Behavioral and Social Science

1

NEIGHBORHOOD CORRELATES OF TOBACCO PRODUCT ADVERTISING IN NEW YORK CITY

Daniel Giovenco, Torra Spillane

Columbia University Mailman School of Public Health

Early Independence Award, 2016

Background: The tobacco marketplace in the United States is increasingly diverse, with products such as small cigars, smokeless tobacco, and electronic nicotine delivery systems (ENDS) growing popular among various subgroups. Importantly, not all tobacco products are equally as risky. Rather, they fall on a continuum of harm, with combusted tobacco posing the greatest health risks to users. This study examines the relationship between neighborhood demographics and tobacco product advertising in NYC retailers.

Methods: Ten percent of licensed tobacco retailers in NYC were selected via stratified, random sampling from 188 neighborhoods (n=879 retailers). In July-October 2017, researchers conducted store audits to document advertising for all tobacco products. Using U.S. Census data, neighborhoods were grouped into 3 tertiles (i.e., low, medium, high) for a variety of demographic and socioeconomic variables (e.g., racial/ethnic distributions, median household income). Cochran-Armitage trend tests assessed associations between neighborhood characteristics and product advertising.

Results: One in five (21.2%) retailers advertised cigars, but this was not evenly distributed across neighborhoods. For example, 32% of retailers in predominantly African American neighborhoods advertised cigars, compared to 16% of retailers in largely White neighborhoods (p.001). Non-combusted products followed reverse trends. As income level and the percentage of White residents increased, so did the prevalence of ENDS and smokeless tobacco advertising (p.001). Notably, ENDS advertisements were significantly more common in neighborhoods wit the highest income levels (33.2%) and a large proportion of White residents (33.1%) compared to nighborhoods with the lowest income levels (19.1%) and that were largely African American (20.9%).

Conclusions: Non-combusted products are commonly advertised in neighborhoods with higher income levels and fewer minority residents, while inexpensive cigar promotions saturate predominantly Black and low income neighborhoods. Given the risk differences between combusted and non-combusted tobacco products, these patterns may widen existing health disparities.

2

DOES SHALE GAS DEVELOPMENT IMPACT INFANT HEALTH THROUGH DRINKING WATER?

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Early Independence Award, 2015

Background: Widespread hydraulic fracturing of shale formations has yielded a range of economic and environmental benefits. However, adverse health outcomes associated with shale gas development (SGD) remain uncertain. This study aims to quantify reproductive health risks associated with SGD via drinking water contamination.

Methods: We build a novel data set that links gas well activity to both infant health outcomes from the universe of birth records in Pennsylvania from 2003 - 2014 and Community Water System (CWS) drinking water contaminant measurements using the geographical coordinates of a mother's residence, gas wells, and public drinking water source locations. We compare water quality and birth outcomes (e.g. low birth weight (LBW); preterm birth (PTB)) for water systems with well bores close (e.g. 1 km) to their source to water systems with well bores further away (e.g. 10 km) using a difference-in-differences design. In sensitivity analyses, we run our models using a subsample of infants born to mothers who are only exposed to gas wells through threatened CWS source locations but do not live near a gas well.

Results: We find that drilling an additional well bore within 1km of CWS source locations increases shale gas-related contaminants by 1 to 3 percent, on average. We find that a standard deviation increase in the number of well bores drilled increases PTB and LBW by about 4 to 11 percent for mothers that do not have a well adjacent to their home. We find evidence that suggests lower-SES mothers experience larger impacts.

Conclusion: Our paper contributes to an increasing body of research that estimates the causal impacts of SGD on the environment and health in order to weigh the extent of these potential costs against its economic and environmental benefits. In addition, this work demonstrates a unique application of economic methods in a public health setting.

3

DOES A PERSONALIZED ULTRAVIOLET PHOTO INCREASE SUN SAFE BEHAVIOR?: EVALUATING AN INTERVENTION IN UTAH HIGH SCHOOLS

Jakob Jensen

University of Utah

New Innovator Award, 2015

Ultraviolet (UV) photos can be used to communicate skin cancer risk. UV photos can either be of the target person (personalized) or of someone else (stock). Students 12 -18 years of age (N = 1,062) at eleven high schools in Utah were recruited to participate in an experiment comparing the relative persuasive impact of personalized and stock UV photos. Fear, threat, efficacy, threat to freedom, and reactance were measured immediately after the intervention. Sun safe behavior was assessed by self-report one month later. Participants in the personalized UV condition reported greater fear than those in the stock UV condition. UV condition was indirectly related to sun safe behavior via fear; personalized

UV photos increased fear which increased sun safe behavior. The indirect effect was moderated by self-efficacy; as efficacy increased the indirect path via fear was larger. The results support the value of personalized UV photos for skin cancer prevention interventions, and the potential importance of fear as a mediator.

4

FORM AND FUNCTION IN HUMAN SONG

Samuel Mehr

Harvard University

Early Independence Award, 2017

Humans use music for a wide variety of social functions: we sing to accompany dance, to soothe babies, to heal illness, to communicate love, and so on. Across animal taxa, vocalization forms are shaped by their functions, including in humans. Here we show that vocal music exhibits recurrent, distinct, and cross-culturally robust form-function relations detectable by listeners across the globe. In Experiment 1, internet users (N = 750) in 60 countries listened to brief excerpts of songs, rating each song's function on six dimensions (e.g., used to soothe a baby). Excerpts were drawn from a geographically-stratified pseudorandom sample of dance songs, lullabies, healing songs, and love songs recorded in 86 mostly small-scale societies, including hunter-gatherers, pastoralists, and subsistence farmers. Experiment 1 and its analysis plan were pre-registered. Despite participants' unfamiliarity with the societies represented, the random sampling of each excerpt, their very short duration (14 s), and the enormous diversity of this music, the ratings demonstrated accurate and cross-culturally reliable inferences about song functions on the basis of song forms alone. In Experiment 2, internet users (N = 1000) in the United States and India rated three "contextual" features (e.g., gender of singer) and seven "musical" features (e.g., melodic complexity) of each excerpt. The songs' contextual features were predictive of Experiment 1 function ratings, but musical features and the songs' actual functions explained more variability in function ratings. These findings are consistent with the existence of universal links between form and function in vocal music.

Bioinformatics and Computational Biology

5

SINGLE CELL DISTRIBUTION SHAPES GOVERN THE DISCOVERY OF PREDICTIVE MODELS FOR TRANSCRIPTION REGULATION

Gregor Neuert

Vanderbilt University

New Innovator Award, 2014

Gene transcription requires that multi protein complexes are coordinated in a temporally ordered process. Despite the fact that most of these proteins have been identified, predicting the response of noncoding and coding RNA transcription upon mutations in these complexes is still impossible. One reason is that mechanistic and predictive modeling in biology remains very challenging and poorly understood. Although randomness and complexity of biological systems play roles in this concern, we hypothesize that significant and overlooked challenges arise due to specific features of single-molecule and single-cell events that control crucial biological responses. Here we demonstrate why modern statistical tools to disentangle complexity and stochasticity don't apply to the non-symmetric distributions that characterize spatiotemporal mRNA fluctuations in single-cells. As an example, we integrate single-molecule measurements of mRNA (RNA-FISH) and advanced computational analyses to explore the evolutionary conserved high osmolarity glycerol (HOG) Mitogen Activated Protein Kinase pathway's osmotic stress induction of multiple stress response genes in thousands of single S. Cerevisiae yeast cells. Through systematic comparisons of the same model to the same data, we elucidate why standard population mean modeling approaches yield non-predictive models for single-cell gene regulation. We further explain how advanced tools recover precise, reproducible, and predictive understanding of diverse transcription regulation mechanisms, including gene activation, polymerase initiation, polymerase elongation, single-cell variability in nascent transcription, mRNA accumulation, mRNA nuclear export, and nuclear and cytoplasmic mRNA degradation. In the future, our single cell modeling approach may provide a framework to systematically model and predict how individual proteins within multiprotein complexes contribute to noncoding and coding RNA transcription regulation.

6

IDENTIFYING DISEASE-RELEVANT CELL TYPES FROM GENOME-WIDE ASSOCIATION STUDY DATA

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Broad Institute of MIT and Harvard

Early Independence Award, 2017

There are many diseases whose causal tissues or cell types are unknown; identifying these tissues and cell types is critical for exploring gene regulatory mechanisms that may contribute to disease. Joint analysis of GWAS data with gene expression data or chromatin data provides one avenue for identifying these relevant tissues and cell types. In this talk, I will present a method called stratified LD score regression (S-LDSC) and I will show that this method can be used to identify relevant tissues and cell types by combining GWAS data with gene expression and/or chromatin data. I'll then present results from application of S-LDSC to a variety of chromatin and gene expression datasets, together with genome-wide association study (GWAS) summary statistics for 48 diseases and traits with an average sample size of 169,331. Many of our results recapitulate known biology; we also find several results that

highlight the ability of our method to distinguish among related tissues and cell types, including an enrichment of inhibitory over excitatory neurons for bipolar disorder, but excitatory over inhibitory neurons for schizophrenia and body mass index; and enrichments of T cells for asthma and eczema, B cells for primary biliary cirrhosis, and myeloid cells for Alzheimer's disease.

7

EVOLUTION OF ENVIRONMENTALLY-ENFORCED, REPEAT PROTEIN TOPOLOGY IN THE OUTER MEMBRANE

Joanna Slusky[a], Meghan Franklin [a], Sergey Nepomnyachiy[bc], Nir Ben-Tal[b], Ryan Feehan [a], Rachel Kolodny[c]

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New Innovator Award, 2017

Outer membrane beta barrels (OMBBs) are the proteins on the surface of Gram negative bacteria. These proteins have diverse functions but only a single topology, the beta barrel. It has been suggested that this common fold is a repeat protein with the repeating unit of a beta hairpin. By grouping structurally solved OMBBs by sequence, a detailed evolutionary story unfolds. A strand-number based pathway manifests with progression from a primordial 8-stranded barrel to 16-stranded and then to 18-stranded barrels. The transitions from 16- to 18-stranded barrels show mechanisms of strand number variation without domain duplication, such as a loop to hairpin transition. This indicates that repeat protein topology can be perpetuated without genetic duplication likely because the topology is being enforced by the membrane environment. Moreover, we find the evolutionary trace is particularly prominent in the C-terminal half of OMBBs which may be relevant to understanding OMBB folding pathways.

8

PHENOTYPE-SPECIFIC ENRICHMENT OF MENDELIAN GENES NEAR GWAS LOCI ACROSS 62 COMPLEX TRAITS

Valerie Arboleda, Malika Kumar Freund, Kathryn Burch, Huwenbo Shi, Nicholas Mancuso, Gleb Kichaev, Kristina Garske, David Pan, Paivi Pajukanta, Bogdan Pasaniuc

UCLA

Early Independence Award, 2018

Although recent studies provide evidence for common genetic basis between complex traits and Mendelian disease genes, a thorough quantification of their overlap in a phenotype-specific manner remains elusive. We hypothesize that disruption of any individual gene with a large-effect coding variant results in severe phenotypes, as in Mendelian disorders, while non-coding variants typical of GWAS loci might act by collectively dysregulating expression of these same Mendelian disease genes, resulting in a nuanced and/or tissue-specific phenotypes. Here, we quantify the shared genetic basis of 62 complex

traits and diseases with their corresponding Mendelian disease gene. For each trait, we used significant genome-wide association study (GWAS) loci to define a set of putative risk genes, and we quantify the enrichment of genes known to cause similar Mendelian phenotypes within this GWAS gene set. We identified 56 pairs with a significant enrichment of Mendelian genes in the GWAS gene set for a phenotype-matched complex trait and 26 pairs of phenotype-unmatched complex traits and Mendelian disorders with significant gene overlap, which suggests the existence of potentially novel shared biological mechanisms. We demonstrated that the effect sizes for SNPs in GWAS complex traits are maximized with the traits phenotype-specific Mendelian gene set. Finally, we identify novel examples of significant GWAS variants in BMI GWAS directly interacting with phenotype-matched Mendelian disease genes CREBBP and CYP19A1, using adipose-specific Hi-C data. Our work demonstrates how leveraging a phenotype-matched approach coupled with functional genomic datasets can identify and prioritize candidate genes dysregulated by distant non-coding GWAS variants.

