 plays an essential role in fibrosis initiated by EMT. Phosphorylation of Smad3 in the C-terminal SSXS motif by type I TGF-β receptor kinase is essential for mediating TGF-β response. Smad3 activity is also regulated by phosphorylation in the linker region. We have been studying Smad3 linker phosphorylation for a number of years. In collaboration with Dr. Akira Ooshima, we show that Smad3 EPSM mutant, which mutated the four phosphorylation sites in the linker region, markedly enhanced TGF-β-induced EMT of Smad3-deficient primary renal tubular epithelial cells, whereas Smad3 3S-A mutant, which mutated the C-terminal phosphorylation sites, was unable to induce EMT in response to TGF-β. Furthermore, immunoblotting and RT-PCR analysis showed a marked induction of fibrogenic gene expression with a significant reduction in E-cadherin in HK2 human renal epithelial cells expressing Smad3 EPSM. TGF-β could not induce the expression of α-SMA, vimentin, fibronectin and PAI-1 or reduce the expression of E-cadherin in HK2 cells expressing Smad3 3S-A in response to TGF-β. These results suggest that Smad3 linker phosphorylation has a negative regulatory role on Smad3 transcriptional activity and TGF-β/Smad3-induced renal EMT. Elucidation of mechanism regulating the Smad3 linker phosphorylation can provide a new strategy to control renal fibrosis.

**Publications:**

Dhar-Mascareno, M., Belishov, I., Liu, F. and Mascareno, E. J. Hexim-1 modulates androgen receptor and the TGFβ signaling during the progression of prostate cancer. *Prostate* 2012 72:1035-44.


Protein Targeting Laboratory

Dr. Peter Lobel trained at Columbia University and Washington University in St. Louis and joined CABM in 1989. He is currently conducting research on lysosomes and associated human hereditary metabolic diseases. This work resulted in identification of three disease genes that cause fatal neurodegenerative disorders. He was a Searle Scholar and received the excellence in research award from the UMDNJ Foundation. Dr. Lobel is Director of the CABM Biological Mass Spectrometry Core Facility.

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.

Publications:


Dixit, S.S., Jadot, M., Sohar, I., Sleat, D.E., Stock, A.M. and Lobel, P. Loss of Niemann-Pick C1 or C2 protein results in similar biochemical changes suggesting that these proteins function in a common lysosomal pathway. *PLOS One* 2011 6:e23677.

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 Assistant Dean of Medical Scientist Training and 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.

The Millonig lab has been studying autism for the last 10 years, focusing primarily on the transcription factor ENGRAILED2. Previous genetic studies by the Millonig lab demonstrated that two EN2 SNPs (rs1861972 and rs1861973) are significantly associated with ASD. These results were observed in 3 separate datasets (P=.00000035; 517 families). Six other groups have also demonstrated association for EN2 with ASD. These data suggest that a DNA variant in EN2 increases ASD risk. Subsequent LD mapping, re-sequencing and association analysis identified the ASD-associated haplotype as the most likely risk allele.

To provide further evidence that the associated haplotype contributes to ASD risk, its function was investigated in primary neuronal cultures. Because the ASD-associated haplotype maps to the intron, potential regulatory effects were tested. In neuronal cultures, luciferase assays demonstrated that the haplotype results in a ~250% increase in expression. Additional analysis determined that both associated alleles are sufficient and necessary for activator function, and two transcription factors, Cux1 and NfIB, mediate haplotype function. Because ASD is a neurodevelopmental disorder, the in vitro analysis was extended to transgenic mice. QRTPCR measured transgene levels for all the lines at 5 developmental time points (E9.5, E12.5, E17.5, P6 and adult).

At all stages the ASD-associated haplotype results in increased expression (P<.001). In addition ISHs demonstrated expanded expression domains at E9.5, E17.5 and in the adult. We then investigated if EN2 levels...
are increased in individuals with autism. 90 cerebellar samples were acquired. Taqman QRTPCR 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; Type 3 tests of fixed effects). These data support EN2 as an autism susceptibility gene and increased levels of EN2 being correlated with autism risk.

Neural Tube Detects (NTDs)
Neurulation is a complex morphogenetic process whereby the neural plate folds into the neural tube. Neural tube defects (NTDs) result from abnormal neurulation. The last step of neurulation is apposition and fusion of the neural folds on the dorsal midline, which is a poorly understood process. To understand the genetic and developmental basis of human NTDs, the lab has been studying the spontaneous mouse mutant called vacuolated lens (vl).

Previous analysis by others determined that the vacuolated lens (vl) mutation perturbs neural fold apposition and fusion. Vl arose on the isogenic C3H background and results in NTD phenotypes with 100% penetrance. Our positional cloning discovered a mutation in the orphan GPCR, Gpr161, is responsible for the vl phenotypes. Gpr161 is specifically expressed in the tips of the neural folds.

When vl was crossed onto other genetic backgrounds to map the locus, we discovered the mutant phenotypes were rescued. QTL modifiers were subsequently mapped (Modifiers of vacuolated lens 1-5: Modvl1-5). Modvl5 congenics were generated and they are sufficient to rescue the vl NTD phenotypes. Previous research by others indicates the retinoic acid (RA) pathway coordinates neural fold development. The RA pathway is down-regulated in Gpr161vl/vl embryos but are rescued by the Modvl5 congenic. Injection of exogenous RA rescues the Gpr161vl NTD phenotypes.

