Mammalian Synthetic Gene Circuits: New Research Tools in Cancer Biology

Awardee: Gábor Balázsi
Award: New Innovator Award
Awardee Institution: The University of Texas MD Anderson Cancer Center

The emerging field of synthetic biology builds gene circuits for scientific, industrial, and therapeutic applications. Adaptability of synthetic gene circuits across different organisms could enable a synthetic biology pipeline, where circuits are first designed *in silico*, then characterized and optimized in microbes, to be finally reimplemented in mammalian settings for practical usage. However, the processes affecting gene circuit adaptability to new cell types have not been systematically investigated. To address this problem, we constructed a mammalian version of a negative feedback-based "linearizer" gene circuit that we previously developed in yeast. The first naïve mammalian prototype was nonfunctional, but a computational model suggested that we could recover function by improving gene expression and protein localization. After rationally developing and combining new parts as the model suggested, we regained function, achieving linearly inducer-dependent gene expression control and low gene expression variability in MCF7 breast cancer cells as previously in yeast.

These results confirmed the adaptability of the yeast linearizer to mammalian cells by a series of well-defined optimization steps, which should be generally relevant for transferring other gene circuits. Following this rationale, we moved additional gene circuits from yeast into cancer cells, establishing cell lines where the expression of specific target genes can be tuned with high nongenetic variability (noise). Using these newly engineered cell lines in parallel with cells carrying the linearizer gene circuit, it becomes possible to test whether nongenetic expression variability of pro-metastatic or metastasis-suppressor protein expression affects cancer progression. Moreover, linear and uniformly tunable protein levels can be used to deliver precise perturbations and thereby unravel the dynamics of regulatory networks driving cancer progression, a crucial step towards rational and efficient cancer treatment design.

Specialized Ribosomes: A New Frontier in Gene Expression and Organismal Biology

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

The regulatory logic for how the one-dimensional genetic code is translated into three-dimensional morphology in a multicellular organism poses one of the greatest challenges to modern biology. Notably, the prevailing dogma has been that the ribosome is an integral but passive participant in directing how the genome is functionally expressed. Our findings unexpectedly reveal that fundamental aspects of gene regulation and mammalian development are instead controlled by "specialized ribosomes," harboring a unique composition or activity, which direct where and when specific proteins are made. For example, we have identified that RPL38, one of the approximately 80 core ribosomal proteins (RPs), acts to establish the mammalian body plan by selectively regulating the translation of homeobox mRNAs, key master regulators of animal development. These findings transform our understanding of gene regulation and suggest newfound specificity to how the genomic template is decoded into proteins to instruct key cell fate decisions. We have also uncovered a second layer of unexpected specificity to the ribosome by performing the first quantitative expression screen for RPs, revealing remarkable heterogeneity in RP expression patterns within different cell types/tissues in the developing vertebrate embryo. Collectively, our ongoing work seeks to define the regulatory basis by which "specialized ribosomes" add a new level of control to gene expression. I will present our recent findings that identify novel *cis*-acting RNA elements embedded genome-wide within mammalian 5' UTRs, which act as regulatory filters that interface with specialized ribosomes. These RNA structured elements functionally act to recruit the 80S ribosome to cellular mRNAs through direct interactions with specific RPs to regulate spatial-temporal gene expression in vivo. We have also undertaken a highly functional approach to define the repertoire of transcripts that rely on specialized ribosome components during cell fate specification. In particular, we have carried out state-of-the-art mass spectrometry to delineate for the first time ribosome heterogeneity during cellular differentiation and to define the repertoire of transcripts that rely on specialized ribosome components for cell fate specification and differentiation. Together, these studies reveal that specialized translational machinery in conjunction with unique *cis*-acting RNA elements within the 5' UTRs of mammalian mRNAs provide a new layer of regulatory control to gene expression that guides evolution and organismal development.

Defining RNA Ligands that Activate PKR during Metabolic Stress

Awardee: Brenda L. Bass
Award: Pioneer Award
Awardee Institution: University of Utah

Co-authors: Osama A. Youssef, Takahisa Nakamura, Sarah A. Safran, Gökhan S. Hotamisligil
Co-authors' Institutions: University of Utah, Cincinnati Children's Hospital Medical Center, Harvard University

The central hypothesis of my Pioneer project is that long, cellular double-stranded RNA (dsRNA) is a previously unrecognized signaling molecule. Since dsRNA binding proteins (dsRBPs) are not sequence-specific, we postulate that dsRBPs that bind viral dsRNA to initiate an immune response also respond to endogenous dsRNA, possibly explaining the inflammatory component of many diseases.

To test this idea, we first focused on the dsRBP PKR. PKR is activated by viral dsRNA, but intriguingly, its kinase activity is also stimulated by metabolic stress (Nakamura et al., 2010). This stimulation requires a functional dsRNA-binding domain, but the cellular RNA required to respond to metabolic stress is unknown. To investigate this, we used mouse embryonic fibroblast (MEF) cells expressing wild-type PKR (PKR_{WT}) or PKR with a point mutation in each dsRNA-binding motif (PKR_{RM}). Cells were incubated with, or without, palmitic acid (PA) to mimic a high-fat or regular diet, respectively, followed by immunoprecipitation of PKR. PKR immunopurified RNAs from two sets of three biological replicates were subjected to high-throughput sequencing. To focus on dsRNA ligands, RNAs enriched in both immunopurified PKR_{WT} and PKR_{RM} after PA treatment were excluded from subsequent analysis.

We identified 122 (Dataset A) and 90 (Dataset B) genes enriched by ≥ 2-fold in PKR_{WT} samples after PA treatment (FDR ≤ 5%). Interestingly, ~40% (Dataset A) and ~80% (Dataset B) of the enriched genes encoded small nucleolar RNAs (snoRNAs). Immunoprecipitation of PKR in extracts of UV-crosslinked cells, followed by RT-qPCR, provided further confirmation that snoRNAs specifically associated with PKR_{WT} after PA treatment.

snoRNAs are noncoding RNAs that act with conserved proteins to modify rRNA and snRNAs. While snoRNAs are highly base-paired, they are not rod-like dsRNA, and their association with PKR was unexpected. To validate that snoRNAs are involved in activating PKR in vivo, we used CHO cells haploinsufficient for the spliceosomal protein SmD3. These cells maintain pre-mRNA splicing, but show reduced levels of snoRNAs that are encoded within introns (Scruggs et al., 2012). We observed that wild-type, but not SmD3-deficient cells, showed increased PKR phosphorylation after PA treatment. Using purified components, we also find that snoRNAs can bind and activate PKR in vitro.

While snoRNAs localize to the nucleolus, most studies show that PKR is cytoplasmic. We are considering a model whereby metabolic stress causes PKR to move to the nucleolus where it binds snoRNAs, and ongoing studies are designed to test this.

Remote Control of Biomolecular Motors Using Light-Activated Gear Shifting

Awardee: Zev Bryant
Award: New Innovator Award
Awardee Institution: Stanford University

Co-authors: Muneaki Nakamura, Lu Chen, Tony D. Schindler
Co-authors' Institution: Stanford University

Cytoskeletal motors perform critical force generation and transport functions in eukaryotic cells. Protein engineering has been used to modify cytoskeletal motors for dynamic control of activity and directionality, providing direct tests of structure-function relationships and potential tools for controlling cellular processes or for harnessing molecular transport in artificial systems. We have previously created myosin motors that can be signaled to switch directions in response to changes in [Ca^{2+}]. Light is a more versatile control signal because it can be precisely modulated in space and time, and is generally orthogonal to cellular signaling. Here we report the design and characterization of a panel of cytoskeletal motors that reversibly change gears—speed up, slow down, or switch directions—when exposed to blue light. Our structural designs incorporate a photoactive protein domain to enable light-dependent conformational changes in an engineered lever arm. We have used in vitro motility assays to confirm robust spatiotemporal control over motor function and to characterize the kinetics of optical gear shifting. Our modular approach has yielded controllable motors for both actin-based and microtubule-based transport. Genetically encoded light-responsive motors will expand the optogenetics toolkit, complementing precise perturbations of ion channels and intracellular signaling with spatiotemporal control of cytoskeletal transport and contractility.

