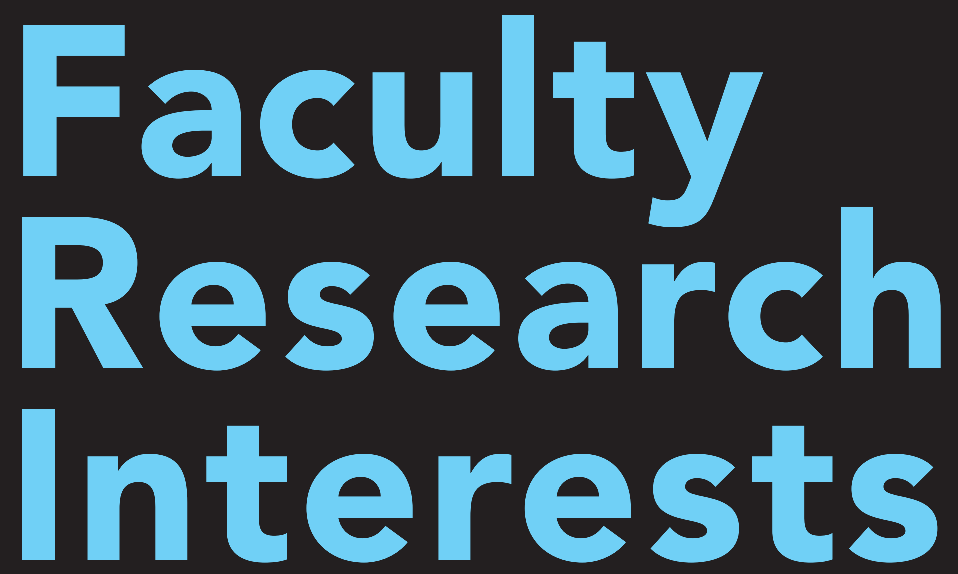
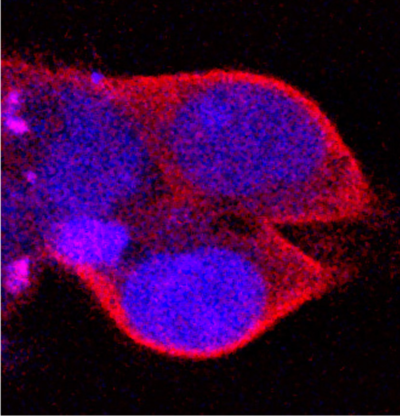


Faculty Research Interests

2010-2011



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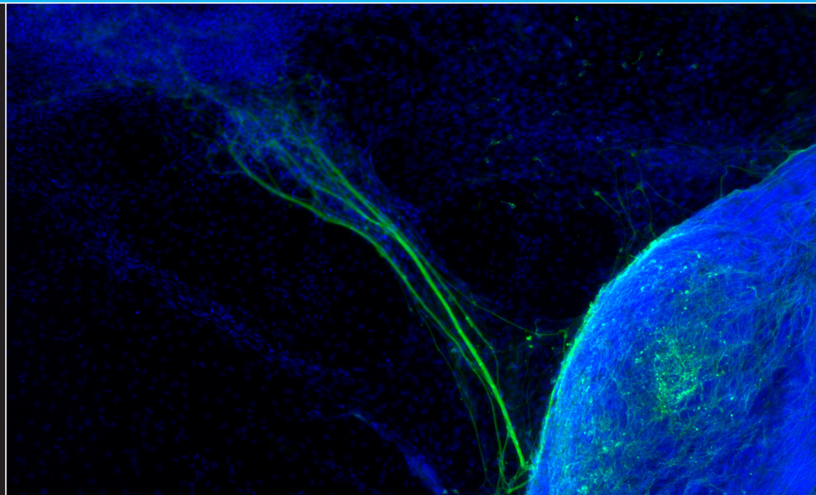

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Photo 1: HIV-1
Electron micrograph depicting HIV-1 budding from the surface of an infected cell.

Jennifer Cano, PhD Student in the Laboratory of Dr. Ganjam Kalpana

Photo 2: NLSS6A
HIV-1 gag assembling at the plasma membrane of an infected cell.

Jennifer Cano, PhD Student in the Laboratory of Dr. Ganjam Kalpana

Photo 3: Neuron cell from iPS
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Ph.D. Program in the Biomedical Sciences

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M.D./Ph.D. Medical Scientist Training Program (MSTP)

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BASIC SCIENCE DEPARTMENTS · Anatomy & Structural Biology · Biochemistry · Cell Biology · Developmental & Molecular Biology · Microbiology & Immunology · Molecular Genetics · Molecular Pharmacology · Neuroscience · Pathology · Physiology & Biophysics · Systems & Computational Biology · CLINICAL · Clinical Investigation

2010-2011 Basic Science Faculty Research Interests
produced by **Einstein Graduate Programs in the Biomedical Sciences**
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email: salvatore.calabro@einstein.yu.edu

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Protein Complexes
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Spinal Cord
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Key Words: ***Autism, human genetics, developmental neurobiology, language***

The Autism Spectrum Disorders: From Genetic to Biology

My work is aimed towards understanding how disorders of human cognition, and the Autism Spectrum Disorders (ASDs) in particular, are influenced by genetic variation. Defined entirely in terms of behavior, the ASDs represent a unique class of clinical conditions involving deficits in language use, impaired social behavior and a circumscribed range of interests.

Work in my lab employs a blend of molecular genetics and developmental neurobiology to identify disease-related genes and understand how they operate functionally. Drawing on both hypothesis-based and discovery-driven methodologies, I'm particularly excited to purse a variety of studies focusing on Contactin-Associated Protein-like 2 (CNTNAP2). In addition to the potential importance of this molecule to ASD biology, we and others have obtained data to support a role for this gene in related disorders of cognition including specific language impairment, intellectual disability, and schizophrenia. And so our findings from cell, mouse, and human-based systems are likely to be of broad interest.

Looking forward, we will direct substantial effort towards understanding how individual molecular variants work alongside one another to modulate risk. New insights around how seemingly distinct molecules converge to shape disease-related processes will prove important in the development of potential therapeutics.

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Key Words: ***Neurotransmitter-gated ion channels, GABA, synaptic transmission, protein structure, malaria, drug resistance, nucleoside transport***

ION CHANNEL STRUCTURE, FUNCTION AND DYNAMICS

Neurotransmitter-gated ion channels are essential components in synaptic transmission. Our work focuses on the GABAA receptor, the major post-synaptic inhibitory neurotransmitter receptor in brain. It is the target for drugs used clinically in the treatment of anxiety and epilepsy, and for general anesthesia and insomnia. GABAA receptors are members of a gene superfamily that includes receptors for glycine, acetylcholine, and serotonin. Our goals are to understand the structural bases for the functional properties of the channel and to understand the molecular interactions by which drug binding modulates structure and channel activity. We use a combination of techniques including site-directed mutagenesis, heterologous expression, covalent chemical modification and electrophysiology. These studies have identified the residues lining the channel, the location of channel blocker binding sites and identified conformational changes occurring during channel gating and modulation by drugs including valium and general anesthetics such as propofol.

MALARIA DRUG RESISTANCE TRANSPORTERS AND PARASITE PHYSIOLOGY

Malaria is a major public health problem in the developing world causing millions of deaths per year. Using heterologous expression systems we have characterized the expression and transport properties of nucleoside transporters (PfENT1 & PfENT4), sodium-hydrogen exchangers (PfNHE) and the *Plasmodium falciparum* chloroquine resistance transporter (PfCRT) involved in drug transport and resistance. We are currently exploring the properties of a family of nucleoside transporters to identify the transporters involved in AMP and Immucillin transport and developing a high throughput assay for PfENT1 inhibitors to identify novel anti-malarial drugs.

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Key Words: ***X-ray crystallography, actin cytoskeleton, signal transduction, tumor metastasis, allergens***

Using the infomation derived from high resolution X-ray crystallography, we exploit the structure of individual proteins to understand the formation and regulation of macromolecular assemblies. In particular, our lab is focused on the regulation and assembly of the actin cytoskeleton, and the general structural features of trans-membrane signal transduction pathways.

We are studying a number of proteins involved in the formation and localization of filamentous actin networks in the cell. We have solved the structure of profilin, which binds to actin monomers and controls the rate of actin filament assembly, and which is also involved in regulating the phospho-inositide signal transduction pathway. Profilin has been directly implicated in a number of normal and pathological processes such as cytokinesis and *Listeria* infection. We are currently solving the structure of fimbrin, one of the major actin binding proteins responsible for cross-linking individual actin filaments to form bundles and networks. This structure will be of general importance, as many actin cross-linking proteins, including those responsible for muscular dystrophy, *Shigella* infection and tumor metastasis, are expected to share extensive structural homology with fimbrin.

We have recently solved the first structures of allergens, which are responsible for the clinical symptoms of allergy, including rhinitis, conjunctivitis and asthma. The structures of these proteins provide novel targets for the design of therapeutic agents which block the primary events in the allergic response. The spatial information derived from these allergens will allow us to obtain novel structural information about the receptor-mediated events which trigger histamine release, and will provide general principles about signaling pathways dependent on receptor clustering events.

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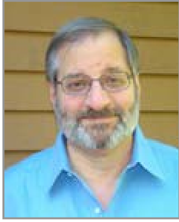

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Key Words*: *Electrophysiology, cortex, monkeys, seizures, neurotoxicology, clinical studies

NEUROPHYSIOLOGIC TECHNIQUES TO EXPLORE NORMAL AND ALTERED FUNCTION IN ANIMAL MODELS

Our laboratory uses a variety of neurophysiologic techniques to explore normal and altered function in both the peripheral and central nervous systems. Experimental procedures include EEG, evoked potentials, ensemble and single unit recordings, current source density, and conduction velocity. For the past several decades, we have focused on the timing and spatial distribution of neuroelectric events in the neocortex of behaving monkeys. Current studies explore the complex auditory processing of components of speech and music in primary auditory cortex. In addition to our "basic science" studies, we have examined biomarkers of nerve damage in a variety of animal models, including transgenic and mutant mice, diabetic neuropathy, seizure disorders, neurotoxic insult and iatrogenic deficits of central and peripheral nerve function. Finally, we have participated in the "translation" of basic neuroscience principles to human clinical studies. We are currently involved in the design and conduct of multicenter Phase 1-4 clinical trials of experimental therapies intended to reduce or prevent diabetic and chemotherapy-induced neuropathies, to improve the treatment of chronic inflammatory demyelinating polyneuropathy and to monitor the treatment-related CNS effects of compounds targeting HIV infection, cancer and depression. In this latter capacity, we have worked with the Centers for Disease Prevention and Control, the Environmental Protection Agency, the National Institute of Occupational Safety and Health and numerous pharmaceutical and biotechnology companies.

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***Key Words:** drug use, HIV, medication adherence, smoking cessation*

I am the Chief of the Division of General Internal Medicine and a Professor in the Departments of Medicine, Psychiatry & Behavioral Sciences, and Epidemiology & Population Health. I am also the Director of Substance Abuse Research for the Department of Psychiatry, and the Coordinator of the Clinical Core for the Einstein/Montefiore Center for AIDS Research (CFAR). I have a long-standing interest in behavioral medicine, including adherence with antiretrovirals and other medications, nicotine dependence, and substance abuse. Since joining the Einstein faculty in 1996, I have established a successful research program focused on HIV infection in drug users and other medical complications of substance abuse.

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Key Words: ***PI 3-kinase, signal transduction, diabetes, cancer***

SIGNALING BY PHOSPHOINOSITIDE KINASES

Phosphoinositide 3-kinases are lipid kinases that mediate signaling by tyrosine kinase and G-protein coupled receptors. PI 3-kinases are important regulators of cellular proliferation, motility, apoptosis, and vesicular trafficking. They are critical for insulin signaling, and are important in clinical diabetes. Mutational activation of PI 3-kinases is commonly found in human cancers. We are interested in the mechanisms that regulate PI 3'-kinase activity and the role of PI 3-kinases in intracellular signaling in diabetes, cancer and aging.

1. Activating mutations of PI 3-kinase in human cancer. The Class IA PI 3'-kinase is a heterodimer composed of a catalytic subunit (p110) and a regulatory subunit (p85). In normal cells, PI 3-kinase is activated when p85 binds to phosphotyrosine residues in receptor tyrosine kinases and their substrates. However, p85 and p110 are frequently mutated in human breast, colon, prostate and brain cancer. Using biochemical and biophysical methods, we are studying the mechanism of PI3K activation in normal cells, and how mutations in the regulatory and catalytic subunits of PI 3-kinase can lead to constitutive activity in cancer cells.

2. PI 3'-kinases in autophagy. Autophagy is a cellular response to nutrient deprivation in which cytosolic contents are engulfed and delivered to the lysosome for degradation. Autophagy is required for the viability of pancreatic beta cells, hepatocytes and neurons and for innate immune responses to pathogens. Downregulation of autophagic degradation has been implicated in neurodegenerative syndromes and in aging. The mammalian Class III PI 3-kinase, hVps34, plays essential roles in both vesicular trafficking and autophagy. We are studying the role of hVps34 in autophagy, and the mechanisms that regulate hVps34 activity, using both cell culture and animal models (mice and zebrafish).

3. PI 3'-kinases in tumor metastasis. Activating mutations of PI 3-kinases are frequently found in human breast cancer and other tumors. Using genetic methods, we have engineered human breast cancer cells to express physiological levels of wild type or mutant PI 3-kinase. We are studying the effects of the mutants on tumor metastasis in vivo, using xenograft tumors in SCID mice. We hope to uncover the mechanisms by which oncogenic PI 3-kinase mutations lead to enhanced metastasis.

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Key Words: ***Development, growth, drosophila, cell cycle, apoptosis***

GROWTH AND DEVELOPMENT OF DROSOPHILA

Much is known about the molecular biology of the cell, but many interesting processes in biology and medicine depend on populations of cells and the interactions between them. The fruitfly Drosophila melanogaster, in which in vivo genetic mosaic techniques are advanced, can be used to investigate how cell-cell interactions contribute to body form and function. We are elucidating the molecular mechanisms of neurogenesis, using molecular and genetic experiments as well as mathematical modeling to understand how certain cells are chose to differentiate into neural fates, and also how this is linked to terminal cell cycle withdrawal that maintains neurons in a post-mitotic state for the remainder of life. These studies focus on the retina as an exemplary neural tissue, and help to understand both neural developmental defects and neurodegenerative conditions, since there is emerging evidence that neurodegenerative conditions including Alzheimer's Disease, Huntington's Disease, Ataxia Telangiectasia, stroke, AIDS-related dementia, and viral encephalitis are associated with inappropriate cell cycle activation by otherwise postmitotic neurons.

We are also seeking a comprehensive understanding of the phenomenon of cell competition, which is revealed when the organism eliminates cells that would survive were the whole organism the same genotype. Even wild type cells can be out-competed by 'super-competitor' genotypes, such as cells that over-express myc, or cells mutated for certain tumor suppressor genes. Cell competition reveals a way that cell interactions can select the most favorable progenitor cells available, something that was not suspected prior to these chimera studies. In rodents, cell competition is thought to explain how liver progenitor cells from young donors replace older host cells when liver regeneration is stimulated in chimeras. Cell competition might occur during the development of cancer, since tumors normally differ genetically from normal cells.

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Key Words: ***Gap junctions, connexin, voltage-dependent gating, electrophysiology, Molecular Dynamics, Charcot-Maire-Tooth disease***

STRUCTURE FUNCTION RELATIONS OF GAP JUNCTIONS

The goal of research conducted in my lab is to define the molecular mechanisms underlying voltage-dependent gating of connexin channels by combining electrophysiological, molecular and computational methods. Membrane voltage is an important parameter regulating the activity of both intercellular and undocked hemichannels. All connexins display two distinct voltage gating processes, Vj- and loop-gating, that are intrinsic hemichannel properties. Voltage closes hemichannels by destabilizing the open state and stabilizing closed states. The recent publication of a high resolution x-ray crystal structure of a Cx26 gap junction has provided us with a starting point to perform Molecular Dynamics simulations of the hemichannel in a membrane system. This approach has refined the open hemichannel structure, identified interactions that stabilize the open state and provided a working model of the voltage-gating. We examine the structural implications and operation of voltage dependent gating by site directed mutagenesis, expression of in vitro synthesized RNA in Xenopus oocytes, Molecular Dynamics simulations of connexin hemichannels imbedded into model membranes and with the solution structure of peptides with NMR. We are extending the results obtained from our investigations of Cx26 and Cx32 to other, more distantly related members of the connexin gene family to determine the generality of the gating mechanisms we have described.

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Key Words: ***longevity genes, aging, insulin resistance, atherosclerosis, lipids, obesity***

Why do some people live much longer than others? What allows these individuals to escape age-associated diseases that contribute to mortality in the elderly? Is this a result of favorable genes or merely a healthy life style? If genes do play a role, what are the mechanisms? To address these questions, we recruited over 1000 Ashkenazi Jews. The Ashkenazi Jewish population is unique as it is derived from a small number (several thousands) of founders. The subjects fall into three groups; probands, subjects with exceptional longevity (1:10000 in the general population); their offspring; and a control group. We studied the genetic and metabolic profile. We found certain physiological characteristics such as high levels of high-density lipoprotein (HDL) as well as significantly larger particle sizes of HDL and low-density lipoprotein (LDL) in the proband and the offspring groups compared to the control group. This phenotype is associated with a lower prevalence of hypertension, CVD, the metabolic syndrome, and homozygosity of the I405V and C(-641)A variant in the CETP and ApoC III genes, respectively. Recently, we discovered a connection between the CETP variant and cognitive function. We showed that the protective genotype of I405V, namely VV, is associated with the highest scores on the MMSE test for cognitive function. Furthermore, we expanded our research to a newly discovered serum protein adiponectin (ADIPOQ) expressed and secreted exclusively by adipose tissue. ADIPOQ plays a protective role against insulin resistance and atherosclerosis. We demonstrate for the first time that exceptionally long-lived probands have markedly higher levels of serum ADIPOQ. We also demonstrated that the distribution of ADIPOQ levels in the offspring group is bimodal, suggesting that a subset of the offspring may have inherited the favorable ADIPOQ trait. The pattern of distribution of ADIPOQ levels in offspring, its significant heritability, and association of a common ADIPOQ polymorphism with ADIPOQ levels and with exceptional longevity suggest that genetic determinants of ADIPOQ may contribute to this rare phenotype of exceptional longevity. Since obesity is associated with low ADIPOQ, these findings shed light on the biology behind the emerging evidence that human longevity is being threatened by the epidemic of obesity. Recently we used novel genetic screens such as analysis of SNP sites (p53, MTP, BRCA1 and IGF-1) and gene chip mapping techniques to identify mutations in new, uncharacterized genes that may be linked to diseases of aging such as cardiovascular disease and cancer. We hope this can explain this rare trait and often-desirable state defined as longevity.

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Key Words: ***Behavior, HIV, adolescent, mental health, health disparities***

(1) Prevention of mental health problems secondary to physical conditions in children and their parents.

(2) Children affected by parental HIV. Project Care (NIMH) was a succession planning/disclosure intervention for mothers with late-stage HIV/AIDS. It provided legal, social and support counseling to mothers in order to arrange for the future care and custody of children, and was designed to assure that every child had a secure placement after parental death. She also completed a study of child caregivers (children under age 16 who are caring for their ill mothers with HIV/AIDS). Her sample was with families in New York City; Dr. Geoff Foster, her collaborator, studied children in Mutare, Zimbabwe.

(3) Prevention of HIV transmission among youth. Project Safe (NIMH) was a three-group randomized trial of 600 youth aged 14-17 that successfully reduced unsafe sexual behavior among high-risk teenagers. She is currently PI of three related studies. StaySafe is a three-group randomized trial (n=600) funded by NICHHD; it targets teenagers aged 13-16 who are not yet sexually active through programs addressing how gender norms increase risk for HIV and STDs. It Takes 2 is funded by NIDA. This two group randomized trial (n=400) is testing a new version of Project Safe which includes gender norms and addresses how relationship characteristics (e.g., love, trust, expectations of monogamy, future commitment) affect risk behavior. Adolescent Relationships and HIV/STD Risk (NIMH) is a basic research study of 360 adolescent couples to identify how love, trust, expectations of monogamy, future commitment power and communication are related to HIV risk reduction.

(4) Community Based Participatory Research. Dr. Bauman is currently PI of Bronx Youth as Partners in Community Based Participatory Research to Reduce Health Disparities (NCMHHD) is a three-year project which is using a community-based participatory action research model to reduce health disparities among African American and Latino youth in the Bronx, NY. This project defines its community as Bronx adolescents, and it proposes to design, implement and conduct a process evaluation of an intervention developed to reduce one health disparity selected by the community. Adolescents are the lead decision-makers. There are two levels of partnership, Albert's Leaders of Tomorrow (A.L.O.T.) and The Bronx Coalition for Adolescent Health (Coalition). A.L.O.T. has the decision-making responsibility for all aspects of the project, and it is composed of 14 Bronx teenagers age 14-19 and 6 adults (researchers, program specialists and physicians). The Coalition is composed of 40 Bronx organizations that have a demonstrated commitment to serving Bronx youth and have agreed to be part of the participatory action research process. A.L.O.T. chose to focus on mental health, and we are in the process of developing a mental health intervention.

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Key Words: ***Head & Neck Tumors, epigenomics, gene expression, cancer***

Head and neck squamous cell carcinomas (HNSCC) constitute an anatomically heterogenous group of neoplasms that share in common a causal association with tobacco and alcohol exposure. The clinical course of these neoplasms is difficult to predict based on established prognostic clinicopathological criteria. Unfortunately, the 5-year survival rate has improved only marginally over the past decade; as a result, it is estimated that 45,641 cases and 11,210 deaths will occur in 2009 in the United States from HNSCC. To date, there are few molecular markers that can be reliably used in either early detection or as indicators of prognosis.

Evidence has now established that aberrant DNA methylation and chromatin remodeling associated with promoters, or first exons of genes, is one mechanism frequently associated with the transcriptional silencing of critical genes in HNSCC and other cancers. One goal of our lab is the genome-wide analysis of aberrant DNA methylation events in head and neck tumor genomes. These “epigenetic signatures” can be used to identify CpG island sequences frequently hypermethylated in HNSCC, and to characterize previously indistinguishable subtypes of this disease. The long-term goal of our research is to identify genetic and epigenetic signatures associated with successful treatment and patient outcome in this disease, as well as those signatures that indicate that drug treatment will not work. This should lead to more unique gene discoveries and, in the future, new targets for anti-tumor drugs.

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Key Words: ***gap junctions, glutamate receptors, ischemia, neurodegeneration, neurotransmitters***

Areas of investigation include: molecular and cellular physiology of glutamatergic transmission; gap junction and hemichannel mediated intercellular communication; mechanisms of delayed neurodegeneration induced by global ischemia; and neuroprotection after ischemia or other insult.

Glutamatergic transmission is the primary mode of excitation in the nervous system. Modifications of synaptic efficacy underlie development and learning and also play important roles in disease processes. NMDA receptors, one class responding to glutamate, mediate some types of long term potentiation, which underlie at least one form of memory. Protein kinases and phosphatases modify single channel properties and trafficking, i.e., movement out from the cell body, insertion into the surface membrane, removal, and recycling or degradation. Regulation involves protein synthesis in dendrites as well as cell bodies. Change in glutamate receptor expression appears to mediate delayed neuronal death in the hippocampal CA1 following global ischemia and in CA3 following kainate induced status epilepticus. In situ hybridization and immunocytochemistry indicate down regulation of GluR2, the AMPA receptor subunit that limits calcium permeability of these receptors, and measurements with Ca2+ indicators demonstrate increased Ca2+ permeability. Increased Ca2+ influx in response to endogenous glutamate may then trigger cell death by apoptosis. REST (RE-1 silencing transcription factor), is upregulated after ischemia and is known to suppress GluR2 expression. In ischemic preconditioning a brief period of ischemia leads to tolerance of a longer lasting and otherwise injurious ischemic episode. We have now shown several changes in gene expression that are responsible for the ischemic tolerance after preconditioning.

Electrical synapses formed by gap junctions are known to synchronize a number of types of inhibitory interneurons widely distributed in the mammalian brain. Junctional conductance can be affected by transjunctional voltage and pH, which provides possibilities for asymmetric intercellular signaling. Gap junction channels are formed by a hemichannel provided by each of the coupled cells; because of their high conductance and permeability, it was thought that hemichannels were closed until docking with another hemichannel. Now there is evidence that hemichannels not apposed to another hemichannel can open under physiological as well as pathological conditions. We are investigating the controlling mechanisms at the level of single (hemi) channels. Hemichannels mediate intercellular signaling by secreted molecules, such as ATP, and may be involved in propagation of damage (or protection) at boundaries between normal and injured tissue.

Several human genetic diseases are caused by connexin mutations, including X-linked Charcot-Marie-Tooth disease and one type of non-syndromic deafness. We are analyzing how the altered biophysics of the mutations leads to the pathology.

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Key Words: ***Systems, evolution, biological networks, molecular genetics, computational biology***

Our research agenda involves multidisciplinary research into quantitative problems of **Systems Evolutionary Biology** that can be approached using a combination of computational, mathematical and experimental tools. The focus of the research program is mainly on quantitative aspects of evolution, gene networks, and modeling developmental processes, however, the relationship between these subjects and experimental molecular genetic studies of evolution and development are an integral part of the research efforts.

In a recent study we examined the relationship between the topology of a biological network and its functional or evolutionary properties. It has been suggested that most, if not all, biological networks are 'scale free.' That is, their connections follow power-law distributions, such that there are very few nodes with very many connections and vice versa. The number of target genes of known transcriptional regulators in the yeast, *Saccharomyces cerevisiae*, appears to follow such a distribution, as do other networks, such as the yeast network of protein-protein interactions. These findings have inspired attempts to draw biological inferences from general properties associated with scale-free network topology. One often cited general property is that, when compromised, highly connected nodes will tend to have a larger effect on network function than sparsely connected nodes. For example, more highly connected proteins are more likely to be lethal when knocked out. However, the correlation between lethality and connectivity is relatively weak, and some highly connected proteins can be removed without noticeable phenotypic effect. Similarly, network topology only weakly predicts the response of gene expression to environmental perturbations. We use evolutionary simulations of gene networks (theoretically/numerically) as well as experimentally, to address these properties in the context of complex gene networks.

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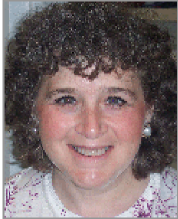

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Key Words: ***Inflammation, HIV, CNS, NeuroAIDS, chemokines, virus-host interactions***

Dr. Berman's laboratory examines the mechanisms that mediate HIV entry into the CNS and how viral and inflammatory mediators damage neurons and other CNS cells. More than 40 million people worldwide are HIV infected. As a result of antiretroviral therapies, HIV infected people are living longer. HIV enters the CNS early after infection and despite therapy, persists within the CNS. Prevalence of NeuroAIDS and its associated cognitive impairment is increasing. An understanding of mechanims that mediate these effects are critical to development of therapeutic strategies.

HIV infection of the CNS can have devastating consequences, often resulting in cognitive impairment and severe neurological complications. The basis of this impairment is poorly understood. Although its development is associated with early viral infiltration of the CNS, the number of activated monocytes/macrophages within the CNS appears to be a better indicator of neurologic compromise than viral load, suggesting that leukocyte infiltration and cognitive impairment are tightly correlated. How infected monocytes cross the blood brain barrier (BBB) and infiltrate the CNS is not well understood. This process is critical to the development of NeuroAIDS as it brings leukocytes into the brain where they activate and infect microglia, and effect damage to the BBB and other CNS cells. The mechanisms of HIV-infected monocyte transmigration across the BBB have only been minimally characterized. We are characterizing several of the steps in this transmigration process using a tissue culture model of the human BBB. We analyze the mechanisms that mediate attachment and diapedesis of HIV-infected monocytes across the BBB to identify markers that contribute to brain infection and BBB disruption, such as adhesion molecules, tight junction and adherens proteins, chemokines and their receptors. In addition, the lab is examines virus-host gene interaction using Chip technology and microRNA analyses. The lab has a major translational component, examining sera and CSF from HIV infected individuals for predictors of cognitive impairment, as well as patient cells for unique markers of this impairment. We examine tissue from HIV-infected individuals for altered proteins. The overall goal is to identify targets for therapeutic intervention to limit the entry of HIV into the CNS.

HIV infected people who abuse drugs have more extensive CNS damage associated with significant cognitive impairment. As many drugs of abuse cause an increase in extracellular dopamine, we examine the effects of dopamine on HIV infection of macrophages. We demonstrated that dopamine increases HIV infection of human macrophages and are addressing the mechanisms by which dopamine causes this increase as well as alterations in macrophage function. We also study the impact of buprenorphine and methadone,therapies for Opiate abuse, in the context of NeuroAIDS.

The laboratory also studies epigenetic regulation of host-viral interactions. Our focus is on identifying microRNA's that mediate macrophage response to HIV infection..

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Key Words: ***B cells, antibody heavy chain genes, DNA rearrangements, transcription factors, CTCF***

EFFECT OF THE 3' REGULATORY REGION ON ANTIBODY HEAVY CHAIN GENE REARRANGEMENTS AND EXPRESSION IN B CELLS

Antibody genes are constructed by DNA rearrangements and are expressed only in B cells. Occasionally, mistakes occur during construction that result in malignant transformation. My long term goal is to understand how normal antibody gene rearrangements occur. Our work focuses on analysis of "ignition" elements - cis-acting control sequences within and flanking the antibody heavy chain (IgH) gene cluster that regulate gene rearrangements and expression, and B-cell-specific "keys"--trans-acting nuclear DNA-binding proteins that activate and regulate these control sequences. We are analyzing an ~40 kb regulatory region that lies immediately downstream of the mouse and human IgH gene clusters for its long range effects on antibody genes. Multiple hypersensitive sites in this region identify enhancers and binding sites for proteins, such as CTCF and Pax5, that play a role in long-range regulation of the heavy chain locus. This region is essential for class switch recombination to generate all different classes of antibodies, and has been shown to contribute to somatic hypermutation that increases the ability of antibodies to bind to different antigens, as well as to high levels of expression of secreted antibodies, and to cis regulation of the locus. We propose that the extended 3' regulatory region also serves as a boundary for DNA rearrangement events affecting heavy chain genes.

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Key Words: enzymology, kinetics, mechanism, protein structure, antibiotic resistance.

One focus of this laboratory is the mechanistic and structural description of enzymes that are essential for the viability of bacterial and parasitic pathogens. Through a combination of recombinant DNA methods, protein purification, kinetic and chemical mechanistic analysis and three-dimensional structural determination, we hope to develop these enzymes into targets for subsequent inhibitor evaluation, and eventual drug design. A major effort is underway to clone, sequence, express, enzymatically characterize and crystallize the enzymes involved in amino acid and vitamin biosynthesis in *Mycobacterium tuberculosis*, the causative pathogen in tuberculosis. We have mechanistically characterized seven of the eight enzymes in the L-lysine biosynthetic pathway, and many of those involved in pantothenate (Vitamin B5) biosynthesis. Future studies are focused on the completion of our functional and structural characterization of the remaining enzymes in these important pathways, as well as enzymes central to the biosynthesis of *L*-arginine and *L*-leucine.

We also have an interest in the mechanisms of by which bacteria become resistant to extant antibacterial compounds. The aminoglycoside class of antibiotics acts by inhibiting prokaryotic protein synthesis. Clinical resistance to aminoglycosides is due to the expression of enzymes that modify the drug, especially those that catalyze the N-acetylation of the drug. We have mechanistically and structurally characterized two such enzymes from *Salmonella enterica* and *M. tuberculosis* that differ in the regioselectivity of acetylation, and have defined the molecular basis for the differing regioselectivity. We have also identified a structurally unique protein from *M. tuberculosis* that causes resistance to fluoroquinolones, a second important class of antibacterials that inhibit DNA gyrase. Finally, we have identified a chromosomally encoded β–lactamase in *M. tuberculosis* whose expression is responsible for the resistance of this organism to the important β-lactam class of antibiotics. We have kinetically and structurally characterized the inhibition of this enzyme by clavulanate, a β-lactamase inhibitor. We have shown recently that the combination of this inhibitor with a very slowly hydrolyzed substrate, meropenem, is effective in killing TB, including those exhibiting an extensively drug resistant phenotype.

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Key Words: ***Epigenetic, gene regulation, stem cells, reprogramming***

HUMAN EMBRYONIC STEM CELLS, EPIGENETICS AND REPROGRAMMING:

Epigenetic is the study of mitotically or meiotically heritable changes in gene function not associated with changes in DNA sequence. Epigenetic regulations are mediated by changes in chromatin structure that alter access of transcription factors to their cognate binding sites, and therefore, expression levels of genes and transgenes. Understanding these regulations is critical for gene therapy, cancer therapy and generally to gain a greater ability to modify mammalian genomes. The main focus of my lab is to understand the molecular bases of some of these epigenetic regulations. We are particularly interested in using massively parallel sequencing technologies to understand the mechanisms of establishment, maintenance, and inheritance of chromatin structures. We have recently developed a method termed TimEX to determine the timing of DNA replication genome-wide. We are using TimEX in conjunction with Chip-Seq and RNA-seq to study epigenome organization.

The second major focus of the lab is to use the epigenetic information obtained above to improve the reprogramming of somatic cells into induced pluripotent stem cells and to develop methods to reprogram somatic cells into other stem or progenitor cells by over-expression of transcription factors without reverting to an embryonic state.

Finally, we are currently developing a system to produce normal human red blood cells with embryonic, fetal or adult characteristics by forced differentiation of human ES cells using a genetic approach. The goal is to generate large amount of genetically homogenous, genetically modifiable normal erythroid cells at different stages of differentiation. This system could allow us to produce red cells for transfusion medicine, to study the silencing of the gamma-globin gene, to develop silencing resistant gene therapy cassettes that will be useful to cure the hemoglobinopathies, and to generally study the establishment and maintenance of epigenetic signals directly in untransformed human cells. We also have active research projects on the role of DNA methylation, DNA replication, histone modification, linker histones, and transcriptional interferences in the determination of expression levels.

FOR MORE DETAILS: http://cellbio.aecom.yu.edu/Lab/Bouhassira/

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Key Words: ***RNA, DNA, ribozymes, riboswitches, DNA mimics, proteins***

CELLULAR REGULATION BY REVERSIBLE MACROMOLECULAR ASSEMBLY AND ASSOCIATION

Biology is a dynamic process. Among the myriad array of reversible association reactions that constitute life as we know it, small molecules bind to proteins, proteins self-associate and bind to other proteins and nucleic acids and nucleic acids fold and bind to each other in elaborate processing, signaling and regulatory cascades. What is common to these processes is the physical chemistry that underlies these interactions. For example, electrostatic interactions mediate both the binding of proteins to DNA and the folding of RNA. DNA binding by proteins may be competitively regulated by other proteins that mimic the electrostatic character of DNA. Our laboratory seeks answers to the following questions by combining quantitative analysis with innovative approaches:

How does RNA fold? Although RNA is an informational intermediate in the central dogma, much of its biological function requires it to fold into unique three dimensional structures. However, large RNA molecules often navigate tortuous kinetic pathways to achieve their biologically active structure. Our group utilizes rapid kinetics that report the time evolution of RNA structure with single nucleotide spatial and millisecond time resolution to illuminate RNA folding pathways. Recently, our interest in RNA structure and folding has explanded to embrace RNA aptamers, small RNA molecules selected to bind to proteins with with high affinity. In collaboration with departmental colleagues Mark Girvin and Matt Levy, we are studying the structure and thermodynamics of aptamer – protein complexes to learn principals that will allow us to build biologically efficacious aptamers.

How do proteins mimic DNA? MfpA is a pentapeptide repeat protein (PRP) of Mycobacterium tuberculosis that confers resistance to fluoroquinolones by binding to DNA gyrase and inhibiting its topoisomerase activity. The novel PRP fold is a right-handed quadrilateral beta-helix of comparable size and shape to B-form DNA. MfpA has appreciable negative surface potential resulting in a structural and functional mimic of duplex DNA. In collaboration with departmental colleague John Blanchard we seek to illuminate the mechanism by which MfpA and PRP proteins in general confer antibiotic resistance by studying their structure, stability, folding and interaction with DNA gyrase.

Our interest in protein – DNA interations has evolved over the years from prokaryotic to eukaryotic gene transcription factors to proteins that play a role in oncology. We are beginning to turn our attention to proteins involved in epigenetic regulation in collaboration with Department of Genetics colleague John Greally.

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Go to **http://www.aecom.yu.edu/home/faculty/profile.asp?id=2584**, click on **'Pubmed Search'**, for an almost complete list of my laboratory's publications.

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Key Words: ***myosin, cell motility, metastasis, cell division, phosphorylation***

Cell migration, cytokinesis and the maintenance of cell morphology are fundamental force-requiring processes of eukaryotic cells. Myosin-II is an essential contractile protein and although myosin-II filament dynamics are under strict temporal and spatial control, the mechanisms controlling filament assembly and disassembly in higher eukaryotes are not known. We are specifically addressing how covalent modification (i.e., phosphorylation) and noncovalent interactions with novel regulatory proteins mediate the subcellular localization, organization and assembly of myosin-II during chemotactic motility. These studies will provide new information regarding the molecular signals regulating myosin-II organization during normal cell physiology as well as the pathologies contributing to tumor cell invasion and metastasis.

Phosphorylation of nonmuscle myosin-II on the heavy chain regulates filament assembly and is attributed to several kinases. We showed that heavy chain phosphorylation regulates the chemotactic motility of tumor cells. Moreover, genes coding for proteins that modulate the myosin-II regulatory pathway are up-regulated in invasive tumor cells. Given these findings, we are examining the intermediary signaling pathways in tumor cells that regulate heavy chain phosphorylation and the subsequent effects on motility and invasion. Our interdisciplinary approach combines biochemistry and structural biology to define the physical and chemical features underlying the regulation of myosin-II assembly, and molecular and cellular techniques coupled with fluorescence microscopy to investigate how phosphorylation regulates myosin-II dynamics in vivo.

We are also studying S100A4, a Ca^{2+}-binding protein that is directly involved in tumor metastasis and regulates tumor cell motility by promoting the monomeric, unassembled state of myosin-II. Thus S100A4 is an excellent target for investigating the mechanisms controlling the localized assembly/disassembly of myosin-II that are relevant to motility, development and metastasis. We are taking a global approach to dissecting S100A4 function; biochemical and structural approaches are being used to identify the mechanisms by which S100A4 regulates myosin-II assembly, intravital imaging studies will evaluate the impact of S100A4 expression on metastasis in live animal models; and a S100A4 knockout mouse has been developed to examine S100A4 function in normal physiology.

S100A4 is not simply a marker for metastatic disease, but rather has a direct role in metastatic progression. These observations suggest that S100A4 is an excellent target for therapeutic intervention. We developed several assays to identify small molecules that disrupt the interaction of S100A4 with myosin-IIA. Our efforts are now focused on obtaining high-resolution x-ray structures of S100A4 bound to small molecule inhibitors to identify the chemical and structural determinants involved in S100A4 inhibition, and biochemical and cell-based analyses to evaluate the selectivity and potency of lead compounds. These studies will provide the biochemical and structural foundation for the design of second generation S100A4 inhibitors.

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Key Words: ***lectins, cell surface carbohydrates, cancer, thermodynamics***

Cell surface carbohydrates have been demonstrated to be involved in a variety of biological recognition phenomena including cellular recognition and adhesion, regulation of inflammation, control of cell growth and metastasis. Although the structures of many of these carbohydrates have been elucidated, relatively little is known about their molecular recognition properties other than their interactions with glycosylases and lectins. Lectins are carbohydrate-binding proteins that are widely found in nature including in plants, animals and pathogenic organisms. Lectins and the cell surface glycans of glycoproteins and glycolipis in metazoans play important roles in cellular homeostasis and innate and adaptive immunity. Our research includes characterizing the biophysical and biochemical properties of lectins and their interactions with multivalent glycans and glycoproteins that are cellular receptors involved in signal transduction processes including cell growth, arrest and apoptosis. Techniques used to explore these interactions include nuclear magnetic resonance spectroscopy, isothermal titration microcalorimetry, x-ray crystallography and atomic force microscopy.

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Key Words: ***Sickle cell disease, sickle cell hemoglobin, red cells, polymerization, translational approach to therapy***

Sickle cell hemoglobin (HbS) forms long, rod-like, polymers when deoxygenated. These rigidify and damage red cells, producing microvascular obstruction and pathogenesis in sickle cell disease. The HbS system has structural, thermodynamic and kinetic properties in common with other long protein polymers (e.g. tubulin, flagellin, actin, tobacco mosaic virus, beta-amyloid) including helical structure, nucleation controlled kinetics, entropy and excluded volume driven reactions, and phase separation. Thus, it is interesting both for its pathogenic significance and its mechanisms.

HbS polymerization and gelation (i.e. polymer cross-linking) have been studied by physical chemical methods (e.g. light scattering, ultracentrifugation, viscometry, NMR), but these methods reflect average properties of vast numbers of fibers without observation of individual fibers as they nucleate, grow and cross-link. Electron microscopy damages gel structure and fails to reveal events in real time. Conventional microscopy does not resolve the thin (200A diameter) fibers. Using differential interference contrast (DIC) microscopy we overcome these limitations and observe individual HbS fibers in real time as they nucleate, grow and cross-link to form the final solid-like gel. These studies demonstrate two mechanisms of nucleation of new fibers, mechanisms of fiber growth and depolymerization, diverse cross-linked and branching structures and their mechanisms of formation.

Our work now has four facets: (1) We are undertaking a pilot clinical study in which we hypothesize that failure of complete polymer dissolution in the lungs exacerbates pathology and also provides a rationale for new, pulmonary assist-based, therapy. (2) We continue to examine mechanisms of the depolymerization of hemoglobin S fibers in laboratory, physical-chemical and imaging studies. (3) Having previously shown that sickle HbS fibers are very stiff, we now characterize gel viscoelasticity and the underlying gel structures and fiber interactions, the basis of red cell rigidity and a major factor in vaso-occlusion. (4) We study changes in red cell membrane viscoelasticity resulting from polymerization-induced cellular damage. Disciplines and methodologies for these studies include physical chemistry, polymer physics, imaging and structural biology, and a pilot clinical trial. (5) We also plan work on the mechanisms by which sickle trait blood inhibits pathogenesis in falciparum malaria.

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Key Words: ***Nod-like receptors, inflammation, cell death, caspase-1, bacterial toxins.***

UNDERSTANDING THE ROLE OF NOD-LIKE RECEPTORS IN MICROBIAL PATHOGENESIS

Nod-like receptors (NLRs) are intracellular surveillance receptors that play a critical role in the innate immune response. These receptors are activated by a series of molecules common to infection or cellular damage. NLR activation has been linked to a diverse array of infectious diseases including anthrax, salmonella, gram-negative shock, as well as the inflammatory diseases gout, silicosis, and infarcts. However, the underlying mechanism by which NLRs activate the immune response and also cause diseases is poorly understood, and the focus of our research. NLR inducers trigger two critical proinflammatory events: cell death and caspase-1 activation. We are using pharmacology and genetics to control these inflammatory events and to test the impact on the immune response and disease.

Role of Cell Death in Immune Responses. NRL inducers trigger a programmed form of cell death (necrosis) in antigen-presenting cells. Cytolysis of antigen-presenting cells by NLR inducers is a powerful trigger of the innate and adaptive immune response. Several groups have demonstrated that cathepsin inhibitors prevent cell death mediated by NLR inducers. Using cathepsin-deficient macrophages we identified specific cathepsins required for cytolysis mediated by specific NLR inducers. To determine the link between cytolysis of specific immune cells mediated by NLR inducers and inflammatory events Using these cathepsin-deficient macrophages and mice allows us to analyze.

Most NLR inducers have been shown to boost the immune response. It is therefore not surprising that many NLR inducers could act as adjuvants, i.e. enhance the immunogenicity of vaccines. To determine the role cell death mediated in adjuvancy, we are testing inflammatory responses mediated by select adjuvants in cathepsin-deficient mice. We are using *in vitro* and *in vivo* assays to determine whether specific cathepsins and NLR signaling are critical for the production of inflammatory cytokines, and B-cell and T-cell responses. These studies will also establish whether cytolysis mediated by NLR inducers and NLR signaling trigger a specific immune response.

Role of NLR signaling in Immune Responses. NLR inducers activate NOD-like receptors resulting in caspase-1 activation and the release of inflammatory cytokines. Using macrophages lacking specific NLRs (such as Nalp3 and Ipaf) and caspase-1/Asc we found that NLR signaling is critical for cytolysis and the release of inflammatory cytokines. In contrast to these NLR inducers, we found that particulate inducers trigger cell death independent of NLR signaling and caspase-1 activation. We are currently testing the contribution of NLR signaling and cytokine release to activation of the innate and adaptive immune response mediated by NLR inducers.

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Key Words: ***Type 1 diabetes, reactive oxygen species, hyperglycemia, diabetic complications***

Dr. Brownlee is the director of the JDRF International Center for Diabetic Complications Research Center. The overall goal of the Center is to develop innovative, effective therapies for preventing the development and progression of diabetic complications in people with Type 1 diabetes. We are a 21st century global research center, bringing together leading research groups from academic institutions on three continents who have complimentary strengths in nearly every aspect of complications research: The Albert Einstein College of Medicine in New York, the Baker Heart Research Institute in Melbourne, and the University of Heidelberg.

The JDRF International Center for Diabetic Complications Research Center has three major objectives:

(1) to determine the primary mechanisms by which hyperglycemia causes both the microvascular and the macrovascular complications of Type 1 diabetes: nephropathy, retinopathy, neuropathy and atherosclerosis

(2) to elucidate the molecular basis for hyperglycemic memory; and

(3) to develop effective therapies for preventing the development and progression of diabetic complications.
The unifying theme of the research is the central role of reactive oxygen species in the activation of pathways mediating hyperglycemic damage, in the transduction of hyperglycemia-induced signals, and in the maintenance of continued post-hyperglycemic damage.

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Key Words: ***nervous system development, extracelluar matrix, C. elegans, heparan sulfate, genetic disease, Kallmann Syndrome***

Genetics of Nervous System Development

My lab uses the small nematode *C. elegans* with its simple and well characterized nervous system as a genetic model. We are trying to understand how growing axons navigate the extracellular space in order to connect to their appropriate partners. The extracellular space is filled with a complex mixture of proteins and proteoglycans e.g. heparan sulfate (HS) proteoglycans which are a particular focus of the lab. We are asking how specific modification patterns of the polysaccharide HS determine the path of developing axons.

We have shown that distinct modification patterns in HS serve specific and instructive functions during neural development leading us to formulate the 'HS code' hypothesis. We propose that defined combinations of modifications in the sugars of HS contain information and generate a molecular map that helps shaping the nervous system. Our goal is to decipher the information contained in HS, determine the factors that create and modulate it and describe the genes that respond to it.

In a related project we are investigating a pathological dimension of HS by studying Kallmann Syndrome, a human genetic disease with specific neurological defects. Using *C. elegans* as a model, we have shown that *kal-1*, the nematode orthologue of the gene mutated in human Kallmann patients, has a role in axon branching and requires HS with specific modifications for these functions. Our goal here is to understand how KAL-1 functions on a molecular level during disease and development. We approach this by conducting genetic screens to identify novel genes that interact with *kal-1*.

In summary, our studies are directed towards an understanding of how heparan sulfate and its modifications (the 'HS code') functions during development and disease of the nervous system.

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Key Words: ***Connexins, De-novo formation and gating of gap junction channels, cell-cell coupling, pathophysiology of diseases related to connexin mutations***

Our major goal is to study *de-novo* formation, gating and regulation of gap junction (GJ) channels and unapposed/nonjunctional hemichannels formed by connexin (Cx) proteins. GJ channels mediate direct cell-cell exchange of cytosolic ions and molecules. By combining electrophysiological, imaging and computational modeling methods, we examine electrical cell-cell coupling, intracellular pH, Ca^{2+} concentration and metabolic cell-cell communication in living cells that express different wild-type Cxs, their mutants and Cxs fused with color variants of green fluorescent protein. We demonstrated that there are two distinct gating mechanisms in GJs, fast and slow/loop, named so because of differences in the kinetics of gating transitions. The fast gate, which is sensitive to transjunctional voltage (V_j), closes the GJ channel to a residual state, which serves as a selective filter that preserves electrical cell-cell signaling but restricts metabolic communication. The slow gate closes GJ channels fully and is sensitive to V_j as well as chemical factors. We propose that the clustering of GJ channels into junctional plaques (JPs) is central to their ability to function. We are testing the hypothesis that JP formation starts with the aggregation of hemichannels into hemichannel plaques (HPs), followed by the superposition of HPs from apposing cells, docking of apposing hemichannels and channel pore opening. We reported that only ~1-15 % of GJ channels that assemble JPs are functional. We also examine conditions under which heterotypic junctions, formed between cells expressing different Cx isoforms, exhibit nearly unidirectional electrical signaling and may function as rectifying electrical synapses. Furthermore, we found that the flux (J_j) of metabolites through Cx43/Cx45 GJs and other heterotypic junctions is affected by ionophoresis and transjunctional voltage (V_j) and that the J_j-V_j dependence is asymmetric. This asymmetry is modulated by differences in the resting potentials of the communicating cells and bursts of high frequency action potentials resembling those that occur during neuronal activity or cardiac tachyarrhythmia. This modulation of intercellular signalling can play a crucial role in many aspects of intercellular communication in excitable and non-excitable tissues. Finally, we study the role of Cxs in the development and cell-to-cell spread of apoptosis and the Cx mutants related to deafness, oculodentodigital dysplasia, X-linked Charcot-Marie-Tooth disease, cardiac arrhythmia and other hereditary diseases.

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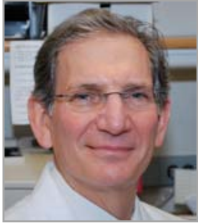

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Key Words: ***Human papillomavirus pathogenesis, methylation & evolution, microbiome evolution & disease, complex genetics (prostate cancer; excessive sweating), VHL***

The main focus of the Burk laboratory is to understanding how evolution of papillomaviruses has created types that are highly pathogenic and cause cervix neoplasia and other cancers in man. These investigations extend from the clinical/epidemiological (i.e., molecular epidemiology), basic molecular virology and molecular evolution focusing on primate papillomaviruses. The papillomaviruses are 8.0 kb double stranded DNA viruses readily amenable to cloning and sequencing, making this system ideal as a model for DNA virus evolution and ecology. Over 150 HPV types exist, further characterization of HPVs infecting the population (i.e., from our large sample repository) have allowed us to explore the virus as a species, the role of viral evolution in the genesis of oncogenic types, and characterization of the frequency and heterogeneity of HPV types and variants in the population. The lab uses phylogenic methods and other analytic strategies to test hypotheses about the relationship and characteristics of the large family of papillomaviruses. Many of these experiments are done in collaboration with investigators (Dr. Rob DeSalle) at the American Museum of Natural History and the National Cancer Institute (Dr. Mark Schiffman). Exploration of natural selection of papillomaviruses has led us to the conclusion that the viruses are evolving through complex means yet to be discovered. The evolutionary studies are also driving experimental studies. The biological activities of specific viral variants with increased oncogenic potential are under investigation using in vitro model systems. In particular, we are examining the activities of HPV E6 and E7 in cells. We are interested in identifying the activity associated with cancer vs. evolutionary developed phenotypes. In addition, we have recently developed a primate model for PV and cervix neoplasia in collaboration of Wake Forest Medical Center.

Most recently, we have started asking questions about the complex ecological community of bacteria, fungi, viruses and other organisms within the reproductive tract of women and how that might act as a cofactor for disease outcomes. These studies employ next-gen sequencing and state of the art bioinformatics. Other interests of the lab include the genetics of complex human disease (prostate cancer, excessive sweating) using large case-control clinical collections of genomic DNA with candidate gene sequencing; and, the cell biology of the primary cilia in renal cancer and other disorders.

The lab is dedicated to creating a warm environment based on collaborative science and teamwork. Our mission is to facilitate each individual reaching his or her potential through learning, experimentation and sharing in pursuit of knowledge to advance the human condition.

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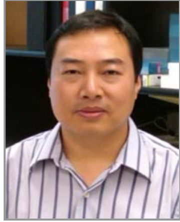

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***Key Words:* Central Nervous System, Obesity, Diabetes, Metabolism, Diseases**

CENTRAL NERVOUS SYSTEM CONTROL OF OBESITY, DIABETES AND METABOLIC DISEASES

Obesity and diabetes represent two of the greatest epidemics and public health problems facing the nation. The major research question in our laboratory is how inflammatory pathways could mediate the central nervous system particularly hypothalamic dys-regulation of systemic energy balance. To address this question, first, we want to profile metabolic challenge-induced inflammation within neural and neuroendocrinal systems, the connections with neural and neuroendocrinal pathways, and the contributions to obesity and diabetes. Second, we plan to identify the intrinsic molecular systems that normally counteract metabolic inflammation and to explore why and how the systems are weakened under nutritional oversupply. In addition, we will take steps from our mechanistic studies to develop cell therapy, aiming to extinguish cellular and molecular inflammation associated with over-nutrition and thereby reverse metabolic dysfunctions in order to control and prevent the spread of both diseases.

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Key Words: ***Protein, structure/function, dynamics, enzymes, protein folding***

The Structures and Dynamics of Proteins. Our work is centered on studying the structural and dynamical properties of proteins in order to understand the molecular mechanisms of protein function. We have developed new spectroscopic methods to obtain the vibrational spectra of specific protein groups and/or bound ligands, even within large proteins. With these techniques, it is possible to determine bond lengths with an accuracy of better than 0.01 Å. We also have developed techniques to monitor atomic motion in proteins on multiple time scales, as fast as picoseconds and out to minutes.

Structure and Dynamics of Enzymatic Catalysis. The primary problem of the lab is to understand the dynamics of enzymatic catalysis at a molecular level. This involves measurement of (1) static structures of enzymes complexes with their ligands and (2) how atomic motion evolves during the catalytic event. Structure is probed with vibrational spectroscopic tools that are capable of determining the Raman and IR spectra of bound substrates and specific protein molecular moieties. Vibrational spectroscopy yields a very high resolution of structure (better than 0.01 Å), and changes on this order are key to understanding enzymatic catalysis. Modern paradigms for enzymatic catalysis all include atomic motion of the catalyst and reactants, either implicitly or explicitly. Binding of a substrate to form the Michaelis complex involves motions: formation of encounter complex(es), movement of the substrate towards the enzyme active site, desolvation of substrate, and often loop or flap closure or domain motion. Once the Michaelis complex is formed, movement of atoms and groups at the binding site occur to bring about the proper catalyzed chemistry and achieve these catalytic states with the incredible rate enhancements approaching 10^{18} relative to uncatalyzed reactions. We have recently developed kinetic approaches that can measure molecular motions in proteins on fast time scales (down to 10 ps), here-to-fore inaccessible to measurement, based on initiating chemical and structural changes via a laser induced temperature jump. Measurements of the evolving structure is probed using optical and vibrational spectroscopies.