Gpr161 is also localized in adult stem cell populations in the brain and spinal cord. Phenotypic characterization of the vl mutation demonstrated that Gpr161 is needed for the proliferation of these stem cell populations. Interestingly RA stimulates adult stem cell neurogenesis. Ongoing studies are investigating the role of Gpr161 in adult stem cell neurogenesis and RA signaling.

Schizophrenia
miRNAs are 18-26bp RNAs that coordinate gene expression. Our collaborator, Linda Brzustowicz MD (Department of Genetics, Rutgers University), performed a genome-wide miRNA expression profile and discovered that 24 miRNAs are dysregulated in the prefrontal cortex of individuals with schizophrenia and
bipolar disorder. My lab has initiated studies to understand how these dysregulated miRNAs affect neurodevelopment so new targeted therapies for these common and debilitating diseases can be developed.

My group selected 4 miRNAs to study in more detail based upon several criteria including expression differences between affected and control groups. To begin investigating the function of these miRNAs in neurodevelopment, we have performed the following experiments. QRTPCR expression analysis determined that all 4 miRNAs are widely and abundantly expressed in the adult and developing mouse brain. These miRNAs have been over-expressed in primary mouse cortical cells. Both proliferation and differentiation phenotypes have been observed. Finally, predicted targets of these miRNAs are enriched for neurodevelopment and include genes previously genetically associated with schizophrenia or bipolar disorder. Ongoing experiments will uncover the molecular and cell biological pathways regulated by these miRNAs. This information will be critical for the development of new-targeted therapies for both schizophrenia and bipolar disorder.

Publications:


Dr. Arnold Rabson’s laboratory studies the molecular basis of cancer and human retroviral infections. His recent research activities are focused in two major areas: 1) the mechanisms responsible for the pathogenesis of retroviral infections, particularly infection with the human T-cell leukemia virus (HTLV-1), and 2) the roles of cellular transcriptional regulation in development and in human cancer.

It is estimated that 15-20 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, suggesting 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 and have also demonstrated this in a mouse model of HTLV-1 latency and gene expression, opening up a new model to study HTLV-1 pathogenesis. Recently, they have discovered a unique mechanism responsible for HTLV-1 activation. T-cell receptor activation in HTLV-1-infected cells increases the stability of the HTLV-1 RNA encoding the HTLV-1 Tax transactivator gene. Rabson’s data support a model for HTLV-1 pathogenesis whereby activation of the T cell receptor in HTLV-1 infected T cells will induce latent HTLV-1 expression through stabilization of the RNA encoding the HTLV-1 transactivator, with more HTLV-1 Tax protein produced. Increased Tax expression ultimately leads to proliferation of subsets of HTLV-1 infected T 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.

Interestingly, induction of HTLV-1 Tax expression in infected T cells results in the production of multiple cytokines, including a number of immunosuppressive cytokines, such as IL-10 and TGF-β. These cytokines may help inhibit the strong anti-HTLV-1 cytotoxic T cell response, allowing infected cells to escape the host immune response and contribute to the pathogenesis of HTLV-1-associated diseases.
The second major area of study in Dr. Rabson’s laboratory continues to be the roles of transcriptional regulation in the pathogenesis of human cancer. In collaboration with Drs. Ruth Steward and Svetlana Minakhina (Waksman Institute), Dale Schaar (CINJ), and Hatem Sabaawy (CINJ), the Rabson lab is investigating the functions of PDCD2, a highly conserved nuclear 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 has shown that the deletion of the *mynd* conserved domain of PDCD2 leads to early embryonic lethality, confirming the prediction that this is an essential functional domain of the protein. Further studies have shown that PDCD2 is essential for the proliferation of mouse embryo fibroblasts, and also likely plays a role in T cell development. 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 on-going.

**Publications:**


Molecular Virology Laboratory

Dr. Aaron J. Shatkin, a member of the National Academy of Sciences, has held research positions at the National Institutes of Health, The Salk Institute, and the Roche Institute of Molecular Biology. He has taught at Georgetown University Medical School, Cold Spring Harbor Laboratory, The Rockefeller University, UMDNJ-Newark Medical School, University of Puerto Rico, Princeton University and other institutions. Shatkin was editor of the Journal of Virology from 1973-1977, founding editor-in-chief of Molecular and Cellular Biology from 1980-1990 and is currently editor of Advances in Virus Research. He has also served on the advisory boards of a number of organizations. In 1989 he was recognized by New Jersey Monthly magazine with a New Jersey Pride Award for his contributions to the State’s economic development. In 1991 the State of N.J. awarded Shatkin the Thomas Alva Edison Science Award, and he was selected as one of New Jersey’s top ten scientists by N.J. Business magazine. He was elected Fellow of the American Academy of Arts and Sciences in 1997 and the American Association for the Advancement of Science in 1999. Shatkin received the 1977 National Academy of Sciences Molecular Biology Award, the 2003 Association of American Medical Colleges Award for Distinguished Research in the Biomedical Sciences, the Edward J. Ill Outstanding Medical Research Scientist Award and from Robert Wood Johnson Medical School the R.W. Schlesinger Basic Science Mentoring Award in 2009 and the Honorary Alumnus Award in 2011.

mRNA 5'-capping

One of the earliest steps in the cascade of events necessary for mRNA formation and function is the addition of a 5'-terminal m7GpppN "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. cerevisiae 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'-triposphatase (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'-triposphorylated 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-
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.