Chemical Biology

9

INTERROGATION OF RIBOSOMAL FUNCTION USING SYNTHETIC BIOLOGY AND CONTINUOUS EVOLUTION

Ahmed Badran

Broad Institute of MIT and Harvard

Early Independence Award, 2017

In nature, fundamental biological phenomena that are central to cellular life are inherently hindered from probing and interrogation, as these dynamic systems cannot be easily decoupled from immediate artifactual disruptions throughout the living cell. One such case is the ribosome, a colossal multi-component protein factory that functions as the nexus for cellular information and signaling events, integrating nutrient availability with growth dynamics and resource allocation. Despite decades of research, this biomolecular assembly remains superficially understood and underexplored, owing to the difficulty associated with decoupling the translational apparatus from cellular viability. Using engineered orthogonal transcription-translation networks, we can robustly monitor ribosome activity in living cells, overcoming challenges associated with ribosomal manipulation in vivo and providing a framework for high-throughput ribosomal interrogation. Our ongoing work has built upon this platform to study various parameters of ribosomal function, including the evolution of the modern ribosome, dissection of putative processivity-fidelity relationships during translation, experimental validation of ribosomal divergence across prokaryotes, and prediction of ribosome-small molecule interactions. This work has the potential to extend our understanding of key factors governing ribosomal function and dynamics, providing potentially general design paradigms for manipulating translation in vivo.

10

CROSSTALK IN CYSTEINE POST-TRANSLATIONAL MODIFICATIONS

Brent Martin

University of Michigan

New Innovator Award, 2014

Cysteine residues carry out diverse functions in proteins, serving as nucleophiles in enzyme active sites, redox sensors, or a platform for a number of post-translational modifications. For example, S-palmitoylation describes the addition of a long-chain fatty acid to select cysteine residues, which promotes membrane tethering, trafficking, and localization across hundreds of peripheral membrane proteins. The cysteine sulfhydryl group is also key target of oxidative stress, and contributes to aging, neurodegeneration, heart disease, diabetes, and cancer. Despite the central role of oxidative stress in human health, our ability to study the precise mechanisms of such modifications has been hampered by a lack of selective chemical and analytical methods. In this presentation I will present recent progress to directly addresses this gap. In one example, we found that sulfinic acids and nitrosothiols react to form a stable thiosulfonate bond. Leveraging this cross-reactivity, we developed both nitrosothiol and sulfinate-linked probes to enrich and annotate hundreds of endogenous S-sulfinated and S-nitrosated proteins, respectively. These methods demonstrate a bi-directional approach to profile select redox cysteine modifications. We have also optimized separation and mass spectrometry methods for direct annotation of S-palmitoylation and other hydrophobic modifications. Based on these studies, we sought to explore the cellular pathways underlying the oxidative stress response. Using a genome-wide cell-based screen, we identified known regulators of proteostasis and the antioxidant response, but also revealed an uncharacterized link between enzymes catalyzing protein S-palmitoylation and resistance to oxidative damage. Altogether, these studies point to an unappreciated interdependence across cysteine modifications, where S-palmitoylation may itself protect cells from oxidative damage.

11

NEW CHEMICAL APPROACHES TO REVEAL THE DYNAMICS OF THE TRANSCRIPTOME

Matthew Simon

Dept of Molecular Biophysics & Biochemistry, Yale University

New Innovator Award, 2014

RNA sequencing (RNA-seq) offers a snapshot of cellular RNA populations but does not provide insight into temporal information about when the sequenced RNA was synthesized. Without temporal information, fundamental aspects of gene expression are masked, including the regulated kinetics of RNA transcription, processing and degradation. One means to reveal these dynamics is through metabolic labeling of new RNAs with 4-thiouridine (s4U) which historically has been used to biochemically purify new RNAs (Russo et al. 2017). This approach has been limited by the inefficient chemistry used to capture the newly synthesized RNAs, difficulty distinguishing bona fide new RNAs

from contaminating old RNAs, challenges normalizing samples, the large amount of input material necessary for biochemical purification, and biases in enrichment. To overcome these challenges, we have developed a series of chemical tools to improve biochemical enrichment of metabolically labeled RNAs using methanethiosulfonate (MTS) chemistry (Duffy et al. 2015; Duffy et al. In revision) and complementary approaches to avoid biochemical enrichment all together through TimeLapse-seq (Schofield et al. 2018), a chemical approach that directly reads out sites of s4U incorporation in a sequencing experiment. TimeLapse-seq is based on RNA-friendly, oxidative-nucleophilic-aromatic-substitution chemistry to convert the s4U residues into cytidine analogues, leading to apparent U-to-C mutations that mark new transcripts upon sequencing. TimeLapse-seq is a single-molecule approach which is internally normalized, revealing rich RNA population dynamics within a single sample. Together these chemistries reveal regulated changes to host immune response (Li et al. 2017), heat-shock-induced transcriptional dynamics and unexpected levels of post-transcriptional regulation, providing deeper insight into the dynamic regulation of gene expression.

12

DISCOVERY OF DRUG-LIKE SMALL MOLECULES FROM THE HUMAN MICROBIOME

Mohamed Abou Donia

Princeton University

New Innovator Award, 2015

The human microbiome includes a complex community of bacteria that are living in symbiosis with the human host. By analyzing genomic data of cultured members of the human microbiome, it was previously revealed that they have a great potential to produce bioactive small molecules that are possible mediators of microbe-microbe and microbe-host interactions. However, no systematic analysis has yet examined the biosynthetic potential of uncultivable, yet-uncultivated, or not-yet sequenced members of the human microbiome. To accomplish this goal, we developed a computational algorithm for the systematic discovery of small molecule biosynthetic gene clusters (BGCs), directly from complex metagenomic sequencing datasets of human samples. This analysis revealed novel BGCs, even for clinically-important structural classes not before known to be encoded by members of the human microbiome. Here, we report the discovery, chemical and biological characterization of the small molecule products of such BGCs, through a hybrid metagenomic-synthetic biology approach. Our study not only implicates the human microbiome as a potential source for drug-like small molecules, but it also illustrates that combining computational and synthetic biology is a powerful strategy towards accessing them.

13

CHEMICAL PROTEOMIC PLATFORMS TO PROBE METABOLIC SIGNALING ACROSS SCALES OF SPACE, TIME AND REACTIVITY

Raymond Moellering

University of Chicago

New Innovator Award, 2017

Biological systems are inherently heterogeneous, both at the molecular level (e.g., encoded proteins existing in distinct posttranslational modification states) and the cellular level (e.g., organization of biomolecules to distinct regions of a cell or distinct cells within a tissue). To understand regulatory mechanisms in these systems under normal or diseased states, we must be able to probe biomolecular function in native environments across scales of space and time. Existing proteomic platforms provide quantitative snapshots of the proteins present in a biological sample, yet these methods typically require homogenization of samples, signal-averaging over thousands-to-millions of cells, and provide no information on protein function. Therefore, innovation in the development chemical probes and technology platforms is needed to study protein activity within complex native environments. In the first part of this talk I will describe the development of new chemical probes and complimentary proteomic technologies to enable quantitative measurements in the proteome in native biological contexts, ranging from subcellular complexes, single cells, primary tissues to live animals. In the second half of this talk I will describe the integration of these platforms to discover new roles for reactive endogenous metabolites as intracellular signals in normal and diseased biological states, as well as the potential to regulate these signals for therapeutic benefit. Both halves of the talk will emphasize the role of these integrated chemical proteomic platforms as a discovery engine to identify novel targets for diagnostic and therapeutic development in human disease.

14

SYNTHESIS AS A RECIPE FOR DISCOVERY IN BIOMEDICINE. CREATING AN ARTIFICIAL LIFE FORM

Steven Benner

Foundation for Applied Molecular Evolution, The Westheimer Institute for Science and Technology, Firebird Biomolecular Sciences LLC

Transformative Research Award, 2017

Much modern scientific research focuses on "descriptive biology", which analyzes in increasing detail the physiology (macro, cellular, molecular) by which today-extant organisms manage the challenges arising in daily life. However, natural physiology is the outcome of what was a narrow range of prebiotic chemical possibilities on the early Earth, and then developed by four billion years of biological evolution having a limited Darwinian search strategy. Therefore, our modern physiologies represent narrow, and largely imperfect, solutions to biological problems.

This creates the opportunity for "descriptive biology" to be complemented by "synthetic biology", which creates alternative ways of solving biological problems. By pursuing a grand challenge in synthesis, scientists are forced across uncharted grounds where they must solve unscripted problems using available theory and technology. When these are inadequate, the synthesis fails, and fails in a way that informs those scientists about the deficiencies in theory and technology, some of which may not have been intuited by the scientist aforehand. Thus, a well constructed synthetic effort can drive discovery and paradigm change in ways that descriptive analysis and hypothesis-based research cannot.

Here, our grand challenge is to construct a living bacterial system that uses a genetic system different from natural DNA and RNA. Instead, the system uses nucleic acids built from at least six different nucleotide "letters", metabolism to construct those nucleotides, and regulatory systems to manage that metabolism and its errors. This presentation will describe how we construct this new biology from the ground up, what we have learned about natural biology in the process, and what new technologies have emerged. 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 approaches to broad-spectrum therapy, and new ways to do biotechnology manufacturing.

Clinical and Translational Research

15

HAPLOINSUFFICIENCY OF A HISTONE MODIFIER, KMT2D, IN A MOUSE MODEL OF KABUKI SYNDROME LEADS TO DIFFERENTIATION DEFECTS IN PLASMA CELLS

Hans Bjornsson

Johns Hopkins University

Early Independence Award, 2013

Kabuki syndrome (KS) type 1 is caused by heterozygous loss of function mutations in the histone modifying protein KMT2D. KMT2D adds histone 3, lysine 4 mono- and trimethylation, which are found at active enhancers and promotors, respectively, suggesting that KS is a disorder of gene regulation. Immune dysfunction, including hypogammaglobulinemia (especially low IgA), splenomegaly, and diminished response to immunizations, is frequently observed in KS patients. To clarify the underlying mechanisms and identify cellular populations for detailed epigenetic phenotyping, we evaluated the Kmt2d+/bgeo mouse model. These mice have splenomegaly (p0.03), and a 4 fold decrease of serum IgA lvels (p0.0002) compared to WT littermates. IgA is a majo component of mucosal immunity and primarily produced in the gut. To investigate the mechanism of IgA defects, we examined the secondary lymphoid tissues of the gut, the Peyer's patches (PP) and mesenteric lymph nodes. PP in Kmt2d+/bgeo mice are strikingly smaller and there are significantly fewer PP's compared to WT littermates (Kmt2d+/bgeo mean=2.4; WT mean=7.2; p0.0001). We detected sinificantly decreased levels (50%; p0.02) of a post-IgA-class-switch-recombination transcriptin the mesenteric lymph nodes and a decrease in mature IgA-producing plasma cells in Kmt2d+/bgeo mice compared to WT littermates, consistent with low serum IgA. However, we identified an increase in immature IgA+ plasma cells in Kmt2d+/bgeo mice, indicating a possible block in differentiation. The B1 cell population of the peritoneal cavity also contributes to IgA-producing plasma cells in the gut. Compared to WT littermates, Kmt2d+/bgeo mice have a decreased B1a cell population. Thus, our data suggest defects in mucosal immunity and widespread defects of the B cell lineage in Kmt2d+/bgeo mice. These data also suggest specific cellular transitions that could be investigated to further understand how a histone modifier, such as KMT2D, leads to disease phenotypes.