The Dynamics of Protein Folding. We also wish to understand how proteins arrive at their three dimensional structure (the protein folding problem). A number of studies are underway to understand the thermodynamics of folding. In addition, the crucial kinetic events of protein folding occur faster than the conventional millisecond time scale of stopped-flow mixing techniques. These early kinetic events in the folding process are being studied using new techniques that can initiate chemistry (like the folding of a protein) on the 10 picosecond time scale and monitor the evolving structural changes using advanced spectroscopic approaches.

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Key Words: ***transport, membrane proteins, sodium/iodide symporter (NIS), cancer radiotherapy***

The main research topic in my laboratory is the study of transport membrane proteins. A central focus has been the Na^+/I^- symporter (NIS), a key membrane transport protein that mediates the active translocation of iodide (I^-) in the thyroid and other tissues such as salivary glands, gastric mucosa, and lactating mammary gland. We are carrying out the detailed characterization of NIS and investigating its medical applications. I^- uptake is the first step in the biosynthesis of the thyroid hormones T_3 and T_4. Thus, NIS plays a crucial role in thyroid hormonogenesis and in the evaluation, diagnosis, and treatment of thyroid disease. For example, NIS-mediated radioiodide therapy is highly effective in the treatment of thyroid cancer metastases. The cDNA clone that encodes NIS was isolated in my laboratory by expression cloning in *Xenopus laevis* oocytes (1). We have also carried out a thorough electrophysiological analysis of NIS activity in oocytes, uncovering an *electrogenic* 2:1 Na^+/I^- transport stoichiometry. We are investigating the molecular mechanism that underlies congenital iodide transport defect (ITD). This is an autosomal recessive congenital condition caused by NIS mutations and characterized (when untreated) by hypothyroidism, goiter, low thyroid I^- uptake, low saliva/plasma I^- ratio, and mental retardation. The loss of NIS function leads to profound physical, physiological, and behavioral changes in patients. Our molecular analysis of ITD-causing NIS mutations has provided significant structure/function information about the symporter, including the finding that the β-carbon hydroxyl at residue 354 [located in transmembrane segment (TMS) IX] is essential for NIS function and that several other residues in the same TMS are involved in the Na^+ translocation pathway (2). We have extensively studied the expression and regulation of NIS in lactating breast. Over 80% of the human breast cancer samples we studied expressed NIS, whereas we found no expression in normal samples. Moreover, we have demonstrated that NIS is *functionally expressed* in human breast cancer metastases. These findings suggest that NIS-mediated radioiodide accumulation may be as effective a tool in human breast cancer as it is in thyroid cancer (3). We have demonstrated *in vitro* and *in vivo* that NIS actively transports the environmental pollutant perchlorate (ClO_4^-), including translocating it to the milk and, strikingly, that the Na^+/ClO_4^- transport stoichiometry is *electroneutral*, revealing that NIS translocates *different substrates* with *different stoichiometries*. That NIS actively concentrates ClO_4^- in the milk suggests that ClO_4^- poses a higher environmental health risk than previously believed, as ClO_4^- would thus directly inhibit the newborn's thyroidal I^- uptake (4).

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Key Words: ***synaptic transmission, plasticity, glutamate receptors, GABA receptors, neurotoxicity, CaMKII, memory***

Our lab studies the molecular mechanisms regulating synaptic transmission in the central nervous system. Specifically we are interested in understanding how changes in neuronal activity can cause the lasting modifications in the strength of synaptic connections that are believed to be important for neuronal development, learning, and memory formation. In order to develop a complete picture of the events involved in such synaptic plasticity, the lab employs a combination of techniques including whole cell electrophysiology, microscopy, biochemistry and molecular biology.

The focus of our efforts is on investigating how rearrangements in the localization of key signaling components, particularly neurotransmitter receptors, may regulate synaptic strength. Our previous results have shown that the induction of one form of synaptic plasticity, long-term depression, is mediated by a rapid internalization of synaptic AMPA-type glutamate receptors, the primary mediators of fast excitatory chemical transmission in the CNS. We continue to examine in depth the mechanisms that modulate AMPA receptor localization at the synapses with an interest in understanding how these play a role in synaptogenesis, plasticity, and neurotoxicity in the brain.

We are additionally investigating how activity at excitatory synapses modulates the trafficking of $GABA_A$-type receptors at physically separated inhibitory synapses. Our studies aim to understand the means by which crosstalk between excitatory and inhibitory synapses can control excitability in the brain. CaMKII appears to plays an important role in this process, as we find this protein can be selectively translocated to and potentiate either excitatory or inhibitory synapses in response to different conditions of excitatory neuronal activation. Ongoing studies seek to understand how the regulation and targeting of this kinase to GABAergic synapses contributes to modulating inhibition under physiological as well as pathophysiological conditions, such as seizures.

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Key Words: ***fungus, antibody response, virulence genes, clinical trials***

The Casadevall laboratory has research projects on broad fields dealing from basic immunology to fungal pathogenesis to the development of antibody-based therapies for infectious disease and melanoma. We work with two organisms: Cryptococcus neoformans and Bacillus anthracis.

The general themes of our research work are listed below.

Mechanisms of fungal pathogenesis: We study several different aspects of *C. neoformans* pathogenesis. In their own unique way, each project advances our knowledge of how *C. neoformans* causes disease and how innate and humoral immunity influences the outcome of infection: these include intracellular replication, exocytosis, and the interaction of virulence factors with the host.

Capsule function: The capsule of *C. neoformans* is one of the main virulence factors of this pathogenic fungus. A main feature of the capsule is that it can dramatically change its size depending on the environment. We are interested in the process of capsule growth, and the influence that such growth has on the interaction with the host.

Melanin structure and function: Melanin production in *C. neoformans*, is associated with virulence. Melanin is a pigment with an undefined chemical structure and tremendous physical stability. This pigment accumulates in the cell wall of *C. neoformans*. We are interested in understanding the fundamental biological process of how melanin in the cell wall is remodeled to allow growth and budding to occur. As an example of the wide reach of melanin research, an antibody to fungal melanin made in our laboratory is currently in evaluation for the treatment of melanoma. In collaboration with Dr. Dadachova we have shown that melanin may be involved in energy transduction mechanisms by which microbes may obtain biologically useful energy from electromagnetic radiation.

Relationship between antibody structure and function: This area of research focuses on understanding the relationship of antibody structure and its biological function. To answer such questions the generation, characterization and molecular analysis of monoclonal antibodies against antigens of *C. neoformans* and *Bacillus anthracis* are necessary. In addition, we are also investigating epitope specificity and idiotypic reactivity of antibodies.

Origin of virulence in pathogenic fungi: The mechanism of acquiring and maintaining virulence by *C. neoformans* is unknown. The evolution of virulence traits is being studied in three different systems. The interactions of *C. neoformans* with the free-living soil amoeba *Acanthamoebae castellanii* and the slime mold *Dictyostelium discoideum* are being characterized. These interactions help us to understand the environmental survival strategy of *C. neoformans* and relate to the emergence of fungal virulence for humans. In addition, mouse-passaged *C. neoformans* strains are also being used to understand how this pathogenic yeast evolves virulence in mammals.

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Key Words: ***synapses, excitatory and inhibitory synaptic transmission, synaptic plasticity, endogenous cannabinoids, retrograde signaling***

Synaptic transmission underlies nearly every aspect of nervous system function and is relevant to most neuropsychiatric conditions. Indeed, how we think, feel, act and learn all rely on information transfer between nerve cells. Several decades of research continue to show that activity-dependent changes in synaptic efficacy are critical to experience- dependent modification of neural function. In our laboratory we study how synapses modify their efficacy, and the functional impact of such changes in a neural network. One of our main goals is to elucidate the specific molecular events that underlie various forms of short-term and long-term plasticity at excitatory and inhibitory synapses. Some of the current projects in our lab are:

Endocannabinoid-mediated synaptic plasticity. The mechanisms underlying changes in synaptic efficacy are more diverse than previously expected. Recently, a new type of use-dependent synaptic plasticity that requires retrograde signaling by endogenous cannabinoids (endocannabinoids) has been identified in several brain structures. Endocannabinoids are produced "on-demand" from neurons, cross the synaptic cleft, and by activating presynaptic cannabinoid receptors, suppress transmitter release in a transient or long-term manner. The mechanisms and physiological consequences of endocannabinoid-mediated synaptic plasticity are not fully understood. We are currently investigating the molecular basis, induction rules, and functional impact of endocannabinoid-mediated synaptic plasticity. Brain areas of interest where we study such form of plasticity include the hippocampus, cerebellum, amygdala and prefrontal cortex.

Activity-Dependent Synaptic Plasticity Expressed by NMDA Receptors. Although most excitatory synapses express both AMPA and NMDA subtypes of glutamate receptors, because excitatory neurotransmission is dominated by AMPARs, excitatory efficacy is commonly associated with the amplitude of AMPAR-mediated synaptic responses. As a result, most studies of the molecular and cellular bases of LTP/LTD have largely focused on AMPAR-mediated neurotransmission and plasticity, while NMDARs are recognized mainly for their role as the most important known trigger for the induction of AMPAR LTP/LTD. Growing evidence now suggests that NMDARs can also express LTP/LTD at several CNS synapses. It is also increasingly recognized that NMDARs themselves contribute significantly to information transfer at synapses particularly during periods of repetitive activity and may also participate in the generation of persistent activity in neural assemblies. Using acute hippocampal slices we are investigating the molecular basis and functional contribution of synaptic plasticity expressed by NMDARs.

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Key Words: ***tuberculosis, reactivation, latency, granuloma, B cells, tumor necrosis factors, vaccine, , mouse models of disease***

We are studying the pathogenesis and host defense mechanisms involved in diseases caused by *M. tuberculosis*. Specifically, we are interested in understanding: i) how *M. tuberculosis* establishes latency and how quiescent infection reactivates. This investigation focuses on the dormancy regulon that is regulated by environmental signals such as nitric oxide and hypoxia. ; ii) the tuberculous granulomatous response, which plays an important role in host defense against *M. tuberculosis*. In particular, we are examining how TNF and B cells, two major components of the granuloma, influence the host immune response; and iii) Mechanisms by which the immunogenicity of mycobacteria can be augmented. To this end, we are targeting B cell immunity and TNF modulating attributes of mycobacterial components for the development of effective anti-tuberculosis vaccines. .

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Key Words: ***Virology, anti-viral therapy, Ebola virus, Marburg virus, viral entry***

All viruses must cross a host cellular membrane to enter the cytoplasm, where the raw materials and machinery necessary for viral multiplication are present. Strategies for antiviral therapy that block cell entry are especially desirable because they provide an opportunity to interdict viral replication even before the virus can commandeer the host cell's resources. However, no successful strategies of this type are currently available for emerging viral pathogens, in part because details of the entry-related virus-host interactions and viral protein transformations remain obscure for these agents.

We are currently focused upon uncovering the mechanisms by which the highly pathogenic Ebola and Marburg filoviruses exploit their host cells to gain entry into the cytoplasm. Our goals are to:

Determine how endosomal cysteine proteases mediate filovirus entry
We have found that specific endosomal cysteine proteases are essential host factors for filovirus entry and novel targets for development of antiviral drugs. Our initial experiments to characterize the role of these enzymes indicate that they act by carrying out extensive proteolytic remodeling of the viral glycoprotein, GP, and that a multistep proteolytic cascade targeting GP within the host cell endocytic pathway is a critical feature of the filovirus entry mechanism. This proposed role for endosomal cysteine proteases is unprecedented for enveloped viruses, which are generally considered to be in a "race" to avoid inactivation by these enzymes during entry. We are using a combination of biochemical, molecular genetic, chemical genetic, and cell biological approaches to test our working hypothesis, which is that filoviruses employ a novel principle, proteolysis, to trigger the entry-related activities of their glycoproteins.

Identify additional host factors and pathways required for filovirus entry
We are carrying out unbiased chemical and genetic screens to identify additional host factors that are necessary for filovirus entry. We are also developing cell-based assays for steps in entry, including binding, membrane fusion, and cytoplasmic delivery of viral nucleocapsids to understand how newly-identified interactions between virus and host contribute to the filovirus entry pathway. Host factors discovered in these experiments will become potential targets for the development of anti-filovirus therapies.

Exploit a new "forward genetic" strategy we have established to select filovirus entry mutants in a biosafety level 2 setting.
While we extensively use pseudotype viruses bearing filovirus GP for entry studies, these agents do not themselves encode GP, and therefore, they do not permit "forward genetic" studies to select mutant forms of the glycoprotein. Indeed, the only systems available for such studies of filovirus entry utilize viruses that must be handled in a biosafety level 4 laboratory. To overcome this limitation, we have established a recombinant virus-based forward genetic system that faithfully mimics the authentic viral entry process and is safe to use. We are utilizing these reagents to dissect the sequence determinants of host factor use, for example, by selecting mutants by passage in inhibitor-treated cells. This technology is opening up new opportunities to examine the functions of GP during filovirus entry.

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Key Words: ***Epigenetics, glucose transporters, glucagon receptor, diabetes, obesity***

MECHANISMS OF DIABETES AND OBESITY PATHOGENESIS

The global epidemics of Type 2 Diabetes Mellitus (T2DM), obesity and the Metabolic Syndrome cannot be explained simply by genetics and/or current lifestyles. Data suggests these adult diseases have their origin in the intrauterine (IU) and early postnatal environment. Using mouse models we have shown that exposure to a maternal high fat diet predisposes offspring to future development of these metabolic diseases. Combined these data strongly suggest epigenetic alterations of the fetal genome are the cause of increased incidence of disease in adults. A systems biology approach is currently underway to address this hypothesis. Specifically, genome wide changes in gene expression, DNA methylation and histone modifications are being characterized in livers at late gestation (e18.5), post-weaning (5 wks) and at 6 mos of age in offspring exposed to maternal high fat diet. Changes in DNA methylation and histone modifications have been identified that are associated with altered gene expression in offspring exposed to a maternal high fat diet. Future studies that incorporate ChIP-Seq and genome wide HELP assays will be used to define the epigenetic basis underlying IU exposure to a maternal high fat diet. We are also interested in identifying the components of the maternal diet that are linked to a poor metabolic phenotype later in the life of the offspring. By varying dietary fat content and supplementing the maternal diet, we hope to alter the disease susceptibility of offspring.

Additional studies are focused on two members of the glucose transporter gene family (GLUT4 and GLUTx1/GLUT8). GLUT4 is insulin and exercise responsive and is the major glucose transporter expressed in cardiac and skeletal muscle and adipose tissue. GLUTx1/GLUT8 is a newly identified member of the glucose transporter gene family that is expressed in many tissues. By using transgenic and gene knockout mouse models we study the role of GLUT4 and GLUT8 in whole body and organ specific glucose utilization in normal and disease states (e.g. diabetes mellitus and obesity). GLUT8 is upregulated in several tissues in response to GLUT4 ablation and may represent a novel anti-diabetic target. We are also studying the potential role of GLUT8 in tumor cell growth and metabolism that may lead to novel chemotherapeutic modalities.

We also study the glucagon receptor. Glucagon elevates serum glucose levels. We are studying the role of glucagon action in the pathophysiology of T2DM using mouse models. Novel insight gained from these studies indicates a role for glucagon in normal development of pancreatic islets and in hepatic substrate utilization, energetics and survival. Our gene knockout studies provided proof of concept that inhibition of glucagon action can lower fasting glycemia and may be useful in treating hepatic insulin resistance in diabetics.

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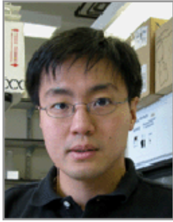

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Key Words: ***obesity, neuropathy, signal transduction, gene regulation, trafficking,***

The long term goal of this research program is to understand the molecular basis of cellular machinery and their relationships to human diseases. A branch of the laboratory focuses on elucidating the interplays between transcription factors and signaling pathways. We ask how does extracellular stimuli communicate with intracellular effectors, and eventually modulate cellular responses in normal and diseased states. For example, we have recently demonstrated that adipokine gene expression mediated by transcription factor NFAT contributes to insulin and glucose homeostasis via a non-cell autonomous mechanism. We have further found that upstream regulators and downstream effectors of the NFAT transcription factor impinge on adipocyte biology at multiple levels. Another branch of the laboratory examines intercellular trafficking and its role in demyelinating neuropathy. A range of biochemistry, molecular biology and cell biology are applied, in conjunction with primary cell culture engineered mice and *C. elegans* genetics, to dissect the molecular basis of cellular machinery. In sum, I strongly believe that elucidating the basis of molecular pathways will provide the most benefits in understanding human diseases.

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Key Words: Obesity, diabetes, leptin, hyperglycemia

Our group is interested in contribution of genetic variation to the chronic diseases of obesity, diabetes, and diabetes complications. We would like to develop an understanding of the various functions of leptin responsive neuronal networks that regulate feeding, body composition, metabolism, and reproduction. Using cell type specific manipulation of gene expression, we have analyzed the roles of POMC, AGRP/NPY, and SF1 neurons within the hypothalamus. We are also characterizing the role of melanocortins in regulating pubertal development and reproductive function. A second interest of our group is to dissect the genetic contribution to the susceptibility/resistance to complications of long term hyperglycemia, particularly effects on the endocrine pancreas and the kidney.

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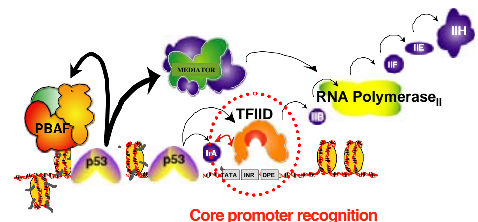
Key Words: ***Eukaryotic transcription, Single Molecule Imaging, p53, mHtt, caner, aging***

Single Molecule Imaging Of Transcription Factors Involved In Cancer And Ageing
The focus of our laboratory is to develop single molecule systems to understand the molecular mechanistic basis of actions of disease driven transcription factors on their respective target genes. Specifically, we are using a powerful combination of single molecule fluorescence, biochemistry, proteomics and molecular biology to understand how these key factors dynamically discriminate their various targets in vitro and also within the complicated milieu of the cell.

Gene expression initiated by mammalian RNA polymerase II (Pol II) involves a highly coordinated assembly of over 100 different polypeptides residing within several multi-subunit complexes to form the pre-initiation complex (PIC). One critical step in gene activation involves direction of the core recognition complexes to specific target promoters. The multi-subunit TFIID complex is a principle component within the transcriptional machinery capable of recognizing and targeting specific promoter DNA. Current models suggest that activators, such as the tumor suppressor p53 protein and the onco-protein c-Jun, can stimulate transcription by targeting factors, such as TFIID, TFIIB, TFIIF, Mediator, and Pol II.

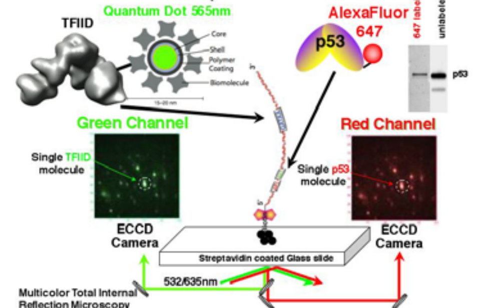
Imaging the dynamic assembly of the transcription PIC in real time
Single molecule-fluorescence has become an advanced and sensitive tool to study protein dynamics of individual molecules in a population in real time. To gain a better mechanistic understanding of how the p53 and c-Jun activators dynamically regulate transcription pre-initiation complex (PIC) formation at the single molecule level, we developed a system to specifically label multisubunit human transcription complexes with Quantum dots to image their real-time assembly on promoter DNA. **T**otal **I**nternal **R**eflection **M**icroscopy is used to examine the recruitment of single quantum dot labeled TFIID and AlexaFluor labeled p53 molecules at the promoter DNA.

Pre-initiation complex (PIC) formation is a multi-step process stimulated by transcriptional activators



Time resolved studies revealed that p53 helps recruit TFIID to the promoter DNA, in addition to affecting a step in the transcription cycle after TFIID has arrived at the promoter DNA. Current efforts are focused on examining the role of p53 in transcriptional elongation by Pol II.

Analyzing the co-localization of multiple fluorescent factors On promoter DNA



Huntingtin's role in modulating the transcription machinery
Huntington's disease pathogenesis involves the in vivo sequestration of various transcription factors by a mutant Huntingtin (mHtt) protein containing polyglutamine expansions. Recent studies have shown that mHtt associates with p53, TFIID and TFIIF to both stimulate and repress transcription at different target promoters thereby disrupting normal cellular function. In order to better understand how mHtt impacts transcription and reprograms gene networks, we are using our single molecule and bulk assays to determine which steps in the transcription cycle are affected on various known in vivo target promoters. In addition, through a strong collaboration with the Liu laboratory here at Einstein we will use high resolution cryo-EM to obtain 3D structures of mHtt bound to p53 and TFIID.

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Key Words*:** ***Cancer, invasion, metastasis, cell motility, imaging

CELL MOTILITY, CHEMOTAXIS AND CANCER

Metastasis represents the major cause of mortality in cancer patients. The unique ability to breach the basement membrane of epithelial barriers and migrate is believed to distinguish non-metastatic from metastatic tumor cells. According to the three-step hypothesis of metastasis, adhesion, proteolysis, and motility are the keys steps involved at the cellular level in the traversal of basement membrane barriers. Blood vessels are crucial in metastasis because they supply the necessary nutrients for tumor growth and, in particular, the mechanism for dissemination of the metastatic tumor cells once the blood vessel wall has been crossed, a process called intravasation. We have shown that macrophages, in particular, are necessary for enhancing the motility of tumor cells, proteolysis, and intravasation in the primary tumor. The tumor cells themselves contribute to invasion and intravasation by responding to signals from macrophages by exhibiting amoeboid chemotaxis and the production of invadopodia, invasive protrusions capable of degrading extracellular matrix. This is a problem because normal cells also exhibit these abilities during cellular mediated immunity, wound healing and embryogenesis. This makes the development of drugs specific for the inhibition of only tumor cell chemotaxis and intravasation difficult to design. The Condeelis lab is focused, in particular, on defining the signaling pathways from tyrosine-kinase receptors to the actin cytoskeleton in carcinoma cells and the molecular markers that can detect these activities in vivo. Several of the markers discovered in the Condeelis lab are now in clinical trials to evaluate their usefulness in the treatment of breast cancer metastasis.

Students can select projects which involve (1) the preparation of animal models of breast cancer that allow interactions between tumor cells and macrophages to be measured in vivo (2) expression and domain analysis of key proteins in the EGF-signaling pathway to the actin cytoskeleton (3) preparation of caged molecules that can be used to test strategies for drug design to prevent tumor cell chemotaxis (4) imaging-based phenotype analysis of tumor cells in vitro and in vivo that have been genetically or drug manipulated to measure the effects of the manipulation on chemotaxis and metastasis; (5) the development of novel imaging technologies for detecting kinase and protein function in vivo. Imaging is performed in the Analytical Imaging Facility and the Innovation Lab of the Gruss Lipper Biophotonics Center of which Dr. Condeelis is the scientific director. The two types of organisms used in these studies are transgenic mouse models of cancer, and cell lines from human and rat/mouse tumors. Both laser confocal and multiphoton imaging are used with live animals.

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Key Words: ***chemotaxis, phagocytosis, macrophages, pathogenesis, intracellular infection, tumor progression***

Macrophages play an important role in host defense against invading micro-organisms and they are also key players in initiating and maintaining an immune response. However, macrophages can also play negative roles, such as in chronic inflammatory disease. Macrophages are therefore a prime target for therapies, but it is important to elucidate the mechanisms by which they are recruited to and activated in tissues.

Phagocytosis via receptors for the Fc portion of IgG (FcγRs) or for complement (CR3) requires actin assembly, pseudopod extension, and phagosome closure. Several human pathogens, such as Cryptococcus neoformans, and Legionella pneumophila and are internalized by macrophages, escape destruction and grow and divide inside the macrophage. The phagocytic processes by which these organisms are internalized are currently unknown. We are currently dissecting the downstream signaling pathways that mediate these processes during phagocytosis.

The directed movement of cells in response to chemoattractants involves several complex, interrelated processes, including directional or chemotactic sensing, polarity, and motility. These processes are mediated by complex, interacting signaling pathways that appear to have many similarities but yet have distinct characteristics depending on the chemoattractant and receptor. We are currently dissecting the signaling pathways required for macrophage chemotaxis towards; 1) CSF-1, a growth factor for macrophage survival and differentiation produced by many tumors and found in high concentrations in arthritic joints and 2) Chemokines that direct monocyte recruitment to different tissues. Understanding the specific signaling components required for migration towards each of these factors will allow us to test the importance of these signaling components in vivo.

It is now increasingly recognized that the tissue microenvironment plays a critical role in tumor progression, but most studies on tumor metastasis involve the use of endpoint assays or in vitro studies on cell lines. Also, tumor-associated macrophages (TAMs), which are present in large numbers in many tumors, appear to play an important role in promoting the progression of solid tumors to an invasive, metastatic phenotype. In vitro we will use a reconstituted paracrine assay between macrophage and carcinoma cells and in vivo interactions of macrophages and tumor cells in the tumor microenvironment will be examined by intravital imaging using multiphoton microscopy. Also, the ability of macrophages, altered in pathways identified in vitro, to migrate into the tissue space will be tested in animal models of peritoneal infection and atherosclerosis.

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***Key Words**: Lysosomes, autophagy, aging, protein degradation, neurodegeneration*

The **main focus** of our laboratory is on understanding how proteins are transported into lysosomes for their degradation and how malfunctioning of the lysosomal system contributes to aging and age-related disorders. A common feature of senescent cells is the accumulation of abnormal or damaged proteins in their cytosol that, undoubtedly, impairs cellular function. Protein accumulation results, at least in part, from impaired protein degradation with age. Among the different systems that participate in the intracellular degradation of proteins, lysosomes are the most affected by age. We have previously identified in many tissues of aged animals a decrease with age in the activity of a selective pathway for the degradation of cytosolic proteins in lysosomes known as chaperone-mediated autophagy. The **main goal of our research** is to identify the defect(s) that lead to the decreased activity of autophagy with age, and to analyze if the correction of those defects and recovery of normal proteolytic activity in old cells leads to an improvement in cellular function.

Chaperone-mediated autophagy is mainly activated under conditions of stress, such as nutrient deprivation or exposure to pro-oxidants. Under those conditions, substrate proteins are selectively recognized by cytosolic chaperones (hsc70 and cochaperones) that stimulate their binding to a receptor in the lysosomal membrane (LAMP-2A). The transport of the cytosolic proteins into lysosomes for their degradation requires also the presence of another chaperone in the lysosomal matrix (lys-hsc70).

Our efforts are currently directed to the:

1. Characterization of the different components involved in chaperone-mediated autophagy and identification of new players for this pathway.- We use in vitro systems with isolate lysosomes from several tissues (liver, kidney and spleen) of mice and rats to reconstitute each step involved in the degradation of substrate proteins: binding to the lysosomal membrane, uptake into the lysosome, and complete degradation once at the lysosomal matrix. For the identification of the new components of the systems we are currently using different immunochemical approaches and proteomic approaches. We have also initiate genetic (RNAi) and chemical screenings for the identification of modulators of CMA. Lastly, we are investigating changes in lipid composition on the dynamics of lysosomal components.

2. Correction of the age-related defect in chaperone-mediated autophagy. We have found that binding of substrates to the lysosomal membrane decreases dramatically with age due to a decrease in the levels of the receptor protein in the lysosomal membrane. We have generated bitransgenic mouse models in which the levels of the lysosomal receptor can be regulated by administration of tetracycline, a common antibiotic, in different tissues. We have shown that restoration of normal CMA in liver results in removal of damaged proteins, improved response to stress and preservation of normal liver function. These exciting findings have motivated us to attempt to restore CMA in other organs such as brain and to explore the effect of upregulation of CMA in the course of detrimental neurodegenerative disorders as Parkinson's disease.

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Key Words: ***eye, development, embryonic stem cells, transcription factors, signaling, chromatin remodeling, crystallin***

GENETIC AND EPIGENETIC REGULATORY MECHANISMS IN MAMMALIAN EYE DEVELOPMENT & OCULAR DISEASES

We are studying molecular mechanism of temporal and spatial regulation of expression of tissue-specific genes during mammalian development. We use an integrative approach to identify and characterize specific DNA-binding transcription factors and their co-activators interacting with proximal and distal regulatory regions of genes whose expression is coordinately regulated during development. We also study the dynamics of covalent modifications of core histones associated with these genes. Finally, we are interested in the developmental roles of ATP-dependent chromatin remodeling enzymes Brg1 and Snf2h and histone acetyltransferases CBP and p300.

Our model system is the ocular lens. Because of its unique morphology, lens is an advantageous tissue to study molecular mechanisms of embryonic induction, cellular differentiation, intercellular signaling and aging. Lens progenitor cells are formed as a result of multiple signals exchanged between the head surface ectoderm surrounding the anterior neural plate, the pre-placodal region, and lateral mesoderm. Lens precursor cells and terminally differentiated lens cells are marked by the expression of crystallin genes. Lens development as well as crystallin gene expression are regulated by a sparse number of genes encoding transcription factors such as Pax6, large Mafs, Prox1, Six3, Sox1, Sox2 and Hsf4 expressed in the lens. These genes act in concert with various signal transduction pathways notably FGFs, BMPs/TGF-b and Wnts. These genes either control the entire process of eye development (Pax6) or its specific stages. Mutations in these genes are responsible for a wide spectrum of human congenital eye diseases (aniridia, early onset cataract, and glaucoma) affecting both the anterior segment (cornea, lens, iris, and trabecular meshwork) and retina. Our primary focus is on the genetic network regulated by Pax6. *Pax6/eyeless* is considered a "master" gene for eye development. Thus, studies of Pax6's function are important for understanding of eye development and evolution of visual systems.

Finally, we recently developed a procedure to generate large quantities of lens progenitor cells from human ES cells. This finding allows us to investigate the molecular mechanisms of lens lineage formation and to develop procedures to differentiate these cells into lentoid bodies. We plan to develop induced pluripotent cells (iPS) from patients with early onset senile cataract and from individual that do not develop cataract between age 65-80 and to use these materials to study mechanism of cataractogenesis. Our long-term goal is to identify genes and pathways involved in this disease and identify genes with protective roles in age-onset cataract.

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Key Words: ***Radiotherapy, melanoma, infectious diseases, viral cancers***

Molecular nuclear medicine plays an important role in the diagnosis and therapy of cancer and infectious diseases. Molecular nuclear medicine agents are compounds which are labeled with radioisotopes. Depending on the properties of a radioisotope, such compounds can be used for diagnostic or therapeutic purposes or both. For example, radioimmunotherapy takes advantage of the specificity of the antibody-antigen interaction to deliver a radioisotope which emits microbicidal radiation to a target, either a cancer cell or a microbe.

Radioimmunotherapy is currently used successfully for the treatment of non-Hodgkin lymphoma in patients who failed all other treatments. We are interested in applying radioimmunotherapy to treatment of metastatic melanoma - one of the few cancers with the increasing incidence for which there is no effective cure once it spreads. This project is already in the clinic with Phase I/II trial on-going in Hadassah, Israel. Another novel application of radioimmunotherapy which has been discovered in our laboratory is treatment of infectious diseases. This is important because of the urgent need for new approaches to treat infectious diseases caused by the increasing prevalence of highly resistant microorganisms and by occurrence of many infections in immunosuppressed individuals in whom standard antimicrobial therapy is not effective.

Yet another novel application of targeted therapy which our laboratory is interested in is radioimmuno-therapy of viral cancers such as HPV-related cervical and head and neck cancers and hepatitis B-related liver cancer. By targeting radiolabeled antibodies to viral antigens which such cancer cell continue to express – we are hoping to achieve exquisite specificity and low toxicity of treatment. All of these projects are carried out in collaboration with Dr. A. Casadevall.

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Key Words: ***Malaria, in vivo biology, virulence mechanisms, parasite and host transcriptional profiling, metabolomics, stress response, drug resistance***

MALARIA PARASITE PHYSIOLOGY AND HOST RESPONSE TO INFECTION

Our primary research interest is in pathogenesis and parasite biology in the natural setting in the malaria parasite *Plasmodium falciparum.* Patients infected with this parasite can be completely asymptomatic or develop severe disease resulting in death. The goal of our research has been to define the molecular mechanisms that underlie this variation in disease outcomes in *P. falciparum.* Toward this goal, we have developed a new pathogenesis model through the analysis of *in vivo* parasite biology and associated host factors using a whole genome approach. We have identified novel parasite biology when it resides in the human host; this biology has not been reported under *in vitro* cultivation and may play a role in enhanced virulence and/or transmission capacity. To further understand the implications of these novel *in vivo* states we will study the parasite under *in vitro* conditions that mimic host blood stream conditions. We are also studying host response to infection using whole genome approaches to identify host factors that associate with severe disease outcomes. We are now defining the small molecule repertoire of the parasite and the changes in human plasma metabolome associated with malaria infection. The long term goal is to identify parasite and host processes involved in disease to serve as targets for vaccine or chemotherapeutic development. We carry out field based translational studies in cohorts infected with malaria in Africa and these inform our experimental work using basic molecular biology approaches in the laboratory.

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Key Words: ***Organic synthesis, chemical biology, biomolecular nanotechnology, molecular imaging, signal transduction, molecular modeling, drug discovery***

The long term goal of our laboratory is to design and synthesize small molecule and foldamers probes to study the biology of signal transduction pathways, with developing embryos (identify new gene or co-factors), and develop new therapeutic and diagnostic agents for diseases induced by the TGF-beta-family, retinoic acid, oxidative stress signaling pathways, and potentially new pathways that we may identify. **To achieve the above objectives, we are using multidisciplinary tools including organic synthesis, chemical genetics, computational modeling, radio synthesis of PET and SPECT ligands and nanotechnology.**

Organic Synthesis: We are developing new catalytic asymmetric reactions using organo catalysts. By using these reactions, we are synthesizing diversity oriented five membered and six membered biologically significant molecules.
Chemical Genetics and Drug Discovery: Chemical genetics is a method of identifying small molecules that alter the function of biological pathways, resulting in the induction or rescue of a specific phenotype. Forward chemical genetics (FCG), analogous to more traditional genetic screens, involves screening a library of compounds to find small molecules that generate consistent phenotypes in biological assays. Reverse Chemical Genetic (RCG) involves screening a library of small molecules to a known target for agonists or antagonists of developmentally important pathways. For FCG, after synthesizing a particular library, we are testing its bio-activity in the context of developing zebrafish embryos to find the phenotypic changes and then to identify the genes and co-factors related to a particular phenotype by functional genomic approach (using biotin avidin affinity columns). For RCG, we are designing chemical libraries *in-sillico* by molecular docking, using known computer-assisted drug design software, and also developing new software, for known targets (TGF-beta receptor family, Retinoic acid receptors family and oxidative stress protein signaling pathways). After synthesizing these *in silico* lead molecules in lab, we are testing their bio-activity. We are iterating this process as needed to get clinically acceptable compounds. We are testing our chemical libraries to identify the mechanism of signal transduction pathways for Alzheimer's, Glioblastoma multiforme, Leukemia, Prostate cancer, Parkinson and Alzheimer's diseases.
Molecular Imaging: Developing new imaging agents (Nuclear probes) for known target (brain cancer, prostate cancer, Alzheimer's disease, Hypoxia agents, Apoptosis agents and Myocardial blood flow agents.
Biomolecular Nanotechnology: Synthesizing non-natural foldamers based nanoparticles for biological application. The goal of our research is to develop a new technology and that will allow the rational design and rapid synthesis of biological functional molecules with molecular weight in the kilo-Dalton range and with dimension on the order of tens of nanometer. Utilizing these approaches our group will synthesize biological proteins (catalytic activity, modulators and information processing capabilities and other). Students in our lab have the opportunity to be trained in (organic synthesis, Molecular imaging, nanotechnology, molecular modeling, biological assays chemical genetics, writing grants and papers, and technical skills for scientific presentations) a wide array of experimental approaches to study the biology of disease and discover new diagnostic and therapeutic agents.

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Key Words: ***HIV-1replication, reverse transcription, restriction factors, elite controllers***

Our laboratory is interested on understanding the mechanism used by restriction factors (endogenously expressed proteins) to restrict HIV-1 replication. The restriction factors TRIM5alpha and TRIMCyp are monkey proteins that potently block HIV-1 during uncoating—the initial step of HIV-1 replication. Our investigations have demonstrated that TRIM5 proteins block HIV-1 replication by potently inhibiting reverse transcription--essential early step in HIV-1 replication that converts the viral RNA in viral DNA and allowing productive infection. We are using these restriction factors to carefully define the uncoating mechanism of HIV-1, which will give rise to new ways to inhibit HIV-1 replication and treat HIV-1/AIDS. We have established that these restrictions factors accelerate uncoating making HIV-1 replication abortive. Our investigations will attempt to mechanistically define the timing of uncoating and reverse transcription during replication. Interestingly but not surprisingly these restriction factors block HIV-1 replication extremely potently and better than any drug known to date. We like to say not surprisingly since these restriction factors were design by nature block HIV-1 replication, and nature knows best.

The main focus of the lab is to define HIV-1 uncoating by using biological and biochemical assays. One of the most important questions in the HIV-1 field is to understand the relationship between uncoating and reverse transcription. We currently have in vivo and vitro assays to follow the uncoating and reverse transcription of HIV-1. These assays had allowed us to better understand how reverse transcription is block during the accelerated uncoating triggered by TRIM5alpha and TRIMCyp. We are also studying the structural biology of these restriction factors attempting to identify the TRIM5alpha domains required to block HIV-1 replication. We have also created mice strains that do not contain TRIM5alpha to understand the in vivo role of TRIM5alpha.

To understand uncoating in a broader manner, we are also studying the molecule transportin-3 (TNPO3) known to be required for HIV-1 replication during the initial steps of HIV-1 replication. We are identifying which proteins of the HIV-1 virus is required to interact with TNPO3, which is a nuclear transporter. We think that this transporter promotes HIV-1 uncoating in the nuclear pore and assist the transport of HIV-1 to the nucleus of the cells allowing productive infection. We have also created mice strains that do not express transporting to better understand the role of TNPO3 in HIV-1 replication using and in vivo model.

Finally, we are studying HIV-1 infected patients known as elite controllers—HIV-1 infected patients that never develop AIDS. We strongly believe that these individuals have novel ways to restrict HIV-1 replication, which will help us to develop new therapies against HIV-1/AIDS. Among this novel ways to restrict HIV-1 replication, we hypothesize the existence of new restriction factors in T cells that block HIV-1 replication. For this purpose, we are currently investigating HIV-1 replication blocks in T cells. We have several T cell lines that block HIV-1 replication at early stages suggesting the existence of a block similar to the one imposed by TRIM5alpha to HIV-1 replication. Overall, our laboratory is interested in defining the uncoating process of HIV-1 replication by using restriction factors such as TRIM5alpha, TRIMCyp and T cell restrictors, or proteins that assist uncoating such us transportin-3 (TNPO3). We are certain that these investigations will lead to novel and unforeseeable ways to treat HIV-1/AIDS.

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Key Words: Cancer, Mouse models, PI3K, Signal transduction; Estrogen

THE PI3K/PTEN/AKT PATHWAY IN EPITHELIAL TUMORIGENESIS

The central theme of my laboratory is the use of genetically engineered mouse models to identify and characterize the specific biological processes that are controlled by the PI3K/PTEN/AKT pathway, a signal transduction cascade altered in most cancers.

Our work revolves around two major, interconnected, areas:

- Deepening our understanding of the pathways regulated by the PI3K/PTEN/AKT axis in epithelial homeostasis, neoplastic transformation and progression, and of its role in controlling stem cell function during these processes;
- Identifying the mechanisms that allow and regulate the crosstalk between the PI3K/PTEN/AKT cascade and hormone-dependent signaling pathways.

Some examples of current projects include:

1- the identification of the specific cellular responses induced by PI3K activation in the very early stages of neoplastic transformation, using expression profiling and proteomics approaches;

2- the elucidation of the role of the crosstalk between PI3K and Estrogen Receptor (ERα) in epithelial homeostasis and in neoplastic transformation of hormone-sensitive tissues;

3- the dissection of the mechanisms through which the senescence response resulting from Ras activation is abrogated by PI3K activation, thus leading to more aggressive tumors;

4- testing the hypothesis that genetic alteration of thyroid stem/precursor cells leads to the development of more aggressive tumors.

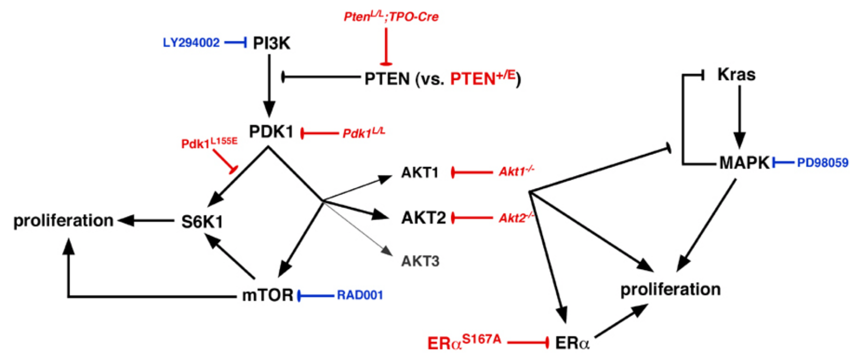

Fig.1. Schematic representation of the molecular network under analysis: chemical inhibitors utilized to dissect relative contributions are indicated in blue, genetic approaches are indicated in red. See text for further details.

Our work is expected to lead not only to a more detailed knowledge of the molecular network that is induced *in vivo* upon chronic PI3K activation, but also to the identification of essential signaling nodes that may be amenable to therapeutic intervention.

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Key Words: ***type 1 diabetes, autoimmunity, immunology, T cells***

T CELLS AS EFFECTORS AND THERAPEUTIC TARGETS IN TYPE 1 DIABETES

Type 1 diabetes is an organ-specific autoimmune disease characterized by T cell-mediated destruction of the insulin-producing beta cells of the pancreatic islets. While insulin therapy allows for continuation of life, it neither cures the disease nor prevents its devastating complications. Studies utilizing the nonobese diabetic (NOD) mouse model of the disease have shown that T cells, recognizing autoantigenic peptides bound to major histocompatibility complex (MHC) molecules, are absolutely required for disease development. T cells specific for beta cell antigens can also be detected in the peripheral blood of type 1 diabetes patients. Our laboratory utilizes an extensive collection of mouse models to investigate the antigenic specificities, pathogenicity, and immunobiology of T cells in type 1 diabetes. These models are also being used to develop and optimize therapeutic strategies, and new, increasingly "humanized" mouse models are continually in development in our group. Access to patient samples from Einstein's clinical affiliates permits translation of research findings to the human disease. The goals of our work are to better understand the underlying immunopathogenesis of type 1 diabetes and to develop improved tools to monitor and manipulate pathogenic beta cell-specific T cells. Our current projects include: (1) Development of a dendritic cell-based therapeutic strategy for type 1 diabetes; (2) Exploration of the molecular pathways responsible for tolerance induction by steady-state dendritic cells; (3) Identification of T cell epitopes of beta cell antigens providing broad population coverage; (4) Discovery of novel beta cell antigens.

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Key Words: ***microglia, astrocytes, lysosomal storage diseases, blood-brain barrier***

Our principal interests lie in the therapy of neurologic diseases and in the field of glial neurobiology. Much of our work has focused on developing effective therapeutic strategies for neuronal storage disorders such as Tay-Sachs disease. The goal here is to find effective means of replacing the lysosomal enzymes that are genetically deficient in these diseases. A current line of investigation stems from studies demonstrating that bone marrow transplantation can treat the central nervous system (CNS) in one storage disease, (-mannosidosis. We are now attempting to delineate the critical cellular mechanisms, responsible for this success. Our studies to date have indicated the importance of donor-derived microglia, their secretion of lysosomal enzymes and neuronal uptake mechanisms in obtaining therapeutic efficacy. Insights gained are helping to direct improvement of clinical strategies for related disorders as yet untreatable.

A new major goal is the development of a novel approach for the treatment of CNS aimed at overcoming the hurdle posed by the blood-brain barrier to potential therapeutic agents. Cell lines from unique transgenic animals are being developed with both brain-targeting and secretory properties to serve as safe and effective vectors for the delivery of such agents from the circulation to neurons within the CNS. This approach may be applicable to a wide variety of diseases with global CNS involvement for which current delivery modalities are inadequate.

Our interest in non-neuronal populations of the CNS has focused in recent years on microglia. The biology of this cell type is relatively unexplored territory. Our projects have included the discovery of the presence a novel opiate receptor in mammalian microglia and astrocytes, and evidence for immediate microglial responses to neuronal injury.

Our experimental approach is largely at the level of cell biology but extends to biochemistry, molecular genetics, histology and transplantation. Cultures of CNS cell populations from different species together with immunocytochemical techniques are used extensively. State-of-the-art approaches utilizing fluorescent probes and real-time laser confocal microscopy are also employed to monitor molecular and subcellular changes in living cells.

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Key Words: HIV, HIV receptors, HIV glycoproteins, HIV fusion/entry

Gp120 binding to the CD4 receptor drives reordering of the gp120 core structure and creates/exposes a co-receptor binding site on gp120. CCR5 and CXCR4 are the most biologically relevant HIV-1 co-receptors. We demonstrated that negatively charged residues and sulfotyrosines in the CCR5 amino-terminal domain (Nt) interact directly with gp120 and are indispensable for HIV-1 fusion and entry. We also demonstrated that CXCR4 co-receptor function depends on similar residues in the Nt and second extracellular loop. Studies with inhibitors of CCR5 co-receptor function showed that regions not directly involved in gp120 binding also play an important role in viral entry. Our continuing goal is to define how HIV-1 envelope glycoproteins interact with fusion co-receptors. To this end, we are studying the role of sulfotyrosines in CCR5- and CXCR4-mediated fusion and entry, identifying the gp120 residues that interact with different regions of the co-receptors, exploring co-receptor interactions with envelope glycoproteins from non-clade B isolates, and characterizing novel compounds that inhibit viral entry by interfering with CCR5 or CXCR4 co-receptor function. Our work will provide a detailed molecular picture of the protein complex that mediates HIV-1 membrane fusion and viral entry, and will facilitate the development of more potent and specific inhibitors that are clinically relevant.

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Key Words: cancer, DNA repair, genetic basis of cancer, meiosis

Genomic Instability and Cancer in Murine Models

The maintenance of genomic integrity in all organisms requires multiple DNA repair pathways that are involved in the processes of DNA replication, repair and recombination. Perturbations in these pathways can lead to increased mutation rates or chromosomal rearrangements that ultimately result in cancer. MMR is one of the repair systems that mammalian cells employ to maintain the integrity of its genetic information by correcting mutations that occur during erroneous replication. Mutations in MMR genes are linked to one of the most prevalent human cancer syndromes, Lynch syndrome and a significant number of sporadic colorectal cancers. At the molecular level tumors that develop in these patients display increased genomic mutation rates as indicated by increased instability at microsatellite repeat sequences (termed microsatellite instability, MIN). MMR in eukaryotes is complex and involves several homologs of the bacterial MutS and MutL proteins. In mammals, the initiation of the repair process requires two complexes formed by three different MutS homologs (MSH): A complex between MSH2-MSH6 for the recognition of single base mismatches and a complex between MSH2-MSH3 for the recognition of insertion/deletions. The repair reaction also requires a complex between the two MutL homologs MLH1 and PMS2 that interacts with the MSH complexes to activate subsequent repair events which include the excision of the mismatch carrying DNA strand and its re-synthesis. These steps are carried out by exonculeases, polymerases and a number of replication associated proteins. In addition to correcting DNA mismatches, the MMR system mediates an apoptotic response to DNA damage and suppresses recombination between non-identical sequences in mammalian genomes. All of these functions are thought to be important for genome maintenance and tumor suppression. We have generated knockout mouse lines with inactivating mutations in all the different MutS and MutL homologs, and also in genes that function in the later MMR steps to study their roles in genome maintenance and tumor suppression. In addition, we have generated knock-in mouse lines with missense mutations and conditional knockout mouse lines that inactivate specific MMR functions and/or model mutations found in humans. Our studies indicate that specific MMR functions play distinct roles in maintaining genome stability and that defects in these functions have important consequences for tumorigenesis and the response of tumors to chemotherapeutic treatment. They have also revealed that some of the MMR proteins play essential roles in the control of meiotic recombination in mammals.

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Key Words: *human papillomavirus, cervical cancer, cervical dysplasia, clinical trials, molecular epidemiology*

Dr. Mark Einstein is an internationally-recognized, board-certified Gynecologic Oncologist whose primary research interests focus on the pathogenesis and therapy for cervical cancer. Specifically, he is studying the numerous pathways involved in the malignant transformation of the cervix starting from the causative infectious agent- Human Papillomavirus. His translational work in cervical cancer is multi-faceted; investigating the host and virally-induced epigenetic, genetic and immune profiles along the malignant transformation path to provide insight into the process of cervical carcinogenesis. Dr. Einstein has developed and has been leading numerous multi-institutional clinical trials in targeting HPV and cervical cancer as well as cervical cancer prevention. He is active in clinical trial cooperative groups as Co-Chair of the Gynecologic Oncology Group Vaccine Committee and sits on the GOG Cervix Committee. He is on the HPV working group of the NCI Aids Malignancy Consortium. He is also an active member and a program leader of the Gynecology Division of the New York Cancer Consortium- a P01 Phase II clinical trial consortium and is the PI of many of its gynecologic cancer therapeutics trials accruing patients throughout New York hospitals. He is active in policy-making regarding cervical cancer prevention participating in the development of the American Cancer Society recommendations for HPV vaccines and is on the working group for the 2011 NCI/ACS cervical cancer screening update. He also was part of the working group for the Society of Gynecologic Oncology's (SGO) HPV vaccine recommendations as well. He also is Chair of the Gynecologic Oncology Foundation's (GCF) National Cervical Cancer Public Education Campaign and sits on their Board of Directors. He is also a Board member of the American Society for Colposcopy and Cervical Pathology. Dr. Einstein is also a consultant to the World Health Organization (WHO), developing their modules on the immunologic basis of HPV vaccines. He has received funding for cervical cancer-related translational research by the NIH, GCF, ACOG and the Berlex Foundation. He has been funded by and named an American Cancer Society Research Scholar. Dr. Einstein is also a Fellow of the American Board of Obstetrics and Gynecology and a Fellow of the American College of Surgeons.

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Key Words: ***connectomics, neural networks, behavior, developmental biology***

CONNECTOMICS: STRUCTURE, FUNCTION, AND DEVELOPMENT OF NEURAL CIRCUITS

How complex neural circuits form and how they function are major unsolved problems in neurobiology. We use the nematode *Caenorhabditis elegans* to study these questions at the cellular and genetic levels. We are currently completing a comprehensive description of the synaptic interactions in the nervous system of the *C. elegans* adult male—the male connectome. We identify synapses and the trajectories of neurons in serial section electron micrographs and construct neural maps using a novel software platform. Our male wiring diagram, together with that of the adult hermaphrodite, which was published in 1986, completes the description of nervous system connectivity for the adults of this species, the only animal species for which this information is available.

We are now investigating how the male circuits generate the male's behavior and how the circuits are genetically specified. The *C. elegans* male nervous system contains a set of circuits located in its tail that generates the male's copulatory behavior. The neural network containing these circuits consists of the processes of some 185 neurons and over 5,000 synapses. We analyze the patterns of connectivity within this network using computational methods to identify pathways that subserve particular steps of behavior. Hypotheses regarding neuron function are experimentally tested by cell killing techniques. We probe the functions of classical and peptide neurotransmitters, their receptors, and gap junctions by genetic methods.

To determine how the network is genetically specified, we make use of transgenes that express fluorescent proteins targeted to specific synapses. We plan to use these synapse-specific labels to identify mutants and genes that affect formation of particular cellular synaptic contacts. In these experiments we hope to uncover the still elusive class of proteins that encode the molecular determinants of synaptic specificity.

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Key Words: ***reproductive neuroendocrinology, signal transduction, neuroprotection, hormones***

One major focus in the lab is the regulation of female reproductive physiology and behavior by the ovarian hormones, estradiol and progesterone. We wish to understand how hormonal modulation of synaptic transmission in specific brain regions coordinates the timing of ovulation with mating behavior (lordosis), thereby maximizing reproductive success. Estradiol acts via a ligand-activated transcription factor, estrogen receptor-α (ERα), to regulate female reproductive function. Recent findings from our lab indicate that hypothalamic insulin-like growth factor-1 (IGF-1) receptors act in concert with ERα to control gondatropin releasing hormone (GnRH) neurons and hence female reproductive function. We are now testing two related hypotheses: (1) that IGF-1 regulates estradiol-dependent afferent signals to GnRH neurons, and (2) that IGF-1 regulates GnRH neuronal responsiveness to afferent input. We also collaborate with Dr. Genevieve Neal-Perry to test the hypothesis that reduced IGF-1 receptor signaling in the aging brain is responsible for attenuated neural responses to ovarian steroids and hence the attenuated luteinizing hormone (LH) surge that characterizes the perimenopause. Other experiments test the hypothesis that the reduced amplitude of the preovulatory LH surge in middle-aged female rats results from changes in the ability of ovarian steroids to modulate excitatory (glutamate and kisspeptin) and inhibitory (GABA) signals that regulate GnRH neurons. Recent findings suggest that intra-hypothalamic infusion of the neuropeptide kisspeptin, a potent activator of GnRH neurons, rescues LH surges in middle-aged females by enhancing local glutamate release.

A second major research topic in the Etgen laboratory is the role of estradiol and IGF-1 in improving outcomes after global ischemia (e.g., in cardiac arrest). This project is being carried out in collaboration with R. Suzanne Zukin. We have shown that physiological levels of estradiol reduce both hippocampal neuron loss and ischemia-induced cognitive impairments in young female rats. These neuroprotective actions of estradiol require co-signaling by brain IGF-1 receptors and are mediated by the ERK/MAPK signaling pathway. We are now investigating whether the middle-aged brain retains its responsiveness to the neuroprotective actions of estradiol and IGF-1, and if the duration of hormone withdrawal influences the efficacy of hormone treatment. Our findings suggest that both estradiol and IGF-1 can rescue hippocampal neurons from ischemia-induced cell death in middle-aged females even after a prolonged interval of estrogen deprivation.

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***Key Words**: Neurotransmitters, synaptic transmission, Mauthner-cell, electrophysiology, intrinsic excitability, Huntington's Disease*

MECHANISMS OF SYNAPTIC TRANSMISSION AND SENSORIMOTOR BEHAVIOR

Our research activities focus on basic mechanisms of synaptic transmission in the central nervous system and factors involved in the regulation of the strength of the synaptic connections between neurons, in the context of the neural networks in which they are embedded. Synapses are the fundamental units of information processing in the brain, and both the release of neurotransmitters and the responsiveness of the postsynaptic cell to that transmitter can be modified by patterns of activity or by endogenous modulators. This plasticity may involve short- or long-term modifications in the function and/or structure of synapses.

The laboratory uses a number of experimental models, including identified neurons in the goldfish midbrain studied in vivo. Electrophysiological, morphological and statistical techniques are used to study the regulation of synaptic transmission at identified central synapses, in addition, the neural network in the goldfish mediates a vital escape response, and we complement the cellular techniques with behavioral studies to investigate not also ask how neurons and neural circuits integrate sensory information and shape the resulting motor behavior.