Publications:


Molecular Neurodevelopment Laboratory

Dr. Mengqing Xiang came to CABM in September 1996 from the Johns Hopkins University School of Medicine where he conducted postdoctoral studies with Dr. Jeremy Nathans. He earned his Ph.D. at the University of Texas M.D. Anderson Cancer Center and has received a number of honors including a China-U.S. Government Graduate Study Fellowship (CUSBEA Program), a Howard Hughes Medical Institute Postdoctoral Fellowship, a Basil O’Connor Starter Scholar Research Award and a Sinsheimer Scholar Award. In 2003 he received the Award in Auditory Science from the National Organization for Hearing Research Foundation. His work is currently supported by NIH.

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.

Foxn4 activates Dll4-Notch signaling to suppress photoreceptor cell fates of early retinal progenitors

Retinal progenitors must acquire multipotency and establish competence for the generation of the full range of retinal cell types. We have shown previously that the forkhead/winged-helix transcription factor (TF) Foxn4 is required by early retinal progenitors to establish the competence for the generation of amacrine and horizontal cells. Loss of Foxn4 function eliminates most amacrine cells and all horizontal cells and causes a progenitor fate-switch to photoreceptors while overexpression of Foxn4 promotes the amacrine cell fate. Thus, Foxn4 acts to not only confer early retinal progenitors with amacrine and horizontal competence but also inhibit the alternative photoreceptor fates. It promotes amacrine and horizontal fates in part by activating the expression of proneural bHLH TF genes Ptf1a, Neurod1, and Neurod4, which are required for the specification of amacrine and/or horizontal cells. How does Foxn4 suppress the photoreceptor cell fates? To address this question, we carried out microarray profiling of global gene expression in Foxn4 control and mutant retinas to identify candidate Foxn4 target genes. This led to the discovery that Dll4, a Notch ligand gene, is positively regulated by Foxn4, thereby genetically connecting a TF to the Notch signaling pathway.

Consistent with the activation of Dll4 expression by Foxn4, there is a dramatic downregulation of Dll4 expression in Foxn4 null retinas and Foxn4 colocalizes with Dll4 in a subpopulation of retinal progenitors. Moreover, Dll4 expression could be specifically induced by misexpressed Foxn4 in developing retinas. Several lines of evidence indicate that Foxn4 is able to directly activate Dll4 expression: 1) Foxn4 can activate reporter gene expression through a 5’ and an intronic enhancer evolutionarily conserved in the Dll4 locus among many vertebrate species; 2) there is in the 5’ enhancer a cluster of Foxn4 binding motifs; 3) site-directed mutagenesis
of the binding motifs causes severe loss of reporter activity; and 4) both mouse and human Foxn4 can occupy the 5’ enhancer as determined by chromatin immunoprecipitation assay.

Notch signaling plays multiple roles during retinogenesis including proliferation and maintenance of progenitor cells, specification of Müller glial cells, and inhibition of the photoreceptor cell fates. As a direct target of Foxn4, Dll4 may act to partly mediate the Foxn4 function in fate specification and proliferation of retinal progenitors. We tested this possibility by conditionally inactivating Dll4 in prenatal and postnatal retinal progenitors. This genetic ablation leads to decreased progenitor proliferation and marker expression but increased cones and rods and upregulated TFs involved in photoreceptor production. Thus, Foxn4 may suppress the photoreceptor competence in retinal progenitors by upregulating Dll4-Notch signaling which in turn represses the expression of photoreceptor TFs to inhibit the photoreceptor fates.

Model by which Foxn4 promotes the amacrine and horizontal cell fates but suppresses the alternative photoreceptor and ganglion cell fates in early retinal progenitor cells (RPCs).

A: Schematic illustration of retinal phenotype in Foxn4 null mutant mice. B: Early RPCs are capable of generating ganglion, amacrine, horizontal, and photoreceptor cells. C: Foxn4 specifies early RPCs into amacrine and horizontal cells by activating the expression of Ptf1a, Neurod1 and Neurod4, three bHLH TFs involved in the specification of these two cell types. It suppresses photoreceptor fates by directly activating Dll4-Notch signaling which in turn represses expression of the Otx2, Crx and TRb2 TFs involved in photoreceptor fate determination and differentiation.

Our data reveal that Foxn4 has an inherent activity to inhibit the alternative photoreceptor fates of early retinal progenitors by activating the Dll4-Notch signaling. Dll4 appears to partly mediate the Foxn4 function by serving as a major Notch ligand to expand the progenitor pool and limit photoreceptor production. It is therefore conceivable that during neurogenesis, like Foxn4, most or all competence and commitment factors may act in concert to not only promote pertinent cell fates but also suppress alternative fates available to multipotent progenitor/stem cells to ensure the highest fidelity of cell differentiation. Ultimately, it may be the balance of positive and negative influences exerted by many neurogenic factors that tips the multipotent progenitor toward a particular fate.
Ebf1 deficiency causes increased retinal Müller cells and abnormal topographic projection at the optic chiasm

The Ebf TFs play important roles in the developmental processes of many tissues. We have shown previously that four members of the Ebf family are expressed during mouse retinal development and are both necessary and sufficient to specify multiple retinal cell fates. In a more recent study, we have investigated the changes in cell differentiation and retinal ganglion cell projection in Ebf1 knockout mice. Analysis of marker expression in Ebf1 null mutant retinas reveals that loss of Ebf1 function causes a significant increase of Müller cells.

