Quantitative real-time characterization of single-cell aging: from phenotypes to lifespan

Awardee: Murat Acar
Award: New Innovator Award
Awardee Institution: Yale University

Cellular aging is the dynamic process of accumulating genetic and molecular changes in cells. Age-associated damage to cellular structures results in the deterioration of physiological functions, leading to cell death. A variety of diseases such as cancer, type 2 diabetes, and Alzheimer's disease are linked to cellular aging; yet, our understanding into the mechanisms of cellular aging and how these mechanisms are coupled to the initiation of various disease states is very limited. For example, we know very little about how chromosome instabilities occur in old cells. A basic understanding on the set of genes and gene networks responsible from directly regulating lifespan and the mechanisms used in this regulation is also missing. This lack of understanding is contributed by the fact that cellular aging is a complex phenotype to measure and comprehensive studies on aging require the application of novel experimental approaches and technological platforms. Using the replicative aging of the yeast *Saccharomyces cerevisiae* as an experimental model, I propose to apply quantitative single-cell and single-molecule tracking approaches with the goal of: (1) uncovering the effect of bistable galactose metabolism on single-cell aging; (2) investigating how chromosomes become unstable with aging; (3) exploring the links between cellular aging and protein misfolding in single cells. To facilitate real-time measurements of replicative lifespan, we will utilize a microfluidics platform that automates the separation of daughter cells away from their mothers. Aging mother cells will be time-dynamically imaged until they no longer produce daughter cells. Results from these projects will broaden our limited understanding on how single-cells age by elucidating which genetic and phenotypic changes accompany or drive the aging process. For several decades, the labor-intensive nature of the conventional micromanipulator-based aging platforms has limited the research progress in the aging field. The inability of the colony-based aging assays to quantify aging phenotypes at the single-cell level was another important deficiency, as there are usually cell-to-cell variations in aging among the cells forming a colony. Using microfluidics platforms to automate single-cell lifespan measurements will overcome these deficiencies and limitations, and has the potential to transform the field of aging.

Innate Immune Pattern Recognition Receptors Connect Genomic Instability with Neurodegeneration

Awardee: Mark W. Albers
Award: New Innovator Award
Awardee Institution: Massachusetts General Hospital

The sequences of genomes present in individual cortical neurons from the same human vary significantly, and the degree of structural mosaicism in genomes increases with aging, which is a critical risk factor for neurodegenerative disease. We hypothesized that the *intrinsic* induction of innate immune signaling cascades in neurons arising from sporadic genomic lesions, such as expanded nucleotide repeats, e.g. *C9orf72*, or monoallelic genomic inversions introduced during repair of double stranded DNA breaks, is an unrecognized mechanism of neurodegeneration.

Olfactory sensory neurons (OSNs) are an excellent model system because they naturally undergo degeneration and regeneration, they are susceptible to degeneration in Alzheimer's disease (AD), and they are arrayed in an orderly anatomy that is genetic tractable, afforded by many genes that are specifically expressed in OSNs. We developed mouse models to elucidate mechanisms of neurodegeneration by overexpressing isoforms of the human amyloid precursor protein (hAPP) exclusively in OSNs. In two novel lines— Nd1 conditionally expressing a pathogenic mutation of hAPP, and Nd2 conditionally expressing a non-pathogenic mutation of hAPP—we find marked neurodegeneration that is dependent on transgene expression, but not the levels of APP or Aβ. RNA-seq of sorted OSNs from the Nd1 line revealed a robust induction (>10x) of a network of genes that comprise an innate immune-mediated signal transduction cascade. RNA fluorescent *in situ* hybridization affirmed that this pathway is markedly induced in OSNs in both the Nd1 and Nd2 lines. Importantly, these innate immune genes remain at basal levels in parallel mouse lines that express the same isoforms of hAPP at the same levels but do not exhibit the neurodegenerative phenotype. Suppression of the expression of either the Nd1 or Nd2 transgene reverses the expression of the innate immune pathway and the neurodegenerative phenotype. A known trigger of innate immune signaling pathways is engagement of dsRNA by pattern recognition receptors (PRRs). Our preliminary data reveal complex chromosomal rearrangements, including inversions, in the Nd1 line and the association of dsRNA expressed from the Nd1 transgene bound to a PRR, the myeloma differentiation antigen 5. Moreover, we found dsRNA arising from the hexanucleotide expansion in *C9orf72* in brains from patients with frontotemporal dementia and ALS, and we found induction of PRRs in *C9orf72* expanded and AD human brains. We suggest that genomic lesions that elicit dsRNAs can mimic viral infections and activate innate immune signaling pathways in neurons to trigger neurodegenerative disease in humans.

Funding NIH DP2 OD006662, Lefler Foundation, Wilkens Foundation

Cortical networks guiding hunger-dependent attention to real and imagined food cues

Awardee: Mark Andermann
Award: Assistant Professor, Harvard Medical School
Awardee Institution: Beth Israel Deaconess Medical Center

The goal of my laboratory is to understand how the needs of the body determine which sensory cues are attended to, learned, and imagined. In particular, we are investigating how natural and experimentally induced states of hunger modulate neural representations of sensory food-associated cues, and the consequences for obesity, binge eating, and other eating disorders. Previous studies support a simple model for hunger-dependent processing of food cues: During states of satiety, food cue information enters sensory neocortex but may not be relayed by cortical areas involved in selective processing of motivationally salient food cues, such as postrhinal cortex (POR). By contrast, during states of hunger, area POR may be attentionally 'primed' such that food cue information spreads from visual cortex through POR to subcortical areas that orchestrate food-seeking behavior. Such motivation-specific priming of cortical sensory representations may be caused by hunger-induced activation of hypothalamic AgRP neurons which, in turn, stimulate reward-related subcortical inputs to cortex.

Consistent with this model, human neuroimaging studies have identified temporal lobe cortical brain areas that respond more strongly to food-associated images than to other images during states of hunger. We are characterizing the influence of hunger on cortical area POR and other homologous brain regions in behaving mice, using novel tools for mapping and manipulating brain activity in identified neurons. We are assessing visual responses to food-associated, aversive, and neutral cues, in the same neurons across multiple daily sessions, each preceded by 24 hours of fasting or of free-feeding. We predict that states of hunger will selectively bias the evoked and ongoing activity of neural representations of food cues but not of other, non-food cues. We are visualizing the activity of hundreds of neurons in early visual cortex and in area POR, using novel methods for long-term, simultaneous two-photon calcium imaging across all cortical layers. We have established methods that will allow us to test whether the effects of fasting and refeeding on cortical activity can be mimicked by (1) rapid and reversible optogenetic activation of hypothalamic AgRP neurons, a manipulation known to trigger rapid food-seeking, and (2) by natural elevations in AgRP neuron activity (using optetrode recordings).

Together, these experiments will provide a novel conceptual and technical framework for investigating the neural pathways that underlie hunger-dependent processing of food-associated cues – a critical step towards novel, cell-type specific therapies targeting excess imagery of, and attention to, foods and food cues.

Trans-generationally inherited RNAs trigger small RNA biogenesis

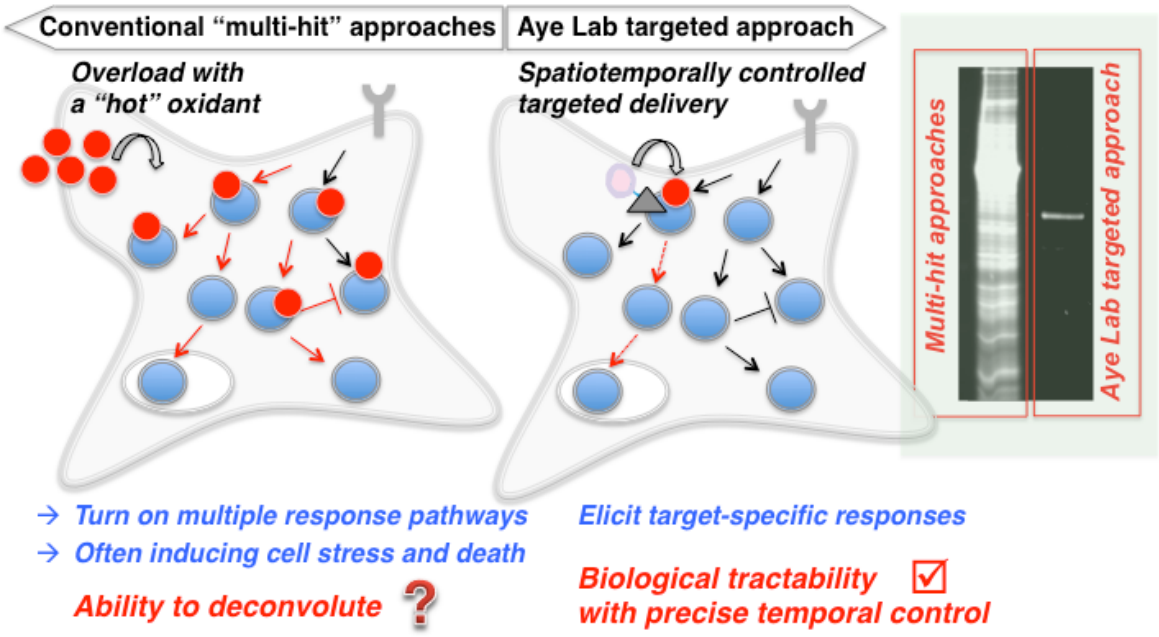
Awardee: Alexei Aravin
Award: New Innovator Award
Awardee Institution: California Institute of Technology

Small non-coding RNAs that associate with Piwi proteins, called piRNAs, serve as guides for repression of diverse transposable elements in germ cells of Metazoa. In *Drosophila*, the genomic regions that give rise to piRNAs, the so called piRNA clusters, are transcribed to generate long precursor molecules that are processed into mature piRNAs. How genomic regions that give rise to piRNA precursor transcripts are differentiated from the rest of the genome and how these transcripts are specifically channeled into the piRNA biogenesis pathway are not known. We found that trans-generationally inherited piRNAs provide the critical trigger for piRNA production from homologous genomic regions in the next generation by two different mechanisms. Inherited piRNAs enhance processing of homologous transcripts into mature piRNAs by initiating RNA processing in the cytoplasm. Second, inherited piRNAs induce installment of the H3K9me3 mark on genomic piRNA cluster sequences. The HP1 homolog Rhino binds to the H3K9me3 mark through its chromodomain and is enriched over piRNA clusters. Rhino recruits the piRNA biogenesis factor Cutoff to piRNA clusters. We propose that trans-generationally inherited piRNAs act as an epigenetic memory for identification of substrates for piRNA biogenesis on two levels, by inducing a permissive chromatin environment for piRNA precursor synthesis and by enhancing processing of these precursors.

Deconvoluting redox biology with targeted chemistry

Awardee: Yimone Aye
Award: New Innovator Award
Awardee Institution: Cornell University

The ability to dissect multicomponent signaling networks has been the bedrock of numerous biomedical breakthroughs. Despite the emerging importance of redox-dependent cell signaling, studying the regulatory roles of reactive diffusible small-molecule messengers remains devilishly difficult. The only general way to study the consequences of redox events has been through "multi-hit" approaches in which the entire specimen is treated with a reactive entity. Multi-hit strategies have yielded important information about stress-associated pathways, but hitting many targets trips many signaling switches simultaneously. Consequently, functional links between upstream modifications on specific targets and downstream response remain unknown. The impact of a single redox event on a single target has thus been completely unaddressed. My laboratory has introduced a new way to selectively flip a single redox switch in cells at a precise time by selective perturbation with redox-derived signaling electrophiles (see Figure). Here we describe an application of our new methodology in target-specific cell activation that triggers single redox events at a specific protein target. We unexpectedly discover that modest modifications on a single target are sufficient to elicit a pharmaceutically important response downstream that is equivalent to whole-cell-stimulated response. The data suggest that our chemistry-driven targeted perturbation approach is an exciting first step to understanding specificity along individual redox signaling trajectories.



Stress-response balance drives the evolution of a newcomer network and its host genome

Awardee: Gábor Balázsi
Award: New Innovator Award
Awardee Institution: Stony Brook University

Newcomer stress response modules are major contributors to drug resistance in microbial infections and cancer. Yet, their evolution is poorly understood, partly because we lack appropriate model systems that allow the development of quantitative, experimentally testable predictions. To address this problem, we used a synthetic gene circuit integrated into the budding yeast genome to model the adaptation of a newcomer stress response module and its host in three different scenarios. In agreement with computational predictions, we found that (i) mutations target and eliminate the module if it gratuitously responds to harmless signals; (ii) mutations inside and outside the module activate the module if it initially fails to respond to stress; and (iii) a select set of intra- and extra module mutations may be required to fine-tune the module's response if initially suboptimal. These findings reveal predictable, environment-dependent routes of stress-response network evolution with implications for drug resistance development in the clinic.

Biocompatible Chemistry: Interfacing Non-Enzymatic Reactions With Microbial Metabolism

Awardee: Emily P. Balskus
Award: New Innovator Award
Awardee Institution: Harvard University

Chemical modifications of small molecule metabolites occur continuously in every organism and play a fundamental role in essentially all biological processes, from the construction of the building blocks for genetic information storage and cellular enzymatic machinery to the continual processing of nutrients that sustains life. Although these reactions have been thoroughly investigated from an observational standpoint, there have been relatively few attempts to alter biological chemistry using tools and approaches from synthetic organic chemistry. My lab is currently developing biocompatible reactions: non-enzymatic chemical transformations that can be used to manipulate the structures of small molecules in the presence of living organisms, altering both metabolite architectures and biological functions. We have shown that biocompatible catalysts can utilize microbial metabolites directly for synthetic chemistry. We have discovered a palladium catalyst that can utilize microbially generated hydrogen gas in alkene hydrogenations. We have also identified an iron catalyst that can perform cyclopropanation reactions using microbially synthesized styrene. In both cases we could apply these reactions for preparative scale chemical synthesis. Simultaneously, we have also demonstrated the feasibility of using non-enzymatic chemistry to control bacterial growth. We have shown that biocompatible ruthenium and iron catalysts can promote chemical reactions that rescue the growth of auxotrophic microorganisms, supplying key nutrients required to support life and rendering growth of the organism dependent on the success of a non-biological transformation. The success of our work lays the foundation for further applications of biocompatible chemistry in synthetic chemistry, synthetic biology, and medicine.

Increasing Healthspan by Rapid and Transient Telomere Extension

Awardee: Helen M. Blau and
Award: Transformative Research Award
Awardee Institution: Stanford University School of Medicine

Co-author: John Ramunas
Co-author Institution: Stanford University School of Medicine

Telomeres are composed of repetitive TTAGGG sequences located at the ends of chromosomes and are essential for the preservation of genome integrity. Telomere shortening can result in genomic instability and the induction of a DNA damage response that may result in either senescence or apoptosis. Telomeres are extended by the enzyme telomerase, which comprises a protein component telomerase reverse transcriptase (TERT) and an RNA component (TR). Mutations in TERT or TR are found in a spectrum of diseases, the telomeropathies, including dyskeratosis congenita (50-60% of patients) and familial idiopathic pulmonary fibrosis (8-15% of patients). More broadly, as we have shown, short telomeres are implicated in other genetic diseases including Duchenne Muscular Dystrophy (1), and in diseases of aging including cancer and heart disease. Telomere extension has been proposed as a means to improve cell culture and tissue engineering, and to treat disease. However, telomere extension by non-viral, non-integrating methods remains inefficient. Here we report that delivery of modified mRNA encoding telomerase reverse transcriptase (TERT) to diverse human cell types increases telomerase activity transiently (24-48 h) and rapidly extends telomeres. Successive transfections over a four-day period extended telomeres up to 0.9 kb in a cell type-specific manner conferring up to 28 ± 1.5 additional population doublings. Notably, unlike immortalized cells, all treated cell populations eventually stopped increasing in number and exhibited senescence markers to the same extent as untreated cells. To deliver TERT mRNA in vivo we have made progress using exosomes. Exosomes are advantageous as they are 40-100 nm vesicles that transport mRNA, microRNA, and proteins naturally between cells in a targeted manner, are non-immunogenic, and release their contents directly into the cytoplasm of target cells. We are developing biomimetic synthetic exosomes that have the advantages of natural exosomes but avoid the endogenous targeting moieties and cargo that may have unwanted effects or sterically hinder loading of therapeutic molecules including modified mRNA. These technologies for rapidly extending telomeres and increasing cell proliferative capacity without risk of insertional mutagenesis should have broad utility in disease modeling, drug screening, and regenerative medicine.

(1) Sacco, A. *et al.* Short Telomeres and Stem Cell Exhaustion Model Duchenne Muscular Dystrophy in mdx/mTR Mice. *Cell* **143,** 1059–1071 (2010)
Mourkioti, F. *et al.* Role of telomere dysfunction in cardiac failure in Duchenne muscular dystrophy. *Nat. Cell Biol.* **15,** 895–904 (2013).

Selective Penicillin-Binding Protein Imaging Probes Reveal Substructure in Bacterial Cell Division

Awardee: Erin E. Carlson
Award: New Innovator Award
Awardee Institution: University of Minnesota

We are working to understand the enzymes involved in the biosynthesis of peptidoglycan (PG), a complex polymeric structure that is a major component of the bacterial cell wall and is essential for survival. PG is a common target for antibiotic therapy, but its structure and assembly are only partially understood. PG synthesis requires a suite of penicillin-binding proteins (PBPs), the individual roles of which are difficult to determine because each enzyme is often dispensable for growth perhaps due to functional redundancy. To address this challenge, we generated fluorescent derivatives of the β-lactam-containing antibiotic cephalosporin C to enable selective examination of a subset of PBPs. These probes facilitated specific *in vivo* imaging of active PBPs in both *Bacillus subtilis* and *Streptococcus pneumonia* and revealed that even PBPs that are located at a particular site (e.g., septum) are not all intermixed, but rather that PBP subpopulations are discretely localized. Presently, we are working to generate probes with other selectivity profiles, which will facilitate the construction of a more comprehensive understanding of bacterial growth and pathogenesis.

Optical trapping of individual human immunodeficiency viruses in culture fluid reveals heterogeneity with single-molecule resolution

Awardee: Wei Cheng
Award: New Innovator Award
Awardee Institution: University of Michigan

Co-authors: Y. Pang, H. Song, J.H. Kim, and X. Hou
Co-author Institution: University of Michigan

Optical tweezers use the momentum of photons to trap and manipulate microscopic objects, contact-free, in three dimensions. Although this technique has been widely used in biology and nanotechnology to study molecular motors, biopolymers and nanostructures, its application to study viruses has been very limited, largely due to their small size. Here, using optical tweezers that can simultaneously resolve two-photon fluorescence at the single-molecule level, we show that individual HIV-1 viruses can be optically trapped and manipulated, allowing multi-parameter analysis of single virions in culture fluid under native conditions. We show that individual HIV-1 differs in the numbers of envelope glycoproteins by more than one order of magnitude, which implies substantial heterogeneity of these virions in transmission and infection at the single-particle level. Analogous to flow cytometry for cells, this fluid-based technique may allow ultrasensitive detection, multi-parameter analysis and sorting of viruses and other nanoparticles in biological fluid with single-molecule resolution. (Supported by NIH 1DP2OD008693-01; WC).

