POSTER ABSTRACTS

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BEHAVIORAL AND SOCIAL SCIENCE

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URBAN SCALING OF MORTALITY IN US CITIES

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2018 Early Independence Award

Introduction: Urban scaling refers to how attributes of cities such as wealth or infrastructure change systematically as a function of city size (population). In this study we examined the scaling properties of mortality in 926 US cities.

Methods: We obtained data from 2013 to 2017 for 926 core-based statistical areas in the US that ranged from 12 thousand to 20 million people. Mortality data was obtained from the National Vital Statistics System with information on county of residence and cause of death, and population data was obtained from the Census Bureau. We classified deaths using the Global Health Estimates categories (24 groups of causes of death). We estimated the scaling coefficient of an OLS regression of log(deaths) on log(population), adjusted for all-cause mortality. A scaling coefficient above (below) 1 represents outcomes that occur more frequently in larger (smaller) cities.

Results: We used data on a total of 11.9 million deaths over 5 years. Cardiovascular diseases (31%), cancer (22%), neuropsychiatric conditions (13%), respiratory diseases (8%), and unintentional injuries (5%), were the most common causes of death.Neuropsychiatric conditions (b=1.04), infectious and parasitic diseases (b=1.04), and cancer (b=1.01) were the only conditions with a superlinear scaling behavior. Cardiovascular, respiratory, digestive and endocrine diseases, and intentional injuries, showed a linear scaling behavior (b=1). All other conditions had a sublinear scaling behavior, with special emphasis on maternal and child mortality: maternal conditions (b=0.58), SIDS (b=0.63), congenital anomalies (b=0.86), and perinatal conditions (b=0.91).

Conclusion: This study has described the emergence of different causes of death with city size. Understanding the mechanisms that lead to an increased frequency of maternal and child health conditions in smaller cities and neuropsychiatric and infectious diseases in larger cities will help identify policy-relevant drivers of health in growing urban populations.

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BRIDGING OBSERVATIONAL STUDIES AND RANDOMIZED EXPERIMENTS BY EMBEDDING THE FORMER IN THE LATTER

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2016 Early Independence Award

Consider a statistical analysis that draws causal inferences from an observational dataset, inferences that are presented as being valid in the frequentist senses; i.e., the analysis produces: 1) consistent point estimates, 2) valid p-values, valid in the sense of rejecting true null hypotheses at the nominal level or less often, and/or 3) confidence intervals, which are presented as having at least their nominal coverage for their estimands. For the hypothetical validity of these statements, the analysis must embed the observational study in a hypothetical randomized experiment that created the observed data, or a subset of that hypothetical randomized data set.

This multistage effort with thought-provoking tasks involves: 1) a purely conceptual stage that precisely formulate the causal question in terms of a hypothetical randomized experiment where the exposure is assigned to units; 2) a design stage that approximates a randomized experiment before any outcome data are observed, 3) a statistical analysis stage comparing the outcomes of interest in the exposed and non-exposed units of the hypothetical randomized experiment, and 4) a summary stage providing conclusions about statistical evidence for the sizes of possible causal effects.

Stages 2 and 3 may rely on modern computing to implement the effort, whereas Stage 1 demands careful scientific argumentation to make the embedding plausible to scientific readers of the proffered statistical analysis. Otherwise, the resulting analysis is vulnerable to criticism for being simply a presentation of scientifically meaningless arithmetic calculations. The conceptually most demanding tasks are often the most scientifically interesting to the dedicated researcher and readers of the resulting statistical analyses.

This perspective is rarely implemented with any rigor, for example, completely eschewing the first stage. We illustrate our approach using an example examining the effect of parental smoking on children's lung function collected in families living in East Boston in the 1970s.

MIND OVER GENOME? LEARNING ONE'S GENETIC RISK CHANGES PHYSIOLOGY INDEPENDENT OF ACTUAL GENETIC RISK

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[1] Stanford University

2016 New Innovator Award

The overarching purpose of this New Innovator research is to move beyond the limited notion of the placebo effect as some mysterious response to an inert substance toward the recognition that our mindsets (e.g., beliefs and expectations) are responsible for these responses. In the current talk I will discuss our research exploring the role of mindsets in shaping the physiological impact of learning one's genetic risk. As precision medicine becomes increasingly popular, millions of people now have access personal genetic risk estimates for diseases such as Alzheimer's, cancer and obesity. While this information can be informative, research on placebo and nocebo effectssuggests that learning of one's genetic risk may evoke physiological changes consistent with the expected risk profile. Here we tested whether merely learning of one's genetic risk for disease alters one's actual risk by making people more likely to exhibit the expected changes in gene-related physiology, behavior and subjective experience. Individuals were genotyped for actual genetic risk and then randomly assigned to receive either a 'high-risk' or 'protected' genetic test result for obesity via cardiorespiratory exercise capacity (experiment 1, *N* = 116) or physiological satiety (experiment 2, *N* = 107) before engaging in a task in which genetic risk was salient. Merely receiving genetic risk information changed individuals' cardiorespiratory physiology, perceived exertion and running endurance during exercise, and changed satiety physiology and perceived fullness after food consumption in a self-fulfilling manner. Effects of perceived genetic risk on outcomes were sometimes greater than the effects associated with actual genetic risk. If simply conveying genetic risk information can alter actual risk, clinicians and ethicists should wrestle with appropriate thresholds for when revealing genetic risk is warranted. Implications of this research for genetic testing as well as healthcare more broadly will be discussed.

BIOINFORMATICS AND COMPUTATIONAL BIOLOGY

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MULTI-TISSUE ANALYSIS REVEALS SHORT TANDEM REPEATS AS UBIQUITOUS REGULATORS OF GENE EXPRESSION AND COMPLEX TRAITS

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2017 Early Independence Award

Short tandem repeats (STRs) have been implicated in a variety of complex traits in humans. However, genome-wide studies of the effects of STRs on gene expression thus far have had limited power to detect associations and elucidate the underlying biological mechanisms. Here, we leverage whole genome sequencing and expression data for 17 tissues from GTEx to identify STRs whose repeat lengths are associated with expression of nearby genes (eSTRs). Our analysis reveals more than 3,000 high-confidence eSTRs, which are enriched in known or predicted regulatory regions. We show eSTRs may act through a variety of mechanisms. We further identify hundreds of eSTRs that potentially drive published GWAS signals and implicate specific eSTRs in height, schizophrenia, and blood traits. Overall, our results demonstrate that eSTRs potentially contribute to a range of human phenotypes. We expect that our comprehensive eSTR catalog will serve as a valuable resource for future studies of complex traits.

TOWARDS A UNIFIED MODEL OF PROTEIN EVOLUTION

Sergey Ovchinnikov

Harvard University

2018 Early Independence Award

I'll describe some progress towards developing a unified statistical model of protein evolution that combines known priors, conservation, coevolution, and phylogenetics. I'll provide an overview of how these models can be used to extract more accurate structural constraints for protein structure determination, functional prediction, and annotation ofunknown protein families found in metagenomic environmental samples.

USING BRAIN DYNAMICS AS A LENS TO ANCHOR PSYCHIATRIC NOSOLOGY INTO BIOLOGICAL FEATURES

Manish Saggar

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2018 New Innovator Award

Despite the accelerated pace of discovery in neuroscience, the pace of development for treatments of mental illness has not only been slow but almost stagnated. This slow pace could be mainly attributed to the lack of accurate and neurobiologically grounded diagnostic nosology (or a disease classification system). The grounding of a diagnosis in biological features can not only provide a reliable and accurate diagnosis but can also provide specific biomarkers to track the illness and test the efficacy of new treatments. With the advent of modern noninvasive neuroimaging modalities, sophisticated methods have been developed to examine both structural and functional activity/connectivity of the brain for characterizing different psychiatric disorders. Nevertheless, several issues remain in developing neuroimaging based diagnostic nosology for mental illness. First, translational applications of current findings are limited due to the nonspecific relationship between the markers and disorders, with a considerable overlap between seemingly dissimilar disorders. Second, given that neuroimaging research almost always requires group-based analysis, it is unclear how neuroimaging-based diagnostic nosology could be translated into clinical environments for diagnosing and treating patients at an individual level. Third, most current neuroimaging methods are limited to providing a statistical characterization of the observed data and cannot provide mechanistic explanations regarding how the underlying neural populations interact and process information differently in patients. Addressing these issues, here, we propose to take a bold step towards computationally modeling the entire landscape of the brain's dynamical organization at an individual level. This modeled landscape could then be used as a "lens" towards (a) characterizing (and stratifying) psychiatric illness and (b) generating biologically grounded mechanistic insights regarding how neural processes interact during ongoing cognition to give rise to different dynamical landscapes in patient populations. We will discuss several challenges associated with our approach, propose solutions, and provide preliminary results.

CHEMICAL BIOLOGY

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TRANSFORMING LIFE SCIENCES: ARTIFICIAL LIFE

Steven Benner

Foundation or Applied Molecular Evolution

2017 Transformative Research Award

Much modern biomedical research focuses on "descriptive biology", which analyzes in ever greater detail the physiology (molecular, cellular, and macroscopic) by which modern organisms manage challenges arising as they survive. However, this physiology is the outcome of a narrow range of prebiotic chemical possibilities on the Hadean Earth, to be later developed by four billion years of evolution having the limited Darwinian search strategy. Thus, modern physiologies are narrow and largely imperfect solutions to biological problems.

This creates the opportunity for the "descriptive biology" programme to be complemented by "synthetic biology", which creates alternative ways of approach biological problems. By pursuing a synthetic grand challenge, scientists are driven across uncharted grounds where they must solve unscripted problems using available technology and theory. When these are inadequate, the synthesis fails, and fails in a way that alerts us to the deficiencies in theory and technology, some of which may not have been seen by the scientists. Thus, a well constructed synthetic effort drives discovery and paradigm change in ways that description and hypothesis-based research cannot.

We will describe our grand challenge, to construct living bacteria that use genetic systems different from natural DNA and RNA. That synthetic system uses nucleic acids built from six or more different nucleotide "letters", a metabolism to construct those, and regulatory systems to manage that metabolism. We have taken major steps to construct this new biology from the ground up. This has taught us much about natural biology and yielded new technologies. The last range from new tools to survey environments for pathogens, new methods to diagnose diseases both on-site and in high-resource environments, new tools to create broad-spectrum therapeutics, and new ways to do use biotechnology in manufacturing.

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TRANSCRIPTIONAL CONTROL OF LEUKEMIA GROWTH BY ENL YEATS INHIBITION

Michael Erb

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2018 Early Independence Award

Pathogenic transcriptional regulatory networks sustain malignant cell phenotypes in human cancers. We have previously identified that the transcriptional co-activator, ENL, is essential for the survival of diverse acute leukemia in both cellular and animal models of the disease but is dispensable for the survival of hematopoietic stem and progenitor cells. Thus, we predict that ENL-targeted anti-cancer agents will possess favorable therapeutic windows and have therefore endeavored to discover small-molecule inhibitors of ENL. The ENL YEATS domain recognizes acylated lysine side chains within amino-terminal histone tails and this function is essential for both the localization of ENL to chromatin and for its ability to sustain leukemic proliferation. We developed an ultra-high-throughput screening (uHTS) assay that reports on the association of a synthetic histone peptide to recombinant ENL YEATS domain and screened a collection of over 250,000 small molecules. Validated hits were identified among false positives using a novel target engagement assay, resulting in the classification of two structurally distinct chemical scaffolds as ENL YEATS inhibitors. Hits were optimized via hit expansion studies and iterative medicinal chemistry to yield selective ENL YEATS inhibitors. These data will support the development of ENL YEATS antagonists as *in vivo*chemical probes and targeted anti-cancer agents.

TAMING UNDRUGGABLE TARGETS WITH NOVEL SYNTHETIC BIOLOGICS

Raymond Moellering

University of Chicago

2017 New Innovator Award

Numerous chemistries have been applied to stabilize specific peptide conformations. Many of these strategies, however, lack the general structural, chemical and environmental compatibility desirable for diverse applications in the synthesis of peptide therapeutics. Most notably, there has been limited progress in applying combinations of bioorthogonal chemistries to template and stabilize peptide and protein tertiary structure, which is often needed to mimic bioactive conformations. We have recently made progress in the development of new bioorthogonal strategies that can be used to synthetically stabilize peptide macrocycles to mimic several biologically important topologies. Furthermore, we have integrated these chemistries with existing reactions to mimic larger protein domains involved in protein-protein and protein-DNA interactions. Applications to inhibit several challenging intracellular protein targets in cancer and the immune response will be presented.