Chemistry-Based Molecular Signature for Central Nervous Sub-System Activity Underlying the Atypia of Clozapine

Awardee: Timothy Cardozo
Award: New Innovator Award
Awardee Institution: New York University

Co-authors: Sergey Shmelkov, Evgeny Shmelkov
Co-authors' Institution: New York University

A functional sub-system in the human central nervous system (e.g., limbic system) consists of the specific network of neural circuits that underlie an observable macroscopic human physiologic phenotype (e.g., memory). At higher resolution, the activity of a specific set of biomolecules within the sub-system likely governs the observed phenotype. Inferring either the neuroanatomical or molecular sub-systems governing specific psychiatric phenotypes has been a particularly difficult challenge, but such inference is critical for understanding the organic basis of psychiatric diseases. Here, we derived an integrated tissue and molecular signature for a psychiatric phenotype, namely the atypical pharmacologic action of the antipsychotic drug clozapine. The signature was derived using the drug's chemical structure as the sole input, and was based on the postulate that the bioactivity of the drug for a particular receptor is only significant in a tissue if the RNA for the receptor is strongly expressed. The results show that dopamine D4 receptors in the pineal gland and muscarinic acetylcholine M1 (CHRM1) receptors in the prefrontal cortex (PFC) are preferentially targeted by clozapine and not Thorazine, suggesting that the action of these receptors in these specific brain tissues is responsible for clozapine's atypical effects. This signature diverges markedly from the consensus view that the ratio of activities against the serotonin 5HT-2a (HTR2A) and dopamine D2 (DRD2) receptors distinguishes atypical antipsychotics. Indeed, our results suggest that the common antipsychotic effect of both drugs derives primarily from 5HT-2a in the PFC and 5HT-2c in the caudate nucleus. From this analysis, clozapine's atypical mood effect derives simply from its action on D4 receptors in the pineal gland, and its activity on positive schizophrenia symptoms (psychosis) that are resistant to chlorpromazine derives from its action on CHRM1 receptors in the PFC. Because these signatures derive exclusively from the chemical structures of these clinically utilized drugs, which have established phenotypes in human subjects, and from gene expression patterns largely in human tissues, the signatures we have identified represent objective candidates for the molecular and neuroanatomical sub-system organic basis of psychosis in humans. D4 and CHRM1 receptors may thus represent new drug targets to advance the treatment of schizophrenia.

Novel Mitochondrially Derived Peptides and Their Role in Health and Disease

Awardee: Pinchas Cohen
Award: Transformative Research Award
Awardee Institution: University of Southern California

Mitochondria are involved in energy metabolism and apoptosis, and are central to the pathogenesis of multiple diseases, including diabetes, cancer, neurodegeneration, and aging. Mitochondria contain nearly a thousand proteins of nuclear origin, but the mitochondrial chromosome only encodes 13 proteins. A decade ago, three labs including our own cloned humanin, a novel 24-amino-acid peptide proposed to be encoded from the 16S ribosomal RNA region of the mtDNA that was shown to be a potent cytoprotective factor that binds and antagonizes Bax and IGFBP-3. Humanin has been shown to be protective, in vitro and in vivo, in models of stroke, amyotrophic lateral sclerosis, and Alzheimer's, and has metaboloprotective activities against diabetes and atherosclerosis. We recently identified an additional six peptides encoded from open reading frames (ORFs) within the 16S rRNA, which we named SHLPs (small humanin-like peptides). Analysis of their expression reveals that they are transcribed in the mitochondria from mtDNA, are detectable in plasma, and exhibit a tissue-specific distribution. SHLPs 1–5 act as potent bioactive molecules acting to induce cell survival and reactive oxygen species (ROS) inhibition (like humanin, via activation of ERK and STAT3 phosphorylation) but with different temporal profiles, suggesting that these peptides may act in concert. SHLP6 has opposing actions, potently inducing apoptosis and inhibiting vascular endothelial growth factor (VEGF) expression and angiogenesis in cancer cells and suppressing the growth of prostate cancer xenografts in severe combined immunodeficiency (SCID) mice. We further identified a novel peptide encoded within the mitochondrial 12S rRNA, which we have named MOTS-c (mitochondrial open-reading-frame of the twelve S rRNA type-c), that acts as a key regulator of metabolic homeostasis. MOTS-c was detected in various tissues and in circulation, suggesting both cell-autonomous and non-cell-autonomous actions. MOTS-c profoundly shifts and coordinates glucose, nucleotide, mitochondrial, and fatty acid metabolism. Notably, MOTS-c causes a >20-fold increase in endogenous AICAR levels, via the de novo purine synthesis pathway, and also activates AMPK signaling, independently of AMP. In mice, MOTS-c significantly activates AMPK in skeletal muscles, and prevents the development of obesity and insulin resistance in response to a high-fat diet. Furthermore, age-dependent muscle insulin resistance was fully reversed by MOTS-c treatment of aged mice. These observations reveal that the mitochondria possess previously unappreciated roles in the regulation of metabolism and cellular function that occur via the production of mitochondrially derived peptides (MDPs). We propose that the mitochondrial peptidome could explain important new aspects of mitochondrial biology and dysfunction with relevance to human biology and disease and that the novel MDPs we describe here may represent retrograde communication signals from the mitochondria.

Accelerated Discovery via a Whole-Cell Model

Awardee: Markus W. Covert
Award: Pioneer Award
Awardee Institution: Stanford University

Co-authors: Jayodita C. Sanghvi, Sergi Regot, Silvia Carrasco, Jonathan R. Karr, Miriam Gutschow, Benjamin Bolival, Jr.
Co-authors' Institution: Stanford University

Whole-cell modeling promises to accelerate biological discovery by prioritizing future experiments based on existing datasets. However, this promise has never been tested. To assess the ability of whole-cell models to make novel and correct predictions, we used a recently developed whole-cell model of *Mycoplasma genitalium* to determine quantitative specific growth rates for all of the single-gene disruption strains, and then compared simulations to new experimental measurements obtained in our laboratory. These comparisons resulted in a comprehensive map of the consistencies and discrepancies between model predictions and experimental observations that covered the entire genome. Further detailed analysis of the discrepancies between simulated and experimental results led to detailed, quantitative model predictions about specific kinetic parameters that had never been previously measured. Our subsequent measurements of these kinetic values corresponded strikingly with the model's predictions. We conclude that whole-cell modeling can make accurate, quantitative predictions about previously unmeasured biological properties, and thereby accelerate biological discovery.

Cellular Mechanotransduction at the Molecular Level

Awardee: Alexander Dunn
Award: New Innovator Award
Awardee Institution: Stanford University

Co-authors: Masatoshi Morimatsu, Armen Mekhdjian, Arjun Adhikari
Co-authors' Institution: Stanford University

Molecular-scale forces direct cellular behavior in diverse circumstances that include stem cell differentiation, cancer metastasis, and tissue growth and repair. In general, however, the mechanisms by which cells sense mechanical cues remain poorly understood. We have recently developed molecular tension sensors (MTSs) that allow us to observe the mechanical forces experienced by single proteins in living cells. Although this technique is broadly applicable, our first application has been to understand how cells sense their environment via integrins, a class of proteins that cells use to adhere to the extracellular matrix. Cells exert traction forces via their integrins during cell migration, and force sensing at integrin complexes is particularly important in directing stem cell differentiation and proliferation. The mechanisms by which integrins and their associated proteins exert and detect mechanical force are thus the subject of intense interest. The enhanced spatial resolution of our measurement allows us to directly visualize forces *within* integrin-containing assemblies, termed focal adhesions (FAs), for the first time. We find that the large majority of integrin molecules experience modest, single-piconewton tensions, suggesting that both adhesion and tension sensing can arise from the collective contribution of relatively weak binding interactions. We also observe striking structural and mechanical heterogeneity within single FAs. Together, these observations suggest the presence of a cellular mechanosensing apparatus on the ~100 nm length scale, whose mechanism is the subject of ongoing investigation.

RNA Memory, Cellular Phenotype, and the Microenvironment

Awardee: James Eberwine
Award: Pioneer Award
Awardee Institution: University of Pennsylvania Perelman School of Medicine

Co-authors: Jai-Yoon Sul, Ditte Lovatt, Brittani Ruble, Ivan Dmochowski, Jaehee Lee, Peter Buckley, Miler Lee, Mugdha Kandalhar, Junhyong Kim
Co-authors' Institution: University of Pennsylvania Perelman School of Medicine

The relative and absolute abundances of cellular RNAs that comprise a cell's expression profile are important for cellular function. We have developed the phototransfection methodology to transfer RNA populations between cells and under the appropriate conditions when transferring populations of RNA, observed the transfer of cellular phenotype as evidenced by altered cellular morphology, antigen presentation, single cell transcriptome, and physiology. Among the cell types that have been modified and generated are neurons into astrocytes, fibroblasts and astrocytes into cardiomyocytes, and human fibroblasts into human liver cells. The idea of RNA memory as practiced by transcriptome-induced phenotype remodeling (TIPeR) has been confirmed and utilized by a number of groups to create various cell types. In analyzing the transcriptomes of single cells for these studies we identified a novel class of RNAs that we call CIRTs (cytoplasmic intron-retaining transcripts) that exist in all cells tested and have been shown to play a role in RNA subcellular targeting, exon selection, and proper protein localization (all dependent upon cytoplasmic splicing). These data have compelled the elaboration of the sentinel RNA hypothesis. To examine the RNA memory idea in vivo we developed a procedure for characterization of the RNA complement from individual cells and subregions of cells that reside in their natural environment. Data from these experiments suggest that the microenvironment is important in elaborating the class of RNAs that are responsible for producing and maintaining proper cellular functioning.