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HUMAN BRAIN CANCER, RATHER THAN BRAIN CANCER CELLS, ON A PLATE

Howard Fine

Weill Cornell Medical Center

Pioneer Award, 2017

The median survival of patients suffering with glioblastoma (GBM) has gone from 12 months three decades ago to a mere 15 months today. We hypothesize that a major reason for the lack of successful therapies in gliomas is a failure to develop experimental systems that accurately model the complexity and resulting emergent properties of the "cancer state". Thus, we have embarked on building a complex ex vivo model of the human brain harboring a growing tumor in a patient-specific manner. We have achieved the first iteration of this initiative by successfully generating human cerebral organoids generated from patient-specific induced pluripotent stem cells that contain most of the correct cell types and neuro-anatomic compartments seen in a 20-week old human fetal brain. Primary patient-derived GBM stem cells grow within their autologous cerebral organoids and form destructive tumors that phenocopy the parental clinical tumor. Additionally, we can form de novo GBMs by introducing genomic aberrations of the patient's original tumor within their own cerebral organoids by using advanced gene editing techniques. The resultant model GBMs phenocopy many of the biological and clinical characteristics of human GBM in situ including diffuse invasion, glioma stem cell proliferation and self-renewal, tumor-mediated cerebral necrosis, clonal heterogeneity, transcriptomic and genomic methylation profiles and tumor-tumor-host cell interconnecting tunneling microtubes that conduct calcium transients through interconnecting gap junctions.

We propose that our *ex vivo* tumor system largely recapitulates many of the complex conditions of GBM growth in vivo but in an experimentally manipulable, biologically (clinically) relevant, logistically pragmatic and scientifically rigorous way. This approach will allow us for the first time to mechanistically study clinically apparent emergent phenomenon of GBM, not previously studied, ultimately leading to novel and more effective strategic therapeutic approaches to this devastating disease.

17

USING ECOG SIGNALS TO DECODE INTENDED MOVEMENTS FOR EXOSKELETON CONTROL

Karunesh Ganguly[a], Nikhilesh Natraj[a], Daniel Silversmith[b], Robert Matthew[b], Edward Chang[a]

[a]University of California, San Francisco, [b]UC Berkeley

New Innovator Award, 2015

Patients with tetraplegia are severely disabled. Surveys have found that restoration of upper-limb function is a high-priority for such patients. Brain-Machine Interfaces (BMIs) can eventually restore upper-limb reaching and grasping function by seamlessly merging the computational power of the brain

with artificial prosthetic systems. A major challenge is robust translation of BMI technology to patient care. Two well-recognized limitations of current approaches are instability of recordings and the lack of proprioceptive feedback signals. Our research proposal aims to conduct a pilot clinical study to test electrocorticography (ECoG) based control of an arm and hand exoskeleton in tetraplegic patients with residual proprioception. We have primarily focused on developing the computational framework to translate ECoG signals into control signals for hand control. Importantly, we have found robust evidence of representations of arm, hand and finger movements in ECoG signals. Moreover, we have refined a wearable upper-limb exoskeleton for arm/hand control. A key area of innovation has been the development of a soft-wearable exoskeleton. We are currently recruiting for the pilot trial to directly test ECoG control of the exoskeleton. Our continued research should advance translational efforts by optimizing control under conditions that maximize neural learning mechanisms and provide natural sensory feedback.

18

CARDIAC NOCICEPTIVE AFFERENTS EXPRESSING TRPV1 PROMOTE VENTRICULAR ARRHYTHMOGENESIS IN CHRONIC ISCHEMIC CARDIOMYOPATHY

Olujimi Ajijola, Koji Yoshie, Pradeep Rajendran, Louis Massoud, Siamak Salavatian, Janki Mistry, Tamer Sallam

UCLA

New Innovator Award, 2017

Sympathetic activation is implicated in ventricular arrhythmogenesis following cardiac injury. Sympathetic drive to the heart may be initiated centrally or peripherally, however, the role of cardiac sympathetic afferent signaling (CSAS), mediated by dorsal root ganglion (DRG) afferent fibers expressing the TRPV1 channel in arrhythmogenesis is unknown. Resiniferatoxin (RTX), a potent TRPV1 activator, can be applied to induce targeted chemo-axotomy. We utilized this approach to dissect the role of this neural circuit in arrhythmogenesis in chronically infarcted porcine hearts, and examined the underlying mechanisms using histochemistry, autonomic modulation, simultaneous cardio-neural mapping, and molecular approaches. Epicardial RTX application resulted in distal axotomy of TRPV1 afferents, demonstrated by loss of epicardial fibers immunoreactive for sensory but not adrenergic markers, and by loss of functional responses to TRPV1 agonists, bradykinin and capsaicin, indicating loss of the CSAS. Animals with chronically infarcted myocardium exhibited profound ventricular arrhythmogenesis, elicited by programmed stimulation, or cesium chloride administration. Depletion of CSAS prevented arrhythmogenesis induced by either method in infarcted hearts. Expectedly, this protection was not explained by differences in myocardial ion channel expression. Mechanistically, loss of CSAS mitigated adverse myocardial remodeling in the border zone, decreasing fibrosis, and preserving myocardial function, resulting in improved cardiac stability and resistivity to stress. In vivo cardio-neural mapping involving simultaneous recordings of epicardial electrograms and extracellular neuronal recordings of cardiovascular-related stellate ganglion neurons, revealed that cardiac injury destabilizes reflex processing within stellate ganglia, increasing basal neuronal firing rates, altering responses to cardiovascular stressors, and causes a loss of integrated reflex processing. Depleting CSAS in this cardiac

injury model normalized these processes. These data collectively indicate that following cardiac injury, chronic CSAS drives adverse cardiac remodeling, and dysregulation of neural processing within adrenergic ganglia, resulting in electrically unstable ventricular myocardium. We propose TRPV1-expressing afferents as a target to reduce arrhythmogenesis following cardiac injury.

19

SUSTAINABLE HEALTH ENGINEERING- TOWARDS OPTIMAL TREATMENT OF INFANT HYDROCEPHALUS IN THE DEVELOPING WORLD

Steven Schiff

The Pennsylvania State University

Pioneer Award, 2015

Hydrocephalus is the most common childhood condition requiring neurosurgery. Of the estimated 400,000 new cases each year, the majority are in the developing world, and the largest single cause appears to be neonatal sepsis. Although sustainability in health care is a concept still being defined, I am building an interlocking strategy based upon control engineering to optimize diagnosing, treating, and preventing the burden of such disease. I will sketch an evolving strategy towards an optimal approach to such complex syndromic disease, fusing genomic microbial surveillance, low-cost diagnostics, and predictive treatment.

20

COMBINING RADIATION AND TUMOR-SPECIFIC ANTIBODY THERAPIES TO ELICIT IN SITU TUMOR VACCINATION

Zachary Morris

University of Wisconsin School of Medicine and Public Health

Early Independence Award, 2017

In syngeneic murine tumor models, we reported a cooperative interaction between radiation (RT) and intratumor (IT) injection of tumor-specific antibody (mAb). Combined treatment with RT + IT-immunocytokine (IC; a fusion of a tumor specific antibody and IL2) markedly enhanced this response and induced an in situ vaccine effect that resulted in tumor-specific T cell memory. Here, we observe that non-fused tumor-specific mAb + IL2 may also elicit an in situ vaccination response following RT, resulting in improved tumor response and animal survival compared to control mice treated with single or dual combinations of RT, mAb, and/or IL2. Following RT + IT mAb+IL2, one-half of mice were rendered disease-free and all of these showed evidence of tumor-specific immunologic memory. In separate studies, we evaluated the impact of well-established, distant, untreated tumor sites on the in situ vaccine response elicited by RT + IT-IC. In this setting, we observed a strong antagonistic effect of untreated tumor sites on the local and systemic anti-tumor immune response to RT + IT-IC. We coined

this “concomitant immune tolerance” (CIT). CIT is tumor-specific and requires regulatory T cells (Tregs). Importantly, CIT can be overcome by delivering RT to all tumor sites. Consistent with a process mediated by temporary depletion of an RT-sensitive, tumor-infiltrating immune lineage (such as Tregs), a 2Gy RT dose can overcome CIT when delivered to all tumor sites. In most clinical scenarios, it is not possible to deliver even 2Gy to all metastatic sites using external beam RT without also triggering lymphopenia. Consequently, we investigated the capacity of molecular targeted radiation therapy (MTRT) to deliver immunomodulatory RT to all tumor sites in the setting of metastatic disease. In preliminary studies, we have confirmed that MTRT can reduce tumor-infiltrating Tregs and overcome CIT when delivered together with an in situ vaccine regimen.

High Throughput and Integrative Biology

21

MICROBIOME PROGRAMMING OF HOST PHYSIOLOGY

Buck Samuel

Baylor College of Medicine

New Innovator Award, 2017

Much attention has been invested in cataloging the microbes that inhabit our bodies (our “microbiome”) in various states of health and disease. Equally pressing is generation of an understanding the molecular pathways that shape composition and function of the microbiome and its influence on our physiology. This process occurs from the moment that the microbiome first colonizes the gut in early infancy, where microbial input is required to complete development of the intestine, immune and nervous systems. These transient interactions shape future ability to maintain proper microbiome function and health, plus can predispose to disease and an inability to regulate microbiome function. Thus, defining the genetic regulators of these events is one of the most fundamental questions in microbiome research. Our goal is therefore to comprehensively identify the molecular pathways that govern host shaping of microbiome form and function.

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EFFECTIVE DETECTION OF VARIATIONS IN SINGLE CELL TRANSCRIPTOME

Chenghang Zong

Baylor College of Medicine

New Innovator Award, 2014

The quantification of transcriptional variation in single cells, particularly within the same cell population, is currently limited by the sensitivity and technical noise of single-cell RNA-seq assays. We developed multiple annealing and dC-tailing-based quantitative single-cell RNA-seq (MATQ-seq), a highly sensitive and quantitative method for single-cell sequencing of total RNA. By systematically determining technical noise, we demonstrate that MATQ-seq captures genuine transcriptional noise in single cells at the whole transcriptome. With total RNA detection ability, MATQ-seq is also suitable for samples where partial degradation cannot be avoided. Using MATQ-seq, we have successfully profiled heterogeneous stroma cells in lung cancer, different sub-states of germinal cell B cells as well as individual pancreatic intraepithelial lesions that are extremely prone to RNA degradation.

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REGULATORY PRINCIPLES GOVERNING ENHANCER SPECIFICITY DURING ANIMAL DEVELOPMENT

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University of California, San Diego

New Innovator Award, 2017

The human genome contains on the order of a million enhancers. These segments of the DNA act as switches to regulate where and when the approximately 20,000 genes are expressed. As such, enhancers provide the instructions for tissue specific gene expression, thus enabling successful development and cellular integrity. Numerous studies have demonstrated that mutations in enhancers can alter tissue specific expression and cause phenotypic variation and disease. For example, a single mutation in a limb bud enhancer leads to aberrant expression of the gene SHH and results in extra fingers and toes. In an enhancer for the membrane protein Duffy, a point mutation results in malarial resistance. Computational analysis suggests that the majority of mutations associated with disease are located within enhancers. Despite the fundamental importance of enhancers for organismal integrity and their discovery over 30 years ago, we lack a broad understanding of how enhancer sequence encodes tissue specific expression. As a result we do not understand which changes in enhancer sequence are simply inert variation between individuals and which mutations lead to phenotypic diversity and disease.

These fundamental questions remain unsolved because we cannot relate enhancer sequence to gene expression patterns and phenotype on a scale sufficient to identify the overarching regulatory principles. The two main challenges in deciphering the relationship between enhancer sequence and tissue specific gene expression are: 1) the complexity of enhancers, and 2) the complexity of organisms. To address these problems, we have developed high-throughput functional assays to test millions of enhancer variants in millions of whole developing embryos. The model organism that enables such in-depth functional approaches is the marine chordate *Ciona intestinalis*. I will discuss our recent findings using these high-throughput functional approaches to identify regulatory principles governing enhancer function and how violations in these principles can pinpoint mutations associated with disease.