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CHEMICAL SYNTHESIS OF FULL-LENGTH PROTEINS USING SEQUENTIAL DNA-TEMPLATED REACTIONS

Nicholas Stephanopoulos

Arizona State University

2018 New Innovator Award

The synthesis of full-length, functional proteins from completely synthetic peptides is a long-standing goal of chemistry and biology, and would allow for the incorporation of multiple non-canonical amino acids (NCAAs) to probe structure and function. Although solid-phase peptide synthesis can routinely yield molecules ~50 amino acids in length, techniques like native chemical ligation (NCL) are necessary to link them into larger molecules. To date, however, NCL has been limited to proteins 150-200 residues in length, and membrane proteins are particularly challenging to make due to their hydrophobic nature. We propose to develop a novel method for stepwise NCL templated by DNA splints, which will enhance the local concentration of every peptide coupling step, and allow for the synthesis of significantly longer molecules than traditional NCL. The N-terminal handle will be attached via a photocleavable thiol auxiliary, and the C-terminal handle via a thioester. When the splint brings the N-terminus of one peptide into close proximity with the C-terminus of the next peptide, NCL will occur spontaneously due to the increased local concentration. Cleavage of the DNA tags with UV light will then leave a fully native amide bond behind. This process can be repeated for each subsequent peptide, and because the DNA will enhance the rate of coupling by co-localizing the termini, there should be no drop-off in yield of the couplings with each step. We will describe the synthetic strategy for making ultra-long DNA-peptide-DNA conjugates, as well as progress towards the synthesis of linkers for the N-terminal photocleavable auxiliary. Our ultimate goal is to use this method to synthesize full-length, folded, and functional G-protein coupled receptors (GPCRs) from synthetic peptide fragments, and determine their structure by cryo-EM via immobilization on a rigid DNA origami scaffold as a fiducial marker.

CLINICAL AND TRANSLATIONAL RESEARCH

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IMMUNOTHERAPY DIRECTED AGAINST SKIN CANCER PRECURSORS PREVENTS SKIN CANCER

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2015 Early Independence Award

Skin cancer is the most common type of cancer. Although ultraviolet radiation has been recognized for decades as its preventable risk factor, the incidence of skin cancer, including squamous cell carcinoma (SCC), has doubled over the last decade in the United States. Besides morbidity and mortality associated with skin cancer, skin cancer treatments represent a rising public health challenge with increasing complications and rising costs. Therefore, skin cancer prevention is urgently needed. To accomplish this goal, we developed topical calcipotriol plus 5-fluorouracil (5-FU) immunotherapy that effectively eliminated SCC precursors called actinic keratosis in a randomized double-blind clinical trial with 131 participants. Three years after the completion of the trial, we performed a blinded prospective cohort study on its participants in order to determine the long-term effectiveness of calcipotriol plus 5-FU treatment for SCC prevention. Calcipotriol plus 5-FU combination induced tissue-resident memory T (T_{RM}) cell formation in face and scalp skin, which was associated with significantly higher erythema scores compared to control groups (Vaseline plus 5-FU, p1500-day follow-up period, and significantly fewer developed SCC on the treated face and scalp within 3 years (2 of 30 [7%] versus 11 of 40 [28%] in control group, hazard ratio 0.215 [95% CI: 0.048-0.972], p=0.032). Interestingly, we found more epidermal T_{RM} cells persisting in the calcipotriol plus 5-FU-treated face and scalp skin compared with control months to years post treatment (p=0.0028). Our findings demonstrate that a short course of a topical immunotherapy that induces T cell immunity and effectively eliminates actinic keratosis can significantly lower the long-term risk of SCC. Our research substantiates a previously unrecognized concept that immunotherapy against premalignant lesions can be used to prevent cancer development and recurrence in high-risk patients.

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HEMATOPOIETIC STEM CELL ONTOGENY AND CLONAL EVOLUTION

Sergei Doulatov

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2018 New Innovator Award

The blood system is a developmental hierarchy maintained by hematopoietic stem cells (HSCs) at the apex. HSCs undergo self-renewal and multilineage differentiation into lineage-committed progenitors, giving rise to all mature blood cells. Our lab is interesting in understanding the origins of HSCs during embryogenesis and their clonal evolution during postnatal life, leading to hematopoietic malignancies.

A long-standing puzzle in the field has been why differentiation of pluripotent stem cells (PSCs) gives rise to hematopoietic progenitors, but fails to generate transplantable HSCs. We have shown that activation of specific transcription factors can promote HSC fate during development. However, the success of these strategies has been limited. We have been exploring a novel paradigm that epigenetic factors oppose HSC fate by specifying closed chromatin at promoters of HSC-specific genes. Understanding the role of negative regulation in fate determination would enable generation of HSCs from PSCs to overcome shortages of donor bone marrow for stem cell transplantation.

During post-natal life HSCs become quiescent and divide infrequently. This longevity makes HSCs susceptible to acquisition of somatic mutations, over time leading to clonal outgrowth and malignant transformation. The patterns of mutation acquisition in individual stem cells and their impact on self-renewal and differentiation remain poorly understood. We show that reprogramming of HSCs from patients with blood malignancies generates induced pluripotent stem cells (iPSCs) from single premalignant cells, directly informing the temporal order of mutations in individual patients. Differentiation of mutant iPSCs into HSCs captures the impact of somatic mutations on stem cell properties. Reprogramming thus enables molecular and functional interrogation of clonal evolution, identifying common mutational trajectories andkey pathways underlying malignant transformation.

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A LMNA VARIANT CAUSES TISSUE-SPECIFIC CHANGES IN SPATIAL GENOME ORGANIZATION AND CARDIOMYOCYTE LINEAGE INSTABILITY.

Rajan Jain

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2018 New Innovator Award

Despite being germline mutations, it remains unclear how variants in *LMNA* result in specific disease phenotypes in a tissue specific manner, such as dilated cardiomyopathy. Moreover, it remains unknown how spatial positioning of the genome is impacted in cell types that develop disease versus those that are not affected clinically. We determined that cardiac myocytes from patients with cardiomyopathy and *LMNA* variants had abnormal nuclear morphology compared to failing controls. We then knocked a mutation into control human induced pluripotent cells (iPSCs) resulting in a point mutation (T10I). Mutant cardiac myocytes (iPSC-CMs) demonstrated a loss of nuclear morphology, which is associated with an increase in DNA damage and impaired calcium transients compared to isogenic control cells. In addition, lineage pathways relevant to alternative, undesired fates lose residence in Lamina Associated Domains (LADs) and their gene expression is upregulated in mutant iPSC-CMs. Surprisingly, we saw a near equal amount of the genome gain residence in LADs in mutant iPSC-CMs compared to control cells. This change in nuclear architecture is, in part, due to a spatial repositioning of heterochromatin, marked by Histone H3 Lysine 9 dimethyl, away from the nuclear lamina. Next, we differentiated mutant and control iPSCs into hepatocytes to understand tissue specific changes in mutant and control tissues. Strikingly, we found that mutant iPSC-derived hepatocytes retained relatively normal nuclear morphology and architecture. Taken together, these data indicate that loss of normal genome organization is an early event in cardiac laminopathies. Further, our studies suggest a critical role for lamina-chromatin interactions is to maintain normal lineage stability during development. Finally, our studies how genome organization changes in a cell-specific fashion to influence in tissue-specific phenotypes observed in *LMNA* mutant tissues.

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CLINICAL AND TRANSLATIONAL APPROACHES TO COGNITIVE IMPAIRMENTS IN BREAST CANCER

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2014 New Innovator Award

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. Cancer-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. Little is known about the biological mechanisms contributing to CRCI development, though studies suggest that increased inflammation may be involved. Additionally, interventions for CRCI are sparse. Our team has been interested in developing models for forward and backward translation of research from pre-clinical to clinical research models. 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 have developed a clinically relevant CRCI mouse breast cancer tumor model (E0771) with adriamycin and cytoxan chemotherapy to study the effects of cancer and chemotherapy on memory function (delayed spatial alternation), as well as the contributing role of key cytokine and neuroinflammation pathways that contribute to neurotoxicity involved in CRCI. Interestingly, our pre-clinical work suggests that both cancer and chemotherapy may contribute to neurotoxicity and neuroinflammation. We are also longitudinally assessing CRCI in breast cancer patients receiving chemotherapy and contributing cytokine factors using a cognitive assessment which has analogous features to our pre-clinical model memory function test (using delayed match to sample), and other neurocognitive tests. We have identified that TNFR levels are correlated with lower performance on the delayed match to sample memory visual working memory test. We have ongoing work assessing other cytokines that are associated with other cognitive domains. To further our understanding of the impact of cancer on CRCI, we have studied the role of chronic lymphocytic leukemia on CRCI showing that risk of disease has a greater impact on CRCI than treatment in that population, which needs to be further studied. We also are completing preclinical studies to assess the role of exercise on CRCI. Our results have provided a model that can be used for pre-clinical and clinical research, and has shed light on mechanisms and possible interventions for CRCI.

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MAPK PATHWAY ACTIVATION IS CORRELATED WITH GLIOBLASTOMA RECOGNITION BY CD8+ T-CELLS: EVIDENCE OF IMMUNOEDITING AND IMPLICATIONS FOR ANTI-PD-1 IMMUNOTHERAPY

J. Robert Kane[1], Junfei Zhao[2], Takashi Tsujiuchi[2], Brice Laffleur[2], Gerson Rothschild[2], Angeliki Mela[2], Rimas V. Lukas[1], Uttiya Basu[2], Fabio M. Iwamoto[2], Raul Rabadan[2], and Adam Sonabend[1]

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2015 Early Independence Award

Immunoediting is a process in which non-antigenic or immunosuppressive tumor clones get selected by, and thus survive anti-tumoral immunity. Given that glioblastoma is resistant to immunotherapy and genetically diverse, we hypothesized that CD8+ T-cells subject these tumors to immunoediting by altering the tumor genotype, phenotype, and microenvironment. We investigated this question by depleting CD8+ T-cells in a transgenic murine glioma model in which gliomas develop *de novo*. Gliomas developed in the absence of CD8+ T-cells were more antigenic, showing elevated expression of MHC-1, and upon transplantation, did not graft into recipients with intact immunity. Exome sequencing revealed that CD8+ T-cell depletion during glioma development was associated with aneuploidy due to chromosomal loss. MAPK, a pathway we recently implicated in glioblastoma patients that respond to PD-1 blockade, was only activated in murine gliomas developed in the absence of CD8+ T-cells. With regard to the tumor microenvironment, CD8+ T-cell depletion during glioma development led to robust macrophage infiltrates. Our results indicate that CD8+ T-cells infiltrate gliomas and impact the genotype, phenotype, and microenvironment during development, selecting against clones that are antigenic and have chromosomal loss. CD8+ T-cells select against MAPK pathway activation—a pathway implicated in glioblastoma response to anti-PD-1 immunotherapy.

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A COMPARATIVE APPROACH TO DE-RISKING NEXT-GENERATION CELLULAR IMMUNOTHERAPIES FOR CANCER AND AUTOIMMUNITY

Nicola Mason[1], Preethi Haran[1], Matthew Atherton[1], Ailian Xiong[1], Christoph Ellebrecht[1], and Aimee Payne[1]

[1] University of Pennsylvania

2018 Transformative Research Award

The remarkable success of genetically modified T cell therapies for hematological malignancies has unleashed investigator creativity to expand their use for other tumor types and other disease processes such as autoimmunity and infection. For rapid, clinical translation of advanced next-generation cellular therapies, immune competent “models” that develop comparable pathologies to human patients are needed. Pet dogs spontaneously develop cancers and autoimmune diseases that show remarkable clinical, biological and genetic similarities to those that occur in humans. Integrating immune competent canine patients into the drug-development paradigm aims to facilitate the advancement of cellular immunotherapies and de-risk their deployment in the human clinic. Chimeric autoantibody receptor (CAAR) T cells express a chimeric receptor that consists of an autoantigen linked to intracellular signaling domains that initiate T cell activation following engagement by autoantigen-specific B cell receptors. The resulting interaction leads to selective T cell mediated depletion of pathogenic, autoreactive B cells. Pre-clinical work in experimental mouse models of pemphigus has demonstrated that CAART can selectively deplete autoreactive B cells and mediate complete histological remission of disease. Using a comparative approach to evaluate the safety and clinical efficacy of CAART in pemphigus, we aim to better understand how CAART performs in vivo in the setting of spontaneous disease. We are developing methodologies to genetically engineer canine CAR and CAAR T cells that will be evaluated in clinical trials performed in client-owned animals with spontaneous pemphigus. Our comparative approach aims to enable “pre-clinical” assessment of next-generation cellular immunotherapies in clinically relevant, canine patient populations thereby accelerating clinical translation to the human clinic.

