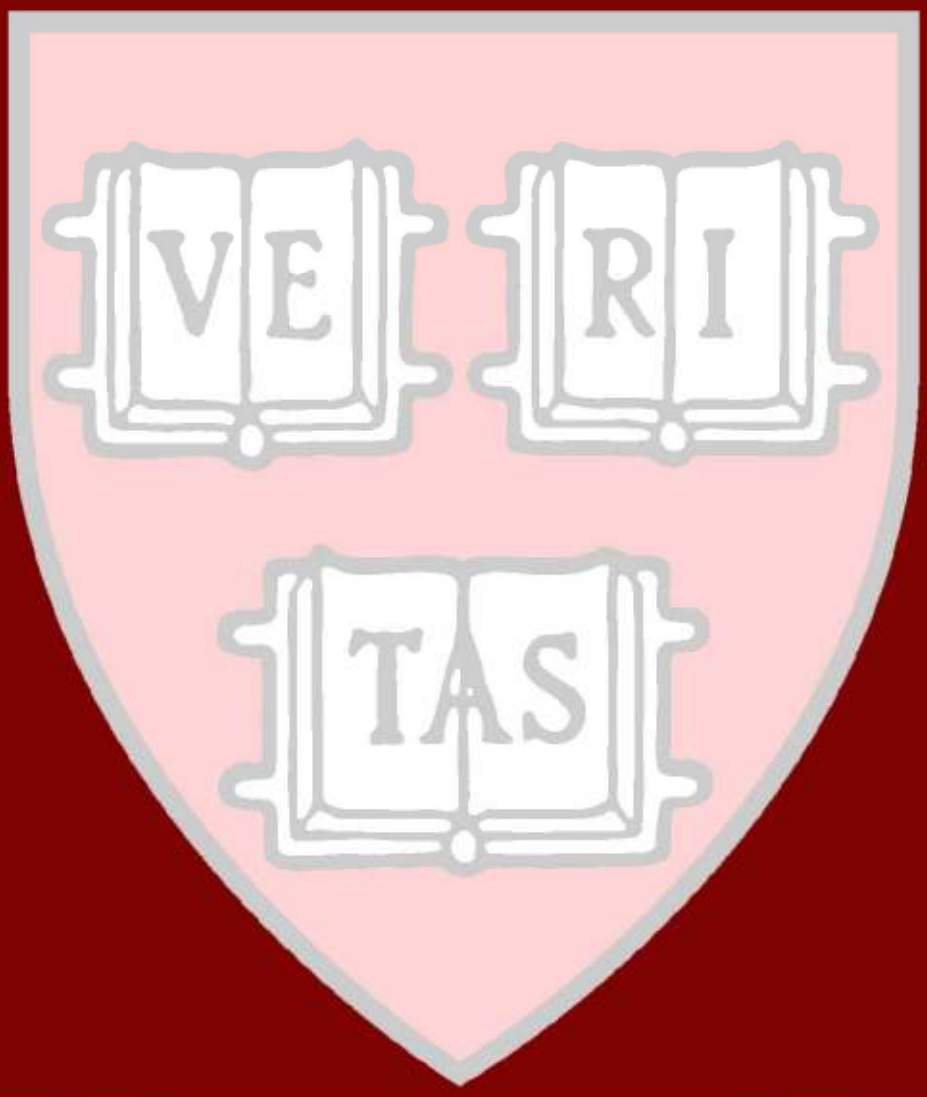

HARVARD COLLEGE

PRISE

PROGRAM FOR RESEARCH IN SCIENCE AND ENGINEERING

Abstracts 2007

Letter from the Director

It is with great pleasure that I write this foreword to the 2007 Harvard College Program for Research in Science and Engineering (PRISE) Abstract Book, a project conceived, organized, and composed by PRISE Fellows to commemorate their summer of research, community, and learning. During the exciting ten weeks of PRISE, the Fellows have spent countless hours working under the guidance of distinguished Harvard investigators across the Faculty of Arts and Sciences, the Harvard Medical School and affiliated hospitals, and the Harvard School of Public Health.

The amazing array of research experience during PRISE is captured in short form within the pages of this book. As one of the main goals of PRISE is to encourage and foster the development of young scholars entertaining careers in scientific research, the abstracts herein are a testimony of the experiential strides made in a relatively brief amount of time. This scientific experience has been augmented by participation in an interdisciplinary residential community of undergraduate scientists at Leverett House during the summer, providing a social environment and the opportunity to interact with peers in a meaningful way. During dinner, late at night in the JCR, and even during trips to Tanglewood or Red Sox games at Fenway Park, it has not been uncommon to hear PRISE Fellows discussing their research findings of the day.

To the PRISE Fellows of 2007, I wish you every success in your growth and trajectory as scientists. Your engagement, enthusiasm, and inclusiveness are inspiring, and I look forward to hearing about your great discoveries in the future.

Gregory A. Llacer, Director
Harvard College Program for Research in Science and Engineering (www.priselink.harvard.edu)

Letter from the Editor

Dear fellow PRISE scholars,

It has been an amazing and unbelievably quick 10 weeks. I find it hard to recall all of the activities we've done together simply because of the shear number of experiences. From Cirque du Soleil's performance of Delirium to whale watching; from Red Sox games to even something as plain as lounging around in the JCR playing N64, I've enjoyed every moment of my PRISE experience. A friend recently posed the question, "what event was the highlight of PRISE?" After considering this for a few moments, I could honestly answer that the PRISE Presentations were the best part. They represented the culmination of over 110 productive summers and, ultimately, were the reason we were chosen as PRISE Fellows. A criterion for PRISE, if you remember, was "A strong dedication to developing or furthering academic interest and excellence in scientific research." The presentations exemplified this quality in all of us.

In order to put the presentations in print, I decided to compile this interdisciplinary abstract book of your research. I trust you will hang onto this book and enjoy reading about each other's research. I also hope that you all continue your PRISE experience by participating in the Harvard Undergraduate Research Symposium (HURS), which is organized by the Harvard College Undergraduate Research Association (HCURA). I look forward to keeping in touch with you all.

Best wishes,

Shiv Gaglani, '10
Editor-in-Chief, PRISE Abstract Book; President, HCURA

Jackie Hsieh, '10
Copy and Layout Editor

Roanna Ruiz, '09
Design Editor

Table of Contents

Margaret Arnold mrarnold@fas.harvard.edu
Dunster 2008
Psychology

Personality Perception in Toddlers: Trait Awareness in Four-Year-Olds as Related to Threat, Consistency, and Behavior

The Spelke Laboratory, William James Hall

The present study, Fact and Faces, examines personality perception and the ways in which young children make judgments about their peers. Four-year-old participants will view a brief slide show in which they are introduced to eight children. For each face presented, participants will be given a trait label and an overt behavior consistent with this trait. Participants will then be shown the faces again and asked to remember the trait label they had been told. The current study will consist of a number of conditions looking at different personality traits in order to determine the types of information 4-year olds attend to and make a priority of remembering. These traits include being nice versus being mean, talking a lot versus being quiet, and playing inside versus playing outside. I predict that 4-year olds will choose which traits to pay attention to according to social relevance, level of threat, group membership, consistency, and contribution to their own survival. They will not pay attention to ambiguous traits that may seem irrelevant or unimportant. This study will be a significant step in reconciling past research on personality perception in infants, toddlers, and adults. The results may also have implications for proper development and the extent to which toddlers' environments can affect their senses of well-being.

Jeffery James (JJ) Blair jblair@fas.harvard.edu
Currier 2009
Astronomy

Serial Guiding for the Large Synoptic Survey Telescope

The Stubbs Laboratory, Laboratory for Particle Physics and Cosmology, Harvard University

Despite the revolutions in astronomy enabled by digital CCD and microprocessor technology now employed in telescopes, our ability to observe the sky as a whole is exceedingly limited. Currently, the largest optical telescope in the world, the Large Binocular Telescope in Arizona has an optical field of view of 10 arc minutes, a tiny fraction of the sky in even one hemisphere. In order to enable a more comprehensive survey of the night sky, the international astronomical community has proposed the LSST. The Large Synoptic Survey Telescope, to be built at the Cerro Tololo Inter-American Observatory in Chile, will have an unprecedented large field of view, amounting to almost 9.6 square degrees of sky, allowing the scope to completely image an entire hemisphere of the sky every three nights.

Due to the enormous cost of imaging equipment for the cutting-edge scope, we are considering new methods for image finding and telescope tracking. The proposed method involves using preexisting serial registers already bound to the CCD detectors in the scope to read off one dimensional slivers of the sky many times a second to create a two dimensional image: one dimension in length, another in time. This will allow subtle shifts in telescope position to be monitored and corrected for before they are even read from the image. Implementation of serial register tracking will reduce construction costs and preserve field-of-view integrity while improving image resolution and eliminating telescope mount distortions.

Further study is needed to determine the efficacy of the proposed tracking changes.

David Bochner dnbochn@fas.harvard.edu
Mather 2008
Biology

Love, War and Fruit Fly Genetics: The Role of Octopamine in Courtship and Aggression

The Kravitz Laboratory, Department of Neurology, Harvard Medical School

In the fruit fly *Drosophila melanogaster*, aggression and courtship are innate behaviors that involve stereotyped patterns of movements (called modules) by one fly and stereotyped responses by another. Each module involves a distinct pattern of actions: for fighting, a series of combat maneuvers (chasing, lunging, boxing), and for courtship, a specific sequence of mating behaviors (tapping, singing, copulation). Sexual dimorphisms also exist for these responses: females fight with a different repertoire of maneuvers, and rarely show courtship behavior beyond accepting or rejecting male advances.

Previous work by our laboratory suggests that the neurotransmitter octopamine, the invertebrate homolog of the vertebrate neurotransmitter norepinephrine, modulates the choice to court or fight another male fly. Male flies deficient in and without octopamine spend more time courting males, spend more time displaying male-male patterns of courtship behavior, and transition back and forth between courtship and aggression over the course of a single encounter. Feminizing octopaminergic neurons in the brains of male flies causes a similar phenotype.

We are examining the hypothesis that this choice between courtship and aggression may be mediated by one or more of the five known subtypes of octopamine receptors, proteins which bind octopamine and convert it into a signal inside the cell. This summer, over the course of the PRISE program, I have been investigating the role of one particular type of octopamine receptor, the Octopamine ß1 receptor, in mediating the decision between courtship and aggression. Based on preliminary data, it appears that eliminating the expression of the ß1 receptor in male flies causes them to spend more time courting males than controls, but in contrast to octopamine-deficient flies, does not increase the length of individual courtship events towards males. These early results support the suggestion that the role of octopamine in *Drosophila* behavior may depend on the type of receptors present in target cells, and implies a complex relationship between the neurotransmitter and its receptors.

Bryant Bonner bbonner@fas.harvard.edu
Kirkland 2009
Biology

Molecular Mechanisms Controlling Birth and Differentiation of Corticospinal Motor Neurons

The Arlotta Laboratory, Center for Regenerative Medicine, Massachusetts General Hospital

During development of the neocortex, neuronal progenitors give rise to several different subtypes of neurons. As of yet, the molecular mechanisms that control the specific differentiation of these neural progenitors remain unknown and difficult to elucidate. Here we try to address these issues within the cerebral cortex in trying to identify the early instructive signals that induce the generation of corticospinal motor neurons from telencephalic progenitors. Fezf2, a transcription factor, has been shown to be necessary and at least in part, sufficient to generate corticospinal motor neurons from neural progenitors, controlling early decisions regarding lineage specific differentiation. By exploiting this Fezf2 mediated fate switch, the lab has begun to better understand the transcriptional level changes that accompany the switch. However, to completely understand this switch, it is also necessary to examine changes at the protein level, especially crucial post-translational changes. Towards this end, we have been setting up an

in vitro system in which Fezf2 is overexpressed in neural progenitors in culture. We have begun this process utilizing both electroporation and retroviral overexpression of Fezf2 in dissociated cortical progenitors isolated from E14.5 mouse embryos, analyzing the efficacy of both. The Fezf2 overexpressing progenitors generated from these experiments with then be used to identify proteomic changes via mass spectrometry. With these results we hope to elucidate the protein changes associated with the Fezf2 mediated induction of corticospinal neuron fate.

Jannis Brea jbrea@fas.harvard.edu
Pforzheimer 2010
Undecided

Can you hear me now?: Determining auditory discrimination in adult female mice

The Hensch Laboratory, Department of Molecular and Cellular Biology, Harvard University

While language is one of the few faculties considered distinctly human, its development relies upon other systems such as hearing or sight. By studying the maturation of such sensory systems, we hope to gain a greater understanding of language acquisition. Our lab examines critical periods in mouse auditory development and in the perception of ultrasonic communication. During the critical period, the plethora of possible neural connections is narrowed and fixed based upon environmental cues. In addition to understanding what happens during this pruning process, we hope to provide a model of the effects of Valium (benzodiazepine) on fetal language development.

In order to determine whether the mice are differentiating between sounds, our project is to train female mice to perform a two-alternative forced-choice task. In this task, the mouse pokes its nose into a central port to trigger one of two tones. Depending on the triggered tone's frequency, the mouse either pokes left or right to receive a water reward. Preliminary results are demonstrating that female mice can perform this discriminatory task successfully.

Once we have validated the task as a reliable method for determining whether mice can discriminate between tones, further work may take a variety of paths. Our lab's main focus will be to verify that auditory discrimination undergoes a critical period in mice. Previous work in the Hensch lab has developed mechanisms by which the critical period's timing may be modified. Specifically, we have identified a set of inhibitory neurons whose development and release of the inhibitory neurotransmitter, GABA, triggers the critical period. Administering benzodizpines enhances the efficacy of GABA and has been found to precipitate the critical period. Consequently, we hope to use our auditory discrimination task to examine the effects of Valium on hearing and communicative development in mice, with an eye toward informing future human studies.

Sarah Brittman brittman@fas.harvard.edu
Quincy 2008
Chemistry

Electrical Properties of Antimony Telluride Nanowires

The Converse Laboratory, Department of Chemistry and Chemical Biology, Harvard University

Semiconductor chalcogenide glasses, typically alloys of germanium, antimony, and telluride, exhibit a reversible phase change between crystalline and amorphous states. Amorphization occurs when the crystalline phase is melted and then quenched to prevent the atoms from organizing into a regular lattice.

Chalcogenides also exhibit threshold switching, which reduces the energy required to induce recrystallization. Basically, applying an electric field above a particular strength to the amorphous state creates a current channel, and the power from this current heats the surrounding material. When the material reaches the glass temperature, the atoms can reorganize into their crystalline lattice.

Because the amorphous and crystalline states have very different diffraction indices and resistivities, one application for phase change materials is binary data storage. DVD technology already makes use of the optical properties of chalcogenide thin films, and companies such as Samsung, Intel, and IBM are developing and testing electronic phase change memory.

These applications fuel interest in the electronic properties of chalcogenides and the mechanisms by which the phase change occurs. Research on chalcogenide thin films has been active for decades, but recently chalcogenide nanowires have been synthesized and electrically characterized. Such work aims to investigate how reducing these materials to the nanoscale in two dimensions affects their properties. The research group of Professor Hongkun Park at Harvard has already synthesized and characterized the electrical properties of germanium telluride nanowires. Current work now focuses on antimony telluride nanowires. The threshold switching behavior of the wires has been confirmed, and nanowire devices switch between states differing in resistance by up to three orders of magnitude. Factors affecting the threshold electric field are also under investigation.

Nevin Britto nbritto@fas.harvard.edu
Mather 2010
Engineering Sciences

A Sensitive Assay to Measure Pancreatic β-cell Growth

The Kulkarni Laboratory, Joslin Diabetes Center

β-cells are insulin-producing cells in the pancreas that are extremely important for the regulation of blood sugar in mammals. The main goal of my project is to design and optimize a functional proliferation assay for the measurement of the replication of beta cells. In diabetes, the autoimmune destruction of β-cells or the ineffectiveness of existing insulin leads to poor regulation of blood sugar levels. My research has focused on using the "hanging drop" technique to measure the growth of β-cells.

The main advantage of the hanging drop technique is that it simulates an in vivo environment since the β-cells are in contact with each other and in suspension in media rather than being influenced by artificial plastic material when adherent to a flask or petri dish. The hanging drop technique also minimizes the use of materials, since I will be suspending approximately 60,000 beta cells in a 30 μl drop and subjecting them to small amounts of stimulants such as Fetal Bovine Serum (FBS), Glucose, Insulin, or Glucagon-like-peptide-1 (GLP-1), which are all known to enhance replication of β-cells. In preliminary experiments, I mainly focused on stimulating β-cells with FBS to test and optimize the assay and to confirm alterations in proliferation by immunostaining for a replication marker. Future experiments will focus on treating the cells with other stimuli such as glucose, insulin, or GLP-1 and fine-tuning the technique for immunostaining and counting the number of cells. I will co-immunostain the cells with a beta cell marker (e.g. insulin) and a proliferation marker (e.g. bromodeoxyuridine or phosphohistone H3) to confirm the number of proliferating cells under different stimulatory conditions. The long-term goal of this project is to use this assay to easily measure the proliferation of β-cells in response to stimulation with serum from mouse models of diabetes (e,g, Liver-specific Insulin Receptor Knockout Mice (LIRKO) or from diabetic human patients. This technique will also be useful for the detection of potentially novel growth factors that enhance beta cell proliferation.