Neural Circuits Underlying Operant Learning in Larval Zebrafish

Awardee: Florian Engert
Award: Pioneer Award
Awardee Institution: Harvard University

We have developed an operant learning paradigm in larval zebrafish where the animal is given the ability to remove an aversive stimulus with a directed tail flick. To that end an infrared laser beam is directed at a head-fixed animal, tail-motion is recorded and analyzed online with a high-speed camera, and the size and direction of the motion is fed back in a closed-loop system to control the power of the laser. We find that fish learn quickly to turn off the laser with a directed tail flick in a specified direction—e.g., to the left—and that they can relearn a reversed

paradigm where the learned behavior is now a tail flick toward the other direction—e.g., to the right. We perform whole-brain two-photon calcium imaging before, during, and after these learning blocks and describe the response properties of various neuronal populations that correlate with and drive the learned behaviors.

Agent_Zero: Toward Neurocognitive Foundations for Generative Social Science

Awardee: Joshua Epstein
Award: Pioneer Award
Awardee Institution: Johns Hopkins University

Under his Pioneer Award, Epstein created a new theoretical entity: "Agent_Zero." This software individual, or "agent," is endowed with distinct emotional/affective, cognitive/deliberative, and social modules. Grounded in contemporary neuroscience, these internal components interact to generate observed, often far from rational, individual behavior. When multiple agents of this new type move and interact spatially, they collectively generate an unprecedented range of dynamics spanning the fields of social conflict, psychology, public health, law, network science, and economics. "Agent_Zero" is a signal departure in what it includes (e.g., a new synthesis of neurally grounded internal modules), what it eschews (e.g., standard behavioral imitation), the phenomena it generates (from vaccine refusal to violence to financial panic), and the modeling arsenal it offers the scientific community. His Pioneer research has produced a book entitled *Agent_Zero: Toward Neurocognitive Foundations for Generative Social Science*, forthcoming from Princeton University Press (2013). The book's Princeton University Press website will also offer a huge library of interactive applets and source code to the scientific community. Epstein will discuss his plans to deepen and scale up "Agent_Zero" at his Center for Advanced Modeling in the Social, Behavioral, and Health Sciences at Johns Hopkins University, founded with the support of the Pioneer Award.

Visualizing Oxygen Tension on the Microscale in 3D *in Vitro* Models of Cancer

Awardee: Conor L. Evans
Award: New Innovator Award
Awardee Institutions: Harvard University, Massachusetts General Hospital

Co-authors: Alexander J. Nichols, Emmanouil Rousakis
Co-authors' Institutions: Harvard-Massachusetts Institute of Technology Health Sciences and Technology Program, Harvard University, Massachusetts General Hospital

Hypoxia is known to play a major role in the poor prognosis and survival of patients with cancer, and is thought to help drive the development of aggressive and metastatic cellular populations. Hypoxia gives rise to a host of therapeutic resistance mechanisms, including cellular quiescence, upregulation of anti-apoptotic factors, and increased expression of DNA repair enzymes, that allow cancer cells to survive treatment with chemotherapeutic agents. Due to the irregular growth of tumors and their leaky, unorganized vasculature, the distribution of oxygen throughout tumorous tissue can be highly heterogeneous on the microscale. It is not entirely understood how this complex oxygenation landscape impacts therapeutic response on the cellular and tumor levels. This lack of knowledge is partially due to the fact that current oxygen imaging tools are focused on providing either large-scale tissue oxygenation measurements or have been designed to report blood oxygenation levels. Both approaches are unfortunately not ideal for microscale mapping of cancer cell subpopulations that lie in hypoxic regions far from the vasculature. Our research has been focused on developing a platform for real-time, cellular-level imaging of oxygen tension deep within tumors. 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 designed to enable cellular uptake even in highly acidic tumor compartments. These new sensors have been tested in a three-dimensional *in vitro* model of ovarian cancer where model tumors grow and develop into large (> 300 μm diameter) nodules that become both hypoxic and acidic. Studies have shown that oxygen sensors built with our sequential click dendrimers readily penetrate throughout large nodules and report oxygenation changes. Current research is focused on modifying these probes for translation to *in vivo* use to visualize, in real time, the oxygenation profile of tumors undergoing therapy.

Identifying and Characterizing Small Molecules from the Human Microbiome

Awardee: Michael Fischbach
Award: New Innovator Award
Awardee Institution: University of California, San Francisco

In complex biological systems, microbe-microbe and microbe-host interactions are often mediated by diffusible small molecules. We set out to study biologically active small molecules encoded by members of the human microbiota. By systematically searching through genomic and metagenomic sequence data generated by the National Institutes of Health Human Microbiome Project, we identified ~10,000 putative biosynthetic gene clusters (BGCs) in the human microbiome. Notably, BGCs in each body site are composed of a small core and a large auxiliary set and are mostly stable over the timescale of months. Remarkably, we find that gene clusters encoding a class of antibiotics that is currently in a phase II clinical trial, the thiopeptides, are widely distributed among the human microbiota. We purified and solved the structure of a new thiopeptide antibiotic, lactocillin, from a prominent member of the vaginal microbiota, *Lactobacillus gasseri*. We show that this antibiotic is potently active against gram-positive bacteria, with a potential role in shaping the composition of the vaginal community and in defending the vaginal flora against pathogens. Moreover, we discovered several thiopeptide antibiotic gene clusters in human gut and oral metagenomic datasets that are not present in any sequenced bacterial strain. Importantly, we show that some of these clusters are expressed in vivo using human metatranscriptomic sequencing data. Our findings illustrate the widespread distribution of BGCs in the human microbiome and show for the first time the production of drug-like molecules by human-associated bacteria.

Custom Redesign of the Human Genome with Engineered DNA-Binding Proteins

Awardee: Charles A. Gersbach
Award: New Innovator Award
Awardee Institution: Duke University

The impact of reverse genetics, synthetic biology, and gene therapy has been restricted by the limitations of conventional genetic engineering technologies. To expand the capacity for genetic modification of mammalian cells, we are engineering artificial DNA-binding proteins, including zinc finger proteins, TAL effectors, and CRISPR/Cas9 to regulate and edit endogenous mammalian genes. For example, we have engineered both protein-based and RNA-guided transcriptional activators targeted to human genes relevant to medicine, science, and biotechnology (Perez-Pinera et al., *Nature Methods*, 2013). Delivery of combinations of transcription factors led to synergistic effects on gene activation and tunable expression levels. This approach recapitulates the previously intractable complexity of natural regulation of mammalian genes that is the product of cooperative actions of many transcription factors. We

have also developed novel methods for controlling the activity of these proteins, such as optogenetic regulation of protein dimerization with blue light (Polstein et al., *Journal of the American Chemical Society*, 2012). In other studies we have engineered synthetic nucleases to stimulate gene targeting to genomic safe harbor sites (Perez-Pinera et al., *Nucleic Acids Research*, 2012). This approach is particularly useful for generating isogenic cell lines. We showed that this method leads to a decrease in the variability of transgene expression within a clonal cell line and between multiple clones relative to conventional techniques. Finally, we have used similar methods to correct mutations causing genetic disease. We engineered synthetic nucleases targeted to the human dystrophin gene that is mutated in Duchenne muscular dystrophy patients (Ousterout et al., *Molecular Therapy*, 2013). When we delivered these nucleases to cells from patients with this disease, the correct gene reading frame and expression of the functional dystrophin protein were restored. We further demonstrated that these nucleases were well-tolerated and did not lead to off-target alterations of the exome in several corrected clonal cell populations. Collectively, these studies demonstrate the potential of engineered DNA-binding proteins to enable new approaches in medicine, science, and technology.

Feel the (Tiny) Force: Mechanical Regulation of Bacterial Pathogenesis

Awardee: Zemer Gitai
Award: New Innovator Award
Awardee Institution: Princeton University

Bacteria, like all organisms, organize their subcellular components to efficiently grow and adapt to constantly changing environments. My lab uses imaging-based approaches to study how bacterial cells use cytoskeletal polymers to establish robust shapes, compartmentalize metabolic networks, and form complex multicellular communities. Recently we discovered that some pathogens, like *Pseudomonas aeruginosa*, a major cause of hospital-acquired infections and cystic fibrosis complications, develop virulence in response to sensing mechanical cues from their hosts. Historically, single-cell organisms were thought to only respond to chemical cues like quorum sensing or nutrient availability, but our data indicate that bacteria also “feel” the presence of their hosts and integrate this information into their developmental response. *P. aeruginosa* can infect virtually every host cell type assayed, so this pathway enables the bacteria to regulate the energetically costly process of virulence using host-nonspecific environmental cues. We also find that mechanical environments can dramatically impact bacterial colonization, as species with polarized motility structures use the regulated retraction of adhesive appendages to migrate upstream against fluid flows. By moving upstream, these "bacterial salmon" colonize niches like catheters and mucosa that flush away most bacterial species. Bacterial mechanosensation requires proteins with homology to mammalian mechanosensors, suggesting that a sense of touch may be deeply conserved.