Moreover, there is an obvious decrease of ipsilateral and retinoretinal projections of ganglion cell axons at the optic chiasm, whereas the contralateral projection significantly increases in the mutant mice. These data together suggests that Ebf1 is required for suppressing the Müller cell fate during retinogenesis and important for the correct topographic projection of retinal ganglion cell axons at the optic chiasm.

Publications:


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Protein Engineering Laboratory

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

In 2010 the lab embarked on a three-year $2.9M 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. We are partnered with two “affinity capture reagent” consortia: 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 can be found at the NIH website: [http://commonfund.nih.gov/proteincapture/index.aspx](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 (cf. 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. 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 expression
success rates 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.

**Figure 1.** Examples of the QC tests of the biotinylated antigens requested by RAN, including Caliper characterization (lower left) and streptavidin bead binding test (lower right). Note that biotinylation via the in vitro approach was virtually quantitative as indicated by the lack of antigen remaining in the supernatant after a streptavidin bead pull-down (lane 4 of SDS gel in lower right panel); separate controls (not shown) indicated that this was biotin-dependent and not due to non-specific binding to the beads.

**Publications:**

Biomolecular Crystallography Laboratory

Dr. Eddy Arnold obtained his Ph.D. in organic chemistry with Professor Jon Clardy at Cornell University in 1982. From 1982 to 1987, he was a postdoctoral researcher with Professor Michael G. Rossmann at Purdue University and was a central member of the team that solved the structure of a human common cold virus by X-ray crystallography. Among the awards and fellowships Arnold has received are a National Science Foundation Predoctoral Fellowship, Damon Runyon–Walter Winchell and National Institutes of Health Postdoctoral Fellowships, an Alfred P. Sloan Research Fellowship, a Johnson and Johnson Focused Giving Award, and a Board of Trustees Award for Excellence in Research at Rutgers. He received an NIH MERIT Award in 1999 and a second consecutive one again in 2009. He was elected a Fellow of the American Association for the Advancement of Science in 2001 and a Fellow of the American Academy of Microbiology in 2006. In 2010 Dr. Arnold was named a Board of Governors Professor at Rutgers University. His laboratory is supported by grants from NIH and by research fellowships. Dr. Arnold is involved in numerous international collaborations aimed at developing drugs for treatment and prevention of global infectious diseases, including HIV/AIDS, flu, and tuberculosis. His team has contributed to the discovery of two drugs used to treat HIV infection.

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 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 target of the 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 an array of crystal structures representing diverse 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.

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 led the clinical trials.

The DAPY drugs are simple compounds that are 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 2011. In what may be unprecedented for a new best-in-class drug, Johnson & Johnson has permitted 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 account 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 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; and 2) influenza virus proteins including the polymerase from 2009 H1N1 pandemic strain.

Publications:


Structural Microbiology Laboratory

Dr. Joseph Marcotrigiano obtained his B.A. from Rutgers with highest honors for research on the structure of HIV-1 RT and integrase as a Henry Rutgers Undergraduate Scholar. He received the National Starch Award and the Bruce Garth Award for highest standing in the chemistry program and was selected for a graduate fellowship at Rockefeller University. He completed Ph.D. studies with Dr. Stephen Burley in 2000 and was awarded the inaugural David Rockefeller Jr. Alumni Fellowship. He conducted postdoctoral research as a Merck Fellow of the Life Sciences Research Foundation in the Center for the Study of Hepatitis C with Dr. Charles Rice at Rockefeller University. Dr. Marcotrigiano joined CABM in 2007.

HCV entry

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.

**Innate immune response to viral replication**

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.

**Insights into alphavirus polyprotein processing and pathogenesis**

Alphaviruses are a family of mosquito-borne pathogens with a positive sense RNA genome. Large outbreaks of Chikungunya virus, a member of the alphavirus family, have been reported in parts of Southeast Asia and several of its neighboring islands in 2005–07 and in Europe in 2007, making it an important emerging pathogen. The alphavirus replication complex consists of four proteins (termed nsP1, nsP2, nsP3, and nsP4) encoded as a single polyprotein (termed P1234), which undergoes self-cleavage by a viral encoded protease within nsP2. Genome replication occurs through a minus strand intermediate, which is made in substantially less amounts than progeny plus strands. Transition between the production of minus and plus sense RNA synthesis is accomplished through cleavage between nsP2 and nsP3 (termed P23). Recently, we have determined the structure of the precleavage form of P23, helping to illuminate these critical steps in the virus life cycle (Shin et al. 2012).
nsP2 and nsP3 share an extensive interface with nsP3 creating a ring structure with a 15-18Å diameter that encircles nsP2 (figure at left). The P2/3 cleavage site is located at the base of a narrow cleft formed by the two proteins that is solvent exposed but is not readily accessible for cleavage by the nsP2 protease. The nsP2 protease active site is over 40 Å away from the P2/3 cleavage site, supporting a trans cleavage mechanism.

nsP3 contains a previously uncharacterized domain with a zinc coordination site within a novel protein fold. Mutation of the zinc-coordinating residues abolishes viral replication. Previously identified mutations in nsP2 that result in formation of noncytopathic viruses, as well as temperature sensitive mutations, cluster at the nsP2/nsP3 interface, highlighting the importance of the interface for replication and pathogenesis. Supporting this notion, structure-based mutations in nsP3 opposite the location of the nsP2 noncytopathic mutations prevent efficient cleavage of P23, affect RNA infectivity, and alter viral RNA production levels. Lastly, a potential surface for RNA binding is proposed based on the location of ion-binding sites and adaptive mutations on a basic surface that encompasses both nsP2 and nsP3. Cleavage at the P2/3 junction may therefore alter the RNA binding properties of this basic surface and contribute to template switching for RNA synthesis.