Can Cenik cancenik@fas.harvard.edu
Pforzheimer 2008
Applied Mathematics

Genome-wide analysis of 5'UTR introns

The Roth Laboratory, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School

Introns are parts of the pre-mRNAs that are removed from the transcript before translation. The evolution and function of introns are one of the fundamental problems in genome biology. Even though most studies focus on the introns that are in the translated regions, nearly 40% of all human genes contain introns in their 5'UTRs.

We investigate the splicing patterns and related functions of 5'UTR introns in humans using computational tools. A high confidence set of 12,087 5'UTR introns has been identified in the RefSeq database. Affymetrix human whole genome tiling array and human exon-junction array data is used to infer expression of all genes and an intron inclusion measure of all 5'UTR introns for each eight human tissues. A thousand introns are identified as candidates for complete/partial intron retention type alternative splicing. A self-organizing map and complete-linkage hierarchical clustering of intron inclusion measures are used to separate tissue specific expression profiles. We will find motifs in these co-clustered introns to identify the determinants of tissue-specific splicing of 5'UTR introns. Future experimental and computational studies will explore the connection between the tissue dependent splicing patterns of these 5'UTR introns and the overall expression of their genes, and also search for pre-splicing effects of 5'UTR introns on transcription by searching within the genomic sequence of 5'UTRs for DNA regulatory motifs correlated with tissue-dependent gene expression patterns.

Christopher Chen chen@fas.harvard.edu
Currier 2010
Chemistry

Characterization of the Roles of *E. coli* N-acetylmuramic L-alanine Amidases

The Kahne Laboratory, Department of Chemistry and Chemical Biology, Naito Laboratory, Harvard Faculty of Arts and Sciences

Beta-lactam antibiotics kill bacteria by binding to penicillin binding proteins (PBPs) and preventing proper peptidoglycan synthesis, resulting in a weakened cell wall that causes the cell to lyse and die. With bacterial resistance to beta-lactam antibiotics becoming increasingly common, scientists have begun studying bacterial proteins other than PBPs for potential as novel drug targets. N-acetylmuramoyl-L-alanine amidases AmiA, AmiB and AmiC are hydrolytic enzymes that aid in bacterial cell division, splitting the septum so that daughter cells can separate. Their biochemical role is to cleave the peptide side chains from the glycan backbone of peptidogylcan. While AmiA, AmiB and AmiC are not essential for cell survival, without these enzymes bacteria are highly sensitive to lysis and cannot survive in most environmental conditions, making these amidases potentially highly attractive drug targets. Little is known about the individual amidases' physiological roles or enzymatic properties. The Kahne Group is working on developing a biochemical assay to elucidate each amidase's substrate preferences, kinetic parameters and domain characterization in an effort to understand their enzymatic behavior and evaluate their usefulness as drug targets.

Serene Chen sichen@fas.harvard.edu
Kirkland 2008
Biochemical Sciences

Visualizing the development of the synaptic basket structure in the mouse submandibular ganglion

The Lichtman Laboratory, Sherman-Fairchild Building

The mature neural circuitry of higher vertebrates is the result of many synaptic rearrangements that take place early on during development. This heavy dependence on environmental input is hypothesized to allow neural circuitry to reflect experiences in the post-natal world—removing unnecessary connections while retaining others that are functionally relevant. While we know that synaptic pruning occurs, the underlying mechanism remains largely unknown. Developmental synaptic rearrangements have been traditionally studied in the neuromuscular junction. It is, however, also important to study neuron-neuron synapses since postsynaptic nerves and muscles likely differ significantly. We examine synapses in the submandibular ganglion (SMG), an autonomic ganglion whose neuron-neuron synapses are accessible and can be labeled for imaging. The post-synaptic neurons lack extensive dendritic arborization, having instead fine rudimentary processes that extend less than one micron from the soma. Presynaptic axon branches form basket-like structures to surround and innervate postsynaptic processes. Despite extensive physiological studies, little is known about the anatomical changes that occur during the elimination of SMG synapses, because of the inability to observe the ultra-structures over the entirety of the post-synaptic neuron. We overcome this impediment by using Serial Transmission Electron Microscopy on neurons from an adult and a neonate mouse SMG. The combined technique of electron and fluorescent microscopy allows the identification of cells in the neonates that receive multiple inputs—a hallmark of synapses during development. Using Blendmont and Reconstruct software to align and rebuild the sections, we are able to reconstruct the entire ganglionic neuron and the innervating axons surrounding the cell and the synapses on the cell. The reconstruction is anticipated to reveal aspects such as spatial distribution of synapses, the morphology and branch patterns of the axons, the arrangements of the filapodial dendrites and the association of the glial cells with the synapses.

Hannah Chung hchung@fas.harvard.edu
Quincy 2009
Chemical and Physical Biology

Distribution of Fitness Effects of Deleterious Mutations

The Murray Laboratory, Department of Molecular and Cellular Biology, Harvard University

Selection drives fitness upwards, while deleterious mutations drive fitness downward. Theories about how what is known as a selection-mutation equilibrium is attained as well as the evolution of phenomena such as senescence, genetic recombination, and sexual reproduction typically call for a better idea of the rate at which spontaneous mutations arise and the magnitude of their fitness effects. For this reason we are interested in finding out the distribution of fitness effects of deleterious mutations in *S. cerevisiae*. In order to do so we mutagenize diploids using UV and the chemical mutagen MMS and conduct large scale tetrad dissections and fitness assays. The measure of fitness in which we are interested is the selection coefficient, *s*, given by the slope of the line of ln(mutated yeast : ancestral yeast) vs. number of generations. If mutated yeast has some fitness advantage, then *s* is positive; if it has some fitness defect, *s* is negative. Determining this distribution of fitness effects can shed light into how equilibrium fitness is reached, as well as lend support to theories which rely upon spontaneous mutation.

Victoria Clark vclark@fas.harvard.edu
Quincy 2008
Biochemical Sciences

Alternative Splicing in Retinal Development

The Cepko Laboratory, New Research Building, Harvard Medical School

The role of alternative splicing in retinal development will be studied by focusing on the activity of alternative splicing factor *Sfrs10* in the developing chick retina. The findings from my Biochemical Sciences 91r project indicate that *cSfrs10* is strongly expressed in retinal progenitor cells, and that this expression is temporal and spatial. In addition, the finding that *cSfrs10* expression is itself regulated by alternative splicing indicates that its levels may have significant biological impact in the developing retina. Therefore, altering the levels of cSfrs10 may have a profound effect on retinal differentiation and cell fate determination. To test the effect of knocking down expression of cSfrs10, a replication competent avian sarcoma (RCAS) virus cSfrs10 RNAi construct will be injected into the optic cup of early chick embryos. This will allow the RNAi construct to spread to many retinal progenitors. To test the effect of misexpressing cSfrs10, an RCAS construct with the cSfrs10 gene downstream of a constitutive promoter will be injected into the optic cup of early chick embryos. If the perturbation of Sfrs10 levels produces a phenotype, the targets of cSfrs10 will be studied to determine the mechanism by which cSfrs10 affects retinal development.

Robert Corty rcorty@fas.harvard.edu
Mather 2010
Chemical and Physical Biology

Structural Determination of HIVGP41 and SIV GP41 including Transmembrane region and Fusion peptide

The Harrison Laboratory, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School

HIV is the virus that causes AIDS in humans. Simian Immunodeficiency Virus (SIV) is a closely-related strain of virus that causes Simian-AIDS in some non-human primates. Both are membrane-bounded viruses that enter host cells through the following process: 1) receptor-mediated attachment to a host cell 2) insertion of a fusion peptide into the host cell membrane, and 3) "zipping up" of the protein which contains the fusion peptide over the course of a few minutes, which pulls the viral membrane into contact with the host-cell membrane. This fusion protein is known as glycoprotein41 (GP41), and has been the target of much pharmacological study, including the development of Fuzeon, a peptide that binds GP41 to inhibit the "zipping up" process.

Needless to say, a detailed structural understanding is required for optimal drug development. Previous crystal structures have omitted the transmembrane region and the fusion peptide region of GP41, but recent research suggests that the final closure of the "zipping up" process relies heavily on the interaction of the transmembrane region and the fusion peptide. We seek to better understand the structure of these parts of GP41 by cloning, expressing, purifying, and folding the protein, including the transmembrane region and fusion peptide, then using X-ray crystallography to determine the structure. This may be difficult because the transmembrane region and fusion peptide are highly insoluble -- we plan to use a variety of detergents to attempt to solubilize the protein. The procedure will be followed on both HIV GP41 and SIV GP41. Our strategy is to express the protein in E. coli in two different ways: 1) With a His tag for purification, and 2) with a Maltose-Binding-Protein extension to aid solubilization and

purification. Expressed in E. coli, the protein will then have to be folded. We also intend to express the protein in insect cells, which should fold and secrete it autonomously.

The HIV virus is the cause of the global AIDS epidemic currently devastating sub-Saharan Africa. Complete, detailed understanding of GP41, so crucial to the HIV life cycle, is one step in developing fusion-inhibiting drugs.

Jennifer DeCoste jdecoste@fas.harvard.edu
Leverett 2009
Biology

Effects of Low Birth Weight on Brown Adipose Tissue Gene Expression

The Patti Laboratory, Joslin Diabetes Center

Low birth weight (LBW), especially when followed by rapid postnatal weight gain, is a risk factor for obesity and type 2 diabetes. Brown adipose tissue (BAT), which is present in substantial amounts in infants, functions to dissipate energy as heat (a process called thermogenesis) and contributes to whole-body energy expenditure. Therefore, functional changes in the BAT of LBW individuals might contribute to development of excess adiposity, obesity, and type 2 diabetes. In this experiment, a murine model of LBW was used to investigate differences in gene expression in the BAT of 24 hour-old LBW mice compared to controls. Mothers were subjected to 50% food restriction during days 12.5 to 18.5 of pregnancy, resulting in an 18% reduction in birth weight. BAT was collected from the offspring at age 24 hours, total RNA extracted, and cDNA produced using reverse transcriptase PCR. The cDNA was analyzed using quantitative PCR to measure the mRNA expression of a number of genes known to be important for BAT function.

Expression of two genes that act in a pathway to induce mitochondrial biogenesis, PGC-1a and ERRa, was decreased in LBW mice. Expression of deiodinase 2, which activates thyroid hormone intracellularly, was also decreased in LBW mice. Since intracellular activation of thyroid hormone in BAT increases thermogenic activity, the expected net effect of reduced deiodinase would be decreased thermogenesis in LBW mice. Consistent with these findings, the b-adrenergic receptor, which responds to epinephrine and norepinephrine by increasing energy expenditure in the BAT, was also decreased in the LBW mice. Finally, C/EBPa, a key regulator of white adipose tissue adipogenesis, was decreased in the LBW mice. Together, these changes would be expected to decrease energy expenditure in neonatal LBW mice, which could contribute to accelerated weight gain during early postnatal life and the obesity phenotype observed later in life in LBW mice.

Ellen De Obaldia mdeobald@fas.harvard.edu
Adams 2008
Biology

Interactions between vascular cells during *in-vivo* vasculogenesis

The Bischoff Laboratory, Childrens Hospital, Harvard Medical School

Vascularization of thick implants remains a barrier to the functionality of tissue engineered (TE) constructs. Vascularization is critical for tissues with volume greater than 2-3 mm^3, since nutrient provision, gas exchange, and waste elimination all depend on diffusion distance. Currently, there are no tissue engineered (TE) constructs with an inherent microvascular bed which may be connected to the

host vasculature. The Bischoff laboratory has shown that when human cord blood-derived endothelial progenitor cells (EPCs) and human bone marrow-derived mesenchymal progenitor cells (MPCs) are combined in Matrigel and subcutaneously implanted into immunodeficient mice, they form a vascular network with functional anastomoses to the host vasculature. This model takes advantage of the intrinsic vasculogenic potential of endothelial progenitor cells, which may be obtained non-invasively.

The purpose of my summer research is to shed light on the cellular assembly process by which blood vessels form *in vivo*. Matrigel implants will be extracted over a time course within seven days following injection. Extracted Matrigel plugs will be serially sectioned, stained using immunofluorescence, and evaluated by confocal microscopy. MPCs used were retrovirally transfected to confer expression of green fluorescent protein (GFP), so that they could be identified. EPCs were identified by using a human-specific antibody against the endothelial marker CD31. By tracking each cell type over this time course, we hope to explain the cellular assembly processes that occur in vasculogenesis. Thus far, we have confirmed the presence of human-specific CD31+ lumens and the perivascular contribution of the MPCs. Ongoing experiments will further investigate underlying processes of vasculogenesis, such as hypoxia response, proliferation, migration, and growth factor expression.

Kimberly DeRose kderose@fas.harvard.edu
Eliot 2009
Astronomy

Star Formation in Embedded Clusters RCW38 and IRAS20050+2720

The Wolk Laboratory, Harvard-Smithsonian Center for Astrophysics

This project involves star formation in two embedded star clusters: RCW38 and IRAS20050+2720. Adaptive optics data taken of RCW38 using the Very Large Telescope in Chile gives us the highest resolution data studied of the region yet. RCW38 is an optically-obscured cluster at a distance of 1700 parsecs with a massive binary system IRS2 at its center whose intense stellar winds have exposed the center portion of the cluster and produced shocked emission. I focused on Class II sources, young stars that still have large disks of dust and gas that might someday form planets. These "protoplanetary" disks cause a reddening effect in the K-band measurements (centered at 2.18 microns). By identifying foreground sources and statistically determining the effective background contamination, I calculated the fraction of stars in the cluster that still have disks (36.5%), telling us about the evolutionary state of the cluster and how star formation takes place in the presence of massive stellar winds. Although photoevaporation of disks has been observed in other clusters when exposed to the far-ultraviolet radiation, there is no significant trend implying that the disk fraction drops as distance to IRS2 decreases.

For IRAS 20050+2720, the Spitzer Space Telescope and Chandra X-ray Observatory were used to take data to assist in identifying the evolutionary state of cluster members. Near and mid-infrared observations helped determine Class I (protostars) and Class II sources while x-rays were used to find Class III objects. Class IIIs are photospheres whose spectral energy distributions mirror that of a normal star, but whose high energy emission indicates youth and thus cluster membership. By studying the distribution of these sources as well as their quantities, we hope to be able to better understand the lifetime of disks in this cluster.

John Edwards jbedward@fas.harvard.edu
Kirland 2008
Neurobiology

A Novel Approach to Deriving GFP-Positive Embryonic Stem Cell Lines

The Maas Laboratory, Division of Genetics, Brigham and Women's Hospital, Harvard Medical School

PURPOSE: Previous studies have shown that green fluorescent protein (GFP)-positive embryonic stem (ES) cell lines can be established from existing GFP-negative ES cell lines by gene transfection or viral infection and subsequent clonal selection or fluorescence activated cell sorting (FACS). In this study, I aim to derive GFP-labeled murine ES cell lines by selecting stably transfected cells from blastocyst stage embryos. METHODS: After removing the zona pellucida of E3.5 blastocyst-stage embryos with Tyrode's acid solution, zona-free blastocysts will be co-cultured with mitotically inactivated mouse embryonic fibroblasts and allowed to attach. At 24, 48, or 72 hours *in vitro*, cells will be transfected with a GFP vector using electroporation, lentiviral infection, or the chemical agent Fugene. Cells will then be cultured into ES cell lines and assayed for (a) efficiency of GFP-positive ES cell line derivation and (b) stability of reporter gene transduction. RESULTS: Our work has shown that previously established ES cell lines tolerate transfection with the GFP vector. While expressing GFP, the cells retain several known stem cell markers including Oct-4. We are currently deriving GFP-labeled murine ES cell lines using the novel approach described in this abstract. IMPLICATIONS: Current methods of deriving ES cell lines require the destruction of the developing embryo at the 100- to 200-cell blastocyst stage. Our research focuses on novel approaches to deriving ES cell lines that preserve the viability and developmental potential of the embryo. By labeling cells before the derivation of an ES cell line, we will be able to determine more conclusively the efficiency and effectiveness of these novel approaches.

Penny Fang pfang@fas.harvard.edu
Eliot 2008
Biology

Using Stem Cells to Model Alzheimer's Disease: Deriving Amyloid Precursor Protein and Presenilin Mutant Neurons

The Eggan Laboratory, Department of Molecular and Cellular Biology, Harvard University

By the time patients with Alzheimer disease are gripped with inabilities to speak, recall personal memories, and recognize loved ones, or even by the time the first signs of memory loss appear, it is far too late to study what went wrong in their brains. Even if it were possible to obtain a small slice of brain from these patients, one would only see the end result of catastrophe: a neuronal mass spotted with amyloid beta plaques and overrun by a thicket of fibrous tangles consisting of hyperphosphorylated tau. In the attempt to circumvent these limitations *in vivo*, mouse and human embryonic stem cell lines with dominant amyloid precursor protein and presenilin mutations were developed to model Alzheimer's *in vitro.* Mouse cell lines were derived from hemizygous transgenic mice, and human cell lines from existing cell lines lentivirally transfected with a construct upregulating amyloid beta 42 expression, the shortened form of amyloid beta found to accumulate in plaques. Cells were then differentiated towards neurons and optimized for survival. Future steps include biasing neuron differentiation towards cholinergic, cortical neurons, those primarily affected in Alzheimer's, and performing a chemical screen to alter disease progression.

Kyle Foreman kforeman@fas.harvard.edu
Leverett 2008
Psychology

Primate Reciprocity: Sense of Fairness and Action-Omission Distinctions

The Hauser Laboratory, Cognitive Evolution Lab, William James Hall

Altruism and cooperation are hallmarks of human interaction, necessary components for the establishment and maintenance of a society such as our own. Yet – perhaps surprisingly – truly selfless behavior is seldom seen in our animal forebears, even our closest primate relatives. This study aims to elucidate what factors animals take into account when deciding whether or not to cooperate with others, giving us insight into how our own moral judgments are formed.