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TRANSLATING CELLULAR IMMUNOTHERAPIES FOR AUTOIMMUNITY TO CANINE CLINICAL TRIALS

Aimee Payne[1], Christoph Ellebrecht[1], Baomei Wang[1], Xuming Mao[1], Adam Alghalith[1], and Nicola Mason[1]

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2018 Transformative Research Award

Autoimmunity occurs when the body's immune system mistakenly attacks normal tissues, leading to disease. Treatments for autoimmunity chronically suppress the immune system, which risks fatal infection; thus the ideal therapy would eliminate only the disease-causing autoimmune cells while preserving normal immunity. We recently developed a novel gene-engineered chimeric autoantibody receptor (CAAR) T cell therapy that uses the autoantigen targeted in disease to direct T cell cytotoxicity against only those B cells that express autoantigen-specific B cell receptors. We have shown that CAAR T cells cause complete histologic and serologic remission of autoantibody-mediated disease in an experimental mouse model of pemphigus vulgaris and engraft to form memory CAAR T cells that may offer lasting protection against disease recurrence. We now seek to establish dogs with naturally occurring autoimmune disease as a system for better understanding the in vivo function of cellular immunotherapies, in a setting that is more physiologic than mice whose disease is artificially induced and treated shortly after disease induction. We will present the results of ongoing work to develop novel canine autoantigen-specific cellular immunotherapies for pemphigus. Synergies between research in cancer and autoimmunity, as well as in humans and canines, offer a unique opportunity to improve our understanding of chimeric immunoreceptor biology and disease pathophysiology through comparative immunology studies. Ultimately, our work could facilitate the clinical translation of cellular immunotherapies for a broad range of canine and human diseases.

SYNERGISTIC EFFECTOR/ENVIRONMENT ENCODING: A NEW PERSPECTIVE ON MOTOR CORTEX AND BRAIN-COMPUTER INTERFACES

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2018 New Innovator Award

Neuromotor disorders can result in devastating loss of volitional movement, greatly reducing independence. Emerging brain-computer interface (BCI) technology offers the possibility of building new artificial links between the nervous system and the external world, bypassing damaged motor pathways and allowing users to control robotic limbs or even reanimate paralyzed muscles with their thoughts.

Every voluntary action engages vast networks of neurons performing complex calculations that are still not fully understood despite decades of research. Current BCI systems typically regard non-motor information as noise, and focus on extracting signals that exclusively represent desired movements. However, motor cortical areas do not simply issue motor commands: they dynamically incorporate sensory information in order to regulate interactions between the body and the external environment. Predicting movement without accounting for the sensory component of motor cortical activity leads to relatively slow, imprecise control and poor generalization across behavioral conditions. Here, we propose an alternative strategy: using artificial vision to gather information about the environment, and using this information as a filter to interpret motor cortical activity more accurately.This holistic approach will allow us understand the role of different cortical areas driving behavior in more detail, and optimize our strategies for extracting movement-related information from cortical circuits.We call this approach synergistic effector + environment (SEE) decoding.

Our preliminary results confirm that individual neurons in motor cortex are strongly driven by visual stimuli and demonstrate how incorporating features of the visual scene can improve movement decoding accuracy. Crucially, our approach will allow us to determine if information derived from visuomotor areas (e.g. dorsal and ventral premotor cortex) can be supplemented or even entirely replaced using artificial sensors. Our results suggest SEE decoding has the potential to make BCI control systems more accurate, faster, and potentially less expensive and invasive.

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3D BIOPRINTING COMPLEX VASCULARIZED TISSUES

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2014 New Innovator Award

The repair of damaged tissues and organs presents a significant clinical problem worldwide. As a promising technique for tissue engineering, 3D bioprinting offers greater precision to control the internal and external structure of a tissue scaffold and cell distribution to better replicate the structural and functional complexity of native tissues. However, one critical challenge in 3D bioprinting tissues and organs is the need to create a highly efficient and perfusable 3D vascular network. Thus, the objective of this study is to 3D bioprint novel complex vascularized tissues for improved neovascularization and tissue formation. Specifically, we successfully developed an integrated method for combining dual 3D bioprinting with regional immobilization of bioactive factors to create a biomimetic vascularized nano bone tissue. A biphasic structure was made by integrating a bone scaffold of high mechanical strength with a perfusable vascular network of high flexibility. After regional immobilization of bone morphogenetic protein and vascular endothelial growth factor peptides, our results showed that human umbilical vein endothelial cells (HUVECs) could self-organize to form both tubular vessels and capillary networks while the human mesenchymal stem cells (hMSCs) were homogeneously distributed on the bone region for osteogenesis in a dynamic culture. We also employed a stereolithographic bioprinting technique to fabricate novel honeycomb-like vascularized cardiac constructs for myocardial regeneration. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), hMSCs, and HUVECs were tri-cultured in our 3D bioprinted constructs for the formation of a vascularized myocardium. The immunostaining and gene expression results showed that greatly improved angiogenesis and cardiomyogenesis were obtained in our 3D bioprinted anisotropic tissue constructs when compared to an isotropic control under dynamic culture. Furthermore, our results demonstrated that the hiPSC-CMs grew favorably on our bioprinted cardiac tissue in vivo and the bioprinted tissue was well integrated into the mouse heart.

HIGH-THROUGHPUT AND INTEGRATIVE BIOLOGY

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LIVE CELL TRANSCRIPTOMICS

Paul Blainey

The Broad Institute of Harvard/MIT

2017 New Innovator Award

Mammalian cell biology is dynamic and responsive. Advances in genomic technology over the last decade made rich datasets accessible to individual biomedical investigators on a routine basis. But today's 'omic approaches require the destruction of cells to access their molecular contents and cannot support measurements of the same cells at different time points. Researchers need tools that can probe living biology in tissue to produce rich time-series data specific to cells of interest that illuminate dynamic phenomena. We are developing a technology that allows cells to self-report their internal states in time series measurements by secreting a portion of their contents, which can be collected for analysis (e.g. by RNA-Seq) without harming the cells. We will show results from technical development work that resulted in a robust protocol and comparison of self-reported versus conventional 'omic data.

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MAPPING THE GENETIC LANDSCAPE OF HUMAN CELLS

Luke Gilbert[1], Jonathan Weissman[1], and Max Horlbeck[1]

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2018 New Innovator Award

Seminal yeast studies have established the value of comprehensively mapping genetic interactions (GIs) for inferring gene function. Efforts in human cells using focused gene sets underscore the utility of this approach, but the feasibility of generating large-scale, diverse human GI maps remains unresolved. We developed a CRISPR interference platform for large-scale quantitative mapping of human GIs. We systematically perturbed 222,784 gene pairs in two cancer cell lines. The resultant maps cluster functionally related genes, assigning function to poorly characterized genes, including TMEM261, a new electron transport chain component. Individual GIs pinpoint unexpected relationships between pathways, exemplified by a specific cholesterol biosynthesis intermediate whose accumulation induces deoxynucleotide depletion, causing replicative DNA damage and a synthetic-lethal interaction with the ATR/9-1-1 DNA repair pathway. Our map provides a broad resource, establishes GI maps as a high-resolution tool for dissecting gene function, and serves as a blueprint for mapping the genetic landscape of human cells.

INFECTIOUS DISEASES AND IMMUNOLOGY

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METABOLIC SYMBIOSIS BETWEEN TUMOR CELLS AND REGULATORY T CELLS PROMOTES IMMUNOSUPPRESSION IN CANCER

Greg Delgoffe

University of Pittsburgh

2017 New Innovator Award

Immunotherapy is front and center in the therapeutic arsenal for cancer, but, despite the promise of durable responses, these therapies only provide a subset of patients with benefit. Thus, it is critical to understand how immunity is regulated within the tumor microenvironment, providing insight into strategies to increase immunotherapeutic response. We and others have shown that the tumor microenvironment is charaterized by a distinct metabolic landscape: a sink of essential nutrients and the build-up of potentially toxic byproducts. For tumor-infiltrating effector T cells, these metabolic barriers induce severe functional defects preventing immunotherapy response. However, not all T cells in the tumor microenvironment are functionally disabled: regulatory T (Treg) cells, suppressive populations tasked with preventing autoimmunity, thrive in the tumor. Treg cells have a distinct metabolic profile, upregulating several downstream components of the glycolytic pathway without taking up glucose. Rather, Treg cells upregulate genes involved in the transport of lactic acid. Tumor microenvironments are rich in lactate, which acts as a potent suppressor of conventional T cell activation and differentiation. However, regulatory T cells thrive in lactic acid conditions. Isotopic flux analysis revealed Treg cells take up lactic acid,not only oxidizing it for energy but also building higher order sugars to support cell proliferation. To determine the importance of this pathway in vivo, we generated mice bearing a Treg cell-restricted deletion of the lactate transporter. These mice are immunologically normal in unperturbed situations, suggesting lactate transport is not generally required for Treg cell function. However, without lactate transport Treg cells fail in the tumor microenvironment, resulting in unleashed tumor immunity and slowed tumor growth. Our studies suggest Treg cels possess a degree of metabolic plasticity permitting survival in various environments, which is exploited in cancer. This metabolic symbiosis represents a therapeutic target to reduce immunosuppression and improve immunotherapy for cancer.

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PARTICLE SIZE VARIATION IS A VIRAL ADAPTATION STRATEGY AGAINST CHANGING EVOLUTIONARY PRESSURE

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2017 New Innovator Award

Influenza A virus isolates from human patients display heterogeneous filamentous form. Virions have a uniform ~80nm diameter and lengths that range from ~80nm for spherical particles to > 30μm for long filaments. The surface of influenza virions is densely decorated with viral glycoprotein hemagglutinin (HA), the viral cell entry/membrane fusion protein. Spherical particles have ~500 HAs, and this number scales with particle length. Each infected cell produces a range of particle lengths, however, the functional purpose of this phenotypic heterogeneity or the role of filamentous particles remain elusive. Filamentous virions package a single genome complement but require immense cellular resources (e.g. membranes and viral proteins) to assemble. In fact, in vitro passaging of influenza viruses readily selects for spherical virions, demonstrating that filaments are disfavored under idealized cell-culture conditions. We have combined *in vitro* single-particle imaging and stochastic simulations of membrane fusion to generate simple predictions allowing molecular level interpretation of experiments in living cells. As a surrogate of evolutionary pressure on HA fusion activity, we used fluorescently labeled antibody fragments (Fabs) targeting the base of HA as quantitative probes. We show that influenza cell-entry is inherently inefficient, and is perturbed by vastly substoichiometric numbers of HA inhibitors. Increased particle length increases both the rate and probability of fusion and consequently compensates for inhibitor effects on either the fusion rate or efficiency. On the other hand, HA mutations that accelerate fusion only compensate for rate effects of inhibitors and would furthermore require a compensatory genetic change once pressure is removed. The phenotypic variation in particle length might thus represent a built-in reservoir of particles where each type is fit for a different set of conditions (presence or absence of antibodies, activating proteases, etc.), allowing the virus to maneuver changing pressure in its environment.

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EMERGENCY HEMATOPOIESIS AND THE STERILE TISSUE INJURY RESPONSE

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2018 New Innovator Award

Sterile tissue injury elicits a vigorous emergency hematopoietic response in the bone marrow that supplies abundant myeloid cells to the affected organ. Most of what we know about this response results from ensemble measurements such as flow sorting, immunostaining, and qPCR. While these have provided some mechanistic insights, the full diversity of the emergency hematopoietic response remains largely unknown. We used single cell RNA-Seq to perform genome-wide transcriptomic profiling of > 50,000 single myeloid cells after sterile tissue injury, from their origins in the bone marrow, through the circulating blood, and into affected tissue, where additional diversification and specialization ensues. We will present recent discoveries and ongoing work defining the origins and consequences of emergency hematopoiesis after sterile injury. Among our findings are an unexpected location for initial pathway activation, involvement of an unanticipated cell type, recognition of unappreciated intercellular communication, and signaling dynamics with surprising consequences. As our cellular and molecular mechanistic work continues, we have begun parallel efforts to examine its relevance to humans and to explore the utility of our findings for diagnosis and therapy.

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2’3’-cGAMP IS AN IMMUNOTRANSMITTER PRODUCED BY CANCER CELLS AND REGULATED BY ENPP1

Lingyin Li

Stanford University

2017 New Innovator Award

2’3’-cyclic GMP-AMP (cGAMP) is characterized as an intracellular second messenger that is synthesized in response to cytosolic dsDNA and activates the innate immune STING pathway. Our previous discovery of its extracellular hydrolase ENPP1 hinted at the existence of extracellular cGAMP. Here, using mass spectrometry, we detected that cGAMP is continuously exported as a soluble factor by an engineered cell line but then efficiently cleared by ENPP1, explaining why it has escaped detection until now. By developing a potent, specific, and cell impermeable ENPP1 inhibitor, we detected cGAMP export in cancer cell lines commonly used for mouse tumor models. In tumors, depletion of extracellular cGAMP using neutralizing proteins decreased tumor- associated dendritic cells and $CD8^+$ T cells and diminished the tumor shrinkage effect of ionizing radiation. Boosting extracellular cGAMP by genetic knockout and pharmacological inhibition of ENPP1 increased tumor-associated dendritic cells, shrunk tumors, and synergized with ionizing radiation and anti-CTLA-4 to cure tumors. In conclusion, cGAMP is an anti-cancer immunotransmitter released by tumors and detected by host innate immunity.