Publications:


Dr. Gaetano Montelione is Director of the Northeast Structural Genomics Consortium of the NIH Protein Structure Initiative, an inter-institutional project in large-scale structural proteomics and bioinformatics. His laboratory develops new experimental and computational methods for determining 3D structures of proteins using nuclear magnetic resonance spectroscopy (NMR), and new approaches for high-throughput production and characterization of protein samples. He has served as a member of the NSF Molecular Biophysics Study Section, NSF Committee of Visitors, NSF Advisory Committee for Biological Sciences (BIO AC) and as an advisor to both the World Wide Protein Data Bank (WW_PDB) and the Biological Magnetic Resonance Database. Dr. Montelione has received the Searle Scholar Award, the Dreyfus Teacher-Scholar Award, the Johnson and Johnson Research Discovery Award, the American Cyanamid Award in Physical Chemistry, the NSF Young Investigator Award, and the Michael and Kate Bárány Award of the Biophysical Society. He is an elected Fellow of the American Association for the Advancement of Science.

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:


X-ray crystal structure of the complex formed between the influenza B NS1 protein and one of its human host targets, interferon-stimulated gene 15 protein (ISG15). This 3D structure, along with mutagenesis studies based on the structure, provide understanding of the sequence-specific recognition of ISG15 by NS1 that determines the host range of the influenza B virus. (Guan R, Ma LC, Leonard PG, Amer BR, Sridharan H, Zhao C, Krug RM, Montelione GT *Proc. Natl. Acad. Sci. USA* 2011, 108: 13468–13473).


Mills, J., Acton, T.B., Xiao, R., Everett, J.K., Montelione, G.T. and Szyperski, T. Solution NMR structure of the helicase associated domain BVU_0683(627-691) from *Bacteroides vulgatus* provides first structural coverage for protein domain family PF03457 and indicates domain binding to DNA. *J. Struct. Funct. Genomics* 2012 [Epub ahead of print].

Protein Design and Evolution Laboratory

Dr. Vikas Nanda joined CABM in September of 2005 after studying as an NIH National Research Service Award postdoctoral fellow in the laboratory of Dr. William DeGrado at the Department of Biochemistry and Biophysics at the University of Pennsylvania School of Medicine. His research focused on studying the molecular basis of transmembrane interactions among integrins involved in regulation of platelet hemostasis. Prior to that, Dr. Nanda received his doctorate in Biochemistry from Johns Hopkins University. His laboratory is supported by federal grants including a recent highly competitive NIH New Innovator Award. In 2012 he was inducted into the Master Educators Guild and received the Foundation of UMDNJ Excellence in Research Award.

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.

**Publications:**


Protein Crystallography Laboratory

Dr. Ann Stock performed graduate work on the biochemistry of signal transduction proteins with Professor Daniel E. Koshland, Jr. at the University of California at Berkeley. From 1987 to 1991 she pursued structural analysis of these proteins while a postdoctoral fellow with Professor Clarence Schutt at Princeton University and Professor Gregory Petsko at the Structural Biology Laboratory in the Rosenstiel Center at Brandeis University. Dr. Stock has received National Science Foundation Predoctoral and Damon Runyon-Walter Winchell Postdoctoral Fellowships, a Lucille P. Markey Scholar Award in Biomedical Science, an NSF Young Investigator Award, a Sinsheimer Scholar Award and an NIH MERIT Award in 2002. She has received the Foundation of UMDNJ Excellence in Teaching Award, the Molecular Biosciences Graduate Association Educator of the Year Award and the designation of Master Educator at UMDNJ. In 2004 Dr. Stock was selected as an Outstanding Scientist by the NJ Association for Biomedical Research. She was elected Fellow of the American Association for the Advancement of Science in 2006 and fellow of the American Academy for Microbiology in 2007. Dr. Stock was an investigator of the Howard Hughes Medical Institute from 1994-2011. She served as Chair of the Board of Scientific Counselors for the NIH-National Institute of Dental and Craniofacial Research in 2008-2009 and currently serves as an Editor of the Journal of Bacteriology and as a member of the Editorial Board of PLoS Biology. She served on the Council of the American Society for Biochemistry and Molecular Biology from 2008-2011 and currently serves as a member of the ASBMB Education & Professional Development and Finance Committees. Dr. Stock serves as an elected member of the RWJMS Faculty Council and the UMDNJ Faculty Senate.

Research in our laboratory focuses on structure/function studies of signal transduction proteins. Effort is concentrated on response regulator proteins that are the core of two-component systems, the most prevalent signal transduction pathways in bacteria. These systems are important for virulence in pathogenic organisms and are targets for development of new antibiotics.