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, Benjamin Theobald, Brandon D'Arcy, Kevin Abney
Co-authors' Institutions: University of South Alabama

Many drugs and procedures help prevent the onset and progression of atherosclerotic cardiovascular disease (CVD). Nonetheless, CVD is still the leading cause of death in the United States, and the cost of CVD is staggering (more than $444 billion; $1 of every $6 spent on health care in 2010). CVD originates from many different aberrations in normal lipid metabolism (some genetic, some lifestyle choices) that result in elevated plasma lipoproteins (principally low-density lipoproteins, or LDLs) and/or 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 (e.g., 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/or increasing HDLs. However, 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. Studies of mycobacteria survival in human macrophages revealed a surprising observation. Mycobacteria feed on cholesterol while contained in the phagosomes of macrophages. Analysis of bacterial cholesterol catabolism revealed key enzymes that catalyze cholestane ring opening. Our hypothesis is that genes encoding key bacterial 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 three key enzymes (cholesterol dehydrogenase, 3-ketosteroid- Δ1-dehydrogenase [KSTD] and 3-ketosteroid-9-αhydroxylase [Ksh]) that together catalyze B-ring opening and aromatization of ring A. Each enzyme has been expressed individually in *E. coli* and human cells. To control the expression of these enzymes, we are developing a hybrid promoter that incorporates elements of the LXR/RXR responsive region contained in the human ABCA1 promoter (induced by elevated cholesterol), and components of SREBP-2 responsive domain from the HMG-CoA reductase promoter (responsive to low cholesterol). To deliver the "cholesterol catabolism cassette," we are adapting a transcription activator-like effector nuclease (TALENs) targeting the AAVS1 locus in the human genome, using a "ribosomal skipping" sequence between proteins.

Bacteria-Human Somatic Cell Lateral Gene Transfer Is Enriched in Cancer Samples

Awardee: Julie C. Dunning Hotopp
Award: New Innovator Award
Awardee Institution: University of Maryland

Co-authors: Karsten B. Sieber, David R. Riley, Kelly M. Robinson, James Robert White, Ashwinkumar Ganesan, Syrus Nourbakhsh
Co-authors' Institution: University of Maryland School of Medicine

There are 10 times more bacterial cells in our bodies than there are human cells, meaning that a subset of our cells have the potential to be in contact with a vast microbial community. Viral DNA is known to integrate in the human genome, but the integration of bacterial DNA into the human genome has not been described. Using publicly available sequence data from the human genome project, the 1000 Genomes Project, and The Cancer Genome Atlas (TCGA), we examined bacterial DNA integration into the human somatic genome. We find that bacterial DNA integrates into the human somatic genome and that such integrations are detected more frequently in (a) tumors than normal samples, (b) RNA than DNA samples, and (c) the mitochondrial genome than the nuclear genome. Hundreds of thousands of paired reads support random integration of *Acinetobacter*-like DNA in the human mitochondrial genome in acute myeloid leukemia samples. Numerous read pairs across multiple stomach adenocarcinoma samples support specific integration of *Pseudomonas*-like DNA in the 5'-UTR and 3'-UTR of four proto-oncogenes that are up-regulated in their transcription, consistent with conversion to an oncogene. These data support our hypothesis that bacterial integrations occur in the human somatic genome and may play a role in carcinogenesis. We anticipate that the application of our approach to additional cancer genome projects will lead to the more frequent detection of bacterial DNA integrations in tumors that are in close proximity to the human microbiome.

Acoustic Tweezers: Manipulating Particles, Cells, and Organisms Using Standing Surface Acoustic Waves (SSAW)

Awardee: Tony Jun Huang
Award: New Innovator Award
Awardee Institution: The Pennsylvania State University

The ability to manipulate cells and microparticles in a biocompatible and dexterous manner is critical for numerous biological studies and applications such as cell-cell communication, biosensing, tissue engineering, and regenerative medicine. Here we summarize our recent progress on an "acoustic tweezers" technique that utilizes standing surface acoustic waves (SSAW) to manipulate particles, cells, and organisms. This technique is capable of manipulating cells and microparticles regardless of shape, size, charge, or polarity. Its power intensity, approximately 10^7 times lower than that of optical tweezers, compares favorably with those of other active patterning methods. Cell viability, proliferation, and apoptosis studies have revealed the technique to be biocompatible. The aforementioned advantages, along with this technique's simple design and ability to be miniaturized, render the "acoustic tweezers" technique a promising tool for various applications in biology, chemistry, engineering, and materials science.

Phototunable Biomaterials to Engineer Complex 3D Cell Microenvironments

Awardee: Andrea M. Kasko
Award: New Innovator Award
Awardee Institution: University of California, Los Angeles

A critical aspect of designing biomaterial carriers for cells and drug delivery is tuning/controlling the material's degradation behavior. In the last decade, there has been considerable interest in using photochemistry to produce biomaterials because of the ability to form scaffolds under physiological conditions, in the presence of tissues, cells, proteins, and DNA. Because photochemistry has been established as an effective method to form biomaterials under physiological conditions, integrating photochemistry as a degradation mechanism should be equally biocompatible, affording spatial and temporal control over the chemical, mechanical, and physical properties of the biomaterial, and allowing for the controlled release of therapeutic agents, and the ability to trigger this release. Photodegradable biomaterials enable strict temporal control of the degradation process by controlling the exposure to and dosage of light, coupled with precise spatial resolution by using photomasks and/or focused laser beams.

We have synthesized a series of photodegradable linkers based on a photolabile nitrobenzylether (*o*-NB) moiety, whose degradation rate is a function of wavelength, molar absorptivity, and light intensity. We incorporated these photodegradable *o*-NB groups as linkers in hydrogel networks and used them to tether drugs into 3D scaffolds. We developed a predictive model of (multistage) photodegradation and release. We have achieved multi-scale (10^{-7} m to 10^{-2} m) control over the physical properties of this system using both single and two-photon photolysis, and enhanced their sensitivity to two-photon photolysis. We created a catalogue of functionalities at the benzylic position (release site), allowing direct conjugation of virtually any therapeutic agent. We demonstrated the incorporation and release of therapeutic peptides (cell adhesive RGD sequence) and proteins (bovine serum albumin, TGF-beta1) from hydrogel depots, and showed that protein activity is unaffected by the incorporation and release processes. We demonstrated the selective, multistage release of multiple model therapeutics incorporated into a single hydrogel depot and the wavelength-biased release of one cell population over another from a hydrogel depot. We have exploited the unique photophysical properties of our material to generate hydrogels with complex topographies and varying modulus, including unique swelling-induced micropatterns. This innovative biomaterials platform allows the creation of complex 3D cell-instructive microenvironments through multistage release of chemical cues and physical patterning to direct cell growth. No other biomaterial platform has the potential to recapture the complex cascade of signals seen during tissue development. The ability to externally control and manipulate the chemical and physical microenvironment of cells represents a transformative approach to biomaterials and tissue engineering.

Direct In Vivo Assessment of Human Stem Cell Graft-Host Neural Circuits

Awardee: Jin Hyung Lee
Award: New Innovator Award
Awardee Institution: Stanford University

Stem cell therapies hold the potential to treat intractable neurological diseases by replacing lost or damaged post-mitotic cells. However, the effect of a neural transplant on host circuitry has never been measured directly, preventing the systematic design and optimization of these therapies. Here, we present an approach that unifies optogenetics, stem cell biology, and medical imaging to directly map stem cell-driven neural circuit regeneration in vivo. Human neurons were differentiated from a living genetic Parkinson's disease patient's induced pluripotent stem cells after being engineered to express channelrhodopsin-2. Following striatal transplantation, high-field anatomical and functional magnetic resonance imaging (fMRI) was used to non-invasively visualize the ensuing regeneration processes. Tumorigenesis was found to depend on the exact stage of stem cell differentiation, and optical stimulation resulted in robust local and global activity. These results demonstrate a novel platform that can be used to develop human stem cell therapies for central nervous system diseases and assess the functional integration of regenerated tissues in vivo.