We also have begun to study membrane properties that determine the intrinsic excitability of mammalian CNS neurons, using brain slice electrophysiology, and we are focusing on ion channels that are differentially regulated in normal and dysfunctional circuits, for example, in Huntington's Disease.

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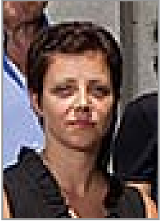

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Key Words: ***Biostatistics; prioritization; epigenomics; gene set analysis; hierarchical models***

PRIORITIZATION AND INTEGRATION OF BIOLOGICAL INFORMATION IN STATISTICAL MODELS

High-throughput technologies allow quantitative measurements of many biological processes. The goal of biostatistics is to summarize that information, providing a deeper understanding of biology and the specific disease under study. An important area of statistical research in genomic studies is how to take a statistical summary of importance and incorporate it into the larger system - where information concerning the functional relationships between loci, genomic context, and prior evidence of importance is known or hypothesized.

Machine-learning and ensemble-based techniques have been successfully applied to many types of genomic data to allow complex relationships between loci to be modeled. This has resulted in significant gains in prediction accuracy, but has also made description of the underlying process and identification of the most relevant loci difficult. In addition, small sample size and high dimension tend to yield a multiplicity of equally predictive models and hundreds of statistically significant loci associated with these models. Gene set methods add higher-level structure using gene ontology (GO) classification, producing more interpretable results with higher concordance across studies of the same phenotype. Using similar higher level information, we are examining methods to select candidate loci in epigenome-wide association studies using a metric that combines the locus-specific statistical summary with higher level biological properties, gene set information, and prior evidence of functionality. Further integration across several types of biological measurements using meta-analytic approaches will facilitate the selection of high confidence candidate loci or regions, with much higher likelihood of true biological importance.

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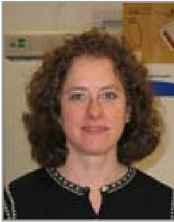

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Key Words: ***fungus, host-pathogen, interactions, pathogenesis***

ASPERGILLUS: HOST-PATHOGEN INTERACTIONS

We are developing antibody-based reagents for prevention and diagnosis of disease due to *Aspergillus*. Invasive aspergillosis occurs mainly in severely immunocompromised hosts and our ability to diagnose or treat this disease with antifungal drugs is limited. Therefore, strategies that enhance the host immune response are an attractive area for development. The laboratory is developing monoclonal antibodies (MAbs) that block or delay germination, the transition from the spore form to hyphal growth that is required for *A. fumigatus* to invade host tissue. Currently, we are focused on one MAb that we made (MAb 318) that inhibits germination *in vitro*, alters alveolar macrophage-conidia interactions and prolongs survival in experimental murine pulmonary infection. MAb 318 binds to three *A. fumigatus* proteins. We are distinguishing which interaction inhibits germination. We also are examining mechanisms by which MAb 318 enhances macrophage function and prolongs survival. Long term goals include determining the suitability of MAbs that bind to this target for passive prophylaxis and of the target itself as a vaccine to prevent disease. We also want to understand the role of this protein during germination and identify additional MAbs that prevent germination, as complete inhibition may require binding to more than one target.

A second area of investigation is development of better antibody-based diagnostic tests for invasive aspergillosis. We currently are employing antibody engineering techniques to enhance the sensitivity and specificity of a test that detects galactomannan, a cell wall and secreted carbohydrate. We also are working to identify novel fungal targets that can distinguish invasive disease from colonization or other forms of aspergillosis. Promising targets will be used to design new assays, with the goal of improving our ability to diagnose human disease.

Fungal products also play important etiological roles in the pathogenesis of asthma. A third area of research is the development of MAbs to fungal or host molecules that can regulate inflammation in response to fungal allergen exposure. Such reagents would have potential for use in combination with currently available asthma therapies, which may allow reduced dependence on some that are associated with broad ranging adverse effects.

We also are developing MAbs to inhibit *Aspergillus*-derived aflatoxins, common contaminants of stored grains that are potent hepatic carcinogens and acutely toxic. No protective MAbs against aflatoxin exposure have been identified. We made a novel aflatoxin-keyhole limpet hemocyanin conjugate that is potently immunogenic in rats. We currently are making MAbs to aflatoxin B_1. MAbs will be tested for their ability to prevent acute toxicity and DNA damage in vitro and in vivo. In the long term, protective MAbs will be assessed for capacity to prevent disease in humans.

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Key Words: Tuberculosis, HIV-1, Mucosal Immunity, Pediatric Vaccine, PMTCT

Developing a Combination Pediatric TB-HIV Vaccine

The overall objective of our research project is to develop a safe and effective oral combination pediatric TB-HIV vaccine. Infants in developing countries where HIV-1 and tuberculosis (TB) are highly endemic are at a high risk both for mother-to-infant HIV transmission or Mycobacterium tuberculosis (Mtb) infection, as well as rapid progression to AIDS or miliary TB after infection. Despite the dramatic impact of antiretroviral and formula replacement programs to prevent mother-to-child-transmission of HIV-1 (PMTCT) in industrialized countries, an estimated 150,000 cases of breast-milk MTCT of HIV-1 occur in resource-limited countries each year.

There is an urgent need for an effective neonatal vaccine against TB or HIV-1 transmission in Africa that is safe for infants at risk for HIV-1. A vaccine for PMTCT of HIV-1 via breast milk, to which vulnerable infants are repeatedly exposed, will require (1) a highly accelerated vaccine schedule and (2) the rapid induction of antiviral immune responses in local tissues of the oral and intestinal mucosa. Intradermal Mycobacterium bovis bacille Calmette-Guérin (BCG) vaccination in healthy newborns rapidly primes strong adult-level Th1-type IFN-gamma CD4+ and CD8+T cell responses against TB and unrelated co-administered antigens. BCG also can be given by the oral route. We previously developed a novel vaccination strategy using recombinant (r) BCG to protect against measles virus (MV) as an alternative to live-attenuated MV vaccination that is ineffective in early infancy; neonatal vaccination with rBCG expressing MV nucleocapsid in rhesus macaques resulted in a significant (1.5 log) reduction in viral load in lymph nodes and in lung pathology compared to controls (P<0.05) after a respiratory MV challenge. Given the large geographical overlap between Mtb and HIV infection in Africa, until recently, BCG vaccination was recommended at birth for all infants and given to >85% of the birth cohort in Africa. However, it now appears that the risk for disseminated BCG disease in HIV-infected infants (~0.42%/year) clearly outweighs the potential benefits of BCG in these children. Alternative methods to control TB in infants with HIV are urgently needed.

Candidate attenuated M. tuberculosis (AMtb) strains that are markedly safer than BCG, and that generate protective immunity against virulent Mtb challenge in murine models have recently been developed by William Jacobs, Michelle Larsen and co-workers at AECOM. We demonstrated recently that single-dose immunization in neonatal mice with recombinant AMtb expressing HIV Env (rAMtb-Env) rapidly generates Env-specific CD8+ T cells among PBMCs at a frequency that is > 15-fold higher than responses after immunization with rBCG-Env. Preliminary studies (in collaboration with Kristina Abel at UC Davis) have recently confirmed the safety of neonatal AMtb immunization in immunocompromised SIV-infected infant macaques.

Using this strain, and its derivatives we are testing the hypothesis that oral administration of rAMtb-HIV is safe and superior to parental vaccination in eliciting local mucosal immune responses in newborn macaques. Opportunities are available for PhD students to participate in designing improved rAMtb vectors and determining the efficacy of these vectors expressing multiple antigens (Gag, Pol and Env) in generating immunity in murine models, or against oral SIV challenge in the infant macaque model of human HIV breast-milk transmission. These studies will generate safety and efficacy data to justify the future testing of this strategy as a novel combination HIV-TB vaccine in human infants.

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Key Words: membrane channels, gating, transport, bacterial toxins

The objectives of the research in this laboratory are two-fold: (1) to obtain a detailed understanding both of the mechanism(s) by which channels are opened and closed (i.e., gated) and of the physico-chemical factors that govern transport through open channels; (2) to determine the relationship of the translocation of proteins and polypeptide chains across membranes to the formation and existence of wide-lumen channels. The methodology employed is the study at both the macroscopic and single-channel level of the size, ion-selectivity, voltage-dependent properties, and stochastic behavior of channels incorporated into planar phospholipid bilayer membranes. These channels include those inserted into membranes by bacterial proteins such as diphtheria toxin, tetanus toxin, botulinum toxin, anthrax toxin, and colicins of the E1 class (E1, Ia, Ib and A). With respect to the first objective: the genes for the channel-forming proteins mentioned above have all been cloned and sequenced, so that detailed models of channel structure and gating can be developed. Moreover, models can be stringently tested by comparing properties of specifically modified channels (formed by site-mutated proteins) with their predicted behavior. With respect to the second objective: all of the above-mentioned bacterial toxins consist of at least three domains, only one of which is necessary for channel formation; one of the other domains is an enzyme that must cross a vesicular membrane to enter the cytosol and thereby cause cell intoxication. Whether and how this enzymatic domain of the toxin crosses planar bilayers in conjunction with the opening and closing of the channels formed by the channel-forming domain is being investigated.

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***Key Words:* comparative protein structure modeling, de novo protein design, sequence alignment, scoring functions, functional sites, protein complexes, bioinformatics**

BIOINFORMATICS AND COMPUTATIONAL BIOLOGY OF PROTEIN STRUCTURES.

The biochemical function of a protein is defined by its interactions with other molecules. The residues that determine these interactions are close in space but are frequently distant in sequence. Consequently, the three dimensional structure of a protein is often more informative than the corresponding sequence.

Research in our lab is focusing on obtaining three dimensional structures of proteins by computational modeling or design and to utilize these structures to understand or design functions. We are developing protein structure modeling techniques using evolutionary principles as a conceptual framework, but incorporating 'ab initio' components in the modeling process. Meanwhile, as an extension of evolutionary events we are developing methods to design new, protein-like architectures from building blocks that were observed already in known structures.

In terms of biomedical relevance, we use our structure modeling methods to build ‘in silico’ evolving networks of transcription factor-DNA complexes, to study structure function relationships in antibodies, membrane transporters and various other biologically relevant systems. Our protein design techniques are used to study the evolution of protein structures and planned to be utilized in redesigning molecular binders such as antibodies, with desired properties, for instance, with the ability to better penetrate tissues or access tight binding sites.

Lab Webpage: www.fiserlab.org

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Our laboratory employs and integrated multi-methodological approach to issues in the cognitive neurosciences, using structural and functional neuroimaging, high-density electrophysiology, imaging genomics, eye tracking, psychophysics and virtual reality to understanding the neural basis of basic sensory-perceptual and cognitive functions. Our work is translational at its core in that we employ an equal mix of basic-science projects in healthy individuals with clinical studies in our patient groups. Our approach is to first develop novel assays of a given cognitive function in healthy individuals, which are then deployed in populations of interest. The mission of the lab is to understand the underlying neurobiology of developmental disorders, as a means to develop more effective treatments interventions, and we have worked extensively in adolescent Schizophernia, Autism Spectrum Disorder, ADHD and Aging. With a $2.8 million grant awarded by the National Institutes of Health, we are examining whether multisensory integration (i.e., the brain's processing of information from different senses) is impaired in people with autism. Another area of major focus for us is the basic neurobiology of attention, with more than 60 of our over 150 papers concentrating on the mechanisms of attentional control. This latter work is supported by a grant from the National Science Foundation (NSF).

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Key Words: ***G protein-coupled receptors, signal transduction, receptor trafficking, Fragile X syndrome***

MOLECULAR CELL BIOLOGY OF METABOTROPIC NEUROTRANSMISSION

Research in the laboratory focuses on elucidating the molecular and cellular underpinnings of metabotropic glutamatergic neurotransmission in the brain, with the ultimate goal of developing a molecular rationale for targeted interventions in neuropsychiatric disorders. Metabotropic glutamate receptors (mGluRs) are G protein-coupled receptors enriched at excitatory synapses throughout the brain where they act both pre- and postsynaptically to regulate glutamatergic neurotransmission. Signaling by mGluRs is critical to synaptic circuitry formation during development and to forms of activity-dependent synaptic plasticity. Dysregulation of mGluR signaling is implicated in neurological and psychiatric disorders linked to abnormal neurodevelopment, including schizophrenia and Fragile X Syndrome, the most common inherited form of mental retardation. We use a combination of molecular biology, biochemistry and imaging techniques to pursue two principal lines of research. First, we are investigating the molecular mechanisms underlying the temporo-spatial regulation of mGluR signaling: current projects examine the role of adaptor proteins and specialized membrane compartments in orchestrating and fine-tuning mGluR activity under physiological conditions and in animal models of Fragile X Syndrome. Second, we are investigating the mechanisms underlying mGluR transport to and from synaptic sites during neuronal differentiation and in response to activity-dependent changes in synapse composition. Current projects examinine the role of recently identified mGluR-interacting proteins in supporting receptor trafficking to synaptic sites.

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***Key Words:** Stem cells, trafficking, nervous system, bone marrow, sickle cell, cancer*

Our laboratory is interested in understanding how hematopoietic stem cells (HSCs) and mature blood cells traffic in vivo. We have uncovered a key role for the nervous system in regulating HSC trafficking, and are evaluating its role in the inflammatory response in diseases such as sickle cell disease. In addition, we are also exploring whether the traffic paradigms uncovered for healthy stem cells applies to cancer cell migration and metastasis.

Molecular and cellular constituents of the stem cell niche. HSCs continuously traffic from the bone marrow to the blood compartment (and vice-versa) under homeostasis. Ongoing studies have focused on the role of the nervous system in the regulation of the HSC niche in the bone marrow. This effort is based on our recent observations suggesting a critical function of adrenergic signals emerging from the sympathetic nervous system (SNS) in HSC egress. While investigating further the mechanisms by which HSCs were mobilized, we have found that exposure to constant light significantly reduced mobilization efficiency following the administration of the hematopoietic cytokine G-CSF. G-CSF is the most commonly used HSC mobilizer in the clinic to harvest stem cells for transplantation. This finding prompted us to assess how HSC are released from the bone marrow under steady-state conditions. We have described the phenomenon and its mechanisms. These studies revealed that stromal cells in the bone marrow are subjected to circadian adrenergic signals transmitted by the ï ¢3 adrenergic receptor that lead to the degradation of the transcription factor Sp1 and diurnal changes in the expression of the chemokine Cxcl12. Recent investigations are focused on the identification and regulation of the stromal target for the SNS. These studies have led to the identification of a nestin+ mesenchymal stem cell as a candidate niche cell required for HSC maintenance in the bone marrow.

Mechanisms of sickle cell vaso-occlusion. This project emerged from our intravital microscopy observations suggesting that sickle cell vaso-occlusion was mediated by the direct interaction between sickle erythrocytes and adherent leukocytes in small venules. Further analyses using novel high-speed multichannel fluorescence microscopy techniques have revealed that E-selectin-mediated activating signals emanating from the inflamed endothelium led to the activation of specific microdomains on the leading edge of adherent neutrophils, which then induce intravascular heterotypic interactions between erythrocytes or platelets with adherent leukocytes. Ongoing studies dissect further the molecular basis of this phenomenon.

Role of the nervous system in cancer. We are exploring the role of the autonomic nervous system in cancer formation and metastasis using xenogeneic and transgenic models of prostate cancer. Ultimately, the goal of these studies is to obtain new insight on the cellular and molecular cues that regulate the tumour microenvironment and allow cancer cells to spread.

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Key Words: peptidases, neuropeptides, obesity, neurodegeneration

This laboratory works on three related projects involving peptides and peptidases.

1) Peptide hormones and neurotransmitters are an important class of extracellular messengers that are involved with a wide variety of biological functions including feeding and body weight regulation, fear, anxiety, pain, circadian rhythms, memory, reward mechanisms, and many others. One project in the laboratory is focused on neuropeptides and the enzymes that are involved with their post-translational processing. We are studying the various peptide processing enzyme by examining the levels and molecular forms of peptides in mice lacking peptidase activity (knock-out mice or naturally occurring mutations); peptides are being measured using a quantitative peptidomics technique. The goal of these studies is to define the physiological role of each neuropeptide processing enzyme.

2) In addition to neuropeptide processing enzymes, several other cellular peptidases are being studied in the laboratory. Current projects use peptidomics and other techniques to identify the physiological function of the peptidase. One of the enzymes being studied is cytosolic carboxypeptidase 1 (CCP1, also called Nna1), which when mutated in a mouse causes neurodegeneration of Purkinje cells and several other cell types. Another enzyme currently being studied is carboxypeptidase A6, which has been implicated in axonal guidance during development. A third enzyme under analysis in our laboratory is carboxypeptidase D; this enzyme functions in the processing of growth factors and in *Drosophila* is necessary for animals to survive beyond the intermediate larval stage.

3) Another area of research is on novel peptides, including classical neuropeptides that are secreted from cells via the regulated secretory pathway, non-classical neuropeptides that are secreted from cells independently of the secretory pathway, and cytosolic peptides that may have functions in regulating cellular function. The goal of these studies is to identify peptides, understand how they are produced and regulated, and finally to determine their function.

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Key Words: ***nanotechnology, translational research, nitric oxide, nitrite, blood substitutes, protein dynamics, water, osmolytes, molecular biophysics***

Nanotechnology and Translational Research

We have developed a novel nanoparticle platform for topical and systemic drug delivery. The platform is capable of sustained delivery of therapeutic levels of nitric oxide, peptides and chemotherapy drugs. The high collaborative research program consists of nanoparticle platform development, platform characterization, preclinical applications including wound healing in the presence and absence of radiation damage, antimicrobial therapies, topical treatment of erectile dysfunction, hypertension and peripheral vascular disease, enhanced MRI imaging and targeted drug delivery. The goal is tissue specific drug delivery with imaging capability.

Protein Dynamics, Reactivity, Osmolytes and Solvent Slaving

We seek to understand how solvent motions modulate functionally important protein dynamics. Our approach focuses on the water surrounding the protein, the different categories of protein dynamics that contribute to functionality and how the interplay between water and protein dynamics is modulated by osmolytes.

Hemoglobin based blood substitutes and the role of NO/nitrite in the control of vasoactivity.

Dr. Friedman is the Director of the Einstein Blood Substitute Program Project which seeks through a multidisciplinary effort to improve the outcome of transfusions by developing hemoglobins and nanoparticles that enhance tissue perfusion and counter toxic effects due to NO scavenging and production of ROS. A major focus is on the biophysical mechanisms through which hemoglobin in the presence of nitrite can generate stable nitrosothiols that cause vasodilation.

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***Key Words:* C. neoformans, cryptococcosis, bioterrorism, toxins, virulence, cytokines, phenotype switching, replicative aging**

The primary focus of my laboratory is the pathogenesis of chronic *cryptococcosis*. We demonstrated that *C. neoformans* manifests multiple phenotypes, which allows the yeast to evade the immune response. Research projects focus on the host response as well as the molecular mechanisms that allow the fungus to change. Using mouse models we established that phenotypic switching occurs *in vivo* and changes the outcome in chronic *Cryptococcus* infection. Microarray analysis of switch variants have determined that phenotypic switching to a hypervirulent switch variant involves epigenetic down regulation of several genes. We have generated knockout variants and this work confirms that a Knockout leads to hypervirulence. Now we will study the contribution of these genes to virulence and investigate molecular mechanisms that regulate this epigenetic silencing. Our work is of general importance because it studies genes that down regulate virulence rather than up-regulate virulence. We also examine an aspect of microbial pathogenesis that has never been studied before: the role of microbial aging in virulence. *C. neoformans* undergoes asymmetric cell divisions. *This process is referred to as generational or replicative aging.* As a consequence older, also referred to as senescent mother cells can be distinguished from younger daughter cells or virgin buds. The ensuing phenotypic changes in the aging mother are such, that they could potentially give older cells a biological advantage *in vivo*, thus promoting their selection. The ability to sample pathogen directly from the CSF, and the availability of serial isolates from CSF samples offers a unique opportunity to investigate this dynamic microevolution within a pathogen population, which replicates in a host with cryptococcal meningitis. In addition, this laboratory works on a bioterrorism related project. We study the relevance of staphylococcal toxins during natural *S. aureus* infection. We have generated monoclonal antibodies to staphylococcal enterotoxin B (SEB). These mAbs can protect SEB injected mice from SEB induced shock and death. In addition we have developed a capture ELISA that allows us to measure SEB toxin in body fluids. Studies now concentrate on making high affinity antibodies and chimeric antibodies that are optimized for toxin neutralization of SEB in vivo.

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Key Words: ***chromatin, DNA replication, Drosophila, nucleosome***

BIOCHEMISTRY AND GENETICS OF CHROMATIN ASSEMBLY

In the eukaryotic nucleus, hundreds of millions of base pairs of DNA are packed into chromosomes. Chromatin, the central nucleoprotein filament of a chromosome, has many different forms and organization levels, which range from the 10 nm oligonucleosome filament to highly condensed metaphase chromatin. Chromatin is the natural state of DNA in the nucleus and the native substrate for nuclear processes such as DNA replication, recombination, repair and transcription. The assembly of DNA into chromatin and dynamic conversion between its different forms are critical steps in the maintenance and regulation of the eukaryotic genome.

The ultimate goal of our research is to understand how chromosomes are assembled and how chromatin assembly regulates the structure and activity of eukaryotic chromosomes. The crucial first step in this direction is a systematic study of factors that mediate the assembly of chromatin. In our work we use biochemical approaches to analyze mechanisms of chromatin assembly by a SWI/SNF-like factor ACF. In addition, we dissect its function in vivo by methods of Drosophila genetics. We also use biochemistry and genetics to identify novel assembly factors in Drosophila. Thus, we are trying to uncover the intricate network of chromatin assembly and remodeling factors and their roles in the hierarchical organization of the chromosome, from the nucleosome to higher-order structures.

1. Molecular Mechanisms of Chromatin Assembly

ACF (ATP-utilizing chromatin assembly and remodeling factor) was identified on the basis of its ability to mediate ATP-dependent reconstitution of chromatin in vitro. We study ACF as a prototype assembly factor to elucidate elementary molecular events that take place during ATP-dependent assembly of nucleosomes.

2. Biological Function of Chromatin Assembly Factors

In Drosophila, ACF is the major (but not the only) ATP-dependent chromatin assembly factor. In order to expose its biological function, we study Drosophila mutants that do not express ACF. Similarly, we analyze respective biological functions of histone chaperones and redundant ACF-like factors, such as CHD1 and NoRC.

3. Higher-Order Chromatin Forms

During chromatin assembly, ACF can mediate deposition of both core and linker histones (H1). Thus, ACF can assemble the 30 nm chromatin fiber in a defined system. To reconstitute other higher-order chromatin forms, we incorporate various components, such as modified core histones, histone variants and heterochromatin proteins. In vitro assembled chromatin vectors can turn into useful tools in research and therapy. These studies will eventually lead to the discovery of molecular techniques to reconstitute functional metazoan chromosomes.

Sperm DNA is compacted with protamines to form enzymatically static sperm “chromatin”. We have begun to analyze protein factors that mediate protamine deposition during spermatogenesis and their removal from DNA after fertilization.

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Key Words: ***Epilepsy, epileptogenesis, development, GABA, chloride cotransporters, infantile spasms, Rett Syndrome.***

ROLE OF $GABA_A$ SIGNALING IN EPILEPTOGENESIS AND BRAIN DEVELOPMENT EFFECTS OF EARLY LIFE EPILEPSY ON BRAIN DEVELOPMENT MODELS OF INFANTILE SPASMS AND EARLY LIFE EPILEPSY PATHOPHYSIOLOGY OF RETT SYNDROME

The maturation of $GABA_A$ receptor-mediated signaling from excitatory to inhibitory is an age-related process controlled by cation chloride cotransporters, such as KCC2. As a result, GABA exerts dual functions, being an important neurotrophic factor during early development and the principal inhibitory neurotransmitter of the mature central nervous system. In our laboratory we have been investigating the age and gender specific mechanisms through which early life stressors and seizures may disrupt the normal patterns of brain development, by disrupting the neurotrophic effects of GABA. We are also studying methods to reverse these adverse processes. Furthermore, we are very interested in understanding how epileptogenesis proceeds in the developing brain and what is the specific role of $GABA_A$ receptors in this process.

To better understand the pathophysiology and design better methods to treat catastrophic early life epilepsies, we are developing and studying new models of early life epilepsy. These include a model of symptomatic infantile spasms that recapitulates most of the features of the human condition (collaborative project with Drs Moshé and Scantlebury). Several projects are under way to elucidate the pathophysiology and treatment of infantile spasms.

Rett syndrome is one of the major causes of mental retardation and epilepsy. Most of these patients have mutations in the MeCP2 gene and also manifest abnormal stereotypic movements and autonomic dysfunction. Despite the devastating course of the disease, two independent laboratories have recently demonstrated that, in mice, phenotypic reversal can be achieved by restoring the normal function of MeCP2. We are using a mouse model of Rett syndrome to determine how pathogenic mutations of MeCP2 may interfere with the function and physiology of structures involved in the control of motor system and seizures, like the substantia nigra and how these processes may be reversed by appropriate therapeutic interventions.

Students interested in these projects will gain exposure to a variety of *in vivo* and *in vitro* techniques that combine molecular biology, *in vivo* and *in vitro* electrophysiology, histological, and behavioral studies and will be involved in projects with direct translational relevance to the clinical practice, i.e. identification of novel therapies.

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Key Words: ***chromatin structure and function, transcriptional regulation, macro domains, macroH2A, cancer, senescence, poly(ADP-ribose)***

THE ROLE OF MACRO DOMAIN-MEDIATED REGULATION OF CHROMATIN STRUCTURE AND FUNCTION DURING CANCER AND SENESCENCE

Macro domains are found in several histone variants, chromatin remodelers, and other transcriptional coregulators (e.g. macroH2A, PARP14, CHD1L) with roles in cancer progression, senescence, innate immune responses, and viral pathogenesis. These protein modules function, in part, as ligand binding domains for NAD^+-derived poly(ADP-ribose), ADP-ribose, and O-acetyl-ADP-ribose. The ability of macro domains to bind these ligands links the function of macro domain-containing proteins (MDCPs) to NAD^+-dependent signaling events catalyzed by NAD^+-utilizing enzymes such as PARP-1, PARG and SIRT1. Our laboratory employs a variety of cell-based, genomic and biochemical techniques to explore the role of macro domains, their ligands and the NAD^+-utilizing enzymes that produce them in transcriptional regulation.

The histone variant macroH2A1 is an MDCP of particular interest to our group. MacroH2A1 incorporates into nucleosomes found in large chromatin domains that occupy a quarter of the genome in human cells. MacroH2A1 exists as one of two splice variants, macroH2A1.1 which can bind to NAD^+-derived ligands, and macroH2A1.2 which cannot associate with these small molecules. Interestingly, while both macroH2A1 variants are present in normal adult cells, macroH2A1.1 splicing is decreased in a variety of human cancers including endometrial, lung, testicular, colon, and bladder cancer. Additionally, macroH2A1.1 can trigger an innate tumor suppressive pathway called oncogene-induced senescence. We are currently exploring the mechanisms that regulate macroH2A1 splicing, the specific roles of each macroH2A variant in transcriptional regulation, and how these processes are perturbed during oncogenesis.

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Key Words: ***cell division, microtubule cytoskeleton, molecular motors, cytoplasmic dynein, bidirectional organelle transport, human pathologies, high-resolution fluorescence microscopy, single-molecule biophysics***

The Gennerich laboratory is interested in the fundamental molecular mechanisms of cytoskeletal motor proteins and their associated biological processes. We use multidisciplinary approaches, integrating high-resolution live cell imaging and single-molecule biophysics, to study how these motors use the energy of ATP hydrolysis to perform mechanical work in cells and to understand how these motors are controlled to facilitate diverse cellular activities.

Current research is focused on the microtubule-based motor protein cytoplasmic dynein and its role in cell division and the long-distance transport of organelles and mRNAs. We combine the development of sub-wavelength resolution and single-molecule microscopy techniques with biochemistry and cell biology tools to explore how dynein works at the molecular and cellular level. The mechanistic insights gained from these studies will lead to a better understanding of the fundamental design principles of motor proteins and their associated cellular functions. Ultimately we want to understand how defects in motor function lead to human diseases such as neural disorders and human cancers.

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Key Words: ***EPR spectroscopy, enzymes, intermediate states***

INVESTIGATION OF PROTEIN/SUBSTRATE INTERMEDIATES USING ADVANCED EPR SPECTROSCOPY

The goal of our research is to characterize protein function through the determination of intermediate state structures generated along a given reaction pathway. These intermediate states involve transient forms of the protein, cofactor and/or substrate. In a variety of enzyme systems, intermediates consist of paramagnetic species in the form of metals, organic radicals, or both metals and radicals. In addition, for systems which lack endogenous paramagnetic species, it is often advantageous to introduce a stable radical "spin label" to serve as a reporter of protein structure. Electron paramagnetic resonance (EPR) spectroscopy is well suited for the characterization of all of these classes of paramagnetic species. Thus our primary tools for structural characterization involve advanced EPR techniques, including electron spin echo envelope modulation (ESEEM) and electron nuclear double resonance (ENDOR). A current focus in EPR development is the construction of a high frequency pulsed EPR/ENDOR spectrometer to extend the capabilities of EPR spectroscopy beyond magnetic field strengths available in commercial spectrometers.

Examples of systems currently under study include:

Glutamate Mutase: This enzyme catalyzes the reversible carbon skeletal rearrangement of *(S)*-glutamate to *(2S,3S)*-3-methylaspartate. EPR spectroscopy of radical intermediates is being used to unravel the mechanism of this rearrangement.

Ribonucleoside Triphosphate Reductase (RTPR):
Because ribonucleotide reductases play a key role in DNA synthesis, their inhibition forms the basis of an important class of anti-tumor drugs. Both the turnover of substrate and the mechanism of inhibition involve organic radical intermediates, in addition to paramagnetic cobalt(II) present in the adenosylcobalamin cofactor. The study of RTPR function and inhibition is an ongoing research topic.

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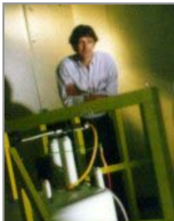

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Key Words: membrane proteins, ATP synthase, multidrug resistance, NMR

Membrane proteins are responsible for signaling, energy transduction, and transport, making them key players in infectious disease, genetic disorders, and cancer. These proteins have resisted routine structural analysis. Hence our overall research goals are to develop and apply new solution conditions and NMR methods to determine structures of membrane transporters and pumps, alone and as drug-protein complexes, in order to understand how they function and guide the development process of new or improved antibiotics. The F_O portion of the F_1F_O ATP synthase is one focus of present efforts in the lab. This complex is responsible for synthesizing the vast majority of cellular ATP - over 80 pounds of ATP per day in the average human. In the ATP synthase, H^+ translocation across the membrane through F_O provides the driving force for ATP synthesis on F_1. A ring of small "c" subunits make up the bulk of the F_O complex, and are responsible for translocating H^+ through F_O. We determined the structures of subunit-c in both its protonation states, providing a picture of the conformational changes linked to energy transduction. The c-subunit of the ATP synthase was recently shown to be a useful, genus-specific target for antibiotics (against *M. tuberculosis* and *Strep. pneumonia*, for example). Hence we are investigating the structural basis for the specificity of inhibitors with the human, *M. tuberculosis*, *S. pneumonia*, and *E. coli* forms of the protein.

We are also intrigued by a simple but important multidrug resistance pump from *S. aureus*. Bacteria use several methods to resist the lethal effects of antibiotics. The broadest resistance results from the action of Multidrug Resistant Pumps (MDRs), which extrude a range of compounds of quite diverse chemical structure. The Small Multidrug Resistance pumps (SMRs) are dimeric proton-drug antiporters that contain the full multidrug transport machinery, stripped to its barest essentials. Hence they are ideal transporters for a comprehensive structural and functional understanding of drug transport and inhibition in a medically important MDR. We are determining the structures of the conformations that make up the functional cycle of an SMR, and identifying the binding determinants for multiple drugs and inhibitors using solution NMR.

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Key Words: ***Regulation, structure/function, physiological and pharmacological roles of a proton-coupled folate transporter, hereditary folate malabsorption***

This laboratory recently cloned a novel proton-coupled folate transporter (PCFT- SLC46A1), required for the intestinal absorption of folates, and demonstrated that PCFT is mutated in the autosomal recessive disorder hereditary folate malabsorption (HFM) – (Cell, 127:917, 2006; Blood, 110: 1147, 2007). Since folates are key one-carbon donors essential for DNA and RNA synthesis and methylation reactions, understanding the properties, role and regulation of this transporter is essential. Structure-function studies of PCFT are geared towards identifying residues required for the maintenance of tertiary structure, the translocation pathway, folate and proton binding, and the carrier's alternative conformational states. These studies are informed by analyses of PCFT mutations in patients with HFM and by site-directed mutagenesis. Transport studies employ both electrophysiological measurements in Xenopus oocytes and radiolabeled folate flux determinations in cell lines. Homology modeling is utilized in structure studies (J Biol Chem, 284:17846, 2009; Am J Physiol Cell Physiol, 297:C66, 2009). PCFT is also required for transport of folates into the brain, essential for neural development in infancy. Studies are exploring the role PCFT plays at the vascular blood-brain-barrier and at the level of the choroid plexus. PCFT is a member of one of the seven families of proton-coupled transporters that operate most efficiently at low-pH. These transporters are all required for the absorption of their substrates (peptides, amino acids, monocarboxylic acids, divalent metal ions, etc) in the acidic microclimate of the proximal small intestine. Proton-coupled transporters also mediate export of their substrates from acidified endosomes during receptor-mediated endocytosis. This is also the case for PCFT (J Biol Chem, 284:4267-74, 2009). Other studies are exploring regulation of PCFT in in a variety of tissues and the impact of methylation on the expression of this gene (Mol Cancer Ther, 8:2424, 2009). As families are identified world-wide with HFM, and studied in this laboratory, the genetics of this disorder and its manifestations are being characterized. Finally, structural analogs of folates are employed for the treatment of cancer and autoimmune diseases. Membrane transport is a key determinant of the effectiveness of these drugs and impaired transport is an important element in drug resistance. A new-generation antifolate, pemetrexed, has a high affinity for PCFT and this transporter preserves the activity of this drug in the absence of all other folate transporters. Studies are exploring the role of PCFT in the activity of pemetrexed and other antifolates (Mol Pharm, 74:854, 2008).

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Key Words: ***AIDS, HIV, hematopoiesis, gene therapy, virology***

We are investigating various aspects of HIV infection using novel murine models we developed that are chimeric for the human immune system. Human thymus and liver implanted in mice with an intrinsic defect in their B cells and T cells (thy/liv-SCID-hu mice) grow significantly and repopulate the periphery of these mice with human T cells. The significant numbers of human T cells present in the periphery of our thy/liv-SCID-hu mice permits the development of disseminated HIV infection in these mice after either intraimplant injection or intraperitoneal inoculation of HIV. In addition, we have developed novel transgenic mice expressing human CD4 and CCR5 or full length HIV that develop HIV infection. These mice are being used to explore the immunopathogenesis of HIV infection including the investigation of the mechanism of HIV dissemination, the effect of HIV on thymic maturation, the effect of cytokines on HIV replication, the emergence of phenotypic variants during in vivo infection and the efficacy of various therapeutic interventions targeted to HIV.

We have also developed a novel model to study in vivo human hematopoiesis and gene therapy. Irradiated SCID mice transplanted with cultured human bone marrow cells (BM-SCID-hu) become significantly engrafted with human lymphoid and myeloid precursor cells in the bone marrow and mediate the population of the peripheral lymphoid tissues with mature human lymphoid and myeloid cells. These mice are being used to understand what cytokines, stromal cells and signals are required for the maturation of human hematopoietic cells in the bone marrow and for the subsequent population of the peripheral tissue. This model system is also being used to investigate gene therapy using human hematopoietic stem/precursor cells by examining the expression of vectors transfected into these human stem cells. In addition, we are using this model to investigate how inappropriate expression of different cell cycle regulatory genes and oncogenes affects hematopoiesis and potential induction of malignant transformation.

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Key Words: ***Cancer, pediatric malignancies, osteosarcoma, antifolate resistance, mesenchymal stem cells, insulin like growth factor receptor***

My clinical interests are focused upon the care of children, adolescents and young adults afflicted with sarcomas. My clinical research activities include Phase 1, 2 and 3 clinical trials as well as supportive care, biology and quality of life studies as institutional, Children's Oncology Group and the Sarcoma Alliance for Research through Collaboration Group studies. The longstanding focus of our laboratory has been the mechanisms of antifolate resistance that are observed in osteosarcoma. We seek to understand how alterations in membrane transport influence sensitivity versus resistance to antifolates and how normal folate requirements are met in the context of these alterations. We are interested in defining the signal transduction pathways that are relevant to osteosarcoma in part to identify key genes involved in osteosarcoma pathogenesis. It is felt that these signal transduction pathways may be amenable to inhibition by targeted therapies enhancing the standard treatment with cytotoxic chemotherapy. Much of our current efforts are directed towards the IGF-1R signaling pathway. We are interested in understanding the cell of origin of osteosarcoma, which may be a mesenchymal stem cell or a more differentiated osteoblast. We are exploring further, the genetic pathways that drive these cells towards an osteosarcoma phenotype. The laboratory performs preclinical drug studies utilizing osteosarcoma xenografts as a site for the National Cancer Institute funded Pediatric Preclinical Testing Program. Our laboratory serves as a national osteosarcoma tissue repository for the national cooperative group, the Children's Oncology Group.

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Key Words: ***Cancer vaccines, listeria-based vaccines, breast cancer, metastasis, curcumin, aging***

Breast cancer is the most common cancer among women around the world, and 40% of the women diagnosed with breast cancer will progress to metastatic disease, which is primarily responsible for patient mortality. Current treatment is not effective against metastases. Recently, our laboratory has developed DNA-based cancer vaccines that are highly effective against metastases. Various approaches are now under investigation to improve cancer vaccination for application in human clinical trials.

LISTERIA-BASED CANCER VACCINES

We have developed vaccines encoding tumor-specific antigens using an attenuated non-toxic and non-pathogenic bacterium *Listeria monocytogenes* (LM). LM delivers tumor-specific antigens with high efficiency directly into antigen presenting cells, resulting in activation of tumor-specific cytotoxic T lymphocytes (CTL) that subsequently kill tumor cells. Very recently, we discovered that Listeria infect and kill tumor cells in vivo through (1) the generation of high levels of reactive oxygen species (ROS), and (2) Listeria-specific CTL-mediated cytolysis. This novel discovery creates complete new directions in the development of cancer therapies, i.e., any DNA sequnce that improves tumor cell death can be included. Various approaches to improve tumor cell death in vivo by Listeria-based vaccines are currently under investigation in our laboratory.

COMBINATION THERAPIES

Most tumors produce factors that inhibit vaccine-induced T cell responses in vivo. One such factor is interleukin (IL)-6. Currently, we are investigating combination therapies of Listeria-Mage-b vaccine and Curcumin. Curcumin is a non-toxic Indian spice that down regulates IL-6.

THE IMPORTANCE OF THE AGE FACTOR IN CANCER VACCINATION

More than 50% of all cancer patients are 65 years or older. However, vaccines are less effective at older age than young age, due to T cell unresponsiveness. Our laboratory found that CD8 T cell anti-tumor immune responses are strongly reduced, but innate immune responses (macrophages and natural killer cells) are still active at old age. Therefore, we are modifying our vaccines using alternative approaches, i.e., Listeria-based vaccines that activate innate immune responses and induce apoptotic pathways in tumor cells at older age.

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Key Words: ***epigenetic, chromatin, cytosine methylation, bioinformatics***

EPIGENOMICS IN HUMAN DISEASE

The genome is used in different ways in multicellular organisms to establish and to maintain cellular differentiation and fates. Transcriptional programming of this type requires some ability to maintain a memory of a differentiation state through cell division, a property usually described as epigenetic. The mediators of such epigenetic regulation potentially include the positioning, types and post-translational modifications of histones within nucleosomes, the methylation of DNA, the influence of short RNAs and chromatin looping in three dimensions.

The malleability of the epigenetic and transcriptional programs in the cell allow adaptation to the environment. However, the same adaptive and flexible processes used to the cell's advantage can acquire errors and lead to pathological changes. As a consequence, genome-wide epigenetic studies (referred to as epigenomic studies) can give insights into disease states, originally exemplified by cancer, but now including everything from diabetes mellitus to Alzheimer's disease.

The Greally lab has interests in both basic science and clinical research, focusing on epigenomic processes. We have a long-standing interest in cytosine methylation, having developed genome-wide assays that interrogate not only promoters of genes but also the other genomic contexts that are usually ignored, finding patterns of regulation in normal cells that serve to guide understanding of changes observed in human disease states. The question of how this methylation mark is read by the cell has prompted us to initiate a program in structural epigenomics with colleagues in Biochemistry, and the development of a new approach to identify R-loop forming DNA in mammalian cells. Our human disease interests include type 2 diabetes mellitus arising as a consequence of intrauterine growth restriction, chronic kidney disease, Huntington's disease, asthma and allergy, breast cancer, and viral infection of cells.

We recognize that computational skills are now an essential part of epigenomics research, making this a major focus of the lab. The same combination of molecular and computational approaches to study basic science and clinically-relevant questions defines Einstein's Center for Epigenomics, directed by the PI, bringing new technological and computational resources to Einstein as a whole.

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Key Words: ***radiobiology, hepatocyte transplantation & tissue regeneration, tumor vaccines, radiation therapy, high intensity focused ultrasound (HIFU), MR spectroscopy***

Stem cell based therapies for radiation-induced gastrointestinal syndrome. The radiation biology laboratory is investigating the molecular pathways of radiation-induced normal tissue injury and their therapies. We have developed murine and rodent models of radiation-induced gastrointestinal syndrome to investigate "late effects" of radiation on DNA repair, senescence and ageing. Our goal is to transplant mesenchymal stem cells and endothelial progenitor cells in order to restore the irradiated host intestinal stem niche and to protect against irradiated-induced cell death, thus providing mitigation from radiation induced gastrointestinal syndrome.

Preparative irradiation for hepatocyte transplantation. We were the first to use liver irradiation as a preparative regimen for liver cell transplantation. Using radiation-based preparative regimen for transplantation of hepatocytes, liver progenitior cells and bone-marrow-derived stem cells, we have ameliorated a variety of metabolic liver diseases and are expanding this strategy to restore liver function in end-stage liver diseases, such as, cirrhosis. Furthermore, we have used a preparative regimen of focal irradiation, delivered by stereotactic radiosurgery (SRS), to ablate parenchymal cells in various organs and create a microenvironment that promotes the engraftment, growth and differentiation of progenitor / stem cell in vivo.

Autologous *in situ* tumor vaccines. Using focal delivery of physical agents, such as, irradiation and therapeutic ultrasound, we have induced unfolded protein response in tumor tissues. Subsequently, irradiated and HIFU-treated tumor cells served as a source of tumor antigens in vivo, where RT- or HIFU-treated tumor cells would provide danger signals and release tumor antigens for DC activation. The long-term goal is to design novel tumor vaccines and amplify the immune response to such vaccines with primary tumor therapy, RT, HIFU and chemotherapy. Various clinical trials are being designed to test these hypotheses.

Development of novel radiosensitizers. Our long-term goal is to screen for drugs that can radiosensitize tumor cells without having undue normal tissue toxicity. Since, majority of tumor cells have aberrant G1 checkpoint, irradiated tumor cells depend upon the G2 checkpoint for survival. Thus, novel drugs that target the cell cycle checkpoints, especially the G2 checkpoint, is being investigated as radiosensitizers. Molecular targets include proteins that participate in the DNA damage surveillance pathway, such as, ATM and Plk1.

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Key Words: ***reactive oxygen intermediates, hypertension, hyperglycemia, diabetes, radio-labeling, leukocytes***

NMR STUDIES OF INTRACELLULAR METAL IONS AND OXIDATIVE STRESS

We have been interested in elucidating the role of intracellular mineral ions and oxidative stress in the pathophysiology of essential hypertension and type 2 diabetes. Our main research tool is NMR spectroscopy, a biophysical technique that combines the advantages of noninvasiveness and high specificity for measuring intracellular mineral ions. Oxidative stress, which may play a contributory role in the pathogenesis of diabetes as well as hypertension, causes generation of reactive oxygen intermediates which can be monitored by magnetic resonance techniques. We have previously demonstrated abnormal intracellular ion handling in hypertension resulting in increased intracellular sodium, free calcium and decreased intracellular potassium and free magnesium. Our results also supported the hypothesis that oxidative stress can induce derangement of intracellular ion balance which may, at least in part, account for the abnormal potassium, sodium and free calcium ion balance observed in hypertension. Our specific aims are (1) to investigate the role of altered renal sodium homeostasis in salt-sensitive hypertension; (2) to demonstrate that oxidative stress, which results in overproduction of reactive oxygen species (ROS), can cause loss of unsaturation in membrane fatty acyl chains; (3) to investigate a possible protective role of magnesium against oxidative stress-induced damage, (4) to investigate increased vulnerability of hypertensive as well as diabetic kidney and myocardium to ischemic damage and its relationship to increased peroxidative degradation of membrane lipids. Investigations of intracellular ions and membrane lipids in hypertension and uncontrolled hyperglycemia may eventually lead to better strategies for the management of these health disorders.

^{64}CU-LABELED LEUKOCYTES FOR PET IMAGING OF INFLAMMATION AND INFECTION

This collaborative project involves radio-labeling WBC with positron emitter ^{64}Cu for PET imaging of inflammation and infection. The aim of our work is to introduce the use of WBC labeled with a positron emitting radio ligand for clinical applications of PET imaging. Advantage of using PET radio tracer Cu^{64} (short half life of 12.7 h), instead of radioactive ^{111}In (half life 67 h) for gamma-ray imaging, is that PET radiotracer approach is more sensitive and provides much better image resolution to detect regions of interest. However, unlike indium, Cu usually leaks out of the cell. We have been successful in labeling WBC with ^{64}Cu, using intracellularly localized metal ion chelators which are introduced in the membrane permeable acetoxy methyl ester form and are hydrolyzed by intracellular esterases to yield highly charged anionic forms that bind Cu^{64} and remain localized within the cell. Our aim here is to find the best approach for radio labeling leukocytes with Cu and testing the radio labeled leukocytes in animal models of infection. As ^{64}Cu-labeled leucocytes adhere to sites of infection and inflammation, their location can be imaged with high sensitivity and resolution using PET scanning.

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Key Words: ***development, C. elegans, ultrastructure, informatics***

The soil nematode *Caenorhabditis elegans* is a model system used to study the genetic control of cellular development. Our laboratory specializes in ultrastructural studies of the nervous system. We use serial thin sections, electron microscopy, electron tomography and immunocytochemistry as primary tools to follow the development of identified neurons, particularly their axon outgrowth and synaptic connectivity. In addition, we are investigating the ultrastructure of many other nematode tissues, including muscle, intestine, and the germline, and processes such as aging, autophagy and necrotic cell death.

Many of our studies involve anatomical defects in genetic mutants or RNAi-induced gene knockdowns. The Hall lab acts in collaboration with outside investigators in most studies. In recent years we have investigated the basis for tubulogenesis in several tissues, synaptic defects and sensory ending defects in mutants and in transgenic strains, defects in the basal lamina, etc. The Hall lab is also working in concert with Dr. Scott Emmons on the complete reconstruction of the wild type nervous system of the adult male. In collaboration with Dr. Adela Ben-Yakar at U. Texas, we are studying neuronal recovery and regrowth after laser-induced breakage of axons and dendrites.

The Hall lab is also responsible for the creation and maintenance of several important websites (www.wormatlas.org; www.wormimage.org; www.gfpworm.org) that teach the anatomy of the nematode. This informatics work is supported by a grant from NIH Division of Research Resources to Dr. Hall. Together with Dr. Zeynep Altun, Hall has recently published a major anatomy text on *C. elegans*.

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Key words: ***Diabetes, insulin resistance, glucose effectiveness, global diabetes***

Dr. Meredith Hawkins is Professor of Medicine and Director of the Global Diabetes Initiative at the Albert Einstein College of Medicine. Her current research interests include the regulation of hepatic glucose production by hyperglycemia per se in type 2 diabetes mellitus, and the effects of nutrient excess on metabolic features of the insulin resistance syndrome. Challenging a generally 'insulin-centric' view of hepatic glucose metabolism, Dr. Hawkins has highlighted the importance of defective 'glucose effectiveness' to suppress glucose production in diabetes mellitus. Significantly, she proved the efficacy of several therapeutic modalities to restore this regulation in humans with diabetes: activating hepatic glucokinase, normalizing circulating fatty acid levels, and inhibiting gluconeogenesis. More recently, Dr. Hawkins has made novel and important observations about the role of fatty acids in systemic inflammation, through effects on adipose tissues macrophages. Her work suggests that adipocyte-derived factors "prime" adipose macrophages to respond to nutritional regulation. Additionally, Dr. Hawkins has tremendous interest in the burgeoning epidemic of obesity and diabetes in the developing world.

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Key Words: ***adhesion, cadherins, growth factors, signal transduction, cancer, cell cycle, metastasis***

REGULATION OF BREAST CANCER METASTASIS BY CELL-CELL ADHESION

Cell-cell adhesion is a primary modulator of morphogenetic events during normal embryonic development. Metastatic dissemination of epithelial tumor cells is also strongly influenced by the activity of cell-cell adhesion molecules, in particular members of the cadherin family. My laboratory has shown that N-cadherin, a cadherin involved in dynamic processes such as cell migration and neurite outgrowth, is upregulated in invasive breast cancer cells and promotes metastasis of breast cancer cell lines. In contrast, E-cadherin, known to promote stable epithelial contacts, is lost during metastatic progression. Our data suggest that cadherin switching during tumor progression has a broader consequence than a simple change in cell-cell adhesion. The shift in cadherin expression also affects proteolytic activity of cells, their migration, invasiveness and metastasis. We and others have shown that even in the presence of E-cadherin, and strong cell-cell adhesiveness, N-cadherin can convert poorly invasive breast cancer cell lines into invasive and metastatic tumors, thus suggesting a dominant role for N-cadherin in this process. These effects of N-cadherin are mainly due to a functional cooperation with the FGF receptor resulting in epithelial to mesenchymal transition, cell signaling and morphological changes leading to metastasis. Thus our hypothesis is that N-cadherin upregulation in tumor cells is a key step in a series of interdependent molecular changes which lead to metastasis.

More recently, we discovered that Retinal cadherin (R-cadherin, a classic cadherin highly expressed in the brain and retina is also present in the mammary epithelium. We showed that similarly to E-cadherin, R-cadherin acts as an invasion suppressor gene which is downregulated in invasive breast carcinomas. Moreover, R-cadherin knockdown in mammary tissue leads to disruption of morphogenesis and gain of metastastic properties. Conversely, R-cadherin expression in aggressive breast tumor cells suppresses invasion and metastasis, and restores glandular morphogenesis.

My lab is currently investigating:
1) the molecular basis for cooperation between N-cadherin and the FGF receptor responsible for metastasis with the goal of obtaining functional inhibitors of metastasis;
2) the signaling pathways downstream of N-cadherin and FGFR responsible for metastasis;
3) the relationship between cell cycle progression and metastasis and,
4) the mechanism for breast cancer suppression by R-cadherin.

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Key Words: ***brain, stem cells, neurogenesis, genetics, development, regeneration***

WHAT FACTORS REGULATE NEURAL STEM CELL FATES IN THE FOREBRAIN?

The Hébert lab is interested in two broad questions: how the forebrain develops and how parts of it can be regenerated in the adult. In particular, we are interested in understanding how a simple sheet of neuroepithelial cells early in embryogenesis can develop into the adult cerebral hemispheres, the part of our brains that we use for our highest cognitive and perceptual functions. Essential to this understanding is the identification of the signals that pattern the early forebrain and regulate the fate of neural stem cells and progenitor cells throughout development and in the adult.

The primary approach we are using to test the roles of candidate signaling molecules in embryos, postnatal animals, and adults is a conditional genetic approach in the mouse. This approach, which uses CRE/loxP technology, allows us to test the function of particular factors by deleting or overexpressing the genes that encode them specifically in the cerebrum. In addition, studies in the adult also require approaches including stem cell transplants and viral delivery of genes to evaluate the feasibility of using genetically modified neural progenitor cells, alone or in combination with modified cellular environments, to achieve regeneration of damaged forebrains.

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Key Words: ***herpes simplex virus, mucosal immunity, inflammation, clinical trials***

The overall goal of the basic work being conducted in our laboratory is to identify the signaling pathways required for HSV-2 invasion and exploit this knowledge to develop novel strategies for prevention. Current work from our laboratory demonstrates that HSV activates calcium (Ca^{2+}) and phosphorylation signaling pathways and that these signaling pathways play critical roles in the establishment of infection.

In related studies, we are also exploring how HSV overcomes mucosal immunity to initiate infection. Work from our lab indicates that cervicovaginal secretions obtained from healthy women protect against HSV infection and substantially reduce viral yields. Mechanistic studies suggest that this intrinsic activity is mediated in part by defensins with contributions from secretory leukocyte protease inhibitor (SLPI) and possibly other proteins. Currently we are expanding these studies and conducting a clinical trial to evaluate mucosal immunity in the genital tract in cycling and non-cycling women, focusing on the intrinsic anti-HSV and anti-bacterial activity found in genital secretions from healthy women.

A second major focus of work in the lab is to identify the components that contribute to this innate protection, and by using purified components and recombinant proteins, defining the mechanism of activity. Additionally, we are testing the hypothesis that HSV triggers changes in the mucosal environment, which allow it to escape cervical secretion defenses, enhance its own infectivity and facilitate HIV co-infection. Our preliminary observations support the paradigm that HSV disrupts the epithelial barrier by targeting tight junction and adherens junction proteins, and interferes with host defenses by triggering an inflammatory response and a loss in protective proteins such as SLPI. These changes could facilitate both its own infectivity and enhance HIV co-infection. We are conducting a clinical study in parallel to examine the mucosal environment among women with active genital herpes and healthy controls.

Results obtained from this bench research are critical to the laboratory's translational studies. The focus of the Translational Microbicide Research Program is to identify optimal combinations of topical microbicides that are safe and target different steps in HIV life cycle, thus reducing the risks of drug resistance and providing greater protection than could be achieved with a single agent, and also target HSV infection. Candidate combinations are evaluated using a multi-tiered approach for anti-viral activity and safety using human cervical cultures, as well as primary T cells and macrophages, in the presence of cervicovaginal secretions and seminal plasma. Leading combinations are then evaluated in human explant cultures (cervical, vaginal) and in murine genital models and a new cotton rat model for anti-viral activity and for the impact on mucosal immunity. If results of these pre-clinical studies suggest that candidate microbicides are safe and effective, the drugs are advanced for regulatory testing, and undergo evaluation in Phase I clinical studies.

Clinical research interests also include prevention of infectious disease complications in transplantation. Members of the research group are involved in studies to optimize pre-emptive prophylaxis for CMV and EBV, vaccine responses in transplantation recipients, and other related infectious complications.