24

SYSTEMS-LEVEL ANALYSIS OF MOBILE GENETIC ELEMENTS IN A POPULATION OF NEUTROPENIC PATIENTS HIGHLY VULNERABLE TO MULTIDRUG RESISTANT INFECTION

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New Innovator Award, 2017

Horizontal gene transfer (HGT) is the predominant mechanism driving the emergence of multi-drug resistant organisms. Given that the human microbiome serves as a vast reservoir for antibiotic resistance genes, it is now thought that pathogens acquire antibiotic resistance determinants through HGT with members of the human microbiome with whom they come into contact. To respond to the relative lack of tools that are capable of examining the mobile gene pool within natural microbial communities, we have developed an experimental and computational pipeline for identifying the bacterial hosts of specific mobile elements and antibiotic resistance genes. We are currently applying methods to a cohort of neutropenic patients who are placed on prophylactic antibiotics for extended periods and who are particularly vulnerable to multidrug resistant infections. Using our experimental and computational approaches, we aim to define the network of gene exchange and use this to predict the flow of mobile genes through bacterial communities.

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THE HUMAN SKIN MICROBIOME: METAGENOMES TO THERAPEUTICS

Julia Oh

The Jackson Laboratory

New Innovator Award, 2017

The human skin harbors an abundant microbial ecosystem with bidirectional metabolic exchanges supporting symbiotic and commensal functions. Sequence- based analysis of microbial community structure and organization of the human microbiome has yielded valuable insight into the microbial diversity and function of its different body niches. Metagenomic analyses of the diverse skin sites in healthy humans demonstrate that contrasting forces of the skin's biogeography and individual characteristics shape the skin microbiome and the dynamics of its bacteria, fungi, and viruses. However, shifts in the ecological properties of the skin microbiome are significantly associated with skin disease, disease severity, and other physiologic host factors such as age or primary immunodeficiency. We have developed new computational and experimental tools to reconstruct the human skin microbiome with unprecedented resolution, including algorithms for rapid and on-the-fly database creation and massively parallelized single cell approaches. The microbial "blueprint" revealed by our microbiome surveys reconstructs genes and genomes of a microbial community and thus fuels our engineering arm, which focuses on rational selection and design of engineered probiotics using synthetic biology. Here, we use skin commensals for microbial chassis for heterologous production of therapeutic proteins and molecules with the goal of both acute and preventative treatment of skin disease. Finally, we address the microbial-host interactome, the mechanisms through which products secreted by different microbial

species and strains interact with the immune system. Such an interactome would provide the foundation for exploiting microbial metabolites for reprogramming the immune system in perturbed states and would help us understand how to reverse the interactions that underlie these wide range of immune-related skin diseases. Taken together, these complementary research areas leverage both technological and computational innovations together with mechanistic understanding of microbial community dynamics and interactions with the host to systematically and rationally implement therapeutic construction.

26

DAISY DRIVE SYSTEMS FOR SAFE, LOCAL, AND REVERSIBLE POPULATION EDITING

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New Innovator Award, 2017

Engineered gene drive systems that offer no fitness benefit to organisms can spread through wild populations by distorting inheritance in their favor, potentially enabling numerous ecological engineering applications. Our models predict that the self-propagating CRISPR-based gene drive systems we originally described and demonstrated will be highly invasive: releasing very few organisms will often result in spread to most populations of the target species. The difficulty of contained field trials and the likelihood of international spread will effectively preclude most applications.

To overcome these problems, we are developing daisy drive, a self-exhausting form of gene drive in which CRISPR components arranged in a daisy-chain are lost over successive generations until inheritance-biasing stops. Our models predict that daisy drive will spread to only local populations before being naturally eliminated. To confine effects within arbitrary political boundaries, daisy drives can swap the positions of two ribosomal genes, created a threshold-dependent effect that selects for engineered genes in areas where they are abundant and swiftly eliminating them in majority wild-type areas. In principle, daisy drive systems could potentially remove arbitrary engineered genes from sexually reproducing populations, a key ethical and safety benefit.

We are studying daisy drive evolutionary robustness and dynamics in laboratory populations of sexually reproducing C. elegans, which can be grown in very large numbers, reproduce every 3 days, and readily propagate in metapopulations linked with programmed gene flow rates by a liquid-handling robot. We are additionally engineering mice in which each daisy drive link corresponds to a coat color gene to create a living family tree that will offer an intuitively appealing visual understanding of how the technology works. All experiments are pre-registered to ensure that people can have a voice in decisions regarding ecological engineering technologies that may one day affect them.

27

CELL CHIRALITY IN DEVELOPMENT AND DISEASE

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New Innovator Award, 2013

The development of the vertebrate body plan requires the establishment of a proper axis of left-right (LR) polarity. Changes in orientation of the LR axis due to genetic or environmental factors can lead to malformations and disease. We demonstrate that the cultivation of cells on micropatterned 2D surfaces and in 3D graded hydrogels reveals an intrinsic cellular LR asymmetry, termed cell chirality. With these novel tools, we further examine the role of cell chirality in the development of organ specific LR asymmetry, cardiac c-looping as well as in the regulation of endothelial permeability. Overall, our results show that individual cells are chiral, and they can organize and form asymmetric tissues both in vitro and in vivo. We propose that in vitro platforms could be used as effective in vitro tools to study the initiation of LR asymmetry, to diagnose disease, and to study factors involved with birth defects in laterality.

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ELUCIDATING CELLULAR MECHANISMS AND THERAPEUTIC STRATEGIES FOR NEURODEGENERATIVE DISEASES WITH CRISPRI AND CRISPRA

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New Innovator Award, 2015

Human genes associated with brain-related diseases are being discovered at an accelerating pace. A major challenge is the identification of the mechanisms through which these genes act, and of potential therapeutic strategies. To elucidate such mechanisms in human cells, we are leveraging a CRISPR-based platform for genetic screening that we recently co-developed. Our approach relies on CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa), in which a catalytically dead version of the bacterial Cas9 protein recruits transcriptional repressors or activators, respectively, to endogenous genes to control their expression, as directed by a small guide RNA (sgRNA). Complex libraries of sgRNAs enable us to conduct genome-wide loss-of-function and gain-of-function screens in mammalian cells. We have adapted this strategy for use in human iPSC-derived neurons and other cell types, in order to elucidate disease mechanisms and therapeutic strategies in patient-derived cells and isogenic control cells. Our pilot screens systematically reveal genes and cellular pathways relevant for neuronal survival and response to oxidative stress.

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GENETIC BASIS OF INDIVIDUAL RECOGNITION IN PAPER WASPS

Michael Sheehan

Cornell University

New Innovator Award, 2017

How does a genome encode knowledge? Prior to taking our first breath, our brains have already been 'programmed' by the instructions in our genome to know how to respond to our environment. Different species are born with different innate knowledge, indicatign that some features of our genome encode specific information. Understanding how the genome encodes knowledge has the potential to reveal new insights into how neural circuits develop and potentially lead to novel treatments. Here we present initial results of work using the novel and genetically facial recognition abilities of the paper wasp, Polistes fuscatus, to begin to identify genetic changes that encode knowledge. This unique species of wasp recognize each other as individuals using facial features, similar to humans, but other closely related wasps do not recognize individuals. The unique biology of wasps also means that it is unusually possible to identify features of the genome associated with recent evolutionary changes. By combining multiple de novo assembled genomes from our focal species and close relatives with populatin genomic data we have identified numerous recently selected regions of the genome that have been under recent selection. In many cases, we can identify specific mutations that are likely to have been the target of recent selection. Ongoing work seeks to link specific changes in the genome to changes in patterns of gene expression and neural development among these unusually tractable wasps. The present study lays the groundwork for identifying the spectrum of genomic features.

Infectious Diseases and Immunology

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ALLERGIC INFLAMMATORY MEMORY IN HUMAN RESPIRATORY EPITHELIAL PROGENITOR CELLS

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New Innovator Award, 2015

Barrier tissue dysfunction is a poorly defined feature of chronic human inflammatory disease. Allergic inflammation in the upper airway barrier can develop from persistent activation of Type 2 immunity (T2I), resulting in the disease spectrum known as chronic rhinosinusitis (CRS): ranging from inflamed mucosa to the severe tissue reorganization seen in nasal polyps. Identifying the principal cell types and states which maintain and propagate disease in humans is vital to treating these conditions. While basal cell hyperplasia is a hallmark of severe disease, how these progenitor cells functionally contribute to clinical presentation and barrier tissue dysfunction in humans remains unexplored.

Profiling twelve primary human CRS samples that span the range of clinical severity with the Seq-Well platform for massively-parallel scRNA-seq, we report single-cell transcriptomes for human respiratory epithelial cell subsets, immune cells, and parenchymal cells from a T2I inflammatory disease, and map key mediators. We find striking differences between non-polyp and polyp tissues within the epithelial compartments, providing key insights into the unique tissue remodeling observed in polyps. Specifically, we identify a global reduction in epithelial diversity in polyps characterized by fewer glandular and ciliated cells, cytokine-mediated education of basal progenitors, and concomitant phenotypic shifts in mature secretory cells. Through combined transcriptomics and epigenomics, we identify and then test key signaling pathways and transcription factors which may lock basal cells from polyp tissue into an uncommitted state. Finally, we validate our mechanisms in vivo through a clinical intervention to modify basal and secretory cell states.

Our data provide the first-in-human evidence for immune effector cytokines acting to rewire tissue stem cells and indicate that T2I barrier dysfunction stems from intrinsically altered epigenetic “memories” in basal cells. Collectively, our results identify a cellular mechanism for the persistence and chronicity of severe human respiratory disease.

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DISCRETE CHROMATIN STATES DEFINE TUMOR-SPECIFIC T CELL DYSFUNCTION AND THERAPEUTIC REPROGRAMMABILITY

Andrea Schietinger

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New Innovator Award, 2017

Tumor-specific T cells in solid tumors are dysfunctional, allowing tumors to progress. The epigenetic regulation of this T cell dysfunction and therapeutic reprogrammability (e.g. to immune checkpoint blockade) is not well-understood. We recently found that T cells in tumors differentiated through two discrete chromatin states: a plastic dysfunctional state from which T cells could be rescued, and a fixed dysfunctional state resistant to reprogramming. We identified novel surface markers associated with each chromatin state that demarcated reprogrammable from non-reprogrammable PD1hi dysfunctional T cells within heterogeneous T cell populations in murine tumors. Importantly these surface markers were also expressed on human PD1hi tumor-infiltrating T cells (TIL) and preliminary data now reveal that these biomarkers also predict reprogrammability of human TIL. In vivo pharmacologic modulation of transcription factors associated with each chromatin remodeling step improved therapeutic reprogrammability of dysfunctional T cells. Our study has important implications for cancer immunotherapy by defining key transcription factors and epigenetic programs underlying T cell dysfunction and surface markers that predict therapeutic reprogrammability.

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FUNCTIONAL INTERROGATION AND MINING OF NATIVELY-PAIRED HUMAN VH:VL ANTIBODY REPERTOIRES

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Early Independence Award, 2016

Next-Generation sequencing has become an essential tool in the analysis of antibody responses in the settings of health, vaccination, and disease. However, immune receptors comprise two chains encoded by separate mRNA strands, and conventional NextGen sequencing fails to identify the native pairings encoded by individual lymphocytes. To overcome this limitation we have applied recent technical advances in high-throughput sequencing and functional analysis of complete antibodies (i.e., paired heavy and light chain sequencing) to generate a comprehensive understanding of the antibody response to vaccination and natural infection. Here we present a new technology to screen natively-paired human antibody repertoires from millions of B cells. Libraries of natively-paired variable region heavy and light (VH:VL) amplicons were expressed in a yeast display platform that was optimized for human Fab surface expression, and the resulting libraries were interrogated for binding to viral vaccine antigens via FACS paired with next generation sequencing. Using our method we identified HIV-1 broadly neutralizing antibodies (bNAbs) from an HIV-1 slow progressor and high-affinity neutralizing antibodies responding to an Ebola virus glycoprotein vaccination. These next-generation approaches are providing detailed molecular feedback on immune receptor responses and are informing the design and discovery of new vaccines and therapeutics.