Eradicating Chronic Infections

Awardee: Kim Lewis
Award: Transformative Research Award
Awardee Institution: Northeastern University

Chronic infections make up approximately half of all cases of infectious disease. These include biofilm infections of prostheses, indwelling devices, and soft tissues; as well as endocarditis, osteomyelitis, latent tuberculosis, and the untreatable infection of patients with cystic fibrosis. One common feature of chronic infections is an inefficient immune response. Surprisingly, chronic infections are mainly caused by pathogens that are not resistant to antibiotics. We set out to resolve this paradox and found that in biofilms, bacterial populations produce dormant persister cells, phenotypic variants of the wild type. Drug targets are inactive in persisters, which accounts for their tolerance to antibiotics. We find that the difficulty of eliminating pathogens can actually be exacerbated by antimicrobial therapy, which selects for high-persister (*hip*) mutants in various pathogens. For example, in *E. coli* isolated from patients with urinary tract infection, gain-of-function mutations in the HipA kinase, a protein synthesis inhibitor, increases production of persisters. *Hip* isolates are analogous to resistant mutants, but rely on a mechanistically different approach to survive antibiotics. Dormancy pathways are redundant, suggesting that the traditional inhibition of targets will not eliminate persisters. We

reasoned that an opposite approach, *activation* of a target such as a protease, may kill persisters. We discovered a natural product, lassomycin, which activates the ATPase of the essential mycobacterial protease ClpP1P2C1, and found that it kills persisters in *M. tuberculosis*. A known activator of the ClpP protease of eubacteria, acyldepsipeptide (ADEP), completely sterilized a biofilm of *S. aureus* in vitro. Proteomic data of persisters shows that ADEP forces the cell to self-digest. ADEP was able to completely eradicate an untreatable chronic *S. aureus* infection in a mouse biofilm model. These findings both identify the culprit of chronic infections, and point the way to disease eradication by activation/corruption of targets.

Nanoelectronics Meets Biology

Awardee: Charles M. Lieber
Award: Pioneer Award
Awardee Institution: Harvard University

Nanoscale materials enable unique opportunities at the interface between the physical and life sciences, and the interfaces between nanoelectronic devices and cells, cell networks, and tissue makes possible communication between these systems at the length scale relevant to biological function. In this presentation, the development of nanowire nanoelectronic devices and their application as powerful tools for the recording and stimulation from level of single cells to tissue will be discussed. First, a brief introduction to nanowire nanoelectronic devices as well as comparisons to other tools will be presented to illuminate the unique strengths and opportunities enabled by active electronic devices. Second, opportunities for the creation of powerful new probes based on controlled synthesis and/or bottom-up assembly of nanomaterials will be described with an emphasis on the creation of nanowire probes capable of intracellular recording and stimulation at scales heretofore not possible with existing electrophysiology techniques. Third, we will take an “out-of-the-box” look and consider what the future might hold in terms of merging nanoelectronics with cell networks in three dimensions to “synthesize” “cyborg” tissues, as well as novel tools for in vivo recording. The prospects for blurring the distinction between electronic and living systems in the future will be highlighted.

Sweet Patterns: Revelations from Systems-Based Glycan Analysis

Awardee: Lara Mahal
Award: New Innovator Award
Awardee Institution: New York University

Glycosylation, which creates a diverse array of carbohydrate epitopes attached to cell surface proteins and lipids, is an inherently complex system that is poorly understood. Carbohydrates play crucial roles in a diverse array of medically relevant biological processes from viral pathogenesis to tumor cell metastasis and stem cell differentiation. Systems-based approaches to biology, in which large datasets are analyzed using bioinformatic algorithms, provide an important avenue for exploring the mechanics of complex systems that cannot be predicted a priori. Using our glycomic analysis platform, lectin microarrays, on the NCI-60, a set of human tumor lines, my laboratory has identified new glycan regulation mechanisms and the role of glycosylation in epigenetic loops controlling cell fate. Specifically, we have shown that miRNA are a major regulator of the human glycome. By integrating our glycomic dataset with miRNA expression data using a multidimensional analysis, we discovered unique associations of miRNA and glycan structures that map onto the miRNA regulation of genes involved in glycan biosynthetic pathways. We have validated these pathways and begun to interrogate further the relationship between miRNA and the glycome. We have discovered a series of glycosylation-related genes, in miRNA-controlled networks, that directly impact cell fate through regulation of epithelial to mesenchymal transition. Our work opens up cell regulatory loops to include glycosylation, which can impact multiple signaling pathways of the cell. This argues both that the miRNA networks can map specific cell glycosylation into known regulatory networks, and that glycans are both outcomes and inputs into these networks, providing a new point of control to target.

A Systems Approach to Identifying ALS Disease Mechanisms

Awardee: Tom Maniatis
Award: Pioneer Award
Awardee Institution: Columbia University

Amyotrophic lateral sclerosis (ALS) is a fatal, adult-onset neurodegenerative disease characterized by the progressive loss of motor neurons. Approximately 5 to 10% of ALS cases are familial, while the remainder are sporadic, with no family history. Genetic studies and whole exome sequencing have identified several genes in which dominant gain-of-function mutations cause ALS, and some of these mutations have been found in sporadic ALS patients, apparently a result of "de novo" mutations. Although some of these "ALS genes" encode proteins thought to be critical for normal motor neuron function, mutations in other genes appear to cause the formation of toxic protein aggregates. In addition, another class of ALS mutations is found in proteins that function in the removal of protein aggregates (components of the ubiquitin/proteosome and autophagy pathways). Thus, mutations that promote aggregation or interfere with protein homeostasis appear to be causative factors in ALS.

ALS is not simply a disease of motor neurons, but is a multicellular disease that also involves astrocytes, microglia, and oligodendrocytes. My laboratory is systematically interrogating the transcriptomes of whole spinal cords and acutely isolated glial cells from ALS mouse models by RNA deep sequencing methods in an effort to identify pathways that are critical to disease progression. In addition, we are analyzing the affects of coculturing astrocytes bearing ALS-causing mutations with embryonic stem (ES) cell–derived motor neurons. We have previously shown that astrocytes bearing ALS mutations adversely affect the viability of cocultured ES cell-derived motor neurons. We analyzed the transcriptomes of purified astrocytes and motor neurons using a sandwich culture method in which the two cell types are cocultured without direct contact and then recovered for RNA-Seq analysis. Analysis of these transcriptomes has revealed striking cell autonomous and nonautonomous changes in gene expression in cocultured motor neurons and glia, indicating that the two cell types profoundly affect each other. In addition, we found a remarkable concordance between the cell culture data and expression profiles of whole spinal cords and acutely isolated spinal cord cells during disease progression.

We are establishing a comprehensive database of ALS mutations derived from whole exome and genome DNA sequencing, and of transcriptomes from different cell types derived from both ALS mouse models and patients. We are interrogating this database with the goal of identifying disease-specific alterations in critical signaling pathways with the ultimate objective of identifying drug targets for the treatment of ALS.

How the Mouse Brain Is Wired: Connectivity Mapping in the Whole Mouse Brain

Awardee: Partha Mitra
Award: Transformative Research Award
Awardee Institution: Cold Spring Harbor Laboratory

Brains and nervous systems derive their unique capabilities from their neural network architecture. Understanding brain function and dysfunction is therefore contingent on understanding how brains are wired. However, even after a century of neuroanatomical research our knowledge of brain connectivity remains quite incomplete. In contrast with completely mapped genomes of multiple species, there has been no comprehensive circuit map available for any mammal. Recognition of this gap has led in the last few years to a modern era of neuroanatomical circuit mapping, with intensifying efforts at multiple levels of spatial resolution.

The goal of the Mouse Brain Architecture Project (http://mouse.brainarchitecture.org) is to obtain a brain-wide connectivity map of the mouse. The project employs a core technique from classical neuroanatomy—injection of a neuronal tracer substance, i.e., a molecule or a virus that is taken up by neurons, and transported along axons away from (anterograde tracer) or towards the cell body (retrograde tracer). We have employed four different tracer substances, two "classical" tracers and two viral tracers, anterograde and retrograde. There are two innovations compared to the classical methodology:

(1) Grid-based Approach: The injections are systematically placed on a grid of injection sites, each site receiving a single injection in a single mouse of an anterograde or a retrograde tracer substance. This grid-based, "shotgun"-like approach allows for the construction of a comprehensive, brain-wide map.

(2) Digital Microscopy and Computational Neuroanatomy: Traditionally, tracer-labeled sections have been visualized under a microscope and analyzed by expert neuroanatomists to draw conclusions. However, this is not a scalable approach and does not lead to digital datasets that can be subjected to computational analysis. We have employed digital slide-scanning microscopes to digitize the entire tracer-labeled mouse brain, and subjected the results to computational analysis in order to extract connectivity information in the context of a geometrical atlas (as opposed to named brain compartments, which are visually identified).

Data have been released pre-publication on the project website, providing a virtual microscope into the whole brain datasets. The process of computational analysis of these data to assemble the brain-wide map is in progress. Data from the project will be presented including

reconstructed three-dimensional projection patterns across the brain, along with computational analyses and inferences about the brain-wide connectivity architecture.