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PREDICTIVE PERSONALIZED PUBLIC HEALTH (P3H): A NOVEL PARADIGM TO TREAT INFECTIOUS DISEASE

Steven Schiff

Penn State University

2018 Transformative Research Award

In recent years, we have demonstrated that it is feasible to predict epidemic disease outbreaks from retrospective seasonal and geographical case data and to show that we can take climate factors into account in our predictive models. We are moving closer to real-time prediction at the population level. But we have never used prediction at point-of-care for treating the individual patient.

Presently, personalized medicine uses delayed results of laboratory testing of individuals. For infectious disease, most of such testing has targeted the pathogen in the host-pathogen interaction. The role of laboratory testing is to modify therapy after a variable period of time delay. Personalized medicine today is therefore reactive. Complicating matters further, many infectious epidemic diseases are strongly dependent on environmental factors and climate. Lastly, we want to name the pathogens we are fighting, but we must know the resistance characteristics to select therapy for patients effectively. Both speciation and resistance can now be determined from molecular data, which can be integrated into point-of-care treatment predictions.

We here propose a radically different approach to the treatment of infectious diseases – predictive personalized public health (P3H) policy at the individual patient level. Our hypothesis is that the alternative to time-delayed and expensive laboratory analysis of specimens from individual patients, is to use predictive modeling to forecast point-of-care treatment. Time-delayed personalized testing can be conducted as optimized sparse surveillance, and that data used for real-time prediction to guide point-of-care treatment. All of this can be wrapped within a rigorous optimal control framework, seeking sustainable patient outcomes under cost constraints.

To ensure implementation, we are partnering with in-country government economic and health planners to evaluate and implement our methods. Our deliverable will be an open-source framework ready for clinical trials testing and adaptation to the public health infrastructure in any country.

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IMAGING IN THE TIME OF PRECISION MEDICINE

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2018 Early Independence Award

The ability to see biologic processes deep within the human body has already transformed modern medicine and practically all patients undergo diagnostic imaging as part of their clinical workup. We are, however, just at the leading edge of molecular imaging technologies that will foster the diagnostic and therapeutic accuracy that the precision medicine initiative launched.

Our lab develops positron emission tomography (PET) radiotracers, which are molecular beacons that when administered to a patient, can seek and report the presence of a bacterial infection. We have enrolled 11 patients in this pilot study, and early results suggest the desired specificity of the radiotracer for bacterial infection over other common pathologies such as inflammation and cancer (Carbon-11 trimethoprim; NCT03424525). The data from our first-in-human protocol mirrors our recent findings in rodent models, further supporting the potential of this translational bacterial imaging strategy (Sellmyer et al. PNAS 2017).

Moreover, these radiotracers can be repurposed and applied to image human cell-based therapies for cancer treatment. By engineering immune cells with the bacterial protein target of trimethoprim, this new class of radiotracers is able to follow the trafficking of CAR T cells to the spleen and subsequently to the tumor target with high sensitivity in rodent models (~10,000 cells per mm^3). Thus, these radiotracers may be applied for long-term monitoring of engineered cells to treat cancer or alternatively for regenerative medicine or gene-therapy applications.

The clinical importance of new molecular imaging tools is clear. Imaging specificity, diagnostic accuracy, and facile therapeutic monitoring leads to better patient outcomes by saving needless biopsies or invasive procedures that may have devastating complications and by allowing tailored, efficient therapeutic interventions. The key is developing incisive molecular and cellular approaches that will usher imaging forward in the time of precision medicine.

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QUANTIFYING THE CONNECTIVITY OF MALARIA PARASITES USING HUMAN MOBILITY AND PARASITE GENETIC DATA

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2018 New Innovator Award

Local and cross-border importation remain major challenges to malaria elimination and are difficult to measure using traditional surveillance data. To address this challenge, we will present work using a range of data sets describing human mobility patterns and parasite connectivity to identify local and imported flows of malaria parasites in Bangladesh and Namibia. Using mobile phone data and travel surveys to quantify human mobility patterns, we find largely consistent results with measures of fine-scale spatial structure in the local parasite populations. We explore both the national and international connectivity patterns of parasites and provide a framework for incorporating genetic and human mbility into malaria surveillance programs.

INSTRUMENTATION AND ENGINEERING

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APTAMER-FIELD-EFFECT TRANSISTOR NEUROPROBES: TOWARDS MULTIMODAL SENSING

Anne Andrews

UCLA

2017 Transformative Research Award

Monitoring neurotransmitters in living tissue necessitates sensors that approach the spatiotemporal resolution of neuronal communication, while differentiating similarly structured neurochemicals with high selectivity. We have engineered sensors based on chemically synthesized oligonucleotide receptors, termed aptamers, coupled to field-effect transistors (FETs) for fully electronic sensing. These sensors show high sensitivity and selectivity for neurotransmitters and other small molecules with femtomolar detection limits *in vitro.* To enable *in vivo* measurements*,* we miniaturized sensor architectures to microfabricate neuroprobes with arrays of aptamer-field-effect transistors. The initial prototype is microfabricated on silicon (version 1.0). Each device has lithographically patterned interdigitated gold source and drain electrodes deposited on ultrathin, semiconducting indium oxide channels. Functionalization of semiconducting surfaces with neurotransmitter-specific aptamers enabled electronic neurotransmitter sensing. Reducing individual FET footprints leads to increases in the numbers of independent transistors on a single neuroprobe for multi-site/multi-target detection capabilities. Individual FETs are electrically addressable for multiplexed aptamer functionalization. Silicon microprobes can be fabricated to include electrophysiological sensors, and optical and chemical stimulation for multimodal function. Prototypes of next generation designs on flexible substrates (version 2.0) have been fabricated on thin polyimide. Neuroprobes will enable *in vivo* detection of neurotransmitters in the brain with high spatial resolution and selectivity.

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A NANOMEDICINE APPROACH TO POLYCYSTIC KIDNEY DISEASE

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2018 New Innovator Award

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited disorder, affecting 600,000 Americans and 12 million worldwide. ADPKD is characterized by progressive kidney cyst growth (i.e. cystogenesis) and enlargement of both kidneys, and is a leading cause of end-stage renal failure, dialysis, and kidney transplantation. Most treatments aim to control symptoms and secondary consequences of the disease, such as high blood pressure, hemorrhage, and urinary tract infections. However, recently, tolvaptan, a small molecule drug used for hyponatremia (low blood sodium levels) received FDA approval and has been repurposed as a therapy to slow cyst formation in ADPKD. Other drugs such as metformin, rapamycin, and pioglitazone that are used to manage diabetes and immunosuppression, have also been proposed. While these therapies show some efficacy in preclinical and clinical studies, they suffer from short half-lives in circulation, poor bioavailability to the kidneys, and adverse side effects. To mitigate these limitations, nanoparticles, such as peptide amphiphile micelles (PAMs), can be used to enhance bioavailability and in vivo half-life of drugs by acting as protective drug carriers. Herein, we describe the development of kidney-targeting PAMs (KPAMs) that 1) are small enough to take advantage of glomerular filtration to enter the kidneys, 2) actively target renal epithelial cells through receptor-binding peptides, and 3) unload therapy at sites of ADPKD. We describe how the characteristics of nanoparticles such as size and charge affect kidney bioavailability, and describe strategies for oral and transdermal delivery. To our knowledge, this strategy represents the first nanomedicine strategy for ADPKD therapy.

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HIGH THROUGHPUT SCREENING OF CLINICALLY APPROVED DRUGS THAT PRIME TRANSFECTION IN HUMAN MESENCHYMAL STEM CELLS

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2017 New Innovator Award

Human mesenchymal stem cells (hMSCs) are under intense research for applications in cell and gene therapeutics due to ease of isolation from multiple adult tissues and unique immunomodulatory properties. While hMSCs unique properties give them great potential in therapeutic applications, hMSCs intrinsic therapeutic properties could be greatly enhanced by gene delivery, the delivery of exogenous genetic material to a cell. Gene delivery through viral transduction is efficient, but suffers from safety issues related to immunogenicity and insertional mutagenesis, however, non-viral gene delivery, while safer compared to viral, suffers from inefficiency and low transgene production, especially in hMSCs. To address the shortcomings of non-viral gene delivery to hMSCs, our lab has demonstrated that pharmacological 'priming' with the glucocorticoid dexamethasone can significantly increase non-viral gene delivery to hMSCs by overcoming barriers to transfection. This work seeks to develop a library of transfection priming compounds for hMSCs by screening 707 FDA approved drugs from the NIH Clinical Collection. Adipose-derived hMSCs (AMSCs) from two donors were primed with NCC compounds at four concentrations 30 minutes prior to delivery of lipoplexes containing plasmid DNA encoding for a fusion protein of enhanced green fluorescent protein and luciferase. Cells were then analyzed 48 hours later, using fluorescent microscopy to image conditions and determine compounds that significantly affected transgene production compared to a vehicle control (VC), without significant toxicity. Compounds that resulted in significant transfection fold-changes over VC, in both donors, were assigned to drug classes according to Chemical Entities of Biological Interest classifications and grouped by these classifications. Among the identified drug classes that increased transfection were glucocorticoids, antibiotics, antihypertensives, and statins. Notably, clobetasol propionate, a glucocorticoid, increased transfection nearly eight-fold over the VC. Finally, drug-set enrichment analysis was conducted and suggest that modulation of transfection-induced stress is vital for increasing non-viral gene delivery to AMSCs.

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IMPLANTABLE CARDIAC ENERGY HARVESTING DEVICES USING GEOMETRICALLY STRUCTURED PIEZOELECTRIC THIN FILMS

Xiaojing Zhang[1], Lin Dong[1], Zhe Xu[1], Andrew Closson[1], Congran Jin[1], Xiaomin Han[1], Zi Chen[1], Meagan Oglesby[2], Danny Escobedo[2], and Marc Feldman[2]

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2016 Transformative Research Award

Harvesting energy directly from the human body offers a new paradigm to power wearable electronics and implantable medical devices without the need to replace batteries. For patients with implantable devices such as cardiac pacemakers, there is an urgent need to improve the quality of patients' post-implantation life through eliminating the risks and costs associated with battery replacement surgeries. Here, we demonstrate the designs, characterizations and pre-clinical studies of compact implantable devices showing significant improvement in the electro-mechanical energy conversion efficiency. The proposed energy harvesting designs combine the thin flim energy transduction materials development with geometric mechanics towards seemless integration with existing medical implants such as the pacemakers. Four energy harvesting device prototypes have been developed using composite piezoelectric films made of mesoporous PVDF-TrFE: (1) helical energy harvesting device coated on a pacemaker lead, harvesting the heart's bending and twisting motion; (2) dual-cantilever porous PVDF-TrFE film utilizing the kinetic energy of pacemaker lead; (3) buckled beam array within a soft tube as a means of harnessing the complex lead motion (bending and vibration); and (4) multi-buckled-beam energy harvester using PVDF-TrFE films. The results based on those four geometrically designed thin film energy harvesting prototypes showed great promise to provide electrical energy for powering implantable devices. Moreover, *in vivo* studies demonstrated clinical translation in porcine models. A sealed energy harvesting device was inserted, together with the pacemaker leads, using standard implantation techniques. Evaluations under different anchoring conditions, varying heart rates, and drug treatment conditions were performed in two different porcine right ventricles. Both *in vitro* and *in vivo* results demonstrate the energy harvesters' capability to provide significant electrical energy directly from the motion of a pacemaker lead. The proposed implantable cardiac energy harvesting strategy can be extended to power other medical implants and wearable devices alike without the limit of the battery replacement.