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NEURON-MICROBE INTERACTIONS IN PAIN AND HOST DEFENSE

Isaac Chiu

Harvard Medical School

New Innovator Award, 2016

Microbes, neurons, and the immune system interact closely at barrier surfaces. We find that bacteria directly activate sensory neurons to produce pain during infections. Sensory neurons in turn modulate the immune response to bacterial pathogens. Therefore, the nervous system plays a direct role in the host-microbe interactions. Understanding the mechanisms by which bacteria activate the nervous system could lead to new approaches to treat pain and infection.

34

IMMUNE SENSING OF HELMINTHS AND ALLERGENS

Jakob Von Moltke

University of Washington

New Innovator Award, 2017

The mammalian immune system encounters an enormous diversity of foreign stimuli, including viruses, bacteria, protozoa, parasitic worms (helminths), and allergenic particles. Determining how these stimuli are sensed and distinguished is fundamental to our understanding of the immune response and accordingly to our therapeutic interventions. The discovery of Toll-like receptors in the 1990s established the paradigm that specific microbial ligands are detected by matching immune receptors. Many ligand-receptor pairs have since been identified, and our understanding of bacterial and viral "type 1" detection is quite advanced. By contrast, very little is known about how the immune system first senses helminths and allergens, which give rise to a "type 2" immune response. We recently reported that the type 2 immune response to intestinal helminth infection requires a specialized epithelial lineage known as the tuft cell. Although tuft cells were discovered more than 50 years ago, their physiologic function remained unclear; we believe tuft cells represent the missing link in initiation of type 2 inflammation. Tuft cells physically bridge the divide between helminths in the intestinal lumen and immune cells in the underlying tissue, and encode a chemical sensing pathway that we found is required for intestinal type 2 immune responses. The goal of this work is to combine in vivo and in vitro screening strategies to identify the ligand-receptor pair(s) that mediates sensing of helminths by tuft cells. We are also developing new techniques for the in vitro culture and analysis of tuft cells. Although focused on helminth infection, if successful this work would establish a novel paradigm for the initiation of type 2 immune responses and should provide insights into the detection of allergens and other type 2 agonists as well. Our findings may therefore uncover novel therapeutic targets for treating both helminth infection and allergic inflammation.

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SLC19A1 IS THE DOMINANT IMPORTER OF 2'3'-CGAMP AND ANALOGS IN PRIMARY MONOCYTES

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New Innovator Award, 2017

2'3'-cGAMP is a second messenger that activates the anti-cancer innate immune STING pathway, and 2'3'-cGAMP analogs are in clinical trials for metastatic solid tumors. However, it is unknown how extracellular 2'3'-cGAMP and its analogs cross the cell membrane to activate intracellular STING. Using a genome-wide CRISPR screen, we identified SLC19A1 as an importer of 2'3'-cGAMP and its analogs, including bacterial cyclic dinucleotides. While different cell types use different 2'3'-cGAMP importers, SLC19A1 is the exclusive 2'3'-cGAMP importer in primary CD14+ monocytes. Remarkably, the

investigative new drug 2'3'-CDAS is a selective substrate for SLC19A1 and only activates STING in cells with high SLC19A1 expression. The cell-type selectivity of 2'3'-CDAS may explain its immunotherapeutic efficacy in mice over other equipotent STING agonists. Finally, our discovery of the extracellular 2'3'-cGAMP-SLC19A1-STING axis in primary monocytes suggests a physiological role for extracellular 2'3'-cGAMP and its bacterial analogs in innate immunity.

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TARGETING BACTERIAL INFECTIONS BY IMAGING ELECTRICAL INTERACTIONS BETWEEN HOST SURFACE AND A PATHOGEN

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New Innovator Award, 2017

Chronic bacterial infections pose dangerous health risks because they often require rigorous treatment regimens or surgeries. Current anti-microbials have little effect against persistent infections. Most drugs target intracellular processes important for bacterial viability, but pathogens rapidly adapt and develop antibiotic resistance. We aim to target extracellular charge interactions important for bacterial virulence. Geobacter sulfurreducens use hair-like filaments called pili as "nanowires" to transfer electrons for respiration and biofilm formation. We will evaluate whether pili of pathogens, which are crucial for lung infections of Cystic Fibrosis (CF) patients, show high conductivity similar to G. sulfurreducens pili. We have developed new tools to directly image and measure electrical charges and electron transfer in pili and living biofilms. By extending these tools to simultaneously analyze the interactions between host surface and living pathogens, we aim to identify the mechanism of infection by investigating three common bacterial survival strategies: (1) Adhesion to host cells is one of the most common microbial survival strategies. Furthermore, bacteria form biofilms, which cause 80% of microbial infections in the body, and result in chronic infection and the need for surgical removal of afflicted areas. By correlated imaging of adhesion force and charge, we will determine the role of charge interactions in bacterial adhesion to the host cell. (2) Iron accumulation and metabolism of pathogens, particularly accumulation of Fe (II) during infection, hinders existing chelation therapies that target chelation of Fe (III). We will evaluate how bacterial charge interactions affect their iron accumulation and metabolism in CF lung. (3) Adaptation by pathogens in dynamic environments is a major factor in the failure of antimicrobial therapies, but the mechanisms of adaptation remain unclear. Using newly-developed tools, we aim to achieve a comprehensive understanding of charge interactions to bring about a major shift in our understanding of bacterial infections.

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MEMBRANE PARTITIONING OF MYCOBACTERIAL PEPTIDOGLYCAN SYNTHESIS

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New Innovator Award, 2017

Antibiotics that target peptidoglycan are potent weapons against many bacterial infections but have historically been of limited utility against tuberculosis. The specter of drug resistance has revitalized interest in alternative strategies to halt Mycobacterium tuberculosis (Mtb) peptidoglycan synthesis. The peptidoglycan polymer is built next to and with precursors from the plasma membrane. While it is well known that the synthetic pathway spans both cytoplasmic and periplasmic compartments, lateral organization within the plasma membrane is an emerging concept. In mycobacteria, including Mtb, intracellular membrane domains (IMD) comprise biochemically and spatially distinct regions within the conventional plasma membrane. We find that MurG, an essential, membrane-bound synthase for peptidoglycan precursors, is enriched in the IMD but that the sequentially-acting flippase MurJ and extracellular polymerases such as PonA1 localize to the conventional plasma membrane. Lipid-linked precursors are detectable throughout the plasma membrane in wildtype bacteria but accumulate in the IMD upon depletion of MurG or MurJ. By contrast, inhibition of peptidoglycan polymerization or depletion of a negative regulator of MurJ alters the distribution of precursors within, but not between, different regions of the plasma membrane. Our data suggest that lipid-linked precursors are made in the IMD but flipped and polymerized in the conventional plasma membrane. We hypothesize that membrane partitioning enables efficient precursor synthesis and precise insertion of these molecules into the growing peptidoglycan polymer. Focal targeting of the IMD may be a new strategy for disrupting Mtb cell wall synthesis.

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INSPIRED BY A MECHANISM: A STORY OF ANTIVIRAL DRUG RESISTANCE

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New Innovator Award, 2017

Enveloped viruses use membrane fusion to deliver their genomes to the cell and initiate infection. For influenza virus, the main player in this process is a viral protein hemagglutinin (HA). More than 500 HA molecules densely decorate the virion surface. A virion engages the target cell membrane by burying more than a hundred HA molecules at the interface with the cell. Here, HAs act independently of one another, but a randomly assembled cluster of 3-5 HAs inserted in the target membrane collaborates to bring about fusion. This process “wastes” a lot of HAs, but results in a very robust overall mechanism with great potential for functional change. Membrane fusion inhibitors, such as Arbidol, inhibit viral infectivity by binding to and stabilizing the pre-fusion HA structure. Based on the fusion model, we predict that membrane fusion inhibitors primarily serve to slow fusion rate rather than decrease fusion efficiency. It would follow that influenza could attenuate the effects of fusion inhibitors by a general mechanism, i.e. by modulating (on the fast viral evolutionary time scale) the fusion kinetics. We probed this notion using a panel of fusion kinetic mutants and a combination of cell-based infectivity assays, single-particle fusion experiments and stochastic simulations. The patterns of resistance to distinct

fusion inhibitors agree with general predictions, but the results reveal unexpected effects and inhibitor-specific subtleties with potentially important consequences for fusion inhibitor design.

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DESIGNED ANKYRIN REPEAT PROTEINS AS THERAPEUTICS AGAINST INFECTIOUS DESEASE

Zhilei Chen

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New Innovator Award, 2018

Clostridium difficile infection (CDI) is a major nosocomial disease associated with significant morbidity and mortality. The pathology of CDI stems primarily from the two C. difficile secreted exotoxins 'toxin A (TcdA) and toxin B (TcdB)' that disrupt the tight junctions between epithelial cells leading to the loss of colonic epithelial barrier function. Here we report the engineering of a series of monomeric and dimeric designed ankyrin repeat proteins (DARPins) for the neutralization of TcdB. The best dimeric DARPin, DLD-4, inhibited TcdB with an EC50 of 4 pM in vitro, which is ~330-fold more potent than the FDA-approved anti-TcdB monoclonal antibody bezlotoxumab. DLD-4 also protected mice upon toxin challenge in vivo. Cryo-electron microscopy (cryo-EM) studies revealed that the two constituent DARPins of DLD-4- 1.4E and U3- bind around the middle and the C-terminal end of the Delivery domain of TcdB. Subsequent competitive ELISA studies showed that DARPin 1.4E and U3 interfere with the interaction between the TcdB and its receptor CSPG4 and FZD2, respectively. Moreover, our cryo-EM studies revealed a new conformation of TcdB (both apo and DARPin-bound at pH 7.4) in which the combined repetitive oligopeptides (CROPS) domain points away from the Delivery domain. This conformation of the CROPS domain is in stark contrast to that seen in the negative-stain electron microscopy structure of TcdA and TcdB at the same pH, in which the CROPS domain bends toward and kisses the Delivery domain. Combined with our biochemical and in vivo data, this new structural insight of the full-length TcdB in complex with DLD-4 should provide a more complete structural framework to accelerate the development of next generation anti-toxin biologics for preventing and treating CDI.

Instrumentation and Engineering

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SPECKLE-MODULATING FREE AND LARGE GOLD NANOROD ENHANCED OPTICAL COHERENCE TOMOGRAPHY FOR BRAIN TUMOR MARGIN DETECTION AND IN VIVO NEUROIMAGING

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Early Independence Award, 2012

Introduction: Optical coherence tomography (OCT) is a technology with the potential to allow for intraoperative detection of brain tumor margins. OCT systems are capable of rapid imaging of large three-dimensional volumes with cellular level resolution. However, OCT imaging has previously been limited by speckle artifact and the lack of suitable contrast agents, limitations that are surmounted in this study.

Methods: We prepared mice with orthotopic U87 glioblastoma xenografts and glass cranial windows. We also created large gold nanorods (LGNR) with plasmonic peaks tuned to the spectral range of the OCT scanner. LGNRs were injected intravenously into tumor-bearing mice and OCT imaging was performed *in vivo* utilizing a novel method for the removal of speckle artifact called Speckle-Modulating OCT (SMOCT). Fresh *ex vivo* human surgical samples were also imaged.

Results: In vivo SMOCT readily distinguished tumor from normal brain with cellular level spatial resolution and to a depth of 1.5 mm. Additionally, SMOCT allowed for the highest resolution ever seen *in vivo* of mouse white matter architecture. Cortical layers were also readily visible in SMOCT in both live mice and in the *ex vivo* human samples, representing a novel ability to interrogate cortical cytoarchitecture across a large field of view. SMOCT was also able to readily identitify the tumor margin of an *ex vivo* human low-grade glioma. Systemically administered LGNRs were tumor specific and provided excellent spectral contrast using SMOCT. *Ex-vivo* hyperspectral and IHC imaging confirmed the localization of LGNRs within the tumor and found that the LGNRs were largely localized within tumor associated macrophages (TAM). TAM movement could then be tracked in real time using SMOCT.