Current research efforts are focused on characterizing representative two-component signaling pathways 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 pathways involved in phosphate assimilation and osmoregulation, two model two-component systems in E. coli. Tools have been developed and data are being accumulated to facilitate the mathematical modeling of the mechanism of regulation in representative two-component systems.
Research with translational potential is focused on 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.

**Publications:**

Dixit, S.S., Jadot, M., Sohar, I., Sleat, D.E., Stock, A.M. and Lobel, P. Loss of Niemann-Pick C1 or C2 protein results in similar biochemical changes suggesting that these proteins function in a common lysosomal pathway. *PLOS One* 2011 6:e23677.


Education, Training and Technology Transfer
Lectures, Seminars and Symposia
Lecture Series 2011-2012
Lectures will be held in CABM Seminar Room 010 unless otherwise noted

October 21, 2011 – RWJMS Main Lecture Hall
(Pre-Registration Required)
25th Annual CABM Symposium

Tuesday, November 29, 12:00
Virginia A. Zakian, Princeton University
“Pif1 Family DNA Helicases: Multi-Functional Helicases That Affect G-quadruplex DNA in vivo
and in vitro”

Wednesday, December 7, 12:00
Matthew D. Scharff, Albert Einstein College of Medicine
“B Cells Hijack Normal Repair Processes to Generate Antibody Diversity”

Wednesday, January 11, 12:00
Richard H. Ebright, Rutgers University, HHMI
“Structural Basis of Transcription Initiation”

Tuesday, February 21, 12:00
Jacques R. Fresco, Princeton University
“Site-Specific Self-Catalyzed Depurination, a Biological Mechanism Used to Create Mutations
and Genomic Sequence Diversity”

Wednesday, March 14, 12:00
Dinshaw Patel, Memorial Sloan-Kettering Cancer Center
“Bioimaging at the Nanoscale: Single-molecule and Super-resolution Fluorescence Microscopy”

Wednesday, April 18, 12:00
Xiaowei Zhuang, Harvard University, HHMI
“Translational Control of Cancer”

Wednesday, May 23, 12:00
Michael Gale, University of Washington
“Innate Immunity”
Postponed

Supported by Sanofi Aventis
Good Questions Drive Good Science

Harold Varmus, MD

Monday, April 16, 2012

Hosted by the Center for Advanced Biotechnology and Medicine
HAROLD VARMUS, MD
Director
National Cancer Institute

Harold Varmus, MD, a co-recipient of the Nobel Prize for studies of the genetic basis of cancer, became director of the National Cancer Institute in 2010. He was president of the Memorial Sloan-Kettering Cancer Center for 10 years and served for six years as director of the National Institutes of Health.

Dr. Varmus is a member in the U.S. National Academy of Sciences and the Institute of Medicine and is involved in several initiatives to promote science and health in developing countries. He was co-chair of President Obama’s Council of Advisors on Science and Technology, a co-founder and chair of the Board of the Public Library of Science and chaired the Scientific Board of the Gates Foundation Grand Challenges in Global Health. Dr. Varmus is the author of numerous scientific papers and five books, including a recent memoir titled The Art and Politics of Science.
CABM RETREAT, June 20, 2012
Cook Campus Center, New Brunswick, NJ

Program

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

8:45 AM  Opening Remarks – Ann Stock

9:00 AM  Session I – Insights into Function through Structure
Chair: Dishaben Patel – Biomolecular Crystallography Lab (Eddy Arnold)

Gregory Kornhaber – Structural Bioinformatics and Proteomics Lab (Guy Montelione)
“Community Outreach Activities of the Northeast Structural Genomics Consortium”

Jim Stapleton – Protein Design and Evolution Lab (Vik Nanda)
“Redesign of the Lipid-Facing Surface of a β-Barcode Membrane Protein”

Fuguo Jiang – Structural Virology Lab (Joe Marcotrigiano)
“How Does RIG-I Recognize Double-Stranded RNA and Get Activated?”

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

Sergio Martinez – Biomolecular Crystallography Lab (Eddy Arnold)
“Inhibition of DNA Polymerization in HIV-1 Reverse Transcriptase by NNRTI Drugs: a Structural Analysis”

10:15 AM Posters and Coffee

11:00 AM  Session II – Cell Structure and Trafficking
Chair: Anna Dulencin – Developmental Neurogenetics Lab (Jim Millonig)

Ling Huang – Protein Targeting Lab (Peter Lobel)
“Unraveling the Intracellular Trafficking of the Niemann-Pick Type C Proteins”

Bo Li – Developmental Neurogenetics Lab (Jim Millonig)
“Type II Cadherins Regulate Congenital Cataract in vacuolated lens (vl) Mouse Mutant”

Min Zou – Molecular Neurodevelopment Lab (Meng Xiang)
“Bnr3a/Pou4f1 Regulates Dorsal Root Ganglion Sensory Neuron Specification and Axonal Projection into the Spinal Cord”

Hisako Masuda – Bacterial Physiology and Protein Engineering Lab (Masayori Inouye)
“YeeU, a Novel Factor that Enhances the Bundling of Cytoskeletal Polymers of MreB and FtsZ in E. coli”
12:00 PM Introduction of CABM SURE Program Leaders and Students – Steve Tuske

12:05 PM Lunch and Break

2:00 PM Posters and Coffee

3:00 PM Session III – Gene Expression and Cell Regulation
Chair: Ian Bezar – Protein Crystallography Lab (Ann Stock)