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***Key Words**: nicotine dependence, schizophrenia, autism, mental retardation, VCFS, 22q11, signaling, genetically engineered mouse model, lentiviral gene transfer*

A major aim of this laboratory is to understand more fully the genetic basis of neuropsychiatric disorders using genetically engineered mouse models. To achieve this goal, the Hiroi laboratory has recently focused on two tractable research topics: nicotine dependence and 22q11 syndrome.

NICOTINE DEPENDENCE

The core nature of nicotine dependence is evident in wide variations in how individuals become and remain smokers. Individuals with pre-existing behavioral traits are more likely to develop nicotine dependence and experience difficulty when attempting to quit. Many genes likely contribute to individual variations in the development of nicotine dependence and behavioral traits in complex manners. However, the identification of such genes has been hampered by the phenotypic complexity of nicotine dependence and the complex ways molecules affect elements of nicotine dependence. We use behavioral paradigms to globally model distinct aspects of nicotine dependence in mice and examine how specific genes contribute to diverse aspects of nicotine dependence and pre-existing behavioral traits. Our studies so far have revealed that the transcription factor FosB, monoamine oxidase A and cGMP-dependent protein kinase (PKG) are required for nicotine cue reactivity. We are currently identifying specific brain regions through which PKG mediates the expression of nicotine cue reactivity using a PKG-carrying lentiviral vector.

22q11 AND NEUROPSYCHIATRIC DISORDERS

A surprisingly large number of kilo- to mega-base copy number variations (CNVs) exist in the human genome and are associated with autism spectrum disorders, mental retardation and schizophrenia. Human chromosome 22q11.2 is considered a hotspot of CNVs. Children and adolescents with 22q11.2 duplications and deletions consistently exhibit cognitive and intellectual impairments during development, and they are often diagnosed with autism, mental retardation and schizophrenia. The association between 22q11.2 duplications/deletions and developmental cognitive impairments is remarkably consistent and replicable. However, because the diagnosis of autism, mental retardation and schizophrenia include variations in diverse cognitive and intellectual capacities, the precise nature of cognitive impairments caused by 22q11.2 duplication/deletions remains unclear. Moreover, duplications/deletions of 22q11.2 encompass 1.5 Mb or larger regions, making it impossible to determine whether segments or single genes are responsible for phenotypes in humans. Our laboratory has identified two small human 22q11.2 segments whose over-expression causes, during development, behavioral phenotypes relevant to neuropsychiatric disorders in mice. Our current work examines the role of each of the genes encoded in the segments in behavioral phenotypes that model elements of neuropsychiatric disorders in mice.

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Key Words: ***Rho GTPase, fluorescence resonance energy transfer (FRET), fluorescent biosensor, live-cell imaging, spatiotemporal dynamics.***

SPATIOTEMPORAL DYNAMICS OF RHO-FAMILY GTPASES IN LIVING CELLS, VISUALIZED BY FLUORESCENT BIOSENSORS

P21 Rho family small GTPases are critically important in many disease processes including malignant cancers, developmental defects, arthrosclerosis and autoimmune dysfunction. This class of signaling molecules is critical in these diseases by impacting directly: cell polarity, motility and migration through their actions on downstream cytoskeleton/adhesion dynamics; and proliferation by intersecting mitogenic and apoptotic signaling pathways. Rho family GTPases regulate these processes by tightly coordinating their activities in response to various environmental cues. Only a very small fraction of GTPases turn on or off at different locations at different times to produce specific effects. Furthermore, most Rho GTPases exist in an interdependent cascade of activation/inhibition pathways resulting in a tight coordination of activation dynamics between each other. It is this coordination of multiple GTPases that is thought to regulate a variety of cellular signaling outcomes. However it has been difficult if not impossible to dissect the spatiotemporal dynamics of signal regulation by conventional imaging or biochemical techniques.

My primary research interest is the development of fluorescent biosensors to visualize and decipher these complex spatiotemporal dynamics of protein activations in living cells in real time. These biosensors enable direct visualization of the spatiotemporal dynamics of protein signaling pathways at high resolution, previously inaccessible by traditional biochemical methods. Knowledge gained from these studies will open a new window into previously unseen, coordinated mechanisms of GTPase signal regulation.

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Key Words: ***HIV-associated neurological disorders, liver cancer, protein dynamics, proteomics, target discovery & biomarkers***

Proteomics provides an unbiased window on the workings of the cell, and can provide cues to functional protein complexes, biomarkers and potential therapeutic targets. Three projects are:

1. More than a quarter of those infected with HIV exhibit some form of cognitive impairment. The Einstein Proteomics Research Center for HIV-Related Neurological and Substance Abuse is investigating the mechanisms underlying the development of cognitive impairment in HIV infected individuals even in the face of modern CART therapy. Quantitative proteomics technologies are being used in animal and tissue culture models and in human subjects, in a collaboration with Drs. Harris Goldstein, Joan Berman, Andras Fiser, Louis Weiss and Julia Arnsten. These studies will help identify new therapeutic targets for preventing the progression neurological consequences of AIDS.

2. Proteomic studies of the development of hepatic neoplasia in rat are being translated into studies with human patients. Serum biomarkers are being identified to help identify patients with early stage hepatocellular carcinoma in a collaboration with Drs. Allan Wolkoff and Milan Kinkhabwala. Our laboratory has also developed proteomic tools for identification of tubulin isotypes and posttranslational modifications. These tools are being used to identify changes in tubulin composition associated with development of metastasis or drug resistance, in collaboration with Dr. Susan Band Horwitz.

3. Our laboratory probes protein dynamics and protein complexes in solution using the tools of hydrogen-deuterium exchange coupled to mass spectrometry and novel technologies for enrichment of crosslinked partners at protein contact sites. The details of molecular structure and interactions reveal mechanistic insights.

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Key Words: cancer, Taxol, drug resistance, microtubules

The research program in Dr. Horwitz' laboratory is focused on: 1) the development of new drugs derived from natural products, such as Taxol, for the treatment of malignancies and 2) the mechanisms by which cells become resistant to antitumor drugs. The mechanism of action of Taxol, an antimitotic agent that enhances the polymerization of tubulin by forming stable microtubules, is being pursued. The novel structure of Taxol, its unique mechanism of action that was first described in her laboratory, and the positive results that have been observed in ovarian, breast and lung carcinomas generated extensive interest in this drug. Although there was no interest in Taxol when she began her studies, today the drug has been given to over a million patients. Her goal is to understand, at a molecular level, the interaction of Taxol with the microtubule and the mechanisms by which the drug induces growth arrest and cell death. Recent evidence indicates that Taxol alters specific intracellular signal transduction events essential for drug-induced cell death. Newly discovered potentially important antitumor drugs, such as the epothilones and discodermolide that are currently in clinical trials and whose mechanism of action is similar to that of Taxol, are being actively investigated. Dr. Horwitz has searched for differences between these agents that could be exploited in the clinic and her laboratory has reported that discodermolide is the first microtubule stabilizing agent that includes a powerful induction of accelerated senescence in its repertoire of tumor cell growth inhibitory mechanisms. Dr. Horwitz and her collaborators are developing quantitative mass spectrometric-based methods to analyze the expression of tubulin isotypes and their posttranslational modifications. This is crucially important since there is accumulating evidence in human cancer cell lines and tumors that specific isotypes exhibit differential sensitivity to Taxol. Therefore there is a need for rapid, sensitive and accurate methods for assessing tubulin composition. In addition, hydrogen/deuterium exchange coupled to liquid-chromatography-electrospray ionization MS is being used to study conformational effects induced by Taxol and other stabilizing agents on microtubules. Dr. Horwitz is committed to using the knowledge gained in her laboratory for the development of therapies for the treatment of human cancer. Taxol-resistant cell lines derived from mammalian tumor cells growing in tissue culture have been developed as model systems for studying drug resistance. Some of these cell lines display mechanisms of Taxol-resistance related to alterations in normal tubulin isotype expression, mutations in alpha- and beta-tubulin, and endogenous proteins such as MAPs and stathmin that modulate drug resistance through their interactions with microtubules. Methodology has been developed that utilizes high-resolution isoelectrofocusing combined with mass spectrometry to analyze tubulin mutations in cell lines and human tumors. In collaboration with Dr. Hayley McDaid, new drug combinations of microtubule stabilizing drugs and signaling inhibitors are being evaluated in xenograft models of human lung cancer.

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Key Words: ***trypanosomes, differentiation, cAMP, pathogenesis, cardiomyopathy***.

PATHOGENESIS OF CHAGAS DISEASE AND MECHANISMS OF TRYPANOSOMA CRUZI DIFFERENTIATION

Studies are aimed to define the pathogenesis of Chagas disease and derive methods and drugs to treat the disease. We propose to examine how the parasite causes cardiomyopathy and vascular pathology. Two pathogenic pathways will be investigated, vasoconstriction/ischemia and inflammation. In addition, the process of remodeling of the infected heart resulted in cardiomyopathy is also under investigation.

The life cycle of *T. cruzi* is complex. Understanding the mechanisms of its differentiation will lead to the design of drugs for the disease. Study in the signal transduction pathways leading to the differentiation is ongoing and cAMP pathway in *T. cruzi* is the focus. Reversible protein phosphorylation is one of the most important biological mechanisms in many organisms. Our research has found that PKA activity is essential for *T. cruzi* and many important substrates of PKA in *T. cruzi* were identified. To better understand the biology of this organism, both global proteomic and bioinformatics approaches will be applied to discover additional PKA substrates. We also found that type I protein phosphatases in *T. cruzi* (TcPP1s) are important in regulating biological processes. Recently, we have identified seven PP1s in the *T. cruzi* genome. Four of these TcPP1s were expressed as enzymatically active. Two TcPP1 regulatory subunits were also identified in the genome. A detailed characterization of all seven TcPP1s and two regulators will be performed, including enzymatic assays and inhibitor profiles with purified recombinant proteins. The mRNA and protein levels will be examined during growth and differentiation. Gene deletion and dominant negative blockade of TcPP1 will be applied to define the importance of each phosphate in the organism. Yeast two hybrid assays and proteomics techniques will be used to identify TcPP1 associated proteins. Overall, these studies will allow us to fully understand the regulating network involved in reversible phosphorylation in *T. cruzi*.

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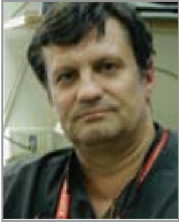

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Key Words: ***connexins, genomic fabrics, intercellular*** Ca^{2+}***- signaling***

CONNEXIN-DEPENDENT NETWORKS IN CONTROLLING THE GENOMIC FABRICS OF VITAL FUNCTIONS

The ability of the biological systems in preserving the vital functions regardless the environment change indicates that the genomic fabrics of these functions are very flexible, with several reserve gene networks on place. We have defined the genomic fabric Ξ of a functional pathway as the triplet (Γ, Π, Θ), where: Γ is the set of the composing genes (γ = number of genes), Π is the transcriptomic profile (set of 95% confidence intervals of expression levels) and Θ is the gene networking within the fabric. The fabric is expected to exhibit characteristic gene composition, expression level, control and intercoordination depending on region, sex and age, respond by transcriptomic tuning to environmental constraints and be sensitive to disease activity.

Our laboratory uses a variety of molecular and computational techniques to identify and characterize the genomic fabrics and the transcellular, connexin-dependent transcriptomic networks that control essential processes such as myelination, heart rhythm, and inflammatory response. Experimental data are then used to develop mathematical models and computer simulation programs of intercellular signaling in complex structures. The connexins (Cx) comprise a family of topologically similar transmembrane proteins that can assemble to form intercellular gap junction channels between cells within vertebrate animals. Such intercellular channels provide cytoplasmic continuity between the interconnected cells and play crucial roles in cell growth, differentiation and synchronous activity within specialized tissues. In connexin-dependent networks, linkage partners are rearranged and strengths modified in both transgenic and diseased animals, in response to pathologic or stressful conditions, as well as during development.

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MOLECULAR GENETIC APPROACHES TOWARDS CONTROLLING MULTI-DRUG RESISTANT TUBERCULOSIS

Tuberculosis, caused by Mycobacterium tuberculosis, causes one in four avoidable deaths in the Third World and kills more adults than malaria, AIDS, and all tropical diseases combined. In recent years, there have been dramatic increases in the numbers of new cases worldwide - one of the consequences of the AIDS epidemic. In addition to these increasing incidences, there have been an emergence of M. tuberculosis strains that are resistant to all seven anti-tuberculosis agents. These alarming trends have caused the World Health Organization to declare tuberculosis a global health emergency, a distinction never accorded another disease. My laboratory has focused its efforts on developing systems to genetically manipulate mycobacteria, particularly M. tuberculosis.

These tools have allowed us to:
1) develop the luciferase reporter phage assay for rapid assessment of drug susceptibilities,
2) analyze the genes involved in resistance to tuberculosis drugs such as isoniazid, ethionamide, and etyhambutol, and
3) to identify specific phenotypic properties associated with a tuberculosis pathogenesis. Current research efforts are aimed at identifying genes involved in the virulence of M. tuberculosis, identifying novel drug targets, and engineering attenuated mutants of M. tuberculosis that can be used as live-cell tuberculosis vaccines.

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MAGNETIC RESONANCE OF MOUSE MODELS OF HUMAN DISEASE

Our lab focuses on developing and applying MRI based methods to study mouse models of human disease. Through synergistic collaborations with other labs we have studied mouse models of cardiac hypertrophy, heart failure, diabetic cardiomyopathy, chagasic cardiomyopathy, obesity, and cancer. We also incorporate other technologies (microPET, non-invasive blood pressure monitoring and ECG) in multimodality studies of mice and are involved in studies aimed at developing targeted MRI and microPET agents. Serial in vivo MRI studies permit the evaluation of the same mouse over a chronic time period. An understanding of the changes in morphology and function that occur in the organs of the living animal during the progression of disease is useful for designing strategies to prevent or limit development of debilitating disease in humans.

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Key Words: ***cell polarity, cell migration, Drosophila, genetics, cancer, RNAi, kinases, cytoskeleton***

PLANAR CELL POLARITY SIGNALING: A MECHANISM FOR CELLULAR POLARIZATION.

To perform many of their functions, most epithelial cells are polarized within the plane of the epithelium, commonly referred to as epithelial planar cell polarity (PCP). The cellular consequences of PCP signaling range from coordinated organization of cytoskeletal elements in single cells to complex migration of groups of cells. Obvious examples of PCP in vertebrates are the ordered arrangement of scales on fish and hairs of mammalian skin. A less visible example is the arrangement of stereocilia in the inner ear, which is essential for hearing. Furthermore, left-right body asymmetry and the complicated movement of mesenchymal cells during gastrulation (called convergent extension) that leads to the elongation and thinning of the body axis also depend on correct PCP signaling. Aberrant PCP signaling leads to neural tube defects such as Spina bifida and cystic kidneys.

PCP signaling is, however, best studied in *Drosophila melanogaster*, mainly because of the versatility of the fly as model system. In *Drosophila*, PCP can easily be seen, e.g. looking at the precisely aligned hairs on wing cells. Genetic and molecular studies led to the identification of a signaling network – the non-canonical Wnt pathway – directing PCP establishment. In recent years it has become apparent that the PCP signaling module is highly conserved from insects to ascidians and humans and is one of the most exciting topics of developmental biology today.

Due to the available tools and the possibility to use a combination of genetic and biochemical approaches, *Drosophila* is ideally suited to further dissect the PCP pathway and define its relationship to the cytoskeleton. My lab is particularly interested in how the Fz-adapter protein Dsh is regulated by phosphorylation. We have identified candidate kinases and a phosphatase in a systematic molecular screen based on RNAi. Current projects further address the functional relevance of these kinases. We also study two additional kinases, Nemo and Rho kinase that have previously been shown to be required for the migration aspect of PCP establishment and – in case of Rock – also for tumor cell migration during cancer progression.

It is our goal to use *Drosophila* as model system to address fundamental questions that are relevant for development and disease.

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Key Words: ***Postsynaptic density, synaptic neurotransmission, proteomics, mass spectrometry, synapse to nucleus signaling, RNA binding proteins***

EXPLORING SYNAPTIC FUNCTION AND ACTIVITY-DEPENDENT SYNAPSE-TO-NUCLEUS SIGNALING

One of the principal questions in neuroscience is how does neuronal activity alter synaptic transmission. This question is critically important since activity-dependent changes in neurotransmission regulate higher order brain functions such as learning and memory. Our lab is interested in understanding how do transient changes in synaptic neurotransmission become long-term. Specifically we are interested in exploring activity-dependent synapse-to-nucleus signaling in neurons. The activity-dependent regulation of nuclear functions is essential for the long-term maintenance of synaptic strengthening and the long-term storage of memories. While the nature of this signaling pathway is widely debated, it is well known that neuronal activity results in the rapid nuclear accumulation of many proteins, including AIDA-1, Jacob, NFATc4 and NF-kB, suggesting that the nucleocytoplasmic shuttling of proteins is a mechanism in nuclear signaling. We seek to understand how synapses relay fast synaptic information to the nucleus and specifically what are the key players in this process, what signals they respond to and what are their nuclear functions. To study this, we use proteomics and mass spectrometry to explore the composition and dynamics of excitatory synapses and nuclei. These methods provide us with a global view of synaptic complexity as well as help us identify novel components of synapse-to-nucleus signaling mechanisms, which we can then further study using reductionist methods in cell and molecular biology as well as biochemistry and imaging analysis. Using these methods we found that a number of synaptic components can shuttle to the nucleus in response to synaptic activity. We also found that a number of nuclear proteins are incorporated into the synapse in response to synaptic activity suggesting that the reverse pathway, nucleus-to-signaling pathway also affects synaptic transmission. Our immediate goal is to study these novel synapse to nucleus signaling messengers and to explore their synaptic and nuclear functions.

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Key Words: HIV, cancer, chromatin, retroviruses, tumor suppressor

ROLE OF INI1/hSNF5 IN HIV-1 REPLICATION AND CANCER

INI1/hSNF5 is a tumor suppressor gene mutated in a majority of atypical teratoid and habdoid tumors (AT/RT), an aggressive childhood malignancy. We originally isolated this gene as an interacting partner for HIV-1 integrase and subsequently demonstrated that it is a component of the SWI/SNF complex that remodels the chromatin in an ATP-dependent manner. We are interested in (i) understanding the role of INI1 in HIV-1 replication; (ii) exploring its potential as a drug target for intervention of AIDS; (iii) understanding the mechanism of tumor suppression by INI1/hSNF5; and (iv) dissecting its role in chromatin remodeling. IV-1 integrase is a virally encoded enzyme necessary for the insertion of viral cDNA into the host genome, an essential step in the life cycle of all the retroviruses. We are developing a novel approach of targeting IN-binding host protein, INI1/hSNF5 to prevent HIV-1 replication by interfering with the function of IN. By using molecular genetic approaches, we found that INI1/hSNF5 protein is necessary for HIV-1 replication, is incorporated into the virions and that a dominant negative mutant of INI1/hSNF5 drastically inhibits HIV-1 particle production.

To study the cellular function and mechanism of tumor suppression by INI1, we are isolating the INI1-interacting cellular proteins, using the two-hybrid system. We found that INI1 interacts with c-MYC, a proto-oncogene, and facilitates its transactivation function. We also have found that INI1/hSNF5 causes cell cycle arrest, consistent with its tumor suppressor function. Furthermore, we have identified a hitherto unsuspected nuclear export signal (NES) in INI1/hSNF5. This NES is masked and appear to mediate the hCRM1/Exportin1-dependent nuclear export of the protein. Studies to further characterize this novel property of INI1/hSNF5 and to understand its implications in HIV-1 replication and tumorigenesis is underway.

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Key Words: *Cardiovascular disease, aging, HIV*

1) Cardiovascular disease in HIV-infected individuals: Patients with long-standing HIV infection appear to have accelerated atherosclerosis and increased risk of clinical cardiovascular events. Our ongoing research is examining whether vascular risks in HIV-infected individuals may be due to side effects of antiretroviral medications, sustained elevations of inflammation markers, coinfections, or other sequelae of HIV infection.

2) Health of Latino populations: Albert Einstein was selected as one of four Field Centers for the 16,000-person SOL project ("Study of Latinos"). This will be a landmark study of heart disease, stroke, diabetes, obesity, and other disorders in Hispanic/Latino adults.

3) Insulin-like growth factors and risk of vascular disease and other age-related conditions: Insulin-like growth factor-1 (IGF-I), a major anabolic hormone, is the main mediator of effects of growth hormone and an important regulator of cell cycle/differentiation and cell survival. We are investigating whether the age-related decline in IGF-I levels may contribute to elevated risks of cardiovascular disease, mortality, declining physical function, and other outcomes among older adults.

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Key Words: ***development, genetics, spinal cord, neurons, mouse, chick, C. elegans***

AXON GUIDANCE AND DENDRITE BRANCHING IN THE DEVELOPING NERVOUS SYSTEM

The research in my laboratory is aimed at identifying mechanisms that establish stereotyped patterns of connectivity in the developing vertebrate and invertebrate central nervous system (CNS). Our vertebrate studies are aimed at understanding how growing axons navigate through intermediate targets/choice points and ultimately connect with their appropriate targets, and how axons, which leave the CNS, choose the appropriate exit point. The invertebrate studies are directed toward identifying molecular mechanisms that control dendrite branching. To achieve these goals, we work with both well-studied and understudied classes of vertebrate spinal interneurons and motor neurons and a unique *C. elegans* neuron that extends highly branched dendrites. Our main focus is on understanding the mechanisms that control the pathfinding of spinal commissural axons, a major class of midline-crossing axons in the developing CNS. In these studies, we use novel *in vitro* assay systems, chick *in ovo* electroporation and a large array of transgenic reporter, as well as, mutant mice to identify guidance cues and their corresponding receptors, which regulate the pathfinding of genetically distinct populations of spinal commissural axons. By manipulating guidance receptor expression in mouse and chick embryos, we are also investigating how dynamic changes in the spatial distribution of these receptors influence various aspects of commissural axon pathfinding within the spinal cord. In addition, we are identifying synaptic targets for genetically distinct commissural axons and elucidating the molecular logic that guides specific subsets of commissural axons from the spinal cord to the brain. A parallel effort is aimed at identifying the molecular logic that regulates the development of spinal accessory motor neurons, a unique class of spinal motor neurons that projects axons to and through discrete and readily identifiable lateral exit points. These studies make use of cell surface markers and reporter mice that selectively label spinal accessory motor neurons and their axons, as well as our previous observation that a particular transcription factor is required for the exit of spinal accessory motor axons from the CNS. In ongoing studies, we are attempting to identify cell surface receptors that may directly facilitate the exit of these axons. In our *C. elegans* studies, we have carried large scale RNAi screens to identify molecules that regulate the branching of dendrites associated with the PVD neuron. Thus far, our results implicate a key role for dynein microtubule motors and associated proteins, as well a protein that interacts with the Fragile-X Mental Retardation protein in regulating the proper formation of dendritic arbors in the *C. elegans* nervous system.

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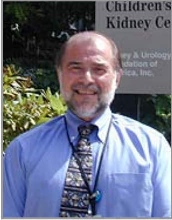

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Key Words: ***progression, kidney failure, hypertension, biomarkers, obesity***

I have been involved in both basic and clinical investigations into the mechanisms of the major kidney disorders in pediatrics. The opportunity to examine the physiology and pathophysiology of normal and abnormal development and function of the kidneys throughout the critical periods of growth and maturation extending into adolescence and young adulthood is unique, especially since the antecedents of adult disease manifest themselves during the pediatric age range. Of the major causes of progressive glomerular diseases, focal segmental glomerulosclerosis is the most common and often devastating entity in pediatric and adult nephrology. We are actively involved in an NIH-funded clinical trial of this condition aimed at defining the most efficacious therapy while investigating the molecular etiologies for its expression. This translational research is further extended into another NIH-supported longitudinal study involving chronic kidney disease in children and its attendant morbidities of abnormalities in: growth and neurocognitive development, and the risk factors for cardiovascular disease and renal progression. Our Division of Pediatric Nephrology has expertise in clinical hypertension and translational research into biomarkers of renal progression, development nephrology, and our collaborations with investigators in Pediatric Neurology and Internal Medicine Nephrology offer an extensive research environment.

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Key words: ***microbicides, HIV, herpes, clinical trials, genital tract mucosal immunity***

Marla Keller, MD is Associate Professor of Medicine (Division of Infectious Diseases), Obstetrics and Gynecology & Women's Health. She directs a clinical research program focused on the clinical testing of microbicides, drugs in development for vaginal application to prevent the transmission of human immunodeficiency virus (HIV) and other sexually transmitted infections. Her work also focuses on defining the factors that contribute to innate mucosal immunity in the adult female genital tract. The ultimate goal of her work is to identify optimal combinations of candidate microbicides to prevent HIV and other STI without deleteriously altering the mucosal environment. Dr. Keller has conducted clinical trials in HIV infected and uninfected women. She is currently conducting clinical trials to evaluate the safety and antiviral activity of candidate microbicides, including tenofovir, dapivirine and Acidform. She recently completed 3 investigator-initiated Phase 1 clinical trials to examine the antiviral activity and effects on mucosal immunity of 0.5% PRO 2000 gel. Her group played a mjor role identifying the importance of examining the impact of semen of the efficacy of candidate microbicides by conducting in vitro and postcoital human studies. She is currently conducting studies in women with genital herpes to examine the factors in cervicovaginal secretions that mediate innate protection against HSV infection and to determine how changes in the mucosal environment increase the risk for HIV transmission or acquisition.

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Key Words: ***Breast cancer, tumor microenvironment, signal transduction***

We use complex three-dimensional tissue culture models of the tumor microenvironment to address important outstanding questions in the molecular oncology of breast cancer.

1. REGULATION OF EGFR LIGAND BIOAVAILABILITY.

Activation of members of the Epidermal Growth Factor Receptor family is a key feature of many solid tumors, including breast cancer. Activation of these receptors requires binding by ligands such as EGF, Amphiregulin or TGFa which are synthesized as transmembrane precursors. We are studying the mechanisms by which the production of these growth factors is regulated at both the transcriptional and post-transcriptional stages. We have shown that a metal-dependent protease, TACE/ADAM17, is a critical regulator of EGFR ligand bioavailability as it cleaves both Amphiregulin and TGFa at the cell surface. This implicates TACE as a druggable therapeutic target upstream of EGFR.

2. INVESTIGATION OF THE MACROPHAGE-TUMOR CELL DIALOGUE IN THE BREAST TUMOR MICROENVIRONMENT.

Cells of the immune system have long been observed in tumors in close association with neoplastic cells. High levels of macrophage infiltration frequently correlated with increased angiogenesis, tumor invasion and poor prognosis. We are developing three-dimension ex vivo models of the breast tumor microenvironment to explore in detail the interactions between macrophages and breast cancer cells. Understanding the dialogue between these cell types should yield potential new therapeutic targets in the tumor microenvironment.

Visit our lab page: http://kennylab.aecom.yu.edu

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Key Words: ***yeast, chromatin, transcription, DNA repair, genome stability, cancer***

HOW DOES CHROMATIN WORK?

Eukaryotic DNA usually resides in a nucleoprotein complex known as chromatin. The basic repeating unit is a nucleosome: ~150bp of DNA wound around eight histone proteins. A simple repeating array would suffice if the only function of the biopolymer was packaging. However, cells need to gain region-specific access to the DNA for replication, transcription, repair or chromosome transmission. Families of enzymes distinguish specific nucleosomes by replacing a major histone with a related variant, or covalently modifying the histones with small chemical moieties. These modifications are then thought to play distinct roles. Current projects in the lab include:

- **(i)** How chromatin controls the usage of inappropriate transcription start sites
- **(ii)** How the acetylation of histone H2A.Z regulates its function
- **(iii)** How cells establish and maintain mitotic chromosome structure
- **(iv)** How genetic interaction networks in yeast can be used to design novel therapies for human cancers

Lab website: http://sites.google.com/site/mckeogh2

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Key Words: ***CNS, motor coordination, cerebellum, basal ganglia, movement disorders,Hereditary Ataxia, Parkinson's disease, dystonia***

The goal of our laboratory is to understand the role of the cerebellum and basal ganglia in motor function and in movement disorders. Of particular interest to us is not only to understand the role of each structure in motor control, but also the manner in which they communicate to coordinate and complement each other. We approach these questions from both basic science and clinical perspectives. We use a combination of techniques, from behavioral studies to imaging and two photon microscopy and electrophysiology (both *in vitro* and *in vivo*). Our studies take advantage of normal and transgenic animal models.

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Key Words: ***Virology, membranes, protein structure/function, membrane fusion, protein traffic, virus assembly***

MOLECULAR MECHANISMS OF VIRUS MEMBRANE FUSION AND VIRUS BUDDING

During infection all enveloped viruses use the essential steps of membrane fusion to enter a cell, and membrane budding to produce infectious progeny viruses. Molecular information on these processes is critical to understanding virus infection pathways and as a key model for cellular membrane fusion and budding reactions.

The research in our laboratory focuses on the molecular mechanisms of virus-membrane fusion and virus budding using alphaviruses such as Semliki Forest virus (**SFV**) and flaviviruses such as dengue virus (**DV**). The flaviviruses and alphaviruses include many important human pathogens such as dengue, West Nile, and chikungunya viruses. DV is currently of particular concern as it has dramatically reemerged to become endemic in >100 countries, with an estimated 100 million cases of dengue infection per year. There are currently no vaccines or antiviral therapies for DV, and new therapeutic strategies for the flaviviruses and alphaviruses are urgently needed. SFV is a highly developed system to study virus fusion and budding, and is an important experimental model for both alphaviruses and flaviviruses.

Both SFV and DV enter cells by endocytic uptake and then fuse their membrane with the endosome membrane in a reaction triggered by the low pH of the endocytic vesicle. The flavivirus and alphavirus membrane fusion proteins are structurally related proteins and refold during fusion to form a homotrimer that mediates virus fusion and infection. In collaboration with Dr. Félix Rey, we determined the structure of the homotrimer conformation of the SFV fusion protein **E1**. This structure is strikingly similar to the DV homotrimer.

Using the structures as a guide, our lab has developed fragments of the SFV and DV fusion proteins that act as dominant-negative inhibitors of SFV and DV fusion and infection. We are currently using expressed fusion protein constructs to characterize the mechanism of trimer formation, to define the stages of the fusion reaction, and to reconstitute trimerization in vitro. We are also developing these in vitro trimers as screens for small molecule inhibitors of virus fusion reactions that will be lead compounds for new antiviral therapies.

E1-membrane insertion and alphavirus fusion are strikingly dependent on the presence of cholesterol in the cell membrane. The control of E1-membrane insertion and the mechanism of E1's cholesterol and pH-dependence are not understood, and we are using biochemistry, fluorescence methods, virus genetics and in vitro mutagenesis of SFV and DV infectious clones to address these questions.

SFV exits by budding through the plasma membrane of the infected host cell. Little is known about budding of alphaviruses or flaviviruses, although it is clear that budding is highly specific and produces very organized virus particles. How does this happen and what are the roles of cellular and viral components? The available data strongly suggest that the exit pathway of the alphaviruses involves unique cellular proteins, and their de novo identification will require a broad-based, unbiased approach. We are testing the role of specific candidate cellular proteins in budding and using fluorescently labeled envelope and capsid proteins to follow budding in real time. We are using an siRNA screening approach to identify novel host proteins involved in the SFV exit pathway.

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***Key Words:** toxoplasma, parasitology, malaria, nucleic acids*

MOLECULAR BASIS OF TOXOPLASMA PATHOGENICITY

Toxoplasma gondii is an important cause of birth defects and opportunistic infections in the immunocompromised including AIDS patients. Our research activities have been devoted to understanding the mechanisms by which this obligate intracellular parasite is able to survive within host cells and cause disease. We use a variety of molecular biology, cell biology and genetic techniques to understand the pathogenesis of toxoplasmosis.

Current projects include:
1. Molecular basis of *T. gondii* latency. Most clinical toxoplasmosis is due to reactivation of the latent bradyzoite form. We are using a molecular genetic approach to determine the mechanism by which *T. gondii* parasites switch from the tachyzoite form to the latent bradyzoite form. This differentiation is stress-induced, and our recent experiments implicate cyclic nucleotide signaling in bradyzoite formation. We are now clarifying the role of protein kinase A and other signaling molecules. This project is in collaboration with Louis Weiss at Einstein.

2. Role of proteinases in invasion. *T.gondii* is an obligate intracellular parasite that must invade host cells in order to survive. Secretion of contents of micronemes is thought to be essential for invasion. Recent studies have shown that serine proteinase inhibitors block host cell invasion. We have identified two microneme serine proteinases that may be involved in processing of secretory organelle contents prior to or during invasion. Because of their potential role in invasion, these proteinases are a potential target for novel chemotherapeutic agents. We are now characterizing the expression, trafficking, and substrate specificity of these proteinases.

3. Role of parafusin in secretion. In collaboration with Birgit Satir at Einstein, we have cloned a *Toxoplasma gondii* homologue of parafusin, a *Paramecium* phosphoglycoprotein implicated in regulation of exocytosis. As in *Paramecium,* this protein is a a phophoglucomutase homologue that localizes to the secretory vesicles.

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Key Words: ***Biostatistics, epidemiologic methods, clinical trials, statistical modeling***

Dr. Kim's research focuses on statistical methods for designing and analyzing clinical trials and epidemiologic studies. Most randomized clinical trials aim to demonstrate superiority of an experimental treatment relative to a standard treatment or placebo. An increasing number of trials, however, are focused on showing that the effects of two treatments on a particular outcome are equivalent, or that one treatment is not inferior to another. These goals are of interest when the new therapy offers benefits such as reduced cost, toxicity, and invasiveness relative to a standard therapy. Dr. Kim is investigating the effects of non-compliance, outcome misclassification and measurement error on the estimates of treatment effects, type I error rate, and power of equivalence trials and non-inferiority studies and developing new approaches for defining the non-inferiority margin.

Her research also includes the development of methods for analyzing interval-censored survival data. Interval–censored data can arise when outcomes are not directly observable but are detected from periodic clinical examinations or laboratory tests. The exact times of the events are not known since the event could have occurred at any time during the interval between the last visit when the subject was determined to be negative for the outcome and the first positive visit. Dr. Kim is developing approaches for the analysis of interval-censored data when multiple outcomes are of interest in the same study, and on evaluating the effect of covariates on the gap times between interval-censored recurrent events.

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Key Words: cell death/apoptosis/necrosis/heart disease/cancer/diabetes

Cell Death: Fundamental Mechanisms and Roles in Human Disease

The most basic decision that any cell makes is to grow (proliferate or hypertrophy), differentiate, or die. Our laboratory is interested in the connections among these processes. A major focus is the fundamental mechanisms that mediate cell death and the role that cell death plays in key human diseases such as heart disease, cancer, and diabetes. We employ a variety of approaches including molecular and cellular biology, biochemistry, mouse and human genetics, and physiology. Key discoveries from the lab include: 1) First delineation of the role of regulated cell death in myocardial infarction ("heart attack") and heart failure. 2) Recognition that death-fold motifs regulate cell death via non-homotypic, as well as conventional homotypic, interactions. 3) Elucidation of molecular mechanisms by which ARC, an endogenous inhibitor of apoptosis, antagonizes both extrinsic (death receptor) and intrinsic (mitochondrial/ER) death pathways. 4) Identification of critical roles for ARC in heart disease, breast cancer, and diabetes. 5) Recognition that Bax/Bak, classical regulators of apoptosis, also mediate necrosis. We are keen to translate these and other basic discoveries into clinical practice through structural anlayses and high throughput chemical screening.

<u>Current areas of investigation</u>

1. Evolutionary and mechanistic connections between apoptotic and necrotic cell death
2. Mechanisms by which Bax/Bak promote necrosis in myocardial infarction
3. Role of necrosis (not apoptosis) in cancer
4. Mechanisms linking death receptor and mitochondrial necrosis pathways
5. Mechanisms that mediate ARC degradation and apoptosis during heart disease
6. Regulation of ER stress-induced pancreatic β-cell death in diabetes
7. Role of ER-mitochondrial tethering by mitofusin 2 in cell death
8. Small molecule therapeutics for heart attacks based around manipulating Bax and ARC

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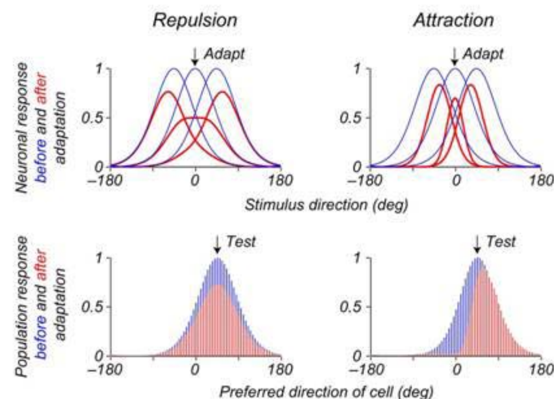
Key Words: ***Vision, plasticity, adaptation, population coding, multielectrode recordings***

Our laboratory studies the neural circuits that underlie visual perception, a general issue that we approach from several directions. For instance, we study how the responsivity and tuning of cortical neurons is altered by recent stimulus history. This form of rapid plasticity--termed adaptation--has strong perceptual effects, allowing us to explore the neurophysiological underpinnings of perceptual phenomena. In addition, we are interested in understanding the functional benefit of adaptation and in learning how adaptation early in the visual system affects subsequent stages of processing. We hope that by understanding the principles of adaptation we will also gain insight into other forms of plasticity such as perceptual learning and recovery from injury. We also study how populations of neurons function together to encode information about the visual world. We record from small populations of neurons simultaneously and measure the correlation of their responses. In particular, we explore how correlation depends on stimulus parameters, recent stimulus history, and cortical location. The primary techniques of the lab are neurophysiological recordings and computational modeling, but also include psychophysics and functional imaging. We hope that employing a range of experimental techniques will help us understand the computations carried out by the visual system and the circuits that perform them.



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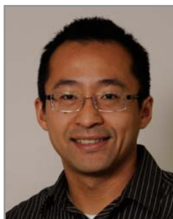

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Key Words: ***Bioorganic chemistry, biophysics, chemical biology, protein engineering, protein-protein interactions, antibody engineering, viral membrane fusion.***

Broadly stated, the objective of our research is to understand principles governing molecular recognition by proteins and antibodies, with the long-term goal of developing new research tools and therapies. Students and post-doctoral fellows can expect to gain expertise in new and traditional biochemical techniques including phage display (library design, synthesis, and screening), protein expression and purification, structural analysis by circular dichroism and X-ray crystallography, and viral neutralization assays. We are currently engaged in two lines of research.

1. Antibody Recognition Explored by Phage Display.

Antibody phage display has emerged as a powerful alternative to hybridoma technology for the generation of monoclonal antibodies and analysis of their interactions with antigens. It is now possible to select high-affinity antibodies against virtually any antigen from phage libraries that bear tailored diversity elements encoded by synthetic DNA ('synthetic antibodies'). This approach obviates the requirement for animal immunization, greatly reducing the labor and cost of antibody production. Selective enrichment of high-affinity binders from phage antibody libraries under controlled conditions enhances the reliability of output antibodies, and permits selection of binding with user-specified stringency. The expression of antibody domains on the surface of bacteriophage was first reported nearly two decades ago, but only recently have synthetic libraries (where diversity is not borne from natural source repertoires) become sophisticated enough for general use. We are developing and testing new synthetic antibody technologies to produce therapeutic, diagnostic, or research agents. Our strategy involves two aspects: first, we use high-throughput mutagenesis to interrogate physicochemical parameters of high-affinity antibody-antigen interactions; and second, we utilize the information obtained from these studies to engineer new synthetic libraries directed against targets that have resisted traditional antibody isolation methods.

2. Dissecting Mechanisms of Viral Membrane Fusion.

The envelope glycoproteins of membrane-bound viruses such as HIV-1, influenza, and ebolavirus all catalyze viral entry into host cells using essentially the same mechanism. Central to this mechanism are well-timed conformational changes of the envelope glycoprotein that result in formation of a six-helix bundle hemifusion intermediate. Formation of this hemifusion intermediate provides the driving force for fusion of the virus and host cell membranes. Small molecules, peptides, or proteins that bind viral envelope glycoproteins and prevent formation of the hemifusion intermediate have been used clinically as antiviral therapies. In addition, antibodies arising from natural infection (or other sources) that prevent the formation of the hemifusion intermediate are able to effectively neutralize the virus, suggesting that conformational mimicry of viral glycoprotein in the prefusion states may serve as an avenue for vaccine development. Using synthetic antibody technologies coupled with traditional biophysical and biochemical approaches, we seek to understand details of the viral membrane fusion process and which steps along the pathway are susceptible to inhibition by antibodies. Information gained from these studies will pave the way for structure-based vaccine design.

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Key Words: ***Antimicrobial immunity, T cell memory, protective immunological responses, in vivo analysis of innate and adaptive responses, microbial virulence***

INVESTIGATING ANTIMICROBIAL CELLULAR EFFECTOR RESPONSES *IN VIVO*

Our work focuses on effector cells from the innate and the adaptive immune system that mediate protective immunity against infections. The development of efficient immune responses against pathogens indeed depends on the induction of such cells. We have mostly been studying memory $CD8^+$ T cells which represent a critical effector arm of the adaptive immune response and mediate intracellular pathogen clearance by expressing various effector mechanisms such as cytolytic activity that lead to the killing of infected cells as well as release of proinflammatory cytokines and chemokines that promote the recruitment of innate immune cells and potentiate their microbicidal activities. Using mice infected with the intracellular bacterium *Listeria monocytogenes*, we recently characterized novel phenotypic and functional features of the memory $CD8^+$ T lymphocytes that are crucial and non-redundant for conferring immunological protection. Induction of $CCL3^+$ memory $CD8^+$ T cells depends upon cytosolic expression of SecA2, an auxiliary secretion system of the bacteria involved in their virulence. Our current projects keep investigating the mechanisms underlying long-term protective immunological memory *in vivo*. We rely on SecA2-deficient and other related mutated bacteria that we have generated, as well as on a range of advanced fluorescent-tracer based methodologies to analyze effector phagocytes and $CD8^+$ T cells. We use cell transfer experiments and have generated novel genetically modified mice in which dynamic cell function can be monitored or which lack specific functional subsets of phagocytes or T cells to dissect the precise sequence of events and the roles of the distinct cell types. Overall, the goal of my laboratory is to improve our understanding of the factors that drive the generation of protective responses against microbes *in vivo*, which will contribute to better preventive and therapeutic antimicrobial strategies.

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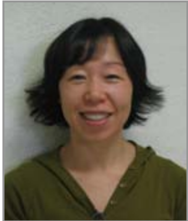

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Key Words: microglia, neuroinflammation, neurodegeneration, cytokines, HIV

Microglia are resident brain macrophages and are the prime instigators of several CNS disorders. My laboratory studies the role of microglia in the nervous system inflammation and neurodegeneration using a model of primary human brain cell cultures and several in vivo mouse models[3]. Pathogenesis-oriented studies and search for potential therapeutic modalities are often the goal. We define the involvement of inflammatory mediators in degenerative human brain diseases including neuroAIDS, multiple sclerosis and Alzheimer's disease. The disease that we currently focus on is HIV-associated neurocognitive disorder, a nervous system dysfunction directly and indirectly caused by HIV infection and inflammatory activation of microglia and macrophages. We study the involvement of cytokines and chemokines in modulating HIV infection, as well as signal transduction pathways that are activated by HIV in these cells. For example, we have recently identified CD45 tyrosine phosphatase as a constitutive protein expressed in microglial cells that can be targeted by an agonist antibody resulting in suppression of HIV replication and microglial activation[1,2]. Another area of long interest is a mechanism of human microglial and astrocyte activation by interleukin-1 and the toll-like receptor (TLR) ligands[4,5]. IL-1 is produced by microglia in response to a variety of different insults and is indispensable for nitric oxide and TNFa production from human astrocytes. IL-1 is responsible for neuronal death during inflammatory degeneration of the human CNS. In contrast, the TLR3 and TLR4 ligands activate a strong anti-viral program, while triggering IL-1 generation. Therefore, we believe that the key to neuroprotection is programming microglia to suppress the production of harmful cytokines while promoting the production of neurotrophic and immunoregulatory mediators. Towards this goal, we have identified a protein factor that enhances the neuroprotective phenotype of microglia by suppressing NF-κB-dependent gene expression while promoting interferon-mediated antiviral pathways. Our long term goal is to understand the mechanism of human glial and neuronal interactions during CNS diseases at the cellular and molecular level, in order to develop molecular therapeutics to promote neuroregeneration.

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Key Words: ***Cancer, genomics, herpesvirus, oncogene, <u>retrovirus</u>***

MOLECULAR GENETICS OF RETROVIRAL DISEASES

Retroviruses are associated with a variety of diseases in humans and other vertebrates including cancer and immunodeficiency. The major goals of the laboratory are focused on understanding the molecular basis of retroviral diseases. Mouse retroviruses cause tumors by a mechanism of insertional activation of oncogenes where the viral DNA integrates adjacent to an oncogene, and enhancer elements in the virus activate transcription of the adjacent host gene. We use these viruses as tools for high-throughput identification of cancer-causing genes in the mouse genome. Using sophisticated PCR techniques combined with massively parallel DNA sequencing, the viruses have been used as molecular tags to identify over 60 different genes that cause lymphomas, many of which have not been associated with a cancer-causing role previously, and we are also interested in the molecular mechanisms by which these genes act. Since retrovirus gene therapy vectors cause tumors in human patients by the identical mechanism, we have developed strategies to prevent retroviruses (or any other gene therapy vector that integrates into the human genome) from activating oncogenes and causing cancer. Our newly developed strategies can block most tumors, are being adapted to human gene therapy use, and we are striving for even greater success.

8% of the human genome is retrovirus DNA. Human endogenous retrovirus K (HERV-K) the newest of all the retroviruses to enter the germline DNA of humans that is transmitted from parents to children. All humans are born with about 20 distinct HERV-K proviruses (the form of retroviral DNA that is integrated into the host genome) in their germlines. We are investigating whether this retrovirus is capable of reinfecting humans today. We have shown that most HERV-K proviruses in the human formed relatively recently in human evolution, long after the divergence of the human ad chimpanzee lineages approximately 6 million years ago. We identified several proviruses that formed so recently that they are not yet fixed in the human genome. We have also identified two HERV-K proviruses that have full length open reading frames for all viral proteins, and are the best candidates to be infectious retroviruses in the human genome today. We are now asking whether HERV-K can indeed replicate in humans today, and whether it might be associated with any diseases.

In collaboration with Drs. Larry Herbst and Robert Burk, we are also studying a herpesvirus and a papillomavirus that are associated with fibropapillomas in endangered and threatened species of marine turtles. We are investigating the evolutionary histories of these viruses, how they are transmitted, the nature of turtle immune responses to them, and the roles of the viruses in causing tumors.

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Key Words: ***directed evolution, aptamers, protein engineering, targeted delivery***

DIRECTED EVOLUTION OF PROTEINS AND NUCLEIC ACIDS

The Levy Lab draws from biological, chemical and combinatorial approaches to understand fundamental biological interactions, as well as to design novel diagnostics, therapeutics and bio-tools. My research focuses on two main areas.

In the first, we are utilizing a new technique, in vitro compartmentalization (IVC), to engineer novel protein-ligand interactions and functions. The method uses water-in-oil emulsions to form femtoliter-sized reaction vessels and thus, avoids many complications and 'bottlenecks' incurred using more traditional in vivo protein evolution techniques. Evolved proteins with altered properties are potentially very useful tools for a variety of biotechnology and nanotechnology applications. In addition, they can provide important insight into the nature of high affinity protein-ligand interactions and substrate specificity. Current work focuses on developing, adapting and optimizing a class of bacterial proteins called sortases for in vivo labeling and imaging applications as well as developing orthologs of the streptavidin-biotin couple.

In the second focus area, we are developing new methods for the identification of cell and tissue specific targeting agents. Of particular interest is the development of cell specific and cell surface receptor specific aptamers. These nucleic acid-based affinity agents hold great promise for the design of novel diagnostics as well as therapeutic agents and offer many advantages over other affinity reagents used for targeting, such as antibodies. We and others have demonstrated that aptamers are capable of directing delivery of cargoes, especially siRNA, to cells. The ability to specifically target and deliver cargoes to cells using nucleic acids opens a new niche in the field of targeting and delivery. Current research in my lab aims to identify novel nucleic acids capable of targeting or delivering cargoes including small molecule drugs, toxins and siRNA to specific cell types and tissues including cancers, pancreatic beta cells, and dendritic cells. In addition, we are developing strategies to perform selections in whole organisms to identify aptamers which can home to specific tissues and organs. In parallel with these efforts, we are further developing the use of aptamers as components in compound delivery reagents. To this end, we are exploring their use for targeted delivery of nanoparticles.

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***Key Words**: sulfate, E.coli, thermodynamics, electron paramagnetic resonance*

In this laboratory we employ a multidisciplinary scientific approach in studying the cellular biochemistry and mechanistic processes of sulfate activation in *E. coli*. Sulfate must be chemically activated before it can be biochemically assimilated. The activation step involves the formation of a phosphosulfate anhydride bond and is catalyzed by the enzyme ATP sulfurylase. This bond is analogous to the phosphoric acid anhydrides found in compounds such as ATP and ADP in that it is an extremely "high energy" anhydride bond. Thus, sulfate is thermodynamically poised, via activation, prior to entering into its subsequent biochemistry.

Our goal is to identify and isolate the critical genetic and biochemical elements in this process and to investigate their mode(s) of action/interaction on the physical chemical level.

Recently, we have discovered that ATP sulfurylase catalyzes hydrolysis of GTP and that this hydrolysis of GTP and that this hydrolysis is coupled to a kinetic stimulation of APS formation. Furthermore, the chemical energy released by the hydrolysis is used to thermodynamically "drive" APS formation. We are pursuing the mechanistic basis of this activation. Other projects include isolation, characterization and cloning of an "effector" protein which stimulated ATP sulfurylase activity and studies of reactant/metal ion complexes which occur on the active site surface of ATP sulfurylase using electron paramagnetic resonance.

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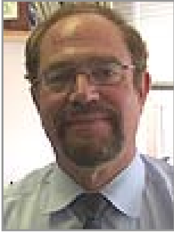

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Key Words:* *Migraine and pain, brain aging and Alzheimer's disease, neuroepidemiology, clinical trials, biomarkers and genetics

As a neurologist and neuroepidemiologist my work has focused in two broad areas: cognitive aging and dementia as well as migraine and other headache disorders. For the past 16 years, I have directed an NIH funded program project, the Einstein Aging Study (EAS) which aims to identify the earliest cognitive, metabolic, anatomic, and neuropathologic markers that distinguish 'normal aging' from early dementia. Our interdisciplinary research team includes neurologists, neuropsychologists-cogntive neuroscientists, epidemiologists, biostatisticians, neuropathologists, neuroradiologists and geneticists among others. Our longitudinal research emphasizes screening for and definitive diagnosis of early Alzheimer's disease in a population-based sample of community- residing older adults. Our long-term goal is to "make Alzheimer's disease a memory" by preventing illness through risk factor modification and treatment. Our strategies include neuroimaging, genetics, biochemical markers and novel cognitive strategies. In addition to our work on dementia, our research team also studies predictors of successful brain aging, the preclinical onset of Parkinson's disease as well gait and motor function in the elderly. Our group is committed both to research and research education. Our trainees have included many CRTP students and K-supported junior faculty members.

As the Director of the Montefiore Headache Center, my interests include classification, natural history, diagnosis and treatment of migraine and other headache disorders. Our group includes neurologists, psychologists, an emergency medicine physician and neuroradiologists among others. Our research has focused on headache epidemiology and genetics, migraine comorbidity, neuroimaging, and biological markers as well as risk factors for and the prevention of chronic daily headache. Our largest current study, the American Migraine Prevalence and Prevention Study is a longitudinal study following 11,000 migraine sufferers from the general population to examine natural history and risk factors for headache progression. We also have a number of clinical trials, diary studies, family aggregation, neuroimaging and biomarker studies. We have a strong interest in migraine genetics, in the role of obesity, inflammation and allodynia in migraine progression.

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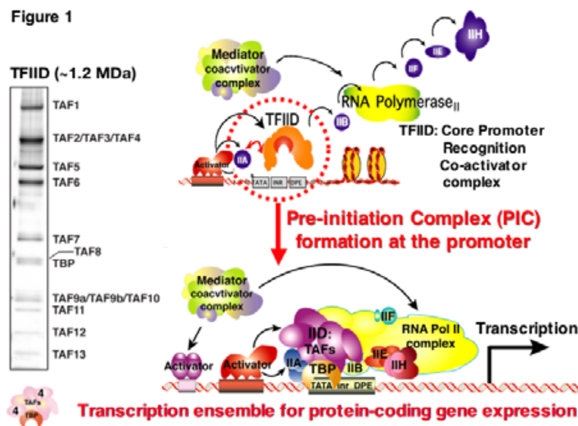
Key Words: ***gene expression, transcription, single particle cryo-EM imaging***

NANOSCALE PROBING OF GENE EXPRESSION

A number of devastating human diseases arise from aberrant protein-coding gene expression in cells. Indeed, precise gene expression in humans is tightly controlled. This process requires highly coordinated interactions between transcription initiation machinery and specific activators to ensure proper response to various physiological stimuli. The development of potent and novel disease treatments demands a fundamental understanding of how these regulatory proteins interact to switch on and off gene expression patterns that meditate cell growth/death, differentiation, aging, etc. in human cells. Therefore, the principal focus of our work is to interrogate how protein-coding genes are transcribed at the early stage of gene expression in human cells in order to express a number of critical factors responsible for diverse cellular processes including stem cell-/cell type- specific differentiation, proliferation, cell cycle arrest, and apoptosis.



To accurately transcribe a gene, a pre-initiation complex (PIC) is required to form at specific regions of the promoter DNA. TFIID is a principal component within the transcriptional machinery responsible for recognizing and binding specific promoter DNA (Fig.1). TFIID directs a recruitment of eight other basal transcription factors including TFIIA and RNA Polymerase II to produce RNA from an accurate, discrete location of a gene. In order to properly respond to various physiological stimuli, sequence-specific DNA binding activators act as key regulators of gene expression to stimulate transcription, in part by targeting TFIID and aiding in its recruitment to promoter DNA.

We currently use a combination of high-resolution single particle electron microscopy (cryo-EM), high-precision single molecule microscopy, and a number of biochemical assays to visualize several distinct human mega-Dalton size macromolecule transcription assemblies during activated transcription initiation involved in tumor suppression, embryonic stem cell differentiation, and ovarian development.

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***Key Words**: gene expression development, transcription factor, liver, development*

Our research on gene expression and transcription factors has focused on the alpha-fetoprotein (AFP) gene, a classical marker of both fetal development and cancer. The studies have three related themes: (1) regulation of liver genes through a specific language of transcription controls, (2) long distance transcription controls that regulate a chromosomal locus, and (3) the transcriptional regulators that control liver development.

After defining and purifying PCF (the "promoter coupling factor"), we cloned a novel homeobox transcription factor, Nkx2.8. Nkx2.8 shows a striking correlation with AFP expression in development and in liver cancer cell lines. In addition, closely related factors are known to regulate organogenesis. Nkx2.8 is therefore a prime candidate to be the transcription factor that triggers the early formation of the liver. The complete mouse and human Nkx2.8 genes have been cloned and sequenced, and we are beginning to develop an Nkx2.8-knockout mouse. This mouse is expected to have distorted liver development and possibly other defects in early endoderm-derived structures.