One of the few concrete examples of altruism and cooperation in animals demonstrates that Cotton-Top Tamarins (*Sanguinus oedipus*) will expend effort to give food to those who have done the same for them previously, an exchange termed reciprocal altruism (Hauser et al, 2003). The present study uses the same methodology to examine whether Cotton-Top Tamarins distinguish between active and passive helping when deciding whether or not to reciprocate. This decision closely mirrors the action-omission distinction, a major component of human moral judgment (Cushman et al, 2006).

If Cotton-Top Tamarins also consider actions and omissions – regardless of outcome – to be of different moral value, then they will cooperate more frequently with other monkeys who actively helped them than with those who did so passively. This will offer interesting insight into how we make our own moral judgments and how the rules for coming to such decisions evolved.

Shiv Gaglani sgaglani@fas.harvard.edu
Mather 2010
Engineering Sciences

Development of TaqMan Assays for Known Drug Resistance Mutations in *Plasmodium falciparum*.

The Wirth Laboratory, Department of Infectious Disease, Harvard School of Public Health

Purpose: Malaria afflicts over 300 million people and results in more than one million deaths every year. Despite immense efforts to develop effective drugs against the malaria parasite, it continues to evolve drug resistance and spread. There are many well-characterized single nucleotide polymorphisms (SNPs) within key genes that are predictive of increasing drug resistance. The purpose of this study was to develop TaqMan assays to detect these SNPs in the most virulent malaria parasite species, *Plasmodium falciparum*. The eventual goal is to study the population genetics of *P. falciparum* and correlate the outputted genotype with the drug resistance phenotype for clinical purposes. The assay may also provide a simple surveillance tool for use in the field.
Methods: Filtered patient blood samples (n = 100) in the form of blood spots on filter paper were obtained from five regions in Senegal (Pikine, Passy, Velingara, Richard Toll, and Thies) in 2005. *P. falciparum* DNA was isolated from these blood spots and quantified using a malaria-specific, real-time polymerase chain reaction (RT-PCR). Each DNA sample was barcoded (another RT-PCR method) at 21 unique SNPs to ensure that it was from a single malaria infection as opposed to a mixed infection. TaqMan assays were ordered from Applied Biosystems to detect 18 drug resistance-related SNPs in six genes: Dhfr (N51I, C59R, S108N, S108T), Dhps (S436A, A437G, K540E, A581G, A613S, A613T), PfATP6

(S769N), Pfcrt (K76Tie, K76Tmn), Pfmdr1 (N86Y, Y184F, S1034C, N1042D), and mitochondrial cytB (Y268N). Each assay consisted of a forward and reverse primer to amplify the region of interest as well as a wildtype TaqMan probe (VIC-labeled) and mutant probe (6FAM-labeled) to discern which SNP was present. The assays were run against sequenced strains with known SNP profiles for validation purposes.
Results: Twelve of the 18 TaqMan assays worked successfully for both the wildtype and mutant alleles. The observed results were very similar to the expected results, suggesting that the genetic technique is robust and specific.
Conclusions: We are currently awaiting TaqMan assay data from the unknown samples. At this point, the assays must be optimized. If successful, this genetic tool could be used extensively to not only diagnose patients with resistant strains of *P. falciparum*, but to characterize the parasite population in that region and recommend changes in drug use.

Jason Gao jasongao@fas.harvard.edu
Leverett 2010
Engineering Sciences

Contact Sensing with Piezofilm

The Howe Laboratory, School of Engineering and Applied Science

The Harvard BioRobotics Lab has developed a compliant robotic hand that allows grasping of objects even within large positioning errors, but improved grasping reliability still requires that the hand be able to detect how it has made contact with its target, so that the hand can be repositioned accordingly in real-time.

Through the use of Shape Deposition Manufacturing (SDM), films containing piezoelectric material were embedded into the soft inner pads of finger linkages to allow dynamic measurements of pressure on a finger linkage. The piezoelectric material generates a voltage when stress and/or pressure is applied, which has been measured and recorded using custom computer software for further analysis and study.

Preliminary results have thus far indicated that detecting when objects are sliding along the finger is difficult; voltages generated by objects sliding along the piezofilm show no remarkable characteristics in a graph of the piezofilm response until the object has been dropped. However, distinct transients are generated in normal and non-normal object contact with the finger surface, allowing fairly reliable identification of initial object contact. With the hand's current architecture of two piezofilm sensors per finger, and a total of four fingers, this provides eight distinct zones of contact that can be uniquely identified and located in order to assess how the hand has contacted the object. The data also indicates that it may be possible to distinguish different areas of contact even within the same piezofilm sensor, by utilizing characteristics of how the piezofilm deforms under surface-lateral and surface-normal contact. Further research is being conducted to study how to utilize these findings in control of the hand.

Erika Geihe egeihe@fas.harvard.edu
Currier 2008
Chemistry

Progress Towards the Synthesis of Novel Proteasome Inhibitors Utilizing an Unusual Intramolecular Allyl Transfer Reaction

The Jacobsen Laboratory, Department of Chemistry, Harvard University

The natural product lactacystin and analogs are important target molecules for total synthesis due to their interesting structures and proteasome inhibition activity. The proteasome, a multi-subunit protein complex, is involved in a variety of cellular processes including oncogenesis, apoptosis, and the immune response. It contains three proteolytic active sites, one of which is preferentially targeted by lactacystin.

We set out to design analogs of lactacystin which would target the other two active sites of the proteasome. We envisioned that we could access these analogs through an intermediate bearing an allyl sidechain. This functionality was installed via an intramolecular allylation reaction. Elaboration of the product to correlate with a known lactacystin analog enabled us to determine the diastereoselectivity of this reaction. The major product of this transformation was the undesired diastereomer. Current work is aimed at developing a method to invert this stereocenter, providing a key intermediate en route to novel analogs.

Brandon Geller bgeller@fas.harvard.edu
Pforzheimer 2008
Biochemical Sciences

Molecular Phylogenics and Evolution of Pheromone Production in the Beetle Family Cerambycidae

The Farrell Laboratory, Museum of Comparative Zoology

Longhorn beetles (family Cerambycidae) occur in nearly every non-marine habitat, exhibit incredible ecological and taxonomic diversity, and are pests of many trees and shrubs. Despite their ecological and economic importance, very little remains known of the relationships among major taxonomic groups of cerambycids, and quite surprisingly, a molecular phylogeny has never been generated for the family. It is my goal to produce a tribal-level phylogeny for the family Cerambycidae using DNA sequence data. In addition to clarifying higher-level relationships across the family Cerambycidae, these data should help reconcile the placement of several intriguing taxa (e.g., *Oxypeltus, Philus*, and *Vesperus*) whose taxonomic placement based on morphological data has remained ambiguous. Once I've used DNA sequences to reconstruct a phylogeny for the Cerambycidae, I propose to use data I've summarized from the literature to reconstruct the evolutionary history of pheromone usage in the family, and to predict the method of pheromone production of unstudied species.

Lauren Gibilisco gibilisc@fas.harvard.edu
Adams 2008
Biology

The Biomechanics of Pectoral Fins of Bluegill Sunfish During Maneuvers

The Lauder Laboratory, Museum of Comparative Zoology

Biological systems used for propulsion differ from manmade devices by using flexible flapping surfaces in contrast to rigid rotating devices such as propellers. Examples of biological propulsors include bird and insect wings, dolphin flukes, and fish fins. Studies of animal movement are important for the design of manmade propulsion devices, because learning from biological systems may provide new insights into how to design more efficient and effective propulsive surfaces. So far, most animal movement research has focused on steady locomotion, even though maneuvering is an important part of the natural locomotor repertoire in animals. A detailed understanding of pectoral fin biomechanics during maneuvers in fishes will have implications in the efficient design of underwater vehicles and robotic fins.

Maneuvers powered by the pectoral fins of bluegill sunfish (*Lepomis macrochirus*) were studied using high-speed, high-resolution cameras. Three cameras recorded maneuvers from three different views simultaneously. From the movies, three-dimensional points on the pectoral fins were calculated and the fin deformation was reconstructed graphically through the duration of the maneuver. From this data, the deformation of the pectoral fins, including change in area, shape, and fin and body angles was available.

It was found that, unlike maneuvers of terrestrial animals such as cockroaches and fruit flies, which require only subtle changes in limb and wing motion to accomplish even significant maneuvers, the pectoral fins of bluegill sunfish deform significantly during turns of even low angular velocities. Furthermore, the movements of the two fins during the maneuvers differ, and these differential deformations between the right and left side fins power the turn. These findings will not only have implications for the efficient design of manmade robotic devices, but also provide greater insight into comparative biomechanics and the hydrodynamics of fish propulsion.

Chelsea Gordon cggordon@fas.harvard.edu
Leverett 2009
Chemistry

Synthesis of Novel Tetracycline Analogs

The Myers Laboratory, Department of Chemistry, Naito Building, Harvard University

Due to the widespread use of tetracycline antibiotics in the treatment of a broad range of bacterial infections, pathogens have developed significant resistance to this series of compounds. As a result, the synthesis of novel tetracycline analogs able to circumvent the microbial resistance pathways is of particular interest. The basic structure of the tetracycline consists of four linearly fused 6-carbon rings A through D. The Myers synthetic route to tetracycline compounds allows for structural variation in the D-ring, thus providing an efficient method of analog production. Here, we utilize the Myers route to produce a common enone intermediate. Coupling of this enone with a variety of D-ring precursors leads to the desired tetracycline analogs. Upon synthesis, these analogs are tested for antimicrobial potency against an array of Gram-positive and Gram-negative pathogens to determine their potential as future antibiotics.

Rishi Gupta rgupta@fas.harvard.edu
Eliot 2009
Mathematics

Lego Robot Swarm

The Nagpal Laboratory, The Self-Organizing Systems Research Group, Maxwell Dworkin, Harvard University

The final product of my summer efforts will be a prototype research tool for testing and demonstrating swarm based algorithms. Swarms are systems of mass-produced, simple and unreliable entities (eg. ants) that can together build big and complicated things (resp. anthills)
without leaders or even global knowledge of intermediate progress. My project is to build a generic, easily replicable "ant" which can be loaded with a variety of sensors and put in controlled environments to demonstrate solutions to anthill-like problems.

The base system is currently built with the Lego Mindstorms kit, which provides easy integration of the microprocessor ("brain"), several sensors, motors, and high-level programming environments. By the end of the summer, I will have 2-3 robots that accomplish simple search
and rescue (eg. together find the five pieces of colored paper in the playpen), and have the ability to follow the border of an existing anthill. The next step is to integrate an RFID (think Charlie Card) reader/writer into the robots, allowing them to leave traces, or "pheromones", on the environment. The project was started in 2006, and will ultimately scale to about 15 robots.

Jackie Havens jhavens@fas.harvard.edu
Leverett 2009
Music

Interactions between Human Cytomegalovirus Processivity Factor UL44 and DNA

The Coen Laboratory, Seeley G. Mudd, Harvard Medical School

Processivity factors bind to the catalytic subunit of DNA Polymerase to aid in long strand DNA synthesis. UL44 is a proposed processivity factor in Human Cytomegalovirus DNA Polymerase. The crystal structure of UL44 shows that it is a C clamp-shaped dimer. Molecular dynamics modeling and biochemical data suggest that UL44 wraps around and interacts with DNA via basic residues in the cavity of the C-clamp and in flexible loops. To further test this interaction, four basic residues in the cavity of the C clamp have been changed to alanines using a QuikChange mutagenesis kit. Mutant proteins were expressed in E. coli as GST fusion proteins, purified on Glutathione Sepharose resin, and cleaved with PreScission protease to remove the GST tag. They were further purified on Heparin and Superdex 200 columns. They were then tested for their binding to DNA using filter binding assays. Cocrystallization of DNA and UL44 is also being attempted by crosslinking a Cysteine residue in the protein with thiol-modified DNA by disulfide bridges.

Gongqi (Gina) He ghe@fas.harvard.edu
Leverett 2010
Biology

The Localization of ClpCP in *Bacillus subtilis*

The Losick Laboratory, Department of Molecular and Cellular Biology, Harvard University

Protein degradation is a major mechanism of cell regulation. *Bacillus subtilis* is a gram-positive bacterium that provides a good model to study proteolysis. Upon starvation, these spore-forming bacteria differentiate into two asymmetrical progenies called the mother cell and the forespore. The main goal is to understand how a bacterium can give rise to dissimilar progeny and the developmental gene expression that governs morphogenesis. The activation of the transcription factor σ^F is essential for sporulation in *subtilis*. While σ^F is produced everywhere in the pre-divisional sporangium, it is activated solely in the forespore by the degradation of SpoIIAB, which is an anti-σ factor that holds σ^F inactive. The process of proteolysis of SpoIIAB by ClpCP protease is important in the understanding of a pathway that could contribute to the cell-specific transcription activation. SpoIIAB is degraded in a ClpCP dependent manner and is determined by its C-terminal amino acids, LCN, which constitute a necessary and sufficient degradation signal. In addition, mutant sporangia lacked a polar septum when they have mutant ClpCP and thus were impaired in asymmetric division. Since the activation of σ^F is dependent upon septum formation, thus ClpCP mutation could inhibit σ^F directed gene expression by directly blocking the degradation of SpoIIAB and indirectly by interfering with polar division. Preliminary work has shown that the protease preferentially localizes in the forespore. This polarization could cause the preferential proteolysis of SpoIIAB in the forespore to lead to compartment-specific activation of σ^F. In order to see if the protease is important for this compartment-specific SpoIIAB degradation, localizations of ClpC and ClpP are studied by making random mutations of the protease to see the effects on the phenotype of the cell. These results will reveal much more about the critical role of proteolysis and hence the process of development.

Jackie Hsieh jhsieh@fas.harvard.edu
Cabot 2010
Neurobiology

Role of miRNA in SIRT1 regulation

The Greenberg Laboratory, Children's Hospital Enders Research Laboratories, Harvard Medical School

The nervous system is influenced dramatically by input from the environment, and thus a study of the sensory stimuli is important for understanding neuronal development. Activity dependent genetic programs are known to have significant effect on neuronal function and neuronal circuitry. One such program is the hypothesized regulation of genes such as SIRT 1, a sequence that correlates with longevity. The gene has been found to be a highly conserved among humans, mice, and other organisms, an evolutionary significance that implies regulation to be just as important. The purpose of the proposed study is to understand post-transcriptional regulation mediated by microRNAs. Using prediction computer programs, specific sequences of miRNA are pinpointed as targeting the SIRT1 gene. Then, molecular cloning is performed to overexpress the miRNA and study its effects on the SIRT1 target gene. Mutated SIRT1 targets are also studied for comparison. Ultimately, if these microRNAs do indeed target the 3'untranslated region of the SIRT1 gene, then activity dependent activity within neurons can be explored using hippocampal cells of the mouse and KCl injections.

Even in the short amount of time since their discovery, miRNAs have been implicated in several human neurological disorders such as Fragile X syndrome, Tourette's syndrome, and various nervous system cancers. Thus, the necessity to study miRNA function is based not only on the important role that they will most likely provide in fundamental nervous system processes such as development and plasticity, but also because miRNAs may provide new insights into the mechanism and possible treatments of different human neurological diseases

Divya Jayaraman djayaraman@fas.harvard.edu
Kirkland 2008
Biochemical Sciences

The role of TopBP1 in the DNA damage response in *Xenopus laevis* oocyte extracts

The Michael Laboratory, Department of Molecular and Cellular Biology, Harvard University

Problems in DNA replication and repair can lead to genomic instability, which has been implicated in cancer. Thus, a fundamental understanding of DNA repair mechanisms can yield insight that might even translate into clinical applications for cancer patients some day.

We are working on a model that will explain how cells respond when replication stalls at a lesion in DNA. TopBP1, a BRCT-repeat-containing protein, is required to initiate DNA replication of undamaged chromosomes. I aim to show that TopBP1 also plays a role in the DNA damage response by facilitating reinitiation of replication of damaged chromosomes. We believe that it does so by recruiting DNA polymerase alpha to a site downstream of a stalled replication fork, thereby temporarily leaving a single-stranded gap in the DNA that is eventually filled in by translesion and processive DNA polymerases. This process prevents the stalled replication fork from collapsing and allows the genome to be replicated, possibly preventing the onset of cancer.

My initial task as a 2006 Herchel Smith Fellow was to detect the presence of these single-stranded gaps in damaged chromatin and prove that these gaps were forming in a replication-dependent manner. To this end, I successfully developed a novel cell-free assay using *Xenopus laevis* sperm chromatin in egg extracts as an experimental system. The key part of this assay involved obtaining a quantitative readout of the gaps in the chromatin by filling them in with radioactively labeled nucleotides. As a 2007 PRISE fellow working on my senior honors thesis, I optimized the assay and then determined the approximate length of these single-stranded gaps using alkaline agarose denaturing gels. I am now using this assay in conjunction with immunodepletion experiments to determine whether TopBP1 is required to regulate the length of these gaps.

Raymond Alexander Jean rjean@fas.harvard.edu
Kirkland, '08
Engineering Sciences

Effects of Substrate Topology in the Growth of Carbon Nanotubes

The Golovchenko Laboratory, Department of Physics, Harvard University

Carbon nanotubes, cylindrical fullerenes of carbon, nanometers in diameter, exhibit extremely good electrical and structural properties. In the development of electronic devices and structural components at the atomic and molecular level, a very controllable process is necessary for the deposition and alignment of carbon nanotubes on substrates. Through the manipulation of silicon substrates, specifically

through changes in surface topology to limit surface area contact, we expect to achieve the growth of longer carbon nanotubes, with some display of directional flow alignment.