Conclusions: SMOCT and LGNR enhanced OCT imaging are promising state of the art technologies for intraoperative tumor margin detection and small animal neuroimaging.

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MECHANISMS OF APTAMER-FIELD-EFFECT TRANSISTOR NEUROTRANSMITTER SENSING

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Transformative Research Award, 2017

We have coupled newly selected high affinity DNA aptamers to field-effect transistors (FETs). We detected not only singly charged neurotransmitter targets, e.g., serotonin and dopamine, but also neutral and zwitterionic small-molecules, glucose and sphingosine-1-phosphate, respectively, under physiological conditions. Fluorescence-resonance energy transfer studies of glucose and serotonin aptamers delineate the significant conformational changes occurring upon target capture, which in the case of glucose, includes repositioning of the aptamer double-helical stems close to FET surfaces. Circular dichroism spectroscopy indicates that some, though not all of these aptamers adopt binding-induced motifs involving G-quartets, which are stabilized by target association. To understand mechanisms further, we systematically altered the distances at which stem repositioning occurs from

the surfaces of FET semiconductor channels by increasing aptamer stem lengths. Longer stem lengths led to decreased FET signal responses. We also altered aptamer densities on surfaces. Higher densities led to lower detection regimes. These findings provide new mechanistic insights into the interactions between aptamers and charge carriers in semiconductors, leading to tunable target sensing. We are identifying high affinity apatmers for additional small-molecule neurotransmitter targets, e.g., glutamate, noreprinephrine, to expand the repertoire of targets amenable to FET sensing. We are also fabricating FETs on silicon neuroprobes for in vivo sensing.

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MICROFLUIDIC SYNTHESIS OF GENE SILENCING CUBOSOMES

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New Innovator Award, 2016

Cubosomes are lipid-based nanoparticles where membranes, instead of enveloping into classic liposomes, intertwine into complex arrays of pores well-ordered in a cubic lattice. We have recently shown that these complex nanoparticles are able to encapsulate large contents of siRNA compared to a liposomal analogue. Importantly, the membranes that form cubosomes have intrinsic fusogenic properties that can facilitate endosomal escape. In this work we show new engineering solutions of microfluidic synthesis of cubosomes loaded with siRNA having biologically relevant sizes and size distribution. We show that the lipid-based nanoparticles display superior gene silencing capacity compared to regular liposomes at virtually no toxicity. This work highlights the importance of tuning a myriad of physical properties of nanoparticles for cellular delivery going beyond surface charge and nanoparticle size. Specifically, we demonstrate that tuning the membrane nanostructure of lipid-based nanoparticles enables a new design handle that exploits facilitated fusion and endosomal escape.

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DEFORMABLE ELECTRONIC MATERIALS FOR TWO-WAY COMMUNICATION WITH BIOLOGICAL SYSTEMS

Darren Lipomi

UC San Diego

New Innovator Award, 2015

The goal of this project is to create a class of electronic materials that can measure signals and interface with the nervous system for two-way communication with biological systems. The project is exploring three classes of materials. (1) Semiconducting polymers with properties inspired by biological tissue. The goal of organic bioelectronics is to detect and treat disease by using signal transducers based on organic conductors and semiconductors in wearable and implantable devices. Except for the carbon framework of these otherwise versatile materials, they have essentially no properties in common with biological

tissue: electronic polymers are typically stiff and brittle, and do not degrade under physiological conditions. Such properties can be realized in a single-component polymer by incorporating biocompatible subunits. We have synthesized a new type of stretchable, biodegradable polymeric semiconductor whose electronic performance is unaffected by the biodegradable components. Such materials have applications in wearable and implantable sensors. (2) Metallic nanoislands on single-layer graphene for cellular electrophysiology and wearable sensors. We have used these materials to measure the forces produced by the contractions of cardiomyocytes using a piezoresistive mechanism. Separately, we have developed orthogonal methods of stimulating myoblast cells electrically while measuring the contractions optically (a modality we nicknamed as "piezoplasmonic"). We have also used these sensors to measure the swallowing activity of head-and-neck cancer patients who have received radiation therapy and are at risk of dysphagia arising from fibrosis of the swallowing muscles. The combination of strain sensing, surface electromyography, and machine learning can be used to measure the degree of dysphagia. (3) We have developed ionically conductive organogels for haptic feedback. Medical haptic technology has myriad potential applications, from robotic surgery and surgical training, to tactile therapy for premature infants and patients with neurological impairment.

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A TARGETED IMMUNOTHERAPY TO ADDRESS ATHEROSCLEROTIC INFLAMMATION

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New Innovator Award, 2015

Cardiovascular disease (CVD) continues to be the leading cause of death in the developed world and is a considerable economic burden. A principle cause of CVD is atherosclerosis, an immunologically complex inflammatory condition within the intima of arterial vessel walls. Current clinical treatments for atherosclerosis focus on lowering serum levels of low density lipoprotein (LDL), with few options available to address the equally critical cell-mediated inflammation. Recent advances in nanotechnology now permit the rational design of targeted nanocarriers for the selective modulation of individual immune cell populations for either therapeutic or investigative purposes. As key regulators of inflammation, dendritic cells (DCs) influence the maturation of atherosclerotic lesions and directly activate T cells contributing to plaque instability and may thus hold promise as targets for CVD. We hypothesized that nanocarriers designed to elicit atheroprotective responses from both systemic and lesion-resident DCs could therapeutically reduce atherosclerotic inflammation. To test this hypothesis, we engineered the surface chemistry and morphology of nanocarriers to selectively target DCs and to transport the bioactive form of vitamin D3 (VD3; 1, 25-Dihydroxyvitamin D3), which is a potent anti-inflammatory and anti-fibrotic when delivered intracellularly. VD3 inhibits pro-inflammatory transcription factor NF-kB via the intracellular nuclear hormone receptor vitamin D receptor (VDR). We found that intravenous administration of vesicular VD3-loaded nanocarriers composed of poly(ethylene glycol)-bl-poly(propylene sulfide) copolymers could dramatically reduce the size of advanced vascular lesions in high fat diet-fed ApoE-/- mice. Furthermore, the LDL/HDL cholesterol ratio was lowered and arterial stiffness significantly decreased, the latter of which was linked to lower systemic levels of TGFβ.

Therapeutic efficacy required an optimized surface density of the P-D2 targeting peptide, which selectively binds the CD11c surface receptor on DCs. Our results highlight the therapeutic enhancement that controlled delivery can have on common bioactives and highlights the efficacy of DC-targeted immunomodulation in cardiovascular disease.

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ACTIVITY-BASED NANOSENSORS FOR EARLY AND NONINVASIVE DETECTION OF ACUTE ORGAN TRANSPLANT REJECTION

Gabe Kwong

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New Innovator Award, 2016

Detecting the onset of transplant rejection is critical for the long-term health and survival of the organ recipient, yet the core biopsy remains the diagnostic gold standard despite its invasiveness, risk of morbidity, and limited predictive power. During acute cellular rejection (ACR), host CD8 T cells damage allograft tissue by releasing the serine protease granzyme B (GzmB) to trigger donor cell death. To develop a noninvasive biomarker of early ACR, we engineer activity-based nanosensors to sense GzmB during rejection by amplifying detection signals in recipient urine for detection. These nanosensors comprise GzmB-specific peptide substrates conjugated to nanoparticles, preferentially accumulate in allograft and secondary lymphoid organs, and sense antigen-specific T cell killing. In a skin graft mouse model of transplant rejection, systemic administration of activity nanosensors result in significant elevation of urine signals at the onset of ACR before features of rejection appear in graft tissue. This approach is noninvasive and may allow routine monitoring of allograft immune health without the risk of a biopsy.

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NANOPILLAR-ASSISTED VIBRATIONAL SPECTROSCOPIC IMAGING FOR DECODING OF CELLULAR MECHANOCHEMISTRY

Ishan Barman

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New Innovator Award, 2017

Advances in live cell imaging have transformed our knowledge of how cells actively distinguish between mechanical microenvironments and regulate their own behaviors in response. Exploring the mechano-chemical coupling can offer mechanistic understanding and reveal the progression profile of difficult-to-treat diseases, such as laminopathy and metastatic cancer. Yet, gaining insights into the nanomechanical-biomolecular interactions has proven to be highly challenging, owing to the lack of non-invasive experimental tools with the requisite mechanical deformation, spatial resolution and molecular

sensitivity attributes. Our laboratory has developed a novel unified platform that provides simultaneous nanometric deformation and real-time measurement of highly localized biomolecular responses in live functioning cells, non-invasively and without using labels. Using this plasmonic nanopillar platform, we have demonstrated Raman imaging of prostate specific membrane antigen localized in the plasma membrane of prostate cancer cells with sub-diffraction limited resolution, and determined differential mechanotype of organ-tropic metastatic breast cancer cells. Beyond its utility as a new "lens" for revealing mechanochemical events, immediate possibilities for our platform abound including stain-free pathology assessment in smears and longitudinal monitoring of therapy response/resistance.

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PHOTOACOUSTIC IMAGING AS A TOOL TO STUDY BIOLOGY

Jesse Jokerst

UC San Diego

New Innovator Award, 2016

Photoacoustic imaging combines the high temporal and spatial resolution of ultrasound with the good contrast and spectral nature of optics. This techniques is "light in/sound out" as opposed to traditional "sound in/sound out" ultrasound. I will present three case studies that highlight the power of photoacoustic imaging to address the needs of the medical community. In the first, I will detail an activatable nanoparticle to image reactive oxygen species. Third, I discuss a mouthpiece for acoustic dental imaging that non-invasively collects pocket depth measurements. Finally, I will detail photoacoustic imaging with a wearable transducer for therapeutic drug monitoring of heparin.

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IMPLANTABLE CARDIAC POWER GENERATION USING FLEXIBLE 3D POROUS THIN FILMS

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Transformative Research Award, 2016

Energy consumption and battery replacement are key to the lifetime and effectiveness of implantable biomedical devices, such as cardiac pacemakers and automatic implantable cardiac defibrillators (AICD). However, there is a lack of promising technologies which can effectively covert the mechanical energy of heart to the electricity, without the risk of interfering with the cardiovascular functions. This project proposes an approach combining thin-film energy conversion materials development with geometric mechanics design, to harness the motion from lead of cardiac pacemaker or defibrillators and convert into electrical power output with orders-of-magnitude enhancement.

Here we present porous PVDF-TrFE composite thin film with enhanced piezoelectric energy harvesting performance. The approach is to engineer the crystalline and surface energy states of flexible piezoelectric polymer porous thin film. Porous size and structure flexibility of PVDF-TrFE films were precisely controlled to increase the piezoelectric output. In addition, four geometric energy harvesting device designs have been demonstrated: (1) helical porous PVDF ribbon on AICD lead, to harvest the bending motion; (2) flexible dual-cantilever porous PVDF film to utilize the kinetic energy of AICD lead; (3) multi-buckled beams within a soft tube to harvest the complex AICD lead motion (bending and vibration); and (4) lead-through-wheel bistable energy harvester inspired by the snap-through motion of AICD lead. The preliminary results showed great promise to provide electrical energy for implantable devices. Furthermore, a mechanical-electro computational model was developed, which coupled mechanical deformations and piezoelectric effects to predict electrical output as a function of key design parameters and materials properties. The successful outcome will provide a design framework to realize a variety of compact and flexible implantable energy harvesters.

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SPATIALLY RESOLVED TRANSCRIPTOMICS ENABLED BY ULTRABRIGHT PDOT PROBES FOR INTERROGATION OF COMPLEX TISSUES

Joshua Vaughan, Daniel Chiu

University of Washington

Transformative Research Award, 2017

The measurement of proteome data at the sub-cellular level on intact tissue or organisms has the potential to revolutionize biology by its ability to identify or classify cell types by protein expression profiles and by its ability to report on complex gene regulation networks in normal or diseased tissue. Among the tissues most in need of high resolution in situ proteomic analysis is the brain due to its wide range of cell-type dependent genes and its intricate cell-cell connectivity. However, in situ proteomic studies of the brain have been severely hindered by limited technical capabilities. We are developing an approach to high-throughput, spatially resolved transcriptomics in complex tissues. Our method is based on the synthesis and use of ultra-bright, spectrally barcoded fluorescent polymer dots to probe mRNA levels as well as the development of an imaging approach combining swellable tissue-hydrogel hybrids and a custom multi-spectral light sheet optical microscope. These tools should be applicable to many types of tissues or small organisms, but the final goal of our project is to demonstrate this method by studying development of the mouse visual cortex.