AFP gene expression is regulated through the interaction between distant enhancers and the promoter. These enhancers can also stimulate the neighboring albumin gene promoter, enabling simultaneous regulation of the two genes by the same enhancers. The AFP - albumin locus is thus a unique model system of developmental regulation through long-distance gene control. This liver-specific locus is quite different from the β-globin family locus, the only other established experimental system of long distance gene control in mammals. In our second major project, we are building this 60-kb locus into a series of expression plasmids, which we are using to define the elements that coordinate regulation of the two genes.

Both of these projects are defining the specific factors and binding sites that establish liver-specific gene regulation. These are elements in the liver "dialect" of the transcriptional control language, which coordinates the normal gene expression as it unfolds through a developmental program. The same controls also coordinate the cell cycle and responses to injury and other stimuli. For our third project, we have developed a unique combination of molecular and computer analysis and are using this approach to fully define the transcriptional control language of liver.

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Key Words: ***Microtubule dynamics, mitosis, microtubule-associated proteins, molecular dynamics***

THEORETICAL STUDIES IN BIOPHYSICS AND CELL BIOLOGY

The research in my group is focused on development and application of novel computational and theoretical methods for studying dynamical processes in biological systems that span over a multitude of spatial and temporal scales. Currently, we are pursuing two major directions.

1. An integrated computational approach for understanding microtubule-associated proteins (MAPs) from the level of the structure and dynamics of individual molecules to the level of their function, regulation and interactions in vivo. MAPs control the assembly dynamics of microtubules and play essential roles in a broad range of important cellular processes such as mitosis, motility, polarization and morphogenesis. We strive to address this problem from both top-down and bottom-up perspectives and established close collaborations with experimental colleagues in cell biology, structural biology and biophysics. The research in this direction consists of four synergistic components: (1) automated image analyses for extracting quantitative and detailed information from experimental data, (2) coarse-grained systems-level simulations of spindle dynamics, (3) simple kinetic model of single molecule dynamics, and (4) atomistic simulations for understanding the molecular mechanism underlying the structure-function relationships of mitotic proteins.

2. Understanding the detailed dynamics of individual biomolecules. Protein dynamics are essential for protein function, but the molecular mechanism of how dynamics impact function remains elusive. Moreover, protein dynamics are strongly influenced by environmental factors such as solvent, confinement and osmolytes. The research along this direction focuses on inter-relationship between solvent dynamics, protein dynamics and protein function. In collaboration with experimental colleagues, we are using atomistic simulations to elucidate the molecular mechanism of the "solvent-slaving" phenomenon (protein dynamics are "slaved" to solvent dynamics), and how to use environmental factors to modulate protein dynamics and function.

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Key Words: ***aging, anergy, autophagy, autoimmunity, cancer, NFAT, regulatory T cells.***

The antigen receptors of T cells recognize not only antigens derived from pathogenic cells and organisms, but also self-antigens expressed on the body's own tissues. In healthy individuals, self-antigens do not elicit a significant immune response. Self-reactive lymphocytes are clonally eliminated during development and cells that survive this process are rendered tolerant in the periphery.

Two of the major of the mechanisms responsible for peripheral T cell tolerance are anergy, an intracellular process in which antigen receptors become uncoupled from their downstream signaling pathways, and regulatory T cells, a population of T cells with the ability to suppress activation of other T cells as well as B cells and dendritic cells. The biochemical pathways that cause inhibition of T cell responses in anergic or suppressed T cells are poorly understood. One of the goals of our lab is to study the molecular mechanisms responsible for the induction of tolerance in T cells. In T lymphocytes, integration of Ca^{2+} and other signaling pathways leads to productive activation, while unopposed Ca^{2+} signaling is associated with establishment of a tolerant state. A major consequence of Ca^{2+} mobilization is activation of members of the NFAT family of transcription factors. We have shown that NFAT1 plays a central role in tolerance induction in T cells. Using in vitro and in vivo mouse models, we have shown that tolerant T cells express a novel set of NFAT-dependent genes, distinct from those characteristics of a productive immune response that cause interference with signaling pathways coupled to antigen receptors, protein degradation and transcriptional modulation. We are currently characterizing the underlying molecular mechanisms involved in the establishment of T cell tolerance and identifying their molecular targets. Furthermore, we are developing potential tools to modulate T cells tolerance with the goal of suppressing T cell responses for the treatment of autoimmune disease, or boost them to potentiate immune responses against cancer cells.

The immune system undergoes age-associated changes. Immunosenescence can be defined as the age-dependent decline in immune function which is responsible for the diminished ability to respond to infections, the lack of success of vaccination protocols and the increased incidence of autoimmune disease and cancer in the elderly. Degradation of proteins in the lysosomes via autophagy plays a key role in maintaining proper cell homeostasis, by reducing the accumulation of damaged proteins and recycling amino acids for new protein synthesis. Evidence suggests that decreased autophagic activity in old organism may be responsible for the deterioration of cell functions with age. We aim to determine how autophagy regulates T cell function, and how the age-dependent dysregulation of this process may account, at least in part, for the defective T cell function during aging.

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Key Words: ***Bioethics, Research Ethics, HIV/AIDS Research, Reproductive Health Research, International Collaborative Research***

My research covers all aspects of bioethics, but focuses on human subjects research, especially on HIV/AIDS and reproductive health research conducted in developing countries. Topics include informed consent, risk-benefit assessments, justice in research, avoiding exploitation of research subjects.

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Key Words: ***cancer, drug metabolism, drug design, drug delivery, nuclear receptors, transcription***

PHENOTYPING ORPHAN NUCLEAR RECEPTORS

Orphan nuclear receptors (those that lack a well defined physiologic ligand) control nearly every major physiologic and biochemical process in eukaryotes - cell metabolism (e.g., cholesterol, energy, bile acids), xenobiotic detoxification, cell differentiation (e.g., gastrulation, retinal development), circadian rhythm, and cancer cell growth and apoptosis (e.g., NURR77). Alterations in the receptor are directly linked to human disease (e.g., NOR1 and extraskeletal myxoid chondrosarcomas). Of these receptors, the steroid and xenobiotic receptor (SXR or PXR) is a key regulator of genes encoding drug metabolizing and transport proteins. In addition, PXR has been implicated in cancer drug resistance, carcinogenesis and pathophysiologic states like osteomalacia. Our laboratory focuses on defining the role of PXR and other orphans in: (i) xenobiotic metabolism and pharmacology (ii) carcinogenesis, organogenesis and anticancer drug resistance.

A. **Transcriptional Determinants of Drug-Drug Interactions**. Our laboratory has demonstrated that the activation of orphan nuclear receptors like PXR may be modulated or inhibited by azole anti-fungal drugs and plant phytoestrogens (e.g., ketoconazole, coumestrol). In this manner, we could control the degree of activation of PXR and subvert unanticipated (or unwanted) PXR mediated drug metabolism (drug-drug interactions). This process would also allow for controlled drug delivery to drug sanctuary sites like the brain etc. We have demonstrated that PXR is "druggable" and that novel site-directed (e.g., AF-2) inhibitors may be developed to control PXR engagement by activating ligands.
B. **PXR controls Blood Brain Barrier (BBB) Function in vivo**. We have developed SCID-PXR KO and humanized mice to help dissect the contribution of PXR in maintaining BBB function in vivo.
C. **PXR controls cancer and stem cell proliferation/metabolism**. We have developed a mechanistic model for PXR mediated cell growth. Future studies in the laboratory will focus on the how and why PXR mediated gene transcription alters - tumor drug metabolism, drug resistance, tumor cell phenotype, migration, and metastasis. We will determine if selective blocking of xenobiotic mediated PXR gene transcription alters some or all of the above tumor cellular phenotype(s) or cell growth. We will perform mechanistic studies exploring ketoconazole mediated suppression of ligand activated PXR gene transcription. The techniques used will include molecular modeling (docking), ligand binding assays, transfection studies with mutant/wild type constructs, site-directed mutagenesis, yeast two-hybrid studies, in vitro protein translation and binding studies, ChIP assays, and real-time PCR.
D. **PXR and Host Defense**. We are studying the role of PXR in gut mucosal defense especially in regards to homeostasis.
E. **Transcriptional and Translational Modifications of PXR**. The laboratory is interested in defining the spectrum of changes that alter PXR transcription and translation. As such, work in progress suggests that certain miRNAs may control and be controlled by these receptors. Post-translational modifications (e.g., phosphorylation) are also important determinants of PXR function and studies are underway to define these mechanisms/modifications.

Visit our lab page: **http://sridharmanilab.googlepages.com/**

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Key Words: oocyte, polarity, follicle cell fate, germline, genetics, zebrafish

The main focus of the lab is to study how cell polarity is established and maintained in the vertebrate oocyte and egg. Although oocyte asymmetries have been documented for over 100 years across phyla, the genes that regulate oocyte polarity are best understood in insects, and are largely not known in vertebrates. The earliest indicator of polarity in vertebrate oocytes is the Balbiani body, an asymmetrically localized aggregate comprised of organelles, proteins, and, in some animals, mRNAs encoding germline determinants and patterning molecules. Where known in vertebrates, the Balbiani body is also the earliest indicator of the animal-vegetal axis. The vertebrate animal-vegetal axis is the first to form, and is a prerequisite for normal development of the later developing embryonic axes, however its specification is poorly understood.

Bucky ball was identified as an essential regulator of oocyte polarity through maternal-effect genetic screens in zebrafish. In *bucky ball* mutants early oocyte asymmetries including the Balbiani body, localization of mRNAs and proteins along the animal vegetal axis, and restriction of animal pole specific follicle cell fates are disrupted. We are currently studying the cellular and molecular mechanism by which Buc regulates ovarian follicle cell and oocyte polarity. We are also pursuing components of the Bucky Ball pathway that are required to establish oocyte polarity and pattern the follicle cell layer using a combination of molecular, genetic, biochemical, and oocyte culture approaches. We exploit the unique genetic access afforded by our *buc* mutant alleles to improve our understanding of Buc function and the mechanisms mediating oocyte polarity and animal-vegetal axis formation in a vertebrate model system. Studies of the genetic and molecular control of axis formation in zebrafish will clarify the mechanisms establishing these earliest oocyte asymmetries, which are common among insects, and vertebrates, including humans, thus this architecture is likely fundamental for germline development and fertility.

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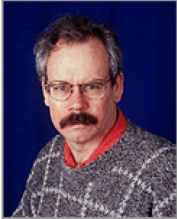

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Key Words: ion channels, sudden death, SIDS, signal transduction, cardiology, parasitology

The research interests of our lab center on investigating the role of ion channel function in normal and disease processes. Ion channels are involved in cellular excitability and signal transduction processes in every type of tissue. Mutations of channel genes that alter their function are an increasingly recognized cause of genetic diseases. We use a multi-disciplinary approach to investigate the normal function of channels and mechanisms of disease-producing mutations.

The main areas of research in the lab are:

1) Mutations in several cardiac ion channel subunits cause sudden death in the inherited disease Long QT Syndrome (LQT) and Sudden Infant Death Syndrome (SIDS). These channels also play important roles in the nervous system, intestine, kidneys and in cancer. We are using a combined approach of cellular electrophysiology, proteomics, protein biochemistry, and structural chemistry to understand how these channels are regulated by protein kinases, and through interactions with other cardiac proteins. Of particular interest is:

a) How mutations alter channel function in a deleterious way.
b) Structural approach to understanding how smaller K^+ channel subunits interact with the channels.
c) Signal transduction pathways that control channel expression and activity.
d) Epigenetic and non-coding RNA factors regulating channel expression and function

2) All cells express membrane ion channels that are evolutionarily conserved. Channels provide selective permeability characteristics of membranes and are essential for normal cell function and viability. We are investigating ion channel candidate genes from intracellular parasites (Malaria, Leshmania, Toxoplasma, Trypanasoma) for their roles as determinants of viability, infectivity and virulence. The long-term goal of this research is to identify essential functional proteins that may serve as pharmacological or immunological targets.

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Key Words: ***neural stem cell biology, brain development, neurodegeneration, epigenetics, cellular re-programming, dynamic tissue remodeling, bioengineering, pharmacoepigenomics***

The primary focus of our laboratory is on defining the regional localization and the biological properties of neural stem cells during embryonic and postnatal development and in the mature and the aging mammalian brain. We are also using stem cells as "biological probes" to elucidate the pathogenesis of a spectrum of complex and poorly understood acquired and genetic nervous system disorders. In these prototypical disorders, distinct profiles of regional stem cells or their more lineage-restricted neuronal or glial progeny undergo irreversible injury and death in response to acute or more chronic injury signals. Further, we are attempting to use the knowledge gained from these multidisciplinary studies to design innovative epigenetic- and stem cell-based regenerative therapies.

We are in the process of defining the dynamic roles of environmental factors, cell-cell signaling pathways and cell autonomous cues in promoting stem cell activation, expansion, lineage restriction, lineage commitment, cell cycle exit and terminal differentiation. We have identified specific transcription factor and epigenetic codes that endow the progeny of specific stem cell subpopulations with their unique cellular properties. These insights have already allowed us to "reprogram" different regional stem and progenitor cells both in vitro and in vivo to acquire the cellular properties of specific neuronal and glial subtypes that are lost in different classes of neurological diseases. We have also utilized embryonic stem cells, both to define initial stages of neural induction and patterning of the neural tube that have previously been difficult to examine experimentally, and as therapeutic reagents for those diseases of the nervous system in which multiple regional neuronal and glial subtypes are targeted.

The ultimate aim of these studies is to identify innovative approaches to brain repair by activation of latent neural stem cell pools throughout the neuraxis to engage in selective regeneration of those cell types and neural network connections that have been compromised in specific disease states. We are utilizing advanced epigenetic reprogramming strategies, including the deployment of multiple novel classes of non-coding RNAs to modulate the dynamic expression profiles of individual genes and integrated functional gene networks through genome-wide targeting of specific DNA motifs/stereoisomers, histone, nucleosome and higher-order chromatin codes and complexes, RNA/DNA editing, and RNA intra-/inter-cellular trafficking. The ability to activate and recruit these latent developmental programs to participate in selective neural regenerative responses will help to reestablish functional neural networks that preserve the integrity of previously acquired informational traces. More importantly, a better understanding of the pathogenesis of individual neurological disorders will allow us to more effectively employ our emerging neural regenerative and epigenomic targeting strategies.

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Key Words: bone marrow failure, cancer, human reproduction, nucleolus, ribonucleoprotein

Our group is studying the mechanism and regulation of nucleolar ribonucleoprotein biogenesis in relation to nuclear dynamics, genetic disease, and human reproduction. Presently we are pursuing two main areas of research:

First, we are analyzing the biogenesis and function of small nucleolar ribonucleoprotein particles (snoRNPs) of the H/ACA class. Human H/ACA ribonucleoproteins (RNPs) are important for many basic cellular processes including protein synthesis, pre-mRNA splicing, and genome integrity. The different functional classes of H/ACA RNPs isomerize some 130 uridines to pseudouridines in ribosomal (r) and spliceosomal small nuclear (sn) RNAs, process rRNA, stabilize telomerase RNA, yield microRNAs, and harbor yet to be determined roles. Each of these functions is specified by one of over 150 H/ACA RNAs, each of which associates with the same four core proteins to form an H/ACA RNP. The central core protein, NAP57 (aka dyskerin or in yeast Cbf5p), is mutated in the predominant X-linked form of the inherited bone marrow failure syndrome dyskeratosis congenita (DC). To understand the stem cell depletion and cancer predisposition characteristic for DC, we are studying the impact of DC mutations on the biogenesis and functions of H/ACA RNPs.

Second, we investigate the function of nucleolar channel systems (NCSs) in the cell and in human reproduction. During the height of receptivity of each menstrual cycle, NCSs transiently develop in the nuclei of endometrial epithelial cells (EECs). They are implicated in the preparation of the endometrium for uterine attachment of the fertilized egg. Although the molecular mechanisms of embryo implantation in humans are poorly understood, NCSs remain unexplored as candidate markers or potential prerequisites for implantation. This can be attributed to the fact that, despite their discovery close to 50 years ago, identification of NCSs is still limited to electron microscopy. We identified a molecular marker of the NCS, the monoclonal antibody mAb414 against nuclear pore complex proteins (nucleoporins), which for the first time allows simple and robust detection of these organelles at the light microscopic level. We are now exploiting our discovery to understand the cellular biology of NCSs and their regulation and function in uterine biology. The latter should have broad applicability to fertility, its regulation, and cancer.

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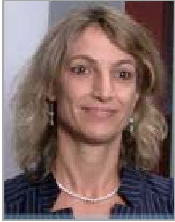

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Key words: sensory neurophysiology; perception; autism; development

At the Cognitive Neurophysiology Laboratory at Einstein we use high-density scalp recorded EEG, intracranial recordings in patients with epilepsy, neuroimaging using fMRI, and psychophysics to understand human perception and cognition. We have 4 high-density EEG booths equipped with high resolution eye-tracking and Presentation software (2 dedicated to recording data in children and two to recording data in adults), housed within the first floor of the Van Etten building.

One arm of my program of research is on multisensory influences on perception and behavior and the underlying brain mechanisms, in typically developing children and adults. Another primary focus of my research is on understanding how individuals with autism process and integrate information differently from typically developing individuals. A major goal of this line of research is to bridge the gap between phenotype and genetics with the development of multidimensional biomarkers. To this end we do extensive in-house phenotyping and we collaborate with scientists in genetics at the Price Center.

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Key Words: ***Cytogenetics, aneuploidy, mouse models, breast cancer***

Genes Regulating Normal Breast Differentiation and Identification of Novel Biomarkers for the Detection and Classification of Breast Cancer

The laboratory focuses on two main areas of research:

Project 1- Role of Septin 9 in Breast Carcinogenesis.
A comparative cytogenetic approach aimed to identify recurrent DNA copy number variations in a panel of murine models for breast cancer resulted in the identification of Septin 9 (Sept9) as potential novel oncogene. The septin family of genes codes for a highly redundant and conserved family of GTP-binding proteins that assemble into filaments and bind to microfilaments and microtubules. Our recent data performed on a panel of human primary tumors reveal that Sept9 is also amplified and over expressed in human tumors. At the locus of genomic amplification deregulation of Sept9 expression occurs by a complex pattern of genetic and epigenetic alterations affecting several Sept9 isoform variants. Our hypothesis is that during malignant transformation, breast epithelial cells undergo genomic amplification of the Sept9 locus and over-express Sept9 mRNA and protein. Additionally, aberrant cytosine methylation occurs in the Sept9 locus resulting in an abnormal pattern of Sept9 isoform variants. We are currently studying how the expression of various Sept9 isoforms is regulated in normal and cancer cells and the functional differences between these isoforms.

Project 2- Stage- and Cell Subtype-Specific Epigenetic Regulation of Mammary Gland Development and breast tumorigenesis.
We are interested in investigating the DNA methylation changes occurring in the development of the normal mammary gland at puberty, adult age, pregnant, lactating and undergoing mammary gland involution. This approach has the final goal of dissecting the molecular processes that mediate methylation changes in the morphogenesis and differentiation of the normal breast to identify "hot spot" loci for gene silencing in breast carcinogenesis.
We performed a genome-wide methylation analysis of cytosine methylation levels during mammary gland development to identify novel candidate loci. This study shows that conserved, non-coding regions around the transcription start sites of a number of genes undergo changes in methylation pattern during mammary gland development. We have selected various candidates that we are currently investigating for their role in determining cell fate during mammary gland development and for their implication in breast tumorigenesis.

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Key Words: birth defects, development, human genetics, chromosome

Our lab is interested in discovering genes required for organogenesis, a process by which organs form during embryonic development, with the purpose to understand the cause of birth defects. Our research begins with collecting DNAs from affected individuals with genetic disorders having known chromosomal gains or losses, and moves to looking at gene function in model organisms. This process runs full circle back to humans to provide at minimum better prenatal screening, ideally, to discover ways to reduce symptoms. The reason for studying chromosomal disorders is that regions in the genome containing large duplications or deletions of DNA, will pinpoint the location of causative genes whose function in organogenesis is sensitive to altered copy number. To identify the responsible ones, among dozens of genes in each interval, we have turned to using mouse and zebrafish model organisms. The model organisms further help to determine gene function.

Our main focus is on a disorder termed chromosome 22q11.2 deletion syndrome (22q11DS). Most affected children have a similar sized 3 million base pair deletion encompassing 40 genes. Children with the syndrome have learning disabilities, cleft palate, hearing loss and cardiovascular defects. One gene in the region termed *Tbx1*, a transcription factor, was found in mouse models, to be responsible for many of the defects in patients with the syndrome. Using knockout and gain-of-function mutant mice, we have begun to understand its function. Since it's a transcription factor, we are interested in genes it can regulate. Our mission is to build a genetic pathway downstream of Tbx1. Since we don't know what pathways are regulated, we took an unbiased gene discovery approach. To do this, we isolated RNA from normal and mutant mouse embryos at various developmental stages and performed microarray gene profiling. From this analysis, it appears that Tbx1 promotes cell survival and restricts differentiation. We are validating these findings by doing real-time RT-PCR and in situ hybridization of probes to genes uncovered on staged mouse and zebrafish embryos. We eventually want to perform molecular studies including chromatin immunoprecipitation followed by next-generation sequencing. To identify genes regulating *Tbx1*, we are screening putative mouse enhancers in zebrafish using the tol2 transposon system. This approach requires injection of enhancers connected to fluorescent reporters into fertilized zebrafish eggs and watching where the reporter is expressed in live embryos.

Although most patients have the same sized 3 Mb deletion, the severity varies dramatically. While some are mildly affected, others are very sick. In order to go full circle with our research program, we are taking genes discovered in the mouse or zebrafish and are seeing if DNA variations in them could alter the overall phenotype in affected individuals. In addition to the candidate gene effort, we are also taking unbiased approaches by performing a whole genome association study using Affymetrix microarrays containing 1.8 million single nucleotide and copy number DNA variations.

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***Key Words:** Epilepsy, brain, hormones, GABA, substantia nigra, infantile spasms*

EPILEPSY AND THE DEVELOPING BRAIN

Clinical and research data suggest that the immature brain is more susceptible to seizures than the mature brain. The focus of the laboratory is to study, in animals, the epileptic process, its modifiers and consequences as a function of age and gender, translating novel findings to clinical applications. Projects include:

1) Identification of specialized subcortical circuits that modify seizures and are involved in the expression of increased seizure susceptibility of the immature brain. Ongoing studies indicate age and sex related differences in GABA function in the substantia nigra, a site critical involved in seizure control. These changes should be taken into account when drugs are developed to treat age-specific and sex-specific disorders in humans.

2) Identifications of factors responsible for the decreased vulnerability of the immature brain to seizure induced brain injury.

3) Identification of surrogate markers that may predict whether seizures may beget seizures or predict the development of an epileptic encephalopathy.

4) Determination of the relation of disorders of carbohydrate homeostasis to epilepsy and its consequences.

5) Animal models of infantile spasms and catastrophic epileptic disorders of infants. Students interested in normal brain function or brain function during disease (epilepsy) can choose thesis projects utilizing a variety of in vivo and in vitro techniques available in the lab. The lab has received consistent funding since 1979.

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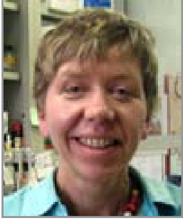

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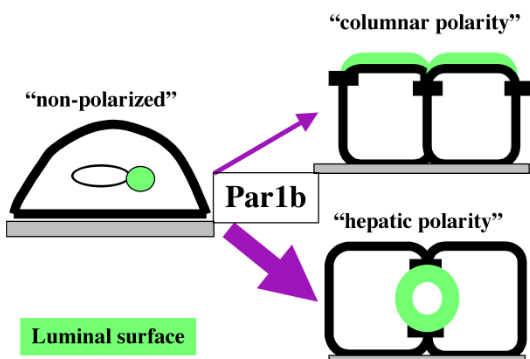
Key Words: ***Epithelial polarity, morphogenesis, cancer, signal transduction, protein trafficking, mammalian cell biology, live cell imaging***

We are using cell biological approaches to understand signaling mechanisms that govern the establishment and maintenance of epithelial cell polarity and their relevance for morphogenesis and cell transformation.

1. Identification of Par1 signaling pathways in epithelial cells
Par1 isoforms are serine/threonine kinases that have emerged as "core determinants" of cell polarity in different contexts, including in mammalian epithelial cells. The relevant PAR1- substrates and signaling pathways, however, are still poorly understood. We are utilizing unbiased screens and candidate approaches to identify Par1 substrates and targets in cultured polarized epithelial cells and elucidate their roles in epithelial morphogenesis. We are also investigating Par1 functions as a tumor suppressor that is targeted by *Helicobacter pylori*, a bacterium implicated in gastric cancer.

2. Mechanisms for the establishment of hepatic versus columnar epithelial polarity
During development, simple (non-stratified) epithelia differentiate along two major lines: they either form lumina at their apices (e.g., columnar epithelia, such as kidney or intestine) or between the lateral membranes of neighboring cells (e.g., liver bile canaliculi -BC). Columnar and hepatic cells also employ different strategies for the biosynthetic protein delivery to the luminal surface. We have recently identified Par1b as the first candidate gene to contribute to this key branching of the epithelial differentiation program (see Fig.). Our goal is to gain insight into the underlying signaling mechanisms for distinct lumen position and trafficking phenotypes in columnar and hepatic cells.



Our repertoire of approaches include the analysis of microtubule-dynamics and of apical surface formation in intact cells by time lapse imaging of fluorescently-tagged proteins, immunofluorescence and confocal laser microscopy to study cell morphology and sub-cellular protein distribution, biochemical assays that measure the kinetics of different protein trafficking steps and biochemical assays that measure protein-protein interactions and enzyme activities in cell lysates,

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Key Words: ***T cells, costimulation, coinhibition, structural biology***

STUDIES ON THE STRUCTURAL BASIS FOR T CELL ACTIVATION AND REGULATION

The research program of the Nathenson lab has followed a simple philosophy, structure follows from function and conversely we can extend structural insights into a testable functional hypothesis. Historically we have used this guiding principle to study the role of MHC in T-cell activation. Biochemical studies using in vitro complex formation of MHC/peptide were used to evaluate the precise mechanism of peptide binding, and in conjunction with the x-ray structure, to determine the structural rules, which govern the selection and binding of such peptides to the MHC molecule. Further biochemical studies were carried out to start defining interactions that are important for recognizing the peptide and the MHC complex by the TCR.

Our initial studies on T cell regulation in collaboration with the Almo laboratory focused on the stimulatory coreceptor CD28 and the inhibitory coreceptor CTLA-4, which, despite their difference in function, bind to the same ligands B7-1 and B7-2. Structural studies suggested that CTLA-4 is capable of forming an extended oligomeric structure, which led to the hypothesis that formation of an extended lattice is necessary for CTLA-4 function but not for CD28. Further biophysical studies using FRET on CTLA-4 and heterodimeric mutants are currently being employed to test this mechanism.

Subsequently we have investigated additional members of the CD28 and B7 receptor families, most notably co inhibitory proteins PD-1 and PD-L1/2. Mutagenesis studies guided by the crystal structure of PD-1 allowed us to generate mutants which either have enhanced or disrupted binding to both the PD-L1 and PD-L2 ligands and more interestingly one mutant that differentially affects ligand binding. Studies with the Nosanchuk lab demonstrated that in the fungal disease Histoplasmosis, the PD-1 pathway is up regulated in order to evade the immune response. Anti PD-1 antibodies reverse the defect in immunity. The mutant PD-1 proteins provide not only reagents to elucidate the signaling pathway but also as a potential therapeutic.

We have recently determined the structures of a number of non-CD28/B7 members of the immunoglobulin and TNF superfamilies with costimulatory activities that have been localized or been postulated to function within the Immunological Synapse. The (SLAM) family includes homophilic and heterophilic receptors that modulate both adaptive and innate immune responses. These receptors share a common ectodomain organization: a membrane proximal IgC domain and a membrane distal IgV domain that is responsible for ligand recognition. We found that three members of the family, NTB-A. CD84, and LY-9 self-associate with a K_d in the nanomolar to sub-micromolar range. These data, in combination with previous reports, demonstrate that the SLAM family homophilic affinities span at least three orders of magnitude, and suggest that differences in the affinities may contribute to the distinct signaling behavior exhibited by the individual family members. These structural data also suggest that, like NTB-A, all SLAM family homophilic dimers adopt a highly kinked organization spanning an end-to-end distance of ~140 Å and are of particular interest in that this kinked geometry can be accommodated by either formation of the same cell (cis) or opposing cells (trans). We believe that the change from cis to trans binding can act as a biological switch to prevent signaling from occurring outside a fully formed immunological synapse.

As more components of the immunological synapse are discovered we will expand our program to not only look at individual receptor proteins but how the network of coreceptors within the immunological synapse interact to ultimately control immunity.

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Key Words: ***vision, synapse, plasticity, glutamate receptors***

MECHANISMS OF SYNAPTIC TRANSMISSION IN THE RETINA

The primary interest of the lab is an understanding of information processing in the retina at the level of individual synapses. Emphasis is on the molecular mechanisms by which synaptic information is modified by short and long-term changes in the visual scene.

The synapse between photoreceptors and an interneuron called the On bipolar cell is a critically important synapse in vision because all visual information flows through it. It has long been appreciated that this is a very different kind of synapse because it is inhibitory despite the fact that glutamate is the transmitter. The On bipolar cell expresses a metabotropic glutamate receptor (mGluR6) that negatively couples to the synaptic channel, recently identified by our group as the novel channel Trpm1 (Shen et al, 2009). In the dark, binding of glutamate to mGluR6 activates a G protein, and one or more G protein subunits then closes the synaptic channel. In the light, glutamate is not released by photoreceptors, and so the synaptic channel (Trpm1) now opens and the cell depolarizes. Thus glutamate mimic an inhibitory transmitter by closing an excitatory synaptic channel. Currently it is not known how the G protein closes Trpm1. A possibility that we are testing, using both molecular and physiological techniques, is that the $G\beta\gamma$ subunit of the G protein can act as an inhibitor of the channel. This would be a novel and exciting finding because G proteins have never been shown to interact directly with Trp channels before now. Our work may also shed light on the underlying cause of congenital stationary night blindness, a disease which has been traced to a mutation in trpm1.

A second project in the lab (a collaboration with Dr. Reed Carroll) is to study synaptic plasticity in On ganglion cells (Xia et al, 2007). We have recently made an exciting new discovery regarding the ability of ganglion cells to "learn" and adapt. Specifically we have found that the visual experience of ganglion cells changes the composition of AMPA receptors from those that contain a GluR2 subunit and are impermeable to Ca2+ to a type of receptor that lacks the GluR2 subunit and is Ca^{2+} permeable. This form of plasticity has never been reported before in the retina, and is similar to the kind of plasticity that is the basis for memory and learning in other brain regions. An important question to be addressed is the contribution of AMPA receptor plasticity to vision. To address this question, we use a combination of physiological, molecular and behavioral approaches. One hypothesis to be tested is that synaptic expression of the Ca2+-permeable AMPA receptor makes On ganglion cells more sensitive to small changes in transmitter release (i.e, increases synaptic gain), allowing for better vision under dim light conditions.

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Key Words: ***Reproduction, aging, ovarian steroids, hypothalmus, GABA***

Dr. Neal-Perry's research focuses on determining the key cellular and molecular mechanisms by which hormones regulate the neuroendocrine axis and female reproduction.

One area of particular interest is the role of the neuroendocrine axis in female reproductive aging, especially the cellular events that alter the brain's responsiveness to ovarian steroids. Reproductive senescence in female rodents and humans is heralded by reduced responsive of the hypothalamus to estrogen positive feedback, resulting in a abnormal luteinizing hormone surges and infertility. There are several candidate neurotransmitter systems and neurotrophic factors that might contribute to age-related LH surge failure and subsequent infertility. Our research suggests that age-related changes in the hypothalamic response to ovarian hormones and the LH surge mechanism are causally related to reduced excitatory neurotransmission mediated by the excitatory neuropeptide kisspeptin. Additional studies have suggested that decreased kisspeptin availability results in an imbalance in excitatory (glutamatergic; decreased) and inhibitory (GABAergic; increased) neurotransmission within the hypothalamus. We have also demonstrated that reduced brain insulin growth factor-1 (IGF-1) signaling in the aging brain impairs hypothalamic responsiveness to estrogen positive feedback conditions. Hypothalamic IGF-1 receptor signaling regulates female reproductive function, hypothalamic kisspeptin expression and nutrient sensing through cellular mechanisms that rely upon autophagy. Current experiments are determining whether compromised hypothalamic IGF-1 receptor signaling in middle-aged females reduces the ability of estradiol to upregulate hypothalamic kisspeptin expression in key areas of the hypothalamus thereby altering the balance of glutamate and GABA neurotransmission in middle-aged females exhibiting abnormal LH surges. We are also investigating the role of autophagy in female reproductive senescence and estrogen dysregulation of gonadotropin release.

Vitamin D receptors are located in the central nervous system, ovaries and uterus and vitamin D is hypothesized to be critical for fertility and reproductive success. Vitamin D deficiency is associated with an accelerated somatic aging phenotype, infertility and/or subfertility. The mechanism by which vitamin D deficiency affects the hypothalamic-pituitary-gonadal axis is unknown. Our lab nvestigates the effects of vitamin D deficiency on autophagy and female fertility as it pertains to hypothalamic-pituitary function, ovarian responsiveness to gonadotropins, embryo cleavage, fertilization and implantation rates.

Our research relies upon expertise in multiple microsurgical techniques, intracerebral microdialysis, intracerebral drug infusion, HPLC, controlled ovarian hyperstimulation, immunohistochemistry, immunoassays, serial blood sampling, *in vitro* fertilization, and a number of molecular biochemistry techniques.

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Key Words: ***basal ganglia, decision-making, electrophysiology in behaving animals, behavioral neuroscience, drug abuse***

NEURAL CIRCUITS UNDERLYING REWARD-SEEKING BEHAVIOR

My lab focuses on understanding the neural circuits responsible for reward-seeking and addictive behaviors. We use a systems-level approach that combines behavioral, pharmacological and electrophysiological techniques in awake, freely moving animals. We begin by identifying a hypothesis regarding the neural circuits underlying a particular behavior. For example, the nucleus accumbens (part of the ventral striatum) projects to motor output structures of the basal ganglia. The accumbens also receives input from limbic structures that have been suggested to process stimuli that predict events of consequence to the animal's well-being. These limbic structures include the basolateral amygdala, which sends glutamatergic axons to the accumbens, and the ventral tegmental area (VTA), which sends a dopamine projection. Therefore, we hypothesized that the amygdala and VTA projections to the accumbens are part of the neural circuit that controls the animal's response to reward-predictive stimuli.

To test this hypothesis, we designed a behavioral task that requires rats to respond, by pressing a lever, to an auditory stimulus that predicts sucrose reward. We then determined that the dopamine projection to the accumbens is required for this behavior by demonstrating that dopamine receptor antagonists microinjected directly into the animals' nucleus accumbens caused animals to cease responding to the stimulus. We also showed that transient inactivation of the amygdala had the same effect. Next, we used multiple simultaneous single-unit recordings of neurons in the accumbens and amygdala to demonstrate that subpopulations of neurons were excited or inhibited by the reward-predictive stimulus. Finally, we established that stimulus-evoked excitations and/or inhibitions in the accumbens are required for the reward-seeking behavior instigated by the stimulus. We did this by inactivating either the dopaminergic VTA neurons or amygdala neurons while recording from accumbens neurons during the stimulus-evoked reward seeking task. Inactivation of either structure selectively abolished the firing of accumbens neurons responsive to reward-predictive stimuli. These experiments established that the convergence of the excitatory projection from the amygdala and dopaminergic projection from the VTA in the accumbens is an important part of the neural circuits that underlie stimulus-evoked reward-seeking behavior. Ongoing experiments seek to determine the nature of the information encoded by the firing of accumbens neurons driven by the amygdala and dopamine projections.

Drugs of abuse can also serve as rewards, often to the extent that drug-seeking (sometimes in response to drug-predictive stimuli) becomes excessive and harmful. A long-term goal of these experiments is to use our increasing knowledge of the neural circuits that control reward-seeking to ask how these circuits produce aberrant behavior (excessive drug-seeking) in addiction.

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Key Words: fungus, histoplasmosis, cryptococcosis, candidiasis, melanin

- ***Histoplasma capsulatum*:** Research on this fungus pertains to the development of active and passive immunotherapeutics. Since individuals with severe histoplasmosis [such as AIDS patients with disseminated disease] often lack effective cell-mediated immune responses, induction of an effective humoral response or passive administration of antibody has tremendous therapeutic potentials. My laboratory is the first to identify protective monoclonal antibodies for the treatment of *H. capsulatum* infection. We are studying the mechanisms of antibody efficacy and are testing the recombinant antigens as a potential prophylactic and therapeutic vaccine. Also, we have embarked with the Nathenson and Almo labs to study the impact of co-stimulation on histoplasmosis and have demonstrated that the PD-1/PDL pathway is critically important to disease pathogenesis. Antibodies can interfere with this negative co-stimulation pathway and prevent lethal histoplasmosis.

- ***Candida parapsilosis*:** This is the newest fungus to the laboratory. The incidence of *C. parapsilosis* infections has exploded in recent years and very little is known about its virulence. We developed the first efficient method for targeted gene deletion for *C. parapsilosis* and are actively pursuing targets to define what makes this fungus pathogenic, with a particular emphasis on secreted hydrolytic proteins and lipid metabolism. We are also targeting virulence associated genes in *C. albicans* and studying their role in pathogenesis.

- ***Cryptococcus neoformans*:** We are primarily using this pathogen to elucidate the impact of melanin in pathogenic fungi. Melanin is a complex polymer of unknown structure that is prevalent throughout the biological kingdoms. Ongoing investigations are examining mechanisms of melanin synthesis and rearrangement by molecular, physical, and immunological methods. This work is in collaboration with Dr. Arturo Casadevall, Departments of Microbiology & Immunology and Medicine.

Additional areas of special emphasis:
-Methamphetamine: This drug has increasingly become a major scourge on our society. Although the behavioral impact of methamphetamine is well understood, there is a dearth of data on the effect of the drug on immune function. We have established that methamphetamine significantly adversely regulates diverse aspects of immunity. We have an ongoing program to further elucidate the mechanisms and impact of this disregulation.

-Nitric oxide releasing nanoparticles: We are exploring the therapeutic potential of this novel compound for the treatment of diverse infectious diseases, including bacterial and fungal diseases. This work is in collaboration with Dr. Joel Friedman, Departments of Medicine and Physiology & Biophysics.

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Key Words: liver cancer; angiogenesis; growth factors

Liver Carcinogenesis: Cellular, metabolic, genetic and proteomic phenotypes of preinvasive and invasive stages of hepatomas as well as cell lineage and metastasis pathways leading to the development of liver cancer are being investigated in chemical-induced hepatocarcinoma rodent animal systems and in transgenic mouse systems considered analogous to hepatocarcinogenesis process in humans. Liver cell types (stem cells, oval cells, bile ductule cells, ductule transitional cells, hepatocytes) will be analyzed for the expression of unique phenotypic properties during carcinogenesis, including asialoglycoprotein receptor, desmosomes, gap junctions, peroxisomes, bile canaliculi, cytoskeletal proteins (microtubules, actin and intermediate filaments), cell proliferation oncogenes, p53 tumor suppressor gene, mRNA sequences of nodule-specific enzymes and retroviral genetic markers. The interactions of carcinogen-altered hepatic cells with extracellular matrix and with endothelium during metastasis will also be investigated.

Endocytosis/Exocytosis: Receptor-mediated endocytosis of asialoglycoprotein/ligands and exocytosis of Herpes simples virus will be studied in rat hepatocytes in culture, mutant liver cell lines deficient in receptor-mediated endocytosis and in human hepatoma cell lines focusing on sorting compartments and mechanisms involved in intracellular trafficking and the interrelations of endosomes, cytoskeleton and motor molecules. Diverse microscopic methods (light-, confocal, laser capture microdissection and electron microscopy) and cytochemical procedures (enzyme-, immuno-, cDNA hybridization, RT-PCR and molecular biology protocols (gene sequencing, cDNA microarrays, proteomics) will be employed.

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Key Words: host-pathogen interaction, Toxoplasma gondii, cell physiology

Our laboratory studies the interaction of host cells with intracellular pathogens, with a major focus on the apicomplexan parasite, *Toxoplasma gondii*. We are interested both in understanding the adaptations employed by the parasite to manipulate its intracellular environment, and also in exploiting these interactions as tools for the investigation of important issues in mammalian cell biology. One area of focus is the modification of host mTOR-dependent functions by *T. gondii*. These functions include the regulation of protein synthesis and cell cycle progression (dependent on mTOR complex-1) and the control of cytoskeletal organization and migration (dependent on mTOR complex-2). We have linked several effects of *T. gondii* on these functions to mTOR pathways, and a goal of our current research is to elucidate mechanisms by which the parasite controls these signals. Additional areas of focus include parasite regulation of host cell autophagy and apoptosis.

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***Key Words**: biophotonics, imaging, quantitative microscopy*

EXPERIMENTAL TECHNIQUES FOR IMAGING THE CELLULAR BASIS OF HUMAN DISEASE

My research is performed in the Innovation Laboratory, a new laboratory that serves as an incubator for the development of novel instrumentation and imaging methods. Within the Innovation Laboratory, physicists and engineers will partner with biologists and chemists in order to push the limits of microscopy as applied to the cellular basis of human disease. Some of the initial biological thrusts include: (1) elucidating the mechanisms governing cell motility and metastasis; (2) visualizing the dynamics of single RNA molecules in living cells and tissues; (3) unraveling the complexities of molecular machines; (4) measuring and localizing the interactions between molecules in live cells; and (5) the analysis of gene expression patterns in single cells. We want to not only visualize and understand the dynamics of cellular events, but we also seek to develop strategies to control their behavior.

At the Innovation Laboratory, we are capitalizing on advances in optoelectronics that are enabling physicists and engineers to develop novel microscopes and to demonstrate the practicality of theoretical innovations. Concomitant with these advances, fluorescence techniques, as applied to the biological sciences, are being extended to new frontiers.

A few of the optical methods that we are currently using include: (1) confocal and multiphoton microscopy including intravital imaging in animals with subcellular resolution ; (2) fluorescence resonance energy transfer; (3) fluorescence recovery after photobleaching; (4) total internal reflection microscopy; (5) rapid spectral imaging; and (6) photon uncaging. In the near future, we will have the capability to use: (1) fluorescence lifetime imaging microscopy; (2) fluorescence correlation spectroscopy; and (3) various forms of interference microscopy.

Projects are available for individuals who wish to apply their quantitative backgrounds and analytical strengths to a compelling biological question. Individuals may become involved with experiments, modeling, theoretical research, image processing or the development of software. So if you think big and you want to explore the nanoscale, please contact us.

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Key Words: ***stem cells, muscle differentiation, regeneration, vertebral segmentation, signaling pathways, transcription factors, mathematical modeling***

REGULATION OF STEM CELL PROLIFERATION, MUSCLE REGENERATION AND VERTEBRAL SEGMENTATION

We focus on two major research areas:

1- Stem cell proliferation, muscle differentiation and regeneration:
Muscle diseases occur due to genetic-, injury- or aging-related causes. It is crucial to discover the gene regulatory network that controls the differentiation of muscle cells to be able to induce adult muscle stem cells to proliferate and differentiate. Moreover, the regulation of metabolism in muscle cells needs deeper understanding to prevent and cure metabolic defects, such as insulin resistance in muscle cells. We are investigating how the proliferation, differentiation and metabolism of the muscle stem cells are controlled by the signaling pathways and their transcription factor targets. We are investigating the roles of these transcription factors by loss- and gain-of-function experiments followed by detailed investigation of the impacts of these perturbations on: muscle stem cell proliferation, muscle differentiation and regeneration, and metabolism in muscle cells. Genome-wide studies, molecular perturbation experiments and imaging will be integrated to achieve these objectives.

2- Systems developmental biology:
The axis of vertebrates is characterized by the iteration of vertebrae. The precursors of the vertebrae are derived from embryonic somite segments. Embryos elongate from their posterior end and a new somite appears at a species-specific pre-determined time. The periodicity of this spatial pattern formation is controlled by a gene-expression oscillator, called the segmentation clock that ticks in the cells residing in the unsegmented tissue. Breakdown of the oscillations disrupts somite boundaries and results in vertebral defects. It is important to identify the genes involved at different stages of segmentation in order to develop future therapies for patients. We aim to identify novel regulators of vertebral segmentation and integrate the experimental data with mathematical modeling to unravel the regulatory network that paces the rhythm of the periodic gene expression and spatial pattern formation.

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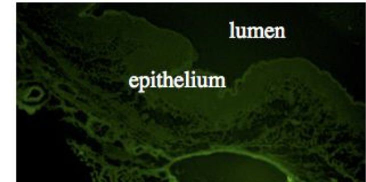
Key Words: RNA interference, gene discovery, therapeutics, HSV-2, immune responses

RNA interference (RNAi) is a mechanism of gene silencing originally described in plants and invertebrates and more recently in mammalian cells. Double-stranded RNAs are cleaved into 21-25mer duplexes, termed small interfering (si)RNAs, and these siRNAs act as a guide to cleave homologous target mRNA, resulting in a sequence-specific decrease in mRNA. Introduction of siRNAs into mammalian cells leads to degradation of specific mRNA.

RNAi has become a valuable tool in gene discovery as well as an attractive therapeutic candidate. We have shown that intravaginal application of siRNAs targeting viral genes protects mice from a lethal herpes simplex virus (HSV-2) infection.



Fluorescent siRNAs introduced into the vagina are observed throughout the genital mucosa.
Adapted from Palliser et al, Nature 2006

These data indicate that siRNAs could be used in a therapeutic setting such as preventing a sexually transmitted disease, e.g. HSV-2. However, this work is in its initial stages and many questions remain: Is the siRNA silencing we observe optimal? What determines duration or efficacy of siRNA silencing? Are there off-target responses associated with the siRNA treatment?

One aim of our lab is to address these questions. For example, we will design constructs that will deliver siRNAs in various ways. By comparing the ability of these reagents to confer specific gene knockdown, we hope to gain an understanding of what components are required to achieve optimal silencing.

A second aim is to utilize a hypothesis-based approach to dissect immune responses in various mouse models using siRNAs. By understanding the immunity associated with infection by a particular pathogen we can potentially adjust immune responses to favor pathogen eradication. In this way, siRNAs could also be used as components in a microbicide, either as an alternative or adjunct to siRNAs targeting pathogen-expressed genes at the infection site.

Lab Webpage: http://palliserlab.googlepages.com/

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Key Words: *transition metals, EPR spectroscopy, heme protein, pulsed EPR, metallo enzyme, prion protein*

Transition metals in biological systems play an important role in oxygen binding, transport, and activation, in electron transfer, and in enzyme catalysis. Research in this laboratory is directed towards understanding the structure and function of metal binding sites in the aforementioned processes, largely through spectroscopic analysis. The tools available include optical, continuous wave, and pulsed EPR spectroscopy. Students wishing to pursue a degree in this laboratory can elect topics related to:

1) the mechanisms of oxygen activation in drug-DNA cleavage reactions and in hemoprotein catalysis;

2) the role of protein structure in modulating catalytic activity and ligand stabilization through hydrogen bonding at metal sites;

3) mapping out active site structures with paramagnetic probes; the mode of substrate binding in metallo enzymes;

4) making distance measurements between bound substrates and metal ions coordinated to biomolecules and between paramagnetic centers; and 5) the determination of nuclear quadrupole, nuclear hyperfine and g tensor orientations at metal centers.

These approaches are being applied to copper- iron-, cobalt- and mangano proteins, in addition to low molecular weight model compounds of known structure.

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Key Words: ***Owl, sound localization, auditory space, experience-dependent synaptic plasticity, electrophysiology***

All auditory information essential for discriminating and identifying sounds ascends through the brainstem auditory nuclei. Our goal is to determine how multiple information dimensions flow and are represented in the avian brain using physiological and theoretical approaches.

A primary advantage of using barn owls for the exploration of auditory processing is the substantial body of behavioral, anatomical and neurophysiological work that has elucidated the mechanisms of sound localization. The main cues owls use to compute sound direction are the interaural level difference (ILD) and the interaural time difference (ITD). Two independent brainstem pathways process ITD and ILD and converge in the midbrain, where a map of auditory space emerges. This computation evokes a head-orienting behavior towards the sound source. Thus, in barn owls, the neural algorithm for sound localization can be viewed as a system in which two input variables (ITD and ILD) are processed in parallel in order to control two output variables (horizontal and vertical coordinates of head saccades). We have used analytical models to describe the neural response in the owl's auditory system. This approach has guided our experiments and aided the interpretation of our findings. Based on behavioral experiments with humans, a similar approach to describe humans' sound localization has also been developed. However, due to a lack of neural data in humans, the predictive power of models of sound localization has been a persistent question. Our studies in barn owls address this issue.

In the auditory midbrain, not only does spatial tuning emerge but it is calibrated through visually-instructed plasticity. The midbrain is thus an attractive structure to perform *in vitro* studies of cellular and synaptic bases of sound localization as well as of experience-dependent plasticity. We have used chicken brain slices to approach these questions. In this preparation, we found endocannabinoid-dependent plasticity mediated by both presynaptic and postsynaptic effects. This process can not only favour synapse-specific modulations but also change the threshold for subsequent synaptic plasticity.

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Key Words: brain, plasticity, synapse, gap junctions, auditory system, calcium

Our laboratory is interested in the properties and dynamics of gap junction-mediated electrical transmission in the vertebrate brain. Perhaps because of the relative simplicity of transmission, electrical synapses are generally perceived as passive intercellular channels that lack dynamic control. Thus, while the study of plasticity of chemical synapses has long been an area of primary interest to neuroscientists, less is known about the modifiability of electrical synapses. In contrast with mammalian electrical synapses that generally have limited experimental access, lower vertebrates have provided with advantageous experimental models in which basic properties of electrical transmission can be more easily study. This is the case of identifiable auditory afferents terminating on teleost Mauthner cells known as "Large Myelinated Club endings". These endings are "mixed" (electrical and chemical) synaptic contacts that offer the rare opportunity to correlate physiological properties with molecular composition and specific ultrastructural features of individual synapses. Gap junctions at these model synapses undergo activity-dependent potentiation and are mediated by connexin35, the fish ortholog of connexin36, which is widely distributed across the mammalian brain.

Our current work focuses on the mechanisms underlying activity-dependent changes in gap junction-mediated electrical synapses by investigating:

Their functional relationship with glutamate receptors in both fish and mammals.

Their interaction with dopaminergic, opioid and endocannabinoid systems.

The molecular mechanisms responsible for changes in electrical transmission, in particular the identification of connexin-associated regulatory proteins.

Thus, while focusing in the properties of electrical synapses, the research of our laboratory explores the complexity of synaptic transmission and signaling mechanisms in general.

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Key Words: ***brain, glutamate, receptors, Alzheimer's, Parkinson's***

SYNAPTIC FUNCTION, MODULATION, AND PLASTICITY

Tight regulation of spike timing and network synchrony are required for normal brain function and pathological conditions such as epilepsy have been linked with excessive synchrony. For example, Absence seizures are accompanied by the onset of low-frequency (3 Hz) spike and wave discharges across broad regions of the brain and increased gamma oscillations have been observed in EEG recordings shortly before and during seizures. Memory acquisition and retrieval is also associated with oscillations in the hippocampus and is in part mediated by multiple types of interneurons. Thus, understanding how spike timing is regulated is a critical step toward improving treatments that modify network activity.
Action potential (AP) timing is a fundamental information unit which neurons use to communicate with each other. Feedback and feedforward inhibition, spike timing-dependent plasticity, and network oscillations are just some of the neuronal functions that have been shown to require the coordinated firing of multiple neurons. This time code can be modulated by subthreshold synaptic input which can either advance or delay subsequent action potentials. The mechanisms underlying this timing modulation may depend on input characteristics, location, and the timing of the input relative to the firing cycle of the postsynaptic cell. We are examining which input characteristics are the most effective for the induction of timing changes. We also examine how interaction between excitatory input and voltage-gated channels and multiple synaptic inputs integrate over the dendritic tree to modulate spike timing. Input-induced changes in spike timing are necessary for establishing synchronous network oscillatory waves which are present during a wide range of cognitive functions. We use a combination of electrophysiology, local photolysis and imaging to address these questions.

Additional ongoing projects examine the role of inhibitory synaptic transmission in sensory processing. The task of combining sensory signals to form a coherent olfactory representation falls mainly on the piriform cortex (PC). Studies have shown that odor identity is represented as spatially dispersed neuronal subgroups in the PC. How these ensembles are generated is still a matter of conjecture and a key step to understanding these codes will depend on the functional connections PC neuronal components make with each other. Of particular interest are synaptic connections made by local interneurons onto pyramidal cells, as these circuits have been shown to tune pyramidal cell output to odor-related inputs from the olfactory bulb. We have determined that interneurons located caudal to a pyramidal cell are more likely to inhibit its spike output than interneurons at more rostral regions**.** Consequently, OB excitatory inputs that activate mostly caudal microcircuits are less likely to elicit spiking in a pyramidal cell than inputs activating mostly rostral microcircuits. We hope to further understand the significance of this rostro-caudal asymmetry by elucidating the cellular and circuit basis for such differential inhibition over PC space.

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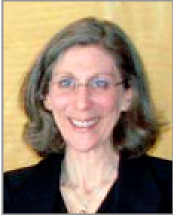

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Key Words: ***infectious disease, Cryptococcus, Pneumococcus, B cells, immunity***

The focus of our laboratory is immunity to encapsulated pathogens, in particular Cryptococcus neoformans (Cryptococcus) and Streptococcus pneumoniae (pneumococcus). The diseases caused by these microbes occur in normal and immunocompromised patients, with pneumococcal disease occurring primarily in young children, the elderly and those with defects in antibody immunity and cryptococcal disease occurring primarily in patients with AIDS and other immunocompromised patients, including those who have undergone solid organ transplantation. At present, antimicrobial therapy for Pneumococcus and Cryptococcus suffers from limitations stemming from antimicrobial resistance and the inability of antimicrobial agents to cure disease in immunocompromised patients, respectively. Pneumococcal vaccines are in use, but have numerous shortcomings, particularly in immunocompromised patients. There is no vaccine for Cryptococcus. Pneumococus is the leading cause of pneumonia in the United States and globally, causing more than one million deaths a year in children under the age of five years. Cryptococcus is a leading cause of fungal meningitis, causing disease in more than 900,000 and death in more than 600,000 people annually, primarily in sub-Saharan Africa. The main virulence factor of both microbes is a polysaccharide capsule that surrounds the organism and poses significant challenges to the development of cellular and antibody immunity and resistance to the diseases they cause. A unifying theme of our work is to identify mechanisms that govern innate and antibody immunity to these microbes with the goal of improving the ability to predict disease susceptibility and developing better therapies and vaccines. Our research involves a multidisciplinary approach to these problems, encompassing immunology, microbiology, molecular biology, genetics, translational research and the use of animal models of disease. Many of the concepts and hypotheses that drive our work are based on the Damage-response framework, a theory of microbial pathogenesis.