MOLECULAR AND CELLULAR BIOLOGY

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THE EFFECT OF MICROENVIRONMENT ON THE DIRECT REPROGRAMMING OF SOMATIC CELLS

Reza Ardehali

UCLA

2014 New Innovator Award

Rationale: Direct reprogramming of cardiac fibroblasts (CFbs) to cardiomyocytes is a promising strategy to regenerate damaged myocardium from endogenous fibroblasts and potentially minimize fibrotic tissue. Despite the therapeutic potential of direct reprogramming, it is not known whether there exists a distinct subset of CFbs with a predisposition towards reprogramming to cardiomyocytes. **Objective:** We employed genetic fate-mapping and transplantation studies to determine if heterogeneity in CFbs influences their reprogramming capacity. **Methods and Results:** Using a combination of CLARITY and light-sheet microscopy, we demonstrated that developmental heterogeneity of CFbs partially influences their anatomical distribution within the heart. We additionally provide compelling evidence that, regardless of their developmental origin, CFbs are able to be successfully converted to beating iCMs through in vitro direct reprogramming. We also show that despite invoking similar levels of proliferation and activation, cardiac injury induces a temporary re-expression of early developmental genes in CFbs that is dependent on their lineage of origin. Finally, when compared to fibroblasts of identical developmental origin from extra-cardiac organs, CFbs generated iCMs with higher efficiency and intrinsically maintain open chromatin at key cardiac transcription factors. **Conclusion:** These data suggest a link between the microenvironment and gene regulation that may influence fibroblast fate conversion more than developmental origin. Moreover, our results underscore the importance of the tissue of residence in relation to direct reprogramming to iCMs, which may be crucial for the development of targeted therapies to promote cardiac repair.

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EWSFLI1 MEDIATED ALTERNATIVE SPLICING OF ARID1A IN EWING'S SARCOMA

Mona Batish

University of Delaware

2012 Early Independence Award

The interaction between post transcriptional processing and epigentic reprograming offer new exciting aveneues for regulating gene expression. In collaboration with Georgetown University, one such interaction has been idenitfied in Ewing's Sarcoma (ES), a pediatric cancer driven by formation of an oncogenic fusion protein EWSFLI1 (EF). Recent work has indicated that EF alters the splicing profile of a number of transcripts and play a role in chromatin regulation through interactions with the chromatin remodelling BAF complex to support its tumor progression. However, the specific BAF subunits that interact with EWS-FLI1 remain unknown. Based on biochemical data, we hypothesized EF leads to alternative splicing of ARID1A, a member of the BAF complex. In this work, we utilized single molecule Fluroscent in Situ hybridization (smFISH) to image ARID1A isoforms and identify their dependency on intercations of EF with splicesome. ARID1A has 20 exons and In normal cells, a large part of exon 18 get removed by splicing to yield short functional ARID1A protein. In case of ES, ARID1A was found to alteranatively spliced to retain entire exon 18 and form a long isoform of ARID1A. We designed two probe sets, one specific to Exon 20 and lother specific to the region of Exon 18 that get altertiabley spliced. In case of longer isoform, both probes will bind and we saw co-localized signal while, for the shorter isoform, only the Exon 20 probes bound. We identified the distribution of long and short isoforms in several normal tissues and also in ES derived cell lines. We treated the cell lines with a drug that inhibit EF dependent splicing. As expected, we found loss of long isoforms upon drug treatment. This study helped to identify the role of splicing modulation in ES oncogenesis. Dissecting this interaction may lead to improved cancer-specific drug targeting.

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TRACING 3D DNA PATHS AND VISUALIZING TRANSCRIPTION IN SINGLE CELLS

Alistair Boettiger

Stanford University

2018 New Innovator Award

Establishment of different cell types during development requires precise interactions between genes and distal regulatory elements. However, our understanding of what these interactions look like in three dimensions, how they vary across cell types in complex tissue, and how they relate to transcriptional state remain limited. I will describe optical reconstruction of chromatin architecture (ORCA), a microscopy approach to follow the path of DNA in intact nuclei in thousands of cells. We have used this approach to the study the structure of the Bithorax-Complex in *Drosophila* embryos with nanoscale accuracy and genomic resolution as high as 2 kilobases. Simultaneous imaging of nascent transcription allowed direct correlation of DNA folding to RNA expression in single cells. We recovered predicted cell-type-specific boundaries between active and Polycomb-repressed DNA and also observed Polycomb-independent boundaries which further partitioned the active, but not repressed, portions of the locus in a cell-type-specific manner. Deletion of these boundary regions corresponded with ectopic enhancer-promoter contacts, aberrant gene expression, and developmental defects. Together, these results illustrate a powerful approach for high-resolution, single-cell DNA domain analysis *in vivo* and suggest a prominent role for 3D chromatin organization in cell identity.

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EXPANSION MICROSCOPY REVEALS A FIBROUS SCAFFOLD OF HUMAN CHROMOSOMES

Hu Cang

Salk Institute of Biological Studies

2014 New Innovator Award

How a human chromosome is folded into an X shape in less than 40 minutes remains elusive since the discovery of mitosis. Chromosome scaffold, a proteinous structure, comprising mainly topoisomerase 2a(top2a) and condensin, spanning the full length at the core of each chromatid, holds the key to the understanding of chromosome folding. However, its structure remains largely unknown. The scaffold was first found fibrous, appearing as an interconnected web of filaments, suggesting that top2a and condensin could oligomerize into filaments on chromatin to re-organize chromatin fibers at the chromosome-scale. However, the filaments were not observed directly, instead, after depleting histones from the chromosomes, leading to debates about whether the observed fibrous architecture was the result of harsh histone depletions. Even whether top2a is an integrated component of chromosome scaffold or merely precipitate during the invasive treatment remains unknown. The difficulty to directly visualize chromosome scaffold reflects the needs for a microscopy with higher resolutions. Here, by integrating STORM/Palm with expansion microscopy, we visualize chromosome scaffold directly, and reveal a fibrous architecture consisting of interlacing top2aand condensin filaments. Furthermore, the fibrous scaffold is found established in two steps. First, top2a and condensin filaments braid together into a single-axis scaffold, which then splits into two, one for each chromatid, giving rise to the iconic X-shape. Lastly, the braid-split process requires cooperation between top2aand condensin, mediated by the C-terminal domain of top2a. Antagonizing the cooperation disrupts chromosome assembly, blocks chrsomatid resolution, and leads to spherical, instead of rod-shaped chromatids. Together, we reveal a braid-split process, through which, filaments of top2a and condensin braid chromatin into a fibrous scaffold, giving rise to a helical order underneath seemingly disordered chromatin fibers.

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PATTERNING THE MICROTUBULE CYTOSKELETON DURING DEVELOPMENT

Jessica Feldman

Stanford University

2015 New Innovator Award

Cells specialize during development in a process of differentiation that is intimately linked to the spatial organization of microtubules. Microtubules are organized by cellular sites called microtubule organizing centers (MTOCs). The vast majority of research on MTOCs has focused on the centrosome, an organelle used by animal cells to organize microtubules during mitosis. However, during cell differentiation MTOC function is reassigned to non-centrosomal sites such as the apical membrane of epithelial cells, down the length of axons and dendrites in neurons, and at the nuclear envelope in myotubes. Despite the importance of these non-centrosomal MTOCs (ncMTOCs) for cell function, little is known about ncMTOC components or the mechanisms that govern ncMTOC establishment in vivo. Using the model organism *C. elegans,* we have developed new technologies to probe the molecular composition of ncMTOCs. We optimized a tissue specific protein degradation system to test the role of conserved microtubule nucleating, stabilizing, and anchoring factors in differentiate cells. Surprisingly, we found that proteins critical for MTOC function at the centrosome are dispensable for microtubule growth and organization at ncMTOCs; microtubules still localize to and grow from the apical surface of *C. elegans* epithelial cells even in the absence of essential factors such as g-TuRC. These data indicate that although they localize similar components, centrosomes and ncMTOCs use discrete proteins to function. To identify novel ncMTOC components, we developed an in vivo proximity labeling technique centered around the promiscuous biotin ligase TurboID. TurboID was fused to the ncMTOC component PTRN-1/Patronin and expressed in intestinal epithelial cells during ncMTOC establishment. Biotin conjugated PTRN-1 proximity interactors were identified using streptavidin pull down followed by mass spectrometry, revealing a number of novel, conserved candidate ncMTOC components. Together, these technologies have revealed fundamental properties of ncMTOCs, an important but understudied area of cell and developmental biology.

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STRUCTURAL BIOLOGY IN CELLULAR ENVIRONMENTS USING SENSITIVITY ENHANCED NMR

Kendra Frederick

UT Southwestern

2018 New Innovator Award

The misfolded proteins associated with neurodegenerative disease can adopt a variety of different conformations, some of which are toxic. Because these proteins have identical amino acid sequences, the cellular environment clearly influences the final state, yet most structural studies do not include the cellular context and, perhaps because we are not studying the correct conformation, not a single therapeutic strategy for these diseases addresses the underlying protein misfolding pathology. Using new sensitivity-enhancement technology for solid state NMR spectroscopy, we study protein structure in native environments - inside living cells - to reveal how both healthy and disease-relevant cellular environments influence protein structure.

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DISSECTING THE MECHANISMS UNDERLYING BACTERIAL SHAPE: HOW TWO DIFFERENT CYTOSKELETAL POLYMERS CREATE ROD-SHAPED CELLS AND DIVIDE THEM IN TWO.

Ethan Garner

Harvard University

2014 New Innovator Award

The shape of bacteria is determined by their cell wall, a crosslinked macromolecule that holds them in shape. To grow and divide in defined shapes, bacteria must regulate the spatial location of material added into this structure. This spatial regulation is mediated small dynamic cytoskeletal filaments bound to the cell wall synthesis enzymes. By examining in vivo dynamics of these systems relative to the other components we revealed fundamental mechanisms underlying bacterial growth and division.

MreB is an actin homolog essential for rod-shaped growth. MreB polymerizes into short, stable membrane-associated filaments. These filaments move around the rod width, powered by the synthesis of the associated enzymes. Our work reveals these inwardly curved filaments act as short-axis sensors, pointing to the most significant inward curvature, orienting the synthesis of the associated enzymes around the rod circumference. Rod shape arises from this circumferential organization: the more MreB mediated synthesis, the more material is oriented the thinner the rods become. This oriented material causes the rod to stretch less across its width in response to internal pressure, maintaining rod shape. Conversely, non-MreB associated synthesis widens cells. Overall, Rod width depends on the balance between MreB-associated and non MreB-associated synthesis. Furthermore, our work also explains how rods form: MreB mediated circumferential synthesis nucleates local rod-like regions that self-propagate via continued MreB orientation within these local regions.

Cell division is regulated by the tubulin homolog FtsZ. In contrast to the filament/enzyme complexes in rod elongation, we find FtsZ movement is independent of enzyme activity: filaments move around the division plane via treadmilling. Filament treadmilling limits both the directional motion of the associated enzymes as well as their synthetic activity. FtsZ treadmilling is a critical feature of cell division, as modulating the treadmilling rate can increase or decrease the rate of cell division.

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ACCURATE GENOMIC VARIANT DETECTION IN SINGLE CELLS WITH PRIMARY TEMPLATE-DIRECTED AMPLIFICATION

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2018 New Innovator Award

Improvements in whole genome amplification (WGA) would enable new types of basic and applied biomedical research, including studies of cellular genetic diversity that require more accurate single-cell genotyping. Here we present primary template- directed amplification (PTA), a new isothermal WGA method that reproducibly captures >95% of the genomes of single cells in a more uniform and accurate manner than existing approaches, resulting in significantly improved variant calling sensitivity and specificity. To illustrate the new types of studies that are enabled by PTA, we developed direct measurement of environmental mutagenicity (DMEM), a new tool for mapping genome-wide interactions of mutagens with single living human cells at base pair resolution. With DMEM, we identified new features of N-ethyl-N-nitrosourea (ENU) mutagenicity, including target base independence of chromatin accessibility by DNAse I, as well as a preference for alkylating thymine that is preceded by adenine. The improved precision and accuracy of variant detection with PTA overcomes the major obstacle required to study genetic diversity and evolution at cellular resolution.

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SPERM CHROMATIN AND ROLE IN DEVELOPMENT

Sue Hammoud

University of Michigan Ann Arbor

2016 New Innovator Award

Sperm and egg cells carry genetic and epigenetic information from parents to offspring, serving as a link between the past, present and future of a species. A striking difference between the sperm and egg is the structural organization of their genomes: oocytes package DNA with histones, whereas sperm package DNA with protamines. This differential packaging traces back > 500 million years, yet its biological and evolutionary significance remains unknown. Protamines have thus far been considered simple, passive structural elements that hypercondense and protect the paternal DNA. Emerging biochemical, evolutionary, and developmental evidence, however, calls for a need to revisit protamine proteins' biological function. To investigate protamines in vivo and circumvent the lack of commercially available antibodies, we have generated novel mouse models in which the endogenous P1 and P2 loci are epitope-tagged. Immunoprecipitation followed by mass spectrometry (IP-MS) has identified numerous potential interacting proteins, including several potential chaperones and chromatin remodelers that may play a role in protamine protein placement in sperm chromatin. Additionally, chromatin immunoprecipitation followed by sequencing (ChIP-seq) allowed us to determine the genome-wide localization of P1 and P2 in mature sperm. Lastly, we have developed modification-specific antibodies as well as PTM point mutant mice to investigate the role of protamine PTMs both in sperm chromatin and during embryonic development, . Together, our moleculat and genetic data point to a potentially overlooked and active role for sperm protamine proteins. Further investigation will undoubtedly uncover unknown functions for these ancient, yet rapidly evolving proteins and may challenge the long-held dogma for protamines as passive, structural proteins.