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RECORDING NEURAL ACTIVITY IN UNRESTRAINED ANIMALS WITH 3D TRACKING TWO PHOTON MICROSCOPY

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New York University

New Innovator Award, 2015

Optical recordings of neural activity in behaving animals can reveal the neural correlates of decision making, but such recordings are compromised by brain motion that often accompanies behavior. Two-photon point scanning microscopy is especially sensitive to motion artifacts, and to date, two-photon recording of activity has required rigid mechanical coupling between the brain and microscope. To overcome these difficulties, we developed a two-photon tracking microscope with extremely low latency (360's) feedback implemented in hardware. We maintained continuous focus on neurons moving with velocities of 3 mm/s and accelerations of 1 m/s2 both in-plane and axially, allowing high-bandwidth measurements with modest excitation power. We recorded from motor- and inter- neurons in unrestrained freely behaving fruit fly larvae, correlating neural activity with stimulus presentation and behavioral outputs. Our technique can be extended to stabilize recordings in a variety of moving substrates.

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PROGRESS ON THE EXPLORER PROJECT: TOWARDS A TOTAL BODY PET SCANNER FOR HUMAN IMAGING

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Transformative Research Award, 2015

Positron Emission Tomography (PET) scanners generate in vivo images of radiolabeled pharmaceutical distribution and kinetics, providing a powerful window into metabolic and physiological processes in the clinic and in research. However, current PET scanners for humans have a short axial field-of-view (AFOV) of 15-25 cm, which require that whole-body surveys are acquired in sequential bed positions and limits their ability to kinetically model drug distributions to small regions of the body. Long AFOV PET scanners address this limitation while improving signal quality and enabling a significant reduction in scan time and radiation dose, thereby offering the promise of a step-change in molecular imaging research and clinical practice.

The EXPLORER Consortium is developing two long AFOV systems: one, academically based at UC Davis, will be capable of total-body imaging at high spatial resolution and will be the first medical scanner of any kind that can image the entire body simultaneously, while the other, academically based at U Penn, will be capable of complete torso imaging at high time-of-flight resolution. In addition, we have developed two mini-EXPLORER scanners that allow us to (1) test all the components of the total-body scanner prior to full scale-up and (2) test a variety of applications in companion animals and nonhuman primates. In this work we report progress in development of the human scanners together with examples of applications developed on the mini-EXPLORER scanners.

Completion of the total-body EXPLORER is expected in May 2018. The PET scanner will have a total axial field of view of 194 cm with expected spatial resolution of ~2.9 mm. The torso EXPLORER scanner is

being fabricated in stages. The first 70 cm is complete and demonstrating a time-of-flight resolution of 250 ps. The final device will have a total length of 140 cm and completion is expected in May 2019.

Molecular and Cell Biology

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REGULATION OF CELL SIGNALING BY ZINC DYNAMICS

Amy Palmer

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Pioneer Award, 2014

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 explores 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 are using a variety of cutting edge technologies from live cell imaging to transcriptomics 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 zinc dynamics give rise to differential gene expression in hippocampal neurons and in infected macrophages. We have also uncovered striking changes in zinc during the mammalian cell cycle and identified that zinc plays a role in the proliferation-quiescence cell fate decision.

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DNA-NANOTECHNOLOGY ENABLED MEMBRANE ENGINEERING

Chenxiang Lin

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New Innovator Award, 2014

Lipid-bilayer membranes form barriers to define the boundaries of a cell and its subcellular compartments. With the help of membrane-associating molecules, they undergo dramatic structural

changes during the life cycle of cells and mediate complex reactions that are vital to cell division, growth and communication. Inspired by such elegance in nature, bioengineers and synthetic biologists have aspired to build artificial membranes to recapitulate the cellular membrane structure and dynamics. In addition, such in vitro preparations provide a complexity-reduced system for cell biologists and biophysicists to study functional interactions between membranes and their associating molecules.

We have made a number of technical breakthroughs in high-precision membrane engineering. Our first approach is to use self-assembled DNA nanostructures as templates to guide the assembly of lipid bilayers and transduce the programmable feature of the DNA nanostructures to the templated vesicles (Yang et al, Nat Chem, 2016). Specifically, we show the assembly, arrangement, and remodeling of liposomes with designer geometry: all of which are exquisitely controlled by a set of modular, reconfigurable DNA nanocages. Tubular and toroidal shapes, among others, are transcribed from DNA cages to liposomes with high fidelity, giving rise to membrane curvatures present in cells yet previously difficult to construct in vitro. Moreover, the conformational changes of DNA cages drive membrane fusion and bending with predictable outcomes, opening up opportunities for the systematic study of membrane mechanics (Zhang et al, Nat Chem, 2017). Our second approach is to deform preformed lipid bilayers by the assembly of DNA nanostructures on membrane. As a proof-of-concept demonstration, we have generated membrane tubules out from spherical vesicles through on-membrane self-assembly of DNA nanosprings with membrane anchors. The tubulation efficiency and membrane tube morphology can be modulated by the design and surface density of DNA nanosprings (Grome et al, Angew Chem, 2018).

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SYNGENICDNA: STEALTH-BY-ENGINEERING TO EVADE RESTRICTION-MODIFICATION BARRIERS

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Transformative Research Award, 2017

Genetic engineering is a powerful approach for discovering fundamental aspects of bacterial physiology, metabolism, and pathogenesis. The problem is the vast majority of bacteria that can be grown in a laboratory remain genetically intractable, beyond the power of genetics for elucidating function or for engineering for human use. The challenge of genetic intractability stymies basic-, synthetic-, and translational-microbiology research and development. Researchers spend years constructing ad hoc genetic systems one species at a time, an arduous and expensive process. But what if every bacterium that could be grown in the laboratory could be quickly and easily made tractable? How rapidly would microbial research progress if every bacterial strain was as genetically accessible as commercially available E. coli?

Here, we introduce SyngenicDNA, a method for rendering cultivable bacterial species genetically tractable irrespective of their taxonomic lineage or genetic barriers. The approach takes advantage of state-of-the-art combinatory genome and epigenome Single-Molecule-Real-Time (SMRT) sequencing technology. It has been designed to prevent non-self DNA (genetic tool) degradation by innate

Restriction Modification (RM) and Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-Cas systems; underlying causes of genetic intractability that exist within most bacteria. SyngenicDNA overcomes these complex bacterial defense mechanisms using a rapid and widely applicable “stealth” based strategy.

The paucity of genetically tractable bacteria is a formidable challenge to deciphering functional attributes of members of the human microbiome. Thus, as proof of principle, we are demonstrating the power of the SyngenicDNA method on bacterial species from the human oral microbiota. We intend to create an initial repository of ~100 model bacterial strains representing anaerobic and aerobic species across eight different phyla within the oral microbiota, each made genetically tractable using the SyngenicDNA method. Our overarching goal is to provide universally applicable methodologies to rapidly render every bacterial species genetically tractable.

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NOVEL STRATEGIES FOR INDUCED IN VIVO TRANSDIFFERENTIATION ACROSS GERM LAYERS

Duc Dong

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New Innovator Award, 2012

The extent to which differentiated cells, while remaining in their native microenvironment, can be reprogrammed into unrelated cell types will reveal fundamental insight into cellular plasticity and impact regenerative medicine. To investigate *in vivo* lineage potential, we developed a novel *in vivo* discovery platform for lineage conversion. Using this platform, we have identified a cocktail of transcription factors that, when mis-expressed in several non-endoderm lineages including skeletal muscle, are able to specifically and cell-autonomously trigger the endoderm genetic program. These endoderm induced muscle cells can proceed to lose muscle gene expression and morphology, while gaining key endoderm organogenesis markers, such as the pancreatic specification genes, *hnf1* and *ptf1a*. Endoderm markers appearing prior to loss of muscle cell morphology, a lack of dependence on cell division, and a lack of dedifferentiation, mesoderm, ectoderm, and pluripotency gene activation, together, suggests that reprogramming occurred by direct lineage conversion. Mechanistic studies reveal novel, broadly applicable strategies for enhancing lineage reprogramming efficiency by leveraging synthetic and xenotic factors, as well as genetic loss-of-function. Our work demonstrates that within a vertebrate animal, differentiated cells originating from one germ layer can be induced to directly adopt a lineage of a different germ layer, suggesting that differentiated cells *in vivo* have unrestricted lineage potential. Our technology may pave the way towards a vast new *in vivo* supply of replacement cells for injuries and degenerative diseases such as diabetes.

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KLF4 BINDING DURING REPROGRAMMING IS LINKED TO ENHANCER REWIRING AND IS CRITICAL FOR THE ARCHITECTURE AND REGULATION OF ENHANCER HUBS

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New Innovator Award, 2015

Cell fate transitions are accompanied by global transcriptional, epigenetic and topological changes driven by transcription factors (TFs), as is strikingly exemplified in somatic cell reprogramming to pluripotent stem cells (PSCs) by OCT4, KLF4, SOX2 and cMYC. How TFs orchestrate the complex molecular changes around their targets in a temporal manner remains largely elusive. Here, using KLF4 as a paradigm, we provide the first TF-centric view of chromatin reorganization during reprogramming and its association to enhancer rewiring and gene regulation. We captured the enhancer connectomes in fibroblasts and PSCs by H3K27ac HiChIP and identified complex 3D enhancer hubs that were strongly correlated with cell-type specific gene expression and coregulation. KLF4-centric conformational analysis at different reprogramming stages revealed that KLF4 is involved in the dissociation of fibroblast-specific and the establishment of PSC-specific enhancer loops concomitantly with repression or activation of linked genes. Moreover, KLF4 occupancy was significantly enriched within highly connected enhancers, suggesting a role in the formation and regulation of complex enhancer hubs. Indeed, disruption of a single KLF4 binding site from a newly identified PSC-specific enhancer was sufficient to reduce expression of multiple genes within the enhancer hub, partly by impairing long-range contact. Our study provides an integrative view of the intricate activities of a master regulator during a controlled cell fate transition and offers novel insights into the order and nature of molecular events that follow TF binding.

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IN SITU GENOME SEQUENCING

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Early Independence Award, 2017

The spatial organization of the genome is an important regulator of cell fate and function by controlling nuclear processes such as gene expression. The nucleus is, in principle, highly amenable to study in its native context via imaging methods, which can capture multiple structural features simultaneously. However, in practice, there exist no tools to image genomic structure at high resolution and high throughput, and investigators must typically rely on 3C-based methods, which provides an indirect measure of genome structure.

We have developed an in situ sequencing method to directly resolve the 3D structure of the genome in its native context within single cells. Here we describe the methodological developments that underpin this new imaging approach. In brief, we first construct a whole-genome sequencing library in situ: a series of enzymatic steps fragments and ligates sequencing adaptors to chemically fixed genomic DNA, and converts those fragments in situ to clusters containing many copies of the original genomic sequence. These amplicons can be interrogated via sequencing by ligation, in which each sequencing

round is read out using fluorescence microscopy. The entire process can be automated using a fluorescence microscope with integrated fluidics. As a proof of principle, we use this approach to spatially resolve thousands of whole-genome sequencing reads per cell from hundreds of individual cells in a single experiment. We expect this platform technology will significantly expand the scope of possible measurements of genomic organization, including high-throughput genome-wide architectural mapping of cis-regulatory elements, higher order chromatin contacts, and chromosomal domains. We anticipate these novel imaging based genomic tools will yield new biological insights about the epigenetic processes that underlie genome structure and regulation.