Deep Sequencing of Circulating Tumor DNA for Personalized Cancer Detection and Monitoring

Awardee: Maximilian Diehn
Award: New Innovator Award
Awardee Institution: Stanford University

Circulating tumor DNA (ctDNA) represents a promising biomarker for non-invasively detecting disease burden in cancer patients while simultaneously providing access to tumor genomes. However, several technical shortcomings of existing methods for ctDNA detection, including lacking the necessary analytic sensitivity, limited breadth of patient coverage, and the need for patient-specific optimization, have prevented routine clinical application. We therefore developed a novel deep sequencing-based approach for quantifying ctDNA that is extremely sensitive and specific and can be broadly applied to nearly every cancer type. Our approach, called Cancer Personalized Profiling by Deep Sequencing (CAPP-Seq), incorporates technical and bioinformatic innovations to ultrasensitively and specifically detect ctDNA. We have successfully applied this method to plasma samples from patients with a wide variety of cancer types, including malignancies such as non-small cell lung cancer, esophageal carcinoma, and lymphoma. CAPP-Seq achieves extremely high analytic sensitivity (<0.01%), and simultaneously detects single nucleotide variants, indels, rearrangements, and copy number alterations. We will provide evidence for the potential application of our approach in a variety of clinical settings, including the detection minimal residual disease, analysis of tumor heterogeneity and subclonal resistance mutations, and distinguishing between post-treatment normal tissue changes and recurrent cancer. Finally, we will describe our work on applying CAPP-Seq for biopsy-free tumor genotyping and early detection of cancer. Our method represents a novel approach to blood-based cancer detection since it provides a personalized biomarker without the need for patient-specific optimization. Future studies will focus on further improving CAPP-Seq's technical performance and documenting clinical utility of ctDNA detection, which promises to facilitate the personalized detection, monitoring, and treatment of cancer.

Essential gene discovery in the malaria parasite *Plasmodium falciparum*

Awardee: Jeffrey D. Dvorin
Award: New Innovator Award
Awardee Institution: Boston Children's Hospital

Human malaria is a leading cause of death and disease worldwide, resulting in nearly one million deaths each year. The most severe forms of malaria result from infection by the *Plasmodium falciparum* parasite, which causes the vast majority of malaria in Africa. Deaths from malaria disproportionately affect children under five years old and pregnant women. Resistance to existing anti-malarial medications is a constant and continually emerging hurdle to the effective treatment of malaria. A molecular understanding of the fundamental biological process of *P. falciparum* replication will provide the necessary tools to develop new anti-malarial therapeutics. Although the genome of *P. falciparum* has been fully sequenced, the function of more than half of the 5,300 genes in the parasite remains unknown. Many of the genes with unknown function have little or no homology with characterized genes from other organisms. Therefore, existing molecular genetic and bioinformatics techniques cannot be used to efficiently determine the function of many of the genes in the parasite. Furthermore, existing technologies cannot predict which genes are essential for survival of the parasite. We hypothesize that these essential genes, and the proteins that they encode, will be attractive targets for the rational design of new anti-malarial therapeutics. A forward-genetic system to investigate the function of essential genes does not exist currently. We have made significant progress to develop two different and much needed forward-genetic systems in *P. falciparum.* Our first forward-genetic system relies upon a robust and tightly controlled inducible expression system to perform saturating transposon-mediated mutagenesis in *P. falciparum*. We utilize next-generation sequencing following saturating mutagenesis to identify essential genes. The immediate goal of this proposal is to generate a complete list of *all* essential genes in the blood-stage of *P. falciparum*. In a second forward-genetic system, we have designed a novel reporter parasite to allow the selection of clones with temperature-sensitive mutations in essential genes. The long-term objectives and public health implications of these studies are to identify novel targets for new anti-malarial therapeutics. This long-term goal will be achieved as a direct result of our identification of novel essential genes in *P. falciparum* parasites.

Photo-chemical activation of Toll-like Receptors for Designing Vaccine Responses

Awardee: Aaron Esser-Kahn
Award: New Innovator Award
Awardee Institution: University of California - Irvine

Our goal is to develop tools that allow researchers to design an immune response. Recent research into the immune system has revealed that foreign pathogens are detected through a series of receptors on antigen presenting cells. These receptors are synergistically activated by multiple pathogen associated molecular patterns. Materials scientists have a large role to play in the coordinated design of vaccines and synthetic activators of the immune system. We report on our development of chemical and polymeric tools for interacting with the immune system that allow us to control the spatial and temporal aspects of innate immune activation. Our methods involve the bio-conjugation of multiple different PAMPs to polymeric scaffolds and the photo-caging and un-caging of TLR agonists. These synergistic PAMP scaffolds have been tested against dendritic cells and we will report on the results. Additionally, we will report on our work coupling these synergistic combinations to cell-surface and other antigen rich environments.

Sensing Oxygen Tension in tissues with Ultrabright "Clickable" Molecular Sensors

Awardee: Conor L. Evans
Award: New Innovator Award
Awardee Institution: Massachusetts General Hospital

Co-authors: Emmanouil Rousakis*, Alexander Nichols, and Zongxi Li
Co-author Institutions: Massachusetts General Hospital, Harvard University, Harvard-MIT Health Sciences and Technology Program

Hypoxia plays a major role in diverse pathologies, ranging from diabetic and pressure ulcers to cancer metastasis and resistance. Hypoxia following complications in wounds, burns, grafts can lead to poor cosmetic and functional outcomes, with long-term hypoxia linked to chronic wounds in patients with abnormal perfusion, such as those with diabetes. In cancer, low oxygen levels have the unwanted effect of triggering pro-survival mechanisms that including cellular quiescence, upregulation of anti-apoptotic factors, and increased expression of DNA repair enzymes that allow cancer cells to survive therapies. Despite the importance of tissue oxygen concentration and the need to understand this complex oxygenation landscape *in vivo*, there are few tools that enable quantitative, dynamic mapping of oxygen tension. Our research has been focused on developing a platform for real-time, cellular to whole tissue imaging of oxygen. First, we have developed a set of bright and highly sensitive planar porphyrin molecular oxygen sensors based on near-infrared phosphorescence quenching. These *meso*-unsubstituted molecules have considerably higher phosphorescence quantum yield than existing commercial probes, enabling rapid oxygen tension sensing and image acquisition. Second, we have developed a simple, but extensible, click-chemistry based scheme that allows for the rapid growth of custom dendrimer layers surrounding these new porphyrin sensors that not only provide an extended oxygen sensing dynamic range, but are also designed to enable cellular uptake. Third, we have incorporated these sensors into liquid bandages that can be applied to the surface of tissue for real-time oxygen tension mapping. These oxygen-imaging bandages can be used to visualize the oxygen dynamics in real-time, as well as provide an assessment of equilibrium tissue oxygenation in burns and grafts.

Using Components of the Circadian Clock to Regulate Stem Cell Fate Decisions

Awardee: Brian Feldman
Award: New Innovator Award
Awardee Institution: Stanford University

Almost all organisms from bacteria to humans have molecular clocks that monitor the Earth's circadian rotation cycles and assure biological rhythms keep pace. The central 'master' clock resides in the superchiasmatic nucleus in the brain where it integrates light signals from the retina to coordinate a variety of vital physiological processes throughout the body. This is exemplified by the fact that perturbations to the clock not only impact sleep/wake cycles but also increase the risk for a number of diseases ranging from diabetes to cancer. Interestingly, peripheral cells also contain clocks that use the same molecular machinery and can be entrained by the master clock to oscillate in sync. While it is clear that master clock entrainment of peripheral cells is critical to systemic homeostasis, targeted uncoupling of peripheral oscillations from the central clock is required to meet the context-specific needs of different tissues. Yet, how this occurs is largely unknown. Metabolic processes exemplify the uncoupling phenomenon, as the content of the diet as well as feeding times appear to override centrally paced entrainment cues. As adipose tissue is integral to both the mediation of and response to systemic metabolism, we examined the role of circadian clock components in the regulation of adipocyte stem cell fate decision to elucidate the mechanism of this regulation. Adipogenesis is a multi-step process resulting in the conversion of undifferentiated progenitor cells into mature adipocytes. We have previously found that the circadian clock component Period3 (Per3) is a negative regulator of adipogenesis in bone marrow-derived stromal through a mechanism that involves interaction between Per3 and PPAR □, a transcription facto necessary and sufficient for commitment to the fat fate. Recent advances in stem cell biology enable the prospective identification and isolation of primary adipocyte stem cells from mouse adipose tissue. We are using this technology to elucidate the mechanisms by which the circadian clock in general, and Per3 in particular, regulates adipocyte fate decisions from these tissue resident adipocyte progenitors. Using a combination of knockout and tissue specific overexpression of Per3 in mouse models, we find that Per3 can toggle adipocyte differentiation signals in tissue resident adipocyte stem cells. Our studies have revealed multiple regulatory pathways downstream of Per3 that likely coordinate the adipogenesis signal. We anticipate that our findings will help elucidate the connection between the circadian clock and stem cell fate decisions.

Imaging the dynamics of neuronal dendrites and synapses in vivo on times scales from seconds to days.

Awardee: Joseph Fetcho
Award: Pioneer Award
Awardee Institution: Cornell University

Co-authors: Joe DiPietro and Dawnis Chow
Co-author Institution: Cornell University

Nervous systems are very dynamic. Their ability to produce adaptive behavior depends on the making and breaking of connections between neurons, which serves both to adjust those networks based on experience and to achieve target levels of excitability by broad homeostatic changes in synaptic strength. Even though the dynamics of dendrites and synapses is crucial, few studies have explored these dynamics in neurons in intact vertebrates. We set out to image the dynamics of entire dendritic trees and associated synapses on time scales of seconds to days to define the pattern of the dynamics and to use that to move toward revealing how the dynamics change with experience and with changes in behavioral state during circadian rhythms and sleep/wake cycles. We imaged dendritic arbors of identified motoneurons in intact, transparent young zebrafish, over both short and long time scales. Filopodial dynamics are rapid with extensions and retractions from dendrites occurring in seconds to minutes. The dynamics are widely distributed and occur at many locations throughout the entire arbor suggesting that these cells are engaged in a widespread search for new inputs. The pattern of dynamics varies between day and night. During the day, the ratio of extensions to retractions is highly variable. At night, the arbor is just as dynamic, however the extensions and retractions occur in about equal numbers. The day/night difference may be a consequence of lower activity dependent stabilization and removal of synapses at night. We have also monitored the dynamics of individual synapses by using fluorescently tagged synaptic markers of receptors and subsynaptic proteins. By tagging receptors with color change proteins, and laser converting receptors at individual synapses, we could monitor the turnover of receptors at particular synaptic sites for the first time in an intact vertebrate. Our observations of the kinetics of glycine receptors reveal time constants of receptor recovery after color change on the order of 4-6 hours, with early evidence suggesting that the time constant varies with cell type and increases in older neurons. In combination with earlier tools we developed to non-invasively image neuronal activity of zebrafish neurons throughout the brain and spinal cord, these approaches set the stage for tying synaptic level events to circuit and behavioral changes in an intact vertebrate. Supported by: NIH DP OD006411

Rapid Synthesis of Multiscale and Multicomponent 3D Tissues by Chemically Programmed Assembly

Awardee: Zev Gartner
Award: New Innovator Award
Awardee Institution: University of California, San Francisco

Co-authors: *Michael E Todhunter, *Noel Y Jee, Alec Cerchiari, Alex J Hughes, Justin Farlow, James C Garbe, Mark A LaBarge, Tejal A Desai (*equal contributions)

Co-author Institutions: University of California, San Francisco and Lawrence Berkeley National Laboratory

Tissue structure underlies the physiology of development and normal tissue function, as well as the pathophysiology of many diseases. Thus, methods for reconstituting tissue structure from single cells will be critical for tissue engineering and regenerative medicine. To construct defined three-dimensional (3D) tissue structures from single cells, across centimeter distances, and in a manner that is amenable to long-term culturing and imaging, we combine microscale direct writing of oligonucleotides with Chemically Programmed Assembly (CPA). The process begins by chemically remodeling the adhesive properties of individual cells using degradable oligonucleotide "velcro," thus allowing cells to rapidly and specifically adhere to materials and other cells coated with complementary DNA sequences. Living cells are thereby rapidly assembled, layer-by-layer, onto a DNA-patterned substrate. Assembled tissues can span several centimeters and are released directly into ECM gels for 3D culture and imaging. We demonstrate schemes for the synthesis of a variety tissues having precise sizes, shapes, cellular compositions, and spatial arrangements fully embedded within ECM gels. These tissues are assembled with single cell spatial resolution and incorporate multiple cell types, providing an unprecedented means to interrogate cell-cell interactions in the context of complex 3D tissue microenvironments. For example, we quantitatively analyze cooperative and competitive interactions between malignant and non-malignant mammary epithelial cells in the same tissue, and observe physical interactions among epithelial, endothelial, and fibroblast cells assembled into a single integrated 3D tissue.

NIH COMMON FUND HIGH-RISK HIGH-REWARD RESEARCH SYMPOSIUM
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POSTER ABSTRACTS – SESSION 1 (DEC. 15, 2014)

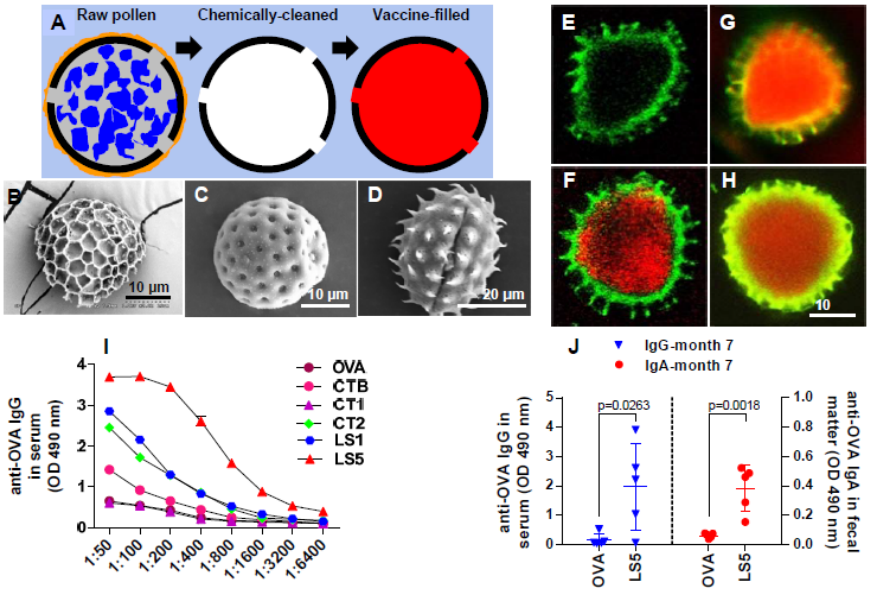
Pollen grains as 'Trojan Horses' for oral vaccination

Awardee: Harvinder Gill
Award: New Innovator Award
Awardee Institution: Texas Tech University

Objective: Oral vaccination can offer a painless, child-friendly, and convenient method of vaccination. Furthermore, in addition to system immunity it has potential to stimulate mucosal immunity through antigen-processing by the gut-associated lymphoid tissues. In this study we propose the concept that pollen grains can be engineered for use as a simple modular system for oral vaccination.

Method and Results: We demonstrate feasibility of this concept by using spores of *Lycopodium clavatum* (clubmoss) (LSs). We show that LSs can be chemically cleaned to remove native proteins to create intact hollow LS shells (**Fig 1A**). While we used LSs (**Fig 1B**), a large diversity in shapes exist as exemplified by pollens of lambs quarters and sunflower (**Fig 1 C,D**). Empty pollen shells were successfully filled with molecules of different sizes (**Fig 1E-H**) demonstrating their potential to be broadly applicable as a vaccination system. Using ovalbumin (OVA) as a model antigen, LSs formulated with OVA were orally fed to mice. LSs stimulated significantly higher anti-OVA serum IgG (**Fig 1I**) and fecal IgA antibodies compared to those induced by use of cholera toxin as a positive-control adjuvant. The antibody response was not affected by pre-neutralization of the stomach acid, and persisted for up to 7 months (**Fig 1J**). Confocal microscopy revealed that LSs can translocate in to mouse intestinal wall.

Conclusion: Overall, this study lays the foundation of using pollens as a novel approach for oral vaccination.



OVA: Ovalbumin; CT: Cholera toxin; CTB: Cholera toxin B subunit; LS: Lycopodium spores
Groups: OVA: 100 µg OVA; *LS1*: OVA(100 µg)+LS(1 mg); *LS5*: OVA(100 µg)+LS(5 mg); *CT1*: OVA(100 µg)+CT(5 µg); *CT2*: OVA(5 mg)+CT(5 µg); *CTB*: OVA(100 µg)+CTB(50 µg).

(A) Schematic illustrating use of pollens for oral vaccination. (B, C, D) Electron micrographs of Lycopodium clavatum spores, Chenopodium album (lambs quarters) and Helianthus annuus (sunflower) pollen grains. Confocal micrographs of chemically-processed lycopodium spores that are (E) empty, (F) filled with sulforhodamine (558 Da), (G) filled with ovalbumin conjugated to texas red (45,000 Da), (H) filled with bovine serum albumin conjugated to texas red (67,000 Da). (I) Titration curve of pooled day 56 mouse serum demonstrating the high anti-OVA IgG stimulated with 5 mg LSs (LS5 group) in comparison to all other formulations. (J) Anti-OVA IgG in individual mouse serum (diluted 1:400), and anti-OVA IgA in individual mouse fecal droppings (diluted 1:5). Use of 5 mg LSs (LS5 group) results in elevated IgG and IgA levels even 7 months after vaccination.

Rapid, Multiscale Sensing Using Acoustic Detection Mechanisms

Awardee: Andrew Goodwin
Award: New Innovator Award
Awardee Institution: University of Colorado Boulder

The overall goal of this work is to design a new class of in vitro and in vivo acoustic biosensors based on the interactions of biomolecules with the surfaces of soft colloidal particles. A technology that can provide a convenient, inexpensive, and portable method for detecting biomolecules without sample manipulation would provide new avenues for measuring both systemic and localized biomolecular concentrations in many different environments and media. For in vitro detection, an in-solution acoustic sensor would obviate the need for sample processing and washing steps, be scalable from microfluidics to batch process, and possess almost no background. For in vivo imaging, development of a contrast agent that can respond to levels of specific soluble biomarkers in a localized environment would provide considerable power to a ubiquitous imaging modality.

The work presented here focuses on two avenues of exploration. First, we have designed microbubbles that are able to change their response to incident ultrasound waves based on specially tuned interactions of biomolecules with their environment. Microbubbles were prepared with DNA oligonucleotides placed as crosslinkers on their surface. Through an aptamer-target interaction, the biomarker thrombin was found to displace the crosslinking strands on the bubble, restoring the bubble's ability to generate highly specific nonlinear echoes. This process resulted in 20 dB (100-fold) activation ratios and was validated in a rabbit thrombosis model, representing the first ultrasound contrast agent that could sense soluble biomarkers in vivo.

In the second approach, we are focusing on the vaporization of superheated liquid droplets specifically in response to interactions with biomolecules. Perfluorocarbon droplets are relatively stable and can be prepared ~ 200 nm in diameter for preferential extravasation into tumor tissue. While by themselves the droplets are poor ultrasound contrast agents, they can be subjected to high intensity pulses that vaporize them in situ into high contrast microbubbles, producing a very large change in ultrasound contrast. Thus a mechanism that could specifically prime the droplets for vaporization into bubbles would represent a mechanism for inducing signal in response to biomolecular challenge. Studies showing the effect of droplet aggregation on ultrasound vaporization will be presented along with future research directions.

Anticipating sudden transitions in biological populations

Awardee: Jeff Gore
Award: New Innovator Award
Awardee Institution: Massachusetts Institute of Technology

Natural populations can shift suddenly in response to small changes in environmental conditions. Examples of such sudden transitions include the collapse of fisheries in response to over-fishing and disease outbreaks in response to falling vaccination rates. Given that these population transitions can have substantial economic and health implications, it would be valuable to obtain advance warning that such a "tipping point" is approaching. Theory from nonlinear dynamics argues that these tipping points should be associated with potentially universal changes in the dynamics of the system resulting from an increase in the time to recover from perturbations. We have used laboratory microbial ecosystems to study these proposed early warning signals of impending population collapse. Yeast cooperatively breakdown the sugar sucrose, meaning that below a critical size the population cannot sustain itself. We have demonstrated experimentally that changes in the fluctuations of the population size can serve as an early warning signal that the population is close to collapse [1]. In particular, we find that the population fluctuations become both larger and slower near a tipping point leading to collapse. In addition, we have demonstrated that in spatially extended populations it may be possible to use the emergence of spatial patterns to anticipate an impending collapse [2]. The cooperative nature of yeast growth on sucrose suggests that the population may be susceptible to cheater cells, which do not contribute to the public good and instead merely take advantage of the cooperative cells. We have confirmed this possibility experimentally and found that such social parasitism reduces the resilience of the population [3,4]. Finally, advance warning of collapse of this cooperator/cheater population must be obtained by measuring the coupled dynamics of the two sub-populations [5].