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Key Words: macrophage, hormone, tumor microenvironment, breast cancer, cell cycle

(1) Tumor Associated Macrophages Promote Tumor Progression and Metastasis. Many hematopoietic cells, particularly those of the innate immune system, populate the tumor microenvironment. Of these both clinical observations and our genetic experiments in mouse models of beast cancer have indicated that macrophages play a pivotal role in enhancing tumor progression and metastatic potential (Joyce & Pollard, 2009). Our mechanistic studies indicate that these tumor-associated macrophages regulate the angiogenic switch required for the malignant transition and also promote tumor cell invasion, migration and intravasation through reciprocal EGF and CSF-1 signaling (Condeelis & Pollard, 2006; Lin and Pollard, 2007; Pollard, 2004, 2008, 2009). Furthermore we have recently identified a sub-population on macrophages that are required for metastatic seeding and persistent growth at distant sites (Qian et al., 2009). The lab is focused upon defining unique sub-sets of macrophages that promote different aspects of tumor progression and metastasis and in elucidating the fundamental mechanisms behind these actions (Qian & Pollard 2010). We are using novel mouse genetic and virus based tools developed in our lab to interfere with signaling pathways in vivo to define the function of these pathways in the metastatic progression of tumors. Further, we are studying the evolution of the immune system during tumor development to determine how the tumors escape from being rejected and focus on the role of macrophages in these processes. The identification of these mechanisms of tumor promotion by macrophages will allow us to novel therapeutic approaches to inhibit tumor progression and malignancy.

(2) Regulation of cell proliferation by female sex steroid hormones. Exposure to estrogen is the major risk factor for endometrial and breast cancer. This carcinogenic effect is thought to be due to the induction by this hormone of continuous cycles of epithelial cell proliferation that allows the fixation of spontaneously occurring oncogenic mutations. In contrast progesterone exposure reduces the risk of these cancers. In the uterus of mice and humans estrogen stimulates epithelial cell proliferation while progesterone completely blocks this estrogen-induced proliferation. We have used biochemical and genetic approaches in mice to identify the mechanisms of action of these sex steroid hormones. We have identified two pathways stimulated by estrogen and inhibited by progesterone that are required for the estrogen-induced mitogenic effect. The first of these is through IGF-1 signaling activating cyclin D1 mobilization into the nucleus while the second involves licensing of DNA replication through the regulation of the function of Minichromosome Maintenance proteins (MCMs) (Pan et al., 2006; Zhu and Pollard, 2007). To extend these studies to humans we have developed a program to obtain endometrial biopsies and have used laser capture microdissection of epithelial tissue to confirm similarities in hormone control of gene transcription between the two species. Further, we have been able to xenograft human endometrial tissue into mice where it forms functional endometrial structures that responds to humans and which allows for biochemical analysis (Polotsky et al., 2009). Using all these techniques, we have identified novel hormone regulated signaling pathways that are deregulated in human endometrial proliferative diseases such as endometriosis and cancer and that may therefore act as therapeutic targets. Current studies are to further elucidate the downstream cascade of transcriptional regulatory proteins that are induced by estrogen and progesterone that are involved in the regulation of cell proliferation. We also have a significant set of studies in understanding the roles of Micro RNAs in the regulation of hormone action. These are pursued at the biochemical and genetic levels and we are currently developing novel genetic tools in which to study sex steroid hormone action (Kuokkanen et al 2010).

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Key Words: ***T cells, tuberculosis, autoimmunity, cancer, vaccine, MHC, CD1, lipids***

IMPROVING T CELL RESPONSES FOR VACCINATION AND DISEASE PREVENTION

Our laboratory studies the control of acquired immune responses by T cells, which we view as the master regulators and key effectors of host defense and immune tolerance. In broad terms, our research can be divided into two interrelated areas. The first is to understand the role of regulatory T cells, with particular emphasis on the activities of a specialized T cell subset known as CD1d-restricted NKT cells. These T cells have the highly unusual property of responding to specific glycolipid antigens, and we are studying ways to control their regulatory functions in various mouse models of autoimmune, infectious or neoplastic disease. The second second major research area is the study of T cell responses against pathogenic microorganisms, especially *Mycobacterium tuberculosis.* We have recently made significant progress in understanding how mycobacteria block effective host T cell responses, and we are now working to incorporate our findings into the rational and intelligent design of a new tuberculosis vaccine. In the short term, we hope to broaden our understanding of how organisms like *M. tuberculosis* successfully evade eradication by the immune system. Our major long term goal is to create genetically or chemically modified live attenuated *M. tuberculosis* strains that will be safe and effective as vaccines against tuberculosis.

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Key Words: ***reverse transcriptase, aptamer therapeutics, HIV associated dementia***

The research focus of our laboratory is directed towards three aspects of HIV - Biology, therapeutics and pathogenesis.

1. HIV-1 Reverse Transcriptase (RT): Multiple projects are ongoing with a focus on delineating structural determinants of critical RT functions and understanding the role of host proteins in HIV reverse transcription. RT is the major drug target for HIV. Thus, a better definition of this drug target is likely to help in the design of new drugs with better efficacy and reduced frequency of drug resistance. Ongoing work includes delineating both physical and functional interactions of RT with other viral proteins (e.g., Integrase) as well as novel cellular proteins.

2. Aptamer therapeutics: We are developing and testing the efficacy of novel, anti-HIV-1 RNA aptamers in inhibiting HIV-1 replication in cell culture. Aptamers are sequences isolated by the iterative process of SELEX that are highly specific to their targets. We have successfully developed aptamers to HIV-1 targets such as RT, Gag, Protease and Nef proteins. The most efficacious aptamers identified in our laboratory, will then be tested in nonhuman primate models, by introducing selected aptamers into hematopoeitic stem cells, which will then be used in bone marrow transplantation followed by challenge with chimeric SHIVs.

3. HIV associated Dementia: HIV associated dementia (HAD) is common among clade-B HIV-infected individuals, but less common among individuals infected with clade-C HIV-1, suggesting that there are clade-specific differences in neuropathogenicity. Understanding clade-specific determinants of neuropathogenesis can shed light on the disease mechanism and help develop targeted drugs for HAD. Therefore, we investigated neuropathogenesis induced by the two HIV-1 clades using SCID mouse HIV encephalitis model in collaboration with Dr. William R. Tyor from the Medical University of South Carolina, who developed this model previously. Introduction of clade B HIV into such mice reproduces the key features of human HAD. Using this model, clade B ($HIV\text{-}1_{ADA}$) or clade C ($HIV\text{-}1_{Indie\text{-}C1}$) HIV-infected macrophages were injected intracranially into SCID mice. Subsequent to injection, in cognitive tests, mice exposed to similar inputs of $HIV_{Indie\text{-}C1}$ made fewer memory errors than those exposed to $HIV\text{-}1_{ADA}$. Furthermore, histopathological analysis of mice exposed to $HIV\text{-}1_{ADA}$ exhibited greater astrogliosis and increased loss of neuronal network integrity. Differences were noted in another key characteristic of HIV-1 that influences HAD, increased monocyte recruitment. $HIV\text{-}1_{Indie\text{-}C1}$-infected macrophages recruited monocytes poorly in vitro compared to that of $HIV\text{-}1_{ADA}$-infected macrophages. Monocyte recruitment was Tat-dependent, in agreement with a striking observation that was previously made by our laboratory, where Tat protein from clade C HIV-1 was shown to be a defective chemokine. This is the first demonstration, since HIV neuropathogenesis was first recognized, that genetic differences in different HIV-1 clades can affect disease severity and that such studies can help identify the key players in neuropathogenesis by HIV-1.

Current studies are focused on the clade differences in cytokine dysregulation, damage to blood-brain barrier and neurotoxicity observed in HIV-infection.

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Key Words: SUMO pathway, ubiquitin, transcription

AT THE INTERSECTION OF THE TRANSCRIPTION, UBIQUITIN, AND SUMO PATHWAYS

How does a cell respond rapidly to changes in its environment? How does an organism develop from a single cell into specialized cell types and into tissues and organs? The answers to these broad questions often boil down to two principal mechanisms: regulation of gene expression and post-translational signaling that alters protein activity. Historically, the long-term interest of our lab has been to understand how the general transcription factors and their regulators combine to generate such exquisitely flexible yet consistent patterns of gene expression. Recent genetic selections in our lab, however, established links between transcription and post-translational modification by the ubiquitin family, drawing our lab into the intersection of the transcription, ubiquitin, and SUMO pathways.

So what are the ubiquitin and SUMO pathways and why are they important? Ubiquitin and SUMO are the two most prominent members of the ubiquitin protein family. Both of these small regulatory proteins (less than 100 amino acids long) are covalently conjugated to other cellular proteins and regulate their activity, analogous to the role that phosphorylation performs in other signaling pathways. Ubiquitin and SUMO are conserved and essential for viability of most eukaryotic cells, and they post-translationally modify many proteins that are important for normal cell growth and human disease. Conjugation with ubiquitin often targets substrates for proteosome-mediated degradation, and it can also serve as a signaling modification that stimulates protein-protein interactions, whereas SUMO conjugation has less predictable biological outcomes. Modification with SUMO can: (1) directly alter protein activity, (2) alter cellular localization, and (3) affect protein stability, but the molecular determinants of these different outcomes are not completely understood. The lack of a clear understanding of the roles and regulation of this essential pathway is one of the major elements that has drawn us to study it further. The ongoing projects in our lab have three goals: (1) to investigate the roles of two newly identified ubiquitin ligases in general transcriptional regulation, (2) to identify downstream effectors of SUMO pathway signaling, and (3) to identify small molecule inhibitors of the SUMO pathway.

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Key Words: cancer, genomics, proteomics, clinical research, translational research

We have developed a multidisciplinary group including surgery, oncology, pathology, molecular biology, protein chemistry, systems biology, epidemiology and biostatistics to study Head and Neck Squamous Cell Carcinoma (HNSCC) (www.aecom.yu.edu/headandneck/). Our studies employ cutting edge genomic and proteomic technologies to elucidate molecular mechanism regulating tumor behavior. Studies on primary human cancer and in model systems have identified candidate signatures that will be tested for use in optimizing initial treatment selection for patients with HNSCC.

Our goals are:

- To develop new diagnostics that will identify optimal treatments at initial diagnosis.
- To assess early genetic changes in smokers to identify individuals at greatest risk for developing cancer and to develop effective interventions.
- To identify potential new targets for drug development.

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Key Words: ***autoimmunity, systemic lupus erythematosus (SLE), anti-DNA antibodies, lupus nephritis***

The laboratory studies systemic lupus erythematosus (SLE), which is the prototypical systemic autoimmune disease. The most characteristic serologic feature of SLE is the presence of antibodies directed against double stranded DNA. While SLE manifests clinically as a disease that can involve many organ systems, it is the injury to the kidney in the form of glomerulonephritis that often determines the prognosis of lupus patients. It has become increasingly clear that not only are anti-double stranded DNA antibodies important in making a diagnosis of SLE, but that these antibodies also play an important role in the pathogenesis of lupus nephritis.

The anti-DNA antibodies present in SLE have the characteristics of antibodies that arise in an antigen-driven response. Lupus associated anti-DNA antibodies are characteristically IgG, somatic mutations are present in the antigen binding regions of the antibody, and they display high affinity for DNA. However, mammalian DNA is not immunogenic, and immunization with DNA does not elicit anti-double stranded DNA antibodies in non-autoimmune mice. The antigen driving the anti-dsDNA antibody response in SLE, as well as the kidney target for these antibodies, is not currently known.

In the laboratory, we are interested in the antigens driving the generation of pathogenic anti-double stranded DNA antibodies, and the cross-reactive target for these autoantibodies in the kidney. This will direct the development of innovative approaches to block the deposition of anti-DNA antibodies in the kidney. Additional projects in the laboratory concern investigation into the molecular mechanisms by which anti-DNA antibodies induce damage in kidney cells, and the role of TWEAK, a novel member of the TNF-ligand superfamily, in the pathogenesis of inflammatory kidney disease.

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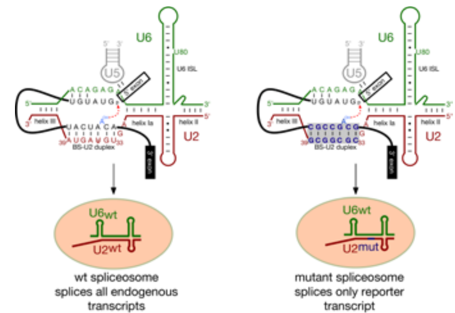
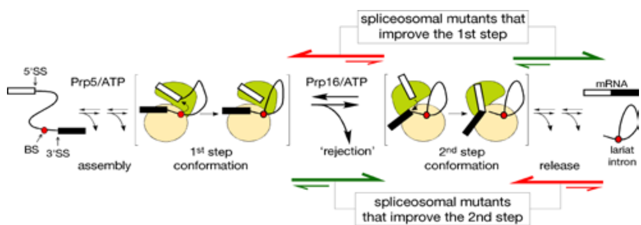
Key Words: RNA, spliceosome, RNA helicase, ribonucleoprotein

Our laboratory is interested in mechanisms by which the spliceosome assembles and catalyzes two related chemical reactions. Recently, we formulated a general model of spliceosome function based on concepts of thermodynamic equilibrium, which impacts our understanding of substrate selectivity and provides general insights into RNP machines whose functions rely on dynamic rearrangements.

Background. Intron removal, an essential maturation step for eukaryotic pre-mRNAs and a control point for regulation, is catalyzed by the spliceosome, a 50-60S complex of five snRNAs and >100 proteins. The spliceosome is both compositionally and conformationally dynamic; each transition along the splicing pathway presents an opportunity for progression, pausing or discard, allowing splice site choice to be regulated throughout both the assembly and catalytic phases of the reaction.



Orthogonal systems for *in vivo* investigation of catalytic center interactions during pre-mRNA splicing. During pre-mRNA splicing, the branch site (BS) base pairs with a universally conserved sequence in U2 snRNA; this interaction is essential for both spliceosome assembly and splicing catalysis. Detailed investigation of the behavior/dynamics of the BS-U2 duplex has been limited by the highly deleterious nature of U2 mutations that disrupt BS-U2 pairing. Thus, we developed an orthogonal system in which a dedicated second copy of U2 with a grossly substituted BS-binding sequence mediates the splicing of a cognate reporter gene. This orthogonal BS-U2 pair produces a non-essential second spliceosome that allows *in vivo* characterization of the BS-U2 helix, positioning of the first-step nucleophile, and its interaction with the spliceosome core, with few constraints. The introduction of further complementary sets of mutations will allow our system to form the basis of a truly orthogonal spliceosome and allow for further mechanistic investigation.



How Can Catalytic Activity Be Modulated? Most splice/branch sites are not optimal sequences. How can they be used for splicing? We have proposed a two-state model of the catalytic spliceosome, in which conformations of the complex supporting the two catalytic steps are in kinetic competition: modulation of the stability of the first and second step conformations results in improvement of one catalytic step to the detriment of the other. This mechanism resembles tRNA miscoding caused by altered equilibrium between open/closed ribosomal conformations; such mechanistic commonality suggests that alteration of rearrangements represents an evolutionarily convenient way of modulating substrate selectivity. Similar modulation of conformational states may explain the ability of mammalian spliceosomes to act on the typically poor splice sites of alternatively spliced introns. In addition, this model poses a large number of testable predictions concerning crucial mechanistic aspects of splicing.

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Key Words: ***community psychology, participatory research, quality of life, health disparities & inequities, cancer detection, HIV prevention, community capacity building***

Bruce Rapkin, Ph.D. is Professor in the Division of Community Collaboration and Implementation Science in the Department of Epidemiology and Population Health at the Albert Einstein College of Medicine and Director of Cancer Prevention and Control Research Program at the Albert Einstein Cancer Center. He received his doctorate in community and clinical psychology from the University of Illinois at Urbana-Champaign. Dr. Rapkin's research focuses on access to care and quality of life for diverse, medically-underserved patients, families and communities. His primary emphasis is on the development of community-academic partnerships to reduce barriers and improve standard of care. He has led several projects to develop strategies to promote evidence-based practice through collaborative research. The first such project was the Family Access to Care Study (R01-MH063045) examined the feasibility of partnerships between frontline providers and health researchers to disseminate mental health interventions for families. The second study, the Queens Library HealthLink Project, is designed to promote community organization, outreach and cancer education to diverse underserved communities, in conjunction with the Queens Borough Public Library System (R01-CA119991). Dr. Rapkin is also principal investigator of two projects involving quality of life appraisal and response shift: The recently completed HIV Choices in Care Study, sponsored by the New York State Department of Health AIDS Institute, employs appraisal methods to ensure a more accurate assessment of patient reported outcomes in a comparison of different Medicaid health service delivery models. More recently, Dr. Rapkin and colleagues initiated a Prospective Study of Quality of Life in Patients with Invasive Bladder Cancer, to examine how differences in appraisal affect quality of life and adaptation to three different options for surgical reconstruction. Dr. Rapkin's collaborative research with community organizations, public health systems and health providers has led to the development of new research designs and assessment methodology to promote evidenced-based interventions in public health. In particular, he has been working on participatory approaches that use both process and outcomes data to support community-based interventions. Dr. Rapkin is on the editorial board of the Journal of Community Psychology, and is a member of the NIMH Consortium on HIV/AIDS and the Family, and serves on the American Cancer Society's National Council for Extramural Research.

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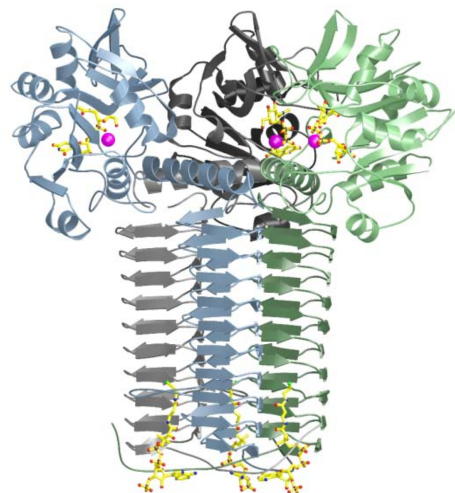
Key Words: ***structure, crystallography, enzymes, drugs, antibacterial***

X-RAY CRYSTALLOGRAPHY OF BIOMOLECULES

Research in this laboratory is primarily concerned with the solution and analysis of protein structure using x-ray crystallography. The solution of the three-dimensional structure of a protein crystal allows for a direct determination of the atomic coordinates responsible for biochemical properties such as protein folding, molecular recognition and chemical catalysis.



In particular, we are interested in bacterial enzymes that are absent from humans and that may represent attractive drug targets for the development of antibacterial compounds. Virtually all important human pathogens are becoming resistant to the antibiotic treatment of choice and bacterial infections due to methicillin-resistant *Staphylococcus aureus* (MRSA), multidrug or vancomycin-resistant *Enterococcus faecium* (VRE), and streptogramin resistant cocci are clinically significant. The effective response to the problem of bacterial resistance requires the identification of novel antibacterial targets and their development from both basic and applied perspectives.

Among the enzymes we are currently studying are a bifunctional acetyltransferase/uridyltransferase capable of synthesizing the UDP-GlcNAc precursor of bacterial cell walls (GlmU), an acetyltransferase responsible for conferring high-level resistance to streptogramin group A antibiotics (VatD), and the key regulatory enzyme of bacterial sulfur assimilation, serine acetyltransferase (SAT).

Projects in the laboratory include (1) basic investigations of the structure and activity of these enzymes to produce detailed descriptions of their function, and (2) applied investigations that utilize high-throughput screening and structure-guided synthetic iterations to identify small-molecule inhibitors of their activities that may be structurally characterized and serve as leads for drug discovery programs.

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Key Words: cancer, hepatitis B, tumor promoters, hepatocytes, topoisomerase, virology

Several human viruses are associated with specific human cancers. However, with hepatitis B virus (HBV), provides the strongest example of a specific virus infection linked to a specific human cancer. The main focus of our laboratory has been to investigate the mechanisms by which members of the HBV virus family, the "Hepadnaviruses", function as causal agents for HCC in their host liver. Studies over the years have demonstrated that persistent infection with hepadnaviruses poses a triple threat for the malignant transformation of hepatocytes.

First, the partial immune response that allows viral persistence, drives a cycle of cell death and regeneration providing an environment permissive for the selective growth of pre-malignant and malignant cells. Second, the viral DNA acts as a direct mutagen for the host genome by integrating into host chromosomes and causing deletions, translocations and in some cases activating endogenous cellular proto-oncogenes. Third, the virus encodes a protein, called the X protein, which can act as a tumor promoter by altering several hepatocyte growth control pathways. Studies on the molecular mechanism of Hepadnavirus DNA integration. In the second area, we have developed an in vitro experimental system to study new hepadnavirus DNA integrations using the Duck hepatitis B virus, DHBV, animal model.

In a series of papers, we demonstrated that DHBV DNA integrations can act as "hit and run" mutagenic agents that cause the deletion of host DNA. We have also shown that agents that increase host DNA damage in infected cells can increase the frequency of viral DNA integration. We have demonstrated that linear DHBV DNA is a preferred integration precursor compared to wild type circular viral DNA. We are continuing to investigate mechanisms of viral DNA integration using the above cell culture system. We have recently obtained data which implicates a cellular enzyme, topoisomerase I in the control of viral DNA replication and possibly also in viral DNA integration. Initial studies on the mechanisms of topoisomerase I cleavage of DHBV DNA have recently been published in Nucleic Acids Research (see reference list). Studies on the role of the hepadnavirus oncoprotein, X in hepatocarcinogenesis. In the third area, we have investigated the natural history and occurrence of the X protein in acute and persistent infections. For these studies we have used the Woodchuck hepatitis virus (WHV) animal model. In two papers, we have been able, for the first time, to acquire quantitative data for the level of WHV X proteins during the progression from normal acute infection through persistent infection to HCC. Hepatocyte transplantation and HBV replication In a series of groundbreaking studies we have developed a hepatocyte transplantation system for the study of hepadnavirus replication in mouse liver. Initially we developed a woodchuck hepatocyte transplantation system in mice in which we are able to replace mouse hepatocytes with woodchuck hepatocytes that are capable of WHV infection. Most recently we have extended this system to include transplantation of human hepatocytes. With this new system we will investigate the replication of a new virus that is becoming a major focus of research, Hepatitis C virus. Molecular characterization of the "Transcriptome" of differentiating liver stem cells.

This is a collaborative project with the laboratory of Dr. Leslie Rogler. Dr. L. Rogler isolated a fetal liver stem cell line that can be regulated to differentiate along the hepatocyte lineage in vitro. In collaboration, our laboratories are using cDNA microarrays to identify genes involved in maintenance of the stem cell phenotype and genes involved in establishing the differentiated hepatocyte phenotype. Using this approach we have identified the Beta Catenin pathway as a key regulatory pathway for establishment of the differentiated state of hepatocytes. This cutting edge approach has led to several very novel and testable hypotheses as to the molecular control of liver stem cell differentiation.

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Key Words: ***Cancer, epidemiology, cervical cancer***

Dr. Rohan is a cancer epidemiologist who studies the role of genetic/molecular, nutritional, and hormonal factors in the etiology and pathogenesis of a wide range of cancers. Much of his work has focused on breast cancer, in relation to which he has a particular interest in the molecular pathogenesis of breast cancer, where his work focuses on identifying molecular changes in benign breast disease tissue that predispose to the development of subsequent breast cancer. Many of his other studies have involved cohort investigations, mostly within the Women's Health Initiative cohorts, the Canadian National Breast Screening Study dietary cohort, and more recently within a new cohort that he established, the Canadian Study of Diet, Lifestyle, and Health. He has published many scientific articles on cancer epidemiology, and he has co-edited books on cancer precursors and on cervical cancer.

In addition to being chair of the department of epidemiology and population health, Dr. Rohan is associate director for population sciences in the Albert Einstein Cancer Center, and he is a member of the Board of Scientific Counselors of the National Cancer Institute. He is on the editorial board of several journals, and is a member of several professional societies.

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***Key Words:* cytochrome, c oxidase, nitric oxide synthase, mitochondria, electron transport, 3-dimensional structure**

In our laboratory the mechanisms and properties of two enzymes, cytochrome c oxidase and nitric oxide synthase, are being investigated as well as the molecular basis of protein folding.

Cytochrome c oxidase is the terminal enzyme in the electron transfer chain. Physiologically, it reduces oxygen to water and utilizes the excess energy to translocate protons across the mitochondrial membrane. The enzyme is responsible for over 90% of the oxygen consumption by living organisms in the biosphere; yet the mechanism of its basic function, the coupling between the redox processes and proton translocation is undetermined. Our objective is to obtain a quantitative description of the manner by which oxygen is reduced to water by exploiting laser spectroscopic methods and rapid mixing techniques developed in our laboratory. These studies will allow us to identify all of the intermediates in the catalytic reaction and thereby establish the molecular basis for one of the most important processes in bioenergetics.

Nitric oxide has been found to play many diverse physiological roles ranging from a neurotransmitter, a vasodilator and a cytotoxic agent. The enzyme that catalyzes the formation of NO from oxygen and arginine is nitric oxide synthase, a very complex enzyme containing several cofactors and a heme group which is part of the catalytic site. We have discovered that NO the enzymatic product, inhibits the enzyme and are now studying the mechanism of the inhibition process. In addition, we are studying a variety of inhibitors of the enzyme to sort between the many mechanisms of inhibition that are possible in nitric oxide synthase. These studies will serve as a foundation for the development of drugs that can be used to treat many different syndromes associated with the under- or over-production of NO.

How a protein folds into its three dimensional structure is one of the central questions in molecular biology and in the biotechnology industry. To advance the understanding of protein folding, it is necessary to determine the structures and the kinetics of the intermediates in the folding pathway. For this, we developed submillisecond mixers in which folding can be initiated in less than 100 microseconds, a time scale that is over an order of magnitude faster than previously possible. This already has allowed us to discover a new model that accounts for the folding of cytochrome c from 100 microseconds to the formation of the native state. Many new experiments with several different techniques will be done to test the generality of this model and its possible role in other proteins.

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Key Words: Hepatocyte transplantation, stem cells, gene-therapy

During the past decade, liver-directed cell therapy and gene therapy for inherited metabolic disorders has progressed to a point where successful clinical application is in sight. Our current preclinical targets are inherited hyperbilirubinemia (Crigler-Najjar syndrome, CN-1), mucopolysaccharidosis VII (MPS-VII) and alpha-1 antitrypsin (AAT) deficiency. We have been pursuing several approaches for liver-directed gene therapy as follows.

Subproject 1. Hepatocyte-based therapies for genetic liver diseases. The conventional source for human hepatocytes is livers from deceased allogeneic donors. Hurdles to broad clinical application of this highly promising approach include the scarcity of usable donor organs and the need for prolonged immunosuppression. To overcome these limitations, we are developing methods to abrogate allograft rejection of hepatocytes by transferring into donor hepatocytes ex vivo specific genes derived from the adenoviral DNA that down-regulate cell surface death receptors, thereby preventing killing by effecter T-cells. In other studies, we are developing methods to induce preferential proliferation of the transplanted hepatocytes by preparative irradiation of the host liver, so that a small number of donor hepatocytes can repopulate the host liver. Our work has been translated into the first successful hepatocyte allotransplantation in a patient with CN-1. Classic AAT deficiency disease arises from the inheritance of the AAT-Z variant that results from substitution of lysine for glutamate 342. This mutation alters the folding and biogenesis of the protein, rendering it prone to polymerization and aggregation within the hepatocyte endoplasmic reticulum (ER). This causes pulmonary emphysema by loss of AAT function. In some patients, accumulation of the misfolded protein causes liver disease as a gain of function. Recently, we have shown that normal hepatocytes transplanted in the livers of mice expressing human AAT-Z spontaneously proliferate, competitively replacing hepatocytes containing globules of PiZ. The mechanism of hepatic repopulation is being investigated in the above models of genetic liver diseases.

Subproject 2. Human embryonic and pluripotent stem cells as sources of hepatocytes: As a novel source of hepatocytes, recently we have started generating differentiated hepatocytes by manipulating human embryonic stem cells and induced pluripotential stem cells in culture. The cells have been used to partially repopulate livers of both immunodeficient mice and immunocompetent rats under cover of tacrolimus, a drug used clinically for liver and kidney allotransplantation. We have shown that human hepatocytes, derived from ES and iPS cells can reduce serum bilirubin levels after transplantation into UGT1A1-deficient jaundiced Gunn rats (model of Crigler-Najjar syndrome, type 1. Current studies are focused on (1) optimization of disease-specific iPS cell development, (2) differentiation of stem cells to hepatocytes and (3) evaluation of metabolic function after transplantation into animal models of human metabolic diseases.

Subproject 3. Gene therapy via recombinant viral vectors and non-viral vehicles: We have developed novel adenoviral vectors that disrupt the costimulatory interaction between antigen-presenting cells and T-lymphocytes and, therefore, can be readministered without stimulation immune response. We are also pursuing in vivo and ex vivo gene therapy based on T-antigen-deleted recombinant SV-40 vectors and lentiviral vectors for the treatment of mucopolysaccharidoses and Crigler-Najjar syndrome, respectively. In a parallel project, we have been developing non-viral vectors comprising liposomes containing specific ligands (such as the F protein of the Sendai virus) for targeted delivery of genes (such as transposition-competent "Sleeping Beauty" vectors).

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Key Words: ***liver, stem cell, Hyperbilirubinemia,, hyperoxaluria, inborn errors of metabolism***

I. Inherited Disorders of Bilirubin Glucuronidation: UGT1A1 is a member of UDP-glucuronosyltransferases (UGT) family of enzymes, which is concentrated in the hepatic endoplasmic reticulum (ER). UGT1A1 mediates the glucuronidation of bilirubin and estrogens. UGT1A1-mediated glucuronidation is required for excretion of bilirubin in bile. We showed that the genetic lesions in any one of the five exons encoding UGT1A1 can abolish or reduce bilirubin glucuronidation, causing potentially lethal Crigler-Najjar syndrome type I (CN-I), or it's less severe variant, Crigler-Najjar syndrome type II (CN-II). We also showed that Gilbert syndrome, a milder form of inherited hyperbilirubinemia, is caused by a promoter variation. We have been studying the regulation of UGT1A1 gene expression.

II. Primary Hyperoxaluria Type 1 (PH1): PH1 is an autosomal recessive disease caused by mutations in the alanine:glyoxylate aminotransferase gene (*AGXT*). In humans, insufficient AGXT activity in liver peroxisomes leads to increased oxalate production that causes calcium oxalate stones in the kidney and then in blood, heart, bones, etc. It is a lethal disease unless combined liver and kidney transplantation is performed. We have developed a mouse model of PH1. Our plan is to cure this disease by (a) gene therapy (b) transplantation of adult primary hepatocytes or (c) hepatocytes derived from human embryonic (hESC) or induced pluripotent stem cell (iPSC). For the latter, fibroblasts from the skin of normal volunteers or patients with PH1will be used to generate iPS.

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Key Words: kinase, phosphatase, cAMP, structure/function

Structure, function and regulation of protein kinases in signaling cascades. A multidisciplinary approach involving protein biochemistry, molecular genetics and cell biology, is used to determine how growth factors and hormones control fundamental biological processes, e.g., gene expression, growth and development. A series of regulatory protein kinases and phosphatases is under investigation. Structure/function relationships are studied in the purified enzymes and genetically engineered mutant enzymes produced in *E. coli* and transfected mammalian cells; the regulation of the expression of protein kinases and their target genes is investigated in cell lines and transgenic animals; anti-sense RNA (CDNA) and over-expression vectors are employed in conjunction with cell-specific promoters to selectively manipulate protein kinase signaling systems and determine the role of these key enzymes in cell differentiation. A partial list of signaling molecules studied includes: protein kinase C, CAMP-dependent protein Kinase (A), the insulin-like growth factor and ligand-activated transmembrane tyrosine phosphatases.

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Key Words: ***Recombination, ubiquitin, immunoglobulin***

V(D)J RECOMBINATION: MECHANISM AND MOLECULAR BIOLOGY.

The chromosomal rearrangement process called V(D)J recombination plays an essential role in the development of the repertoire of immunoglobulins and T cell receptors. In a precise and highly regulated manner two proteins, RAG1 and RAG2, perform many of the biochemical steps of the early phase of the recombination reaction. The current model of the reaction, derived from the work of others in the field as well as our own contributions, would suggest the the RAG proteins participate at several mechanistic steps. These include: Site recognition of the recombination signal sequences, Synapsis of one of each RSS, Cleavage at the coding end in two steps. The RAG proteins may also contribute later in the reaction by positioning the DNA-ends for subsequent joining, recruiting the repair proteins and possibly remaining associated with D segments to reactivate them for a second recombination cycle.

In order for these proteins to shepherd the DNA substrate through the appropriate steps, a series of protein-DNA contacts and protein -protein contacts must occur in a well choreographed manner. Our ongoing research is directed toward illuminating those interactions. In the larger picture, I would anticipate that the interactions with double strand break repair proteins would also relate this work to general DNA repair and may also provide insights into errors of DNA recombination that result in chromosomal translocations. These latter events are considered the incipient change in as many as half of the leukemias of childhood. We have recently found that a separate domain of RAG1 can function as a Ubiquitin Ligase. This suggests an additional regulatory role for the RAG protein that may relate to cell cycle progression and chromatin organization. As a result the lab has become interested in ubiquitin and other post-translational protein modifiers that may act at the rearrangement stage of B-cell or T-cell development.

Interactions between RAG1 and other proteins suggest that the RAG complex may function in other aspects of cell physiology in addition to acting as a nuclease. We are currently pursuing a transcriptional regulatory activity.

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Key Words: antigen presentation, dendritic cells, cytokines, endosomes

Dendritic cells terminally differentiate into the most powerful antigen-presenting cell. This process is accompanied by a complex morphological and functional re-organization of the late endosomal compartments. Late endosomal/prelysosomal compartment are the origin of specialized organelle where antigen processing and MHC class II loading occur. At the moment, even though morphological differences are observed in these compartments (electron dense, multilamellar or multivesicular) the unique composition of each of these compartments and their specific contribution to antigen processing and MHC loading is not yet defined. Another important aspect of their biogenesis which is still not defined is how such compartments originate and specialize during the developmental maturation along the myeloid lineage from a pre-immature to an immature dendritic cell. Pro and anti-inflammatory cytokines, as well as growth factor such as GM-CSF, or bacterial products such as LPS, differentially affect the endosomal maturation and ultimately their functionality in antigen processing/degradation and MHC class II loading/presentation. The focus of my research is to understand how molecules which are involved in late endosomal trafficking and in maintaining endosomal morphology (i.e. AP-1, AP-2, dynamin Alix) are differentially affected by the differential cytokine treatment. Also ultimately how differences in trafficking and endosomal composition affect dendritic cells role in adaptive immunity.

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Key Words: ***Nanotechnology, primary cilium, dynein, kinesin, oncogenesis***

ADVENTURES WITH MOLECULAR MOTORS IN CELL AND CILIARY BIOLOGY

Dynein and kinesin are families of microtubule-motor molecules that are key players in endocytosis, in vesicular trafficking, in mitosis, in cell growth and differentiation, as well as in ciliary growth and ciliary motility. My laboratory is interested in two major new projects involving molecular motors: (1) developing and fabricating nanomachines utilizing the molecular motors, for drug or toxin delivery and (2) probing signal transduction processes in primary cilia. For the first project, dynein and kinesin can be functionally studied by in vitro translocation assays using computer-assisted image analysis techniques that measure the rate of movement of microtubules or of motor-bearing isolated vesicles along microtubules when ATP is added. Both motors and microtubules are being engineered to utilize these movements in self-contained triggerable nanoscale machines that could be utilized to destroy individual target cells. The second project involves the new discovery that primary cilia, which are present on most human cells, are important cell sensors, whose failure to develop or function in various tissues results in polycystic kidney disease, retinitus pigmentosa, pediatric cardiac abnormalities, respiratory disease, situs inversus, and cancer. The signaling pathway begins with receptors in the ciliary membrane, whose placement and stability depend on special kinesin and dynein motors. Current projects on which incoming students may work are:

- Cloning and knockdown of dynein and kinesin motors and the effects of knockdown
- Structural localization of components of signaling systems in the ciliary axoneme.
- Signalling pathways of fibroblast primary cilia in the control of cell division, migration and wound healing
- Role of primary cilia in human embryonic stem cell differentiation
- Engineering and testing components of axoneme, dynein, or kinesin based nanomachines

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Key Words: ***signal transduction, bioinformatics, exoocytosis, toxoplasma***

STUDIES OF THE REGULATION OF SIGNAL TRANSDUCTION AND BIOINFORMATICS IN EXOCYTOSIS

The overall goal of my laboratory's research is to understand the specific sequence of events and molecules involved in the signal transduction that leads to membrane fusion, exocytosis and the release of secretory products. This specific process is called exocytosis and involves membrane fusion of the plasma and secretory vesicle membranes with subsequent release of the vesicle content. One of the regulatory molecules of particular interest in this process is an evolutionarily conserved cytosolic phosphoglycoprotein, parafusin.

This protein is a member of the phosphoglucomutase superfamily but with no phosphoglucomutase enzymatic activity. Parfusin is found associated with the membranes at the exocytic site and it surrounds the secretory vesicle as a component of the secretory vesicle scaffold. It undergoes two covalent cyclic modifications phosphoglucosylation/dephosphoglucosylation) and (dephosphorylation/rephosphorylation) during the exocytic process.

The molecular characterization of this molecule, and its relation to other proteins and enzymes (kinases, glucotransferases, phosphatases, phosphodiesterases) that interact with it are being examined. In addition, other components involved in the membrane fusion event such as receptor-operated calcium channels in the plasma membrane and molecules (proton pumps and Ca2+/H+ or Na+ antiports) associated with the secretory vesicles are also being characterized. Several model systems are being studied, including the liver, pancreas, islet cell lines, and the unicellular eukaryote, Paramecium and the obligate protozoan parasite Toxoplasma gondii. In the latter cell system we are focusing on the events associated with host cell invasion. These studies focus on cell and membrane biology using bioinformatics, biochemical, structural, molecular and mutant analysis techniques.

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Key Words: ***astrocytes, gap junctions, connexin43, pannexin1, purinergic receptors, calcium signaling***

Gap junction proteins (connexins and pannexins) and ATP purinergic (P2Y and P2X) receptors are two components mediating calcium signaling in glial cells. These intracellular calcium transients modulate several cellular functions, including neural progenitor cell migration, release of transmitters from glial cells, and cell-to-cell signaling. A broad range of approaches are employed encompassing behavioral and cellular analyses using a variety of techniques (fluorescence imaging, electrophysiology, biochemistry, luminescence, immunochemistry, molecular biology). Current projects of the laboratory include the:

(1) Contribution of gap junctions and P2 receptors to CNS development, with emphasis on neural progentior cell migration and differentiation, *in vitro* and *in situ*.

(2) Determination of the pathways (exocytosis and pannexin channels) of ATP release from cultured progenitor and mature astroytes.

(3) Contribution of pannexin1 channels to seizures and neurodegenerative disorders.

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Key Words: B cells, immunoglobulin, mutation, recombination, lymphoma

Our laboratory is studying how antibody-forming cells respond to antigen by undergoing somatic hypermutation and class switch recombination so that they can produce higher affinity antibodies with more useful effector functions. The molecular and biochemical mechanisms of antibody variable region hypermutation and class switch recombination is being studied in mice that have mutations in various repair proteins in collaboration with Dr. Winfried Edelmann. In order to examine detailed molecular mechanisms, we are also studying how mutation is targeted to antibody genes and some oncogenes in human Burkitt's lymphoma cell lines which are undergoing variable region mutation in culture. These cell lines and genetically defective mice are being used to study the role of activation induced deaminase (AID), mismatch repair and error prone polymerases in the variable region hypermutation and isotype switching. The analysis of these events also involves the examination of AID activity biochemically and, in collaboration with Drs, Aviv Bergman and Thomas MacCarthy, computationally to analyze and simulate the details of the mutational activity that leads to the generation of antibody diversity.

The highly mutagenic processes required to generate antibody diversity also leads to B cell lymphomas so we are trying to understand how AID causes mouse B cell lymphomas and human Chornic Lymphocytic Leukemia (in collaboration with Dr. Nicholas Choirazzi) and how mismatch repair protects B cells from undergoing malignant transformation while also contributing to the generation of antibody diversity.

We are also using somatic mutation and isotype switching to generate better monoclonal antibodies that will protect the host from lethal toxins and emerging infections. Monoclonal antibodies to such toxins and viruses are generated and the impact of affinity and isotype are determined *in vitro* and *in vivo*.

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Key Words: ***EBV, DNA replication, immunoglobulin, B cells***

Our laboratory studies the molecular genetics of replication and the cell cycle in the mammalian genome. One of our long term interests is to determine what prevents reinitiation of DNA replication that could lead to the amplification of cancer causing genes. We are currently intensively studying two important model systems for DNA replication in the human genome. First, we have detected a sequence specific initiation site in the genome of the Epstein-Barr virus (EBV) that has been implicated in causing many forms of human cancer. This virus uses the same enzymes to replicate its DNA as the human genome. EBV is an oncogenic human herpesvirus and is associated with Burkitt's lymphoma and Hodgkin's disease. EBV also causes infectious mononucleosis and is closely associated with nasopharyngeal carcinoma, one of the most common forms of cancer in Asia. The EBV genome persists extrachromosomally in a latent state in a small number of B lymphocytes in most humans and is maintained throughout their entire lifetime. In individuals infected with HIV or in immunosuppressed transplantation patients, EBV-associated, lymphoproliferative disorders can occur at high frequency. Recently a newly discovered Herpes virus has been strongly implicated as the cause of the Kaposi's sarcoma that frequently occurs among AIDS patients. Thus, there is increasing interest in understanding the replication of human herpesviruses.

Second, we have developed an exciting new approach to map the location of replication initiation sites within the extensively sequenced and cloned human and mouse immunoglobulin heavy chain loci. Importantly, we have demonstrated for the first time in mammalian cells that a gradual transition between early and late replicated domains in the Igh locus is achieved by a single replication fork. This raises the possibility that the transition between other early and late replicating regions is also achieved in this manner. In a non-B cell line in which immunoglobulin genes are not expressed, we have identified a region of about 50 kb in which replication of the Igh locus initiates. We now plan to focus on this initiation region and determine if this very important replication initiation site is sequence specific. We plan to determine whether the mammalian Origin Recognition Complex (ORC) binds to this site and to determine whether there is a pre-replication complex at this site. In pre-B cell lines, we are identifying a developmentally regulated replication origin for the Igh locus that appears to be used only when the locus is expressed. We will also determine whether specific proteins in cells of the B lineage play a role in regulating usage of this developmentally regulated replication origin, possibly by interacting with ORC in the pre-replication complex. We are developing an approach to use fluorescent antibodies to study replication initiation in pure populations of isolated EBV genomes. We will then use this approach to focus on the developmentally regulated replication origin in cells of the B lineage. We will use the EBV genome as well as the Igh locus to identify proteins that bind to mammalian replication origins. Targeted integration mediated by homologous recombination will be used to modify chromosomal origins and to determine the sequences critical for origin function. The spatial and temporal organization of DNA replication in mammalian nuclei is also being studied.

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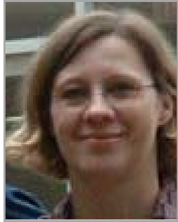

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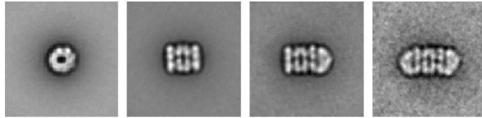
Key Words: ***protein degradation, proteasome regulation, proteasome targets, protein structure, metabolic signaling pathways, aging, dietary restriction***

THE PROTEASOME: REGULATION AND CELLULAR FUNCTIONS



We are studying the major proteolysis pathway in the cytoplasm and nuclei of eukaryotic cells, the ubiquitin-proteasome system (UPS). It plays a pivotal role in eukaryotic protein homeostasis and its activity is required for four major functions: 1) Elimination of damaged proteins (Protein Quality Control), 2) Generation of antigenic peptides for MHC Class I presentation (Immune surveillance), 3) Recycling of amino acids during starvation (Metabolic Function), and 4) Regulated degradation of specific proteins (Regulatory Function). The latter function represents an important mechanism for the regulation of cellular events, such as cell cycle control, receptor function, transcription, organelle function, kinase signaling, apoptosis, etc. Due to the broad scope of cellular pathways regulated by the UPS, loss or decline of proteasome activity has been associated with human diseases such as cancer, neurodegenerative or immune-related disorders. Thus, therapeutic modulation of proteasome activity represents a novel avenue for clinical intervention.

Research in the Schmidt lab is focused on three aspects of proteasome biology:

1) The molecular mechanism of proteasome regulation by activators. The proteasome is a sophisticated molecular machine and its activity is highly regulated. Understanding proteasome regulation at the molecular level should facilitate the rational design of novel proteasome-related drugs. Our investigations focus on the regulation of proteasome activity by the conserved monomeric proteasome activator Blm10, which binds to the proteasome core to enhance its proteolytic activity. These projects involve protein purification, mutagenesis, enzymological and structural approaches.

2) Novel proteasome functions in metabolic adaptation. Under nutrient limiting conditions, cells mount a strong response mediated by Tor and PKA signaling pathways. Their major downstream targets are mitochondria and ribosomes. Our data provide evidence that, in parallel to kinase signaling, the proteasome has a regulatory function during metabolic adaptation. Deletion of *BLM10* in *S. cerevisiae* leads to mitochondrial defects and altered ribosome activity upon nutrient depletion. We have identified the mitochondrial fission protein Dnm1/Drp1 and the transcriptional activator for ribosomal protein gene expression, Sfp1, the yeast analog of c-Myc, as novel proteasome targets. We are studying the regulatory mechanisms that mediate proteasomal turnover of these proteins and have evidence for communication between the proteasome and metabolic kinases. We are furthermore interested in identifying novel proteasome targets related to metabolic adaptation. These projects involve transcriptional analysis, live cell fluorescence microscopy, analysis of post-translational modifications and the investigation of signal transduction pathways involved.

3) Impact of proteasomes in aging cells. Pathways that are required for metabolic adaptation play important functions during aging processes. Our results demonstrate that reduced proteasome levels and activity shorten chronological and replicative life span. Furthermore, proteasome function appears to be essential for life span extension under caloric restriction. We use deletion or over-expression mutants affecting individual aspects of proteasome activity and are testing their contribution to proteasome-related effects in aging cells utilizing a high-throughput quantitative analysis. The identification of the individual components that are required for the protective function of the proteasome in aging cells will allow for screening of chemicals which influence their function and thus might reveal strategies to manipulate lifespan.

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Key Words: ***epidemiology, biostatistics, clinical investigation, clinical research, population studies***

Dr. Ellie Schoenbaum is the director of the new PhD track in Clinical Investigation and Professor of Epidemiology & Population Health.

The goal of this PhD track is to provide rigorous advanced training for highly motivated PhD and MD/PhD students to become clinical/translational investigators. It is expected that, with receipt of the PhD, these scientists will be prepared for independent research careers and to meaningfully contribute to improving the health and welfare of our society using clinical and translational research methods.

The PhD track in Clinical Investigation (PCI) is designed for Einstein pre-doctoral students enrolled in the Graduate Division (including the MD-PhD Program) who wish to pursue careers in clinical research. This track adheres to the regulations and general requirements that pertain to the Einstein PhD. The Institute for Clinical and Translational Research (ICTR) acts as the sponsoring entity within the Graduate Division, and oversees the curriculum and progress of the PCI students. The ICTR also provides an academic home for students, with seminars, support for local and national meetings and other resources. Students choose a mentor who is an accomplished clinical researcher on our faculty, with whom they work closely to complete their original research and write the dissertation. Students take coursework that provides a foundation in clinical research methods (i.e., epidemiology, biostatistics, bioethics, data analysis, grant writing). The mentor guides further education and career development. The PCI leads to a PhD in Biomedical Sciences.

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Key Words: ***Inhibitor design, malaria, cancer, autoimmune disorders, purines, polyamines***

Enzymes catalyze virtually all of the chemical transformations necessary for biological life. Knowledge of the transition-state structure of enzymatic reactions permits the design of powerful inhibitors. Methods have been developed in this laboratory for the experimental determination of the geometric and charge features which characterize enzymatic transition states. This information is then used for the logical design of transition-state inhibitors which have the potential to be new biologically active agents. Specific projects include:

Human genetic deficiency of purine nucleoside phosphorylase causes a specific T-cell insufficiency. Our inhibitors of this enzyme are powerful anti T-cell agents. Two inhibitors are now in human clinical trials against human T-cell cancers and autoimmune disorders. Three T-cell cancer indications for these drugs have received orphan drug status from the FDA and several phase II trials are in progress. A phase II clinical trial has been initiated for psoriasis using our second-generation inhibitor. Third-generation and fourth-generation inhibitors are now being characterized.

Angiogenesis is required for tumor growth. One angiogenetic factor is thymidine phosphorylase, an enzyme that synthesizes deoxyribose 1-phosphase, a precursor to deoxyribose, the angiogenic molecule. We have solved the transition state structure of this enzyme and are now designing transition state analogues. It is hypothesized that such inhibitors will be useful as anticancer agents.

Purine salvage is essential for growth of parasitic protozoa. A family of powerful inhibitors has been prepared against these enzymes from the malaria parasite. Promising results have been obtained in cell culture studies. One of these inhibitors stops the growth of malaria parasites in primate malaria. Plans are underway to initiate human trials in the next few years.

Experimental cancer chemotherapy uses plant toxins coupled to a recognition element for cancer cells. The transition state structure of ricin has been determined to guide the design of inhibitors. These will limit the side-effects of the toxin molecules remaining in the circulation or released from lysed cancer cells. Inhibitors are being synthesized and tested for efficiency, and constructs the plant toxins ricin, saporin and gelosin are being investigated as anticancer agents.

Additional projects involve S-adenosylmethionine recycling and methyl transfer reactions in bacterial quorum sensing, cancer and DNA methylation reactions.

Students in this laboratory can receive training in enzymology, catalysis, protein expression, inhibitor design, computer modeling, inhibitor synthesis, and in drug metabolism studies in cells and animals. Active collaborations occur with laboratories specializing in NMR, X-ray crystallography, mass spectroscopy, synthetic organic chemistry, cancer and medicine. Projects can be designed to include several of these research approaches through active collaborative research programs.

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Key Words: prostaglandins, transport, cardiovascular disease, nonsteroidals

Prostaglandins (PGs) are context-dependent signaling molecules that signal diverse and important biological functions. In addition to their well-known roles in fever, inflammation, ulcer protection, etc, they are very important in both promoting (thromboxane) and inhibiting (prostacyclin) cardiovascular disease.

Our laboratory discovered the first known membrane carrier (PGT) for PGs. PGT is broadly expressed in cell types that synthesize and release PGs, including endothelial cells, kidney glomeruli and collecting tubules, the prostate gland, and platelets. PGT is energetically poised for active PG uptake across the plasma membrane via lactate/PG exchange. We have shown that PGs are released from cells by simple diffusion; this is followed by carrier-mediated reuptake and intracellular oxidation. We are currently using a high-affinity PGT blocker to study the role of PGT in cardiovascular disease (hypertension, arterial thrombosis, and atherosclerosis).

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Key Words: ***Brain, neural systems, computational models, vision, perception, behavior, sensory processing***

We continually interact with stimuli, such as images and sounds, and make inferences about a complex world. How our brain represents and processes the information internally is an intriguing and fundamental issue at the interface of neuroscience and computation. Our lab employs tools of computational and theoretical neuroscience, to study systems from the neural level and through to perception and behavior.

We develop computational models of sensory neural processing based on the hypothesis that images and sounds have predictable and quantifiable regularities to which the brain is sensitive. The models are constructed through interplay with physiological and psychophysical data, and posit functional roles about neural processing. Additionally, a critical way to make progress is utilizing computational tools directly in experimental design and analysis. For example, we have worked extensively on spike-triggered approaches, leading to richer, non-linear characterization of neurons in retina and cortex.

Current specific interests include:
(1) how neurons and percepts are affected by contextual information: spatially, what surrounds a given feature or object; temporally, what we have observed in the past, i.e., adaptation; (2) how neurons represent information hierarchically from one level of neural processing to the next; (3) how populations of neurons work together to achieve perception and behavior; and (4) how we decide where to look next in images.

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Key Words: ***theoretical biophysics, enzyme, catalysis, mechanism***

THEORETICAL STUDIES IN BIOPHYSICS AND BIOCHEMISTRY

The research in my group has three broad aims. First, we wish to develop approximate methods that allow the practical prediction of the dynamics of chemical reactions in biomolecular systems. Because even in large biological systems, many events are governed by quantum mechanics, we seek to develop methods that allow the study quantum processes in complex systems. In biological systems, some enzymatic reactions, which involve the transfer of a hydrogen atom, seem to proceed almost entirely by quantum mechanical tunneling. Our research has focused on the use of modern techniques of theoretical physics such as Feynman Path Integrals, transition path sampling, and the stochastic separatrix to study these complex systems. Using such approaches, we have uncovered the extraordinary possibility that in certain enzyme systems, evolution has developed a catalyst that directs thermal energy to a specific protein vibrational mode that promotes chemistry. This concept of directed protein motions, which we have termed promoting vibrations, is now an area of international research focus.

The second aim of my research is to develop and apply theoretical methods to complex protein systems in humans. A current focus of the work is to provide an understanding of how the thin filament in cardiac muscle tissue regulates heart function, and how mutations in the proteins that comprise the thin filament lead to cardiac diseases, in particular Familial Hypertrophic Cardiomyopathy. We apply both fully atomistic and coarse-grained molecular dynamics to this problem in collaboration with our collaborator Jil Tardiff in the Department of Physiology and Biophysics.

The third aim of research in my group is to develop theoretical methods that will allow us to understand the basic dynamics of condensed phases. In particular, we seek to understand how liquids behave, how they influence chemical reaction, and finally how these effects are made manifest in biological systems.

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Key Words: ***Transcription, chromatin, cell growth, oncogene function, drosophila development***

TRANSCRIPTIONAL CONTROL OF CELL GROWTH AND DEVELOPMENT

My lab is interested in understanding the role of chromatin in the transcription of developmentally important processes such as cell growth using the model organism *Drosophila melanogaster*.

There are currently three main projects in the lab:

(1) The function of the histone demethylase Lid in animal development. JmjC domain-containing histone demethylases were first described in 2005, making them the newest class of chromatin modifying enzyme. We are interested in understanding the function of Little imaginal discs (Lid), a JmjC domain-containing protein whose human orthologs are found misregulated in cancer and mental retardation patients. We are currently pursuing the role of Lid's demethylase activity in regulating gene expression during development in addition to characterizing the function of Lid's other domains implicated in chromatin and/or control of gene expression. Our studies characterizing Lid will enable us to gain insight into the role that dynamic covalent changes to chromatin play in regulated gene expression in vivo and will be directly relevant to understanding how this goes awry in cancer and mental retardation.