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APOLIPOPROTEIN E (APOE) TIGHTLY REGULATES THE RATIO OF α- AND β-SECRETASE THROUGH DISRUPTION OF LIPID RAFTS

Scott Hansen[1] and Hao Wang[1]

[1] The Scripps Research Institute

2013 New Innovator Award

Neurodegeneration in Alzheimer's disease (AD) is associated with mutations in amyloid precursor protein (APP) and apolipoprotein E (ApoE). APP is a highly conserved protein that is cleaved by α- and β-secretases (proteases). β-secretase cleaves APP to beta-amyloid (Aβ) which can lead to Aβ aggregation and fibrils. Aβ is dynamic and cycles high in the brain prior to sleep. Endogenous roles for Aβ continue to emerge including antimicrobial activity. Cholesterol levels affect APP hydrolysis and genetic mutations in ApoE (a cholesterol transport protein) are associated with early-onset of the disease. How cholesterol regulates APP is poorly understood. Here we show that neurons harness lipid heterogeneity to regulate APP using direct stochastic optical reconstruction microscopy (dSTORM) super-resolution imaging. Under low cholesterol conditions, ApoE removes cholesterol from neurons causing APP to translocate out of lipid rafts (away from the enzyme β-secretase) and interact with α-secretase. When presented cholesterol, ApoE causes translocation of APP into lipid rafts increasing its association with and hydrolysis by β-secretase. We conclude that lipid raft integrity and disruption is an endogenous mechanism to regulate the production of APP in cellular membranes. Furthermore, we conclude APP is tightly regulated by substrate presentation (i.e. access of APP to either α- or β-secretase). The known conserved nature of APP's amino acid sequence combined with the tight biological regulation shown here further confirm an endogenous function for APP in cell membranes.

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HIBERNATION IN A DISH: CELL-AUTONOMOUS RESPONSE OF MAMMALIAN CELLS TO LOW TEMPERATURES

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2015 Early Independence Award

Hibernating mammals can reduce body temperature to near freezing for weeks at a time. This reduction in body temperature corresponds to physiological changes such as a change in energy source to stored fat and a significant reduction of metabolic rate. The underlying mechanism of how hypothermic hibernating mammals thrive at cold temperatures, while non-hibernators perish, remains a mystery. One main difficulty in fully understanding hibernation physiology is an incomplete understanding of how cold temperature affects processes at the cellular level. Using cultured cells from hibernating and non-hibernating species, we sought to develop a basic understanding of the cell-autonomous response to the cold. Contrary to expectation, we found that cultured cells retain viability for weeks at a hibernation-like temperature of 6 °C, and that optimum viability requires temperature-dependent adjustment of gas phase carbon dioxide to control growth medium pH. Cold exposure inhibits protein translation and cell cycle progression, and results in changes in a broad range of metabolic pathways. Rewarming allows rapid recovery and resumption of cell proliferation. Cold-exposed cells from hibernating (meadow jumping mouse) and non-hibernating (laboratory mouse) organisms exhibit similar changes in common pathways, including reductions in the metabolite pools of energetically costly pathways such as nucleotide and amino acid biosynthesis. These changes, together with the cessation of protein translation, may help to explain the greatly reduced metabolic rates observed in hibernating mammals.

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REGULATION OF STEM CELL HEALTH DRIVES MUSCULAR ATROPHY DURING AGING

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2018 New Innovator Award

Sarcopenia (age-associated skeletal muscle atrophy) afflicts ~10% of individuals over 65yo and is highly correlated with mitochondrial dysfunction in myofibers. Deep sequencing of mitochondrial genomes (mtDNA) from muscle revealed clear evidence of age-associated pathogenic mutations and deletions. Intriguingly, we observed similar mtDNA mutation signatures in the satellite cell populations isolated from aged mice. Satellite cells are a stem cell population of skeletal muscle, best known for their ability to regenerate new myofibers in response to injury. We hypothesized that aged satellite cells are a source of mtDNA damage in elderly individuals, and therefore investigated the consequences of mitochondrial dysfunction in satellite cells (using conditional alleles of electron transport chain components combined with a lineage-specific Pax7-Cre^{ERT2} allele). Affected animals displayed a rapid decline in satellite cell numbers, accompanied by severe regenerative defects. Strikingly, lineage tracing revealed that mutant satellite cells are rapidly absorbed into existing myofibers via direct cell-cell fusion, thereby inducing a myopathy that histologically and functionally phenocopies sarcopenic atrophy. Mechanistically, elevation of reactive oxygen species was necessary and sufficient to trigger satellite cell absorption via induction of actin network re-organization, and anti-oxidant treatment or genetic inhibition of cell fusion blocks myopathic changes. We therefore propose that mtDNA-damaged satellite cells are actively fated away from a regenerative lineage - thereby inducing atrophy in existing myofibers but limiting the formation of de novo myopathic fibers. These results provide mechanistic insight into the regulation of stem cell health, as well as the consequences of maintaining damaged stem cells during aging. Specifically, our model proposes that damaged satellite cells serve as a cell-of-origin for sarcopenia, and suggests actionable targets to combat this process.

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CAN YOU CLAP TO THE BEAT? FINDINGS FROM THE FIRST GENOME-WIDE ASSOCIATION STUDY OF A MUSICAL RHYTHM TRAIT IN 606,825 INDIVIDUALS

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2018 New Innovator Award

Across musically trained and non-musically trained individuals, there is substantial variability in the ability to perceive and produce rhythms accurately. Individual differences in musical rhythm have been linked to a subcortico-cortical network of brain regions, involving primarily auditory, motor, and subcortical/basal ganglia circuitry. Family-based studies demonstrate a moderate genetic contribution to rhythmic ability. However, understanding the molecular basis of rhythm necessitates genome-wide interrogation in a large well-powered sample. Here we applied Genome-Wide Association Study (GWAS) methodology to identify common genetic variants associated with musical rhythm, collected from N=606,825 research participants from the personal genetics company23andMe. Individuals responded to the question “Can you clap in time with a musical beat?”. To validate this single question, we also conducted a separate (behavioral) study using Mechanical Turk in N=734, and showed that individuals who answered Yes (vs. No) to this self-report question also performed better on a musical rhythm perception task (p= 0.0006). In the genetic cohort, preliminary GWAS revealed 68 independent loci that surpassed the threshold for genome-wide significance. We found two loci on chromosome 4 (4q34.2 and 4q22.1), replicating prior findings of linkage to musicality in this region, as well as new loci including 16p11.2 (a known locus of neurodevelopmental disorders), 2p16.1 (a region linked to mental health and sleep phenotypes) and 17q21.31 (previously associated with cortico-basal degeneration and intracranial volume). GWAS results held after conditioning the analyses on known markers of IQ, using mtcojo, revealing independence of genetic markers of rhythm and IQ. LD-score regression showed 5% SNP-based heritability of the rhythm phenotype.Taken together, these findings provide promising evidence of genetic architecture that may be involved in rhythmic ability in humans.

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THE Wtf4 MEIOTIC DRIVER UTILIZES PROGRAMMED PROTEIN AGGREGATION TO ENACT TARGETED GAMETE KILLING

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2018 New Innovator Award

Alleles that force their own inheritance into more than half of the functional gametes generated by a heterozygote are known as meiotic drivers. Natural meiotic drivers are found throughout eukaryotes and can play significant roles in the origins of infertility and in shaping genome evolution. The power of meiotic drive is also being exploited with engineered 'gene drives' designed to control or eliminate natural populations, such as pest species. Despite the importance and potential of drive systems, the molecular mechanisms used by natural drivers are largely unknown. We previously identified *wtf4* as a meiotic driver in fission yeast. *wtf4* kills meiotic products (spores) that fail to inherit the gene from heterozygotes using two proteins: a spore-killing poison protein and an antidote protein that rescues only the cells that inherit the gene. We now show that the Wtf4^{poison} and Wtf4^{antidote} proteins function in vegetative budding yeast. We employ this highly tractable system to determine that both proteins self-assemble into two types of aggregates. The small Wtf4^{poison} oligomers spread throughout the cell and cause cytotoxicity. The large, Wtf4^{antidote} aggregate is vacuole-associated and non-toxic. When co-expressed, the two types of Wtf4 proteins complex via homotypic interactions. This interaction causes the Wtf4^{antidote} to sequester the Wtf4^{poison} at vacuole-associated inclusions, thus neutralizing the cytotoxicity. This work provides insight into the molecular mechanisms by which meiotic drivers can act and highlights the potential of *wtf*-derived gene drives for use outside of fission yeast.

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REGULATION OF CELL SIGNALING BY ZINC DYNAMICS

Amy Palmer

University of Colorado Boulder

2014 Pioneer Award

The focus of this Pioneer Project was to explore the role of zinc in regulating cell signaling. 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, including > 700 transcription factors, that comprise the zinc proteome bind zinc constitutively. This Pioneer Project has explored 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.We have used cutting-edge technologies from live cell imaging to transcriptomics, ATAC-seq and proteomics to define zinc dynamics and the downstream consequences of these dynamics in neurons, infected macrophages and during the mammalian cell cycle. We have discovered that sub-nanomolar zinc dynamics alter expression of hundreds of genes in neurons, that zinc influences chromatin accessibility and transcription factor activity, and that zinc plays a role in the proliferation-quiescence cell fate decisions, regulating the mammalian cell cycle in two different phases. As with many exploratory high-risk projects, this Pioneer project also extended in an unexpected direction to develop a new tool called Riboglow to track single molecules of RNA in living cells.

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LOCALIZED PIEZO1 CA2+ FLICKERS ARE EVOKED BY MYOSIN-II MEDIATED TRACTION FORCES

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2018 New Innovator Award

Piezo channels transduce mechanical stimuli into electrical and chemical signals and in so doing powerfully influence development, homeostasis, and regeneration. While much is known about how Piezo1 responds to external forces, its response to internal, cell-generated forces remains poorly understood. Here, using measurements of endogenous Piezo1 activity and traction forces in native cellular conditions, we show that actomyosin-based cellular traction forces generate spatially-restricted Ca^{2+} flickers in the absence of externally-applied mechanical forces. Although Piezo1 channels diffuse readily in the plasma membrane and are widely distributed across the cell, their Ca^{2+} flicker activity is enriched in regions proximal to force-producing adhesions. The mechanical force that activates Piezo1 arises from Myosin II phosphorylation by Myosin Light Chain Kinase. We propose that Piezo1 Ca^{2+} flickers allow spatial segregation of mechanotransduction events, and that diffusion allows channel molecules to efficiently respond to local mechanical stimuli.

Note: KE, AC, JH and JN are co-first authors.

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THE ROLE OF A NOVEL MICROVASCULAR NETWORK IN CANCER PROGRESSION AND RELAPSE

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2017 New Innovator Award

Angiogenesis provides tumor cells with oxygen and nutrients that are essential for cancer expansion and spread. The development of anti-angiogenesis drugs originally held great promise. Most of these target Vascular Endothelial Growth Factor (VEGF), which is secreted by the cancer cells, or it's receptor VEGFR, thought to be expressed by all endothelial cells. Yet angiogenesis inhibitors have had limited success in the clinic, and in some instances, cancers treated with these drugs initially respond but surviving cells grow back more aggressively than before treatment. Why cancer cells that rely on angiogenesis for growth can survive, and even thrive, when treated with VEGF/VEGFR inhibitors is a cancer research paradox that has never been fully explained and has significant clinical implications. Using zebrafish models, we have found a microvascular network that is comprised of endothelial cells but, surprisingly, is not VEGFR-positive. The vessels do not carry erythrocytes, so cannot oxygenate tissues, but are leaky and can deliver nutrients and macromolecules that are essential for cell survival. In the marrow, these vessels are in close proximity to hematopoietic stem cells, suggesting that they are part of the stem cell niche. Recent research in normal and cancer stem cells has shown that hypoxia, or the lack of oxygen, may create a metabolic program in stem cells that promotes self-renewal over differentiation. We hypothesize that this novel, deoxygenated vascular network is an important component of the cancer stem cell niche, in that it delivers nutrients to allow cells to survive in a hypoxic environment. Anti-angiogenesis inhibitors that kill normal blood vessels may expand this hypoxic microvascular network and increase the cancer stem cell population. The current focus of our research is to define the role of this novel vascular network in hematopoiesis and cancer progression, determine how these vessels differ from the canonical VEGFR-positive vessels, and identify FDA-approved drugs capable of eliminating these vessels. Overall, this research will define a completely new component of the cancer microenvironment, and has the potential to have a rapid and important impact in the cancer clinic.