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New tools for understanding the blood brain barrier

Awardee: Chenghua Gu
Award: Pioneer Award
Awardee Institution: Harvard Medical School

The central nervous system (CNS) requires a tightly controlled environment free of toxins and pathogens to provide the proper chemical conditions for synaptic transmission. This environment is maintained by the 'blood brain barrier' (BBB), which is composed of highly specialized blood vessels whose endothelium display specialized tight junctions and unusually low rates of transcellular vesicular transport (transcytosis). While BBB breakdown has recently been associated with various neurological disorders, an intact BBB also poses a major obstacle for drug delivery to the CNS. Little progress has been made on manipulating the BBB due to a significant knowledge gap in understanding how BBB function is regulated and identifying the essential molecular constituents governing its processes. This limited understanding has also thwarted our ability to therapeutically manipulate the BBB.

The major impediment to understanding the BBB is identifying its essential constituent and unraveling the mechanism by which these key regulators control BBB function. However, the current *in vitro* models rely on fully differentiated endothelial cells, which already contain unique properties that prevent their use in reconstitution studies. Similarly, the main technique to study the BBB has been EM, however its static snapshots do not provide information on active and dynamic vesicular transport, directionality, or their specific routes to allow investigators to interrogate the key molecular mechanisms that regulate BBB integrity. Recently, we mapped the precise timing of BBB formation and then identified molecules with possible roles in BBB function from simple transcriptome comparisons between CNS and peripheral endothelial cells. Surprisingly, we also found that instead of a physical buildup or disruption of structurally important tight junctions as previously thought, transcytosis regulation seems to be the more likely the major mechanism underlying BBB integrity. In characterizing these developmental properties, I realized that these findings are just the tip of the iceberg and that truly fundamental questions remain in identifying the core pathway and understand how they regulate BBB function. New tools thus are needed for understanding the BBB. Here we propose first to develop a new stem cell-based system to allow reconstitution of a functional BBB *in vitro*, and then to develop a genetic-optical system for monitoring the functional integrity of the BBB *in vivo* at subcellular resolution in real time. This integrated approach will address fundamental questions about the regulation of the BBB, which will then lead to more effective therapeutic strategies and specific targets for BBB restoration and manipulation.

Nanoscale Hyperspectral Microscopy Enables 17-Color Imaging and Benchmarks Endolysosomal Maturation in Living Systems

Awardee: Daniel A. Heller
Award: New Innovator Award
Awardee Institution: Memorial Sloan-Kettering Cancer Center

Low-dimensional nanomaterials can be made sensitive to disease biomarkers and other bioanalytes. To build optical probes using these materials, we developed a new spectroscopic imaging technique that resolved up to 17 distinct near-infrared "colors" of carbon nanotubes with single nanotube spatial resolution in live mammalian cells, in murine tissues ex vivo, and in live zebrafish endothelium. We used this capability to precisely quantify the number of fluorescent nanotubes and their emission changes in a single live cell and within individual endosomes.

The approach facilitated the transient and quantitative imaging of endosomal maturation. Nanotubes monitored vesicular fusion by the coalescence of endosomal contents—nanoparticles could be counted in single endosomes during the fusion process. Concomitantly, nanotube emission was modulated by changes in the endosomal redox environment during endosomal maturation. The fluorescence changes could be reversed by inhibitors of the maturation process—thus providing a measure of drug action.

An Accommodative Contact Lens for Presbyopic Correction

Awardee: Hongrui Jiang
Award: New Innovator Award
Awardee Institution: University of Wisconsin–Madison

Co-author(s): Xuezhen Huang, Xi Zhang, Hewei Liu, Aditi Kanhere, Yen-Sheng Lu, Seyyed Mohammad Moghimi, Jayer Fernandes, Yinggnag Huang, Guangyun Lin, Aliresa Ousati Ashtiani
Co-authors' Institution(s): University of Wisconsin-Madison

Presbyopia is the most common ocular affliction and presents an extraordinary public health issue. Our goal is to correct presbyopia by developing a new type of contact lens called an accommodative contact lens (ACL) that incorporates a tunable lens for accommodation and devices to convert light energy to electricity and store it *in situ* for the operation. We first demonstrate different types of flexible lenses based on electrowetting on dielectrics, dielectrophoretic force, and Fresnel zone plates. These lenses are fabricated onto soft polymers for ultimate integration and embedment into contact lenses. We then report on light energy harvesting devices that can simultaneously achieve storage within the same single device structure. Our approach is to incorporate polyvinylidene fluoride (PVDF) into dye-sensitized solar cells (DSSCs). To improve the charge storage capacity, we developed a novel hydrothermal process to prepare porous hierarchically nanostructured tungsten trioxide (WO_3), and then applied WO_3 to fabricate flexible supercapacitors as a storage device. Compared with traditional carbon electrodes, WO_3 nanomaterials significantly enhanced energy storage capability. In order to improve the light-harvesting efficiency of our device, we introduced a light-trapping structure in the photoelectrode via a femtosecond laser ablation technique. The processed photoelectrode was then used to fabricate DSSCs to enhance the photon-harvesting efficiency (η) by up to 13.5%. Lastly, we report on a fabrication platform to integrate the accommodative liquid lens, control electronics, and energy harvesting and storage device into the soft contact lens for presbyopic correction.

Requirement of a novel dose-dependent X-linked factor in X-chromosome inactivation

Awardee: Sundeep Kalantry
Award: University of Michigan Medical School
Awardee Institution: University of Michigan Medical School

Co-authors: Srimonta Gayen, Emily Maclary, and Michael Hinten
Co-authors Institution: University of Michigan Medical School

The disparity in X-chromosomal dosage between male and female mammals is remedied via transcriptional inactivation of most genes along one of the two X-chromosomes in females, in a process referred to as X-chromosome inactivation. Once inactivated early in embryogenesis, replicated copies of that X-chromosome are transmitted as inactive through many rounds of cell division, essentially for the life of the individual. X-inactivation therefore serves as a paradigm of epigenetic regulation. Conventionally, X-inactivation is thought to initiate via the upregulation of the Xist long non-coding RNA (lncRNA). Xist is transcribed only from the inactive-X and physically coats the chromosome in *cis*. In so doing, Xist recruits protein complexes that are posited to epigenetically silence gene expression. Here we analyze the sufficiency of Xist RNA coating to cause X-inactivation in male and female stem cells. We find that Xist induction in male mouse epiblast stem cells (EpiSCs) and embryonic stem cells (ESCs) is innocuous: in a vast majority of cells, Xist RNA coating does not lead to X-inactivation in males. On the other hand, in female EpiSCs and ESCs, Xist RNA induction almost always coincides with silencing of genes in *cis*. We also confirm this difference in X-inactivation states between males and females in mouse embryos that ectopically express Xist. Together, our *in vitro* and *in vivo* results therefore show that Xist lncRNA is insufficient to trigger X-inactivation in males, and implicate a sex-specific activity in triggering X-inactivation in females. We propose that a novel X-chromosomal factor that escapes X-inactivation is required to initiate X-inactivation in females. Its increased dosage in XX females relative to XY males triggers Xist induction and X-inactivation only in females, and is also the means by which the cell 'counts' its complement of X-chromosomes prior to inactivating one. By profiling EpiSCs via allele-specific RNA-Seq, we have compiled a short list of candidate X-inactivation escapees required to trigger Xist and X-inactivation. We will present data testing the role of these candidates in X-inactivation.

Combating Bacterial Drug Resistance by Targeting the Enzymes of Evolution

Awardee: Rahul Kohli
Award: New Innovator Award
Awardee Institution: University of Pennsylvania

The combination of a rising tide of bacterial drug resistance and the lack of novel therapies makes for a particularly dire situation in infectious diseases. In response to this need, we are interested in altering the paradigm for combating antibiotic resistance by targeting the very pathways that allow bacteria to mutate, adapt and evolve. Adaptation and the acquisition of drug resistance are tied to the pathway that governs stress responses, known as the SOS pathway. Activation of the SOS response is regulated by a key repressor-protease, LexA, which undergoes auto-proteolysis in the setting of stress, resulting in de-repression of adaptive SOS genes. Targeting LexA's self-cleavage therefore represents an attractive approach for slowing acquired antibiotic resistance by preventing adaptation and acquired drug resistance. Building towards this goal, we have dissected the unique active site architecture of LexA's protease domain, providing a basis for the rationale design of probes or inhibitors of the SOS pathway. This biochemical analysis demonstrated that the rate of LexA self-cleavage can be altered by mutations surrounding its scissile bond. By generating bacterial strains which span the full gamut of activation rates for LexA, our studies have also suggested that modulating the cleavage rate of LexA can tune bacterial evolution. Tunability in the SOS response overturns the notion of the SOS pathway as an on-or-off response and provides evidence for evolution is a dynamic and regulatable process in bacteria. These studies provide additional insight into bacterial evolution in response to antibiotics and further support the viability of targeting the SOS pathway as a novel approach to combating bacterial pathogens.

Engineering Biomaterials to Modulate the Immune Response to Implants

Awardee: Wendy F. Liu
Award: New Innovator Award
Awardee Institution: University of California, Irvine

Immune cells are central regulators of wound healing, and play an important role in the host response to implanted materials. In particular, macrophages are innate immune cells that help orchestrate this response by performing a variety of functions in response to biochemical and physical cues in their microenvironment. Our laboratory seeks to better understand how microenvironmental cues regulate the behavior of macrophages in order to design better biomaterials for medical devices and tissue engineering. In one approach, we leverage the ability of immune cells to distinguish self from non-self via specific molecules expressed on the surface of host versus foreign surfaces. We found that decorating biomaterials with an endogenously expressed immunomodulatory self-antigen, CD200, inhibits macrophage activation in vitro and inflammatory cell infiltration in vivo. Current work is focused on promoting the longevity of these surfaces, and testing the effects of CD200 on biomaterials including poly(ethylene glycol) and poly(lactic-co-glycolic acid). In a complementary approach, we are engineering biomaterial surfaces with physical cues that modulate the inflammatory versus wound healing behavior of macrophages. We recently discovered that the geometry of adhesion, and specifically the elongation of macrophages promotes their polarization towards an alternatively activated, pro-healing phenotype. Elongation was synergistic with cytokines that stimulate a pro-healing response, but protected cells from inflammatory stimuli. Interestingly, surface topographies, such as grooves, that lead to changes in cell shape can also control the polarization state of macrophages, and thus influence the host response to implanted materials. Continued work focuses on better understanding the mechanism by which macrophages sense the physical microenvironment to regulate their behavior. Together, these studies aim to design materials to control the host immune response and thus improve strategies for wound healing and regeneration of tissue surrounding implanted materials.

Diverse Epigenetic Roles of Protein Methyltransferases via Nonhistone Methylation

Awardee: Minkui Luo
Award: New Innovator Award
Awardee Institution: Memorial Sloan-Kettering Cancer Center

Epigenetic regulations are involved in establishing cell-lineage diversity and the errors in these processes have been linked to many diseases including developmental abnormalities, neurological disorders and cancer. Among the key biochemical modifications in epigenetics is protein methylation, a process orchestrated by over 60 human protein methyltransferases (PMTs) with *S*-adenosyl-*L*-methionine (SAM) as a cofactor. Defining the targets of the PMTs is pivotal toward elucidating their roles in normal physiology and disease states. To address this situation, the Luo laboratory recently developed BPPM (Bioorthogonal Profiling of Protein Methylation) technology for profiling the histone and nonhistone targets of multiple PMTs inside living cells. Here, human SAM synthetase was engineered to process metabolite mimics (terminal-alkyne-containing methionine analogs), thus allowing *in situ* production of the corresponding SAM analogues. We have successfully implemented the BPPM approach to > 20 human PMTs and showed that each of the PMTs can readily methylate 200 ~ 2000 nonhistone targets, whose functions can associate with most essential biological pathways such as DNA replication, RNA processing, other posttranslational modulars and metabolic enzymes. Here we particularly followed up the functional roles of RBM15 methylation by PRMT1. The single methylation event of RBM15 is sufficient to recruit E3 ligase (CNOT4) and program RBM15 to degradation. We further showed that acute megakaryoblastic leukemia (AMKL, M7) depends on the high level of PRMT1 to down-regulate RBM15 and thus RBM15-involved splicing. PRMT1-RBM15 pathway in AMKL leads to multiple alternative splicing isoforms such as RUNX1, c-Mpl and GATA1, which suppress differentiation and promote differentiation. Our results imply that targeting PRMT1/RBM15 pathway might have therapeutic potential in AMKL.

Seeing molecular vibrations: optical imaging of small molecules for biology and medicine

Awardee: Wei Min
Award: New Innovator Aware
Awardee Institution: Columbia University

Fluorescence is the most popular contrast mechanism employed in modern optical imaging. A number of versatile fluorescence-based techniques, such as single-molecule spectroscopy, two-photon excited fluorescence microscopy and super-resolution imaging, have flourished and transformed the way modern life sciences are conducted. However, fluorescence imaging faces fundamental limitations for studying the vast number of small bio-molecules such as metabolites (e.g., amino acids, nucleic acid, fatty acids and glucose), secondary messengers, neurotransmitters and drugs. This is so because (1) most of the small bio-molecules are intrinsically non-fluorescent and (2) labeling of these small molecules by the relatively bulky fluorescent probes (either organic dyes or fluorescent proteins) would strongly perturb or even destroy the native biochemical activities of these small bio-molecules inside cells. Therefore, how to visualize and study these vital chemical species inside living cells represents a grand challenge. Novel imaging techniques that accomplish this goal would enable researchers the unprecedented ability to map out distributions and to follow dynamics of a wide variety of important small bio-molecules, transforming our ability to study biochemistry and biophysics in living systems. To address this challenge, we have developed a novel optical imaging platform by coupling the emerging stimulated Raman scattering (SRS) microscopy, which is capable of producing concentration maps of chemical bonds in biological samples, with three distinct classes of small vibrational tags with characteristic Raman transitions, including alkyne moieties (i.e., C≡C triple bond, Figure), targeting these vibrational tags, SRS microscopy is well suited for probing in vivo metabolic dynamics of small bio-molecules which cannot be labeled by bulky fluorophores [1-5]. Physical principle of the SRS microscopy and emerging biomedical applications such as imaging lipid metabolism, protein synthesis, DNA replication, protein degradation, RNA synthesis, glucose uptake, drug tracking and tumor metabolism [1-5] will be presented.

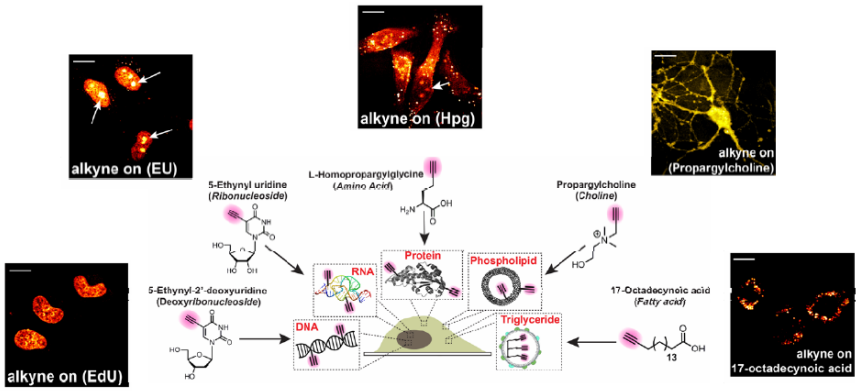

Figure. SRS imaging of *de novo* synthesis of DNA, RNA, proteomes, phospholipids and triglycerides, respectively, through metabolic incorporation of alkyne-tagged deoxyribonucleoside (EdU), ribonucleoside (EU), amino acid (Hpg), choline (propargylcholine) and fatty acid (17-octadecynoic acid) into live mammalian cells [1].

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Challenging the conventional view of zinc in biology and establishing zinc as a regulator of the transcriptional and metabolic program in cells

Awardee: Amy E Palmer
Award: Pioneer Award
Awardee Institution: University of Colorado

Zinc is absolutely essential to all forms of life. It is a crucial building block of cells and has been implicated in many fundamental functions, such as DNA synthesis, transcription, metabolism, and apoptosis. For organisms, zinc is required for growth, development and immune function, and perturbation of zinc is associated with numerous pathologies. Given the centrality of zinc in cell biology and human health, it is astounding that at the most fundamental level we still don't understand how zinc status and availability impact basic cellular functions, and the proteins that sense changes in zinc in order to regulate cellular processes remain a mystery. The traditional model of zinc in biology asserts that the ~ 2000 proteins that comprise the zinc proteome bind zinc constitutively. This Pioneer Project will explore a fundamentally different model where zinc acts as a cellular signal and direct regulator of transcription and metabolic processes by titrating occupancy of the zinc proteome. The central premise is that in a low zinc state the vast repertoire of zinc binding proteins will only be partially saturated with zinc, giving rise to reduced output from zinc-dependent transcription factors and enzymes. When zinc levels increase, the fractional saturation of zinc binding proteins will increase, leading to increased output. The consequence of this model is that regulation of zinc dictates occupancy of the zinc proteome, providing a link between dynamic metal regulation and a wide swath of cellular signaling responses. Thus, changes in zinc status – either dynamically during physiological signaling, or permanently as a consequence of disease – could fine-tune the activity of hundreds of zinc-dependent proteins, establishing zinc as a major regulator of cellular function. This challenges the conventional view that zinc is constitutively and stably bound to the proteins and enzymes that constitute the zinc proteome, and establishes a new paradigm for understanding the impact of metal homeostasis on physiology and health. To test this hypothesis, we will 1) Develop methodology for defining zinc occupancy of the proteome and how it changes with: (i) systematic perturbation of metal availability, (ii) zinc dynamics induced by calcium signaling, and (iii) different disease states; 2) Define the downstream consequences of partial occupancy on both the transcriptional and metabolic program of cells; and 3) Define the consequences of altered zinc homeostasis in cellular models of disease.

Adaptive translation as a mechanism of stress response in biology

Awardee: Tao Pan
Award: Pioneer Award
Awardee Institution: University of Chicago

Co-authors: Michael Schwartz and Xiaoyun Wang
Co-author Institutions: University of Chicago

Genetic diversification is a fundamental requirement for adaptation to changing environments. This process is generally regarded to be time consuming and beyond the scope of the biochemistry within individual cells, which cannot deliberately alter their genetic code. However, recent evidence has revealed that cells can circumvent their genetic confines by making diversified proteins which do not strictly adhere to the amino acid sequence specified in DNA. This process is mediated by the incorporation of nongenetically encoded methionine residues in specific amino acid positions, which is accomplished by misacylation of nonmethionyl-tRNAs with methionine (1). Misacylation of non-Met-tRNAs with Met is highly regulated and can range from 0.01-10% of the Met-tRNAs charged with Met in the cell. Met-misacylation occurs in all three kingdoms of life. We hypothesize that “adaptive translation” can adapt the proteome of an organism for optimal function in varying environments and may be advantageous in conditions for which there is no effective transcriptional response. We are testing this hypothesis in all three branches of life. In *E. coli*, adaptive translation is regulated by specific post-translational modification of the methionyl-tRNA synthetase; chromosomal tRNA synthetases mutant strains that can no longer mischarge are deficient in responding to many types of environmental stresses. In an extreme archael thermophile, adaptive translation is regulated by temperature; Met-substituted mutant enzymes may perform better at the corresponding growth temperature. In human cells, we show that specific Met-substituted mutant enzymes can indeed show very distinct reactivities and cellular localization. Our investigation aims to establish that deviation from the central dogma has evolved to facilitate adaptation to varying natural conditions and in stress response.