Rong Gao – Protein Crystallography Lab (Ann Stock)
“Expression Levels of the Two-Component PhoR/PhoB System are Optimized for Maximal Fitness of Bacteria”

Rudra Dubey – Molecular Chronobiology Lab (Isaac Edery)
“Mapping of Post-Translational Modifications in PERIOD Protein by Mass Spectrometric Analysis”

Steve Anderson – Protein Engineering Lab (Steve Anderson)
“Update: the Human Transcription Factor Antigen Project”

Fang Liu – Growth and Differential Lab (Fang Liu)
“Repression of CD44 Expression by Smad3 and p53 in a Breast Cancer Progression Model”

Poonam Molli – Tumor Virology Lab (Céline Gélinas)
“CAPER alpha Promotes Breast Cancer Cell Invasion and Metastasis”

HsinsChing Lin – Viral Pathogenesis Lab (Arnold Rabson)
“Adaptation to Histone Deacetylase Inhibitors Attenuates Cancer Cell Growth”

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

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CABM

CENTER for
ADVANCED BIOTECHNOLOGY
and MEDICINE

Annual Report 2012 63
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

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  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”

11:40 pm  Jianping Ding, PhD
          Professor, Shanghai Institutes for Biological Sciences, PR China
          “The Regulation of the Histone Acetyltransferase Activity of hMOF Based on
          Structural, Biochemical and Cell Biology Studies”

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

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 Lecturer, 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  Break

SESSION IV:

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

3:50 pm  Joseph Marcotrigiano, PhD
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
Doctoral Degrees Granted 2011/2012

Asli Ertekin
Order and Disorder in Proteins
Advisor: Gaetano Montelione

Daniel Hsieh
Modeling Insertion of Transmembrane Bet-Barrels for
Designing Outer membrane Proteins
Advisor: Vikas Nanda

Binchen Mao
Improving the Quality of Protein NMR Structures by
Rosetta Refinement and its Application in Molecular Replacement
Advisor: Gaetano Montelione

Peng-Ling Sun
ACP5, an Enzyme Responsible for Mannose 6-phosphate Removal from
Lysosomal Proteins and its Biotechnological Application
Advisor: Peter Lobel

Su Xu
Investigation of Late Infantile Neuronal Ceroid Lipofuscinosis:
Lysosomal Storage and Enzyme Replacement Therapy
Advisor: Peter Lobel

Min Zou
Roles of the Brn3 POU Domain Transcription Factors
During Dorsal Root Ganglion and Spinal Cord Development
Advisor: Mengqing Xiang
Undergraduate Students

Arnold Lab  
Matthew Mudrick (SURE 2012)

Edery Lab  
George Ghanim (SURE 2011)  
Yee Chen Low  
Kaiwal Patel (SURE 2012)  
Jash Vakil (SURE 2011)

Gelinas Lab  
Nikita Ekhelikar (SURE 2011)

Inouye Lab  
Sehrish Asmal  
Shantanu Baghel  
Vishal Parikh (SURE 2012)

Lobel Lab  
Mohamed Albana (SURE 2011)  
Dana Betts  
Rishi Jaggernauth (SURE 2012)  
Sharlina Keshava  
Tapan Patel  
Samantha Schlachter  
Alan Tran

Central Lab Services  
Monika Kapadia

Marcotrigiano Lab  
Alicja Cygan (SURE 2012)

Millonig Lab  
Aziz Karakhanyan  
Keiry Rodriguez  
Sammy Taha (SURE 2011)

Nanda Lab  
Alexander Davis (SURE 2011)  
Aisha Jasani  
Kaiser Loell (SURE 2012)  
Priyesh Patel

Rabson Lab  
Chrystal Chang  
Timothy Dinh  
Jordan Hedvat  
Tiffany Tsang

Shatkin Lab  
Frank Zong (SURE 2011)

Stock Lab  
Jose Valverde (SURE 2011)

Xiang Lab  
Sarah Finkelstein (SURE 2012)  
Benjamin Hanft (SURE 2011)

Montelione Lab  
Ashley Aya  
Alexander Bendik  
Amyukta Bengeri  
Nicole Brunck  
Katherine Carrino-Bednarski  
Ya-Min Chi  
Dhaval Gandhi  
Emily Grasso (SURE 2012)  
Sultan Khawam  
Kaustubh Kulkarni (SURE 2012)  
Hy Le  
Joseph Miller  
Patrick O’Connell (SURE 2012)  
Srushi Raja  
Janna Sloand  
Chelsea Stahl  
Amy Suhotliv (SURE 2011)  
Aparna Tatineni (SURE 2011)  
Charmi Vakaria

Administration  
Daniel Yoon
Provisional Patents
“Design of an Influenza A Non-structural Protein 1a Inhibitor and Assay”
Provisional patent filed February 2013.

Marcotrigiano J, Whidby J, Khan AG, Basant A, Altman J, Shires J.
"Mammalian Cell Culture System for Large-Scale Expression of Recombinant Proteins"
Provisional patent filed June 2012.

Montelione GT, Xiao R, Nei Y, Xu Y.
“Stereospecific Carbonyl Reductases”
Provisional patent filed December 2011.

Acton TB, Anderson S, Montelione GT, Huang YJ.
“System for High-level Production of Proteins and Protein Domains”
Provisional patent filed November 2011.