Chemical vapor deposition (CVD) on grating samples yielded nanotubes on the order of 5 to 10 times longer than flat silicon samples of similar preparation, with nanotubes "jumping" from peak to peak over very long distances. It is believed that limited surface interaction, due to the small contact area on the top of the peaks, allowed nanotubes to grow longer than they normally would on flat substrates, where strong surface-nanotube interaction inhibited prolonged growth during CVD. These results indicate the increased ability to control and reliably predict the chemical vapor deposition of carbon nanotubes, a critical part to the development of nanotube-based molecular-scale devices.

Michelle Jung mjung@fas.harvard.edu
Cabot 2008
Biochemical Sciences

Understanding the role of LKB1 in murine skeletal muscle

The Goodyear Laboratory, Department of Metabolism, Joslin Diabetes Center

Diabetes is characterized by abnormally high blood glucose levels. The disease results from faulty insulin production, decreased insulin sensitivity, or a combination of the two, which impairs glucose uptake. There are several different types of diabetes: Type 1 diabetes is an autoimmune disease that affects the beta cells of the pancreas, the major production site for insulin. Type 2 diabetes, the most prevalent version of the disease, results from insulin resistance.

Exercise is important in the treatment of diabetes. Studies conclude that muscle contraction activates the molecule AMP-activated protein kinase (AMPK), which in turn promotes glucose uptake. AMPK also stimulates fatty acid oxidation in liver and skeletal muscle. *In vitro* cell culture and *in vivo* temporal knockout studies have demonstrated that LKB1 regulates AMPK.

Recently, muscle-specific LKB1 knockout (MLKB1 KO) mice were generated. These mice demonstrated impaired contraction-stimulated glucose uptake. However, they also had enhanced glucose metabolism and improved insulin sensitivity and glucose uptake compared to wild type controls. The reasons behind this phenotype have yet to be fully elucidated. The purpose of my research is to develop a better understanding of the genomic and proteomic basis of the MLKB1 KO phenotype using microarray technology.

Peter (Geon) Kim geonkim@fas.harvard.edu
Leverett 2010
Biology

The role of hedgehog signaling during hematopoietic differentiation from mouse embryonic stem cells.

The Daley Laboratory, Childrens Hospital, Harvard Medical School

We studied the role of Indian hedgehog (Ihh), a highly conserved protein that is critical for hematopoiesis, in the differentiation of murine embryonic stem cells (mESC). Because Ihh regulates hematopoiesis in a time- and dose-dependent manner, we analyze the Hh expression patterns in the parental cell line Ainv15 during embryoid body (EB) differentiation. To enhance the specification of mESC to blood lineages, we test the effect of induction of Hh pathway using recombinant Ihh and an inducible cell line. Conversely, we test the inhibition of the pathway using Hh inhibitor cyclopamine.

Minjae Kim mkim@seas.harvard.edu
Currier 2010
Engineering Sciences

Fabrication of a 6-legged Walking Microrobot

The Wood Laboratory, Harvard Microrobotics Laboratory, School of Engineering and Applied Science

Following the trend of miniaturization in engineering, many researches have been made in robotics to create functional millimeter-to-centimeter-scale walking robots. However, many of these robots, using either traditional materials or MEMs technology, were too simple or limited in maneuverability. In this research, a new method of fabricating a robot with 12 degrees of freedoms and 6 legs is explored, using Smart Composite Microstructure (SCM) techniques to provide rigid mechanical links and flexure-based joints. Actuation is provided by shape memory alloy (SMA) wires to emulate tendon-muscle like contraction that exists in actual biological systems. The design of the legs is taken from bugs and insects, especially from cockroaches. Legs are designed differently to perform different tasks in providing locomotion. Also, fabrication of effective leg spines—mechanisms that increases frictional resistance in one direction while yielding in the other direction—is also attempted to give the robot better obstacle climbing ability. Overall, the robot is design to be robust in a multi-terrain environment while relatively simple to fabricate.

Kipyegon Kitur kipyegon@fas.harvard.edu
Adams 2009
Chemistry

Andrimid Biosynthesis

The Walsh Laboratory, Harvard Medical School

The current bloom of multidrug-resistant bacterial pathogens threatens to drag our society back to the 19 th century. Therefore, there is a pressing need of synthesizing antibiotics with new and improved modes of action. Andrimid (*adm*) is a promising antibiotic that can replace the current antibiotics which bacterial pathogens have developed resistance to. Adrimid is an inhibitor of bacterial acetyl-CoA carboxylase, a necessary enzyme in the formation of prokaryotic fatty acids. Recently, it was reported that AdmF, a new transglutaminase-like protein, catalyzes the first amide bond formation in andrimid biosynthesis. We are evaluating the promiscuity of AdmF, in an effort to discern its selectivity. After making different variants of octratrienoyl-chain and installing them on carrier proteins, it was found out that AdmF can catalyze their bond formation with beta-Phe- *S*-AdmA without discrimination. This finding implies that we can make variants of andrimid with improved effectiveness which can combat the current threatening rise of antibiotic resistant bacteria.

Ivan Kotchetkov kotchetk@fas.harvard.edu
Currier 2008
Neurobiology

Developmental Anatomy of Corticostriatal Projection Neurons

The Macklis Laboratory, Harvard Stem Cell Institute, Department of Neurosurgery, Massachusetts General Hospital

Understanding how neurons develop normally is a critical step toward repairing or supporting those that degenerate or are damaged in disease. Corticostriatal projection neurons (CStrPN) are the cortical efferent neurons of the cortico-basal ganglia circuitry. Degeneration of CStrPN has been implicated in the pathophysiology of Huntington's disease (HD), cerebral palsy (CP), and Parkinson's disease (PD), among others. Thus, increased knowledge regarding the anatomic development of CStrPN may be critical, both in understanding the development and organization of this circuitry, and toward potential therapeutics.

We are investigating the anatomic development of CStrPN at distinct stages of mouse development by retrograde labeling from the striatum and other structures. Our current data suggest that there is a period of exuberance in projection pattern of the CStrPN, during early development, followed by pruning of axons, and maturation of their dendritic organization. Multiple approaches are being used to more fully investigate the cellular anatomy of CStrPN. The study of the stage-specific anatomy of CStrPN may enable identification of mechanisms that define, specify, and control differentiation and maturation of cortico-basal ganglia circuitry.

Rosen Kralev rkralev@fas.harvard.edu
Adams 2009
Mathematics

Optimality of scalar solutions and binary alphabet in low-intensity unicast networks

A network is defined to be an acyclic oriented graph with some nodes serving as senders of information and others as receivers; messages can be transmitted only along the direction of the edges, and each node sends messages which are functions of the ones that it received. A network is solvable if there exists a way to transmit all the messages to the corresponding receivers, given restrictions on the edges' capacity and types of functions allowed. The problem that the current project starts off from is whether the solvability of networks is decidable. Through looking at small cases, partial results are achieved on assessing where linear functions and a binary alphabet give the optimal information flow. The method of proving the main conjecture is to break down the general situation into multiple cases, thus providing an algorithm for determining the solvability of a certain class of unicast (one receiver per sender) networks.

Warakorn (Pete) Kulalert kulalert@fas.harvard.edu
Leverett 2010
Molecular and Cellular Biology

The Role of Inositol Phosphate in the mTOR Signaling Network

The Blenis Laboratory, Harvard Medical School

The Mammalian Target of Rapamycin (mTOR) has been identified as a crucial component of the signaling pathways that regulate various biochemical processes in the cell including protein synthesis, ribosome biogenesis, and cell cycle progression. Inappropriate regulation of mTOR leads to a variety of pathological

disorders such as cancer, obesity, and diabetes. Elucidating the mechanisms underlying the mTOR pathway is thus necessary for finding therapeutic targets and effective treatments of these diseases.

siRNA has recently emerged as a valuable genetic tool in mammalian cells, allowing for the specific inhibition of gene expression by targeting the corresponding mRNA for degradation. To develop a better understanding of the TOR network, the Blenis Lab has performed a screen of the human druggable genome using siRNA. This method allows us to investigate the role of each protein in the TOR signaling cascades. The data from the screen identified siRNAs targeting a group of inositol phosphate kinases as 'hits' that downregulate ribosomal protein S6 phosphorylation, a downstream target of mTOR. In order to validate the results of the screen, we performed Western Blot analysis to verify that the knockdown of these kinases by siRNA leads to the reduction of rpS6 phosphorylation. We transfected the cells with four groups of siRNAs—targeting either ITPKC, ITPK1, IHPK3, or IPMK—stimulated the cells, and generated lysates at appropriate time points to assess rpS6 phosphorylation levels. These lysates were subjected to Western Blot analysis, probing for phosphorylated S6 and other known protein players in the pathways. The data from this analysis provided further evidence that these inositol phosphate kinases regulate the mTOR pathway as the knockdown of these proteins resulted in downregluation of S6 phosphorylation and the inhibition of many components both upstream and downstream to TOR.

In order to fill inositol phosphate kinases in the meshwork of the mTOR system, we hypothesize a number of possible mechanisms through which these kinases may regulate mTOR. The possibilities include the regulation of calcium signaling, the dynamic turnover between membrane phosphoinositides and soluble inositol phosphate, and the effect of inositol phosphate on membrane recruitment of particular protein players in the mTOR pathway. Further experiments will be performed in order to test each hypothesis and develop a more complete understanding of inositol phosphates control of mTOR signal transduction.

Dana Lazarus dlazarus@fas.harvard.edu
Quincy 2009
Engineering Sciences

Isolating manganese oxidizing microorganisms (bacteria and fungi) on Cape Cod

The Hansel Laboratory, School of Engineering and Applied Science

Manganese oxides play an important role in the environment. For example, manganese oxides act as an electron acceptor in cell respiration for bacteria, offer protection for microbes from powerful UV rays, are a powerful oxidizing agent, and can absorb toxic metals and elements. Manganese oxidation is kinetically limited in most environments, and it has been demonstrated that the majority of manganese oxidation is facilitated by microbial activity. Currently, the reasons why bacteria oxidize manganese are not well-understood: although the oxidation of Mn(II) to Mn(III) and Mn(IV) is thermodynamically favorable, there is no evidence that bacteria actually derive energy from the reaction. Furthermore, it is currently accepted that manganese oxides are reduced in the presence of light and manganese oxidation is enhanced in the dark by bacteria. However, it has recently been discovered that certain organisms have increased rates of manganese oxidation in the light. My research project is focused on isolating light-enhanced manganese oxidizing bacteria and fungi from field sites on Cape Cod, with the eventual goal of understanding this newly identified metabolic pathway.

In this study, I collected water and sediment samples from two field sites on Cape Cod – Ashumet Pond and Waquoit Bay. These sites were chosen because of the high levels of manganese(II) in the groundwater and water column. Over the summer, I enriched for bacteria and fungi from these field sites and isolated those that oxidized manganese. Using these isolates, we will compare growth rates of microorganisms with and without the presence of light to identify those exhibiting light-enhanced oxidation. The confirmed light-enhanced manganese oxidizing bacteria and fungi will be used for further study.

Nathan Leiby nleiby@fas.harvard.edu
Pforzheimer 2010
Psychology

Linguistic Computational Capacities in Cotton-Top Tamarins

The Hauser Laboratory, Cognitive Evolution Lab, William James Hall

Though it is clear that animals have methods of communication, they differ from human language in four significant ways: (i) animals do not form novel combinations of multiple concepts in order to represent new meanings; (ii) animals use a limited repertoire of calls and signs; (iii) animals do not use abstract categories in communication, such as tense and part of speech; and (iv) animal communication responds only to functionally related context. (Hauser, Barner, O'Donnell, in press)

However, recent studies suggest that some computational capacities underlying language may not be unique to humans. This study probes to what extent cotton-top tamarin monkeys (Saguinus Oedipus) are able to exhibit an understanding of two grammatical rules, A(x^n)A and A(x^n)B. We chose these artificial grammars to investigate several capacities that may underlie language: identity, category, dependency, variable content, and variable distance.

This study uses operant conditioning methods; the monkeys are trained to choose between grammatical and ungrammatical strings presented in the visual domain. After repeating training until achieving a predefined performance level, the tamarins are then presented with novel stimuli. These novel stimuli are designed to specify each of the aforementioned capacities to asses the tamarins' ability to process them. If they perform well on the novel stimuli, this will suggest that they have acquired an abstract grammatical rule which the are using to make their decision- and thus that they possess some of the same computational capacities underlying human language.

Caitlin Lewarch clewarch@fas.harvard.edu
Lowell 2010
Neurobiology

Testosterone, Aging, and Seasonality in the Toba of Northwestern Argentina

The Ellison Laboratory, Reproductive Ecology Laboratory, Anthropology Department, Peabody Museum

The Toba are an indigenous people in Northern Argentina undergoing a transition to a market economy. Previous studies have focused on eastern Toba women living near Formosa, but little work has been done with men or the western groups that experience marked seasonal variation in food availability. Two of the three seasons, *Nakabiaga* and *Kap*, are periods in which plant food is extremely limited and the population is energetically constrained. *Wo'e*, however, is a season of relative abundance, particularly for the harvest of the staple *algarroba* bean. Our study of western Toba men investigated the influence of these seasonal changes in energy availability on testosterone levels. Sensitivity to such variation has been previously documented in women but not in men.

A group of 153 Toba men living in Northwestern Argentina were recruited for this study. Morning and evening saliva samples were taken on three days during each of the three seasons. The samples were analyzed using radioimmune assays (RIA) to determine testosterone concentrations. Of the 153 men initially recruited, 46 had complete enough data sets to be included in the analysis. These subjects ranged from approximately 17-67 years of age at the time of the study.

A preliminary analysis of the data showed that testosterone levels during *Wo'e* were significantly higher than those during less abundant seasons. This relationship existed across all age groups and suggests a strong correlation between seasonal energy availability and testosterone levels. The western Toba also exhibited the expected pattern of aging, with the youngest third of the men exhibiting consistently high testosterone levels compared to the middle and oldest age groups. Overall testosterone levels were lower than those seen in similar studies of Western populations.

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Lowell 2009
Chemical and Physical Biology

Synthesis of a potent and selective type II kinase inhibitor for the Ser/Thr kinase b-raf

The Gray Laboratory, Harvard Medical School

B-raf is a member of the ERK signal transduction pathway, which responds to extracellular growth factors to activate transcription of genes necessary for cell growth and proliferation. With over 30 identified mutations related to human cancers, it is an important target for the design of small-molecule inhibitors. B-raf was found to be susceptible to type II kinase inhibitors, in which the small-molecule inhibitor binds both to the ATP pocket as well as to an adjacent hydrophobic site. This binding mode tethers the kinase in an inactive conformation and prevents it from phosphorylating downstream targets.

The general pharmacophore for type II kinase inhibition involves a flat, heterocyclic 'head' region that can hydrogen bond to hinge residues of the kinase and make hydrophobic interactions with the ATP pocket, and a 'tail' region that contains a hydrogen bond donor-acceptor pair and a hydrophobic region to bind to the allosteric site. A library of type II inhibitors was synthesized and screened in a cell-based assay, and a lead compound was identified that inhibited b-raf (IC50=0.8362μM), PDGFR (IC50=0.03822μM), c-Kit (IC50=0.0306μM), and bcr-Abl (IC50=1.759μM). Optimization was chosen to proceed for b-raf because of its biological importance as a cancer target as well as the current dearth of potent and selective b-raf inhibitors. The lead compound consists of an oxazole head group and a tail containing a phenyl ring bonded to a solubilizing imidazole, linked by a phenyl group and two amide bonds. Analogues of this compound were made in by substituting related head and tail groups for those of the lead compound and reversing the amide conformations. Initial results from cell-based screens yielded surprising results. None of the analogues showed significant inhibition of b-raf (IC50>7μM) but were both potent and selective for c-Kit. Additional compounds are planned that alter the central phenyl ring and utilize urea linkages rather than amide bonds.

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Lowell 2010
Undecided

Processing of Artificial Grammars in Cotton-Top Tamarins (*Saguinus oedipus*)

The Hauser Laboratory, Cognitive Evolution Lab, William James Hall

While many species have elaborate forms of communication, language is unique to humans. One major aspect of language is its reliance on grammars—rules that allow for the generation of infinitely many different, logical statements. Though other species do not use grammars in their communication, the question of their capacity to comprehend grammars is nonetheless relevant to understanding how language evolved in humans. Members of our laboratory's colony of cotton-top tamarins (*Saguinus*

oedipus), a species of New World Monkey, were previously shown to master some simple grammars in habituation experiments. Our study investigated whether tamarins could understand another basic grammar, that of identity—identity defined as two identical objects (AA), in contrast to two non-identical objects (AB).

Our study involved the combination long call (CLC), a vocalization that is the tamarins' natural response to isolation from the colony. Previous studies have shown that tamarins will significantly modify CLC vocalizations in response to their acoustical environments. They have been shown to initiate calls in silence and shorten their calls to avoid noise.

In each trial, the tamarins were placed in an empty chamber and played a ten-minute sound file. The sound file contained white noise interrupted by silences of two different lengths: one just long enough to fit a CLC, one too short for the call. The long silences were preceded by two identical sounds (AA), whereas the short silences were preceded by two non-identical sounds (AB). Preferential calling in the long silences would have suggested their ability to distinguish identity.