(1) Netzer, Goodenbour et al.: *Nature* 462, 522-526 (2009).

Wnt/Notum spatial feedback inhibition controls stem cell activity to regulate planarian brain size in a program of reversible regenerative growth

Awardee: Christian P. Petersen
Award: New Innovator AwardEric Hill and
Awardee Institution: Northwestern University

Co-author: Eric Hill
Co-author Institution: Northwestern University

Signaling pathways that influence growth have been described in detail, but the developmental mechanisms that direct organ size and proportion are incompletely understood. Animals capable of adult regeneration perfectly restore their form after injury, providing an informative model for identification of processes that enable attainment of appropriate size. The planarian *Schmidtea mediterranea* can undergo whole-body regeneration due to a population of adult pluripotent stem cells as well as information that coordinates their activity, and is capable of reversible and proportional growth over an order of magnitude in size by regulating cell number. Using quantitative assays of regeneration extent, we find that planarians proceed from divergent initial tissue compositions created by injury toward a final form with appropriate organ proportions. We find that planarian *notum* and *wntA*, expressed at opposite axes of the planarian brain, exert opposing functions on brain proportion in contexts of both regenerative growth and degrowth. Notum proteins are conserved secreted hydrolases that antagonize Wnt signaling through an unknown mechanism. Double RNAi and expression analyses suggest that *notum* engages in feedback inhibition of *wntA* to control a set-point of brain size attained by divergent regeneration programs. This signaling does not modulate injury-induced cell death or global proliferation but instead directs the neural specification of neoblasts, a cell population that contains adult pluripotent stem cells. These results indicate spatial feedback inhibition can regulate organ size through stem cell control and establish planarians as a system for studying the developmental mechanisms that enable proper growth.

Engineered Environments to Understand Breast Cancer Spread and Drug Resistance

Awardee: Shelly Peyton
Award: New Innovator Award
Awardee Institution: University of Massachusetts Amherst

Metastasis is the leading cause of fatality for women diagnosed with breast cancer. The most common anatomical sites of distant tumor growth include the brain, lung, liver, and bone, and it is well known that this metastatic spread in breast cancer is not random. Rather, different clinical subtypes of breast cancer exhibit unique patterns of metastatic site preference, called tissue tropism. Given the physical and chemical diversity of these secondary tissue sites, my lab hypothesizes that there is a relationship between the biophysical and biochemical properties of the tissue, and the ability of cells within a particular subtype of breast cancer to adhere, migrate, grow, and respond to chemotherapeutics at these secondary sites.

To quantify this relationship, we have created biomaterial microenvironments, which capture the integrin-binding, stiffness, and stem-cell recruitment properties of the secondary site tissues often recipient of breast cancer spread (brain, lung, and bone), and quantified cell adhesion, polarization, motility, and response to chemotherapeutics. This platform consists of a new class of hydrogels with an extremely wide range of mechanical properties that combine poly(ethylene glycol) (PEG), and phosphorylcholine (PC) zwitterions. Cell response to EGF and integrin antibodies was tissue specific, and, excitingly, this “tissue mimic sensitivity” reflects the known clinical site-specificity in human patients.

Separately, we have observed that tissue stiffening increases the resistance of carcinoma cells to sorafenib by nearly three-fold. Toward rational identification of combinatorial treatments, we used a bead-based ELISA (MAGPix, Luminex) and have identified JNK as a promising phospho-protein target, which provided survival signals during substrate stiffening. Dosing carcinoma cells with JNK inhibitor and sorafenib was the one combination, which eliminated stiffness sensitive resistance. We propose that these types of biomaterial environments can be used to predict tissue-specific spread, and may serve as a system that pharmaceutical companies can use to rule out false positives and potentially save billions of dollars in the drug development pipeline.

Illuminating the Mechanistic Pathways of Polymer-Mediated Nucleic Acid Delivery

Awardee: Theresa M. Reineke
Award: New Innovator Award
Awardee Institution: University of Minnesota

The wealth of information being obtained from genomic, proteomic, and glycomic research is allowing researchers to unravel the intricate genetic and epigenetic mechanisms associated with human health and disease. The intracellular delivery of nucleic acids to study these processes offers unprecedented promise for revolutionizing biomedical research and novel drug development. However, the nucleic acid delivery vehicle plays a central yet elusive role in dictating the efficacy, safety, mechanisms, and kinetics of gene regulation in a spatial and temporal manner; thus having a far-reaching impact in health-related research. We have developed several novel carbohydrate-based cationic polymers that have shown outstanding affinity to bind and encapsulate oligonucleotides and plasmid DNA into nanocomplexes (polyplexes) that facilitate highly efficient intracellular delivery while minimizing toxicity. We aim to further understand our wide-range of polymer delivery vehicles for their mechanistic pathways and kinetics of nucleic acid encapsulation and intracellular transport from the cell surface to their final intracellular destination. Our New Innovator Award Program has been driven by three specific goals: 1) to unravel the molecular-level interactions between structurally-diverse yet analogous polymeric delivery vehicles and differing nucleic acid types and to correlate these interactions with the biological stability and mechanisms of the subsequent polyplexes, 2) to understand the interactions of these various polyplex types with cell surface glycosaminoglycans and compare polyplex structure to receptor selectivity and mechanisms of cellular uptake, and 3) to decipher the intracellular trafficking pathways in a spatial and temporal manner from uptake to the final destination for each polyplex form. Glycopolycation vehicles have been synthesized that vary in carbohydrate type and cationic functionality. We show that the polymer structure significantly impacts the ability of the vehicle to bind and compact various nucleic acids (siRNA and pDNA) into polyplexes and polyplex structure dictates nanocomplex colloidal stability. The role of polymeric chemistry on intracellular trafficking and in vivo delivery has been studied via several methods. Polyplexes were found to associate with heparan sulfate on the cell surface and are internalized by endocytic mechanisms involving both caveloae and clathrin. Immunofluorescence images indicate that some polymer vehicles traffic nucleic acids to the Golgi and endoplasmic reticulum (ER), and colocalization experiments indicated retrograde transport of polyplexes *via* COP I vesicles from the Golgi to the ER. Multiple high resolution tomographic images of whole cells were captured via confocal microscopy at various timepoints. The images were reconstructed to visualize and quantify trafficking kinetics *in situ* in a spatio-temporal manner.

Circuit principles of neuronal processing in larval drosophila melanogaster thermotaxis

Awardee: Aravinthan Samuel
Award: Pioneer Award
Awardee Institution: Harvard University

Co-authors: Bruno Afonso, Mason Klein, Matthew Berck, Ivan Larderet, Marc Gershow, James Truman, Simon Sprecher, Albert Cardona, and Marta Zlatic

Co-author Institutions: Harvard University, Howard Hughes Medical Institute Janelia Farm

The goal of systems neuroscience is to understand the computational process by which neural circuits use sensory information to generate adaptive behaviors. *Drosophila* larvae avoid excessively cool temperatures using a small set of sensorimotor transformations that regulate the frequency and outcome of navigational decisions. Navigational decisions separate successive periods of forward movement. During each navigational decision, larvae sweep their head from side to side, gathering thermal information that informs the choice of a new direction for forward movement.

Automated trajectory and posture analysis of individual animals navigating linear temperature gradients enables us to quantify each navigational decision along the trajectory of each animal. Statistical analysis of transgenic strains with defined lesions to specific parts of the larval nervous system from the Rubin Gal4 collection allowed us to define neurons that participate in information processing during thermotaxis.

We have identified two distinct groups of projection neurons that when inactivated exclusively modulate individual navigational decisions, such as turn direction and run length. We mapped the upstream partners using electron microscopy reconstruction and found they receive direct synaptic inputs from the cold sensing neurons. Furthermore we show these neurons respond to cooling using calcium imaging. We are currently characterizing in more detail the computational dynamics of these neurons by measuring and manipulating neuronal activity in freely moving and/or restrained animals using novel methods in optical neurophysiology. Combining behavioral analysis, EM reconstruction of behaviorally important neurons and functional imaging will allow the complete identification of circuits underlying thermotaxis from sensory inputs to motor outputs.

The Better THAN Study: Targeting Heavy Alcohol use with Naltrexone

Awardee: Glenn-Milo Santos
Award: Early Independence Award
Awardee Institution: San Francisco Department of Public Health

Background:
Binge drinking (defined for men as drinking five or more drinks on one occasion), also known as heavy episodic drinking, is highly prevalent in the US. Binge drinking accounts for more than half of the 80,000 annual deaths attributed to excessive alcohol consumption. In 2006, the economic costs of binge drinking exceeded $170 billion in the US. National HIV Behavioral Surveillance data indicate that 57% of men who have sex with men (MSM) reported binge drinking (past 30 days).

Binge drinking among MSM has been independently associated with unprotected sex and HIV infection. Binge drinking is by far the most prevalent exposure attributed to HIV infections among MSM, who comprise over half of the 56,300 new HIV infections in the US in 2006. Thus, effective interventions to reduce binge drinking among MSM may function as an important HIV prevention intervention by reducing alcohol-related sexual risk behaviors. Despite the high prevalence of binge drinking and the continued domestic HIV epidemic among MSM, few alcohol interventions have been proven to be effective in this population.

Oral naltrexone is a low-cost FDA-approved medication for alcohol dependence with few toxicities. Naltrexone is a μ-opioid receptor antagonist that attenuates the rewarding effects of alcohol. The standard daily regimen for oral naltrexone hampers compliance and alternate regimen schedules have been proposed to increase effectiveness of the drug and expand the population that may benefit from this pharmacologic intervention. One promising approach is the intermittent, targeted administration of naltrexone, whereby individuals take the medication as-needed, in anticipation of heavy drinking.

Research Design:
This project, entitled The Better THAN Study, will evaluate the efficacy of targeted dosing of oral naltrexone among non-dependent binge-drinking MSM at risk for acquiring or transmitting HIV. This is a double-blind, placebo-controlled trial of 120 binge-drinking MSM to 12 weeks of naltrexone 50mg, to be taken in anticipation of heavy drinking. MSM will be seen weekly for alcohol-metabolite urine testing, study drug dispensing and brief counseling for alcohol use. Safety assessments and behavioral surveys will be completed monthly. Efficacy on alcohol consumption and alcohol-associated sexual risk behhaviors (Aims 1-3) will be assessed using weekly time-line follow-back, screening for ethyl glucuronide (EtG)-positive urines, and computer administered monthly interviews. Tolerability and acceptability (Aim 4) will be assessed through tracking of adverse events and medication adherence. GEE models will be fitted to estimate treatment effects on repeated study outcomes.

An *in vivo* sensor for detection of cancer metastasis

Awardees: Lonnie Shea and Vadim Backman
Award: Transformative Research Award
Awardees Institution: Northwestern University

Co-authors: Samira M. Azarin, Ji Yi, Robert M. Gower, Brian A. Aguado, Megan E. Sullivan, Ashley G. Goodman, Eric J. Jiang, Shreyas S. Rao, Yinying Ren, and Jacqueline S. Jeruss
Co-author Institutiions: Northwestern University, Northwestern University Feinberg School of Medicine, Robert H. Lurie Comprehensive Cancer Center of Northwestern University, University of Michigan, University of Minnesota, and University of South Carolina

Breast cancer is a leading cause of death for women, with mortality resulting from metastasis. Currently, metastases are often detected once tumor cells affect the function of one or more solid organs, with a high disease burden limiting effective treatment. Herein we report a method for early detection of metastatic cells using a biomaterial scaffold implant to recruit and capture metastatic cells *in vivo*, which achieved high cell densities within the scaffold and a reduced tumor burden within solid organs. Recruitment to the scaffold was mediated, in part, by the local immune cells. The presence of metastatic cells in the scaffold was identified through a label-free detection system using inverse-spectroscopic optical coherence tomography (IS-OCT), which identifies changes to nanoscale tissue architecture associated with the presence of tumor cells. If applied at the time of tumor resection or completion of primary therapy for patients at risk for the recurrence of malignancy, this technology has the potential to identify metastatic disease at the earliest stage. This early identification could then enable initiation of life-preserving therapies while the disease burden is low, favorably impacting disease course.

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A rationally-designed fluorogenic protease reporter visualizes spatiotemporal dynamics of apoptosis *in vivo*

Awardee: Xiaokun Shu
Award: New Innovator Award
Awardee Institution: University of California, San Francisco

Fluorescence resonance energy transfer-based reporters have been widely used in imaging cell signaling. However, their *in vivo* application has been difficult due to poor signal. While fluorogenic reporters overcome this problem, no such reporter of proteases has been demonstrated for *in vivo* imaging. Now we have designed a fluorogenic reporter that becomes fluorescent upon protease activation and requires no exogenous cofactor. To demonstrate biological applications, we have designed a fluorogenic executioner caspase reporter (iCasper) to image apoptosis in vivo, which plays fundamental roles in animal development and disease. iCasper visualizes spatiotemporal dynamics of apoptosis during embryonic development of *Drosophila*, which suggests spatiotemporal coordination between apoptosis and morphogenesis. iCasper also reveals dynamics of apoptosis during tumorigenesis of the larval brain, which may suggest overproliferation induced apoptosis followed by evasion of apoptosis. iCasper will thus be an important tool in understanding biological function and molecular regulation of apoptosis during development and may shed light on molecular mechanisms of tumorigenesis that require evasion of apoptosis. The designed scaffold can be used to engineer genetically encoded fluorogenic reporters of other proteases, which play important roles in a diverse range of biological processes and disease. Our work opens a new door in designing bio-reporters by harnessing unique protein-chromophore interaction.

Induction of cancer cell death by selective DNA misincorporation

Awardee: Derek Taylor
Award: New Innovator Award
Awardee Institution: Case Western Reserve University

Telomeres cap and protect the ends of all human chromosomes. In healthy adult tissue, telomeres shorten with each round of cell division as part of the natural aging process. By limiting human cells to a finite number of divisions before induction of programmed cell death, telomere erosion functions as a tumor suppressor. Conversely, in cancer cells, an enzyme called telomerase is upregulated to synthesize telomere DNA and, thus, nullify the limited number of cell divisions. The upregulation of telomerase in ~90% of metastatic tumors is a primary contributor to the cancer cell's unlimited proliferative properties. Due to this unique and critical role in cancer biology, telomerase provides a novel target for innovative therapeutics. As such, direct telomerase inhibitors are currently being developed, with several compounds showing promise in treating a wide range of human cancers. However, the primary shortcoming with this methodology is that even after telomerase inhibition, the cancer cells must go through multiple rounds of division before telomere attrition results in replicative senescence. This delay allows cancer cells to develop other mechanisms of survival, such as alternative lengthening of telomere mechanisms, to overcome the effects of telomere shortening caused by telomerase inhibition.

This project is designed to explore a novel mechanism to use telomerase to deliver small molecule drugs to cancer cells specifically. Telomere DNA is bound and protected by specialized proteins including telomere repeat binding factors 1 and 2 (TRF1 and TRF2) and protection of telomeres 1 (POT1) proteins. TRF1/2 and POT1 bind telomere DNA with high specificity, such that a single change in telomere DNA sequence drastically reduces the binding efficiency. Abrogation of POT1 or TRF1/2 binding to telomeres induces an immediate DNA damage response. We are investigating whether the misincorporation of non-native nucleotide analogs by telomerase into telomeric DNA will abrogate POT1/TRF1/TRF2 binding. The inability of telomere proteins to bind and protect the telomeres should elicit an immediate DNA damage response and initiate cell death specifically within cancer cells. Measuring potency and selectivity against telomerase-positive and telomerase-negative cancer cells is used to validate the cell-killing potential, and molecular mechanism, of these non-native nucleotide compounds.

A robust platform for engineering multi-input and multi-output (MIMO) circuits in mammalian cells

Awardee: Wilson Wong
Award: New Innovator Award
Awardee Institution: Boston University

Synthetic genetic circuits that can integrate multiple input signals and deliver multiple outputs are increasingly important in cell-based therapy and animal model development. However, synthetic biology remains mostly a microbial-centric discipline, and complex genetic circuits remain challenging to engineer, regardless of the host. Here, through the use of site-specific recombinases (SSRs) and their corresponding recombination sites, we have constructed, in mammalian cells, a suite of multi-input and multi-output (MIMO) circuits, such as a six-input AND gate, half adder, half subtractor, 2:4 decoder, full adder, full subtractor. Moreover, we also created a field-programmable read-only memory (FPROM) device that can select between sixteen two-input logic gates based on four inputs. More importantly, we were able to create all of our computation circuits in a single transcription unit —no linkages between different transcription units are required to achieve the desired functionality. This development bypasses one of the most difficult challenges in synthetic circuit design, and allowed all of our circuits to be successfully implemented on first attempt without optimization. In fact, we have created more than 100 functionally distinct 2-Input-2-Output circuits. This platform provides a powerful tool for high-level mammalian cells reprogramming for animal models development. Furthermore, our circuit design platform minimizes the typical “design-build-test” cycle that plagues the progress of most synthetic biology projects, and illustrates a new paradigm in synthetic genetic logic circuit design.

In Vivo Rescue of the Hematopoietic Niche by Induced Pluripotent Stem Cells

Awardee: Joy Y. Wu
Award: New Innovator Award
Awardee Institution: Stanford University

Co-authors: Rhiannon Chubb, James B. Oh, Alyssa Riley, Xiaojing Huang, and Sean M. Wu
Co-author Institutions: Massachusetts General Hospital, Harvard Stem Cell Institute, and Stanford University School of Medicine

Mesenchymal progenitors and cells of the osteoblast lineage play critical roles in supporting bone marrow hematopoiesis, and cells at specific stages of differentiation contribute to distinct hematopoietic niches. For example, mesenchymal stem cells support hematopoietic stem cells, while osteoprogenitors are crucial for the differentiation of B cell precursors. However, understanding the molecular mechanisms underlying these stage-specific contributions is limited by the inability to prospectively harvest defined mesenchymal populations in large numbers. Induced pluripotent stem (iPS) cells, like embryonic stem (ES) cells, are capable of self-renewal and differentiation into cell types of all three germ layers. Our goal is to use iPS technology to enrich for defined populations along the osteoblast lineage, and to evaluate their distinct roles in supporting hematopoiesis in vivo. To assess the osteogenic potential of iPS cells in vivo, we have employed a model of skeletal complementation. Mouse embryos lacking Runx2, a transcription factor required for differentiation of bone-forming osteoblasts, display a failure of osteoblast differentiation, absence of bone formation, and lack a hematopoietic bone marrow. We have introduced wild-type ROSA-YFP iPS cells into Runx2 null blastocysts and assessed YFP^+ cell contribution by whole-mount fluorescence and histological analysis. We observed the presence of YFP^+ iPS cells in resulting chimeric embryos, with partial skeletal formation that increases in proportion to iPS contribution. Furthermore, these iPS cells were associated with areas of mineralization and restored bone marrow hematopoiesis. In a second model, targeted ablation of osteoblasts with dipheria toxin in chimeric embryos harboring wild-type iPS cells revealed grossly normal skeletal morphology in embryos where iPS contribution exceeds 30%. In summary, iPS cells can undergo osteogenic differentiation in vitro and partially reconstitute an osteoblast-deficient skeleton with hematopoietic marrow in vivo. Further investigation using genetically modified iPS cells with stage-specific fluorescent reporters will enable us to investigate the individual contributions of mesenchymal progenitors, osteoprogenitors, and maturing osteoblasts to distinct hematopoietic niches in vivo.