(2) The role of Lid in Myc-induced cell growth. We isolated Lid in a genetic screen in *Drosophila* to identify novel effectors of Myc oncoprotein function. Based on the clinical importance of understanding the mechanisms by which Myc acts in tumorigenesis, we investigated the role of Lid in Myc-induced growth and showed that it is a novel co-factor required for dMyc-dependent transcriptional activation. Significantly, this occurs independently of Lid's demethylase activity, leaving the mechanism by which Lid function in this context unknown. We are taking both genetic and cell biological approaches to determine the mechanism by which Lid functions in Myc-mediated activation of transcription.

(3) The mechanism by which Myc induces genomic instability. Human cancer cells exhibit many chromosomal abnormalities (deletions, inversions, translocations etc) that are generated through genomic instability. Myc overexpression can lead to double-stranded DNA breaks, although the mechanism by which this occurs has remained elusive. In collaboration with Jan Vijg's lab, we are investigating this question using lacZ mutation reporter transgenes. Using *Drosophila*, we aim to define precisely how Myc acts to promote genomic instability, a process that is key to understanding how Myc acts during tumor formation and subsequent metastasis.

Please visit our website for more information http://web.me.com/secombe_lab

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Key Words: ***chemotaxis, EGF receptor, metastasis, intravasation, intravital imaging***

INVASION AND METASTASIS

For most cancers, the cause of patient death is invasion and metastasis. For head and neck cancer and glioblastoma, surgery limitations make local invasion a serious concern. For breast cancer, the major cause of death is metastasis- dissemination of tumor cells from the primary tumor to many parts of the body followed by formation of new tumors at those distant sites. By understanding the mechanisms by which tumor cells invade and metastasize, we will have better chances of developing appropriate therapies. Tumor cell motility and the orientation of tumor cells by chemotaxis make important contributions to invasion and metastasis.

We are studying the molecular mechanisms of invasion in tissue culture and in mouse models. By studying cells in tissue culture, we are learning about how cells can detect a concentration difference in an external molecule, and respond by moving towards higher concentrations. Such concentration differences at the border of a primary tumor can orient tumor cells to invade the surrounding connective tissue and blood vessels during metastasis. The tissue culture studies of invasion and signaling are combined with analysis in mice. Two *in vivo* systems are being used: 1) injection of tumor cells into the appropriate primary tumor site (mammary fat pad, floor of mouth, or brain for breast, head and neck, and glioblastoma, respectively) and 2) for breast cancer, formation of tumors in transgenic mice using oncogenes. The injection assays allow the rapid molecular manipulation of cell lines to identify important signaling pathways that contribute to metastasis. The transgenic mouse systems provide a more clinically relevant model in which tumors develop from a mammary ductal epithelium directly. Tumor cell motility can be visualized directly using expression of green fluorescent protein (GFP) in the tumor cells combined with in vivo imaging of the tumor cells around the primary tumor.

Web site http://www.einstein.yu.edu/segalllab/page.aspx

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Key Words: ***multiple sclerosis, mouse models of disease, receptor tyrosine kinases and cell signaling, oligodendrocyte and neuronal survival, neurodegeneration***

Multiple Sclerosis (MS) is a debilitating neurologic disease affecting young adults. A central issue for the treatment of MS is how cell signaling pathways regulate oligodendrocyte cell survival during and after the inflammatory response. Oligodendrocytes are the myelinating cells of the central nervous system and their survival is paramount for normal neurological function. We have used a molecular approach to identify several genes implicated in signaling pathways that regulate oligodendrocyte survival including the genes that encode a newly identified family of receptor tyrosine kinases know as Tyro3, Axl, and Mer (TAM). The ligand, growth factor growth arrest-specific protein 6 (gas6) is concentrated along the plasma membrane in resting endothelial cells and is expressed in and secreted by neurons. We have demonstrated that gas6 protects human and wildtype (WT) rodent oligodendrocytes against growth factor withdrawal, and TNFα-induced toxicity; and that this protection was lost in oligodendrocyte cultures established from Axl-/- mice, but not oligodendrocyte cultures established from WT or Tyro3-/- mice (1). Additional studies in human and rodent oligodendrocyte cultures showed that gas6's protective effect against TNFα was mediated by Akt activation; the effect was blocked by treatment with the PI3 kinase inhibitor LY294002 (2). Also, following gas6 stimulation PI3 kinase directly binds to Axl (3). These results suggest that a major function of gas6/Axl/PI3 kinase/Akt signaling is to maintain cell survival under conditions of stress. This has major implications for MS where in lesions both progenitor and mature oligodendrocytes and neurons are at risk for cell injury. We are using several mouse models of disease to examine the role of Axl and Tyro3 in the CNS. During cuprizone toxicity, Axl-/- mice have delayed clearance of apoptotic oligodendrocytes and myelin debris resulting in axonal damage and delayed recovery (4). Ongoing studies are examining the TAM receptors during MOG-induced EAE, and in established brain lesions of patients with MS (5). Ongoing projects include applying techniques of molecular & cell biology, biochemistry, immunocytochemistry, confocal and electron microscopy to address questions concerning the structure, function and regulation of normal spinal cord and brain development and using these studies to gain insight into disease states. The role of gas6 in treatment for demyelinating diseases is being explored.

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Key Words: liver, transplantation, gene therapy, stem cells

Liver Stem/Progenitor Cells for Transplantation and Gene Therapy

Our research has focused on regulation of liver gene expression, cell growth control, liver regeneration and liver reconstitution thru cell transplantation.

Currently, there are four major projects:
1) Isolation and characterization of liver stem cells
2) Repopulation of the liver by transplantation of fetal liver stem cells and identifying genes that regulate this process by a mechanism of cell competition
3) Identifying genes that increase liver replacement in aging rats by augmenting cell competition
4) Treatment of inherited metabolic disorders by *ex vivo* gene therapy using stem cells.

A number of years ago, we developed a cell transplantation system to follow the proliferation, lineage fate and repopulation capacity of liver stem/progenitor cells, using a marker gene, dipeptidyl peptidase IV (DPPIV). Under selected conditions in this system, we have achieved greater than 99% liver replacement by transplanted hepatocytes and have restored serum albumin levels to normal in albumin deficient rats. This cell transplantation system has also been used to identify stem cells in the fetal liver that are bipotent, proliferate extensively for up to one year after they have been transplanted, differentiate into both hepatocytes and bile duct cells, form completely new liver lobules and replace 25% of hepatic mass in normal adult rats. Liver replacement occurs by cell competition, a mechanism originally described in Drosophila during embryonic wing development more than 30 yrs ago. We are currently conducting laser capture microdissection studies in conjunction with microarray analysis to identify the specific genes that regulate cell competition in liver repopulation, compared to those genes regulating cell competition in Drosophila.

Liver regenerative capacity decreases with aging and we have recently discovered that liver repopulation by fetal liver stem cells increases dramatically with the age of the rat. We have found that aging of the rat liver is associated with increased expression of cellular senescence genes, most notably p15INK4b, and we are studying expression of cell cycle signaling pathway genes, ie, cyclins, cyclin dependent kinases and cyclin dependent kinase inhibitors, to determine precisely how cell cycle progression is regulated in fetal liver stem/progenitor cells vs adult and aging hepatocytes. Finally, we have purified both rat and mouse fetal liver stem/progenitor cells and by microarray analysis are defining unique genes expressed by these cells that control their proliferative potential.

In other studies, we have transplanted human cord blood stem cells into NOD/Scid mice with conversion of some of these cells into hepatocytes. We are also studying the ability of human and mouse embryonic stem (ES) cells to differentiate into hepatocytes and to repopulate the liver and are planning to study liver repopulation of transplanted iPS cells. We are also using laser capture microdissection to define the gene expression program of differentiating ES cells by Q-PCR and microarray analysis. Finally, we have transduced fetal liver stem/progenitor cells and mature hepatocytes with lentiviruses containing a marker gene, GFP, and are characterizing this system in conjunction with cell transplantation as a method for *ex vivo* gene therapy in rodent models of Wilson's disease and α_1-antitrypsin deficiency, with the ultimate aim of human application.

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Key Words: ***Mitosis, chromosome, spindle, drosophila***

MECHANISM OF MITOSIS

During mitosis, sets of duplicated chromosomes must be equally segregated between the daughter cell products of cell division. This process occurs on a bipolar protein machine termed the mitotic spindle, an extremely dynamic intracellular structure that utilizes microtubules and force generating enzymes known as microtubule-associated motors to drive its formation and to coordinate chromosome movements with cell division. The focus of the lab is to understand how motors and microtubules work to drive mitotic movements.

Currently, we are examining how motors are utilized to position and move chromosomes throughout mitosis in Drosophila early embryos. Specifically, we are analyzing the specific functions of and functional inter-relationships existing between motors positioned on kinetochores (multi-protein structures that form on centromeric regions of chromosomes) and chromosome arms (non-kinetochore regions of chromosomes). The results of our initial studies suggest that kinetochore motors generate forces directed toward the spindle poles while motors on chromosome arms generate away from the pole or plateward forces and that a balance of these poleward vs. plateward forces tells chromosomes precisely where they lie on the spindle. When these forces precisely balance, a steady state structure forms and the position of chromosomes is maintained. When this balance is tipped, via the up- or down regulation of subsets of motors or changes in spindle structure, specific movements occur as chromosomes move to acquire a new balance position. Thus, the progression through mitosis may occur as the spindle and associated chromosomes transit through a series of transient steady-state structures.

The use of Drosophila embryos as our experimental system allows us to perform real-time quantitative analysis of mitotic movements in vivo using confocal microscopy and to examine spindle structure at high spatial resolution using electron microscopy. Moreover, because these embryos are amenable to both genetic and transgenic manipulation as well as the micro-injection of fluorescent compounds and function blocking agents, we have available a wide range of tools to manipulate motor activity and label specific spindle components for analysis. Since defects in mitosis resulst in numerous diseases such as cancer and trisomy, the results of our studies also have medical implications and we are currently developing potential therapeutic agents.

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Key Words: ***histones, chromatin, epigenetics, egg***

EMBRYONIC CHROMATIN AND THE BIOCHEMISTRY OF EPIGENETIC INFORMATION

Our research interests are focused on a "bottom-up" understanding of chromatin, the complex of DNA, histones, and other proteins that constitute the physiological form of the genome. In particular, our interest is in determining the role of oocyte and egg histone post-translational modifications, histone variants, and histone storage chaperones in establishing an embryonic epigenetic state. Epigenetics is a phenomenon important for an overall increase in the complexity of the genome without changes in gene sequence. Post-translational modifications of histones, and deposition of histone variants, establish a "histone code" of activation or repression of transcription and other chromatin-mediated transactions, and constitute a major part of the epigenome. Epigenetic information is information content "on top of" the DNA-encoded genetic material. In a sense, epigenetic information can be viewed as the landscape on which the dynamic usage of genetic information is encoded.

We primarily utilize extracts of oocytes and eggs of the frog *Xenopus laevis* as well as recombinant proteins and cultured cells in our studies. The oocyte and egg extracts are potent cell-free systems for the biochemical study of the establishment and writing of the epigenetic histone code on remodeled/reprogrammed somatic nuclei.

We are pursuing a number of specific research avenues to understanding embryonic chromatin, including:

- Determining the role of the egg-specific arginine-methyltransferase complex PRMT5/MEP50-catalyzed H2A, H4, and nucleoplasmin methylation in specifying an embryonic epigenetic state
- Understanding the function of early-embryo specific histones (H2A.X, B4) and storage chaperones (Nucleoplasmin and N1/NASP) in establishing embryonic chromatin
- Deciphering the molecular phenomena involved in remodeling/reprogramming of somatic nuclei in egg extract, in particular the changes in the histone code, changes in chromatin-trans acting factors and increased competency for DNA replication.

These studies are designed to probe the molecular role of chromatin components in the establishment of the embryonic state and have direct bearings on understanding basic events in induced-pluripotency and somatic-cell nuclear transfer. This approach provides a unique "bottom-up" molecular understanding of the role of egg components, such as pre-deposition histones, histone modifications, and histone chaperones, in writing the embryonic chromatin state. Embryonic chromatin, as the physiological form of the genome, is a crucial determinant of pluripotency and proper development of the organism.

Our interests are especially important in light of recent discoveries in somatic-cell nuclear reprogramming and particular in the production of "iPS" (induced-pluripotent) stem cells. Our bottom-up biochemical approach will specifically address how the molecular determinants of chromatin potency (i.e. components of the epigenetic code) are established in the egg.

For more information, please visit our website at www.shechterlab.org.

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Key Words: ***Seizure disorders, febrile seizures, epilepsy, childhood, epidemiology,***

Dr Shinnar is well known for his research on a variety of topics relating to childhood seizures and language regression, including when to initiate and discontinue antiepileptic drug therapy, prognosis following a first seizure, prognosis following discontinuation of medications in children with seizures, status epilepticus, febrile seizures, and language regression and it's relationship to autism and seizures. He has been the principal investigator and co investigator on a variety of NIH-funded research studies. He is the Principal Investigator of a large multicenter study "Consequences of Prolonged Febrile Seizures in Childhood". He is also a member of the executive committee and co-investigator of a large multicenter NIH funded study "Childhood Absence Epilepsy: Rx, PK-PD-Pharmcogenetics". He has also been involved in industry-sponsored trials of new medications. Dr Shinnar is a recipient of the Research Recognition Award of the American Epilepsy Society. He has authored over 150 papers and is the senior editor of the book Childhood Seizures and coeditor of the recently published Febrile Seizures. Dr Shinnar has served as a reviewer and editorial board member for a variety of journals. He has mentored students, fellows and junior faculty members interested in clinical research.

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Key Words: ***Cardiovascular disease, wound healing, inflammation, growth regulation, mouse models***

CONTROL OF VASCULAR CELL GROWTH, DIFFERENTIATION, AND INJURY RESPONSE

Vascular disease, the greatest single cause of morbidity and mortality in developed societies, results from interactions between circulating inflammatory cells, the endothelium that lines the vasculature, and underlying vascular smooth muscle cells (VSMCs) that comprise most of the arterial wall. We want to identify new factors and pathways that regulate disease-associated activation of these cell types.

The underlying pathogenesis is complex: factors that impinge on these cell types include reactivated developmental pathways, innate and acquired immune responses, and changes in cell function that result from physical stresses, perturbed blood flow, and biochemical stimuli. Our general approach is to characterize these responses at the molecular level, in cell culture, and in mouse models that reflect specific types of vascular injury, including atherosclerosis, restenosis after angioplasty, saphenous vein graft disease, and transplant-associated arteriosclerosis.

One project focuses on the atypical cadherin adhesion molecule Fat1, which is strongly induced in injured arteries. Fat1 is a member of the ancient Fat cadherin subfamily – in Drosophila, these proteins are important regulators of growth and planar cell polarity. Our work in mammalian VSMCs shows that Fat1 interacts with and limits the transcriptional activity of beta-catenin, the major downstream mediator of Wnt signaling, a core developmental pathway that regulates many aspects of metazoan embryogenesis. Fat1 also interacts with Atrophin proteins to control VSMC directional migration. Our findings suggest that Fat1 is an important regulator of VSMC growth and differentiation in injured vessels.

A second project involves the allograft inflammatory factor-1 (AIF-1), a 17kD Ca2+-binding protein expressed primarily in activated macrophages. We have found that AIF-1 promotes macrophage migration, phagocytosis, survival, and selected cytokine production. We are currently evaluating how AIF-1 contributes to integrated immune responses, and how loss of AIF-1 function in the mouse affects the pathogenesis of multiple inflammatory diseases, including atherosclerosis, in which this factor is normally strongly induced.

In collaborative work with the Stanley lab, we have characterized a role for colony stimulating factor-1 (CSF-1), the main regulator of macrophage survival, proliferation, and differentiation, in control of transplant-associated arteriosclerosis, the major barrier to longterm success of organ transplants. Surprisingly, this effect appears to involve VSMC-associated CSF-1 in an autocrine/juxtacrine mechanism that is largely independent of macrophages.

Ongoing work in these areas involves defining the molecular bases for these effects. By identifying such novel mechanisms, we hope to find new targets for therapeutic intervention to regulate VSMC activities and improve vascular disease prevention and treatment.

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Key Words: ***Cerebellum, topographic circuit development, pattern formation, mouse molecular genetics, movement disorders, hereditary ataxia, autism***

The major goal in our laboratory is to understand the role of specific genes in patterning functionally distinct circuits in the cerebellum. The cerebellum controls are number of neural functions including motor learning, motor control, balance, and specific aspects of cognition. It is thought that genetic based diseases such as hereditary ataxia and autism result from abnormalities in the construction of complex cerebellar circuits. We are interested in determining the cellular and molecular mechanisms that are affected by genetic insults that alter the development of topographic cerebellar circuits.

Our laboratory uses a combination of techniques drawn from multiple disciplines including sophisticated mouse molecular genetics, biochemistry, neuroanatomy, *in vivo* electroporation of engineered DNA, stereotaxic surgery, classic embryology, and imaging. Since our lab has expertise in designing and generating transgenic mice, we are excited to further develop animal models of human diseases in order to understand the mysteries of developmental neurological diseases.

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Key Words: ***Children, adolescents, prevention, sexual behavior, risk, chronic illness, mental health***

Much of my current work focuses on sexual risk behavior in urban adolescents. This includes evaluating several preventive STD/HIV interventions with Dr. Laurie Bauman and a recently funded project of my own to examine the feasibility and acceptability of combining a program for 14-17 year old urban teens with educational workshops for their parents. I also have a pilot grant from the Einstein Global Heath Center to extend work on relationships and HIV risk to college students in 2 major cities in India. In addition, I am a member of the Social and Behavioral Research Faculty at the Einstein-Montefiore Center for AIDS Research. I also am a co-investigator on a study using community participatory research methods to learn about health disparities for Bronx minority youth, through which we are evaluating a mental health intervention for teens that is being delivered in three community-based organizations. My other previous and current work has examined risk and resilience factors influencing children's health and psychological adjustment, including studies of the impact of childhood chronic illness on other family members and factors affecting access to health care for these children. I am currently involved in other prevention studies focusing on asthma, obesity, and early child development with investigators in various pediatric primary care sites.

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Key Words: ***Obesity, Type 2 diabetes, metabolism, triglyceride***

The overriding theme of my research program is to determine the molecular mechanisms by which mammals adapt to nutrient deprivation or fasting through regulating both glucose and lipid metabolism. This research theme is particularly relevant to metabolic diseases such as obesity and type-2 diabetes since in those pathological states, both glucose and lipid metabolism is dysfunctional.

Two major avenues of enquiry are aimed at answering fundamental questions related to the regulation of lipid and glucose homeostasis:

(1) Our primary interest involves determining the biochemical mechanism by which organisms package fat into cytosolic lipid droplets. This is a fundamental question pertaining to the regulation of energy balance in all eukaryotes. We recently identified a two-gene family of multi-transmembrane proteins we named "Fat storage-inducing Transmembrane" Protein 1 and 2 or FIT1 and FIT2 (1, 2). FIT1 and FIT2 are evolutionarily conserved from *S. cerevisiae* to human, but have no homology to known protein motifs or domains, and are localized exclusively to the endoplasmic reticulum, the site of triglyceride biosynthesis and proposed site of lipid droplet biogenesis. We are currently determining the biochemical mechanism by which FIT proteins mediate triglyceride droplet formation as well as their physiological roles in proof-of-principal mouse models.

(2) A second interest is to identify non-thermogenic biochemical pathways that regulate energy expenditure in mammals.
(3) To identify novel proteins regulating fatty acid and triglyceride metabolism.

These combined research efforts are aimed at providing fundamental mechanistic insight into how an organism maintains energy balance. Our laboratory is mechanistically driven, using molecular biology, biochemistry, structural biology, metabolomics (mass spectrometry), mouse gene-targeted and transgenic models, as well as in vitro cell culture models to carry out our goals. In addition, we are actively engaged in translational research using tissues from insulin resistant humans to determine the roles of novel proteins involved in lipid and glucose metabolism identified by our laboratory. Students working in the lab can expect to acquire skills in molecular biology, basic protein biochemistry, lipid biochemistry, generation of genetically engineered mice, and in vitro cell culture assays.

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Key Words: Imaging, gene expression, and mRNA transport and localization

Our work is focused on the travels of RNA within the cell: from the site of its birth to its ultimate biological destiny in the cytoplasm where it makes proteins in specific locations. All we have learned results from the development of new technology, known as in situ hybridization, to visualize specific nucleic acid sequences within individual cells. Using our approach, synthetic nucleic acid probes are labeled with a variety of detectors such as fluorochromes or antigens. Subsequently these molecules are hybridized to the cell and detected using high resolution digital imaging microscopy. This enables the detection of specific nucleic acid molecules within the structural context of the cell. We have developed imaging methodologies and algorithms capable of detecting a single RNA molecule within a cell. As a result of this approach, we have found that specific RNA sequences are located in particular cellular compartments. An example is the messenger RNA for beta-actin which is located in the periphery of the cell where actin protein is needed for cell motility. These transcripts are not free to diffuse. The transcripts may be associated with a cellular matrix or skeleton from the moment of their synthesis through translation. We are investigating how this spatial information is encoded within the gene and how the RNA transcript is processed within the nucleus and then transported to its correct compartment in the cytoplasm resulting in asymmetric protein distribution. We have recently discovered that RNA localization also occurs in yeast. During budding, a nuclear factor represses mating type switching asymmetrically, only in the daughter cell. This is because the factor is synthesized only in the bud because the mRNA was transported there by an actomyosin system. This discovery allows us to investigate the genetic mechanism responsible for this RNA's travels. In addition, we have constructed genetically altered yeast and vertebrate cells carrying chimeric genes modified by recombinant DNA techniques to elucidate the sequences responsible for mRNA localization. A reporter gene can be "delivered" to a variety of cellular compartments by using specific sequences, or "zipcodes", from the mRNAs found in those compartments. These "zipcodes" consist of short sequences in the 3' untranslated region of the mRNA. We have isolated and cloned proteins which bind to the zipcode and decode this information. Recently we have developed technology that allows us to visualize RNA movement in living cells. Currently our efforts are to develop imaging methods to see fast movements in order to characterize the motors driving RNA.

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Key Words: ***Autophagy, lipid metabolism, hypothalamus, energy homeostasis, aging***

Autophagy or "self-eating" is an in-bulk lysosomal degradative pathway that plays a crucial role in protein and organelle homeostasis. Autophagy occurs at basal levels in all cells and is induced following conditions such as stress or nutrient-deprivation. Briefly, the process of autophagy requires the *de novo* formation of a double-walled limiting membrane that engulfs cellular cargo and then seals upon itself to form an autophagosome. The delivery of the engulfed cargo to the lysosome occurs by fusion of the autophagosome with the lysosome leading to cargo degradation. We have recently demonstrated a novel role of autophagy in the mobilization and degradation of intracellular lipid stores thus pointing to a possible function of autophagy in energy homeostasis.

The **primary focus** of the lab is to examine the organ-specific roles of autophagy in the regulation of lipid metabolism and energy homeostasis using biochemical, immunochemical, radiochemical and image-based approaches *in vitro* and in conditional knockout mouse models. Our efforts are currently focused on the function of autophagy in the hypothalamus and in the white and brown adipose tissues.

We are interested in:

1. Elucidating the role of hypothalamic neuronal autophagy in the regulation of food intake and energy homeostasis.
2. Dissecting the upstream nutrient sensing signal cascades that regulate the induction or shut down of hypothalamic autophagy in response to circulating nutrients.
3. Examining the metabolic and regulatory functions of autophagy in white and brown adipose tissue biology.

Studies have demonstrated that aging is associated with reduced autophagic activity. The **second focus** of the lab is to examine the effect of aging-induced reduction of hypothalamic and adipose autophagy in the development of the metabolic syndrome of aging.

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Key Words: ***Leukemia, proliferation, differentiation, chromatin, transcription***

Our laboratory is interested in understanding the mechanisms controlling mammalian development and cell differentiation. Our approach is to investigate systems in which these processes are disturbed, either by malignant transformation (leukemia) or by directed gene inactivation in mice and Drosophila. Currently there are three major projects underway in the lab.

Molecular Mechanisms of Leukemia: In this project we are investigating the molecular mechanisms for a block to differentiation present in blood cell tumors (leukemias). We have traced the cause of the differentiation block to a transcription factor called PU.1. We are trying to learn how dysregulation of PU.1 expression causes the leukemia cells to stop differentiating and start proliferating in an uncontrolled manner by studying the effect of PU.1 on other gene products, including, other transcription factors like GATA-1 and co-factors like RB that promote differentiation, and cyclins, cyclin-dependent kinases (cdks) and cdk inhibitors that promote proliferation. This project includes genome-wide approaches involving chromatin immunoprecipitation and high throughput sequencing (ChIP-Seq) and gene expression profiling with microarrays.

Role of H1 Linker Histones and Chromatin Remodeling Factors in Chromatin Structure, DNA Methylation, Gene Expression and Development in Mice and Drosophila. Recent studies show that posttranslational modifications of core histones (H2A, H2B, H3, H4) (the Histone Code) play a very important role in control of gene expression. The H1 linker histones are more diverse than the core histones. Mice contain 8 H1 histone subtypes including differentiation-specific and tissue-specific subtypes, whereas Drosophila has only one type of H1. H1's are thought to be responsible for the final level of packaging DNA into the compact chromatin structure but we know very little about their role in gene expression and development. We are studying the functional roles of H1 linker histones by inactivating (knocking-out) specific H1 genes in mice and the single H1 in Drosophila. We are also reintroducing mutant H1 linker histones into H1 depleted mouse cells and flies, to perform structure-function studies. We have also established a new connection between H1 histones, DNA methylation and genomic imprinting and we are learning how H1 regulates DNA methylation. We also have a new knock-out mouse for the SNF2H chromatin remolding ATPase, which assembles H1 histone into chromatin.

Human ES Cell Proliferation and Totipotency. Continuous cell proliferation is required to maintain human embryonic stem cell totipotency. We have found that certain central regulators of the cell cycle also control differentiation decisions in the hematopoietic system. We are investigating which cell cycle regulators control human ES cell proliferation and whether these molecules also control their totipotency.

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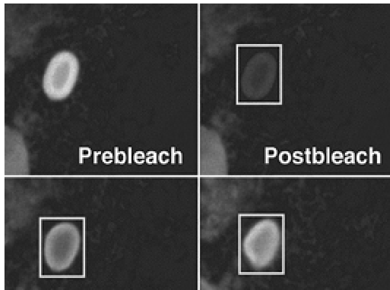
Key Words: ***endoplasmic reticulum, protein misfolding diseases, HIV, imaging, stress, chaperone, GFP, Huntington's Disease, microscopy***

The focus of our laboratory is to understand the cell biology of secretory protein folding and how stress and disease can perturb the folding environment. Specifically, we are using a combination of live cell imaging, biophysical fluorescence methods, and molecular biology to study the organization and dynamics of protein chaperones. We couple quantitative fluorescence microscopy with photobleaching methods (FRAP and FLIP), FRET, and FCS to study protein mobility, protein complex size, a protein's environment, protein concentration, protein-protein interactions, and membrane dynamics in living cells.



FRAP and recovery of GFPSec61 g fluorescence into Organized Smooth ER whorl.

The endoplasmic reticulum (ER) is the largest eukaryotic organelle and carries out multiple functions including: 1) membrane and secretory protein biosynthesis, and export, 2) protein glycosylation and disulfide bond formation 3) lipid biosynthesis, and 4) calcium storage and regulation. The extremely crowded environment of the ER lumen presents a problem for folding proteins. Molecular crowding promotes the misfolding of newly synthesized proteins. Several protein-misfolding diseases, such as cystic fibrosis, initiate in the ER. To prevent protein misfolding, the ER contains chaperones.

Organization, dynamics, and function of lumenal ER chaperone complexes. Analysis of the human genome has revealed that one fourth of all proteins are either membrane or secretory proteins. These proteins enter the ER through a large multi-protein channel termed the translocon. As the nascent peptides co-translationally insert into the translocon, the peptides encounter chaperones, which promote proper folding, disulfide bond formation, prevent protein aggregation, and form the basis of ER quality control.

We are studying how these activities are affected by aging, oxidative stress, Huntington's Disease, Dystonia, Alzheimer's Disease, and HIV infection. In addition, we are developing new fluorescent tools to study the secretory pathway and to serve as alternatives to GFP.

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Key Words: ***molecular motors, kinesin, ATP, microtubules***

STRUCTURE AND FUNCTION OF MOLECULAR MOTORS

In my laboratory we are interested in elucidating the structural basis of the mechanism of action of molecular motors with particular emphasis on motors of the kinesin superfamily. There are three main super-families of linear biological motors: myosins, kinesins and dyneins. These motors use the energy of ATP hydrolysis to move along cytoskeletal filaments, actin filaments in the case of myosins and microtubules in the case of dyneins and kinesins. The kinesin superfamily consists of more than 100 different proteins that power intracellular motile processes such as organelle transport and cell division. An understanding of the similarities and differences among kinesin motors may help developing more specific therapies against cancer. Today several anticancer drugs control the growth of cancer tissue by targeting microtubules.

These drugs have undesirable side effects, as microtubules are an integral part of all eucaryotic cells. A central problem in molecular biophysics is to understand the mechanism by which molecular motors convert the energy of ATP hydrolysis into mechanical work. However, a full understanding of the conformational changes that allow kinesin stepping along microtubules is lacking. It is also not clear what conformational differences account for the different behavior observed between members of the kinesin superfamily. Some kinesins move towards the microtubule plus end while others move toward the minus end. Some kinesins move processively (they are able to take many steps without dissociating from the microtubule) while others lack this ability. Also it is not clear how the kinesin motors interact with their cargoes. In my laboratory we seek to answer these problems using a combination of cryo-electron microscopy and fluorescence spectroscopy. Cryo-electron microscopy is an ideal technique to obtain medium to high resolution information of big macromolecular complexes such as the one formed by the kinesin motors and the microtubules. To trap different structural intermediates we use non-hydrolyzable ATP analogues and rapid mixing techniques. To detect conformational changes in aqueous solutions as the proteins work, we developed a fluorescence polarization microscope that allows determining the orientation of a single fluorophore.

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Key Words: ***gene regulation, genetics, epigenetics, biomarkers, lung disease***

The goal of the Spivack laboratory is to understand inter-individual differences in human gene regulation, and translate these insights into useful biomarkers, to identify individuals at particularly high risk for lung diseases such as cancer, asthma and COPD. This effort would enhance prevention and early detection efforts. The laboratory is currently exploring individual susceptibility markers by exploring quantitative gene (mRNA) expression phenotypes, DNA sequence, and CpG methylation, microRNA, and other epigenetic features potentially underlying those expression phenotypes, *in vitro* and in human populations, in the setting of tobacco, and chemopreventive agent exposures. The overall aim is to develop informative non-invasive risk profiling, preventive, and early disease detection and prevention strategies for the lung in human populations.

There are both mechanistic and translational components to the studies:

Mechanistically, the role of promoter sequence and epigenetic variation in the regulatory regions of carcinogenesis and oxidant pathway genes is being explored in vitro, using human genomic DNA reporter constructs, and native gene regulation models. Unique technologies include the realtime quantitation of native mRNA by the laboratory's RNA-specific strategy, quantitative RNA-specific microRNA-PCR, microRNA affinity pull-down assay, and our tagged-bisulfite genomic sequencing strategy to determine CpG methylation status at high resolution. A novel reporter construct assay for analysis of high resolution methylation function is imminent. Individual mechanisms of chemopreventive responsiveness are being explored. Genome wide assays at the methylome, micronome, and transcriptome levels have commenced.

Translationally, biomarkers of risk and lung disease itself are being established by (a) our fresh frozen lung tissue biobank, (b) pairing laser capture microdissected human lung specimens and several unique, non-invasively collected surrogate specimens developed in the laboratory, such as mRNA expression signatures from brush-exfoliated buccal mucosa cells, and DNA methylation analyses from exhaled breath condensate, both new airway biomarker classes at time we reported them. These airway-derived specimens continue to accrue from our sampling (currently n>650) of a population assembled in a lung cancer case-control context. The specimens are being studied for quantitative gene expression in the carcinogen and oxidant metabolism, cell cycle and other pathways, by both the RNA-specific RT-PCR approach and by the tBGS DNA methylation sequencing approach, by microRNA-PCR, and others. Phytochemical library screening for desired molecular and phenotypic effects is ongoing, These expression, genetic, and epigenetic data are being linked to precise measurements of tobacco and chemopreventive exposure, as an approach to putting a real metric to gene-environment interaction.

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Key Words: ***gap junctions, stem cell, heart, nervous system, liver, connexin***

ROLES OF GAP JUNCTIONS IN EXCITABLE AND INEXCITABLE CELLS

Research of our laboratory is centered on physiological and cell/molecular biological studies of gap junctions, the intercellular channels that allow cells to directly exchange ions and metabolites. In the nervous system, gap junctions form electrotonic synapses, permitting synchronized excitation of coupled cells, and they couple glia into a complex interconnected network. We are studying the plasticity of expression and function of electrotonic synapses between neurons in culture and between astrocytes, and a major effort of the laboratory is to examine functional roles of gap junctions within sensory ganglia in mouse models of chronic pain. In the heart and vasculature, gap junctions allow impulse propagation and second messenger diffusion throughout the tissues; current research interests on heart include mechanisms of arrhythmias resulting from chronic parasitic infection (Chagas' disease) and use of stem cell therapy in treatment in a mouse mode of this disease. Studies on endothelium focus on mechanotransduction through the glycocalyx. In embryonic tissues and in liver and lens, gap junctions presumably play a role in metabolite exchange; of interest are the conductance and permeability of individual channels, conditions that determine expression of these channels and the extent to which the channels are open or closed, and whether certain pathological states are ascribable to alteration in gap junction function. These studies utilize a variety of preparations, including primary cultures of cells from transgenic mice with altered expression of connexin and other genes and transfection of wildtype and mutated connexin sequences into communication deficient cell lines, where small high resistance cells permit structure-function analysis at the single channel level. Techniques include intracellular recordings with conventional and ion-selective microelectrodes, photomanipulation such as FRAP, optical monitoring of intracellular ionic activities (especially Ca^{2+} and propagated Ca^{2+} waves), patch clamp recording of single channels and whole cell currents and standard molecular biological and immunological methods such as Northern and Western blot analyses, immunostaining and RT-PCR and expression profiling using microarrays.

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Key Words: ***signal transduction, hematopoiesis, macrophage, cancer, growth factors***

GROWTH FACTORS AND SIGNALING IN DEVELOPMENT

CSF-1 Biology: Colony stimulating factor-1 (CSF-1) is a glycoprotein growth factor which regulates the production of mononuclear phagocytes, Langerhans cells, Paneth cells, osteoclasts, and the function of certain non-mononuclear phagocytic cell types in the female reproductive tract. Its effects are mediated via a receptor tyrosine kinase, the c-*fms* protooncoprotein. CSF-1-deficient mice are osteopetrotic, due to a lack of osteoclasts, have poor fertility and several other defects related to CSF-1 regulation of macrophages that have critical scavenger and trophic roles in the development, maintenance or function of tissues in which they reside. CSF-1 receptor (CSF-1R)-deficient mice have a more severe phenotype, consistent with the recent discovery of a second CSf-1R ligand, interleukin-34 (IL-34). The developmental and physiological roles of CSF-1 and IL-34 are being studied using transgenic approaches and CSF-1- and CSF-1R-deficient mice. As regulation via the CSF-1R is also important in innate immunity, inflammatory diseases, atherosclerosis, obesity, leukemia and within tumors, for tumor progression and metastasis, similar approaches are being taken to investigate the role of the CSF-1 and IL-34 in disease development.

CSF-1 signal transduction: Since phosphorylation of specific CSF-1R intracellular domain tyrosine residues initiate particular signaling pathways, detailed structure-function studies of the CSF-1R are being carried out in macrophages. In the analysis of very early post-receptor events, ~180 proteins that are rapidly phosphorylated in tyrosine in response to CSF-1, or that are associated with them, have been identified by mass spectrometry. A combination of genetic, proteomic, biochemical and analytical imaging approaches are being used to elucidate the roles and interactions of these signaling proteins in the immediate post-receptor events in CSF-1 signal transduction. Among the proteins studied in detail are the cbl proto-oncogene product, that negatively regulates CSF-1 proliferation signaling by enhancing CSF-1R endocytosis; macrophage F-actin associated and tyrosine phosphorylated protein (MAYP/PSTPIP2), that regulates the actin cytoskeleton, macrophage morphology and motility and downstream of kinases (Doks)-1, -2 and -3, that regulate signaling and motility.

Signaling by the Shark tyrosine kinase: Embryonic dorsal closure in *Drosophila* is a series of morphogenetic movements involving the bilateral dorsal movement of the epidermis (cell stretching) and dorsal suturing of the leading edge cells to enclose the viscera. The Syk family tyrosine kinase, Shark, is expressed in the epidermis and plays a crucial role in this Jun kinase-dependent process, where it acts upstream of JNK in leading edge cells. Mutations in the genes for *shark* gene and Shark-interacting proteins, coupled with cell biological approaches are being used to define Shark function and the Shark signaling pathways.

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Key Words: ***Notch signaling; glycobiology; cancer***

GLYCAN FUNCTIONS IN DEVELOPMENT, CANCER AND NOTCH SIGNALING

The post-translational modification of proteins is a critical factor in determining their biological functions. Glycosylation is the most abundant and varied post-translational modification. The complement of glycans on cell surface glycoproteins changes during embryonic development, immune cell development and transformation into a cancer cell. Cell surface changes that lead to metastasis and spread of cancer cells are also correlated with the appearance or disappearance of particular sugar residues. Specific glycans on Notch receptors modulate signal transduction by Notch ligands. This is a new paradigm of signal transduction whereby the transfer of a single sugar residue alters the ability of Notch receptors to signal. We are using CHO cell glycosylation mutants, a co-culture Notch signaling assay, glycosyltransferase gene knockout mice, and biochemical approaches including MALDI-TOF mass spectrometry, to identify biological functions of cell surface and Notch receptor sugars, and the underlying mechanisms by which sugars mediate and modulate Notch signaling. Notch receptors span the cell surface membrane. When a Notch ligand like Delta or Jagged on an apposing cell binds to Notch, it induces cleavages that release Notch intracellular domain into the cytosol. The Notch intracellular domain complexes with transcriptional and other factors and translocates to the nucleus where it activates target genes that ultimately lead to a change in cell fate or cell growth control. We have shown that Notch receptors require *O*-fucose to function during mouse development and are investigating the consequences of conditional inactivation of global Notch signaling in specific cell types. We have shown that mice expressing a Notch1 mutant that cannot add O-fucose to the ligand binding domain have defective T cell development, and are investigating T and B cell development and functions in mice lacking glycosyltransferases that act on Notch receptors.

In other work we have shown that mice lacking the bisecting GlcNAc on complex *N*-glycans exhibit enhanced progression of mammary tumors induced by a viral oncogene. The mechanism involves altered interactions of growth factor receptors that carry the bisecting GlcNAc with the galectin lattice at the cell surface, thereby affecting growth factor signaling. Mice with a null mutation in the *Mgat1* gene die at mid-gestation. We generated female mice in which only oocytes lack *Mgat1* and found that oogenesis is compromised; male mice with spermatogonia lacking *Mgat1* are infertile. We are investigating the mechanistic bases of these phenotypes. We are also discovering new factors that affect protein glycosylation using our panel of CHO glycosylation mutants. Gain-of-function mutants identify novel aspects of glycosylation and are of special interest. CHO cells and the glycosylation mutants are also being used as hosts to characterize orphan glycosyltransferases identified in genome databases, to develop assays for determining biological roles for sugars in cell-cell and cell-pathogen recognition, for glycosylation engineering of recombinant glycoproteins used in anti-cancer and anti-inflammation therapies, and to develop models of defects observed in patients with congenital disorders of glycosylation and muscular dystrophies.

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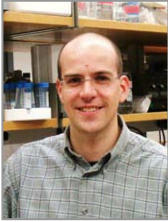

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Key Words: ***leukemia, stem cells, hematopoiesis, cancer stem cells, transcription, chromatin remodeling, epigenetics***

TRANSCRIPTIONAL REGULATION OF NORMAL AND CANCER STEM CELLS IN HEMATOPOIESIS AND LEUKEMIA

Acute myeloid leukemias (AML) are malignant diseases that originate from a single transformed cell which has progressively acquired critical genetic changes that disrupt key growth-regulatory pathways. Despite the established use and optimization of regimens applying polychemotherapy and the development of multiple new agents that are effective at reducing the tumor burden, relapse continues to be the most common cause of death in AML. Newer experimental evidence demonstrates that AML arises from a small population of cancer stem cells / leukemia stem cells (LSC). Similar to normal hematopoietic stem cells (HSC), LSC are quiescent in terms of cell cycle and thus, conventional cytotoxic therapies are not effective against LSC in the majority of cases. However, therapeutic eradication of the LSC within the leukemia clone will be essential for a cure of disease. Therefore, an improved understanding of the molecular pathways that suppress the formation and maintenance of LSC is required for the development of therapies that target LSC rather than the bulk tumor cells (leukemic blasts). Recent findings from our own group and others demonstrate a critical role of transcriptional master regulators (e.g. PU.1) in the genesis and function of LSC in AML, and that transcription factors are already deregulated in the early stem cell compartment.

The goal of our research is to identify critical mechanisms that drive leukemia stem cell (LSC) development and function, and to better understand the mechanisms of how transcriptional regulators (e.g. transcription factors and chromatin-remodeling factors) cause formation of LSC.

To identify implicated pathways we are utilizing rigorously defined stem and progenitor cell subsets isolated by means of multi-parameter high-speed fluorescence-activated cell sorting (FACS). Identified target genes are biochemically and functionally tested using lentiviral gene transfer and in vitro as well as in vivo assays for leukemia stem cell self-renewal and differentiation, including colony-forming assays, serial replating assays, and transplantation models that allow for assessing their function in LSC formation and maintenance. Our studies aim at providing the basis for development of targeted, LSC-directed therapies.

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Key Words: ***host-pathogen interactions, bacterial pathogens, Legionnaires' disease, pathogenic mechanisms***

ENVIRONMENTAL INFLUENCES ON THE ACQUISITION OF VIRULENCE PHENOTYPES BY MICROBIAL PATHOGENS

Legionella pneumophila is a fresh water bacterium and causative agent of Legionnaires' disease, a potentially fatal pneumonia. *L. pneumophila* can invade and replicate in aquatic amoebae which can form cysts that capture the internalized Legionella. Legionnaires' disease is spread by inhalation of Legionella-containing amoebae cysts that are aerosolized from air conditioning systems, shower heads and humidifying devices.

A type four secretion system (T4SS) is required for *L. pneumophila* to replicate within and evade killing by amoebae and by macrophages in the lung. The Legionella Dot/Icm T4SS is required if *L. pneumophila* is cultured in laboratory media. My laboratory demonstrated that if *L. pneumophila* is incubated in water and encysted in an amoeba host, *Acanthamoeba castellanii*, the Dot/Icm T4SS is in fact dispensable for invasion, delay of phagosome acidification and intracellular multiplication. Our data implicate an alternative T4SS, the Lvh T4SS, in those virulence phenotypes when Legionella is exposed to mimics of its aquatic and amoebae environmental niches. In sum, our data suggest that the Lvh T4SS is critical for the environmental phase of the Legionella life cycle, in which the bacterium associates with amoebae prior to infection of humans.

Our long term goal is to characterize the molecular mechanisms by which *L. pneumophila* transitions from a fresh water bacterium into a human pathogen via an amoeba intermediate. Our specific focus is the Lvh T4SS implicated under conditions that mimic the environmental niches of *L. pneumophila*. We use genetic, biochemical, cell biological and, with collaborators at the University of Washington, animal model studies to identify and characterize the molecular agents.

Other human microbial pathogens, *Mycobacterium tuberculosis*, *Francisella*, *Vibrio cholerae* and *Cryptococcus neoformans*, invade and are protected from killing by the host when sequestered within environmental amoebae. Our studies suggest that by mimicking the environmental niches of these pathogens, new insights on microbial virulence mechanisms may be identified. Moreover, our work suggests that control of amoeba encystment may be an untapped avenue for limiting the spread or the persistence of pathogens in the environment.

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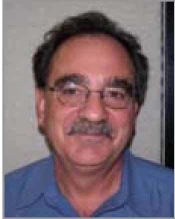

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Key Words: ***brain, speech, auditory processing***

The broad objective of this project is to elucidate neural mechanisms associated with complex sound processing relevant for the perception of speech, music and auditory scene analysis by examining electrophysiological responses within monkey auditory cortex. There are many similarities between monkeys and humans in their auditory cortex organization and in their ability to perform phonetic and complex sound discriminations, highlighting the utility of primates as a reasonable electrophysiological model. Direct recordings in monkey auditory cortex offer the opportunity to investigate neural bases of complex sound encoding with a detail that is unobtainable by non-invasive studies in the human. Our studies will clarify normal mechanisms of speech and other complex sound encoding, and serve as a benchmark for evaluating hypotheses regarding dysfunctional processes associated with abnormal speech and hearing development.

Recent speech-related work has focused on the cortical processes involved in the encoding of the voice onset time and place of articulation phonetic parameters. Music-related studies have concentrated on auditory cortical encoding of pitch and timbre, as well as the neural response features associated with consonance and dissonance of musical intervals. Temporal and spectral streaming relevant for auditory scene analysis are also being actively investigated. Cortical responses are described using 3 complementary, concurrently recorded measures of neuronal ensemble activity; multiunit activity (MUA), auditory evoked potentials (AEPs) and the derived current source density (CSD). CSD analysis characterizes the temporal and laminar distributions of current sources and sinks that reflect net synaptic activation and inhibition, whereas phasic MUA patterns determine changes in the net firing rate of neuronal ensembles. These recording procedures yield stable measures of the synchronized neuronal activity required for complex sound encoding. Through their relationship with the AEP, monkey intracortical responses can be directly linked with homologous responses in humans.

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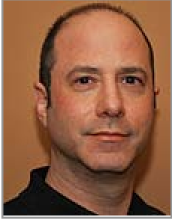

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Primary research interests involve viral and cancer epidemiology, especially as it relates to:
The viral causes of cancer such as human papillomavirus (HPV) and simian virus 40 (SV40)

1. Immunogenetic factors that influence viral infection with HPV, human immunodeficiency virus (HIV), and hepatitis C virus (HCV)
2. The effects of growth factors (e.g., IGF) on viral infection and on tumorigenesis (including cervical, breast, endometrial, and colorectal cancer)

Our studies involve collaborations in large multi-institutional prospective cohort investigations as well as the development of new (targeted) cohort and cross-sectional studies.

Recent Observations:

1. Highly active antiretroviral therapy (HAART) is associated with reduced burden of HPV and cervical neoplasia in HIV-positive women.
2. Specific HLA class I and II alleles are associated with increased risk of HCV viremia
3. Women with low IGFBP-3 levels have ≥50% reduction in risk of incident clinical AIDS
4. IGF-I is associated with increased risk of HPV persistence and HCV disease progression
5. High insulin levels explain the relation of obesity with postmenopausal breast cancer
6. Endogenous estrogen levels are associated with risk of colorectal cancer

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Key Words: ***Aging, genomics, genome-wide, high-throughput analysis***

FUNCTIONAL GENOMICS OF AGING

Our long-term research goal is to investigate the genetic components of aging and aging-related disease using functional genomics approaches. We focus on the identification of gene sequence variation, i.e. single nucleotide polymorphisms (SNPs), in candidate genes and the assessment of their potential functional impact on aging-related phenotypes. Candidate genes include categories of genes implicated in the modulation of common causes of aging, e.g. free radical production, antioxidant defense, genome maintenance, and apoptosis, or more targeted pathways involved in specific aging-related diseases such as breast cancer. Any genetic variation found to be significantly associated with one or more defined aging phenotypes is then further investigated in specific functional tests, utilizing in silico modeling, in vitro cell culture models, and mouse models. This should ultimately result in an integrated approach to study the genetics of aging at different levels ranging from genetic determinants in the form of DNA sequence variations, through cell type- and tissue-specific gene expression profiles, to molecular and cellular endpoints in tissues, to impacts on quality and duration of life span. The results are expected to lead to the identification of functional pathways that control basic aging processes and the onset of age-related diseases.

Four systematic multidisciplinary studies are currently underway. *First,* we have initiated a population-based association study to test genotype-phenotype correlations of genome maintenance genes in a breast cancer cohort. We currently focus on the tumor suppressor BRCA1, which is involved in double strand break repair with broad effects on cellular physiology and genomic stability. We have established a high-throughput mouse embryonic stem cell transgenesis to knock-in human BRCA1 haplotype variants for functional analysis in vivo. *Second,* in a cohort of longitudinal study of aging, we are testing the hypothesis that genetic variation at loci involved in genome maintenance mechanisms (e.g., DNA repair, antioxidant defense, cell cycle control, and apoptosis) can be related to individual differences in the rate and severity of aging-related phenotypes. *Third,* we are focusing on identification of functional SNP haplotypes of genes involved in the Growth Hormone/Insulin-like Growth Factor-1 (GH/IGF-1) pathway. Down-regulation of the GH/IGF-1 pathway is well-known to extend life span in model organisms varying from worms and flies to mice. We are investigating whether this evolutionarily conserved pathway play a role in human longevity using Ashkenazi Jewish centenarian cohorts. *Fourth,* we are studying mouse models that harbor human gene variations in DNA repair/genome maintenance and as a consequence manifest premature aging phenotypes. Our results from transcriptome analysis delineate a complex genetic network of cellular responses to endogenous DNA damage and suggest it as the cause of the premature aging phenotypes in these mice.

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Key words: ***auditory, cognitive neuroscience, electrophysiology, attention, memory***

THE NEUROBIOLOGICAL BASES OF AUDITORY SCENE ANALYSIS

My research is in the field of Cognitive Neuroscience and is focused on understanding the neural basis of auditory information processing in healthy adults and children, and how the process breaks down in impaired populations. Our laboratory's research uses a combination of non-invasive recordings of human brain activity (event-related potentials [ERPs]) and functional magnetic resonance imaging (fMRI), in conjunction with measures of behavioral performance, to specify the processes and brain structures that contribute to the organization, storage and perception of a coherent sound environment.

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Key Words: ***Kidney disease, fibrosis, genomics, epigenomics, biomarkers, notch signaling, Wnt/beta catenin pathway, tissue progenitor cells,***

MOLECULAR MECHANISM OF KIDNEY DYSFUNCTION

Twenty million Americans suffer from chronic diabetic and non-diabetic kidney diseases that cause the kidneys to fail. When the kidneys fail, the average life expectancy is just over two years and the survival depends on costly and disabling dialysis or transplantation treatments.

Work in my laboratory is aimed towards the understanding of renal fibrosis and chronic kidney disease development. We are performing translational (hypothesis generating) and mechanistic studies. The aim of our translational research work is identify novel, genetic, genomic and epigenenomic biomarkers of chronic renal disease. We collected large number control and diseased human kidney tissue samples, which we are using for genome wide transcriptome and epigenomics (mainly cytosine methylation) analysis. We hypothesize that integrative analysis of epigenetic and genetic settings in diseased cells can provide a rational basis for more accurately modeling the critical biological pathways involved in mediating the progressive phenotype in patients. We also predict that epigenomic integrative analysis can be used to determine the identity of chromatin and transcription factors that contribute mechanistically to aberrant transcriptional programming in chronic kidney disease, and that this information can be used for designing therapeutic strategies.

We use genetic approaches and mice as a model organism to test the role of candidate signaling molecules directly in vivo. The Cre/loxP and tet inducible transgenic technologies allow us to analyze the function of particular factors by deleting or overexpressing genes that encode them in specific cell types in the kidney. Specifically, we are working on determining the role of the Notch and Wnt/beta-catenin pathway in chronic kidney disease development, renal epithelial cell homeostasis, renal stem or progenitor cell function and differentiation. Our recent results highlight the role of embryonic programs in adult disease development.

Visit our website: **www.susztaklab.com-a.googlepages.com**

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Key Words: ***Serotonergic systems, antidepressant targets, genetics***

This research program investigates the genetic basis of the regulation of synaptic transmission of the neurotransmitter serotonin. Drugs that target the serotonergic system are the most commonly prescribed therapeutic agents for the treatment of a wide spectrum of behavioral and neurological disorders, from depression to eating disorders, autism, schizophrenia and Parkinson's disease. Using mouse and *C. elegans* as animal models, our laboratory is undertaking genetic dissection of the genes and biochemical pathways in serotonin signaling and characterizing therapeutics that can alter them.

One project is to identify serotonin deficient mutants in *C. elegans*. We have isolated a set of neuron-specific serotonin deficient (*nss*) mutants through unbiased genetic screens. The *nss* mutants offer us a unique opportunity to elucidate genetic pathways and biochemical mechanisms that regulate the development and function of specific serotonergic neurons.

A second project is to identify and characterize antidepressant-resistant genes. Using chemical mutagenesis and RNA-interference (RNAi) technology, ongoing experiments search genome-wide for mutations that confer resistance or hypersensitivity to selective serotonin reuptake inhibitors (SSRIs) in *C. elegans*. This screen will broadly explore SSRIs targets distinct from the known serotonin transporter and reveal downstream pathways regulated by serotonin signaling. We will translate genetic leads from *C. elegans* into functional analysis in mouse models.

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*Key Words: **Chagas disease, Trypanosoma cruzi, thromboxane, adipose tissue, adipocyte***

Chagas disease, caused by the protozoan parasite, *Trypanosoma cruzi*, is the most important cause of heart disease in many areas of Latin America. For over three decades our laboratory has investigated the pathogenesis of Chagas disease. We have found that the parasite can synthesize thromboxane A_2 which causes platelet aggregation and vasoconstriction. We have also demonstrated that the parasite has a thromboxane synthase and we are interested in cloning the gene for this synthase. In addition, we have been examining the presence of the thromboxane receptor (TP) on mammalian cells and the possibility that the TP also is present on the parasite itself. We are interested in examining what signaling pathways are activated by TP.

Our group has examined the consequences of *T. cruzi* infection on adipose tissue and adipocytes. Adipocytes exert their influence through the synthesis and release of adipocyte-derived secretory proteins termed adipokines. The intense pro-inflammatory potential of adipose tissue suggests that it plays an important role in the innate immune response during infections and in the pathogenesis of cardiomyopathy. Adipose tissue from *T. cruzi*-infected mice and infected cultured adipocytes display upregulation in markers of inflammation and *in vivo* adipose tissue is infiltrated by leukocytes and macrophages. We plan to extend our previous investigations by examining the consequences of manipulation of adipose tissue on the pathogenesis of Chagas disease. In addition, since fat and glucose metabolism are interrelated, we will also explore the role of hyperglycemia and insulin resistance and leptin signaling on *T. cruzi* infection.

Finally, our laboratory group has pioneered the application of echocardiography, cardiac MRI and microPET to study the cardiomyopathy of Chagas disease in the mouse.