The tamarins, however, did not exhibit significantly higher calling rates in the long silences. We are currently working on other, simpler grammars to test on the subjects. By first beginning with more basic rules, they may later become sensitive to the grammar of identity.

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Leverett 2010
Undecided

Persuasion Games in Social Networks

We model group opinion formation as a Markov process, in which individuals take on the opinions of their neighbors in a social network (or reject these opinions) with probability depending on network weights. We analyze several games played by external parties where strategies are manipulations of the network topology, and characterize response functions in such games. Furthermore, we model such games with delayed response times; we provide an injective mapping from Nash equilibria in games with slow responses to those with quick responses, e.g. we show that equilibrium behaviors in slow games correspond to those in fast games in a relatively simple sense.

We also characterize the power that manipulation of social networks has in opinion formation. In particular, consider an issue in which opinions are well-ordered (e.g. by intensity) and in which individuals holding particular opinions are partitioned into social groups (for example, political parties). Then we provide (weak) conditions that are necessary and sufficient for there to exist at least one social group such that, purely through social network manipulation (e.g. choosing a board or committee within the group), the group as a whole can be caused to form consensus at *any* opinion.

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Winthrop 2010
Undecided

Structural Studies of Reverse Transcription

The D'Souza Lab, Sherman Fairchild, Harvard University

Many devastating and lethal diseases, including acquired immunodeficiency syndrome (AIDs), leukemia, and tumors, are caused by retroviruses. Although much research is being conducted to better

understand these retroviruses, current drug strategies still target only individual proteins, and resistant strains have prevented development of a long-term effective cure. Focusing on crucial viral RNA-protein and protein-protein interactions offers a more promising approach in treating these diseases. Our research interest lies in using nuclear magnetic resonance (NMR) and other biophysical and biochemical methods to examine the structural basis for reverse transcription initiation in human immunodeficiency virus type-1 (HIV-1) and Moloney murine leukemia virus (MLV) life cycles.

The complex required for initiation of retroviral reverse transcription includes several RNA and protein components: a primer binding site, tRNA primer, nucleocapsid (NC) protein, and the enzyme reverse transcriptase (RT). Histidine-tagged HIV-1 RT and MLV RT were cloned using pET vectors, and overexpression and purification of RT expressed in *Escheria coli* was optimized. Testing for proper RT protein folding will be conducted using NMR technology.

Large quantities of reverse transcriptase are required in the initial stages of constructing the viral NC protein-tRNA-primer binding site RNA complexes formed during reverse transcription initiation. After the HIV-1 and MLV RNA complexes have been mapped using NMR, we will study their binding with their respective reverse transcriptases.

Vectors derived from MLV are currently being used to treat severe combined immunodeficiency and are in trials for the treatment of some cancers. Understanding of MLV RNA structures and events such as reverse transcription will facilitate the development of these vectors as a better agent for human gene therapy. Likewise, knowledge of the reverse transcription initiation mechanism in HIV could lead to the development of more successful antiviral drugs.

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Cabot 2010
Biology

Cling-E-coli

The Viel Laboratory, Undergraduate Research Laboratory, Department of Molecular and Cellular Biology, Harvard University

Quorum sensing allows bacteria to regulate physiological activities through the detection of cell-population density. In the *Vibrio fischeri lux*IR quorum system, *lux*I catalyzes the production of an acylated homoserine lactone, a small molecule that diffuses to nearby cells, dimerizes with luxR, and activates transcription of a reporter gene through upregulation and subsequent positive feedback of a luxpR promoter. By insertion of these genes into *Escherichia coli,* quorum sensing was activated by targeting the *Escherichia coli* to calmodulin using random peptide libraries expressed on the outer membrane protein AIDA; in addition, previously characterized histidine and streptavidin tags were used to target bacteria to nickel and biotin, respectively. This targeting was also harnessed for use in the wildtype Fec signaling system; by the computational insertion of a random library in loops 7 and 8, we hoped to allow the bacteria to produce a reporting signal upon the binding of the target. As a proof of concept, targeting, subsequent quorum activity, and Fec activity were measured through fluorescence of reporter GFP and RFP, as well as quantifying cell density through optical density measurements. Being part of the International Genetically Engineered Machines competition (iGEM), one of the major successes of the project was the characterization of the biological parts (Biobricks) used. Furthermore, a significant contribution of new Biobricks to the repository was made, including a single-cell system that allowed for the targeting and quorum-instigated fluorescence of *Escherichia coli,* in addition to many components of the Fec two component system. The potential usages of targeting, subsequent cell-population detection, and signal transduction are extensive, especially given the versatility of reporter genes that could be inserted at the end of the Fec pathway or in coordination with the quorum-sensing *luxIR* system.

Cesar Lopez cjlopez@fas.harvard.edu
Quincy 2009
Chemical and Physical Biology

Evaluation of Dried Blood Spot Specimens for Early Diagnosis and Quantitative Monitoring of Simian Immunodeficiency Virus Infection in Rhesus Macaques

The Letvin Laboratory, Beth Israel Deaconess Medical Center, Harvard Medical School

Though antibody diagnostic testing has become the most widely employed method for Human Immunodeficiency Virus Type1 (HIV-1) testing, diagnosis during early infection is difficult due to the fact that antibody conversion typically occurs one to several months following HIV infection. Furthermore, antibody testing is limited in that it can only yield a positive or negative status, i.e. no data regarding viral burden can be determined.

The aim of this study is to evaluate the use of dried blood spotting (DBS) as a rapid and high throughput method to both monitor and quantify Simian Immunodeficiency Virus (SIV) RNA in rhesus macaques at the early most stages of viral infection.

Whole blood and DBS samples spotted onto biological transport cards were collected from five male macaques at multiple time-points following SIV infection. Viral RNA loads were quantified by real-time RT-PCR in whole blood samples at the time of collection, while DBS viral RNA loads were quantified following a six-month storage period at room temperature.
We found that viral RNA loads in whole blood and DBS samples were comparable at each time-point, thus demonstrating that viral RNA loads obtained from DBS are reliable indicators of viral RNA burden in the peripheral blood during HIV infection. This technology provides an easy, inexpensive means of specimen storage for HIV nucleic acid testing under field conditions in underdeveloped countries. Importantly, DBS technology also allows for diagnoses of HIV status up to four weeks earlier than conventional antibody testing and quantifiable monitoring of viral RNA loads in the setting of therapy.

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Adams 2008
Physics

Computational analysis of genome-wide SDC3 binding sites in *C. elegans*

The Liu Laboratory, Dana-Farber Cancer Institute/Harvard School of Public Health

Chromatin immunoprecipitation coupled with DNA microarray analysis (ChIP-chip) has emerged as a powerful technique for identifying the *in vivo* binding sites of DNA-binding proteins such as transcription factors (TFs) and histones. With the advent of whole-genome tiling microarrays, biologists have been able to generate massive amounts of data capturing putatively all of the binding sites in the genome for a particular DNA-binding protein. However, this creates the challenge of developing computational tools to analyze and annotate the most significant biological patterns from the data.

In this work, we develop and implement such computational tools to study genome-wide protein-DNA interaction patterns. Specifically, we develop a peak detection algorithm called MA2C for locating binding sites. Additionally, we implement an annotation module called CEAS.py that dynamically retrieves the most up-to-date genome annotation information and produces the genomic distribution of ChIP-chip peaks from an experiment. Applying this machinery to study factors mediating somatic dosage compensation and X-chromosome silencing in the model organism *C. elegans*, we make an intriguing

discovery regarding the binding pattern of dosage compensation factor SDC3—initially thought to bind only the X chromosome, we discover many additional binding sites of the factor in autosomal regions of *C. elegans*, suggesting a possible biological role and mechanism of SDC3 never before considered.

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Leverett 2010
Engineering Sciences

Effects of chemical and biomechanic cues on mesenchymal stem cell differentiation

The Mooney Laboratory, School of Engineering and Applied Science

Due to the therapeutic potential of stem cells in curing a wide range of human diseases and the connection between stem cells and early organismal development, understanding the mechanisms governing stem cell growth and differentiation is imperative. Chemical factors have been well characterized as playing an important part in directing the differentiation of stem cells from a naïve to a committed state. However, the role of biomechanical factors presented by the extracellular matrix, such as the concentration of adhesion peptides and the stiffness of the surrounding substrates, has been largely neglected, particularly *in vitro* studies conducted on 2D polystyrene tissue culture plates. Using previously characterized chemical factors, the D1 murine mesenchymal stem cell line was demonstrated to be able to differentiate into adipocytes and osteocytes, as verified by the presence of neutral lipid droplets and glycerol-3-phosphate dehydrogenase activity in adipogenic cells, and alkaline phosphatase expression and calcium secretion in osteogenic cells. Moreover, the D1 cell line showed the potential of undergoing transdifferentiation from an osteogenic to an adipogenic state. Currently, the early and late gene expressions of D1s are being characterized via real time polymerase chain reaction (PCR) as a method of conducting powerful high throughput differentiation assays. Future studies include optimizing real time PCR reactions, assaying stem cell differentiation on alginate hydrogels capable of emulating the extracellular matrix, and examining the transdifferentiation potential of this mesenchymal stem cell line.

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Adams 2010
Molecular and Cellular Biology

Fat Formation and Parkinson's Disease: Two Separate but Cool Subjects

The Cowan Laboratory, Harvard Stem Cell Institute, Massachusetts General Hospital

In my lab I have been working on two separate projects:

The development of fat cells is not fully understood. My lab is using zebrafish as a model to understand fat development and find relevant promoters. The goal is to find a promoter that is only expressed in zebrafish fat cells. With such a promoter, one could drive genes that lead to changes in fat formation. By studying these changes, we would be better able to understand how fat cells develop. Our method for finding a fat specific promoter is via microinjection (injection of DNA into one-cell zebrafish embryos) of promoters thought to be important for fat development, such as leptin. After microinjection of promoters driving GFP, transgenic fish are analyzed to determine the presence of GFP in their fat cells.

The neurodegenerative disease called Parkinson's is the most common degenerative movement disorder. Due to a lack of patient material, it is not understood on a cellular level. My lab is differentiating human embryonic stem cells into dopaminergic neurons (the type affected in Parkinson's) in order to create an in

vitro model of Parkinson's disease. The goal is to use a cellular model to test chemicals that could be used as drugs to treat Parkinson's. Our method for creating stem cells that contain a hereditary form of Parkinson's disease is to first make a plasmid containing the genetic defect associated with a specific form of Parkinson's. Along with the genetic defect, the plasmid contains a switch which will turn on Parkinson's disease in the cells when a certain chemical is added. The cells will also contain a reporter to assist with quantification of dopaminergic neurons. Genetic constructs will be introduced to the human embryonic stem cells via electroporation to create stable transgenic clones.

Firth McEachern fmmceach@fas.harvard.edu
Kirkland 2008
Earth and Planetary Science

Shocking Ice

The Hoffman Laboratory, Shock Compression Lab, Harvard University

Ice is a common material on planetary surfaces and other solar system bodies, especially among so called "icy bodies" in the outer solar system. In order to accurately predict the outcome of collisions, whether between two Kuiper Belt Objects or a smaller body impacting a planet, physical parameters such as temperature and the speed at which shock waves propagate through the material must be known. Thus, we conduct experiments whereby projectiles are fired at samples of different materials at high velocity in order to deduce these parameters. In this particular study, we look at the data of one particular collision experiment (Shot #50), between steel and ice. The kinematics and pressure of the shock wave was determined by a technique called "impedance matching." Meanwhile, a signal in volts was recorded with respect to time, and converted to radiance, a measure of luminosity. The nature of this data indicated there were two sources of heat in the ice sample: one from the shock itself, and one from hotspots generated by the shock. We employed two methods to derive these temperatures as well as the residual temperature of the ice after being shocked. The two methods agree to some extent, giving a shock temperature between 750-850 C. The results for the hotspot temperatures, however, are inconclusive. Finally, using a similar procedure, the residual temperature in the sample after the shock decays was estimated to be about 420 C. These results help constrain the equation of state of ice, i.e. how ice behaves under different conditions of temperature and pressure, and ultimately provide clues as to what happens when icy bodies collide in "real life."

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Eliot 2009
Mathematics

Complexity of Hardness Amplification

We are interested in finding average-case hard functions in restricted classes of circuits for which we can exhibit worst-case hard functions. To accomplish this, we would like to apply hardness amplification within these classes, so we ask: what is the most restricted class of circuits in which the worst-case to average-case reduction can be placed? We conjecture that if we want to amplify to ½- epsilon (for some epsilon > 0) and we insist that the reduction be by constant depth circuits, then the size of the circuits must be polynomial in 2^poly(1/ epsilon).
Most recently, Dan Gutfreund and Guy Rothblum proved the conjecture for a special case. By viewing the reduction not as a circuit but as a Turing Machine that takes non-uniform advice, they limited the advice to polynomial in 1/epsilon. In this special case of limited advice, they showed that the circuits size must be polynomial in 2^poly(1/epsilon). The question is, if we allow the advice to be exponential in

1/epsilon, must the circuit size still be polynomial in 2^poly(1/epsilon)? If we could avoid such a trade-off between wanting small circuit size and small epsilon, we could get a strong enough reduction to construct pseudorandom generators that fool circuits in AC^0 with parity.

Rachel Moore rmoore@fas.harvard.edu
Quincy 2008
Biology

Epigenetic Control of Variant Surface Antigens in the Malaria Parasite, *Plasmodium falciparum*

The Duraisingh Laboratory, Department of Infectious Disease, Harvard School of Public Health

Purpose: There are an estimated 350-500 million cases of malaria annually worldwide, causing over 1 million deaths. The causative parasite, *Plasmodium falciparum*, is able to evade the human immune system by employing clonal antigenic variation, in which it periodically switches the expression of variant erythrocytic surface proteins encoded by its subtelomeric *var* genes. The histone deacetylase Sir2 has been implicated in the control of *var* expression. Here we further investigate the two Sir2 proteins found in *P. falciparum,* PfSir2-13 and PfSir2-14. We also investigate two families of small proteins, containing Alba and Macro domains, which may interact with Sir2 to assist in the epigenetic gene control. Alba-domains have been well-characterized in archaeal chromatin proteins, where they form stable interactions with the archaeal Sir2 homolog. Also, bioinformatics links Alba with two eukaryotic protein families that have roles in RNA metabolism, suggesting a possible role in translational repression through RNA sequestration. Macro-domain containing proteins have been shown to bind the Sir2 metabolite O-acetyl-ADP-ribose. Genes in microbial and fungal parasites contain a Sir2-like domain in a tandem configuration with a macro domain, suggesting that macro may serve as a receptor for Sir2 metabolites.
Methods: We amplified the three Alba-domain containing proteins and one Macro-domain containing protein from the 3D7 strain of *P. falciparum.* The genes were tagged with HA and transfected into the 3D7 attB strain of *P. falciparum,* together with a plasmid encoding the Bxb1 mycobacteriophage integrase, which facilitates the genomic integration of transgenes in this strain. We also worked to tag the endogenous Sir2-14 gene with HA, to create an episomal GFP-tagged Sir2-13 line under both the endogenous promoter and a calmodulin promoter, and to create a double knockout line lacking both functional Sir2-13 and Sir2-14.
Results: Successful expression of Alba8 was confirmed by Western blot, with results for the other two Alba-domain containing genes pending. Results are not yet available concerning Macro, tagged Sir2 lines or the double knockout due to the lengthy process of making genetically modified *P. falciparum* lines.
Conclusions: Further investigation is needed to determine the roles of alba and macro domain containing proteins in *P. falciparum,* and to further elucidate the role of Sir2. However, a large number of useful tools have been created which will help us address these questions in the coming months.

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Currier 2008
Biochemical Sciences

Identification and Characterization of Small Noncoding RNAs under Sporulation Control in *Bacillus subtilis*

The Losick Laboratory, Department of Molecular and Cellular Biology, Harvard University

Only a small fraction of the RNA in the cells gets translated into proteins. Amongst the untranslated RNAs, the small noncoding RNAs regulate the gene expression of target genes by interfering with their transcription and translation. The search for small noncoding RNAs (sRNAs) in Gram negative bacteria, such as *Escherichia coli* has been particularly fruitful, and more then 100 members were identified by high-throughput screens. In contrast, Gram positive bacteria remain largely unexplored for sRNAs. The current work aims to isolate and characterize sRNAs from *Bacillus subtilis*, a Gram positive soil bacterium that exhibits an intriguing mechanism for overcoming nutrient deprivation. Our screen is triggered by preliminary results from microarray experiments, which detected strong expression signals in several intergenic regions of the *Bacillus subtilis* genome – these regions may contain novel sRNAs. In order to confirm their existence, Northern blots of the complete RNA fraction expressed in sporulation conditions is probed with chemiluminescently-labeled RNA probes from seven of the candidate regions (*yddM-yddN*, *yhfA-yhgB*, *ygeN-comEC*, *yrhK-yrhJ*, *yxbC-yxbB*, *yocL-yokM*, and *yybS-cotF*). In case of positive results, the genomic location of the candidate sRNA genes will be mapped through 5'- and 3'-RACE PCR assays. We will use comparative phylogenetic methods to look for homologs of the identified sRNAs in closely-related, spore-forming bacteria, e.g. *B. licheniformis* and *B. anthracis* to reconstruct the evolutionary history of the genes of interest, and compare it to the published rRNA-based phylogeny of the *Bacillus* phylotypes to make inference about the origin of these genes (e.g., compare divergence vs. horizontal gene transfer). Expression of the sRNA candidates will be scored by measuring β-galactosidase activity in *lacZ* fusion strains bearing the promoters of the identified sRNAs during sporulation conditions. We plan to further characterize the phenotype of the potential sRNAs through overexpression and complete and partial knock-out approach. The described experimental approach may give some hints for the potential targets of the identified sRNAs.