Hunt JF, Price WM, Acton TB, Montelione GT.
“Methods for Altering Polypeptide Expression”
Provisional patent filed September 2011.

Roth M, Schneider W, Montelione G, Inouye M.
“Independently Inducible System of Gene Expression”
Provisional patent filed April 2011.

Awarded Patents
United States Patent 8,277,800 October 2, 2012
“Methods of Treating a Deficiency of Functional Tripeptidyl Peptidase I (CLN2) Protein”
Inventors: Lobel P, Sleat D.

“Method of Expressing Human Lysosomal Protein in Brain Cells”
Inventors: Lobel P, Sleat D.

United States Patent 8,029,781 October 4, 2011
“Methods of Treating a Deficiency of Functional Tripeptidyl Peptidase I (CLN2) Protein”
Inventors: Lobel P, Sleat D.

“Single Protein Production in Living Cells Facilitated by a Messenger RNA Interferase”
Inventors: Inouye M, Zhang J, Suzuki M.
Administrative and Support Staff

Administrative Assistants
Darlene Bondoc
Jane Kornhaber
Elaine Simpson Lear
Janice Nappe
Barbara Shaver

Central Information Technology
Carmella Luczak
Shelley Waltz
Keith Williams

Business Office
Lynnette Butler
John Drudy
Madeline Frances
Sharon Pulz
Kristin Rossi
Diane Sutterlin

Central Lab Services
Ramon Dugenio
Alba LaFiandra
Sally Marshall
Harren Mercado
Minoti Sinha
Angela Sowa
Scientific Advisory Board

OUTSIDE ACADEMIC MEMBERS

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

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

Dr. Thomas Eugene Shenk
Elkins Professor-Department of Molecular Biology
Princeton University

INDUSTRY REPRESENTATIVES

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Vice President, Discovery Chemistry
Roche

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Site Lead, Infectious Disease – HIV
Merck Research Laboratories

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Corporate Vice President
Corporate Office of Science & Technology
Johnson & Johnson

Dr. William S. Somers
Assistant Vice President
Global Biotherapeutic Technologies
Pfizer

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Director, Bridgewater Functional Genomics
Sanofi
UMDNJ MEMBERS

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Director, The Cancer Institute of New Jersey
Associate Dean for Oncology Programs
Professor of Medicine
UMDNJ-Robert Wood Johnson Medical School

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

Dr. Arnold B. Rabson, M.D.
Professor and Director, Child Health Institute of New Jersey
UMDNJ-Robert Wood Johnson Medical School

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Linus C. Pauling Professor
Dean of Biological Sciences
Vice President of Health Science Partnerships

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William M. and Myrle W. Garber
Professor of Cancer and Leukemia Research
Professor of Chemical Biology

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

Dr. 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
Center for Advanced Biotechnology and Medicine
Grants and Contracts
FY 2012: July 1, 2011 - June 30, 2012

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### Center for Advanced Biotechnology and Medicine
#### Grants and Contracts
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<td>Characterization of New Toxins (YmgD &amp; YdfD) from E. Coli, Targeting Cell Wall (NRSA Postdoctoral Fellowship)</td>
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**Total**

- Direct Costs: $23,557
- Indirect Costs: 0
- Total Costs: $23,557

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**Total**

- Direct Costs: $40,000
- Indirect Costs: 0
- Total Costs: $40,000

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**State of NJ**

- Direct Costs: $416,593
- Indirect Costs: $18,182
- Total Costs: $434,775
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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>Nexomics Biosciences, Inc.</td>
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<td>8/17/07 - 7/31/12</td>
<td>$1,451,279</td>
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<td>$287,487</td>
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<td>NIH</td>
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<td>$1,451,279</td>
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<tr>
<td>Targeting the NS1 Protein for the Development of Influenza Virus Antivirals (University of Texas subcontract)</td>
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<td>Structural Genomics of Eukaryotic Domain Families</td>
<td>$37,950,426</td>
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<td>Membrane Protein Production using the Yeast SPP System (UMDNJ subcontract)</td>
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<td>Computational Design of a Synthetic Extracellular Matrix</td>
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<td>Structure-Based Engineering of Allergens to Enhance Digestibility</td>
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**Shatkin, Aaron**  
UMDNJ

| | | | | |
| **Corporation** | | | | |
| Shatkin, Aaron Corporation | $26,250 | $26,250 | $0 | $26,250 |
| Pharmaceutical Companies | | | | |
| **CABM Symposium and Lecture Series** | | | | |
| Shatkin, Aaron Corporation | $26,250 | 7/1/11 - 6/30/12 | $26,250 | $0 | $26,250 |
| **Total** | | | | |
| | $26,250 | $0 | $26,250 |

**Sleat, David**  
UMDNJ

<p>| | | | | |
| | | | | |
| <strong>Foundation</strong> | | | | |
| Shatkin, Aaron Corporation | $25,000 | $20,833 | $0 | $20,833 |</p>
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<td>Computational Design of Beta-Barrier Membrane Proteins (NRSA Postdoctoral Fellowship)</td>
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|   | CABM Total | $11,811,578 | $3,929,455 | $15,741,033 |

**Total**:
- **Total Award**: $3,929,455
- **Direct Costs**: $15,741,033
- **Indirect Costs**: $11,811,578
- **Total Costs**: $35,482,043