Contrast-Enhanced and Image-Guided Surgery for Lung Cancer

Awardees: Shuming Nie and Sunil Singhal
Award: Transformative Research Award
Awardees' Institutions: University of Pennsylvania, Emory University

Co-author: May Dongmei Wang
Co-author's Institution: Georgia Institute of Technology

This Transformative Research Award project aims to develop new and innovative technologies for intraoperative cancer detection and image-guided surgery. Its primary goal is to help the surgeon to delineate tumor margins, to identify diseased lymph nodes and micrometastases, and to determine if the tumor has been completely removed. The technologies under development are broadly applicable to many types of solid tumors, but concerted efforts are currently directed toward lung cancer, one of the most aggressive human malignancies and a worldwide health problem for both men and women. In addition to the development of surface-enhanced Raman scattering (SERS) and pH-activated fluorescent nanoparticles for image-guided cancer surgery, we have performed intraoperative detection of tumor margins and image-guided resection of satellite lesions, metastatic lymph nodes, and micrometastases in large animal models (canines) with naturally occurring lung tumors. Importantly, we were able to initiate a clinical trial ahead of schedule due to the highly promising results from spontaneous canine tumors (considered most relevant to the human disease), and we were able to enroll more lung cancer patients than the originally goal because more patients consented to the image-guided surgical procedure. To date, we have enrolled 33 patients undergoing surgery for removal of a mass with image-guided surgery. All patients successfully underwent a standard-of-care operation. There were different histological subtypes including lung carcinomas. In one case, the surgeons made discoveries due to tumor fluorescence that warranted an additional resection. In another patient, tumor fluorescence from a resected specimen identified a close margin (<1 mm) that required immediate re-resection. There were no associated toxicities and, to date, no patients have developed a local recurrence. Future studies will include image-guided cancer surgery under minimally invasive conditions (endoscopic and robotic surgery) as well as the enrollment of more patients for statistical assessment of recurrence and survival.

Designing Plasmonic Lenses and Nanostructures for Bioimaging

Awardee: Teri Odom
Award: Pioneer Award
Awardee Institution: Northwestern University

We will describe our progress on using metal nanostructures to image and resolve sub-cellular structure. First, we will discuss a new approach to design plasmonic lenses using a genetic algorithm. This strategy enabled us to pre-select or encode information into the lenses based on the desired focusing properties. Gold thin films perforated with different arrangements of nanoholes, but with the same overall footprint size, could be focused to any location in three dimensions. Moreover, a single lens could produce multiple focal points. This property has potential for simultaneous (diffraction-limited) imaging of features in thick samples. Second, we will demonstrate that gold nanostars can be used as a nanoscale probe to resolve how oligonucleotide drugs interact with the cancer cell nucleus. We found that DNA aptamers grafted to the surface of the nanostars could bind to a cell-surface marker and shuttling protein for transport near the nucleus. The DNA drugs were found to produce severe, local changes in nuclear phenotype based on the location of the drug-loaded nanostars.

Giga-pixel Nanoscopy on a Chip: A Computational Wide-Field Look at the Nanoscale without the Use of Lenses

Awardee: Aydogan Ozcan
Award: New Innovator Award
Awardee Institution: University of California, Los Angeles

My research focuses on the use of computation/algorithms to create new optical microscopy, sensing, and diagnostic techniques, significantly improving existing tools for probing micro- and nano-objects while also simplifying the designs of these analysis tools. In this presentation, I will introduce a new set of computational microscopes which use lens-free on-chip imaging to replace traditional lenses with holographic reconstruction algorithms. Basically, 3D images of specimens are reconstructed from their "shadows" providing considerably improved field-of-view (FOV) and depth-of-field, thus enabling large sample volumes to be rapidly imaged, even at nanoscale. These new computational microscopes routinely generate >1–2 billion pixels (giga-pixels), where even single viruses can be detected with a FOV that is >100 fold wider than other techniques. At the heart of this leapfrog performance lie self-assembled liquid nano-lenses that are computationally imaged on a chip. These self-assembled nano-lenses are stable for >1 hour at room temperature, and are composed of a biocompatible buffer that prevents nano-particle aggregation while also acting as a spatial "phase mask." The field-of-view of these computational microscopes is equal to the active-area of the sensor-array, easily reaching, for example, >20 mm^2 or >10 cm^2 by employing state-of-the-art CMOS or CCD imaging chips, respectively.

In addition to this remarkable increase in throughput, another major benefit of this technology is that it lends itself to field-portable and cost-effective designs which easily integrate with smartphones to conduct giga-pixel tele-pathology and microscopy even in resource-poor and remote settings where traditional techniques are difficult to implement and sustain, thus opening the door to various telemedicine applications in global health. Some other examples of these smartphone-based biomedical tools that I will describe include imaging flow cytometers, immunochromatographic diagnostic test readers, bacteria/pathogen sensors, blood analyzers for complete blood count, and allergen detectors. Through the development of similar computational imagers, I will also report the discovery of new 3D swimming patterns observed in human and animal sperm. One of this newly discovered and extremely rare motion is in the form of "chiral ribbons" where the planar swings of the sperm head occur on an osculating plane creating in some cases a helical ribbon and in some others a twisted ribbon. Shedding light onto the statistics and biophysics of various micro-swimmers' 3D motion, these results provide an important example of how biomedical imaging significantly benefits from emerging computational algorithms/theories, revolutionizing existing tools for observing various micro- and nano-scale phenomena in innovative, high-throughput, and yet cost-effective ways.

Nano-Bio Interfacing and Single-Cell Transcriptomics: Innovative New Tools for Cell and Neurobiology

Awardee: Hongkun Park
Award: Pioneer Award
Awardee Institution: Harvard University

In this presentation, I will describe three new experimental platforms—vertical silicon nanowires, single-cell transcriptomics, and nanodiamond-based sensing—that have been developed in my laboratory during my Pioneer Award period and discuss their applications in various biological inquiries, especially in relation to immunology and neuroscience. First, I will describe a vertical nanowire platform that enables high-throughput interrogation and manipulation of living cells by providing multiplexed chemical and electrical interfaces to a cell's interior in a minimally invasive fashion. Using this platform to deliver surface-bound molecules into the cell's cytosol, we are investigating intracellular molecular circuits that govern the behavior of various immune cells and neurons. We are using the same nanowire platform to gain direct electrical access to neurons, thus controlling and monitoring of neuronal activity in a multiplexed fashion. Second, I will describe a newly developed pipeline for single-cell transcriptomics that is broadly applicable to various cell types, and discuss how we are using it to discover variations between individual immune cells in both the abundance and splicing of RNA transcripts. This new profiling method provides a powerful new tool in immunology and neurobiology, enabling the elucidation of functional diversities between cells and the discovery of distinct cell states and circuits. Finally, I will end my talk by describing a new biological sensing platform based on nitrogen-vacancy centers in nanoscale diamonds and discuss how we are using it to sense temperature and electric/magnetic fields associated with the activities of living cells.

Fetal Brain Damage: A Placental Disorder

Awardee: Anna Penn
Award: New Innovator Award
Awardee Institution: Children's National Medical Center

Co-authors: Danielle Leuenberger, Marianna Kiraly, Florian Ermini, Marina Abramova, Anca Pasca, Karen Chisholm
Co-authors' Institution: Stanford University

The placenta is a vital component of healthy pregnancy. Compromised placental function has been linked to diverse developmental disorders including cerebral palsy, autism, and schizophrenia. We are investigating changes in placental hormones that shape fetal brain development. As an endocrine organ, the placenta produces a wide array of neuroactive hormones. This endocrine function can be disrupted in many ways—by abnormal gene expression, infection, prematurity—resulting in long-term damage. Preterm birth, affecting one tenth of all deliveries, provides the most extreme case of hormone loss, but we hypothesize that it is just one of many cases in which placental dysfunction leads to brain damage. We have developed novel animal models in which individual hormones are specifically removed from the placenta to test this hypothesis directly. For our first target, we have focused on manipulation of the neurosteroid allopregnanolone (AP or 3α, 5α tetrohydroprogestrone), which is made in the placenta from progesterone. AP has significant CNS affects, including GABAergic modulation and promotion of neural survival. Using shRNA lentiviral-mediated infection of mouse blastocysts to suppress the synthetic enzyme for AP, 3αHSD, we have shown specific disruption of cortical progenitor production during gestation. Concurrently, we have assessed neural circuit formation in the neonatal cortex and hippocampus following fetal AP manipulation and find that AP suppression may delay circuit maturation. In human placental samples, measurements of steroid-converting enzymes show changes in expression across gestation, as well as differential changes with specific pathologies. Preclinical investigation of neurosteroid replacement as a therapeutic treatment for preterm brain injury is now underway. We are also extending our studies to small placental peptides, such as oxytocin, VIP, and secretin. Taken together, our experiments provide a direct demonstration that hormone production by the placenta (not from the maternal or fetal circulation) is needed for normal anatomical and functional development of the fetal brain. These findings open the door for new therapeutic approaches to developmental brain disorders through replacement of one or more hormones to which the fetus or neonate is normally exposed to prevent or ameliorate developmental brain injury.