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Currier 2010
Undecided

Evaluating the role of DEAD box Helicases in *P. falciparum* gene regulation

The Wirth Laboratory, Department of Infectious Disease, Harvard School of Public Health

Although gene expression levels differ at various stages in the life cycle of Plasmodium falciparum, promoter regions are scarce and poorly characterized in the genome of the parasite, and conventional regulation at the level of transcriptional initiation is unlikely to explain the differences in RNA levels over time in the P.falciparum. The purpose of this study was to examine five members of the DEAD box helicase superfamily (PFB0445c, PFC0915w, PFD1070w, PF14_0563, PF14_0665) which is highly conserved across all eukaryotes. We hypothesize that these proteins interact with plasmodium RNA and are integral for regulation of parasite gene expression across the life cycle. We successfully amplified and cloned PFD1070w and PFB0445c from genomic and cDNA, respectively, using the 3D7 strain of P.falciparum and transformed the plasmids into E.coli for expression of the 6xHis tagged proteins. The successful expression of these proteins will allow us to generate antibodies which will jumpstart studies of the localization of the proteins in parasite cells. The purified protein can also be used to characterize the

binding of the helicases to RNA using electropheretic mobility shift assays. We will also be able to perform binding assays and structural studies to determine the role that DEAD box helicases play in the post-transcriptional regulation of gene expression in P.falciparum.

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Lowell 2009
Chemical and Physical Biology

An Investigation of Intrahelical Lesion Recognition by Human 8-OxoGuanine Glycosylase 1

The Verdine Laboratory, Department of Molecular and Cellular Biology, Harvard University

DNA is constantly exposed to damage from many sources both outside and inside the cell. Unless the damage is controlled by the cell, it will often cause mutations, which can cause a host of other diseases, notably cancer. One common source of damage is oxidative damage from reactive oxygen species generated during cellular respiration. My project concerns a particular kind of oxidative damage, the oxidation of the guanine nucleotide to oxoguanine. In humans, the cell contains an protein, human 8-oxoguanine glycosylase 1 (hOgg1), that recognizes oxoguanine and excises it from the DNA. In particular, we are studying how hOgg1 locates oxoguanine.

How hOgg1 searches for oxoguanine is a particularly compelling problem because although it is known how hOgg1 can distinguish between oxoguanine and guanine while the bases are flipped out of the helix, it is not known how hOgg1 distinguishes between the two bases while they are still within the helix, since the two bases appear almost identical while intrahelical. In order to understand how hOgg1 distinguishes between the two bases, my lab is solving crystal structures of hOgg1 bound to DNA with intrahelical guanine and oxoguanine. To ensure that the protein binds to a particular base, we introduce a sulfur containing carbon chain into the DNA and form a disulfide bond between the DNA and a cysteine residue in hOgg1 that keeps the protein in place. My specific project is to screen different locations for the disulfide bond in order to ensure that formation of the disulfide bond between the DNA and protein does not artificially constrain the system in an unnatural conformation and eventually solve the structure of hOgg1 bound to DNA containing an intrahelical guaninine or oxoguanine.

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Dunster 2009
Biochemical Sciences

Variability in an artificial tandem repeat

The Verstrepen Laboratory, FAS Center for Systems Biology, Harvard University

Considerable parts of most eukaryotic genomes consist of consecutive iterations of short sequences, called Tandem Repeats (TR's) or "satellite repeats". For example, "CAGCAGCAGCAG" is a TR with 4 units of "CAG". Originally considered to be "junk" DNA by genome researchers and often omitted from genomic sequencing and analysis, it is now known that tandem repeats have significant evolutionary value and are linked to myriad human diseases, including Huntington's disease and fragile X syndrome. The most notable property of TR's is their *variability*, caused by addition or removal of one or more repeat units, which causes these sequences to be some of the most polymorphic regions of genomes. However, the principles and mechanism behind this phenomenon are not well understood.

To observe how sequence properties affect variability, artificial TR's varying in number of units, overall length, and purity were inserted after the start codon of the marker gene *URA3* in *S. cerevisae.* As the number of repeats mutated, the marker gene was shifted in and out of frame, which could be observed by plating on media selecting for and against *URA3*. Fluctuation assays were used to measure the switching rate and thus infer the variability of the artificial TR. Additionally, several recombination pathway genes were deleted and the resulting effects on TR variability were observed.

A positive exponential correlation was observed between TR variability and each of the three sequence properties: TR unit size, overall TR length and TR purity. This validated the results of statistical analysis conducted by Legendre and Pochet et al. (*under review*) which found similar trends in *S. cerevisae* genomic data. Additionally, unit size appears to affect the changes in variability caused by deletion of recombination genes; further analysis will hopefully bring insight into the pathways that affect TR variability and the mechanisms underlying this evolutionary mystery.

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Lowell 2008
Biology

Immunomodulatory effect of copolymers at the microglia:T-cell interface in the CNS

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Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) with a presumed autoimmune mechanism. Pathogenesis is characterized by blood-brain barrier (BBB) breakdown, CNS leukocyte infiltration, and reactive gliosis, leading to chronic demyelination and axonal damage throughout the brain and spinal cord. The view of MS as an autoimmune disease is strongly supported by studies in the animal model experimental autoimmune encephalomyelitis (EAE), a CD4+ T cell-mediated disease in which peripheral T-cells reactive against CNS self-antigens are stimulated to cross the BBB and initiate an immune response in brain parenchyma. In MS and EAE, the interaction between self-reactive T-cells and microglia [the CNS-resident antigen presenting cell (APC)] has emerged as an important pathogenic factor and therapeutic target. Copaxone (an FDA-approved immunomodulatory treatment for MS) is an amino acid copolymer with well-documented therapeutic efficacy, whose mechanism of action (still unknown) is speculated to involve T-cells, APC, or both. In the present study, we utilize the mouse EAE model to assess effects of copolymer treatment on microglia:T-cell interactions in the inflamed CNS. Induction of EAE in SJL mice by subcutaneous injection of myelin proteolipid protein 139-151 activated microglial cells to present antigen as measured by MHC class II and costimulatory molecule CD86 expression, but this effect was significantly reduced in mice treated with copolymer. Reduced microglial activation correlated with reduced leukocyte infiltration upon histological analysis, as well as improved neurologic function on daily clinical exam. These data suggest a mechanism by which the observed amelioration of disease in EAE mice may be mediated through microglial cells, principally via a reduced capacity for presentation of self-antigen to pathogenic T-cells within the CNS.

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Lowell 2009
Physics

Shock Properties of Ice

The Hoffman Laboratory, Shock Compression Lab, Harvard University

Impact events are a fundamental part of planet formation and evolution. Many planets, comets, and meteoroids contains significant quantities of ice, so shock experiments on H2O ice and ice mixtures provide insight into their history. Using a 40mm single-stage gun-powder launch system, the Shock Compression Laboratory propels planar steel and molybdenum projectiles at velocities from 1.9 km/s to 2.7 km/s towards ice targets held near 173 K and <100 mTorr vacuum. Upon impact, planar shock waves generate peak pressures on the order of 10 GPa in the ice samples. A four-channel infrared pyrometer collects radiance data as the shock wave propagates through the sample, which can be converted into temperature data using a black-body assumption. Simultaneously, the post-shock velocity of the free surface is measured using an interferometer system. The first trial on a sample of polycrystalline, columnar ice impacted at ~2200 m/s showed a peak pressure of 10.6 GPa with peak and post-shock temperatures of 775 K and 415 ± 20 K, respectively. These were in good agreement with predictions of 790 K and 450 K at 11 GPa. Together, these data provide information about phase transitions and heterogeneities within the samples. Further experiments on porous ice and ice-quartz mixtures are to be conducted soon.

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Leverett 2010
Organismic and Evolutionary Biology

Controlling Cultures For Synchronous Plasmid Supercoiling

The Kleckner Laboratory, Department of Molecular and Cellular Biology, Harvard University

Plasmids undergo topological changes in bacteria throughout the cell cycle. However, this is difficult to observe because the bacteria in a culture are growing at different rates and proceeding through different phases of the cell cycle. It is fact that plasmids coil and uncoil during replication (otherwise known as supercoiling) but little is known beyond that in terms of when and to what degree plasmids supercoil during the cell cycle.

Past experiments have devised two dimensional gel methods that very clearly depict the supercoil densities of different plasmids. The secret here is that we are modeling after these past experiments while tying in the baby cell columns used in the Kleckner lab.

Using the baby cell column and two dimensional gel analysis bacterial culture cell cycles may be synchronized and studied in an experiment controlling for cell age, allowing us to catalog when and how much a plasmid supercoils during the cell cycle.

Philip Roebuck roebuck@fas.harvard.edu
Pforzheimer 2008
Anthropology

Salivary testosterone levels during ultimate frisbee tournaments: Parallels to repeated dominance contests,

The Ellison Laboratory, Reproductive Ecology Laboratory, Anthropology Department, Peabody Museum

A large body of research has been conducted investigating the relationship between testosterone (T) and competition. Considering human competition akin to dominance contests commonly observed among nonhuman primates and other animals, Mazur's Biosocial Model of Status predicts that before a competition T levels will rise to encourage individuals to compete hard for status, and that afterwards T will drop fairly quickly in losers while staying high in winners. Though some studies support Mazur's model, others suggest that variables besides competition outcome play important roles in affecting T response. This study builds on previous research in order to investigate which factors associated with competition have the largest impact on T levels.

18 subjects were recruited from Harvard's men's ultimate frisbee team. Subjects collected a saliva sample before and after each game at 5 weekend tournaments (4-8 games were played per tournament). At the time of each sample collection, subjects also filled out a short questionnaire to record how well each thought he performed in the previous game, and how difficult he expected the subsequent game to be. A longer questionnaire at the beginning of the study documented subjects' age, BMI, experience with ultimate and other sports, relationship status, and competitiveness index scores. Saliva samples were frozen 9 days after each tournament, and testosterone concentration was later measured using radio immuno assays.

Results are still incomplete, but this study should help clarify the relationship between T and competition. Regression analyses will show whether it is competition outcome or other variables, such as subjects' competitiveness, anticipated difficulty of the impending game, playing experience, or individual performance, that has the biggest impact on T levels. The tournament format will also enable researchers to analyze the effect of previous games on T responses later in the day.

Caitlin Rottman rotman@fas.harvard.edu
Adams 2009
Earth and Planetary Science

Gakkel Ridge

The Hoffman Laboratory, Harvard University

Beneath Earth's calm seas the seafloor is ripping apart and new ocean crust, in the form of underwater chains of volcanoes, is being continuously generated at mid-ocean spreading ridges. One example is the Gakkel Ridge in the Arctic Ocean, which until recently has been largely unexplored due to the thick perennial ice cover. However, in 2001 the Arctic Ocean ice was breached by two ice-breakers in a multi-national expedition to investigate in detail the bathymetry, hydrothermal activity, and basalt chemistry of this unique mid-ocean ridge. In addition to being a relatively unexplored geologic environment, the Gakkel Ridge provides a unique natural laboratory because it has the slowest spreading rate of any ridge on Earth. This will enable direct comparison of ridge characteristics and ocean crust formation across the entire spreading rate range of mid-ocean ridges.

As an integral part of a larger ongoing project, my research focuses on the generation of two complementary data sets: 1) trace element concentrations in basalt glasses (quickly quenched magmas) extruded from the Gakkel Ridge volcanoes, and 2) petrographic thin section descriptions of these basaltic rocks. These new data sets will shed light on the dynamics of new ocean crust formation at very slow spreading rates, and are comparable to similar data sets from other mid-ocean ridges. Trace element concentrations will provide information about both the composition of Earth's upper mantle (i.e., the source material that melts to form basaltic magma) and the processes that affect these magmas as they rise from the mantle to the seafloor (e.g., degree of melting, crystallization). Petrographic characterization helps trace the extent of crystallization in the sampled rocks and the overall evolutionary history and is directly related to the composition of the basalts. Together these data sets are crucial for analysis of spatial and temporal variation both along and across the ridge axis. The data will not only help unscramble the complex systematics of mid-ocean ridges, but also allow testing of models formed from study of previously explored ridges.

Roanna Ruiz rchruiz@fas.harvard.edu
Adams 2009
Engineering Sciences

Femtosecond Laser Nanosurgery of C. Elegans Neurons

The Mazur Laboratory, Gordon McKay Laboratory of Engineering and Applied Science, School of Engineering and Applied Science

Accurately studying and understanding the biological dynamics of neurons has challenged researchers in many fields, ranging from biology and neuroscience to chemistry and applied physics. The difficulty lies in the complexity of their interaction, and, more specifically, the precision needed to systematically decode the range of neural pathways and behaviors that can be achieved. To investigate this problem, we use femtosecond laser nanosurgery to ablate the neurons of the model organism known as *Caenorhabditis elegans*.

By focusing laser pulses inside the body of a c. elegans worm, we selectively ablate fluorescently-labeled neuronal dendrites, axons, and cell bodies through nonlinear processes. In general, the peak intensity of a femtosecond laser pulse is very high and material disruption is possible through nonlinear absorption and plasma generation. The pulse duration is very short, and this allows the laser to reach an intensity of optical breakdown with only nanojoules of energy per pulse. The low energy deposition and high spatial localization of nonlinear absorption can produce non-invasive ablation (with several hundred nanometer resolution) of one dendrite of one neuron in a c. elegans worm without damaging neighboring structures or inducing collateral damage in the living organism.

Our research focuses on using a titanium-sapphire femtosecond laser to study the behavior and potential regrowth of c. elegans neurons responsible for controlling vulva function and entrance into the dauer state. With nano-scale precision we cut neurons and carefully monitor and re-image the worms under a UV lamp at various post-surgery time intervals. Much research remains to be done, but it is possible that femtosecond laser nanosurgery investigations on c. elegans neurons can further our understanding of neuron regrowth, interaction, and behavioral effects in larger life forms such as mice, rats, and even humans.

Sammy Sambu sambu@fas.harvard.edu
Leverett 2008
Engineering Sciences

Cell Surface Re-engineering

The Viel Laboratory, Undergraduate Research Laboratory, Department of Molecular and Cellular Biology, Harvard University

Cell surface reengineering involves the modification of the cell membrane for the achievement of unique purposes not afforded by the native cell membrane. We modified two well-characterized *E. coli* surface proteins -- AIDA1 and OmpA. In both cases, we set out to fuse random peptide sequences, a histidine tag and a streptavidin binding peptide to the N and C termini of AIDA1 and OmpA respectively. We performed fluorescence-activated and magnetism-activated cell sorting to test the effectiveness of the presentation of the streptavidin binding peptide (strep2) and the polyhistidine-tag. In addition, we set out to fuse a streptavidin-binding tag and polyhistidine-tag (our positive controls) to a portion of the first loop of OmpA that *in situ,* is in extracellular space.

We purposed to use the bacterial display of a polyhistidine-tag and strep2 to localize bacteria to specified targets and signal downstream events through a bacterial quorum sensing mechanism. Bacteria that display the polyhistidine-tag preferentially associate to cobalt or nickel and their derivatives. Similarly, bacteria displaying strep2 will associate with streptavidin conjugated elements. Hence, we can localize cell surface modified bacteria thereby establishing a quorum. Bacteria will then initiate a quorum sensing mechanism involving chemical signaling between members in the quorum. A subgroup within the team created an artificial chemical signaling pathway dependent on the release of a homoserine lactone (N-[3-oxohexanoyl]-L-homoserine lactone, OHHL). The team designed the production of OHHL in the 'sender' bacteria to trigger the expression of green fluorescent protein (GFP) in 'receiver' bacteria.
Lastly, the team intends to adapt the bacterial display to the identification of ligands for metal ions, proteins of interest and possibly, the transduction of signals across the bacterial membrane. The initial corollary would enable the team to identify consensus sequences for these targets by substituting the streptavidin and histidine tags with random peptide sequences of any prescribed length.

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Pforzheimer 2008
Biochemical Sciences

Primordial germ cells require insulin-like growth factor signaling for proper migration

The Wood Laboratory, The Vincent Center for Reproductive Biology, Massachusetts General Hospital

Insulin-like growth factor (IGF) signaling plays a crucial role in vertebrate growth and development, during both fetal and adult stages, largely through stimulating cell proliferation and survival. Accordingly, gene knockout studies in mice have revealed that a loss of IGF signaling results in proportional growth restriction, in addition to significantly reduced fertility. The latter effect is associated with reduced numbers of germ cells in the adult gonad, but the precise role of IGF signaling in germ cell development has not been identified. To explore the role of IGF signaling in germ cell development, we examined the consequences of suppressed IGF signaling in the zebrafish embryo. Whole-body suppression of IGF receptors (IGF1R) resulted in reduced somatic growth, in addition to mismigration and death of primordial germ cells (PGCs), which are the precursors to eggs and sperm. These observations suggest that in addition to promoting cell proliferation, IGF signaling also promotes the migration of PGCs from their site of origin to the developing gonad. What remains unclear from these studies is which cell

population(s) directly requires IGF signaling to ensure proper migration of PGCs. We hypothesized that PGCs are intrinsically dependent upon IGF signaling for proper migration. To test this, we overexpressed a mutant IGF1R (dnIGF1R) specifically in the PGCs, thereby suppressing IGF signaling in PGCs, while retaining normal IGF signaling in all other cell types. In accordance with our predictions, zebrafish embryos overexpressing mutant IGF1R exhibited normal body development, but had significantly fewer PGCs migrating to the developing gonad, and a corresponding increase in the number of mismigrated PGCs. These data indicate the PGCs intrinsically require IGF signaling through IGF1R to correctly migrate to the developing gonad. A loss of IGF signaling could thus predispose an individual to compromised fertility later in life, by interfering with germ cell migration.