DNA probes for highly multiplexed, precisely quantitative, ultra-resolution imaging

Awardee: Peng Yin
Awards: New Innovator Award and Transformative Research Award
Awardee Institution: Harvard University

Living organisms are complex molecular systems. Imaging provides a natural and direct way to investigate such systems and is thus becoming a central tool for biomedical science. However, due to limitations of current microscopy, scientists face three crippling changes when attempting to visualize biology on the molecular scale: *blurred vision* (i.e. inability to visualize individual molecules clearly, especially for crowded targets), *(partial) color blindnes*s (i.e. only a small number [typically 3 or 4] of colors are used to simultaneously track distinct molecular species), and *ambiguous quantification* (i.e. the inability to precisely count the number target molecules in a resolution limited area). Using programmable fluorescent DNA probes (*Nature Methods*, 11:313, 2014; *Science* 344:65, 2014), we present a highly multiplexed (10× demonstrated), precisely quantitative (>90% precision), and ultra-high resolution (sub-5 nm) optical imaging method that simultaneously addresses these challenges, and hence promises to broadly transform biomedical research.

Circulating tumor cell (CTC) enrichment and analysis enabled by a flexible micro spring array (FMSA) device

Awardee: Siyang Zheng
Award: New Innovator Award
Awardee Institution: The Pennsylvania State University

The dissemination of circulating tumor cells (CTCs) implicated in the metastatic spread of cancer accounts for the majority of cancer-related deaths. CTCs have been established as a prognostic biomarker and are associated with worse survival outcomes in various cancer types. Microfiltration can be an efficient and antigen-independent method for CTC enrichment. We have developed several generations of microfabricated filtration devices for CTC enrichment. The latest device is a flexible micro spring array (FMSA) device for label-free viable mechanical enrichment of CTCs from whole blood samples obtained from patients with advanced and metastatic carcinoma. Unlike previous microfiltration devices, flexible structures at the micro scale minimize cell damage to preserve viability, while maximizing throughput to allow rapid enrichment directly from whole blood with no need for sample pre-processing. The FMSA device can enrich tumor cells with 90% capture efficiency, higher than 10^4 enrichment, and better than 80% viability from 7.5 mL whole blood samples in less than 10 minutes on a 0.5 cm^2 device. CTCs were enriched and analyzed qualitatively and quantitatively based on immunocytochemical determination of phenotype and morphological characteristics. Follow up samples were analyzed during the course of therapy and CTC results were correlated to patient survival and tumor burden. FMSA enriched CTCs obtained from a blood sample may prove to be a prognostic biomarker for advanced cancer. The FMSA device as a versatile platform is capable of viable enrichment and analysis of CTCs from clinically relevant volumes of whole blood.

The Road to the Structure of CFTR and Pharmacological Correction of the Major Cystic Fibrosis Defect: Is There an Actual Binding Pocket?

Awardee: Stephen G. Aller
Award: New Innovator Award
Awardee Institution: University of Alabama at Birmingham

The Cystic Fibrosis (CF) Transmembrane Conductance Regulator (CFTR) is a unique member of the ATP-Binding Cassette (ABC) superfamily in functioning as an ion channel rather than a classical transporter. Loss-of-function mutations of CFTR directly cause the cystic fibrosis (CF) disease. The most prevalent mutation, accounting for up to ~70% of all defective CFTR alleles, is a deletion (F508del) in the first nucleotide binding domain (NBD1), resulting in rapid degradation of virtually all CFTR protein. In contrast, normal CFTR contributes to cholera toxin-associated secretory diarrhea that kills thousands worldwide annually. CFTR has been extensively characterized since the original cloning of the gene in 1989 by Francis Collins and colleagues. Electrophysiological assays, mutagenesis, crosslinking, biophysical studies, cellular localization and low-resolution three-dimensional (3D) structures have shed light on CFTR folding, gating and channel processing. Recent achievements in developing small molecule correctors and modulators showed that CFTR defects can be partially repaired through direct drug-binding to the protein, likely in several distal locations on the molecule. However, progress in answering several critical questions about CFTR will remain limited until high-resolution three-dimensional structure can be achieved, such as: 1) What is the precise amino acid composition and structure of the chloride pore? 2) How well defined is the chloride selectivity filter? 3) How does the physical contact between the regulatory (R) region and rest of the channel regulate channel activity? 4) What is the role of Phe508 in CFTR folding? 5) How does phosphorylation and nucleotide binding regulate chloride gating? and 6) Where do CFTR correctors bind and how do they help fold CFTR-F508del? We are now able to express and purify functional CFTR protein from yeast in sufficiently large amounts for x-ray crystal structure determination. A portion of the innovative proposal employs novel synthetic antigen-binding fragments (SynFabs) to address issues specific to CFTR for forming well-ordered crystals, including immobilizing known regions of disorder and trapping channels in discrete conformations. Taking advantage of existing structures of related ABC proteins, we have created a special homology model to understand problems surrounding the folding of CFTR-F508del. The homology model is sufficiently accurate to allow structure-based *in silico* drug screening for pharmacological "correctors" of CFTR-F508del in primary human CF airway epithelial cells. Results will help us overcome the current efficacy barrier of CF drugs and increase lifespan of CF patients.

Identifying Isolation Methods for Persistent Bacteria by Linking Transient Molecular Events to Stable Genetic Signals

Awardee: Kyle Allison
Award: Early Independence Award
Awardee Institution: Columbia University Health Sciences

Bacterial persistence is a particularly concerning form of population heterogeneity in which a transient fraction of bacterial cells in a population tolerates severe antibiotic treatment while the majority of the population is eliminated. These 'persisters' can contribute to chronic infection and are a major medical problem. Despite their medical and scientific importance, persisters are poorly understood. One of the greatest challenge in studying bacterial persistence results from a lack of methods to isolate persisters from the heterogeneous populations in which they occur. As a result, systems-level analysis of persistent cells has been impossible and even basic questions cannot be answered. Hence, a major challenge in the field is to develop a method to isolate persisters which allows for their analysis by systems-wide, molecular techniques. We have devised a strategy for identifying transient signals that are predictive of persistence, by linking transient molecular events to stable genetic signals that can be quantified by DNA sequencing. To this end, we have developed an approach to analyzing population heterogeneity at the single-cell level using a genome-scale library of fluorescent reporters for promoter activity in *E. coli*. We have applied this method to measure the single-cell expression distributions of 1,637 distinct promoters at high resolution, and we analyzed these distributions in order to combine the 1,637 strains into seven distinct sub-libraries using a threshold-based strategy. This strategy ensures that, within in each pooled sub-library, strains are present at equivalent abundances for a specified threshold used for flow-cytometry sorting. We can therefore sort cells in mixed populations containing hundreds of promoter-reporter strains and to select only cells with expression within the top 5% of their distribution. Sorted populations, containing mixed populations of transient phenotypic outliers, are treated with antibiotics to select for persisters. Surviving populations are enriched with strains that harbor promoters that, when highly expressed, predict persistence. Reiterating this selection approach will lead to further enrichment of predictive signals, and the abundance of strains in resulting selected populations can be determined by sequencing from DNA flanking the promoters. This work will identify highly predictive transient signals for persistence, which will then be used to isolate persistent bacteria. These methods will allow researchers to perform systems-wide characterization of persistence at the molecular level and will accelerate development of new antibiotics treatments.

Structural Basis of the Vinculin–F-actin Interaction

Awardee: Gregory M. Alushin
Award: Early Independence Award
Awardee Institution: National Heart, Lung, and Blood Institute
Co-authors: Kim, L.Y., Thompson, P.M., Campbell, S.L.
Co-authors institutions: National Heart, Lung, and Blood Institute, University of North Carolina at Chapel Hill

The essential protein vinculin is a key component of cell–matrix and cell–cell adhesions, acting as a linker between these connection points and the actin cytoskeleton. Accumulation of vinculin is necessary for adhesions to sustain high traction forces, and its direct interaction with F-actin is required for mechanotransduction. Due to its physiological significance, the molecular determinants of this interface have been under scrutiny for some time, yet conflicting structural models have been reported, largely because of the absence of high-resolution analysis. To address this issue, we have obtained a subnanometer-resolution (8.5 Å, gold-standard FSC 0.143) cryo-EM reconstruction of vinculin's C-terminal "tail" domain (Vt) bound to F-actin, sufficient for constructing an unambiguous pseudo-atomic model of the interface by flexible docking of crystallographic structures of the components. Our findings support a recent experimentally derived model of the interaction (Thompson et al., Structure 2014). Additionally, we find that Vt undergoes a substantial conformational change upon actin binding, characterized by a twisting of helices 4 and 5 and unfolding of helix 1 from the bundle, a segment of which contacts the surface of the actin filament. We postulate that this structural transition enables additional interactions between vinculin and binding partners upon actin engagement. Additionally, we observe a similar conformational transition in the actin-bound structure of the cardiomyopathy-associated vinculin splice variant metavinculin. This suggests that disease mutations harbored in metavinculin helix 1 (H1') may disrupt function by modulating the H1' – actin interface.

Specialized Ribosomes: A new frontier in gene regulation, organismal development, & evolution

Awardee: Maria Barna
Award: New Innovator Award
Awardee Institution: Stanford University

The ribosome is generally considered to translate mRNAs similarly across cell types and developmental stages. However, recent studies suggest greater ribosome-mediated regulatory control in mammalian development and transcript-specific translation. In particular, our findings reveal that fundamental aspects of gene regulation and formation of the mammalian body plan are controlled by what we have termed "specialized ribosomes," which have a unique activity or composition, that direct where and when specific protein products are made. An outstanding question raised from these studies is how ribosome-mediated control of gene expression is encoded within mRNA sequence. I will discuss my lab's recent findings revealing how this regulation is achieved in *cis* and *trans*. In particular large-scale efforts that identify hundreds of unique RNA regulons embedded within the 5'UTRs of key developmental regulators within the vertebrate genome. These elements act as essential RNA "switches" in converting key transcripts into proteins. I will further discuss how the presence a novel Translational Inhibitory Element (TIE) within these same transcripts blocks generic cap-dependent translation, thereby enabling a unique mode of translation initiation. Together, these findings suggest that similar to the complex and highly regulated system of transcriptional control, *cis*-acting RNA regulons in conjunction with more specialized ribosome activity provide newfound regulatory control to gene expression critical for mammalian development.

Histone deacetylase inhibition recovers neurological dysfunction in a mouse model of Kabuki syndrome

Awardee: Hans Tomas Bjornsson
Award: Early Independence Award
Awardee Institution: Johns Hopkins University
Co-authors: Joel S. Benjamin, Li Zhang, Jacqueline Weissman, Elizabeth E. Gerber, Yi-Chun Chen, Rebecca Vaurio, Michelle C. Potter, Kasper D. Hansen, Harry C. Dietz
Co-authors institutions: Johns Hopkins University School of Medicine, Kennedy Krieger Institute, Bloomberg School of Public Health, Howard Hughes Medical Institute.

There are currently few forms of intellectual disability known to be treatable in postnatal life. Kabuki syndrome (KS) is a rare genetic disorder that presents with mild to moderate intellectual disability, as well as some unique physical abnormalities, and is known to be caused by mutations in either of two genes, *KMT2D* or *KDM6A*. KMT2D is a histone-modifying enzyme that adds the open chromatin mark H3K4me3, and KDM6A is a histone demethylase which removes the closed chromatin mark H3K27me3. Given the complementary functions of these two genes, we hypothesized that some of the pathogenesis of KS may relate to an ongoing imbalance between open and closed chromatin of a subset of disease relevant target genes. If this notion is true, any ongoing deficiency may be affected by promoting open chromatin states through HDAC inhibition even in post-natal life. To this end, we have characterized a novel mouse model of KS that has a heterozygous loss of function mutation in *Kmt2d,* which leads to a global deficiency of H3K4me3 as seen by ChIP-seq. Characterization of this model has revealed behavior deficits indicative of hippocampal memory dysfunction in the Morris water maze ($P < 0.005$) and novel object recognition test ($P < 0.05$), as well as both decreased H3K4me3 ($P < 0.05$) and neurogenesis ($P < 0.001$), as measured by number of doublecortin positive cells in the dentate gyrus granule cell layer (GCL), a critical area of adult neurogenesis. After treatment with the HDAC inhibitor AR-42, both the neurogenesis ($P < 0.05$) and H3K4me3 ($P < 0.001$) deficits of the GCL were significantly reversed in the *Kmt2d* mutant mice, as well as the overall global H3K4me3 deficiency. Additionally, the treatment significantly increased the performance of the mutant mice ($P < 0.05$), and normalized their performance in platform crossings during the probe trial of the Morris water maze when compared to wild-type littermates. This work suggests the GCL may be a potentially relevant cell population for some aspects of the intellectual disability seen in KS and perhaps other causes of intellectual disability. Furthermore, manipulating epigenetic marks appears to be a potential therapeutic avenue for KS and related Mendelian disorders of the epigenetic machinery.

Optical Prediction of Metastatic Outcome Using Second Harmonic Generation

Awardee: Edward Bernard Brown
Award: New Innovator Award
Awardee Institution: University of Rochester

Following a diagnosis of invasive breast cancer, the primary tumor is removed and hormonal therapy is begun. At this point a significant decision must be made: should the patient receive additional systemic chemotherapy to attack cells that have escaped the tumor? In the majority of cases the cancer has not yet spread to the adjacent lymph nodes ("N0"), and the choice is unclear. Current data suggests that the majority of N0 patients that are systemically treated would *not* have developed metastases, did *not* need to suffer the toxic effects of systemic therapy, and were "overtreated." Hence there is a pressing need to predict who will (and will not) develop metastases, to minimize overtreatment. Current attempts to meet this need are primarily based upon genomic methods and hence focus on tumor cells, while less attention is paid to the extracellular matrix through which metastasizing cells travel. We and others have studied an optical signal called second harmonic generation (SHG) that is intrinsic to fibrillar collagen. We have demonstrated that tumor collagen structure, as measured with SHG, influences tumor cell motility and that manipulation of tumor SHG signatures alters metastatic outcome. This suggests that SHG may provide prognostic information about metastasis that is complementary, or even superior, to current cell-focused methods. Therefore we explored one SHG measure of collagen structure, the forward- to backwards-scattering ratio (F/B), in 125 ER+ untreated patients with Invasive Ductal Carcinoma. We found that F/B is a significant prognostic indicator of time to metastasis in these patients (based upon 10 year followup data). It is also a prognostic indicator in 60 ER+ IDC patients treated with tamoxifen. Finally, F/B is a significant prognostic indicator in 69 patients with Stage I colon adenocarcinoma. This suggests that F/B may be useful as a rapid and inexpensive way to predict metastatic outcome in several tumor types, to reduce "overtreatment" of cancer patients.

Punctuated evolution of a complex ancestral cell cycle by a viral protein

Awardee: Nicolas Buchler
Award: New Innovator Award
Awardee Institution: Duke University

Cell division is an essential process that has been occurring in an uninterrupted chain for billions of years. Thus, one expects strong conservation in the regulatory network controlling the eukaryotic cell division cycle. Comparison of fungi with animals previously suggested that a simple ancestral eukaryotic cell cycle was driven by mitotic cyclin-dependent kinase activity, and that complex G1 control evolved later. To address the question of cell cycle regulation in the last common eukaryotic ancestor, we examined the evolutionary history of an entire regulatory network with a vast amount of genomes covering most of eukaryotic diversity including plants, protists, animals, and fungi. This allowed us to reconstruct the network of the last eukaryotic common ancestor, which had similar G1 regulators to animals and has been maintained in nearly all eukaryotes. Thus, the ancestral cell cycle network is far more complex than previously anticipated.

In contrast, evolution along the fungal lineage was punctuated by the acquisition and entrainment of the SBF transcription factor, likely of viral origin. We propose that SBF hijacked cell cycle control by activating genes targeted by the ancestral cell cycle regulator E2F. Consistent with the hijacking hypothesis, we show that SBF can regulate promoters with E2F binding sites in budding yeast and that this cell-cycle dependent regulation requires both E2F cis-regulatory sequence and SBF regulator. Last, we show that cell cycle evolution in fungi proceeded through a hybrid network containing both the fungal SBF and the animal E2F, which is still maintained in many basal fungi. The ancestral components were subsequently lost in the fungal lineage. Our analysis supports the hypothesis that hybrid networks are a general mechanism through which core conserved regulatory networks can dramatically evolve.

Autonomously Assembling Nanomaterial Scaffolds for Treating Myocardial Infarction

Awardee: Nathan Gianneschi
Award: New Innovator Award and Transformative Research Award
Awardee Institution: University of California San Diego

The goal of targeted therapeutics and molecular diagnostics is to accumulate drugs or probes at the site of disease in higher quantities relative to other locations in the body. To achieve this, there is tremendous interest in the development of nanomaterials capable of acting as carriers or reservoirs of therapeutics and diagnostics *in vivo*.[1] Generally, nanoscale particles are favored for this task as they can be large enough to function as carriers of multiple copies of a given small molecule, can display multiple targeting functionalities, and can be small enough to be safely injected into the blood stream. The general goal is that particles will either target passively via the enhanced permeability and retention (EPR) effect, actively by incorporation of targeting groups, or by a combination of both.[2] Nanoparticle targeting strategies have largely relied on the use of surface conjugated ligands designed to bind overexpressed cell-membrane receptors associated with a given cell-type.[3] We envisioned a targeting strategy that would lead to an active accumulation of nanoparticles by virtue of a supramolecular assembly event specific to tumor tissue, occurring in response to a specific signal. The most desirable approach to stimuli-induced targeting would be to utilize an *endogenous* signal, specific to the diseased tissue itself, capable of actively targeting materials introduced via intravenous (IV) injection. We present the development of nanoparticles capable of assembling *in vivo* in response to selective, endogenous, biomolecular signals. For this purpose, we utilize enzymes as stimuli, rather than other recognition events, because they are uniquely capable of propagating a signal via catalytic amplification. We will describe their development and utility as a multimodal imaging platform, and discuss their potential as carriers capable of targeting tissue via a new mechanism.

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Mechanism and function of autosomal analog of X inactivation

Awardee: Alexander Gimelbrant
Award: Transformative Research Award
Awardee Institution: Dana Farber Cancer Institute

Autosomal monoallelic expression (MAE) is a recently discovered epigenetic phenomenon that controls the relative expression of maternal and paternal alleles in a large fraction of human genes. The way the active allele is randomly chosen and then stably maintained due to MAE resembles X chromosome inactivation, though MAE affects genes in both male and female cells. MAE can profoundly affect cell fate, causing two sister cells within the same individual to perform in diametrically opposite ways, depending on whether the normal or mutant allele of the gene is active. Understanding the function and mechanism of MAE should significantly contribute to revealing the precise link between specific gene variants and susceptibility to a variety of disorders.

Genes subject to MAE are implicated in major diseases including cancer, autism, and Alzheimer's disease, promising that MAE research will have a significant impact on multiple fields of biomedicine. However, progress in understanding mechanistic and functional aspects of MAE has been hindered by the inadequacy of traditional technological approaches, which don't allow for systematic analysis of a mechanism that inherently generates enormous cell-to-cell variation. This epigenetic heterogeneity masks variation in allelic expression in contexts where cells are analyzed in bulk, such as most genome-wide and high-throughput research strategies. As a result, researchers have lacked basic knowledge or even the tools for efficiently generating this knowledge.

In response, we have developed and validated several pioneering methods that circumvent this barrier, and enable accurate and precise assessment of MAE in human cells and tissues and allow us to conduct systematic functional, mechanistic, and genetic studies of MAE. We use several novel technologies to directly address critical questions about MAE biology. We will dissect the molecular mechanisms involved in MAE initiation, development, and stable maintenance over multiple cell divisions in human cells, opening the door to targeted manipulation of allelic activity. We also address the following fundamental functional questions about effects of MAE: How prevalent is MAE in an organism *in vivo*? How does it vary between individuals? What are the functional consequences of widespread MAE?