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Key Words: ***cardiac muscle, cardiovascular, contractile protein, familial hypertrophic cardiomyopathy***

The research in my lab is currently focused on a basic issue in cardiac muscle biology, namely, how do specific alterations in contractile protein structure impact on overall cardiovascular function. Changes in sarcomeric function due to genetic mutations, post-translational modifications and isoform switching have been implicated in a number of primary cardiovascular disorders including: post-infarct remodeling, congestive heart failure and both dilated and hypertrophic cardiomyopathies. Despite extensive study, however, the primary relationships between these alterations and the subsequent complex cardiac phenotypes remains unclear. We have begun to address these issues by developing a model system based on a well-defined set of mutations in the cardiac Troponin T gene that have previously been linked to the pathogenesis of Familial Hypertrophic Cardiomyopathy (FHC). Cardiac Troponin T mutations cause a particularly malignant clinical syndrome marked by early cardiac sudden death in the absence of significant ventricular hypertrophy. This dissociation between the degree of ventricular hypertrophy and the clinical severity suggests that the primary effects of these mutations may be on myocellular function. Because the phenotypes are conferred via a dominant negative mechanism we have utilized a transgenic mouse approach for a subset of the known cTnT-related FHC alleles. To date, we have successfully established a series of eight independent transgenic mouse lines which express a myc-tagged murine cTnT gene in cardiac tissue and carry mutations in both of the main protein functional domains. In addition, these transgenic animals have been shown to recapitulate many of the histopathological and physiologic findings associated with FHC in patients. One of the most striking findings has been that the observed murine phenotypes are allele-specific. This suggests that some degree of the observed clinical heterogeneity is due to specific changes in cTnT function. In addition, it had previously been suggested that FHC-related missense mutations resulting in a charge change cause a more malignant clinical phenotype. By generating a set of transgenic mice based on the known mutational hotspot at Codon 92 (all of which result in a charge change) we have recently shown that even more subtle changes in protein structure can cause significant and different alterations in cardiac function that are evident at both the cellular and whole-heart levels. We are currently extending our studies to the myocellular and computational level and have recently succeeded in isolating functional adult cardiac myocytes from several of our transgenic lines with the long-term goal of elucidating many of the downstream alterations in cell function caused by these mutations including: Ca^{2+} regulatory processes, ion channel function (possible effects on arrhythmia thresholds), differential gene expression using gene array methodologies and myocyte energetics. Our hope is that a better understanding of these processes may eventually lead to novel theraputic targets for this complex disorder.

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Key Words: infantile spasms, idiopathic generalized epilepsy, hypoglycemia, seizures

ANIMAL MODEL OF CRYPTOGENIC INFANTILE SPASMS; ROLE OF Brd2 GENE IN IDIOPATHIC GENERALIZED EPILEPSIES; MECHANISMS OF GLUCOPENIC SEIZURES

During infancy and childhood, devastating epilepsy syndromes may develop. Among those Infantile Spasms occur between 3 months up to 2 years of age with the incidence of 1 per 3000 live births. The syndrome of infantile spasms is impairing the child's progress, is therapy-resistant and has unfavorable prognosis. ACTH therapy is one of very few partially effective treatments suggesting that hypothalamus-pituitary-adrenal axis derangement may have happened pre- or perinatally. An animal model of cryptogenic infantile spasms responding to ACTH has been developed in the laboratory, consisting of a prenatal insult aimed at the hypothalamus-pituitary-adrenal axis and the spasms are then triggered in infant rats. Autoradiography imaging, and immunohistochemistry revealed hypothalamic areas involved in the control of spasms. These areas were investigated for molecular changes constituting a condition for increased susceptibility to spasms and for ACTH efficacy. Identified molecular targets are tested as possible new therapeutical approaches with efficacy superior to ACTH and with fewer side effects. Patch clamp studies are used to determine changes in the hypothalamic circuitry as a prerequisite for the occurrence of spasms. Brain function is tested postnatally in a variety of available in vivo tests such as reflex behavior, motor skills, learning and memory, and seizure susceptibility. Brain morphology is assessed using light and fluorescent microscopy, metabolic imaging studies, and binding essays.

In humans, BRD2 gene has been connected with idiopathic generalized epilepsy (about 30% of all epilepsies) by repeated association and linkage studies. A mouse knockout, heterozygous in Brd2 (+/-) is investigated for seizure susceptibility, spontaneous seizures, and their mechanisms. Our results indicate that spontaneous seizures occurring in Brd2+/- mice detected by 24/7 EEG/videomonitoring arise from impaired GABAergic inhibition in the basal ganglia.

Extracellular glucose is an essential energy source and also has a signaling function. In diabetic patients, seizures frequently occur as a complication of hypoglycemia. Studies are performed both in animal models in vivo and in brain slices in vitro to determine the effects of extracellular glucose on synaptic activity and seizure susceptibility. We use extracellular and intracellular recordings in vitro, single cell activity recordings in vivo, combined EEG/video recordings in vivo, precise brain microinfusions of substances affecting glucose metabolism and signaling, immunohistochemical and histological techniques. Our results indicate that deep midbrain structures may play a significant role in the development and control of hypoglycemic (glucopenic) seizures.

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*Key Words: **hormones, epilepsy, brain, development***

Hormonal regulation of neuronal excitability during physiological and pathological conditions.

The laboratory is interested in mechanisms by which sex hormones influence the formation of memory as well as their effects on seizures using rat models:

1. Estrogens affect brain development and ongoing modulation of the nervous system. We are interested how estradiol affects the formation of synaptic plasticity, especially the long-term potentiation (LTP). We observed a biphasic effect of estradiol on LTP. We are now in process to explore the underlying mechanisms for this effect.
Another example of neuronal plasticity is the seizure activity.. We ar specifically interested in Temporal lobe epilepsy (TLE), which is one of the most common epileptic syndromes in adult patients. Extensive neuronal loss in the hippocampus known as hippocampal sclerosis is the most common pathology associated with the TLE. Neuronal loss leads to reorganization of hippocampal axon circuits, which then contribute to further seizure genesis and intractability. Women with TLE often have reproductive endocrine disorders. Restoration of normal ovulatory cycles in these female patients is also an effective seizure therapy. This observation suggests involvement of female sex hormones in TLE. Results from our studies show that β-estradiol has neuroprotective effects on seizure-induced damage. Our studies are focusing on the mechanisms, by which the estrogen produces these neuroprotective effects.

2. Several brain regions are different between males and females (sexual dimorphism). Some of the sexually dimorphic structures are also involved in seizure generation (amygdala, hippocampus, hypothalamus) or seizure control (substantia nigra). Our studies show that testosterone influences the substantia nigra. Our goal is to determine the effects of testosterone on development and maturation of the nigral seizure-controlling network.

Studies are performed both in vivo and in vitro, utilizing a variety of techniques including intracranial electrical and chemical stimulations, electrophysiology in vitro (extracellular and patch clamp recordings), behavioral studies, EEG recordings, deoxyglucose autoradiography, immunohistochemistry, Western blot, and anatomical tracing techniques. Students interested in brain function in normal or prenatally compromised brain can choose thesis projects utilizing any of the techniques we are currently using in the lab. Our general approach is to use the best-suited technique for the questions asked.

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Key words: ***dementia, gait, falls, cognition, frailty***

Dr. Joe Verghese graduated from St. Johns Medical College, Bangalore, India in 1989. He then completed postgraduate training in Internal Medicine and Neurology in United Kingdom. He completed his Neurology residency at the Albert Einstein College of Medicine, Bronx, NY in 1998. He did fellowship training in Neurophysiology as well as Aging & Dementia in 1999 at the same institution. He received a Master of Science degree in Clinical Research Methods with Distinction in 2001. Dr Verghese is board-Certified in Neurology. Dr. Verghese is Professor of Neurology at the Albert Einstein College of Medicine; Murray D. Gross Memorial Faculty Scholar in Gerontology and Director, Division of Cognitive and Motor Aging. He is also Clinical Director of the Einstein Aging Study, a NIH funded longitudinal aging study. Dr. Verghese is a recipient of the Beeson award from the National institute on Aging and the Outstanding Scientific Achievement for Clinical Investigation Award from the American Geriatrics Society. His research interest is the effects of disease and aging on mobility and cognition in older adults, and he has had several publications in this area. His current projects include studying the influence of cognitively stimulating activities on reducing risk of dementia and the role of divided attention tasks such as walking while talking in predicting outcomes such as disability and cognitive decline, and global dementia issues.

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Key Words: ***biophotonics, GFP, fluorescent proteins, biosensors, chromophores***

The **long-term goal** of our laboratory is development of a collection of chromophore containing molecular nano-tools based on fluorescent proteins, which could be employed for analysis, manipulation or modification of biochemical processes in living cells, tissues and organisms with light photons. This growing field was termed as a molecular biophotonics reflecting its essence: interaction between photons and biomolecules. Cloning of homologs of a green fluorescent protein (GFP), which emit not only green but also yellow, red and far-red fluorescence, provided a powerful boost for labeling and detection technologies due to availability of colors and biochemical features never before encountered in GFP variants. Recent studies in evolution of GFP-like proteins suggest that the spectroscopic and photochemical properties of the known fluorescent proteins represent just a fraction of the naturally occurring diversity, and it is very possible to stumble upon proteins with completely new combinations of useful features.

Design and characterization of molecular biophotonic tools require highly interdisciplinary research including molecular biology, structural biology, computer modeling, analytical and organic chemistry, and living cell microscopy. Methods from all of these fields we extensively use in our laboratory.

Photoactivatable and **kindling fluorescent proteins** (PAFPs and KFPs) are irreversible and reversible photoactivatable probes, respectively. They are capable of switching from a dark to a fluorescent state in response to the irradiation by a light of the specific wavelength, intensity and duration. KFPs and PAFPs are excellent tools for the precise optical labelling and tracking of proteins, organelles and cells within living systems in a spatiotemporal manner. They bring a new dimension to the kinetic microscopy of living cells, which has been traditionally associated with fluorescence recovery after photobleaching approaches.

Molecular biosensors consisted of GFP variants fused with sensitive domains, such as specific binding peptides or scaffolds, made significant progress last years. However, GFP-based fusions have a low range of fluorescence contrast. In this respect, KFPs with their capability to drastically change fluorescent intensity represent promising templates for the next generation of biosensors. Our results show that besides the light-irradiation, a partial loosening of KFP structures also results in the chromophore triggering between the dark and fluorescent states. KFPs fused with sensitive domains will result in biosensors that exhibit the fluorescence changes of two orders of magnitude and thus will allow spatiotemporal visualization of extremely low levels of intracellular signalling.

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***Key Words**: cytokines, hematopoiesis, growth factors, cell lineages, anemia*

Cytokines play important roles in the regulation of normal hematopoiesis and a balance between the actions of hematopoietic growth factors and myelosuppressive factors is required for optimal production of different hematopoietic cell lineages. We study the role of MAP kinases in the regulation of hematopoiesis and have shown that the p38 MAPK signalling pathway is the dominant cytokine regulated inhibitory pathway in human hematopoiesis. Studies are underway to determine the upstream regulators and downstream effectors of this pathway. The myelodysplastic syndromes (MDS) are collections of heterogeneous hematologic diseases characterized by refractory cytopenias due to ineffective hematopoiesis. These preleukemic disorders are common causes of anemia in the elderly and are rapidly increasing in incidence.

Development of effective treatments has been impeded by limited insight into any unifying pathogenic pathways. We have shown that the p38 MAP kinase is constitutively activated in MDS bone marrows. Such activation is uniformly observed in varied morphologic subtypes of low risk MDS and correlates strongly with enhanced apoptosis observed in MDS hematopoietic progenitors. Most importantly, pharmacological inhibition of p38a by a novel small molecule inhibitors can stimulate hematopoiesis in this disease. In addition to studying the mechanism of this important effect by a variety of approaches, we are also studying various other novel small molecule inhibitors in MDS. Ongoing studies involve search for MDS autoantigens and generation of murine models of this disease.

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Key Words: ***connexins, gap junctions, hemichannels, signaling, deafness***

Connexins (Cxs) comprise a family of membrane proteins that form gap junction (GJ) channels, which provide an important pathway for intercellular signaling in many tissues. Each GJ channel is a multimer of Cx subunits that is formed by the docking two pre-assembled hemichannels, one from each of two apposed cells. Mutations in the *GJB2* gene that encodes the Cx26 GJ protein are one of the most common causes of inherited deafness in the human population. A subset of these mutations leads to syndromic forms of deafness in which sensorineural hearing loss is accompanied by severe, inflammatory skin disorders, such as keratitis-ichthiosis-deafness (KID) syndrome. The underlying basis of syndromic deafness appears to be aberrantly behaving hemichannels, a relatively new mechanism identified among Cx-related disorders. These hemichannels do not participate in the formation of intercellular GJ channels, but rather remain undocked and function as large, ion channels in the plasma membrane. The mutant hemichannels behave in a "leaky" manner leading to compromised cell function and cell death. We use a combination of molecular, biophysical and imaging approaches to investigate the mechanisms by which Cx hemichannels are dysfunctional in KID syndrome. The mutations notably cluster in two domains, the N-terminus (NT) and the first extracellular loop (E1), which we identified to be principal components of the Cx channel pore and to play essential roles in Cx hemichannel gating by voltage and regulation by extracellular Ca^{2+}. Using a recently published crystal structure of Cx26, we are examining specific models of inter-subunit interactions involving E1 and NT residues that mediate hemichannel regulation by extracellular Ca^{2+}, the most prevalent regulatory mechanism that is dysfunctional in KID syndrome mutants. We also identified a mechanistic link between Ca^{2+}, pH and a distinct form of voltage gating, we term loop gating, which robustly regulates opening and closing of Cx hemichannels. Using cysteine-substitution accessibility, we established that two KID syndrome mutants are pore-lining and are investigating whether permeabilities to key signaling molecules, such as ATP and Ca^{2+}, are significantly altered. One of these pore-lining mutants, G45E, leads to a particularly severe, often fatal form of KID syndrome and our initial studies suggest increased permeability to Ca^{2+}, rather than Ca^{2+} dysregulation, may be the key contributing factor. We extend our studies to keratinocytes isolated from transgenic animals carrying the G45E mutation driven under an inducible keratinocyte-specific promoter. Finally, some KID mutants fail to function as GJ channels despite functioning as hemichannels and we are investigating whether these mutant hemichannels exhibit an impaired ability to dock. Together, these studies explore the mechanistic bases of hemichannel dysfunction in Cx26 that lead to severe disorders in humans. These studies should also shed light on a growing list of disorders ascribed to hemichannel dysfunction that includes atherosclerosis, stroke, neuropathy and congenital cataractogenesis.

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Key Words: ***Genome dynamics, DNA damage and repair, aging, functional genomics, transcription deregulation***

GENOME INSTABILITY, AGING AND TRANSCRIPTIONAL DEREGULATION

Genome instability has since long been implicated as the main causal factor in cancer and aging. Exactly how loss of genome integrity may lead to increased cancer risk and loss of organ and tissue function with age remains unknown. We study genome instability as a function of age in various model organisms, including mouse and fruit fly, and its consequences in terms of alterations in tissue-specific patterns of gene regulation.

We developed transgenic reporter systems in mouse and fruit fly, which allows us to determine tissue-specific frequencies of various forms of genome instability, e.g., point mutations, deletions, translocations. By crossing the mutational reporter animals with mutants harboring specific defects in various genome maintenance pathways, the relevance of these pathways for the accumulation of specific forms of genome instability is assessed, in relation to the pathophysiology of aging.

We have now also begun to apply next-generation sequencing approaches to analyze aging cells and tissues for alterations in the genome, epigenome and transcriptome. These same assays are applied for studying single cells, which will give us a handle on the stochastic component of the aging process. By using advanced bioinformatics tools, partly developed in our group, we correlate cellular function with genome/epigenome integrity. This work is done in mouse and human liver and brain as a function of disease and aging as well as in differentiating mouse and human stem cells.

Finally, as a spin-off from our more basic research we are developing, in collaboration with clinical departments, novel assays for measuring subtle genetic changes in single cells or very small number of cells, such as tumor needle biopsies.

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Key Words: ***type 2 diabetes, risk perception, medication adherence, behavioral intervention***

Elizabeth A. Walker, PhD, RN, is the director of the Prevention and Control Division for the Diabetes Research and Training Center at the Albert Einstein College of Medicine. Dr. Walker is currently the principal investigator for two large NIH-funded behavioral intervention studies in minority diabetes populations, using telephonic interventions in Spanish and English to promote medication adherence, lifestyle change, screening for complications, and other self-management behaviors. She is a behavioral scientist and co-investigator for the multi-center Diabetes Prevention Program Outcomes Study, and she chairs the DPP medication adherence workgroup. Dr. Walker is co-PI of a community-based study, Los Caminos, in which a culturally-sensitive diabetes self-management program is developed and evaluated using peer educators in the Bronx. Through the Prevention and Control Cores of the DRTC she provides or facilitates various intervention and evaluation services to multiple health disparities grants in the community. She collaborates in research with the New York City Department of Health and Mental Hygiene. Dr. Walker is a diabetes nurse specialist; she has been a certified diabetes educator since 1986. She is currently co-chair of a CDC Expert Panel on Risk Perception and Decision Making in Chronic Disease. In 2000, she served as the national President, Health Care & Education, of the American Diabetes Association.

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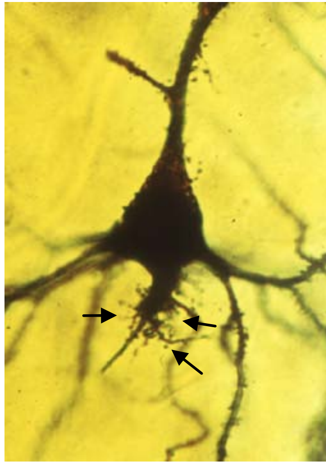
Key Words: ***lysosome, endosome, autophagy, ganglioside, intellectual disability***

PATHOBIOLOGY AND TREATMENT OF LYSOSOMAL DISORDERS OF BRAIN



The research interests of my laboratory are concerned with analysis of pathogenic cascades and development of therapeutic strategies for genetic disorders of the endosomal-lysosomal system. Examples of lysosomal diseases include Tay-Sachs, Hurler, Sanfilippo, Niemann-Pick, and Batten disorders, all of which are characterized by insidious onset and progression of neurological dysfunction, including severe intellectual disability, following an initial period of normal development. Primary proteins implicated in storage diseases include not only lysosomal hydrolases but also soluble and membrane-associated proteins often of unknown function. Animal models of storage diseases include both spontaneous conditions in a variety of species and gene knockout models in mice, both of which are used in our studies. Neurons affected by storage diseases often display remarkable abnormalities, including growth of ectopic dendrites (see figure at left, arrows), formation of axonal defects, compromise in autophagy and salvage systems, and selective vulnerability to premature death. Our studies are focused on the link between the primary protein defect and the abnormal accumulation of substrate (gangliosides, glycosaminoglycans, cholesterol, etc) and with subsequent induced changes in trafficking and signaling events within affected neurons. Therapeutic strategies are primarily focused on small molecule therapy directed at reducing substrate storage, so-called substrate reduction therapy.

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Key Words: ribosome, signal transduction, transcription, RNA-splicing

The ribosome is a molecular machine composed of 4 RNA molecules and 80 different proteins. The construction of a ribosome involves the integration of fundamental cellular processes: the transcription and processing of ribosomal RNA, the transcription, processing and translation of the mRNAs for ribosomal proteins, the assembly of the ribosomal proteins with the ribosomal RNAs, etc. In the yeast *Saccharomyces cerevisiae*, the synthesis of ribosomes consumes an extraordinary proportion of the cell's resources, accounting for >70% of all transcription, about 50% of all Pol II transcription initiation events, and >90% of all pre-mRNA splicing. We have used the genetic/biochemical approaches uniquely available in this organism to study the multiple levels of regulation that control this process. Our current research emphasizes two aspects of ribosome synthesis that will contribute to our understanding of the fundamental aspects of cell growth and regulation:

1) *Quality Control and Degradation*: We recently showed that the insufficient supply of a single ribosomal protein can have a strong effect on the regulation of ribosome synthesis. This led us to realize that the partial deficiency of a single ribosomal protein must lead to the production of many defective ribosomes which must be (A) detected and (B) degraded. Little is known about either process, although the exosome (for the RNA) and the proteasome (for the protein) are likely candidates. We are using Synthetic Genetic Analysis (SGA) to determine the proteins involved in each process, and to understand the biochemical and biological basis of the extreme balance that ribosome synthesis implies.

2) *Ribosome Synthesis and Disease:* It has become increasingly clear that ribosome synthesis, and its surveillance, plays an important part in cellular control. Thus, many insults to the assembly of ribosomes lead to accumulation of p53 and resultant apoptosis. When this control goes awry, tumors can develop. It is my contention that the massive amount of transcriptome data acquired from microarrays and modern sequencing has the potential to reveal far more about the role of aberrant ribosome synthesis in human disease. Therefore, we are learning and developing tools to mine such data, looking for unexpected relationships between the products of the 80 ribosomal protein genes, the 2000 ribosomal protein pseudogenes and the ~200 genes encoding ribosome assembly factors in healthy and diseased cells.

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***Key Words:** cardiovascular disease, lifestyle risk factors, postmenopausal women's health, hormone therapy, long-term observational studies, clinical trials, blood biomarkers of stroke, diet, depression, dementia, Hispanic health*

My research has spanned both cancer and cardiovascular disease, and both these areas of investigation have been brought together in my role as the Principal Investigator in the Women's Health Initiative (WHI). The WHI is a multi-center, multi-part national study of the major causes of morbidity and mortality in older women. It consists of several interrelated clinical trials, and a long-term observational study whose overall objectives are to prevent cancer, heart disease and osteoporosis in post-menopausal women and to identify biomarker, genetic, and lifestyle risk factors for these and other diseases in postmenopausal women. My current research also includes studies on the effects of hormone therapy on dementia and cognition, of blood biomarkers and risk of stroke, and of diet, depression and other psychosocial variables and cardiovascular risk. My research also includes another landmark prospective study, the Hispanic Community Health Study (HCHS), which is looking at cardiovascular health and disease in different Latino subgroups, and also includes research on cognition, genetics, hearing and dental conditions, pulmonary health and mental health.

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Key Words: HIV, nervous system, development, myelination

Human immunodeficiency virus type-1 (HIV) infection is associated with alterations in nervous system function in children. Affected children show loss of or failure to acquire various developmental milestones accompanied by bilateral pyramidal tract signs, progressive to spastic paraparesis or quadriparesis. Post-mortem examination reveals degeneration of the corticospinal tract (CST) in 50-75% of these children. In addition, neurologic signs and symptoms indicative of spinal cord disease are also present in at least 24% of adults with AIDS, and autopsy reveals vacuolar myelopathy (VM) in 17-31% of adult AIDS cases. The mechanisms underlying CST degeneration and VM are still uncertain.

My laboratory is investigating the hypothesis that exposure of the human fetal central nervous system to HIV-1 may cause alterations in normal myelination. These changes may be a part of the basis for the neurologic dysfunction characteristic of pediatric AIDS. The presence of white matter disease during the period of active myelination suggests that HIV-1 infection may contribute to major disturbance in the differentiation of oligodendroglia and their precursors. The in vivo arm of this project examines myelination in fetal CNS tissue exposed to HIV-1 in utero in a qualitative and quantitative manner, and compares the results to others derived from studying control CNS tissue from fetuses of uninfected females. In addition, organotypic cultures of human fetal spinal cord are used to determine the effects of HIV-1 on oligodendrocyte development and myelination in vitro.

Specific projects include:
(1) Characterization of differentiating oligodendrocytes and their immediate precursors in vivo with fixed tissue sections and/or in vitro using organotypic cultures. Immunohistochemistry and electron microscopic techniques are employed.

(2) Another project compares myelination in infected and uninfected brains using immunohistochemical techniques.

(3) The effects of cytokines and antibodies that recognize specific myelin and oligodendrocyte epitopes is being determined on myelinating human tissues.

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*Key Words: **toxoplasmosis, microsporidiosis, parasitology***

Toxoplasmosis: *Toxoplasma gondii* is a well described ubiquitous Apicomplexan protozoan parasite of mammals and birds. CNS toxoplasmosis ranks among the 10 most commonly occurring opportunistic infections and malignancies in AIDS patients, and may well be a greater direct cause of morbidity and mortality than other more common opportunistic infections. Despite recent progress in understanding the biology and antigenic structure of the rapidly replicating form (tachyzoite), very little is known about the cyst form (bradyzoite). The bradyzoite stage of Toxoplasma gondii plays a critical role in maintenance of latent infection. Differentiation of tachyzoites to bradyzoites appears to be stress mediated. We had previously identified a bradyzoite specific gene BAG5 (BAG1/hsp30) encoding a small heat shock protein and have characterized a T. gondii knockout of this gene. The identification of cyst wall specific protiens as well as stress related proteins and their effects on bradyzoite development is proceeding in the laboratory.

Microsporidiosis: The phylum Microspora consists of organisms collectively known as microsporidia, that are "emerging" human and veterinary pathogens. In humans microsporidia are etiologic in several disease syndromes including diarrhea, keratoconjuctivitis, sinusitis and disseminated infection. A microsporidian-specific organelle, the polar tube, is involved in invasion. While the description of the polar tube as a unique microsporidian structure occurred over 100 years ago, the biochemical components of this structure and the mechanism of its formation during invasion remain to be definitively determined. The laboratory is focused on projects invovling the: (1) characterization of the important functional elements of the major polar tube protein [PTP]; (2) characterization of polar tube associated proteins [PTAPs] of *Enc. hellem* and *Enc. cuniculi* and their interaction with PTP; (3) characterization of the function of PTP in the assembly and polymerization events that occur during formation of the polar tube; and (4) the idenficiation of therapeutic targets for the treatment of microsporidiosis.

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Key Words: ***cardiovascular disease, obesity, menopause, atherosclerosis, metabolism, metabolic syndrome***

I am particularly interested in the cardiovascular consequences of obesity and its metabolic sequelae, particularly as they occur within the context of ovarian aging/menopause. As such, my current research projects focus on understanding the role of adipose tissue in metabolic dysregulation, and the extent to which adipose tissue and subsequent metabolic disorders contribute to vascular aging and cardiovascular disease events. I am further interested in examining the role of adipose tissue in regulation of ovarian again and endogenous sex hormones, as well as the role of ovarian aging in adipose-tissue associated vascular disorders. My examination of the cardiovascular and ovarian aging effects of adipose tissue include examination of the relationships between body composition, inflammation, and glucose metabolism with cardiovascular disease, including both hard events such as stroke, as well as subclinical atherosclerosis as measured non-invasively.

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Key Words: ***transcriptional regulation, RNA polymerase, signaling, genetic networks***

TRANSCRIPTIONAL REGULATION AND SIGNALING PATHWAYS

Our laboratory is conducting basic research on the mechanisms of eukaryotic transcriptional regulation. We are especially interested in defining the signaling pathways and the mechanisms that regulate transcription of ribosomal components and transfer RNAs since these processes are critically important for controlling cell growth. Deregulation of cell growth control is widely recognized as a key event in cell transformation and tumorigenesis and is relevant to a broad range of human diseases. In addition, as the synthesis of new protein synthetic machinery constitutes >85% of nuclear gene transcription in growing cell populations, the tight coordinate control of this process, which involves all three nuclear RNA polymerases, is considered to be critical for metabolic economy and ultimately for cell survival. Our research programs span genetics, molecular biology, and biochemistry and utilize budding yeast, mammalian cells and mice as model experimental systems. Much of our current focus is on Maf1, a structurally and functionally novel protein that integrates the outputs of diverse signaling pathways and regulates transcription by all three nuclear RNA polymerases. Maf1 is also being studied because of its potential role as a tumor suppressor. The conservation of Maf1 along with the signaling pathways that regulate Maf1 function enables the reciprocal translation of knowledge between yeast and mammalian systems and facilitates the discovery of new biology.

GENETIC NETWORKS AND FUNCTIONAL GENOMICS

Synthetic genetic array analysis and other systematic genome-wide genetic approaches such as synthetic dosage lethality and suppression are being applied using robotic pinning and colony image analysis to produce, replicate and analyze high density arrays of yeast strains. This technology enables the mapping of genetic interaction networks, defines the function of genes and establishes functional relationships between biochemical pathways. These genetic array-based approaches are being applied to a range of biological processes including transcriptional regulation, cellular signaling and lipid metabolism. The integration of genetic interaction data with other large scale datasets such as DNA microarray, ChIP-sequencing and protein-protein interaction data is used to inform testable hypotheses of the systems level behavior of genes and their products.

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***Key Words:** virology, HSV, herpes viruses, viral entry*

ASSEMBLY AND FUNCTION OF THE HERPES SIMPLEX ENVELOPE

Herpes viruses are associated with a wide range of diseases; Burkitts lymphoma and nasopharyngeal carcinoma (Epstein-Barr virus), cytomegalic inclusion disease (Cytomegalovirus), chickenpox and shingles (Varicella-Zoster virus) and oral or genital mucocutaneous lesions (Herpes simplex virus, HSV types 1 and 2). Their ability to remain latent, then reactivate means they present a serious source of infection following immune suppression in organ transplant, chemotherapy or AIDS patients. The appearance of acyclovir-resistant HSV strains in CD4-suppressed subjects, and the role of a new herpes virus in development of Kaposi's Sarcoma, underline the importance of developing new therapeutic strategies to combat herpes virus infections.

Little is known of how herpes viruses enter and leave cells. Envelope/plasma membrane fusion during infection requires a large number of viral proteins, and the molecular role of each is unknown. Similarly, the pathway by which newly assembled progeny capsids become enveloped then released from the cell remains unclear. Molecular dissection of these phenomena will reveal much about the basic biology of the herpes viruses and yield a wealth of new virus-specific drug targets. Current laboratory projects include the following (visit our lab home page for more information).

A molecular dissection of HSV-1 envelopment. The assembly and intracellular trafficking of HSV is being studied in perforated cells and in cell-free extracts. Our ultimate goal is to reconstitute the assembly of infectious HSV in a defined biochemical system. Proteins necessary for envelope formation and virion movement will then be identified and characterized.

To identify regions of the viral envelope glycoprotein gH required for viral entry into cells. gH is a virally encoded membrane protein essential for fusion of the viral envelope with the cell plasma membrane during infection. We are constructing genetically engineered viruses expressing mutant forms of gH, and testing the effect of these mutations upon gH biosynthesis and viral infectivity.

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Key Words: ***liver, transport, kidney, intracellular anions, endocytosis, microtubules***

A major function of the hepatocyte is the removal of xenobiotic and endogenous organic anionic compounds from the circulation. Much of this transport activity resides in the hepatocyte. The focus of our research has been elucidation of two interrelated, physiologically important hepatocellular transport mechanisms, one for anionic drugs and the other for receptor-mediated endocytosis.

We have identified and cloned members of what has turned out to be a new family of organic anion transport proteins (oatps). They have 12 transmembrane domains and similar biochemical characteristics. Although evidence suggests that the oatps are important in clearance of drugs from the circulation, little is known regarding the mechanism by which they act, their oligomerization state, or mechanisms for subcellular trafficking. In recent studies, we found that many of the oatps have PDZ consensus binding domains and interaction of oatp1a1, a major oatp of the hepatocyte, with PDZK1 is required for its expression on the cell surface. We have found that oatps and several other important drug transporters cycle on microtubules between the cytosol and cell surface, regulated by transporter-specific kinases, nanomotors, and accessory proteins such as Rabs. Elucidation of these novel mechanisms may provide an important link between trafficking of these transporters and alterations in their function that could result in drug toxicity.

These studies of mechanisms of transporter trafficking relate to our other studies of receptor-mediated endocytosis, characterized by internalization of ligand-receptor complexes into an endocytic vesicle (endosome). Subsequently, these complexes dissociate as the endosome acidifies, and ligand and receptor segregate into separate compartments. Ultimately ligand traffics to the lysosome where it is degraded, while receptor recycles to the cell surface where it is reutilized. Our previous studies have shown that this segregation process requires integrity of microtubules. We are investigating the role of microtubules in providing a directed path for these processes and the potential importance of microtubule-associated motor molecules such as kinesins and dynein in providing the force for vesicular movement. To accomplish this, we have devised a cell free *in vitro* system to dissect the functional components of these processes. In this system, endocytic vesicles on microtubules can be viewed using microscopy technologies that permit quantitation of direction and rates of movement. We have reconstituted vesicle fission and segregation, and identified regulatory proteins. Using a proteomics-based approach on highly purified endocytic vesicles, we have discovered a number of novel vesicle-associated proteins and are pursuing studies to define their role in the endocytic process.

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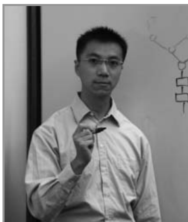

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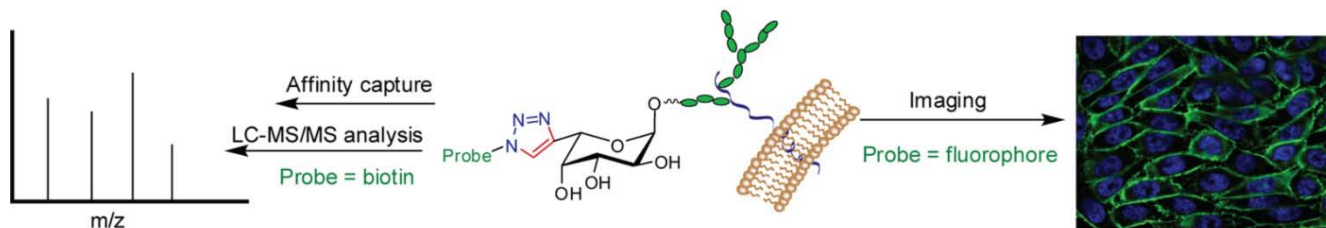
Key Words: ***chemical biology, glycobiology, click chemistry, host-parasite, schistosomiasis***



The research in my lab integrates synthetic chemistry with glycobiology to explore the glycosylation of human pathogens and its relevance to the host immune response. Evidence from numerous studies indicates that cell surface and secreted glycoconjugates play key roles in many infectious diseases such as malaria and schistosomiasis, two of the most prevalent tropical diseases in the world. Our goal is to develop chemical tools to study the biosynthetic machinery that produces the antigenic glycoconjugates and to elucidate the roles of glycans in pathogenesis. Areas of current focus include:

- **Development of new chemical tools for glycobiology**
- **Investigation of *Schistosoma mansoni* (*S. mansoni*) fucosylation**

In the technology development arena, our efforts are directed at discovering new bioorthogonal chemical reactions that can be utilized to probe protein glycosylation in living systems. Bioorthogonal reactions are non-native, non-perturbing chemical reactions that can incorporate an exogenously delivered probe to a target biomolecule in a highly selective manner in a cellular environment or in complex cell lysates. Therefore, these reactions are powerful tools for detection or isolation of the tagged biomolecules, including proteins, lipids and glycans. Additionally, we are also interested in developing new chemoenzymatic methods to synthesize fucoside-containing glycoconjugates. In the host-parasite interaction arena, we are investigating fucose metabolic pathways that allow schistosomes to persist in the human host. We are also interested in discovering specific inhibitors of *S. mansoni* glycan biosynthesis and processing.

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Key Words: ***Nutrition, Lifestyle, Epidemiology, Clinical Trials, Obesity***

My research has focused on the role of nutrition in chronic disease prevention and control and factors related to disparate rates of obesity and health risks. As a result, I have been collaborated in multicenter clinical trials and other studies that address translation of care recommendations into health care for people with diabetes, heart disease, cancer, and obesity.

My current investigator-initiated research includes:
1) a randomized controlled clinical trial to evaluate how a comprehensive approach to family weight management affects cardiometabolic biomarkers
2) a cross-sectional examination of acculturation in relation to biomarkers of cardiovascular risk among Chinese immigrants
3) translation evaluation of simplified tools to promote addressing obesity in primary care and community settings
4) examination of nutrition-related questions in existing databases

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Rajpathak SN, **Wylie-Rosett J.** High prevalence of diabetes and impaired fasting glucose among Chinese immigrants in New Yorkk City. Journal of Immigrant and Minority Health . 2010 Jun 9. [Epub ahead of print]

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Key Words: gene expression, fatty acid and cholesterol metabolism, metabolic syndrome

The sterol regulatory element -binding proteins (SREBP) are critical regulators of both fatty acid and cholesterol homeostasis. SREBP proteins are synthesized as inactive precursors that are tethered to the endoplasmic reticulum membrane. In response to decreased cellular levels of sterols, SREBP precursors are proteolytically processed to mature forms of transcription factors that migrate into the nucleus and activate transcription of target genes, which encode the rate-limiting enzymes in synthesizing fatty acids and cholesterol. Our laboratory is interested in understanding how SREBP-mediated transcription is regulated. Co-activators, such as the Mediator complex, are involved in activating transcription of SREBP target genes. The Mediator is a multi-subunit protein complex. Biochemical and genetic approaches are being taken to study the role of the Mediator subunits in SREBP-mediated transcription. In addition, molecular mechanisms of nuclear SREBP degradation are being studied. Recently, we have observed that the NAD-dependent protein deacetylase SIRT1 orthologs negatively control the expression of SREBP target genes in multiple model systems, consistent with the inverse relationship between SIRT1 and nuclear SREBP protein levels during fasting and in metabolic diseases. SREBP protein stability can be regulated by acetylation. Thus, SIRT1 may play a key role in mediating down-regulation of nuclear SREBP proteins. We have also found that SIRT1 is physically and functionally associated with multi-functional protein complexes, suggesting the novel functions of this putative longevity protein. We are currently studying the functions of SIRT1 complexes. With the ultimate goal of identifying targets for preventing or treating metabolic syndrome, the aim of our research is to advance our knowledge of how lipid homeostasis is regulated at the molecular levels.

Selected Publications:

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Key Words: Mathematical, computational, statistical modeling; immune repertoires; CTL killing; dynamics of infections

Our research relates to immunology, the within-host dynamics of pathogens, and connecting the epidemiology of disease (between-host dynamics) with immunological (within-host) processes. We use mathematical, computational and statistical modeling to address quantitative problems in these areas. Some of our work is purely theoretical. We also collaborate extensively with experimental biologists and use real data, often from dedicated experiments, to search for mechanistic explanations of biological phenomena. Just a few examples of the topics that interest us currently are:

The population biology of immune repertoires: generation, structure, quality and persistence
How flexible is our capacity for immunological memory? What determines the longevity of our protective memory to a particular infectious agent or vaccine? How does our naive T cell repertoire develop throughout childhood? How are our immune repertoires reconstituted in lymphopenic conditions? How do lymphocytes integrate signals from their environment, and what determines the size of any particular T or B cell clone? What happens to the clonal structure and functional integrity of our naive and memory lymphocyte populations as we age? What governs the ratio of CD4 to CD8 T cells emerging from the thymus? What are the rules for the incorporation of recent thymic emigrants into the mature naive T cell pool?

The dynamics of CTL killing
There is currently much interest in developing vaccines that elicit strong cytotoxic T lymphocyte (CTL) responses as well as humoral immunity. However, in contrast to the soluble, rapidly diffusing antibodies constitutively produced by plasma B cells, CTL need to undergo reactivation, migrate to an infection site, rapidly survey potential targets and identify and kill infected cells efficiently. Understanding the spatio-temporal dynamics of CTL activity is essential for understanding T cell vaccine efficacy. Structured population models can be used to study the effect of CTL control on viruses with different reproductive strategies, including HIV, to provide a minimal estimate of the numbers of T cells required to protect against HIV infection.

The within-host dynamics of infections
What are the relative contributions of immunity and resource limitation in controlling acute infections? Why and how do many infections persist? What programs do immune cells follow in response to these infections and how are these self-limited or subverted? Much of what governs the life-histories of cells participating in an immune response is poorly defined, particularly in the later stages of infections. TB, Malaria, HIV, tumours and grafts are diverse examples or analogues of chronic infections, and a deeper understanding of the dynamics of persistently stimulated immune cell populations is crucial for understanding and treating the associated pathology. Addressing such questions requires linking the dynamics of processes within cells (for example, differentiation and senescence) with the population biology (ecological dynamics) of lymphocytes.

Selected References:

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Visualizing recall immune responses with multi-photon microscopy (commentary)**. Andrew Yates.** Immunology, 128(4):461-2 (2009)**.**

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Key Words: ***cancer, oncogenes, B cells, cell signaling,***

THE BCL-6 PROTO-ONCOGENE: FUNCTION IN NORMAL AND MALIGNANT B CELLS

Non-Hodgkin's lymphoma (NHL) is the 5th most common type of cancer in the U.S. and the only major cancer type with a rising diagnosis rate. From the genetic point of view, NHL is distinct from non-hematopoietic cancers in that most lymphomas carry recurrent chromosomal translocations, while mutations in the classic tumor suppressors, e.g. p53, Rb, p16, are infrequent. The majority of the mature B cell lymphomas derive from germinal center (GC) B cells that normally reside in the secondary lymphoid organs and are responsible for generating high affinity humoral immune response as well as B cell memory. A hallmark of the GC reaction is the ability of B cells to highly diversify their immunoglobulin genes through a sophisticated enzymatic system followed by selection for desirable antibody specificity and affinity. These processes are subject to genetic control and intertwined with B cell activation and differentiation events. Recent studies have lent strong support to the theory that lymphoma development often arises as the collateral damage of the antibody diversification process.

The main research theme in my laboratory is transcription regulation and cell signaling control in normal GC B cells and B cell lymphomas. The philosophy that drives our research endeavor is that identification of valid therapeutic target can only be feasible when we obtain adequate understanding of the mechanisms that govern the normal cell counterparts of these tumors as well as the nature of dysregulation that derails the normal processes. As such, our work situates at the crossroad of B cell differentiation, cancer genetics, transcription regulation, and cell signaling, and we constantly draw upon the most recent advances in these perspective fields, testing and integrating new paradigms in our investigations. Current research projects, which are funded by the NIH as well as the Leukemia & Lymphoma Society, aim to **a)** uncover unique genetic mechanisms that are responsible for malignant transformation of GC B cells, **b)** characterize the cell-cell and intracellular signaling events that shape the immunological outcome of the GC response, **c)** investigate the molecular mechanisms underlying relapse and drug resistance of DLBCL.

Selected Recent Publications:

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Key Words: ***protein folding, structure/function, hemeproteins, laser spectroscopy***

STRUCTURE, FUNCTION, FOLDING AND DYNAMICS OF HEMEPROTEINS

Proteins are the building blocks for all life forms. They are produced in ribosomes as non-structured nascent polypeptides, which subsequently fold into functional proteins. The first goal of my research program is to understand the general principle underlies protein folding reactions. The current interest is to dissect the folding pathways of cytochrome c, myoglobin and fatty acid binding proteins. The second goal of my research focuses on the studies of the structure-function relationships in bacterial hemoglobins, human indoleamine dioxygenase and bovine cytochrome bc1. In my research program, a wide array of spectroscopic tools, including optical absorption, fluorescence, circular dichroism and UV / VIS resonance Raman scattering, are utilized to study various biological processes. With the state-of-the-art rapid solution mixing technique developed in my laboratory along with conventional stopped-flow apparatus and laser flash photolysis systems, we are able to follow biological reactions from nanosecond to hours.

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Key Words: *T lymphocyte, costimulation/coinhibition, cancer, autoimmunity, infection*

T cell costimulation and coinhibition: ***From genomics to gene knock-out/transgenic mice to patients***
T lymphocytes play a central role in the initiation and regulation of the adaptive immune response to antigen, whether foreign or native. The outcome of T cell engagement of antigen is determined by both positive costimulation and negative coinhibition, generated mainly by the interaction between the B7 family and their receptor CD28 family. We have recently discovered the newest members of the T cell costimulatory/coinhibitory B7 family, and are using a variety of experimental approaches (gene knock-out mice, transgenic mice, monoclonal antibodies, etc) to understand how new B7 family members regulate T cell activation and tolerance. Current emphasis in the lab is placed in the following areas: 1) Functions of B7x and B7-H3 in T cell responses in vivo; 2) Cancer-associated B7x and B7-H3; 3) Roles of B7x, B7-H3, and PD-L1 in autoimmune diseases; 4) Relationship between B7x and B7-H3 and infection; and 5) New members of the immunoglobulin superfamily. Our goal is to elucidate the mechanisms by which costimulation and coinhibition regulate T cells in peripheral non-lymphoid organs, and to translate the lessons learned in these studies towards developing new therapeutic strategies for immune-mediated diseases such as cancer, autoimmune disorders, infectious diseases, and transplantation rejection.

More information of our research can be found on our lab homepage:
http://www.einstein.yu.edu/zang/page.aspx

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Key Words: ***bioinformatics, genomics, computational biology, evolution, neurodegenerative diseases***

Decoding the human genome and the genomes of many model organisms has been one of the major challenges in the post-genomic era. Where are the biological functional DNA elements located in these genomes? How do they coordinately and dynamically work together to direct gene expression and regulation, cell and organism development? How are their functions altered by epigenetic modifications? How do various classes of DNA elements emerge and evolve? And where are the information encoded in the human genome that distinguishes human from other species? These are just few in the long list of important questions that are currently under extensive studies using experimental and computational approaches.

The research field of my group is Computational Genomics and Bioinformatics, with a strong focus of mining large-scale experimental genomic data to address the above questions. We develop and apply computational techniques for integrating data of comparative genomics and functional genomics (and epigenomics) to decode the structure, function, and evolution of the human genome. More generally, we are interested in bioinformatic and statistical approaches for exploiting novel and biologically significant patterns in high-throughput genomic data. Recently, we have become highly interested in the expression, regulation, and evolution of human genes (coding or non-coding) that are involved in the development, specification, maturation, and maintenance of human neural systems. Working extensively with experimentalists, our study will contribute important information to neurodegenerative diseases and many other brain diseases.

Please see our website for more details:
http://dain.aecom.yu.edu/zhenglab

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Key Words: ***cardiovascular development, mouse genetics, signal transduction, transcriptional regulation***

Our research focuses on molecular mechanisms in heart development and congenital heart disease. We apply an integrated approach including transgenic mouse model, developmental embryology, cell and molecular biology to study endocardial cell (the endothelial cells of the heart) specification and differentiation, transcriptional regulation of heart development, and molecular signaling in coronary vessel patterning.

Endocardial Cell Lineage Specification and Differentiation: Our molecular model for studying endocardial cell specification and differentiation is the transcription factor NFATc1 (Nuclear Factors in Activated T-cells-1). It is the only known transcription factor specifically expressed by the endocardial cells during heart development. We have generated several endocardial specific Cre and lacZ transgenic mouse lines. We are now using the 'Cre-loxP' system to trace the evolution of endocardial cell lineages during heart development. Our data indicate that the endocardium is the origin of cardiac mesenchyme and coronary vascular endothelium, and that VEGF, Notch, and Calcineurin pathway regulates the endocardial to endothelial transition and coronary vascular formation.

Transcriptional Regulation of Cardiac Development: Our study of NFATc1 regulation has led to the discovery of an important auto-regulatory loop via a transcriptional enhancer during cardiogenesis. We are currently characterizing this enhancer paradigm by identifying its upstream regulators and downstream key components using DNA affinity pull-down, Mass-Spec, and cDNA microarrays. We are also using ES cell differentiation, early mouse embryos, and CHIP-Seq to identify the *cis*-elements for the early endocardial expression of NFATc1 when cardiac cells are specified. These studies will define the transcriptional hierarchy of endocardial specification.

Modeling of Cardiovascular Disease: Congenital heart valve disease is a common birth defect whereas senile aortic valve stenosis is a common disease in the elderly. We are generating and characterizing mouse models of congenital heart valve disease or senile aortic valve stenosis by ablation of endocardial cells or genes in the endocardium. These mouse models will allow us to better understand the endocardial role in these diseases.

Epigenetic and genetic Mechanisms of Coronary Vessel Pattering: Coronary vascular formation is a developmental mystery in terms of its origin and patterning. We are interested in the regulatory role of hypoxia and VEGF signaling in coronary vessel development. We generated a mouse model of coronary anomalies by deletion of VEGFA in the myocardium. We also created a hypoxic-responsive mouse model revealing that the myocardium surrounding the forming main coronary arteries is hypoxic. This model permits us to address the role of hypoxic myocytes and their hypoxic-dependent VEGFA production in coronary vessel development. In addition, we have begun to study epigenetic regulation of heart and coronary vascular development focusing on the hypoxia-inducible factor function.

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Key Words: ***cancer, tumor suppressor, cell cycle, proliferation, cell death, mouse models***

CONTROL OF CELL PROLIFERATION, DEATH, AND DIFFERENTIATION BY THE TUMOR SUPPRESSOR pRb, The E3 UBIQUITIN LIGASE Skp2, AND THE CYCLIN-DEPENDENT KINASE INHIBITOR p27Kip1

The retinoblastoma protein pRb is a prototype tumor suppressor whose function is inactivated in a wide spectrum of cancer. Intense research has been carried out to understand how pRb functions to suppress tumorigenesis; and many targets of pRb have been identified, the best established being the E2F transcription factors. In these studies, mouse models of Rb gene mutation have played critical roles in provided insights of pRb function in vivo. Using pRb mutant tumor cell lines in which we introduecd a wide type pRb to block cell cycle progression in G1, we idenfied the E3 ubiquin ligase Skp2 as a new target of pRb (Ji et al. 2004, Molecular Cell). More recently, we used various pRb mutant mice to study the significance of Skp2 in tumorigenesis following loss of pRb. In these studies, we found that Skp2 and its function in the E3 ubiquitin ligase for p27 are essential for cell survival after pRb function is inacitvated (Wang et al. 2010, Nagure Genetics). These studies identify an pRb-Skp2-p27 pathway that is essential for pRb's tumor suppressor function. We are now working on extending the pRb-Skp2-p27 pathway further downstream.

Specific cancers currently under study in our lab include prostate cancer and liver cancer. In prostate cancer, we focus on the mechanism of its androgen-dependence and have found that Skp2 is an target of androgen-androgen receptor signaling (Wang et al. 2008, Journal of Cell Science). In liver cancer, we are comparing the role of p27Kip1 in hepatocyte regeneration and in hepatocyte carcinogenesis (Karnezis et al, 2001, Journal of Clinical Investigation; Sun et al, 2008, Molecular Carcinogenesis).

We have recently discovered a role of pRb in regulation of energy metabolism and type 2 diabetes, and consequently initiated a new line of study in the lab. In many of these studies, we are developing improved methods to achieve gene knockdown (Sun et al, 2006 BioTechniques).

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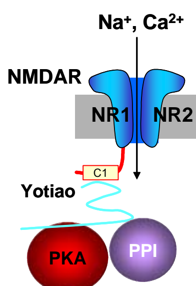
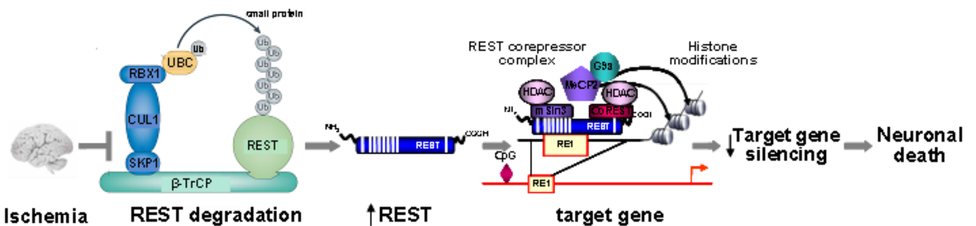
Key Words: ***NMDA receptors, synaptic plasticity, epigenetics, neuronal death, stroke, autism, Fragile X syndrome, Alzheimer's disease***

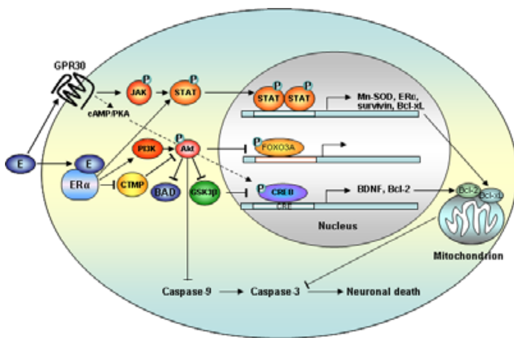


There are four major lines of ongoing research in the Zukin lab. *First, we are studying the mechanisms that regulate trafficking and targeting of NMDA-type glutamate receptors to and from synaptic sites.* We have found that protein kinase C regulates NMDA receptor trafficking and gating. We identified SNAP-25 as the molecular target of PKC phosphorylation critical to insertion of new NMDA channels. We found that calcium influx through NMDA receptors in dendritic spines is under the control of cAMP/PKA signaling and that PKA is critical to NMDA receptor-dependent long term potentiation at hippocampal synapses. Recently, we discovered that the gene silencing transcription factor REST acts *via* epigenetic mechanisms to regulate the switch in NMDA receptor phenotype during brain development. New questions are: What is the molecular target of PKA? Can fear-inducing stimuli activate PKA and regulate calcium signaling through NMDA receptors? How do maternal deprivation and schizophrenia dysregulate the switch in NMDA receptor subtype during brain development? Our interest stems from the fact that NMDA receptors play a central role in cognitive functions such as learning and memory and formation of neural circuitry. NMDAR dysregulation is implicated in Alzheimer's disease, Huntington's disease, AIDS dementia, stroke and schizophrenia.

Second, we are studying the molecular and cellular mechanisms that underlie the neuronal death associated with stroke and epilepsy. We discovered that neuronal insults such as ischemia and seizures activate the gene silencing transcription factor REST, critical to renewal of pluripotent stem cells, in adult hippocampal neurons and that REST is critical to death of hippocampal neurons. The AMPA receptor GluR2 subunit is a target of REST. Silencing of GluR2 leads to expression of calcium permeable AMPA receptors and neuronal death. We have found that REST promotes epigenetic remodeling of AMPA receptors in response to neuronal insults. We have initiated studies to examine epigenome-wide dysregulation in animal models of stroke, Huntington's disease and Alzheimer's disease. Our goal is to identify novel strategies to protect the brain from injury in stroke, epilepsy, ALS and spinal cord injury.



A third area of interest is that of estrogen neuroprotection in animal models of stroke, including global ischemia. Recently, we together with the Etgen lab found that a single, acute injection of estradiol administered after an ischemic event ameliorates hippocampal injury and cognitive deficits. We also found that estrogens act *via* estrogen receptors ERα and GPR30 and JAK/STAT signaling to protect cells. Objectives are to identify mechanisms by which estrogen rescues neurons. Studies address STAT3 and CREB-induced epigenetic remodeling and transcription of target genes. Our interest stems from data that estrogen reduces the risk of cardiac arrest and stroke in humans.



A fourth area of interest is that of RNA trafficking and local protein synthesis in Fragile X. Fragile X syndrome is a leading genetic cause of intellectual disabilities and autism. We found that mTOR signaling is overactivated in Fragile X mice. We showed that dysregulation of mTOR is related to impaired synaptic plasticity in these mice. We also found that targeting of AMPAR mRNAs to synapses is dysregulated in Fragile X neurons. We are using the lentivirus expression system to genetically manipulate key proteins in the mTOR pathway and examine the impact on spine morphology, synaptic plasticity and cognition in the Fragile X mouse. It is hoped that these studies will accelerate the development of novel therapeutic strategies to ameliorate cognitive deficits in humans with Fragile X Syndrome.

Positions for graduate students and postdoctoral fellows are available in all four areas of research. Independent researchers and ideas are welcome, while well-defined and achievable projects are waiting for motivated, young investigators.

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Graduate Programs in the Biomedical Sciences

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