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MASS-SPECTROMETRY OF SINGLE MAMMALIAN CELLS QUANTIFIES PROTEOME HETEROGENEITY DURING CELL DIFFERENTIATION

Nikolai Slavov

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2016 New Innovator Award

Cellular heterogeneity is important to biological processes, including cancer and development. However, proteome heterogeneity is largely unexplored because of the limitations of existing methods for quantifying protein levels in single cells. To alleviate these limitations, we developed Single Cell ProtEomics by Mass Spectrometry (SCoPE-MS), and validated its ability to identify distinct human cancer cell types based on their proteomes. We used SCoPE-MS to quantify over a thousand proteins in differentiating mouse embryonic stem (ES) cells. The single-cell proteomes enabled us to deconstruct cell populations and infer protein abundance relationships. As expected, proteins forming protein complexes are strongly correlated to each other, including most ribosomal proteins (RPs). However, a small subset of RPs covaries as a distinct cluster this might reflect ribosome remodeling and specialization in the distinct lineages, i.e., variation among the RP stoichiometry across the cell lineages that contributes to specialized translation functions. Comparison between single-cell proteomes and transcriptomes indicated coordinated mRNA and protein covariation. Yet many genes exhibited functionally concerted and distinct regulatory patterns at the mRNA and the protein levels, suggesting that post-transcriptional regulatory mechanisms contribute to proteome remodeling during lineage specification, especially for developmental genes. SCoPE-MS is broadly applicable to measuring proteome configurations of single cells and linking them to functional phenotypes, such as cell type and differentiation potentials.

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ALS/FTD-ASSOCIATED TOXIC PEPTIDES INHIBIT UPF1-MEDIATED RNA DECAY

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[1] Yale School of Medicine

2018 New Innovator Award

Expansion of an intronic GGGGCC repeat region within the C9orf72 gene is the major cause of familial amyotrophic lateral sclerosis and frontotemporal dementia (c9ALS/FTD). The expanded repeats are transcribed in both directions, producing two repeat RNAs, which are translated into several dipeptide repeats (DPRs). While the pathophysiological mechanism(s) by which repeat expansion ultimately leads to neurodegeneration remains highly debated, RNA processing defects have been implicated in both C9orf72-associated and other types of familial ALS/FTD. By comparing the transcriptome profiles between c9ALS, sporadic ALS, and control post-mortem brains, we discovered that the RNA substrates of UPF1-mediated decay (UMD) mechanisms were specially up-regulated in c9ALS brains. In vitro, ectopic expression of either poly(GR) or poly(PR) peptides could acutely inhibit UMD in human cells and cause neuronal cell death, suggesting that these two toxic DPRs may cause UMD inhibition in c9ALS/FTD patients and allow deleterious RNAs to accumulate. I will describe our ongoing effort in understanding the molecular and cellular mechanism by which arginine-rich DPRs inhibit UMD. Our results suggest that restoring UMD activity may represent a promising therapeutic approach.

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GENETICALLY ENCODED TOOLS FOR MANIPULATION OF BIOENERGETICS

Denis Titov

University of California, Berkeley

2018 New Innovator Award

Classical biochemical studies have demonstrated that bioenergetic ratios like NADH/NAD+ and NADPH/NADP+ are key regulators of energy metabolism. These ratios change with diet, exercise, disease states and the aging process itself. However, the causal relationship between changes in these crucial bioenergetic parameters and downstream effects of diet, exercise and aging is currently unknown. A key bottleneck to a better understanding of the role of changes in NADH/NAD+ and NADPH/NADP+ ratios is a lack of methods for direct manipulation of these parameters in vivo. Here, I will describe the use of water-forming oxidases, LbNOX and TPNOX, as genetically encoded tools for compartment-specific manipulation of the NADH/NAD+ and NADPH/NADP+ ratios in human cells. I will discuss several applications that demonstrate the utility of using LbNOX and TPNOX for studying the role of redox changes in regulation of physiological processes.

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OPENING WINDOWS INTO PARKINSON'S DISEASE: REVEALING THE IN SITU STRUCTURE OF A PATHOGENIC MUTANT OF LRRK2

Elizabeth Villa

University of California San Diego

2016 New Innovator Award

Mutations in the leucine-rich repeat kinase 2 (LRRK2) are the major cause for familial Parkinson's disease (PD). LRRK2 is a large protein composed of kinase and GTPase domains surrounded by protein-protein interaction domains. Most pathogenic mutations are found in the catalytic domains, suggesting modulation of GTPase and kinase activities play a crucial role in pathogenesis. Although being a promising drug target, LRRK2 has eluded structural determination. Furthermore, while numerous studies showed that pathogenic mutations or loss of the LRRK2 gene affect various cellular processes, the precise function of LRRK2 at the molecular level remains elusive.

Various pathological mutants of LRRK2 have been shown to form filamentous structures surrounding microtubules (MTs). This phenotype has also been observed wild-type LRRK2 after pharmacological kinase inhibition or by addition of a non-hydrolyzable GTP analogue. This suggests that LRRK2 is conformationally arrested on MTs, emphasizing the need of detailed structural information for development of PD therapeutics.

We used correlative cryo-fluorescent microscopy, cryo-focused-ion-beam milling and cryo-electron tomography to reveal the structure of a pathogenic mutant LRRK2 (I2020T) decorating MTs inside cells. We found mutant LRRK2 oligomerizes into a double helix along MTs. Interestingly, mutant LRRK2 preferentially decorates unusual MTs composed of 12 and 11 protofilaments over the canonical 13-protofilaments majorly found in cells. We determined the first *in situ*structure of LRRK2, revealing the interaction between LRRK2 domains and MTs, and the dimerization interfaces that lead to this putative pathogenic state. Upon mapping known genetic and sporadic mutations, our structure will help in the design of inhibitors, and to understand the mechanistic details of LRRK2 function. Our findings suggest that pathogenic mutant LRRK2 could drastically alter cellular MT organization and structure, likely affecting its dynamics and/or roles for cellular processes mediated by MTs that ultimately lead to the pathological phenotypes found in PD patients.

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SUPER-RESOLUTION DISPLACEMENT MAPPING OF UNBOUND SINGLE MOLECULES REVEALS NANOSCALE HETEROGENEITIES IN INTRACELLULAR DIFFUSIVITY

Ke Xu

University of California, Berkeley

2018 New Innovator Award

Intracellular diffusion underlies vital processes of the cell. However, it remains difficult to elucidate how an average-sized protein diffuses in the cell with good spatial resolution and sensitivity. Here we report single-molecule displacement/diffusivity mapping (SM*d*M), a super-resolution strategy that enables the nanoscale mapping of intracellular diffusivity through the local statistics of instantaneous displacements of freely diffusing single molecules. We thus show that diffusion in the cytoplasm and in the nucleus to both be spatially heterogeneous at the nanoscale, and such variations in local diffusivity correlate strongly with the ultrastructure of the actin cytoskeleton and the chromosome, respectively. Moreover, we identify the net charge of the diffuser as a key determinant of diffusion rate: intriguingly, the possession of positive, but not negative, net charges significantly impedes diffusion, and the exact degree of slowdown is determined by the specific subcellular environments.

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A MEMBRANE TRANSPORTER IS REQUIRED FOR STEROID HORMONE UPTAKE IN DROSOPHILA

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[3] Howard Hughes Medical Institute

2018 New Innovator Award

Steroid hormones are a group of lipophilic hormones that are believed to enter cells by simple diffusion to regulate diverse physiological processes through intracellular nuclear receptors. We recently challenged this model in *Drosophila* by demonstrating that a membrane transporter that we named Ecdysone Importer (EcI) is involved in cellular uptake of the steroid hormone ecdysone. *EcI* encodes an organic anion transporting polypeptide, Oatp74D, a member of the evolutionarily conserved solute carrier organic anion superfamily. *In vivo*, *EcI* loss-of-function causes phenotypes indistinguishable from ecdysone- or*ecdysone receptor* (*EcR*)-deficient animals, and *EcI* knockdown inhibits cellular uptake of ecdysone. Furthermore, *EcI* regulates ecdysone signaling in a cell-autonomous manner and is both necessary and sufficient for inducing ecdysone-dependent gene expression in culture cells expressing *EcR*. Altogether, our results challenge the simple diffusion model for cellular uptake of ecdysone and may have wide implications for basic and medical aspects of steroid hormone studies.

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REAL-TIME VISUALIZATION OF THE INCEPTION OF DRUG TOLERANCE IN SINGLE MELANOMA CELLS

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2018 New Innovator Award

Although a non-genetic, reversible drug-tolerant state has been reported both in the clinic and cultured cells, little is known about the rapidity of cancer cell adaptation to drug and the signaling plasticity that enables drug tolerance. Here we use time-lapse imaging and single-cell tracking to monitor the first four days of drug treatment in individual melanoma cells and thereby uncover dramatic heterogeneity in drug response: the majority of the cells stop proliferating and enter quiescence, while a subset of cells initially enters quiescence but then escapes drug action to reactivate CDK2 and occasionally proliferate in drug. CDK2 reactivation is mediated by the reactivation of ERK 3 hours prior, and can be suppressed by further inhibition of ERK activity or by knockdown of key regulators uncovered by single-cell RNA-seq. The signaling plasticity that enables occasional cycling in drug also enables acquisition of bona fide genetic mutations and development of permanent drug resistance.

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‘EAT ME’ OR ‘FUSE ME’: A STORY OF A LIPID SCRAMBLASE IN TROPHOBLAST FUSION AND BEYOND

Huanghe Yang

Duke University

2017 New Innovator Award

Cell-cell fusion or syncytialization is fundamental to the reproduction, development and homeostasis of multicellular organisms, but its mechanistic underpinnings are still largely elusive. We demonstrate that transmembrane protein 16F (TMEM16F), a Ca^{2+}-activated phospholipid scramblase (CaPLSase), plays an indispensable role in placental trophoblast fusion by externalizing phosphatidylserine (PS) to cell surface. The placentas from TMEM16F-deficient mice exhibit defects in syncytialization, which result in placental insufficiency and perinatal lethality. Furthermore, TMEM16F overexpression renders non-fusogenic HEK293T cells capability to spontaneously form multinucleated syncytia, while overexpression of PS receptors further enhances TMEM16F-mediated cell fusion. Our findings thus identify a common cell-cell fusion mechanism in which CaPLSase-dependent externalization of PS can serve as a fuse-me signal to attract PS receptors thereby facilitating syncytialization.

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NEW EXPANSION MICROSCOPY TOOLS TOWARDS WHOLE ORGANISM NANOSCALE IMAGING AND HIGHLY MULTIPLEX NANOSCOPY

Aleksandra Klimas[1], Walter Schneider[2], Clayton Wiley[2], Greg Delgoffe[2], Simon Watkins[2], Edward Burton[2], Alan Watson[2], and Yongxin Zhao[1]

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2018 New Innovator Award

In modern biology, diffraction-limited microscopy is a powerful tool to observe microscopic structures and processes of biological specimens in both health and disease. However, diffraction-limited microscopy is unable to resolve nanoscale configurations of biomolecules below the diffraction limit (~250 nm), which severely limited its capability of analyzing intricate and subtle biological/pathological changes. Recently, Expansion Microscopy (ExM) has emerged as a ground-breaking new principle for scalable, nanoscale optical imaging of biological specimens. Rather than optically magnifying samples, ExM works by embedding biological tissue into a water-swellable polyelectrolyte hydrogel, enzymatically homogenizing them, and then isotropically expanding the tissue-hydrogel physically in pure water. Typical ExM protocols expand tissues by ~100 folds in volume, thus enabling nanoscale optical imaging with resolution ~60 nm using diffraction-limited microscopes. However, most ExM methods cannot process formaldehyde-fixed thick tissue, and they include an enzymatic digestion step that destroys most of the tissue proteins, obscuring fluorescent imaging of more than 4 markers.

To address this, our lab has been developing a suite of new ExM methods with a new biomolecule anchoring strategy and an enzyme-free homogenization method. The new tools enable isotropic expansion of formaldehyde-fixed thick tissue and whole organism by ~6 folds in each dimension and by more than 200 folds in volume in pure water. The expanded tissues can be imaged by a conventional optical wide-field or confocal microscope with effective resolution better than 45 nm. In combination with a reflectance confocal imaging system, we performed large volume, nanoscale imaging of the human optic nerve and the whole 5dpf zebrafish embryo. In addition, the new technique enables expansion of a wide range of human tissues while conserving endogenous DNAs, proteins, and carbohydrates even after multiple rounds of serial imaging, paving the way to highly multiplex, nanoscale, *in situ* molecular profiling of human tissue samples using only commercially available reagents.