Successful completion of this project will provide crucial knowledge for precise interpretation of genotype-phenotype relationship in the context of human normal development and disease. It will also bring new understanding of cell-to-cell and between-individual variability. These insights may be translated into diagnostic, preventative, and therapeutic treatments in the context of personalized medicine.

How does Nature change lifespan?

Awardee: Vadim N. Gladyshev
Award: Pioneer Award
Awardee Institution: Brigham and Women's Hospital

Understanding the mechanisms that control lifespan is among the most challenging biological problems. Many complex human diseases are associated with aging, which is both the most significant risk factor and the process that drives the development of these diseases. It is clear that the aging process and the maximum lifespan of species can be regulated and adjusted. For instance, mammals are characterized by >100-fold difference in lifespan, which can both increase and decrease during evolution. We employ this diversity in mammalian lifespan and the associated life-history traits to shed light on the mechanisms that regulate species lifespan. For this, we utilize methods of comparative genomics to sequence and examine the genomes of exceptionally long-lived species and carry out analysis of lifespan across a panel of mammals. We sequenced the genomes of several mammals with exceptional lifespan, including the naked mole rat, the Damaraland mole rat, and the Brandt's bat, and identified genes that contribute to their longevity. We also work with the longest lived mammal, the bowhead whale. In addition, we apply RNAseq and metabolite profiling approaches to characterize the molecular basis for adaptations associated longevity across mammals. These studies point to both lineage-specific and common processes involving various pathways and provide the first insights into how Nature changes species lifespan. It is our hope that a better understanding of molecular mechanisms of mammalian lifespan control will lead to a better understanding of human diseases of aging.

A new β-catenin/Tcf binding site in DNA mediates robustness in gene regulation

Awardee: Lea Goentoro
Award: New Innovator Award
Awardee Institution: California Institute of Technology
Co-authors: Jae Hyoung Cho, Thomas Hilzinger, Bryan Ryba, Marc W. Kirschner
Co-authors institutions: California Institute of Technology

Fate patterning in embryonic development relies on activation of specific genes at the right time, place, and dose. One important mode of regulating gene expression in development is through activation of signaling pathways. A major signaling pathway in early development is the Wnt/β-catenin pathway. Activation of Wnt signaling in early *Xenopus* embryo leads to *siamois* expression, which is necessary and sufficient to specify the dorsal-anterior cell fate. We have previously observed that dorsoanterior patterning in *Xenopus* and *siamois* expression are buffered against variation in the biochemical parameters of the Wnt pathway (e.g., concentration of Axin, concentration of GBP, activity of GSK3β) (1). Here we demonstrate that the buffering can be recapitulated by a 1-kb region upstream of *siamois*. Using promoter bashing and directed mutagenesis, we identified a distal 11-bp element that is necessary for the buffering. Using electrophoretic mobility assay, biochemical assays, and chromatin immunoprecipitation, we demonstrate that the 11-bp element competes with the canonical Tcf sites by binding directly to the β–catenin/Tcf complex. Thus, a new, dynamic circuit interprets Wnt signaling: β-catenin/Tcf acts through a new DNA binding site that interacts with the established canonical site in an incoherent feedforward fashion, and mediates the robustness in gene expression and *Xenopus* development. In the context of our previous work (1,2), this circuit serves as a putative mechanism of Weber's Law detection, by which cells compute the ratio of β-catenin level after to before stimulation. As Weber's Law has now been found in a growing number of metazoan signaling pathways (3-5, and our unpublished results in Tgfβ pathway), this finding may have broad implications.

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The neural basis of odor-driven behavior in skin-penetrating parasitic nematodes

Awardee: Elissa A. Hallem
Award: New Innovator Award
Awardee Institution: University of California, Los Angeles

My lab studies chemosensory neural circuits in the context of human parasitism. We use skin-penetrating parasitic nematodes as a model system for understanding how parasites use chemosensory cues to locate and infect hosts, and how differences between the chemosensory systems of parasitic and free-living animals enable parasitic behaviors. Skin-penetrating parasitic nematodes are intestinal endoparasites that infect approximately one billion people worldwide and are responsible for some of the most common neglected tropical diseases. The infective larvae of skin-penetrating nematodes are thought to search for hosts using sensory cues, yet their host-seeking behavior is poorly understood. We recently conducted an in-depth analysis of host seeking in the skin-penetrating human threadworm *Strongyloides stercoralis*, and compared its behavior to that of other parasitic nematodes. We found that *S. stercoralis* is highly mobile relative to other parasitic nematodes and actively cruises for hosts to infect. *S. stercoralis* shows robust attraction to a diverse array of human skin and sweat odorants, most of which are also mosquito attractants. The respiratory byproduct carbon dioxide (CO_2) is not attractive for *S. stercoralis*, but is required for parasite development inside the host. Olfactory preferences of *S. stercoralis* vary across life stages, suggesting a mechanism by which host seeking is limited to infective larvae. A comparison of odor-driven behavior in *S. stercoralis* and six other nematode species revealed that parasite olfactory preferences reflect host specificity and infection mode rather than phylogeny, suggesting an important role for olfaction in host selection. Building on these results, we are now elucidating the neural circuitry that mediates odor-driven host seeking. We are comparing the functional architecture of the *S. stercoralis* olfactory circuit to that of the free-living worm *Caenorhabditis elegans* to gain insight into how similar neural circuits support different behaviors, and how the specific features of the *S. stercoralis* olfactory circuit enable human parasitism. Our results may enable the development of new strategies for combating harmful parasitic nematode infections.

Mapping the Human Toxome by Systems Toxicology

Awardee: Thomas Hartung
Award: Transformative Research Award
Awardee Institution: Johns Hopkins University
Co-authors: The Human Toxome Consortium

Technological advances allow high-resolution biological phenotyping of the responses of cells and organisms for the elucidation of mechanisms of toxicity; these include the various omics, high-throughput and high-content technologies. Some of these information-rich tools have the potential to provide a molecular understanding of toxicological mechanisms and are the focal point of our transformative research project. The NIH Project "Mapping the Human Toxome by Systems Toxicology" (NIEHS grant R01ES020750) is a collaboration of Johns Hopkins Bloomberg School of Public Health, Brown University, The Hamner Institute, Georgetown University, U.S. EPA National Center for Computational Toxicology and Agilent; it aims to establish a workflow for the systematic identification and annotation of pathways of toxicity (PoT). A Human Toxome knowledge database and its governance shall be established. The project uses untargeted mass spectrum-based metabolomics and gene array-based transcriptomics. The pilot model is the estrogenic response of MCF-7 cells, a well-established and pre-validated test for endocrine disruption.

In line with the project work plan, the model was established in two laboratories and standard operation procedures were developed. The feasibility of transcriptomics and metabolomics were demonstrated. Priority was given to a number of quality assurance measures and the seminal dataset defined by the consortium is being generated, which comprises several hundred gene arrays and several thousand metabolomics profiles representing different concentrations and time points of treatment. Tools for combined multi-omics data analysis, data integration and visualization are being developed, since the bioinformatics tools associated with the PoT concept represent a core deliverable. In this ambitious project, a number of challenges arose and were tackled by the Human Toxome consortium. The concept of distinct and conserved PoT valid for different cell systems and hazards is a hypothesis to be verified. A working PoT definition was established and will be further refined. A key question is, how many PoTs there are? Challenges include also the quality and standardization of the toxicological test systems (especially *in vitro* systems) and the omics technologies. The bioinformatics tools for identification, annotation, proof of causality and validation, as well as the link of PoT to adversity are not yet fully available. A number of workshops, organized in the frame of this project, as well as commissioned white papers complement the consortium technical work to answer these questions.

Enabling Cholesterol Catabolism in Human Cells

Awardee: Richard E. Honkanen
Award: Transformative Research Award
Awardee Institution: University of South Alabama
Co-authors: Mark R. Swingle, Alla Musiyenko, Brandon D'Arcy, Kevin Abney
Co-authors institution: University of South Alabama

Cardiovascular disease (CVD) is the leading cause of death in the United States, and the cost associated with the medical management of CVD is staggering (>$444 billion in 2010[a]). CVD is complex and can originate from many different aberrations in normal lipid metabolism (some genetic, some lifestyle choices). However, nearly all forms of CVD are associated with elevated plasma lipoproteins (principally LDLs) often in combination with low levels of high-density lipoproteins (HDLs). For many people, CVD is an age dependent, progressive disease that is largely undetected or ignored until an event (i.e. myocardial infarction) occurs in the later stages of disease. Therefore, current therapies focus on preventing a second event (or a primary event in high risk individuals) by reducing the circulating levels of LDLs and increasing levels of HDLs. At a biochemical level, the inability of human cells to degrade the cholestane ring of cholesterol is a fundamental component of CVD. More precisely, if macrophages had the ability to degrade cholesterol, they would not become engorged with cholesterol/cholesterol esters and elicit the maladaptive immune response that leads to the onset and progression of atherosclerosis. The surprising observation that chronic *Mycobacteria* survival in human macrophages was aided by their ability to use phagosome cholesterol as a carbon and energy source lead us to a novel hypothesis: genes encoding bacterial ring opening enzymes can be humanized and used to transformation human monocyte derived macrophages, enabling the degradation of phagosome-cholesterol. To test this hypothesis, we have humanized several key enzymes, including cholesterol dehydrogenase, 3-ketosteroid-Δ1-dehydrogenase (KSTD), 3-ketosteroid-9-αhydroxylase (KshA/B), and cholest-4-en-3-one-Δ^1-dehydrogenase. Together these enzymes can catalyze B-ring opening and aromatization of ring A. To control the expression of the enzymes in our "cholesterol catabolizing gene cassette," we are developing two expression systems for comparison. One is a transcription activator-like effector nuclease (TALENs) targeting the AAVS1 locus in the human genome. The second is a Cas-9 nuclease based system designed to introduce the cassette into the ABCA1 promoter (a lipid flipase that is induced by elevated cholesterol). To coordinate the expression of this multi-enzyme system, we have adapted a "ribosomal skipping" sequence between proteins. This eliminates the need for multiple promoters to control the expression of each protein, which greatly reduces the size of the expression cassette.

[a]http://www.cdc.gov/chronicdisease/resources/publications/AAG/dhdsp.htm

NIH COMMON FUND HIGH-RISK HIGH-REWARD RESEARCH SYMPOSIUM
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Indigenous bacterial species from the gut microbiota regulate host serotonin biosynthesis

Awardee: Elaine Y. Hsiao
Award: Early Independence Award
Awardee Institution: California Institute of Technology
Co-authors: Jessica Yano, Kristie Yu, Gregory P. Donaldson, Gauri G. Shastri, Phoebe Ann, Liang Ma, Cathryn R. Nagler, Rustem F. Ismagilov, Sarkis K. Mazmanian
Co-authors institutions: California Institute of Technology and The University of Chicago

There is growing evidence that the microbiota fundamentally regulates the development and function of the nervous system, but the mechanisms underlying indigenous microbe-nervous system interactions are largely unknown. We explore fundamental interactions between the indigenous microbiota and mammalian host that regulate the bioavailability of neuroactive molecules, including neurotransmitters and neuropeptides. In particular, we reveal that a striking ~60% of peripheral serotonin (5-hydroxytryptamine, 5-HT) and ~20% of hippocampal 5-HT is regulated by the microbiota. We have identified a limited microbial consortium that sufficiently and reversibly modulates host serotonin biosynthesis in specific cell subtypes of the gastrointestinal tract, and that corrects enteric and hemostatic abnormalities related to serotonin deficiency in germ-free and genetically-altered mice. We further identify particular microbial metabolites that confer this serotonergic effect of gut microbes, representing the first account of the molecular mechanisms by which a limited bacterial consortium from the mouse or healthy human microbiota modulates host serotonin levels and serotonin-related disease phenotypes in mice.

Clinical and Translational Approaches to Cognitive Impairments in Breast Cancer

Awardee: Michelle Janelsins
Award: New Innovator Award
Awardee Institution: University of Rochester

Background: While chemotherapy has greatly improved survival for cancer patients, the side effects of this treatment can lead to substantial detrimental effects on quality of life that can be debilitating. Chemotherapy-related cognitive impairment (CRCI) is characterized by difficulty in memory, attention, concentration and executive function. CRCI is most pronounced and severe during chemotherapy (in up to 80% of patients); however, it can last for years following treatment in up to 35% of survivors. With over 13 million cancer survivors in the US, it is estimated that up to 4 million survivors could be living with long-lasting effects of CRCI. CRCI is particularly significant because long-term cognitive impairment can develop, CRCI negatively impacts quality of life, and CRCI can affect treatment adherence. Little is known about the biological mechanisms contributing to CRCI development, though studies suggest that increased inflammation may be involved. **Methods:** This research involves a novel combination of animal modeling and human research to address the role of inflammation in CRCI, and also uses animal modeling to develop interventions that will lead to clinical research studies. We are proposing a clinically relevant CRCI mouse breast cancer tumor model with Adriamycin and Cytoxan chemotherapy to study the effects of cancer and chemotherapy on memory function using cognitive assessment and neuroimaging, as well as the contributing role of key cytokine- and chemokine-mediated immune pathways that contribute to neurotoxicity involved in CRCI. We will use the immune and neurotoxicity factors identified in the CRCI animal model to assess whether they are relevant to the human condition in a breast cancer cohort also receiving the same chemotherapy paradigm and who will also receive similar cognitive assessment and neuroimaging as in the animal study. We will also utilize our mouse model to develop and test interventions for CRCI (including anti-inflammatory target agents, supplements, and physical activity) which will allow us to move forward with the most successful interventions for testing in clinical research. **Results and Outcomes:** The goal of this work is to understand the role of inflammation and other neuro-immune factors in CRCI; develop a clinically relevant animal model of CRCI; and to develop, test, and optimize interventions for CRCI in animal models in order to move them into clinical research protocols.

The nuclear periphery acts as a regulator of recombinatorial potential

Awardee: Megan C. King
Award: New Innovator Award
Awardee Institution: Yale University
Co-authors: Dongxu Lin, Bryan Leland, Kristen Swithers, Rebecca Swartz, Na Liu
Co-authors institutions: Yale University

Repetitive regions of the genome are prone to recombination and thus can lead to genome instability, but this same property may also support molecular diversity and adaptation. Little is known about cellular mechanisms that may control the recombinatorial potential of repetitive DNA. Since many repetitive DNA elements are preferentially associated with the inner nuclear membrane, we hypothesized that their association with the nuclear periphery could serve as an input to genome stability by regulating the likelihood that recombination occurs. To test this concept, we examined recombination in the repeat-rich cell surface adhesin genes of the fission yeast, *S. pombe*. This gene family is repetitive intragenically (repeat modules encode a repetitive peptide domain) and intergenically (related genes reside at distinct genomic loci). We show that the adhesin genes are found associated with the nuclear envelope; this association is dependent on the function of proteins bound to proximal transposons and LTRs. Using assays designed to measure intragenic and intergenic recombination, we found that recombination rates are sensitive to internal repeat number but not transcriptional status. Further, disrupting their nuclear envelope tethers weakened the association of adhesin genes with the nuclear periphery and led to an increase in recombination rate. Release from the nuclear periphery and the concomitant increase in recombination could also be recapitulated in response to specific environmental inputs. Our data suggest that the recombinatorial potential of repetitive elements can be tuned by their nuclear compartmentalization, which may provide a mechanism to balance genome integrity with adaptation in response to a changing environment. This fundamental mechanism may also contribute to pathogen evasion of the host immune system.

Life, death, and function: the primate-specific long non-coding RNA transcriptome

Awardee: Leonard Lipovich
Award: New Innovator Award
Awardee Institution: Wayne State University

The ENCODE (Encyclopedia of DNA Elements) Consortium has highlighted the extraordinary abundance of long non-coding RNA (lncRNA) genes in the human genome, while revealing through its Gencode catalog that protein-coding genes comprise less than one half of the human gene count. In contrast to the better-understood microRNAs, lncRNAs act through diverse, heterogeneous mechanisms, epigenetically and post-transcriptionally, as both positive and negative regulators of gene expression. A remarkable and largely unique property of lncRNAs is their low interspecies conservation: at least 5,000 human lncRNAs are not conserved beyond primates. With a custom high-density oligonucleotide microarray, we examined the estrogen responsiveness of 4,312 full-length lncRNAs in the human MCF7 estrogen receptor alpha (ERα) positive cell line, a key breast cancer model. We identified 127 significantly estrogen-regulated lncRNAs. RNAi of 15 estrogen-induced and overexpression of six estrogen-repressed lncRNAs demonstrated concordant post-perturbation phenotypes across six phenotypic assays: MTT (cell proliferation), trypan blue, BrdU incorporation, PARP western blots and TUNL (apoptosis), and crystal violet staining (cell count). Our estrogen-responsive lncRNAs, which had consensus ERα site sequences and ChIP-seq evidence for ERα binding, shifted breast cancer cells along the apoptosis-proliferation axis. Reduced cell growth and increased cell death were consistently observed upon RNAi of estrogen-induced, and overexpression of estrogen-repressed, lncRNAs. These functional lncRNAs frequently exhibited primate-specific exonic sequences, and relied on primate-specific splice sites and polyadenylation signals to delineate their gene structures and boundaries. One primate-specific estrogen-repressed lncRNA reduced ERK1 and 2 phosphorylation in two ERα positive breast cancer cell lines, consistent with the accompanying decrease in cell viability, and suggesting that non-conserved lncRNAs may provide regulatory inputs into the MAP kinase pathway. These lncRNAs likely originated after the prosimian split, and represent candidate novel targets for cancer therapeutics, because, as new outlier nodes linked by sparse edges to gene regulatory networks, they can be perturbed without impacting conserved network hubs. These lncRNAs' evolutionary histories expose the limitations of nonprimate animal models of cancer, and contribute to the genomic basis of human disease.

In the next phase of this work, I will extend the six functional assays to all 127 leads. I will employ second- and third-generation RNAseq, instead of microarrays, to impute the complete MCF7 estrogen-responsive lncRNAome. I will identify novel primate-specific lncRNAs from RNAseq data and subject them to the same system perturbations and phenotypic assays. Having demonstrated ectopic translation of specific lncRNAs within the framework of the ENCODE Consortium lncRNA proteogenomics effort, I will next test whether primate-specific functional lncRNAs act directly as RNAs, not via translated peptides, and will for the first time

jointly use RiboSeq and mass spectrometry to determine whether human lncRNA ectopic translation is hormone-responsive. The results will address a fascinating new question with the potential to initiate a paradigm shift in cancer biology: Is human cancer, to an extent, a primate-specific disease caused by non-conserved lncRNAs? Implementation of this proposal will yield a conservation-unbiased, high-throughput assignment of cellular functions to primate-specific lncRNAs in a major nuclear hormone receptor pathway relevant to cancer therapeutics.

I very gratefully acknowledge the NIH Director's New Innovator Award program for supporting this research through 1DP2CA196375-01.

Understanding the signaling architecture of calcium-dependent protein kinases in apicomplexan parasites

Awardee: Sebastian Lourido
Award: Early Independence Award
Awardee Institution: Whitehead Institute for Biomedical Research
Co-author: Lucas D. Tilley
Co-author institution: Whitehead Institute for Biomedical Research

Identifying the substrates of different protein kinases remains a major obstacle to understanding the signaling pathways that regulate eukaryotic cells. The complexity and plasticity of these pathways makes it difficult to distinguish the contribution of individual kinases in global studies, and yet *in vitro* assays frequently lack many of the cellular features that dictate kinase-substrate specificity. By engineering kinases that accommodate bio-orthogonal ATP analogues, others have previously demonstrated the feasibility of monitoring the activity of individual kinases in complex lysates. We have improved on these methods using pore-forming toxins to semi-permeabilize cells and quantitative phosphoproteomics to identify the substrates of two related kinases, CDPK1 and CDPK3, in the human parasite *Toxoplasma gondii*. We have previously demonstrated that both calcium-dependent protein kinases (CDPKs) contribute to the regulation of parasite motility and are essential for the infectious cycle. By preserving cellular ultrastructure, we are able to determine the contribution of subcellular localization to kinase specificity. Furthermore, analysis of the substrates has extended our understanding of the cellular processes regulated by these kinases. This includes the previously neglected role of CDPK3 in the regulation of ion homeostasis and the preparation of parasites for extracellular survival prior to exiting the infected host cell. Beyond its relevance to human health, *T. gondii* serves as a model for other apicomplexan parasites, including *Plasmodium spp.*, the causative agents of malaria. We hope that our work will serve as the basis for comparing calcium-regulated signaling within the phylum, and as a model for understanding the role of subcellular localization in the evolution of kinase families.