Regulation of Opren Chromatin in Stem Cells and Development: the case of Vitamin C in DNA Demethylation

Awardee: Miguel Ramalho-Santos
Award: New Innovator Award
Award Institution: University of California, San Francisco

Our lab is interested in the mechanisms that underlie the decondensed chromatin state of pluripotent cells, focusing on embryonic stem (ES) cells, early embryos, and the germline. We investigate the role of histone variants, histone marks, chromatin remodelers, and DNA methylation. We recently gained novel insights into the regulation of DNA methylation, a heritable epigenetic modification involved in gene silencing, imprinting, and the suppression of retrotransposons. Global DNA demethylation occurs in the early embryo and the germline and may be mediated by Tet (ten-eleven-translocation) enzymes, which convert 5-methylcytosine (mC) to 5-hydroxymethylcytosine (hmC). Tet enzymes have been extensively studied in mouse ES cells, which are generally cultured in the absence of Vitamin C (VitC), a potential co-factor for Fe(II) 2-oxoglutarate dioxygenase enzymes like Tets. We found that addition of VitC to ES cells promotes Tet activity leading to a rapid and global increase in hmC. This is followed by DNA demethylation of numerous gene promoters and up-regulation of demethylated germline genes. Tet1 binding is enriched near the transcription start site (TSS) of genes affected by VitC treatment. Importantly, VitC, but not other antioxidants, enhances the activity of recombinant Tet1 in a biochemical assay, at the physiological concentrations of Vitamin C in human plasma and cells. Moreover, the VitC-induced changes in hmC and mC are entirely suppressed in Tet1/2 double knockout (Tet DKO) ES cells. VitC has the strongest effects on regions that gain methylation in cultured ES cells compared to blastocysts and in vivo are methylated only after implantation. In contrast, imprinted regions and intracisternal A-particle (IAP) elements, which are resistant to demethylation in the early embryo, are resistant to VitC-induced DNA demethylation.

Collectively, our work establishes that VitC is a potent and direct regulator of Tet activity and DNA methylation fidelity in ES cells. We are following up these findings with analyses of how histone marks may also be affected by VitC, using ChIP-Seq. We can already document that the heterochromatin mark H3K9me3, and potentially other marks, are also significantly reduced upon VitC treatment. These results suggest that VitC may be a broad “eraser” of epigenetic information. The discovery that a well-known essential nutrient has entirely novel functions with profound effects in gene regulation raises several interesting questions for future work in the fields of developmental biology, reproductive health, nutrition, regenerative medicine, and cancer research.

Reconstructing Regulatory Circuits: Lessons from Immune Cells

Awardee: Aviv Regev
Award: Pioneer Award
Awardee Institution: Massachusetts Institute of Technology, Broad Institute

Molecular circuits are the information processing devices of cells and organisms, transforming extra- and intra-cellular signals into coherent cellular responses. Past studies to chart key circuits required decades of serial work to identify and connect a few components or interactions at a time. While there has been hope that genomic approaches would make it possible to systematically reconstruct circuitry, genomic studies have largely been observational and rarely involve large-scale testing and refinement of models.

With the help of the Pioneer Award, we developed a systematic computational and experimental approach to reconstruct circuitry by profiling the circuit's output or internal state along a relevant time course, learning a computational model that explains the observed data, perturbing every key component proposed by our model, and repeating the process, until data and model converge. All circuits are dynamic, and rewire in response to perturbation, at time scales from minutes to eons, as cells respond to new environmental conditions, differentiate, or evolve. We focus on a carefully selected model system at each time scale: the regulatory circuit controlling the response of primary mouse dendritic cells to pathogens (short term); differentiation of hematopoietic cells, especially in the T cell lineage (longer term); and the rewiring of nutrient responses in a yeast phylogeny spanning 300 million years and 15 species (evolution).

In this talk, I will focus on our study of the regulatory circuit governing the differentiation of Th17 cells, pro-inflammatory immune cells implicated in many autoimmune diseases. We measured transcriptional profiles along a time course of Th17 differentiation, and developed a model with dozens of regulators embedded within a time-varying protein-protein, protein-DNA network. We used nanowires to deliver RNAi to test and refine our model, showing the network is organized into two coupled, self-reinforcing, yet mutually antagonistic modules, explaining how balance is maintained in T cell differentiation. The nodes defined by our model have identified new pathways essential in Th17 differentiation and autoimmunity, for example proposing that the inducible kinase SGK1 is critical for the Th17 cell maintenance circuit, a prediction we validated in vitro and in vivo in mouse models of multiple sclerosis. Because SGK1 is induced by high salt, we hypothesized a novel role for a high-salt diet in promoting Th17 pathogenesis and autoimmunity; we have demonstrated this experimentally in mice. Our work explains the global principles of balance in a complex differentiation system and open new avenues for therapeutic intervention.

Complete Circuits for Understanding Complex Behavior: The *Drosophila* Larva

Awardee: Aravinthan D. T. Samuel
Award: Pioneer Award
Awardee Institution: Harvard University

The major goal of systems neuroscience is to achieve an understanding of complex behavior in terms of the structure and dynamics of an entire nervous system from sensory input to motor output. The fruit fly *Drosophila*, with its strong homologies to vertebrate models, is an excellent system for mapping the connections between neural circuit properties and animal behavior. Its larval form, advantages notwithstanding, has been relatively understudied. We have used funding from the Pioneer program to build a framework and assemble a toolbox for analyzing the neural basis of larval navigational behaviors including phototaxis, chemotaxis, and thermotaxis. We are now beginning to map the components of navigational decision-making to the physiological properties of specific neurons and circuits in the live animal, combining the latest advances in whole brain connectomics, optogenetics, and multineuronal recording using optical probes. With the larva, it should be possible to link the algorithms that organize whole animal behavior to the structure and function of entire brain circuits from sensory input to motor output without gaps.

A Cognitive Framework for Understanding Cellular Behavior

Awardee: Saeed Tavazoie
Award: Pioneer Award
Awardee Institution: Columbia University

A defining characteristic of cellular life is the capacity to deal with environmental change. Microbial behaviors have long been viewed from the perspective of homeostasis, where the response to a stimulus is interpreted as a restoring force that counteracts the immediate intracellular consequences of a change in the environment. However, native environments are highly structured in both space and time, exposing microbes to recurring correlations in environmental parameters over many timescales. We have shown that this correlation structure imposes strong fitness constraints on behavior, selecting for regulatory networks that internalize dynamical models that enable microbes to make predictions about the future trajectory of the environment. These predictive internal representations are analogous to those implemented by neurobiological associative learning since they show plasticity to novel environments over the course of laboratory experimental evolution. Our observations challenge the dominance of the homeostatic framework and emphasize the role of perception and prediction in interpreting cellular behavior. In particular, traditionally defined “stresses” may be important to the organism not because of their immediate and direct fitness consequences, but in the *information* that they convey about the overall state of the

environment and its future trajectory. This perspective has fundamental implications for understanding microbial behaviors and their adaptation to novel environments. Indeed, we have shown that much of the fitness deficit of bacteria exposed to extreme environments is due to regulatory constraints that, although adaptive in the native setting, can substantially repress requisite biochemical capacities in foreign environments. Through fitness profiling and experimental evolution we have shown that loss of function mutations in regulatory and metabolic genes can rewire cellular networks to allow rapid adaptation to extreme environments. Our studies have revealed principles of regulatory network evolution that are critical for understanding cellular behavior and adaptation.

Protective Immunity to Diverse Influenza Viruses Elicited by Synthetic DNA Vaccines Targeting the Globular

Awardee: David Weiner
Award: Transformative Research Award
Awardee Institution: University of Pennsylvania

Co-authors: Jian Yan, Daniel O. Villarreal, Trina Racine, Jaemi S. Chu, Jewell N. Walters, Matthew P. Morrow, Amir S. Khan, Niranjan Y. Sardesai, J. Joseph Kim, Gary P. Kobinger
Co-authors' Institution: University of Pennsylvania

Despite an intensive vaccine program influenza infections remain a major health problem, due to the viruses' ability to change its envelope glycoprotein hemagglutinin (HA), through shift and drift, permitting influenza to escape protection induced by current vaccines or natural immunity. We have been developing a synthetic strategy to target diverse seasonal as well as *newly emerging HA variants* through synthetic mico consensus design. By this strategy, a small collection of as few as 4 HA1 immunogens induces protective HAI against 110 years of diverse H1 viruses as well as providing a stockpile already in place against future emerging HA1 viruses which would be constrained by the biology of HA1 viruses. We provide evidence that this approach is protective against new emerging HA1 viruses as well as historic viruses. As a second example we applied this approach to the newly emerging H7N9, a virus family which has caused global concern. There have been more than 130 laboratory-confirmed human infections of H7N9 resulting in an alarmingly high death rate (32.3%). Currently, no vaccines or drugs are effectively able to target H7N9. Here, we demonstrate the rapid development of a synthetic consensus DNA vaccine (pH7HA) which elicits potent protective immunity against the new family of H7N9 viruses. We show that pH7HA induces broad antibody responses that bind to divergent HAs from multiple new members of the H7N9 family. These antibody responses result in high-titer HAI against H7N9. Simultaneously, this vaccine induces potent polyfunctional effector CD4 and CD8 T cell memory responses. Animals vaccinated with pH7HA are completely protected from H7N9 virus infection and the morbidity associated with lethal challenge. These studies establish that the synthetic consensus DNA vaccine approach represents a new tool for

targeting influenza infection inducing diverse immunity that is apparent in the standard HAI assay allowing a small collection of immunogens to provide broad protection against both prior as well as newly emerging seasonal variants with high accuracy allowing stockpiling. Furthermore the simplicity of design and development without viral material allows for vaccine design, testing, and development into seed stock for vaccine production in a few days in pandemic influenza settings.