NEUROSCIENCE

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ELUCIDATING THE PATHOGENIC ROLE OF EBF3 LOSS-OF-FUNCTION IN NEURODEVELOPMENTAL DISORDERS

Hsiao-Tuan Chao[1], Darrion Nguyen[1], Dongwon Lee[1], Hugo Bellen[1], and Mingshan Xue[1]

[1] Baylor College of Medicine

2018 Early Independence Award

Neurodevelopmental disorders (NDD) encompass many conditions such as intellectual disability, autism spectrum disorders, epilepsy, and psychiatric disorders. These conditions often co-occur, suggesting that there may be common underlying mechanisms of disease. One potential mechanism arises from findings that disrupted inhibitory signaling often occurs in many of these conditions. However, despite significant advances in our understanding of the genetic underpinnings of inhibitory neurobiology, there remains a knowledge gap regarding how genetic alterations perturb neural circuits and lead to neurologic impairments. This gap needs to be addressed in order to bridge molecular functions to pathogenic mechanisms and expand our understanding of inhibition in health and disease.

Recently, we found that deleterious variants in *EBF3* (*Early B-cell Factor 3*), encoding a Collier/Olf/EBF (COE) transcription factor, cause developmental delay, language impairments, autistic features, and cerebellar hypoplasia. This neurodevelopmental disorder is now known as the autosomal dominant Hypotonia, Ataxia, and Delayed Development syndrome (HADDS) (MIM #617330). In mice, Ebf3 has been shown to be a critical mediator of inhibitory neuronal development and migration. Therefore, deciphering the EBF3-dependent regulation of inhibition will advance our understanding of inhibitory neurobiology in health and disease.

To examine the pathogenic role of EBF3 loss-of-function, we generated a mouse *Ebf3* null allele by deleting exons 2-4. Multiple genetically distinct *Ebf3* null alleles were established. All genotypes are born at the expected Mendelian ratio with perinatal lethality observed in homozygous *Ebf3* null mice. Loss of Ebf3 expression was confirmed by Western blot and immunohistochemistry studies. Morphological analysis reveals that loss of *Ebf3* perturbs neurodevelopmental processes in multiple brain regions.

We will integrate fly genetics with mouse neurophysiology and behavior to uncover how EBF3 dysfunction perturbs inhibitory signaling in neural circuits and reveal the associated molecular drivers. These findings will broaden our understanding of inhibitory neurobiology and advance therapeutic strategies for NDD.

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BUILDING A BRAIN: SYSTEMATIC EXAMINATION OF THE LOGIC OF BRAIN CONNECTIVITY IN *C. ELEGANS*

Daniel Colon-Ramos[1], Mark Moyle[1], Leighton Duncan[1], Titas Sengupta[1], Lin Shao[1], William Mohler[2], Anthony Santella[3], Ryan Christensen[4], Hari Shroff[4], Smita Krishnaswamy[1], and Zhirong Bao[3]

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[4] NIH

2018 Pioneer Award

How is a brain wired during development? While molecular genetic studies have contributed significantly to the identification of conserved signaling pathways that regulate distinct steps during neurodevelopment, we lack a systematic understanding of the logic governing the coordinated decisions of the system as it self assembles into a functional brain. We have decided to take an orthogonal approach to understand how, during the development of the *C. elegans* brain, system's level neurodevelopmental decisions influence precise and stereotyped connectivity by restricting the degrees of freedom of single neurons within circuits. Using probabilistic network models to analyze connectomics data, we provide evidence that the nematode brain is composed of domains of nerve bundles with related functional connectivity. We then use light-sheet microscopy in *C. elegans* embryos to identify pioneer neurons and elucidate a hierarchical ordering of neurodevelopmental decisions that underlie the functional circuits identified. By linking connectomic data, probabilistic network models and developmental studies, our approaches allow us to dissect the top-down logic of brain neurodevelopment in a simple nervous system.

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THE ROLE OF DUPLICATED GENES IN HUMAN BRAIN EVOLUTION AND DISEASE

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2018 New Innovator Award

The human cortex exhibits dramatic anatomical and cognitive differences from those of closely related primate species. Despite a few potential success stories, the underlying genetic contributors to unique human adaptive traits remain undiscovered. We posit that human-specific segmental duplications (HSDs; genomic regions > 1 kbp in size with > 98% identity) may be a source of neurological innovation and disease that have remained largely understudied. Two HSD genes, *SRGAP2C* and *ARHGAP11B*, have been previously implicated in cortex expansion. Using a human haploid-derived (CHM1) BAC resource, we previously performed Pacific Biosciences long-read sequencing to correct the largest, gene-containing HSDs, fixing over 18.2 Mbp in the current human reference build and identifying over 30 additional HSD gene families. Of these, we honed in on a set of ten duplicate gene families with the propensity to be functional today based on their presence in all of humans tested (thousands) and exhibiting gene expression in adult post-mortem tissues from GTEX. To refine our genes to those important in neurodevelopment, we are employing a multifaceted functional approach using cell lines, zebrafish, and mice. Understanding that genetic variation segregating in modern human populations can also inform on if a gene is functional (e.g., an excess of truncating mutations may indicate loss of function), we are leveraging sequence data of HSD gene paralogs. Unfortunately, only 1.7% of HSDs are accessible for variant calling using whole-genome shotgun short-read (Illumina) data from 1000 Genomes Project. Furthermore, 78% of HSD regions are completely depleted for common variants (dbSNP). As such, we are performing targeted long-read sequencing in diverse human populations to accurately detect variants in these typically inaccessible regions. Though a work in progress, if successful, the results of these studies will offer important insights into if/how HSD genes contribute to innovative neurological features that distinguish modern humans from related great ape species.

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BUILDING BRAINS IN OUR SLEEP: EVIDENCE FROM FRUIT FLIES

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2018 New Innovator Award

Sleep during critical developmental windows is thought to be important for brain maturation. However, examination of a function for sleep in the earliest periods of nervous system development, when neurons are being born, has been limited by the lack of a tractable experimental system. The adult fruit fly is a widely studied model organism for sleep, but the major wave of neurogenesis in *Drosophila* begins during larval life and ends prior to adulthood. It has remained unknown whether larvae sleep. We developed new approaches for long-term monitoring of larval behaviors, leading to identification of a sleep state in *Drosophila* larvae. We are now exploiting this system to understand the function and regulation of sleep in a nascent nervous system. Our results indicate that both the neural and genetic control of sleep/wake are partially distinct in a developing brain compared to mature adulthood. A neural activation screen has identified 2 sleep-promoting and 4 wake-promoting neurons out of ~10,000 neurons in the larval nervous system. Ongoing work aims to characterize these cells, determine how the circuits are wired, and examine whether these neurons have a conserved function in the adult brain. Using real-time behavioral monitoring in a closed-loop sleep deprivation system, we have also found that sleep deprivation during larval stages attenuates neural stem cell proliferation. We aim to define how sleep loss and neurogenesis are coupled at a transcriptional level, and determine the impact of early life sleep loss on adult learning and memory. Collectively, this work will generate novel tools and approaches for studying the role of sleep in the developing nervous system.

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SYANPTIC REPROGRAMING OF DEVELOPING AND ADULT NEURONS

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2016 New Innovator Award

Much attention has been dedicated to solving the 'connectome,' or street map of the brain. Equally important is an understanding of the road signs and signals that keep traffic (information) moving. This process occurs at synapses, and these connections give meaning to the map. However, because they are small, heterogonous and functionally diverse, little is known about the processes that regulate synaptic integrity. Due to the large burden of adult cognitive disease, we argue that deciphering adult synaptic regulators remains one of the greatest challenges in neuroscience. **Our goal is thus to identify structural and molecular mechanisms that regulate synaptic integrity and instruct the formation of connections with new partners.** Toward this goal, we have uncovered a key role for the metabolic regulator LKB1 in instructing synapse localization in development and aging and shown that manipulating LKB1 can help restore proper wiring even after synapses decline. Further, we have developed a pipeline for the identification of further novel neural and synaptic integrity genes by high-throughput retinal screening (INSiGHT) that analyzes candidate expression, vascular patterning, cellular organization, and synaptic arrangement. Using this system, we examined 102 mutant mouse lines and identified 16 additional unique retinal regulatory genes. Fifteen of these candidates are identified as novel retina regulators, and many (9 of 16) are associated with human neural diseases. We are now testing whether manipulating these signaling pathways can modulate neural connectivity using 3D nanoscopic imaging PAINT and STORM methods that we have developed for visualizing whole neurons and their synapses in CNS tissue. These results expand the genetic landscape involved in circuit organization and help open the door to neuron-specific interventions aimed at preserving cognitive function.

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A NEW TARGET TO STOP THE GLOBAL EPIDEMIC OF MYOPIA

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2015 New Innovator Award

The fast rise in myopia incidence over the last 4 decades has been attributed to a variety of environmental factors, including time spent indoors away from bright sunlight, the spectrum of fluorescent lighting, the use of nightlights, and reading with the page or screen too close to one's eyes, but a lack of basic understanding of the physiological basis of refractive disorder has prevented major progress on interventions to stop this epidemic.

Previous work has demonstrated that the retina measures the average defocus of images during development and uses this signal to regulate the growth of the eye. Despite this amazing discovery, the cellular basis of the defocus signal has remained elusive, so there has been no reliable target for clinical interventions to stop the dysregulation of this pathway that is responsible for the development of myopia in children.

Work in my lab has revealed a specific cell type in the retina that is exquisitely sensitive to defocus in an image. I will discuss the properties of the retinal defocus detector and my long-term plans to translate this discovery into a clinical target.

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SYNAPTOMES OF MOUSE AND MAN

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2014 Transformative Research Award

Many neurodevelopmental, psychiatric and neurodegenerative disorders are rooted in abnormalities of the brain's vast and highly heterogeneous synapse populations. One reason such disorders have been difficult to understand and treat is because we have lacked adequate tools to measure these synapse populations at the level of individual synapses. In addition, too many of the population-scale single-synapse tools now available are applicable only to experimental animals, not to humans. With the support of a 2014 Transformative Research Award, we formed a multidisciplinary consortium comprising biologists, biophysicists, clinicians, mathematicians and computer scientists to develop, utilize and disseminate new tools to enable precise single-synapse measurement across diverse synapse populations in both experimental animals and humans. The present poster describes selected results of our development of new high-throughput array tomography tools and of studies using these new tools to deepen our understanding of both mouse and human synapse populations.

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IDENTIFICATION OF A PUTATIVE VOCALIZATION COMMAND CENTER

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2016 Early Independence Award

Even a seemingly effortless behavior like vocalization requires the coordination of about 40 muscles. To produce this harmony gracefully and robustly, it has been hypothesized that a group of neurons in the brainstem are situated atop the hierarchy to command, directly or indirectly, all of the muscles for speech. Furthermore, this proposed vocalization central pattern generator (CPG) must interact with the control neurons for other vital behaviors like breathing, ensuring the execution of speech during the correct phase of the breathing cycle. We sought to discover the brainstem vocalization CPG by identifying the neurons that indirectly control the key structure for speech, the larynx. Using the monosynaptic rabies virus, we identified a cluster of dozens of medullary brainstem neurons that control murine laryngeal motoneurons. This group of neurons remains autonomously rhythmically active without synaptic neurotransmission and their oscillatory behavior is synchronized with and re-timed by breathing. Future experiments are designed to determine the necessity and sufficiency of this rhythmic central pattern generator for vocalization.

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CORTICAL CONTRIBUTION TO LINGUAL KINEMATICS AS THE TONGUE REACHES FOR, AND MISSES, TARGETS

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2015 New Innovator Award

Chewing, licking, vocalizing and swallowing can require precise control of the tongue, but neural control of tongue kinematics remains poorly understood. Here we combine kilohertz frame-rate imaging and a deep-learning based artificial neural network to track tongue kinematics in mice performing a directed lick task. Cue evoked licks exhibited variably-timed submovements en route to the spout, resembling a hand searching for an object. When licking left or right, submovements exhibited a directional bias towards the spout. ALM photoinhibition impaired cue-evoked licks by abolishing intra-lick submovements, resulting in stereotyped, hypometric licks unable to correct for spout undershoots or misses. Performance deficits during ALM inactivation were 'rescued' by placing the spout very close to the mouth. Our results identify corrective submovements within licks, establish their cortical dependence, and reveal limb-like dynamics of the tongue as it reaches for, misses, and targets.