Porphyrin–phospholipid liposomes permeabilized by near-infrared light

Awardee: Jonathan Lovell
Award: Early Independence Award
Awardee Institution: State University of New York at Buffalo

The delivery of therapeutic compounds to target tissues is a central challenge in treating disease. Externally controlled drug release systems hold potential to selectively enhance localized delivery. Here we describe liposomes doped with porphyrin–phospholipid that are permeabilized directly by near-infrared light. Molecular dynamics simulations identified a novel light-absorbing monomer esterified from clinically approved components predicted and experimentally demonstrated to give rise to a more stable porphyrin bilayer. Light-induced membrane permeabilization is enabled with liposomal inclusion of 10 molar % porphyrin–phospholipid and occurs in the absence of bulk or nanoscale heating. Liposomes reseal following laser exposure and permeability is modulated by varying porphyrin–phospholipid doping, irradiation intensity or irradiation duration. Porphyrin–phospholipid liposomes demonstrate spatial control of release of entrapped gentamicin and temporal control of release of entrapped fluorophores following intratumoral injection. Following systemic administration, laser irradiation enhances deposition of actively loaded doxorubicin in mouse xenografts, enabling an effective single-treatment antitumour therapy.

Multiscale Chemical Approaches to Map Oxidative Stress

Awardee: Brent Martin
Award: New Innovator Award
Awardee Institution: University of Michigan

Radical species are an unavoidable consequence of respiration and the environment, and are tightly buffered by small molecule antioxidants and redo detoxifying enzymes. Oxidative stress emerges when an imbalance develops between the levels of reactive oxygen species and the cell's ability to readily eliminate the reactive intermediates or to repair the resulting damage. Aberrant oxidative signaling is perhaps one of the most important factors contributing to aging, neurodegeneration, heart disease, diabetes, and cancer. In order to induce a phenotypic change, oxidative stress must induce biochemical alterations to the genome, proteome and/or metabolome. Crystallographic analysis revealed that DJ-1 harbors a stabile sulfinic acid, and this oxidative modification is required for the suppression of mitochondrial oxidative stress. We have now shown that this sulfinic acid can react with nitrosothiols to form a thiosulfonate linkage, which can then be reduced by cellular thiols. This provides a potential mechanism for DJ-1 function, which will be further explored with this award. We extended this approach to develop biotin-linked sulfinates for the direct detection and enrichment of endogenous nitrosated proteins. In preliminary experiments, this method led to the identification of hundreds endogenous nitrosated proteins, and establishes a robust new platform to functionally interrogate the dynamics of S-nitrosation. In addition, we describe a new methodology for the selective enrichment of sulfinic acids based on orthogonal alkylation reagents, and propose to identify novel functional sulfinates in the proteome. Finally, we present a new class of ratiometric fluorescent probes for live-cell imaging and ^{19}F-NMR of protein sulfenation *in vivo*. Despite the central role of oxidative stress in human health, our ability to study the precise mechanisms of such modifications is hampered by a lack of selective chemical and analytical methods. In this proposal, we present a series of innovative chemical approaches to study oxidative damage across experimental scales, from live-cell imaging to in vivo imaging, in addition to proteome-wide annotation of oxidative post-translational modifications. Furthermore, we present a likely mechanism for the Parkinson's disease-linked redox chaperone DJ-1, and present new mechanism-based probes to functionally annotate and profile S-nitrosation (R-SNO), S-sulfenation (R-SOH), and S-sulfination (R-SO_2H).

3D Printed Nano-Bionic Organs

Awardee: Michael C. McAlpine
Award: New Innovator Award
Awardee Institution: Princeton University

The development of approaches for multidimensional integration of functional electronic components with biological tissue and organs could have tremendous impact in regenerative medicine, smart prosthetics, and human-machine interfaces. However, current electronic devices and systems are inherently two dimensional and rigid, thus prohibiting seamless meshing with three-dimensional, soft biology. The ability to three-dimensionally interweave biological tissue with functional electronics could enable the creation of bionic organs for restoring impairments, or enhancing human functionalities over their natural limitations. Current electronics are inherently two-dimensional, preventing seamless integration with biology, as the processes and materials used to create synthetic tissue constructs vs. conventional electronic devices are very different. Here, we present a novel strategy for overcoming these difficulties via additive manufacturing of biological cells with various classes of functional electronic nanomaterials. Recently, we have generated a functional bionic ear via 3D printing of a cell-seeded hydrogel matrix in the precise anatomic geometry of a human ear, along with an intertwined conducting polymer consisting of infused silver nanoparticles. This allowed for the *in vitro* culturing of cartilage tissue around an inductive coil antenna in the ear, which subsequently connects to cochlea-shaped electrodes. The printed ear exhibits enhanced auditory sensing for radio frequency reception, and complementary left and right ears can listen to stereo music. Here, we propose extending this approach to new functionalities – such as ultrasonic acoustic reception and vasculature – and new bionic organs. Overall, our approach presents a disruptive and paradigm-shifting new method to intricately merge biology and electronics via 3D printing. The work outlined here thus constitutes a novel, highly interdisciplinary investigation to addressing outstanding questions in the generation of bionic organs, and we anticipate that this work will represent a paradigm-shift in both tissue engineering, as well as 3D interweaving of functional electronics into biological systems.

Adaptor-Layer Integration of RTK Signaling: Basic Understanding and Application to Prediction of Targeted Drug Resistance

Awardee: Aaron Meyer
Award: Early Independence Award
Awardee Institution: Massachusetts Institute of Technology

Receptor tyrosine kinases (RTKs) play a central role in regulation of cell response during development and homeostasis, and dysregulation contributes to diseases such as cancer. RTK-targeted therapies have been applied successfully in cancer treatment, though with limited effectiveness as activity of non-targeted RTKs can enable cells to become resistant. While redundant signaling is now appreciated as a common mechanism of acquired and innate resistance, the exact signaling that is essential to resistance, and whether it is conserved or varies across cancer contexts, has not been addressed. RTKs lead to a common set of downstream signals, but in vastly different quantitative combinations, and differ in their ability to confer resistance in a context-dependent manner. A fundamental, rigorous understanding of resistance is necessary if we are to develop better therapies to overcome this redundancy. TAM receptors (Tyro3, AXL, MerTK) are a family of RTKs that have attracted interest for their widespread roles in tumor resistance and metastasis. However, while the ligands for these receptors have been identified, we lack even a basic understanding of the contexts that lead to activation of these receptors.

RTKs work by auto- and trans-phosphorylation, recruiting adapter proteins, and then phosphorylating those adapters and other associated proteins. Systems biology has concentrated on easily measurable factors such as phosphorylation, but comparisons of signaling between receptors are not easily accomplished, as phosphosites between receptors do not readily equate. The amount of receptor-bound adapter molecules is one quantity that should be directly comparable however. Thus, I plan to develop techniques to measure RTK interaction quantitatively and across the multiple potential interactions within a cell simultaneously with the intention of more completely capturing signaling from these receptors. I will use these techniques combined with quantitative modeling to examine interactions during receptor activation and understand how different RTKs can provide redundant signaling leading to targeted cancer treatment resistance. These resistance and interaction models will then be applied to more specifically understand resistance conferred by the TAM family of RTKs. Through development of mechanistic models for ligand-dependent and independent signaling, linked to adapter interaction, downstream signaling, and tumor cell resistance, I plan to develop an integrative understanding of resistance. This will provide necessary information to develop therapies bypassing this problem.

A novel approach to improve clinical decision support and make possible real-time epidemiology for infectious disease outbreaks using mobile technology

Awardee: Eric J. Nelson
Award: Early Independence Award
Awardee Institution: Stanford University
Co-authors: Clea Sarnquist, Yassar Arain, Nancy Federspiel, Felicia Bill, Gary Schoolnik, Firdausi Qadri, Farhana Haque
Co-authors institutions: Stanford University School of Medicine; International Centre for Diarrhoeal Disease Research, Bangladesh; Institute for Epidemiology, Disease Control and Research, Bangladesh

Introduction. Technology is making possible new approaches to overcome old public health challenges. Conventional epidemiologic approaches are often limited because the transmission of devastating diseases like cholera outpaces current paper based techniques. Cellular networks are now ubiquitous and offer opportunities for novel high-yield interventions.

Approach. Our research strategy requires two phases. In phase one, we are asking how might we develop a mobile technology platform to provide improved clinical decision support and make possible real-time epidemiology to rapidly identify actionable public health interventions. We are using cholera outbreaks in Bangladesh as a model system to explore current clinical and epidemiologic approaches to outbreak response. We are then developing solutions to the problems we discover using a process called human centered design. Phase two of the project merges the solutions developed at the clinic level with larger community based networks of first responders (e.g. community healthcare workers and pharmacists). This abstract focuses on phase one.

Development. Based on opportunities discovered during the preliminary studies, we have designed and built a toolkit for forty dollar smartphones manufactured in Bangladesh that has two primary modalities coined the Rehydration Calculator and Outbreak Responder. The Rehydration Calculator provides rapid decision support to rehydrate patients with life-threatening dehydration from diarrheal disease. The calculator provides recommendations for fluids and medications based on WHO guidelines. The Outbreak Responder is for outbreak response teams or hospitals with high diarrheal disease caseloads. It gathers demographic and clinical data and provides a concise assessment and plan with a safety checklist. Clinical information includes vaccination status which allows for crowd-mapping vaccine efficacy. GIS data include the place of treatment and the patient's residence gathered via a touchscreen map. Follow-up instructions and a call back number are sent directly to the patient's cell phone via text message. A web-based interface receives the data in a secure manner and provides interactive real-time data visualization.

Next steps. The prototype will be evaluated and iterated in three stages: small design sessions with standardized cases, a controlled pilot study, and a cluster randomized controlled trial. We look forward to exploring partnerships to test and implement these tools globally for the betterment of patients and to improve our fundamental understanding of infectious diseases like cholera. We also seek support and dialogue on scaling the project to other epidemic infections, including emerging outbreaks like Ebola.

Decoding the Noncoding Genome: lncRNA Dynamics and Function in Single Cells

Awardee: Gregor Neuert
Award: New Innovator Award
Awardee Institution: Vanderbilt University

One of the greatest enigmas in biology today is the function of the pervasively transcribed noncoding genome. Of the numerous long noncoding RNAs (lncRNAs) found in eukaryotic cells, only a small percentage have been functionally investigated. Of those that have, many have been shown to mediate key biological processes during cellular reprograming, cell cycle progression and mammalian development, in addition to causing human cancers and neurological diseases, underscoring the need for further study of this class of molecule. Interestingly, the majority of lncRNAs are found to exist in pairs with mRNAs or other lncRNAs in both yeast and mammals, an organizational pattern that has proven functional in some cases. We are interested in further elucidating the functional relevance of lncRNAs by focusing on their gene regulatory roles and mechanisms of action within pairs. We propose to investigate the regulatory function and control mechanisms of convergently and divergently transcribed lncRNA-mRNA pairs in yeast. Based on our previous observation of mutually exclusive expression of lncRNA-mRNA pair members in single cells, we hypothesize that the physical orientation of each member of the pair in relation to its partner underlies a binary switch that is 'flipped' by environmental inputs. Specifically, these inputs may induce the expression of one member of the pair, leading to the repression of the other member, and vice-versa. To address this hypothesis, we propose to use a single-cell/single-molecule approach combined with predictive modeling and genetic gain- and loss-of-function methods to investigate the spatial-temporal dynamics of convergent and divergent lncRNA-mRNA pairs. Understanding the pattern of lncRNA-mRNA expression in single cells in response to various environmental inputs is of fundamental importance in identifying lncRNA function and control mechanisms. Our single-cell/single- molecule approach is well-suited to this task because of its ability to spatially and temporally resolve individual molecules of multiple lncRNA/mRNA species at high resolution within the same cell and correlate them to each other and to the observed phenotype. The expected outcome of our studies is an improved understanding of lncRNA function and lncRNA-mediated mechanisms of gene regulation that could not otherwise be achieved using more conventional cell population or *in vitro* studies. The positive impact of this research is the development of a novel, robust and highly informative methodology to interrogate lncRNA function and differentiate between different transcriptional models, independent of the species or specific cellular pathway, which is expected to open new avenues of research in the field.

Proteolysis of a Human Host-Defense Peptide Unmasks Innate Immune Function

Awardee: Elizabeth M. Nolan
Award: New Innovator Award
Awardee Institution: Massachusetts Institute of Technology
Co-author: Phoom Chairatana
Co-author institution: Massachusetts Institute of Technology

Defensins are small, cysteine-rich, ribosomal peptides produced by eukaryotes. These host-defense peptides participate in the human innate immune response. Human α- defensin 6 (HD6) is synthesized and stored in small intestinal Paneth cell granules. Its biophysical properties and physiological function are both remarkable and warrant extensive investigation. Whereas other human α-defensins exhibits broad-spectrum antimicrobial activity, HD6 exerts negligible antibacterial activity *in vitro* and provides host-defense by an alternative mechanism. In the small intestinal lumen, this 32-residue peptide self-assembles into higher-order oligomers or "nanonets" that entrap bacteria and thereby prevent bacterial invasion into host cells.[1] The distribution of hydrophobic residues in the HD6 primary sequence differs from that of other human α-defensins, and our recent biochemical and biophysical investigations revealed that hydrophobic residues are essential for HD6 self-assembly and innate immune function.[2] Here, we present the results from investigations designed to elucidate how the Paneth cell stores HD6 as well as how and where the HD6 nanonet forms. Analysis of human mRNA indicates that HD6 is biosynthesized as prodefensin 6 (proHD6) exhibiting a 49-residue N-terminal region.[3] Our studies reveal that proHD6 neither self-assembles into higher-order oligomers nor prevents the human gastrointestinal pathogen *Listeria monocytogenes* from invading epithelial cells. Moreover, trypsin-catalyzed proteolysis of proHD6 unleashes mature HD6, unmasking immune function. In analogy to zymogen activation, these insights provide a working model whereby the Paneth cell synthesizes, packages and stores inactive proHD6. Proteolytic processing of the propeptide occurs during or following vesicular release, which triggers HD6 self-assembly and allows for nanonet formation in the intestinal lumen.

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Therapeutic Modulation of Host Arginine-Associated Metabolic Pathways Disrupts Viral and Host Processes Required for Pathogen Replication and Manifestation of Disease

Awardee: Augusto C. Ochoa
Award: Transformative Research Award
Awardee Institution: Louisiana State University Health Sciences Center
Co-authors: Timothy P. Foster, Maria Dulfary Sanchez
Co-authors institution: Louisiana State University Health Sciences Center

Since their inception five decades ago, most antivirals have been engineered to disrupt a single viral process or protein that is essential for viral replication. The nature of this approach has limited the overall therapeutic effectiveness and applicability of current antivirals due to restricted viral specificity, a propensity for development of drug resistance, and an inability to control deleterious host-mediated inflammatory responses. To overcome these limitations, we have developed a host-targeted antiviral, peg-ArginaseI (peg-ArgI), rather than pursuing traditional pathogen-targeted approaches. peg-ArgI modulates arginine-associated metabolic pathways that are essential for replication of most intracellular pathogens and for induction of inflammation-associated disease processes. RNASeq whole transcriptome analysis indicated that peg-ArgI treatment of primary epithelia induces a cellular environment that is not conducive for viral replication. peg-ArgI treatment down-regulated cellular processes required for efficient viral genomic replication and spread, while up-regulating transcription of cell-intrinsic antiviral processes. Consistent with these findings, unlike current antivirals that are specific for a given pathogen, peg-ArgI ablated productive replication of a diverse range of important human pathogens including, Chlamydia, HSV-1, HSV-2, Adenovirus, RSV, Parainfluenza, and Influenza. Whole viral transcriptome analysis, as well as subsequent biological and virological assays, indicated that unlike current drugs that target a single viral replicative process, peg-ArgI exerts multiple mechanisms that collectively inhibit HSV-1 replication and transmission. Indeed, other than viral entry, every aspect of the HSV-1 lifecyle was affected. peg-ArgI inhibited HSV-1 genomic replication, select viral protein expression, viral assembly, viral envelopment, intracellular transport, and cell-to-cell spread. In addition, host transcriptome analysis indicated that peg-ArgI could suppress some virus-induced host processes that are associated with development of disease (*e.g.* pathological inflammation and vascularization). In congruence with these results, in two rabbit eye models of virus-induced ocular keratitis, peg-ArgI treatment resolved deleterious inflammation and severe ocular disease processes initiated by either HSV-1 or Adenovirus infection. Collectively, this data demonstrates the viability of therapeutically targeting host metabolic pathways to broadly inhibit replication and transmission of a broad-range of viral pathogens, while simultaneously suppressing deleterious host-mediated inflammatory responses.

Understanding the Biological Importance of Ether-Linked Lipids in Membrane and Organismal Aging

Awardee: Carissa Perez Olsen
Award: Early Independence Award
Awardee Institution: Fred Hutchinson Cancer Research Center
Co-authors: Robin Drechsler, Shaw-Wen Chen
Co-authors institution: Fred Hutchinson Cancer Research Center

Plasmalogens are a specialized class of phospholipids that are prevalent in the membranes of eukaryotes but whose biological function is not yet understood. The key structural difference in plasmalogens is the presence of a vinyl-ether bond as opposed to the ester bond used to link fatty acids in typical phospholipids. These ether-linked species are highly abundant in mammals, particularly in the brain where they comprise more than 40% of the phospholipids. In addition to having an association with many disease states including peroxisomal biogenesis disorders, Alzheimer's disease, and obesity, these lipids have been show to decrease with age as well as alter the properties of the membrane. Therefore, a characterization of these ether-linked lipids will allow us to better understand membrane structure in old animals as well as the biological importance of this lipid species in aging.

The presence of an ether bond in plasmalogens has been proposed to protect the membrane from damage by consuming reactive oxygen species and preventing damage to other cellular components. In fact, plasmalogen-deficient cells are highly susceptible to oxidative stress, and because plasmalogen abundance decreases with age, we hypothesize that these ether-linked lipids play an important role in stress response and organismal aging. However, explicit tests of the impact of plasmalogens on membrane maintenance and aging have not been completed, largely because of a lack of an adequate animal model for high-throughput studies. Recently, we have established biochemical tools that allow us to map membrane composition and dynamics in the model organism, *Caenorhabditis elegans.* Using these HPLC/MS-based methods, we have found that plasmalogens make up approximately 20% of the total phospholipid population in the nematode, similar to their abundance in humans. Additionally, the biosynthesis pathway for plasmalogens is conserved in the nematode, positioning us to begin to interrogate the biological importance of this unique lipid species *in vivo*. Indeed, in support of our hypothesis, we find that depletion of plasmalogens via RNAi results in animals that are sensitive to oxidative stress and have a shortened lifespan. Here, we seek to quantify how plasmalogen dynamics change with age and to establish the nematode as a model for defining a role for plasmalogens in aging.