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Pforzheimer 2008
Chemical and Physical Biology

The Test-Tube Clock: KaiC Mutants in a Model of Ordered Phosphorylation

The O'Shea Laboratory, FAS Center for Systems Biology, Harvard University

The cyanobacterial proteins, KaiA, KaiB, and KaiC, when combined with ATP, can reconstitute circadian oscillation in isolation – no bacteria, only in a test tube. It was discovered that the total level of phosphorylation of KaiC, a large, cruller-shaped protein, oscillates with a near twenty-four-hour period. KaiC is an autokinase and autophosphatase, so it is able to attach and remove phosphate groups from itself. Its activity is modulated by the other two Kai proteins, KaiA and KaiB. KaiA promotes KaiC's autophosphorylation and KaiB inhibits KaiA.

Our lab has recently discovered the fundamental underlying biochemical feature of the circadian oscillator reaction that allows it to oscillate. There are two specific locations on KaiC that are phosphorylated, a threonine and a serine residue. Further, these locations are phosphorylated in an ordered, periodic pattern that coincides with the overall phosphorylation pattern of the protein. Thus there are four states – unphosphorylated, singly phosphorylated on threonine, singly phosphorylated on serine, and doubly phosphorylated at both locations – that make the clock tick. From measurements of the rates the transitions from one state to the other, we have developed a mathematical model that recreates the essential features of the clock: stable oscillations, the correct period, and ordered phosphorylation.

The goal of my research is to biochemically analyze mutated forms of KaiC to corroborate our model of ordered phosphorylation and explore the functional implications of the structure of KaiC. Essentially, I will purify mutant KaiC proteins and measure their abilities to autophosphorylate and autodephosphorylate in the presence or absence of KaiA. Those measurements can then be used as inputs into the mathematical model, which, if robust, would then correctly predict the characteristics of the mutant oscillation. Also, the mutations can be related to the surfaces of KaiC, providing clues about the function of different parts of the protein.

Charlotte Seid cseid@fas.harvard.edu
Leverett 2010
Chemical and Physical Biology

Functional Properties of Mutations in the Cyanobacterial Clock Protein KaiA

The O'Shea Laboratory, FAS Center for Systems Biology, Harvard University

The cyanobacterial circadian oscillator is a system of three proteins, KaiA, KaiB, and KaiC, that maintain an approximately 24-hour cycle in the phosphorylation state of KaiC. Surprisingly, this simplest known circadian oscillator can be reconstructed *in vitro* from purified proteins and ATP. KaiA is known to enhance KaiC autophosphorylation and is inhibited by KaiB, but the precise molecular mechanisms remain unknown.

KaiA in particular is crucial to the underlying enzyme kinetics. A homodimer, KaiA stimulates KaiC autophosphorylation at low relative concentrations, implying a transient yet powerful interaction. By perturbing the clock with KaiA mutants and studying their biochemical properties, the physical interactions among the three proteins may be elucidated.

Three mutations, reported to lengthen the clock period *in vivo*, were selected to represent different domains of KaiA. In contrast to the *in vivo* studies, none of these mutants produced complete oscillations *in vitro*. I9T (N-terminal, implicated in environmental input and phase resetting) produced an initial phosphorylation peak followed by dephosphorylation of KaiC to basal levels; as it was unable to restore phosphorylation, this mutant may have been permanently inhibited by KaiB. M241T (buried residue of the C-terminus, which binds KaiC) showed monotonic dephosphorylation of KaiC, perhaps due to misfolding and loss of function. A mutation at the C-terminal dimer interface, C273Y, prevented total phosphorylation from reaching basal levels once it had peaked. It is possible that this mutation stabilizes a particular transition form of KaiC to prevent further phosphate transfer.

Although preliminary, these results suggest that each mutation impairs a different step in the phosphorylation cycle, and thus different regions of KaiA may correspond to specific functional roles in the clock. Additional experiments, especially a series of KaiA-KaiC partial reactions to determine the mutants' binding curves, are expected to illuminate further the structural basis of this remarkable oscillator.

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Pforzheimer 2008
Engineering Sciences

Development of a four-winged microrobotic flying insect

The Wood Laboratory, Harvard Microrobotics Laboratory, School of Engineering and Applied Science

Small-scale robotic flying insects fall under the category of Micro Air Vehicles (MAVs), whose development facilitates potential applications in search and rescue, espionage, and hazardous environment exploration. Building on the previous success of a two-winged microrobotic flying insect, this study sought to prototype a four-winged microrobotic insect. The study of two-winged insect flight considers insect morphology in addition to manipulation of wing parameters such as stroke amplitude, stroke cycle, angle of attack, and stroke plane. Four-winged insect flight differs significantly from that of the two-winged insect in the additional consideration of wake interference between the fore- and hindwings. Damselflies and dragonflies are able to control the fore- and hindwings independently; the kinematic phase shift and

proximity between the two wing pairs are believed to be responsible for lift production associated with different flight modes such as hovering or forward flight.

All components of the robot were fabricated using Smart Composite Microstructures (SCM), a process that enables the construction of desired 3D microstructures through the manipulation of 2D laser-micromachined lamina (usually by folding). Fabrication techniques were refined to achieve alignment of critical features with micron accuracy as well as consistent and accurate fold angles. The robot is actuated via piezoelectric actuators, whose motion is coupled to transmissions to produce flapping motion.

At the time of submission, the prototype nears completion. Upon the successful flight of the robotic insect, efforts will be made to quantify the aerodynamic forces on the wings for various flight modes and kinematic phase shifts. Further design iterations and improvements are expected to result from an analysis of the data.

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Eliot 2008
Psychology

Goal-Appreciation versus Disgust:: Understanding the Empathy Blocking and Deliberate Self-Harm Puzzle

The Wegner Laboratory, William James Hall

Deliberate Self-Harm (DSH) refers to the deliberate damage of one's body tissue without suicidal ideation. This can include a wide range of behaviors from cutting, picking, rubbing, or burning the skin to ingesting toxic materials. Most of these acts are described as repeated impulsive behaviors with self-cutting being the most common form. While reasons for DSH are often varied, most of the current literature points to the general use of DSH as an emotional coping strategy.

Existing research on self-harmers has focused on potential risk factors, preventive measures, treatment approaches, or reasons for engaging in DSH. However, there is little to be said on the way that others relate to self-harmers. Recent studies carried out in Professor Wegner's lab at Harvard University indicate that knowledge of intentional self-harm blocks a person's empathic response towards a described deliberate self-harmer. The results of these studies have shown that subjects are more likely to remember, after reading a vignette about a girl named Jenny, superficial traits about Jenny, rather than her goals or ambitions, when she is described as a deliberate self-harmer than when she is said to have accidentally cut herself. Participants in the DSH condition also had a harder time explaining other actions performed by the target. A psychophysiological follow-up of the study showed a similar trend: lowered physiological response when watching a described deliberate self-harmer, Jenny, in pain.
However, it remains unclear why this empathy blocking occurs. In previous studies, as the reason behind Jenny's deliberate self-harming action was not explained, it is uncertain what role goal appreciation as opposed to disgust play in blocking the empathic process. By clarifying Jenny's self-harming goals we expect be able to induce a stronger empathetic response in participants towards the deliberate self-harming target. Overall, research in this area can improve the quality of social interactions that deliberate self-harmers have with peers and family and treatment that they receive from health-care professionals.

Daniel Stolper dstolper@fas.harvard.edu
Lowell 2008
Earth and Planetary Science

Environmental Proteomics: Developing Novel Protein Extraction Protocols in Order to Investigate the Metabolic Activity of Deep Sea Sedimentary Microbes

The Girguis Laboratory, Department of Organismic and Evolutionary Biology, Harvard University

Though we do not see them in our daily lives, microscopic single celled organisms make up the majority of the biomass on the Earth. In the past few years, it has been found that a large number of these microbes do not even live on the surface of the Earth or in the oceans. Rather, it has been conservatively estimated that approximately 10-30 percent of the Earth's biomass exists in a kilometer of sediment beneath the ocean floor. However, the physiology of these microbes remains largely unknown. For example, while it has been speculated that they are involved in the regulation of the release of methane from hydrates and in sulfate reduction, the extent and magnitude of these processes are uncharacterized. The regulation of these processes, it is thought, has serious implications for oceanic carbon cycling, which has strong implications for the climate. One way to examine these bacteria and understand how they are living is to investigate their proteins. My project this summer has entailed creating a protein extraction protocol that would effectively extract proteins from deep-sea environments. This protocol entails a cell suspension that concentrates cells from large amounts of sediment and a freeze thaw cycle to extract proteins. In designing this protocol, sediments from the Monterey Bay anoxic basin were experimented on and were used to demonstrate the effectiveness of the protocol. These proteins were run out on both 1-d and 2-d gels and we are currently in the process of testing mass spectrometry techniques to identify the proteins. The next phase is to use this technique on Ocean Drilling Project cores of interests that come from areas rich in methane hydrates.

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Dunster 2010
Chemical and Physical Biology

Genetic variation in the *MAL* network of S. cerevisiae

The Verstrepen Laboratory, FAS Center for Systems Biology, Harvard University

Yeast are ubiquitous in our world. They have been isolated from such diverse environments as the waxy surfaces of grapes to agricultural soil to beetles guts to the human mouth and gastrointestinal tract. In the brewing industry, yeast is added to beer wort, composed mainly of maltose and maltotriose from grains, to start the fermentation process that eventually produces ethanol and carbon dioxide. However, due to continued growth on glucose-rich media, most lab strains of yeast have lost the ability to metabolize maltose. As a result, little is known about the genetic and epigenetic regulation of the *MAL* network and the dynamics of maltose metabolism despite its industrial importance.

The *MAL* network in *Sacchromyces cerevisiae* is composed of a three gene complex that includes an Activator, a Permease, and a Maltase. Regulation of the network seems to exist on three levels: positive feedback through activator, catabolite repression on both the transcriptional and post-transcriptional levels by intracellular glucose and epigenetic control due to subtelomeric location. Differences in expression of the *MAL* genes between strains could account for the differences in fermentation efficiency.

We are interested in investigating the regulatory dynamics of the gene network, the importance of the multiple alleles of each gene, and any subtelomeric silencing at the locus. My focus has been the

epigenetic control due to the telomere positioning effect. Constructing strains with a URA3 marker replacing each *MAL* gene, we can use a *Mal+* diploid with these transformants to test for stochastic switching at each gene. Strains that express URA3 will be able to grow on URA- media, but not 5FOA media. Conversely, strains that have 'turned off' URA3 expression will not be able to grow on URA- but will grow on 5FOA media. Yeast transformations have had very low efficiency, possibly because of chromosomal remodeling at the locus that folds the DNA in an inaccessible conformation. At the moment, I am still constructing the necessary strains and will continue the experiment into the year.

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Kirkland 2009
Mathematics

Decision Making, Reaction Time, and Intertemporal Choice

National Bureau of Economic Research

Difficult decisions take longer and similar options are difficult distinguish. Across a wide variety of tasks, numerous psychology and neuroscience experiments have found a very strong positive correlation between reaction time (RT) and difficulty, as measured by the degree of similarity between options. In this paper we study the relationship between RT and intertemporal choice. In three distinct experiments, subjects are given a set of twenty-seven binary choice problems of the following form: "$X upon completion of the experiment or $Y in τ days." A well accepted fact in psychology and economics is that people discount future rewards (Strotz 1955, Loewenstein 1992, Mazur 1987), and recent work has shown these discount functions to follow a hyperbolic shape (Laibson 1997a). No work, however, has yet been done linking time-preferences, delayed rewards, and RT. Our analysis is threefold: First, using maximum likelihood and nonlinear least-squares techniques we use behavioral data to calibrate discount functions for both pooled and subject-specific data. Second, we use these models to calculate net-present-values of delayed rewards and show that difficulty of choice, as measured by a logit transformation of net-present-value difference between options, can predict subjects' RT. Third, we reverse this analysis and show that discount functions can be estimated using RT data alone, yielding parameters nearly identical to the ones derived from behavioral data. Monte Carlo simulations show that discounting models fit using RT data can predict behavior almost as well as models fit by the actual behavioral data. In our continuing work, we hope to show that by linking both behavioral and RT data we can create models with greater predictive power than those calibrated using behavioral data alone. If successful, this will challenge the revealed-preference approach to economic decision making.

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Leverett 2009
Computer Science

CitySense: An Urban-Scale Wireless Networking Testbed

The Welsh Laboratory, School of Engineering and Applied Science

Research in wireless in the last decade has been astounding; systems have grown in computational resources and scale to generate 'real-world' networking conditions. However, most research groups must be content with simulations or small-scale, homegrown test deployments to evaluate their ideas. CitySense is an open, urban-scale wireless networking testbed with the goal of supporting the development and evaluation of novel wireless systems that span an entire city; our current target city is

Cambridge, MA. CitySense is currently under development but will ultimately consist of 100 Linux-based PCs that will communicate entirely through wireless.

This summer, the first Harvard-based CitySense notes were deployed. I have addressed challenges regarding urban-scale network design, wireless communication, and peer-to-peer networking. Since CitySense is an open testbed, I have been developing the administrative backend for managing CitySense user accounts, in addition to contributing to a system from Microsoft Research that visualizes users' data. I will outline the various engineering challenges of deploying such a testbed as well as the research challenges that we face in building and supporting such a system.

Melissa Tjota mytjota@fas.harvard.edu
Quincy 2008
Biochemical Sciences

Tissue Specific Antigen (TSA) Expression and Presentation in Mesenchymal Stem Cells (MSCs)

The Turley Laboratory, Department of Cancer Immunology & AIDS, Dana-Farber Cancer Institute

Several years ago, a new source of stem cells was discovered in the amniotic fluid that surrounds developing fetuses: amniotic fluid derived stem cells (AFSCs). An important aspect of these cells is that they exhibit promiscuous gene expression, which is the aberrant expression of otherwise strictly tissue-specific proteins. Preliminary RT-PCR analysis of these cells has shown that the mRNA for several peripheral tissue antigens (PTAs) is present including Gad67, α-fetoprotein, and myelin basic protein (MBP). The implication of promiscuous gene expression in T-cell tolerance is particularly relevant to studying autoimmune diseases. In cases where there is a loss of immunological tolerance, an adaptive immune response develops against self-antigens, and it can lead to chronic inflammatory injury to tissues. Multiple sclerosis (MS) is an autoimmune disease in which the immune system destroys the myelin coating the axons of neurons. One possibility to treat MS would be to reestablish self-tolerance and stop the immune system from attacking self-antigens. To this end, a great deal of attention has been directed to understanding how immune responses to myelin basic protein (MBP), a predominant protein component of myelin, are regulated. In particular, the goal of this project is to determine whether or not AFSCs can present MBP peptide-MHC complexes to $CD4^+$ T-cells and re-establish tolerance in MS patients. This study is currently ongoing and there is still a great deal of research that must be carried out, but preliminary results have shown that MBP is transcribed and translated in AFSCs. Future experiments will involve looking at whether or not AFSCs are capable of presenting antigen to T-cells and eliciting a response from them.

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Eliot 2009
Chemistry and Physics

Synthesis of Pyrones as Ligands for Fe Catalysis

The Ritter Laboratory, Mallinckrodt, Harvard University

Pyrones present an interesting framework for low-valent iron complexes due to the potential stabilization imparted by their pyrilium resonance forms. Such iron complexes could thus catalyze cycloadditions and C-C couplings (among other reactions) currently inaccessible through iron catalysis. To this end, the synthesis of two novel 4-pyrone derivatives and their iron(II) complexes is reported. The compounds,

2,6-bis((methyoxyimino)methyl) 4-pyrone and 2,6-bis((2,6-diisopropylbenzimino)methyl)-4-pyrone, were synthesized in 8-10% yield from commercially available 2,6-dimethyl 4-pyrone. The Fe(II) dichloride and dibromide complexes of these ligands were also prepared in near quantitative yields. So far, attempts at structural characterization of these complexes have failed; nevertheless the synthetic route allows for the possibility of synthesizing a wide range of bis-imine and bis-oximate derivatives. Research into the preparation of related compounds (such as the bis-oxazoline) and into the characterization of the iron complexes is ongoing, as well as an initial investigation into their catalytic activity.

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Cabot 2010
Chemical and Physical Biology

The role of cytochrome b5 in granzyme A-mediated cell death

The Liberman Laboratory, CBR Institute for Biomedical Research, Harvard Medical School

The granzyme A (GzmA) pathway allows cytotoxic lymphocytes to induce caspase-independent target cell death. Although GzmA-mediated cell death, unlike classical apoptosis, does not demonstrate mitochondrial outer membrane permeabilization or rely on double-stranded DNA damage, it shares numerous apoptotic morphological features. In particular, the generation of reactive oxygen species (ROS), albeit by an unknown mechanism, causes the nuclear translocation of the SET complex, a key event that leads to single-stranded DNA degradation. We have identified cytochrome b5 (cytb5), a membrane-bound electron carrier hemoprotein in the P450 monooxygenase system, as a likely GzmA substrate whose cleavage may be responsible for ROS generation. After verifying that GzmA cleaves cytb5 both directly and when loaded into target cells by perforin (PFN), we investigated the effects of GzmA on both mitochondrial and ER cytb5 through the use of overexpressed tagged isoforms. Preliminary results suggest that PFN-delivered GzmA cleaves target mitochondrial cytb5 in a dose- and time-dependent manner, but does not cleave the ER isoform. Using FACS and a chromium-release assay to test if overexpressed mutant uncleavable mitochondrial cytb5 protects against GzmA-mediated cell death, we plan to collect data further elucidating the role of cytb5. We will also assess the effect of GzmA on P450 monooxygenase activity. Ultimately, our work may clarify ambiguous steps in the GzmA pathway and provide additional insight on this essential mode of cell death.