Implantable Multi-Analyte Sensors for the Continuous Monitoring of Body Chemistries

Awardee: Natalie Wisniewski and Mike McShane
Award: Transformative Research Award
Awardee Institution: PROFUSA, Inc. and Texas A&M University

Co-authors: Kristen Helton, Soya Gamsey
Co-authors' Institution: University of Washington

Continuous, multi-analyte, in vivo sensors have the potential to dramatically change medical treatment by paving the way for decentralization of health care delivery and shifting the focus away from reactive treatment to preventative maintenance. The largest hurdle in developing implantable sensors that enable long-term continuous monitoring of health status is the foreign body response (FBR). Here we show for the first time that porous, tissue-integrating sensor scaffolds enable long-term oxygen sensing in rats for 6 months. Tissue integrating sensors showed significantly improved response time and sensitivity compared to current conventional sensors. The three dimensional sensor scaffolds promote capillary in-growth throughout the interior of the sensor rather than the typical FBR fibrous capsule formation (100 vs. 0 microvessels per $mm^{2,}$ $P < 0.01$). The tissue-integrating approach allows intimate access to metabolic markers and other analytes of interest without the use of drugs or other tissue response modifiers. Analyte-specific luminescent sensing chemistries were embedded in these hydrogel scaffolds and monitored wireless through the skin with near infrared (NIR) light. The tissue-integrating strategy has the potential to transform current biosensing paradigms by utilizing highly miniaturized, fully implantable, multi-analyte sensors composed of luminescent tissue-integrating materials for continuous monitoring of body chemistries.

Programming Nucleic Acids Self-Assembly

Awardee: Peng Yin
Award: New Innovator Award
Awardee Institution: Harvard University

I will discuss my lab's research on engineering synthetic, nucleic acid-based nanostructures and their applications in biosensing, imaging, nanofabrication, and tissue engineering.

We have recently invented a general framework for programming the self-assembly of short synthetic nucleic acid strands into prescribed target shapes or demonstrating their prescribed dynamic behavior. Using short DNA strands, we have demonstrated the modular construction of sophisticated 1D (*Science*, 321:824-826, 2008), 2D (*Nature*, 485:623-626, 2012) and 3D (*Science*, 338:1177-1183, 2012) structures on the 100-nanometer scale with nanometer precision. Using reconfigurable DNA hairpins, we have demonstrated diverse, dynamic behavior such as catalytic circuits, triggered assembly, and autonomous locomotion.

By interfacing these synthetic, nucleic acid nanostructures with functional molecules, we are developing a diverse range of applications. In biosensing, we have constructed robust and ultraspecific probes for detecting single-base changes in a single-stranded DNA/RNA target. In bioimaging, we have engineered geometrically encoded fluorescent barcodes for highly multiplexed single-molecule imaging and dynamic fluorescent probes for super-resolution imaging. In nanofabrication, we have collaboratively developed a versatile framework for producing inorganic materials (e.g. graphene, silicon dioxides silver, gold) with arbitrarily prescribed nanometer scale shapes. In tissue engineering, we have developed a general strategy to engineer DNA directed self-assembly of biocompatible hydrogel bricks into complex architectures.

I also will discuss my lab's ongoing work on moving the DNA nano-structures from test tubes to RNA based systems living cells, in particular a reconfigurable RNA hairpin system for implementing complex in vivo logic.

In Vivo Reprogramming of Astrocytes to Neuronal Precursors and Functional Neurons in the Adult Brain

Awardee: Chun-Li Zhang
Award: New Innovator Award
Awardee Institution: University of Texas Southwestern Medical Center

Co-authors: Wenze Niu, Tong Zang, Yuhua Zou, Sanhua Fang, Derek Smith, Robert Bachoo
Co-authors' Institution: University of Texas Southwestern Medical Center

Injuries to the central nervous system lead to irreversible neuronal loss. Neural regeneration from endogenous cells could be an ideal approach to replenish the lost neurons and repair the damage. Astrogliosis is a hallmark of neural damage. These reactive astrocytes are initially beneficial by restricting damage spread but detrimental for long-term recovery and repair of the injured central nervous system. We examined ways to change the fate of these astrocytes to neurons by in vivo screens in the adult mouse brain. Through multiple genetic lineage-tracing approaches, we show that the single transcription factor SOX2 is sufficient to reprogram resident mature astrocytes into proliferative neuronal precursors in the adult mouse brain. These induced neuronal precursors, also called neuroblasts (iANBs), persist for months and can be generated even in aged mouse brains. When supplied with neurotrophic factors or treated with a small molecule, iANBs develop into electrophysiologically mature neurons, which functionally integrate into the local neural network. Our results demonstrate that adult astrocytes can be reprogrammed to show remarkable plasticity in vivo, a feature that might have important implications in the regeneration of the central nervous system using endogenous patient-specific glial cells.

Novel Neuronal Structures Revealed by Super-Resolution Fluorescence Imaging

Awardee: Xiaowei Zhuang
Award: Transformative Research Award
Awardee Institution: Harvard University

Dissecting the inner workings of a neuron requires imaging methods with molecular specificity, molecular-scale resolution, and dynamic imaging capability such that molecular interactions inside the neuron can be directly visualized. Fluorescence microscopy is a powerful imaging modality with both molecular specificity and dynamic imaging capability. However, the spatial resolution of light microscopy, classically limited by diffraction to a few hundred nanometers, is substantially larger than molecular length scales in cells. This limitation has severely hindered investigations of many sub-neuronal structures. We recently developed a super-resolution fluorescence microscopy method, stochastic optical reconstruction microscopy (STORM), which breaks the diffraction limit. This approach allows multicolor and three-dimensional (3D) imaging of living cells with nanometer-scale resolution.

In our applications of STORM for imaging neurons, we recently discovered a periodic, sub-membrane cytoskeleton structure in axons that is made of actin, spectrin, and associated molecules. Actin and spectrin are essential for many important neuronal functions, but little is known about the organizations of these molecules in neurons. We utilized STORM to study the 3D organization of actin and spectrin in neurons. Surprisingly, we found that actin forms ring-like structures that wrap around the circumference of the axons. These actin rings are evenly spaced along the axons with a periodicity of ~180–190 nm, forming a quasi-one-dimensional lattice structure with long-range order. Adjacent actin rings are connected by spectrin tetramers. Functionally, this periodic cytoskeleton structure places sodium channels into a previously unknown periodic pattern, which may impact how action potential is generated. This periodic cytoskeletal structure could also provide the stable and elastic support needed for the axons, as well as a structural basis for many other neuronal functions.

Gene Length Matters in Autism

Awardee: Mark J. Zylka
Award: Pioneer Award
Awardee Institution: University of North Carolina at Chapel Hill

Topoisomerases are expressed throughout the developing and adult brain and are mutated in some individuals with autism spectrum disorder (ASD). However, how topoisomerases are mechanistically connected to ASD is currently unknown. We found that topotecan, a Topoisomerase 1 (TOP1) inhibitor, dose-dependently reduced the expression of extremely long genes in mouse and human neurons, including nearly all genes >200 kb. Expression of long genes was also reduced following knockdown of *Top1* or *Top2b* in neurons, highlighting that each of these enzymes is required for full expression of long genes. Pharmacological inhibition of topoisomerases reduced expression of long genes in other cell types, suggesting this length-dependent transcriptional effect was fundamental to all mammalian cells. Genome-wide RNA Polymerase II (Pol II) ChIP-seq experiments in neurons revealed that this length-dependent effect on gene expression was due to impaired transcription elongation, with TOP1 regulating the transition to productive elongation at long genes. We noticed that many high confidence ASD candidate genes are exceptionally long, including *Cntnap2*, *Nrxn1* and *Cntn4* (2,241 kb, 1,059 kb and 1,021 kb, respectively). Expression of these genes and at least 46 other ASD candidate genes were dose-dependently reduced following TOP1 inhibition. Taken together, our study reveals that topoisomerases facilitate expression of long genes, including genes associated with synaptic function and neurodevelopment, via a length-dependent transcriptional mechanism. Our findings suggest that diverse chemicals and genetic mutations that impair topoisomerases, and possibly other components of the transcription elongation machinery, could commonly contribute to ASD and neurodevelopmental disorders.