Characterizing Lymphatic Micrometastases

Awardee: Timothy P. Padera
Award: New Innovator Award
Awardee Institution: Massachusetts General Hospital

Metastasis remains the major cause of cancer mortality, but breakthroughs in our understanding of the molecular and cellular mechanisms regulating metastasis have yet to be broadly translated into improved survival rates in patients with metastatic disease. The challenge is how to treat cancer cells that have spread to lymph nodes or distant organs in order to prevent their growth and ideally eradicate them from the body. Most cancer therapies are developed against the primary tumor growing in its native microenvironment. However, it is clear that the local microenvironment in which tumor cells grow greatly affects the growth rate, metabolism, vascularization, and ultimately the response to therapeutic intervention. For instance, antiangiogenic therapy to date has failed to improve overall survival in cancer patients when used in the adjuvant setting. The presence of lymph node metastases dictate treatment decisions, however their reliance on angiogenesis for growth has not been described. Here, we introduce a novel chronic lymph node window (CLNW) model to facilitate new discoveries in the growth and spread of lymph node metastases. Using the CLNW in multiple models of spontaneous lymphatic metastases in mice, we reveal the surprising lack of sprouting angiogenesis during metastatic growth, despite the presence of hypoxia in some lesions. Treatment with two different antiangiogenic therapies showed no effect on the growth or vascular density of lymph node metastases in our models. We confirmed these findings in clinical specimens, including the lack of reduction in blood vessel density in lymph node metastases in patients treated with bevacizumab. We provide pre-clinical and clinical evidence that sprouting angiogenesis does not occur during the growth of lymph node metastases, which reveals a mechanism of treatment resistance to antiangiogenic therapy in adjuvant settings. The targets of clinically approved angiogenesis inhibitors are not active during early cancer progression in the lymph node, suggesting that inhibitors of sprouting angiogenesis as a class will not be effective in treating lymph node metastases.

Maternal CD4+ T cell responses are critical for fetal survival following acute cytomegalovirus infection in a novel rhesus macaque model of transplacental cytomegalovirus transmission

Awardee: Sallie Permar
Award: New Innovator Award
Awardee Institution: Duke University
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Introduction

Transplacental transmission of human cytomegalovirus (CMV) is the leading infectious cause of brain damage in infants worldwide, accounting for approximately 25% of all infant hearing loss. In this study, we sought to develop a nonhuman primate model of congenital CMV transmission to define the maternal immune responses that are critical to CMV transmission and fetal outcome.

Methods

To develop a nonhuman primate model of congenital CMV infection, four rhesus CMV (rhCMV)-seronegative pregnant rhesus monkeys were inoculated intravenously with rhCMV during first trimester one week after systemic administration of an anti-CD4 depleting antibody. The fetal outcome and maternal immune responses were compared to that of three rhCMV-seronegative, immune competent females infected at the same gestational time point, as well as three rhCMV-seropositive pregnant rhesus monkeys who were depleted of CD4+ T cells at the end of first trimester. Transmission outcome was determined by amniotic fluid, placental, and infant plasma and mucosal fluid rhCMV qPCR, as well as placental immunohistochemistry. Maternal rhCMV-specific T cell and antibody responses were assessed throughout acute and chronic infection.

Results:

In the CD4+ T cell-depleted group, 3 females underwent spontaneous abortion 3 weeks following rhCMV inoculation, whereas the 4th female bore a full-term infant. In contrast, all monkeys in both the immune competent, acutely rhCMV-infected group and the CD4+ T cell-depleted, chronically rhCMV-infected females carried their infants to term, indicating neither rhCMV infection nor CD4+ T cell-depletion alone resulted in high abortion rates. Congenital rhCMV infection was confirmed in the CD4+ T cell-depleted monkeys by immunohistochemical and PCR detection of rhCMV in 2 placentas and aborted fetuses, in addition to neutropenia and detectable rhCMV DNA in the saliva and urine of the surviving infant. In contrast, rhCMV transmission was only confirmed in 1 of 3 infants born to the immune competent, acutely rhCMV-infected monkeys by detection of CMV in urine at birth. While the kinetics and avidity of the CMV-binding IgG responses were similar between the immune competent and CD4+ T cell-depleted groups, the functional CMV neutralizing antibody

response was considerably delayed in the CD4+ T cell depleted group at the time of congenital CMV transmission and fetal loss.

Conclusion

Maternal CD4+ T cell responses are critical to fetal survival following primary maternal CMV infection, potentially due to their contribution to the development of CMV-neutralizing antibody responses. This nonhuman primate model for congenital CMV transmission provides an important tool for studies of maternal CMV vaccine candidates.

Phosphorylation is dispensable for Hsf1 activation and the heat shock response

Awardee: David Pincus
Award: Early Independence Award
Awardee Institution: Whitehead Institute for Biomedical Research

Heat shock factor (Hsf1) is a transcription factor conserved in all eukaryotes that controls expression of genes encoding chaperone proteins and other adaptive responses to maintain protein-folding homeostasis in the cell. Environmental stress such as heat shock, genetic stress such as cancer and age-related metabolic stress such as oxidative damage all activate Hsf1. Despite its central role in cellular adaptation, stress resistance and disease, the mechanisms that regulate Hsf1 activity remain unclear. A conspicuous feature of Hsf1 that has been maintained over evolution is that it becomes phosphorylated during shock. Individual sites of phosphorylation have been implicated in both transcriptional activation and repression in a stress- and gene-specific manner. Here we provide evidence that in budding yeast, Hsf1 displays condition-specific patterns of phosphorylation and can be phosphorylated on 73 unique sites. Yet, we find no relationship between phosphorylation and either activation or repression. By *en masse* mutational analysis we show that Hsf1 can tolerate 152 simultaneous point mutations – which remove all phosphorylation – and retain its essential and heat shock inducible activities genome wide and normal attenuation kinetics. However, phosphorylation does destabilize Hsf1 which leads to substantial cell-to-cell variability in Hsp90 levels. In the absence of phosphorylation, decreased cell-to-cell variability translates into reduced acquired resistance to the antifungal drug fluconazole. Phosphorylation is not the switch that activates or deactivates Hsf1 but promotes noise in the heat shock response that enables the evolution of new phenotypes.

Memory network dysfunction as an early marker of preclinical Alzheimer's disease

Awardee: Yakeel Quiroz
Award: Early Independence Award
Awardee Institution: Massachusetts General Hospital

Finding a disease-modifying treatment for Alzheimer's disease (AD) is one of the greatest challenges of our generation. There is a consensus in the scientific community that the key to success in treating AD is to begin therapies as early as possible before significant brain damage occurs. Thus characterizing preclinical biomarkers and early detection paradigms, which is the focus of my work, is paramount.

A distributed network of brain regions, including the hippocampus, adjacent cortical regions in the medial temporal lobe, and other brain regions subserve memory function. Paired associative memory tasks that rely on this network have been shown to be sensitive to subtle deficits in the preclinical stages of AD. I propose to build upon my previous research with autosomal dominant AD to establish conceptual frameworks and comparisons with late-onset sporadic AD with an emphasis on the analysis of memory network disruption as an early marker of preclinical AD. To this end, I will leverage my access to two extraordinarily rich preclinical AD groups, 1) the Colombian kindred with Presenilin 1 E280A (Glu280Ala) mutation, estimated to have 1,500 mutation carriers, and 2) a group of asymptomatic older individuals who are participants in the Harvard Aging Brain Study (HABS) at Mass General Hospital and are considered at high risk (by molecular pathology imaging) to develop late onset sporadic AD.

The primary goals of this proposal are to: (i) investigate abnormalities of associative memory as a sensitive cognitive marker of preclinical AD; (ii) investigate brain hyperactivity/hyperconnectivity as a marker of early AD pathophysiology; and (iii) examine the role of tau and amyloid−β aggregation in memory network dysfunction. The research proposed will use cognitive measures, fMRI and PET imaging to examine the hypothesis that memory network dysfunction occurs in early preclinical stages of Alzheimer's disease. This research will provide insight into the interaction of cognitive and brain function biomarkers in preclinical AD. In particular, this work will provide new understanding of how amyloid and tau pathology impact memory function very early in the disease process, and their role in subsequent neuronal death and cognitive decline.

Toward Coupled Multiphysics Models of Hemodynamics on Leadership Systems

Awardee: Amanda Randles
Award: Early Independence Award
Awardee Institution: University of California-Lawrence Livermore National Laboratory

The goal of this project is to develop a method to simulate flow of realistic levels of cells through the circulatory system, thereby gaining insight into mechanisms that underlie disease progression and localization, or to inform the design of next generation drug delivery systems. Building a detailed, realistic model of human blood flow is a formidable mathematical and computational challenge requiring large scale fluid models as well as explicit models of suspended bodies like red blood cells. This will require high resolution modeling of ~20-30 trillion cells in the blood stream, and necessitate significant computational advances. To date, we have efficiently scaled our algorithms to run on up to 294,912 processors and are working to extend this scalability to allow the study of large regions of the circulatory system. Building on HARVEY, a parallel fluid dynamics application designed to model hemodynamics in patient-specific geometries, we are working to further validate the results through rigorous comparison with in vivo and in vitro measurements. Through the Early Independence Award, we are working to expand the scope of projects to address not only vascular diseases, but treatment planning and the movement of circulating tumor cells in the bloodstream. Development of a precise understanding of cell movement through the vascular system and the likelihood of penetration of the vessel wall is likely critical to achieving the ultimate goal of reliably predicting the vascular regions most likely to incur secondary tumor sites on a per-patient basis. A patient-specific method to predict these patterns will assist in cancer staging, enable identification of unknown primary sites, and inform next-generation treatment therapies that target cancer cells in circulation. We have developed a multiscale computational fluid dynamics model for assessing hemodynamics in image-based arterial geometries, and demonstrated its ability to accurately predict macroscopic quantities related to disease localization and progression. Based on this preliminary data, we hypothesize that (1) cell deformability impacts movement through the vasculature; (2) *in vitro* measurements can both quantify the range of cell-specific parameters and physiological states that should be used in assessing likely metastatic patterns and validate the computational models; and (3) case-specific simulations can assist in the prediction of vascular regions at risk for secondary tumor sites. Here, we will present the current application of HARVEY to the study of cardiovascular disease and discuss the aims designed to test the aforementioned hypotheses as focus is shifted to the study of cancer metastasis and full body circulatory simulations.

Targeting the neural injury via astrocytic phenotypic alterations

Awardee: Sarah E. Stabenfeldt
Award: New Innovator Award
Awardee Institution: Arizona State University
Co-authors: William Marsh, Seong Song
Co-authors institution: Arizona State University

Over 1.7 million persons sustain a traumatic brain injury (TBI) in the U.S. alone. Current diagnostic techniques for TBI are excellent in detecting gross morphological alterations; however, they do little to detect the immediate molecular/cellular alterations. Therefore, there is a critical unmet need to develop targeting motifs that are sensitive to the heterogeneous molecular pathologies associated with TBI. The mechanical insult from TBI initiates immediate cellular death (i.e. primary injury) and stimulates a broad range of complex deleterious signaling cascades (i.e. secondary injury) including a phenotypic switch in astrocytes from basal to reactive phenotypes. This nuanced astrocyte phenotypic alteration is an ideal candidate to develop the next generation molecular targeting strategies diagnostic and therapeutic applications. In this study, we employed phage display biopanning with single chain variable fragment ('nanobody') libraries against viable basal and reactive astrocyte cultures (*in vitro*). Here, we report identification and characterization of novel nanobodies that (1) distinguish viable unfixed astrocytes over other cell types and (2) preferentially bind reactive astrocytes over basal astrocytes. Such targeting motifs will be critical in developing future diagnostic and therapeutic strategies for neural injury that are sensitive to the injury microenvironment.

Interception of host angiogenic signaling limits mycobacterial growth

Awardee: David M. Tobin
Award: New Innovator Award
Awardee Institution: Duke University Medical Center
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Pathogenic mycobacteria induce the formation of complex cellular aggregates called granulomas that are the hallmark of tuberculosis. Here we examine the development and consequences of vascularization of the tuberculous granuloma in the zebrafish-*Mycobacterium marinum* infection model characterized by organised granulomas with necrotic cores that bear striking resemblance to those of human tuberculosis. Using intravital microscopy in the transparent larval zebrafish, we show that granuloma formation is intimately associated with angiogenesis. The initiation of angiogenesis in turn coincides with the generation of local hypoxia and transcriptional induction of the canonical pro-angiogenic molecule VEGFA. Pharmacological inhibition of the VEGF pathway suppresses granuloma-associated angiogenesis, reduces infection burden and limits dissemination. Moreover, anti-angiogenic therapies synergize with the first-line anti-tubercular antibiotic rifampicin as well as with the antibiotic metronidazole, which targets hypoxic bacterial populations. Our data suggest that mycobacteria induce granuloma-associated angiogenesis, which promotes mycobacterial growth and increases spread of infection to new tissue sites. We propose the use of anti-angiogenic agents, now being used in cancer regimens, as a host-targeting TB therapy, particularly in extensively drug-resistant disease where current antibiotic regimens are largely ineffective.

Inhibition of Cell-Cell Adhesion Randomized Epithelial Chiral Morphogenesis on Micropatterned Surfaces

Awardee: Leo Q. Wan
Award: New Innovator Award
Awardee Institution: Rensselaer Polytechnic Institute
Co-authors: Kathryn E. Worley, David Shieh
Co-authors institution: Rensselaer Polytechnic Institute

The development of the vertebrate body plan with left-right (LR) asymmetry (also known as handedness and chirality) requires the emerging chiral morphogenesis of epithelial cells at specific embryonic stages. In this process, cell-cell adhesions coordinate cellular organization and collective cell migration, and are critical for the directional looping of developing embryonic organs such as chicken cardiac tubes. However, the underlying biophysical mechanism is not yet well understood. Here we modeled normal and delayed epithelial LR symmetry breaking with patterned epithelial chiral morphogenesis on microscale lines with various widths. The patterned cells exhibited biased migration wherein those on opposing boundaries migrated in different directions. Disrupting adherens junctions with ethylene glycol tetraacetic acid (EGTA) resulted in a decrease in velocity difference in opposing boundaries as well as the associated biased cell alignment, along with an increase in the overall random motion. Altering the distance between the opposing boundaries did not significantly alter alignment, but significantly disturbed the velocity profile of the cell migration field. The further examination of cell polarity indicated that disruption of adherens junctions did not affect cell polarization on the boundaries, but decreased the transmission of chiral bias into the interior region of the epithelial cell sheet. Overall, our results demonstrated the dependence of the scale of collective cell migration on the strength of cell-cell adhesion, and its effects on the chirality of a multicellular structure through mediating cell polarity in the vicinity of geometric boundaries. This study demonstrated that our 2D microscale system provides a simple yet effective tool for studying LR symmetry breaking, and possibly for fetal drug screening to prevent birth defects.

Re-Beat Whole Decellularized Mouse Heart with Human iPS Cell-Derive Cardiovascular Progenitors

Awardee: Lei Yang
Award: New Innovator Award
Awardee Institution: University of Pittsburg at Pittsburg

Introduction: Approximately one person dies in every 34 seconds as a consequence of heart diseases in the United States, and over 50% of patients with heart disease do not respond to regular medications. Thus heart disease therapy requires novel therapeutic strategies, such as personalized medicines, new resource of cells for replacement therapy, or donor hearts for transplantation. Heart tissue engineering holds a great potential for future heart disease therapy by building patient and disease-specific heart tissues for those applications.

Material and Methods: C57BL6/J mice were euthanized and the ascending aorta was cannulated with a blunted 20-gauge needle that was sutured to allow retrograde coronary perfusion, followed with a decellularization process with enzyme and detergent treatments [1]. In the meanwhile, human induced pluripotent stem (iPS) cells, which were maintained on mitotically inactivated MEFs in human ES cell medium, were induced for cardiovascular differentiation using our established protocol [2,3]. Approximately 1.0 $X10^7$ human iPS cell-derived multipotential cardiovascular progenitors (MCPs) [1,2], were delivered into one acellular mouse heart through the connected cannula. After 20 days culture, the engineered heart constructs showed contractions, followed with histological, electrophysiological and drug response analyses.

Results and Discussions: In this report, we engineered human heart tissues by repopulating whole decellularized mouse hearts with human iPS cell-derived MCPs. MCPs represent the earliest heart progenitors during human cardiogenesis. The seeded MCPs differentiated *in situ* into CMs, smooth muscle cells (SMCs) and endothelial cells (ECs) with high efficiency, which reconstructed the decellularized mouse hearts. The recellularized mouse hearts exhibited myocardium and vessel-like structures, contracted spontaneously with a rate of 40 to 50 beats/min, exhibited intracellular Ca^{2+} transients (CaiT) and responded as expected to various drug interventions. In addition, we found mouse heart ECM could promote proliferation, specific cell differentiation and myofilament formation of cardiomyocytes from the repopulated human MCPs.

Conclusion: This study at the first time utilized a novel patient-specific cell resource, which is the iPS cell-derived MCPs, for engineering patient-specific heart constructs that could be beneficial to study heart development, and future preclinical applications.

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Integrating 3D Bioprinting and Biologically Inspired Nanomaterials for Complex Tissue Regeneration

Awardee: Lijie Grace Zhang
Award: New Innovator Award
Awardee Institution: The George Washington University

Cells within the human body are in intimate contact with a 3D nanostructured extracellular matrix composed of numerous organic and inorganic components. As a result, one of the revolutionary changes in the field of biomaterials and tissue engineering is to develop biologically inspired nanomaterials and advanced 3D biofabrication techniques to create complex tissue construct mimicking native tissue. However, related studies are limited. As an emerging technique for custom fabricated tissue constructs, 3D bioprinting holds great potential to create highly functional tissues and organs with spatiotemporally organized bioactive cues, desirable patient-specific geometry, and well-controlled architecture. Therefore, the main objective of our research is to develop novel biologically inspired nanomaterials and advanced 3D bioprinting techniques to fabricate the next generation of nano tissue scaffolds for complex tissue regeneration (such as vascularized bone and osteochondral tissues). For this purpose, we designed and synthesized innovative biologically inspired nanomaterials (i.e., DNA based self-assembly nanotubes, nanocrystalline hydroxyapaptes and core-shell nanospheres with bioactive factors). Through 3D printing in our lab, a series of biomimetic tissue scaffolds with nano and micro features were fabricated. Our results show that these 3D printed nanocomposite scaffolds have not only improved mechanical properties but also excellent cytocompatibility properties for enhancing various cell growth and differentiation, thus promising for complex tissue regeneration.

A comprehensive and quantitative thalamocortical circuit diagram at the mesoscopic level

Awardee: Haining Zhong
Award: New Innovator Award
Awardee Institution: Oregon Health and Science University

As recognized by the NIH Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Initiative, the precise wiring diagram of brain circuits is the missing link between our understanding of individual gene function and behavioral outcomes, serving as a focal point of current neuroscience research efforts. It is increasingly appreciated that brain circuits are organized around individual functional subdivisions and different neuronal subtypes. However, the complexity of brain subdivisions and cellular heterogeneity has presented major challenges in dissecting the precise circuit mechanisms underlying specific animal behaviors.
We have established a comprehensive mesoscopic circuit diagram, including both the sub-region and cell type components, of the thalamocortical pathway in mice. The thalamus relays both sensory and motor information to the cortex and is an integral part of all cortical executive functions. Dysfunction along this pathway plays a major role in schizophrenia and frontotemporal dementia. We employed a systematic, high-throughput viral approach to visualize thalamocortical axons with high sensitivity. We then developed algorithms to directly compare injection and projection information across animals. By tiling the mouse thalamus with 254 injections, we constructed the first densely-sampled comprehensive map of thalamocortical projections that described the thalamic subdivisions that target specific cortical sub-regions. Using cluster analysis, we identified new circuit properties that could only be revealed by such a large scale, complete dataset. We verified that the characterized projections formed functional synapses using optogenetic approaches. As an important application, we determined the optimal stereotaxic coordinates for targeting specific cortical sub-regions and expanded these analyses to localize cortical layer-preferential projections. This dataset will serve as a foundation for functional investigations of thalamocortical circuits. Our approach and algorithms also provide an example for analyzing the projection patterns of other brain regions.