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Leverett 2010
Engineering Sciences

Aerosol Delivery of the Tuberculosis Vaccine BCG

The Edwards Laboratory, School of Engineering and Applied Science

Tuberculosis (TB) killed 1.6 million people in 2005, more than 90 percent of who live in developing countries. The primary vaccine for TB is the freeze-dried preparation of Bacille Calmette-Guerin (BCG) but its efficacy varies wildly in developing countries, ranging from 0-80%, due to a host of factors, including dramatic transportation and storage temperature fluctuations.

We are creating a spray-dried form of the BCG vaccine that is not frozen and have shown that it has greater stability at room (25C) and tropical temperatures (40C). We also are engineering the lipid content of the mycobacterial wall and cell membrane to reduce osmotic stresses during spray drying creating a more viable vaccine. We found that adding lipids adonitol and glutamic acid shows log factor

improvement in the number of colony forming mycobacterium, a measure of viability, at temperatures ranging from 4C to 40C.

We are comparing the aerosolizability of the dried vaccines with and without lipids using a model lung, known as an Anderson Cascade Impactor, and its potency *in vivo*. Our research extends to antibiotics for tuberculosis, the vaccine for yellow fever, and anti-malarial drugs and is funded by the Bill and Melinda Gates foundation.

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Leverett 2010
Neurobiology

PirB in Synaptic Plasticity

The Shatz Laboratory, Department of Neurobiology, Harvard Medical School

The human brain can be thought of as the most complex machine in the known universe. Over time, it changes to remember new languages, to appreciate the differences between fine wines, or to recover from injury. One of the most important events that occur in the brain in order to accommodate new experiences is the ability to change the strength of its trillions of synapses, called synaptic plasticity.

It was also originally thought that the brain was "immunologically privileged"—that is, that it did not seem to have proteins involved in eliciting an immune response. One such group of proteins, however, called Major Histocompatibility Complex Class I (MHCI) was discovered in neurons, and is regulated by such things as experience-dependent activity during development.

The receptor to MHCI, Paired Immunoglobulin-like Receptor B (PirB) was then shown to restrict synaptic plasticity. We hypothesize that phosphatases that normally interact with PirB cause the subsequent processes required for this restriction. To investigate this, we have created a quadruple point mutant of PirB in order to more specifically investigate the properties of PirB and its effects on synaptic plasticity. The ability to induce or impede the activity of PirB in subsets of neurons would create therapeutic opportunities for increasing or decreasing the plasticity of certain neural circuits, such as the visual system, the auditory system, the motor cortex, or language-processing areas such as Broca's area, Wernicke's area, or the arcuate fasciculus. (Isaiah 35:5-6)

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Lowell 2010
Chemical and Physical Biology

Chromosome Movement During the E.coli Cell Cycle

The Kleckner Laboratory, Department of Molecular and Cellular Biology, Harvard University

While we have a good understanding of chromosome segregation or splitting in eukaryotes (known as mitosis), there is still much to learn about the process in prokaryotes or bacteria. Unlike eukaryotes, bacteria lack a spindle structure or any obvious mechanism for dividing the replicating chromosomes. Due to the relatively small size of prokaryotes, our ability to visualize the process is limited and conclusions must be drawn indirectly. By studying the movement of the chromosome through the *E.coli* cell cycle, we hope to gain new insights into chromosome segregation.

This project used the baby cell column technique that produces populations of bacterial cells synchronized to the same point in the cell cycle. Previously created strains containing fluorescent tags at certain loci in the *E. coli* chromosome were synchronized using this method. These populations were then visualized using a fluorescence microscope to track the motion of the tagged position on the chromosome. The experiment takes z-sections (cross sectional slices) using phase contrast and red fluorescence channels to identify the position of the cell and fluorescent tag in 3 dimensions. Then, consecutive images were taken in order to quantify the degree of mobility of the fluorescent focus over 15s. This data was collected for cells in the synchronized population and repeated throughout the 2 hour cell cycle at 15 minute intervals.

Upon analysis of the data, it was the discovered that the location of cell boundaries and the position of the fluorescent focus were difficult to accurately define. It is possible that the limitations of microscope resolution and the dimness of the foci prevent accurate data collection. The use of different microscopes and mathematical fitting techniques may solve the problem and the creation of new strains with two separate fluorescent tags in different chromosomal loci opens new avenues for exploration.

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Kirkland 2008
Chemistry

Capturing AlkA in Action: X-ray Crystallography of a DNA Repair Glycosylase with Unusually Broad Substrate Specificity

The Conant Laboratory, Department of Chemistry, Harvard University

AlkA is a bacterial DNA repair glycosylase that possesses broad substrate specificity. Unlike most other enzymes of the glycosylase family, which target only a single type of DNA base damage, AlkA recognizes and processes a diverse array of nucleobase lesions. To elucidate the structural basis underlying AlkA's unusual substrate selection paradigm, we aimed to characterize the chemical interactions between AlkA's active site and various nucleobase substrates by X-ray crystallography. We synthesized lesion-containing, depurination-resistant DNA for co-crystallization with AlkA protein. Initial results revealed structures of AlkA:DNA complexes where the DNA lesion was not bound in the enzyme active site, a finding that has enabled the use of AlkA as an interface for crystallizing many lesion-containing DNA duplexes. To promote lesion extrusion into the active site cavity (the interesting binding conformation), we developed disulfide crosslinking strategies to covalently join protein to DNA in high yield. The engineered disulfide crosslink extends the duration of productive AlkA:DNA binding to time scales amenable to crystallographic study. To date, we have obtained preliminary crystals of AlkA bound to ethenoadenine, hypoxanthine, and *N7*-methylguanine; we are currently using salt and small molecule additives to screen for diffraction-quality crystals.

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Lowell 2010
Chemical and Physical Biology

Developing Targeted Transgenic Zebrafish

The Schier Laboratory, Department of Molecular and Cellular Biology, Harvard University

Transgenesis, the insertion of foreign DNA into an organism, has greatly enhanced our understanding of certain genes and what they do. However, the process presents many problems, including relatively low

efficiency and random insertion. Scientists have recently discovered that integrase from a *Streptomyces* phage, PhiC31, will induce recombination between two DNA fragments: one, attP, in the target organism, and the other, attB, on the gene to be inserted. This method has been successfully tested for efficiency in many other organisms - including fruit flies, frogs, silkworms, even mouse and human cells - but not yet in the zebrafish, an organism prized for genetics and developmental research for its clear embryos, short maturation time, and easy storage. We thus attempt to test and adapt this method for use in zebrafish.

Alexa Weingarden aweingar@fas.harvard.edu
Lowell 2008
Earth and Planetary Science

Microbial Diversity at a Hydrothermal Vent

The Girguis Laboratory, Department of Organismic and Evolutionary Biology, Harvard University

Microbial life at hydrothermal vents plays an important role in global biogeochemical cycles, such as the sulfur, carbon, and nitrogen cycles. These unique environments may also have been an ideal location for life to begin. By studying the microbial diversity over time and within different vent environments, I hope to shed light on both of these subjects. I am amplifying and sequencing 16S rRNA gene sequences of bacteria, archaea, and eukaryotes from a sulfide incubator, representing 12 months of microbial growth at a vent, as well as from original vent material, representing a mature microbial vent community. I am also examining the change in microbial populations spatially within the vent; locations deeper within the vent are characterized by higher temperatures and more reductive water chemistry, which may be reflected in the microbial community. My data will be combined with sequences obtained from other incubators in the same vent to create a broad picture of vent microbial life over time.

Stefan Wernli swernli@fas.harvard.edu
Cabot 2008
Computer Science

Control and Analysis Programs for Scanning Tunneling Microscopy

The Hoffman Laboratoryy, School of Engineering and Applied Science

I have been working in the lab of Professor Jenny Hoffman, whose primary goal is the investigation of high temperature superconductors. The lab is currently engaged in the construction of several microscopes to analyze the behavior of vortices in these superconductors. The overarching importance to this research relates to the use of high-Tc superconductors; by understanding the reaction of vortices to applied currents and changes in crystalline structure, we can learn more about how to pin these vortices and decrease the energy loss they cause. At the current time, the lab is constructing both an Spin Polarized Scanning Tunneling Microscope and a Magnetic Force Microscope, as well as testing a low temperature Scanning Tunneling Microscope at room temperature and about to begin vacuum and cool down testing.

My role in the lab has primarily been with the team testing the STM, writing code related to both the movement control of the STM tip and the analysis of data received from the microscope. Most recently I have been testing a program to control coarse STM movement that I translated from the LabView programming language into the Igor language in order to make it compatible with out data acquisition software. I used this code in conjunction with extant code to move the STM while taking data from an

integrated capacitor in order to help characterize the size of coarse movement relative to several variables in the system. I have also added new features to the code that allows researchers to view and manipulate the Fast Fourier Transform of data from the STM and use it to factor out noise from the image, which we used to analyze the first atomic resolution images received from the microscope. I also spent time integrating a real-time scope into the data acquisition program in Igor, allowing a user to monitor voltages and ffts of those signals during operation.

Jennings Xu jxu@fas.harvard.edu
Pforzheimer 2008
Biology

Sexual Dimorphic Behavior and Ultrasonic Vocalizations in Mice as Regulated by Pheromone Input

The Dulac Laboratory, Department of Molecular and Cellular Biology, Harvard University

In mice, pheromone detection is mediated by the vomeronasal organ (VNO) and the main olfactory epithelium. Male mice that are deficient for Trpc2, an ion channel specifically expressed in VNO neurons and essential for VNO sensory transduction, are impaired in sex discrimination and male–male aggression. Our project shows that female mice deficient for Trpc2 show a reduction in female-specific behavior, including maternal aggression and lactating behavior. Strikingly, female Trpc2 deficient mice also exhibit typically unique male sexual and courtship behaviors, including mounting, pelvic thrust, solicitation, anogenital olfactory investigation, and emission of complex ultrasonic vocalizations towards male and female conspecific mice.

Ultrasonic vocalizations are characteristic of a broad spectrum of rodents, but whose purpose in adult mice is relatively unclear. Current research has shown that under specific conditions, both male and female mice produce ultrasonic vocalizations (USV), though males typically produce a significantly greater quantity and density. These USV occur primarily when the mice are engaged in sexual activities, which are heavily influenced by pheromones detected through the VNO. The male-specific behaviors exhibited by Trpc2 deficient mice are highly correlated with the increased production of USV, to the point of matching or exceeding normal male USV levels. Our current ongoing project further characterizes these vocalizations in a variety of contexts and postulates possible communicative roles for their production.

Our findings provide evidence that functional neuronal circuits underlying male-specific behaviors exist in the normal female mouse brain, and suggest that VNO-mediated pheromone input to female brains act to repress male behavior and activate female behaviors.

Some of the results of our study have been published and are available at:
Kimchi T, Xu J, Dulac C. *Nature* doi:10.1038/nature06089 (2007).

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Leverett 2010
Biology

Dissecting the Role of Gfi-1 and Gfi-1b in Normal Hematopoiesis and Leukemia.

The Hock Laboratory, Center for Cancer Research, Simches Research Building, Massachusetts General Hospital

The formation of all known blood cell lineages stems from a small population of multipotent hematopoietic stem cells (HSCs). HSCs have the ability to both differentiate into mature blood cell types and duplicate themselves without differentiation, a process called self-renewal. Differentiation and self-renewing capacity in HSCs must be strictly regulated in order to ensure that the number of mature blood cells, progenitors, and HSCs does not fluctuate above or below what is necessary. One way in which these two processes can be controlled is through the use of transcription factors.

The Gfi-1 family of zinc-finger transcription factors has been shown to be a key player in maintaining the delicate balance between differentiation and self-renewal. There are two members of the Gfi-1 family, Gfi-1 and Gfi-1b. Studies using conventional knockout mice were previously completed and indicated that normal hematopoiesis during embryonic development is heavily dependent on the expression of Gfi-1. Our study involves the generation of conditional knock-out and transgenic mice to assess the role of the Gfi-1 family in adult HSC function. We hope these novel mice strains will help us understand whether or not expression of Gfi-1 and Gfi-1b in normal, adult HSCs is necessary to restrict their proliferation and maintain their quiescence and whether their expression has a role in the self-renewal ability of leukemic HSCs. In addition, because Gfi-1 conventional knockout mice were observed to have elevated levels of Gfi-1b mRNA, we plan to generate Gfi-1/Gfi-1b double knockout mice in order to investigate a possible compensation effect between the expression of Gfi-1 and Gfi-1b.

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Kirkland 2010
Biology

Target Genes of MeCP2 in Rett Syndrome

The Macklis Laboratory, Harvard Stem Cell Institute, Department of Neurosurgery, Massachusetts General Hospital

Rett syndrome is a neurodevelopmental disorder first described by Austrian physician Andreas Rett. It is the second most common cause in autism in girls, who develop normally for the first 6-18 months of life but then suddenly regress, exhibiting decelerated head growth, loss of motor skills and speech, and hand-wringing motions.

A major challenge we face with many autism spectrum disorders is that their pathology cannot be traced to a defect in a single gene or pathway. With Rett, however, we have been very fortunate. In 1999, it was found that Rett syndrome is caused by mutations in *methyl CpG binding protein 2 (MECP2)* on the X chromosome. MeCP2 is a putative transcriptional repressor that, when defective, leads to the dysregulation of the genes that it normally controls. Currently, the nature of these genes is unknown. Thus, one significant next research goal is to identify the target genes of MeCP2, because their abnormal expression very likely causes Rett.

To identify MeCP2 target genes, our lab has chosen to employ two complementary techniques, microarrays and chIP-on-chip. Using these methods, we identified 18 genes upregulated in Mecp2 mutant neurons, one of which is *MeCP2 target gene 2 (Mtg2)*. To confirm that *Mtg2* is a target gene of MeCP2, we examined the methylation status of its promoter region, as MeCP2 binds mainly to methylated CpGs in promoters. We found that the *Mtg2* promoter is highly methylated (more than 50%). These data provide reasonable evidence that *Mtg2* is in fact a target gene of MeCP2. In the coming months, further studies will be done to investigate the effects of *Mtg2* overexpression *in vitro* and *in vivo*. We hope that our results will eventually lead to the development of viable treatments for Rett patients.

Ching Zhu chingzhu@fas.harvard.edu
Dunster 2009
Biochemical Sciences

Probing Calmodulin-Proteasome Interaction in Yeast

The Finley Lab, Department of Cell Biology, Harvard Medical School

Protein turnover in higher organisms is crucial to biological processes ranging from cell cycle regulation to immune response. The ubiquitin-proteasome system is the major pathway for intracellular protein degradation in eukaryotes. In the classical model, protein substrates are marked for destruction by the attachment of a polyubiquitin chain that is required for subsequent recognition and breakdown by the proteasome. However, new information about this essential process suggests that the proteasome itself may perform more than just passive ubiquitin recognition and substrate breakdown functions. Recent research has revealed that important components within the proteasome, such as the ubiquitin ligase Hul5 and the deubiquitinating enzyme Ubp6, participate in active remodeling of ubiquitin chains on protein substrates. Thus, the proteasome itself may be involved in dynamic regulation of substrate commitment to degradation. The precise mechanisms of this regulation remain to be elucidated. Given these properties of Ubp6 and Hul5, it is of great interest that they, along with the proteasome component Rpn11, contain evolutionarily conserved calmodulin binding sequence motifs. Calmodulin is a calcium-binding protein present in all tissues and cells and responsible for modulating calcium-dependent signaling pathways. To better understand the nature of the putative calmodulin-proteasome interaction, we will create yeast strains containing proteasomes that are specifically defective in their ability to bind calmodulin. Characterization of the proteasome phenotypes of these yeast mutants may contribute to a new model of protein turnover that is far more sophisticated and interesting than previously imagined.

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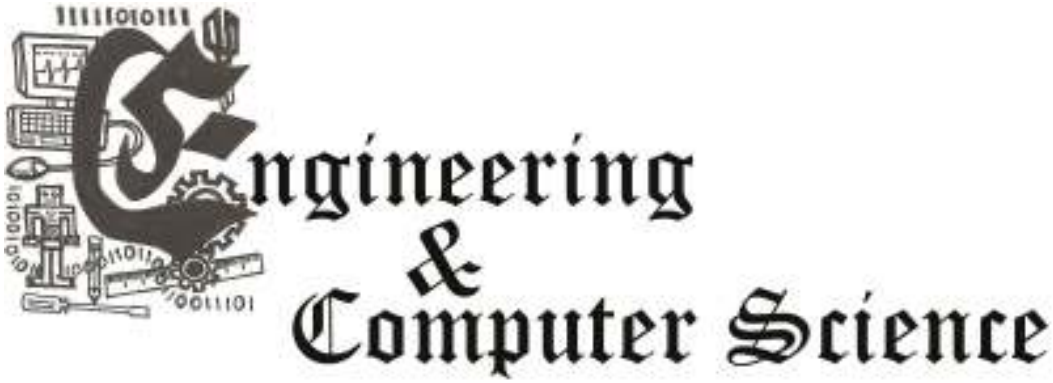

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