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TEMPORAL, SPATIAL, AND GENETIC REGULATION OF MEIOTIC RECOMBINATION PATHWAYS

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New Innovator Award, 2015

Meiotic recombination is made up of multiple pathways to repair DNA breaks. These pathways generate crossovers, which exchange homolog arms, and noncrossovers, which are patch-like repairs. Recombination promotes homolog pairing and the obligate crossover, which is required for accurate chromosome segregation. In mammals, most crossovers are MutLgamma-dependent and most noncrossovers form via synthesis-dependent strand annealing. In order to determine when recombination pathways act during meiosis, we highly synchronized mouse spermatogenesis to monitor recombination in depth at hotspots. We find half of noncrossover recombination is distributed evenly across hotspots and completed early. The remaining noncrossovers are located in the central 200bp of hotspots and form coincidentally with MutLgamma-dependent crossovers in mid-prophase. By analyzing recombination in the absence of MutLgamma, we observe long noncrossovers that suggest dissolution of a common crossover intermediate such as a double Holliday junction. We also document residual Holliday junction resolution by structure-selective endonucleases. Consistent with their proposed roles as back-up repair pathways, they act at the end of meiotic prophase. The different recombination profiles provide an opportunity to investigate the roles of two pro-crossover E3 ligases, RNF212 and HEI10. Our results support a model that HEI10 plays a biphasic role to antagonize crossover designation by RNF212 early and to promote crossover maturation in mid-prophase. Finally, we find that the pattern of noncrossover recombination is influenced by the absence of RNF212 and MLH3 such that central noncrossovers are under-represented. Our findings reveal substantial integration between recombination pathways that regulate the distribution, timing, and pathway choice to ultimately ensure accurate chromosome segregation.

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IDENTIFICATION OF THE CONFORMATIONAL DYNAMICS BEHIND EGFR FUNCTION

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New Innovator Award, 2017

In 60% of drug delivery, membrane-bound receptor proteins bind ligands to initiate microscopic motions. Despite the importance of these motions, their temporal and spatial dynamics have been inaccessible with existing tools due to limitations in resolution, sensitivity, or experimental conditions. Indeed, membrane proteins are notoriously challenging to study due to the difficulty of maintaining a physiologically relevant environment while conducting experiments. Because of this challenge, they are often at the heart of unanswered questions. We overcome this challenge by developing well-controlled synthetic membrane-protein systems that mimic biological environments while developing and applying single-molecule techniques to monitor microscopic motions. Of particular interest is the mammalian receptor tyrosine kinase, epidermal growth factor receptor (EGFR). The EGFR family of receptors is the primary target of cancer drug. These receptors contribute to the development and maintenance of tissues. However, their overexpression and aberrant activation plays a key role in the progression of epithelial and brain tumors. We have created a model membrane system containing functional fluorescently-labeled full-length EGFR. Through this platform, we explore conformational dynamics and interactions under near-physiological conditions to map out the spatiotemporal dynamics that underlie this important therapeutic target.

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APOBEC-COUPLED EPIGENETIC SEQUENCING PERMITS LOW-INPUT, BISULFITE-FREE LOCALIZATION OF 5-HYDROXYMETHYLCYTOSINE AT BASE RESOLUTION

Hao Wu

University of Pennsylvania

New Innovator Award, 2017

Here we present APOBEC-Coupled Epigenetic Sequencing (ACE-Seq), a bisulfite-free method for localizing 5-hydroxymethylcytosine (hmC) at single-base resolution with low DNA input. The method builds upon our observation that AID/APOBEC family DNA deaminase enzymes can potently discriminate between cytosine modification states, and exploits the non-destructive nature of enzymatic, rather than chemical, deamination. ACE-Seq yields high-confidence hmC profiles with at least 1000-fold less DNA input than conventional methods. We apply ACE-Seq to generate the first base-resolution map of hmC in tissue-derived cortical excitatory neurons. We find that hmC is almost entirely confined to CG dinucleotides, and resolving C, mC and hmC reveals that regions appearing heavily methylated can be highly enriched for hmC. Enzymatic deamination overcomes many challenges posed by bisulfite-based methods and expands the scope of epigenome profiling by permitting characterization of scarce samples that will open new lines of inquiry regarding the role of cytosine modifications in genome biology.

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TARGET OF RAPAMYCIN COORDINATES PLANT GROWTH BY DYNAMICALLY REGULATING CELL-CELL TRANSPORT

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Early Independence Award, 2016

In plants, the coordinated redistribution of sugars from mature “source” leaves to support the growth of developing “sink” leaves requires tight regulation of sugar transport between cells through plasmodesmata (PD). PD are nanoscopic membrane-bound channels in the cell walls of plants that connect adjacent cytoplasts, allowing the transport of cytoplasmic molecules up to ~80kDa, as well as viruses, between cells. Although fundamental to plant physiology, the mechanisms that regulate PD transport and thereby support the development of new leaves have remained elusive. Several mutants identified in a forward genetic screen for altered PD transport led us to discover that the sink-to-source restriction of PD transport is promoted by the conserved eukaryotic glucose-TOR signaling hub. We demonstrate that TOR activity dramatically increases as leaves mature from "sinks" to "sources". Genetic, chemical, and physiological treatments directly and indirectly targeting TOR activity support this model. We identify a TOR effector, the bZIP transcription factor ABI5, that contributes to the restriction of PD transport in source leaves. We conclude that plant cells regulate intercellular transport in response to changing carbohydrate availability monitored by the TOR pathway.

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OPTOGENETICS FOR INTRACELLULAR CODEBREAKING: HOW ERK ACTIVITY IS INTERPRETED TO CONTROL GENE EXPRESSION AND CELL FATE

Jared Toettcher

Princeton University

New Innovator Award, 2016

Every cell exists in a complex and changing environment. To deal with their complex surroundings, cells have evolved diverse systems to sense external cues and create an internal representation of this information. However, we are still largely in the dark about how external information is stored in patterns of protein activity, and how this information is decoded into specific cell fate decisions. I will describe our efforts to overcome these challenges using cellular optogenetics: the delivery of precise spatial and temporal activity patterns to a signaling protein of interest. We have developed a suite of optogenetic tools to precisely control MAP kinase (MAPK) signaling. Coupling this system to reporters of MAPK target genes enables us to dissect how signaling dynamics are transmitted to target genes, leading to defined changes at the transcript and protein levels. Applying these tools in the Drosophila embryo further revealed how a model cell fate choice - differentiation into posterior midgut endoderm - is controlled by specific patterns of MAPK activity.

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CELLULAR STRATEGIES FOR CONTROLLING PROTEIN HOMEOSTASIS

Kai Zhou

Buck Institute for Research on Aging

Early Independence Award, 2017

Proteostasis is the guardian of the proteome to ensure proper protein folding, protein-protein interaction, and consequently the organization of the macromolecules and organelles within a cell. Loss of proteostasis and the consequent accumulation of aggregated proteins represent a major hallmark of aging and many age-related neurodegenerative diseases. Research in my lab focus on understanding the cellular strategies used to maintain proteostasis during environmental stresses and physiological aging. My recent works in this direction revealed unexpected roles of mitochondria in proteostasis through controlling the aggregation and degradation of damaged proteins. The mitochondrial import and degradation of misfolded non-mitochondrial proteins is a major protein degradation pathway that allows the cells to restore proteostasis after stress.

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EXPLOITING KNOWLEDGE OF DEVELOPMENTAL BIOLOGY TO GENERATE PURE POPULATIONS OF DESIRED HUMAN CELL-TYPES FROM DIFFERENTIATING EMBRYONIC STEM CELLS

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Early Independence Award, 2017

A quintessential but as-of-yet unrealized goal of regenerative medicine is to artificially generate a pure population of a given human cell-type, with the goal of using such cells to eventually replace missing or dysfunctional cells in patients. The unique ability of human embryonic stem cells (hESCs) to develop into all the hundreds of cell-types in the body has made them prime candidates for regenerative therapies. However, prevailing methods to differentiate hESCs into desired cell-types often yield heterogeneous cell populations containing a number of contaminating lineages. These impure cell populations are poorly suited for regenerative therapies or basic research. To more precisely differentiate hESCs into pure populations of desired cell-types, we have mapped out the stepwise lineage choices through which hESCs progressively differentiate into 20+ human cell-types belonging to the endoderm, mesoderm and ectoderm lineages. Using this knowledge, we can efficiently differentiate hESCs into a pure population of a desired cell-type by inhibiting their differentiation into "unwanted" cell-types. Through this approach, we have generated enriched populations of human liver, bone and heart progenitors from hESCs, each of which is capable of engrafting mouse models and regenerating their cognate human tissue in vivo. The newfound ability to precisely guide hESC differentiation into pure populations of desired human cell-types provides a foundation for regenerative medicine and reiterates the

importance of understanding developmental biology to achieve control over stem-cell differentiation in vitro.

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OLFACTION REGULATES FAT STORAGE DYNAMICS THROUGH NEURONAL ASYMMETRY

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Baylor College of Medicine

Pioneer Award, 2016

The sense of smell is essential for detecting environmental cues to adjust survival strategies and long-term metabolic decisions. Despite its well-known impacts on chemotaxis and feeding behaviors, whether and how olfaction relays signals to directly regulate peripheral fat metabolism remain unknown. Here, we report that environmental odors can dynamically shift the balance between fat storage and mobilization, which is specifically mediated by their corresponding olfactory circuit and associated neuroendocrine signaling. We discovered that specific Caenorhabditis elegans olfactory neurons regulate peripheral fat storage without changing food intake or physical activities. We also uncovered a neuroendocrine pathway linking the olfactory neural circuit and peripheral fat storage tissues, in which a specific neuropeptide acts together with its peripheral receptor and signals through a specific isoform of FOXO transcription factor/DAF-16 to regulate fat metabolism. Furthermore, we found an environmental odor that relies on this neuroendocrine mechanism to promote fat accumulation in a rapid and reversible manner. Together, our work reveals that olfaction directly regulates fat metabolism and suggests its significance in fine-tuning systemic metabolic health dynamically responding to environmental variables. The key components of this novel neuroendocrine pathway are well conserved in mammals, and thus our studies lay the groundwork for further investigation of the impact of olfaction on human metabolic health.

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ANTIGEN-SPECIFIC T-CELL BIOFACTORIES AS VECTORS FOR IN VIVO PROTEIN SYNTHESIS

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New Innovator Award, 2016

The systemic drug toxicity limits the options for diseases that evolve in vivo (e.g. cancer, viral infections, autoimmune disorders). We have developed an artificial cell-signaling pathway that capitalizes on T-cell's innate extravasation ability and transforms the cell into an in vivo living vector (antigen-specific T-cell Biofactory) for synthesizing engineered proteins in situ upon interacting with target cells. The T-cell Biofactory has three constant and two variable domains. The constant domains include- the Receptor that mobilizes the DNA-based Actuator to synthesize engineered proteins and the Secretor for

transporting them into the extracellular space. The variable domains provide a broad applicability and include- a Sensor, part of the Receptor, to identify the disease-specific biomarker; and an Effector transgene with the potential to neutralize the pathology that triggered the T-cell Biofactory. The feasibility was validated by measuring the reporter enzyme activity, the DNA template for which can be replaced with that of the protein with desired therapeutic function. OVCAR3 and A2780cis, with or without endogenous expression of Folate Receptor alpha (FRa) and Mesothelin (MSLN), were used as target and non-target cells. The T-cell Biofactory upregulated the reporter ~35X upon stimulation by the FRa+OVCAR3 compared to when stimulated by FRanegA2780cis. The expression was proportional to the target cell number. It was statistically increased within 1 hr and continued to stay elevated for at least until 10 days in vitro and 3 days in vivo. The platform nature of the T-cell Biofactory was demonstrated by reprogramming the Sensor to target MSLN expression. The FRa-specific and MSLN-specific T-cell Biofactories were used to detect and compare the expression of FRa and MSLN on different cell lines with endogenous (OVCAR3, A2780cis) and engineered (FRa+MSLNnegA2780cis, FRanegMSLN+A2780cis) antigen expression and to demonstrate the activation of T-cell Biofactory with spatiotemporal resolution.

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DISCOVERY OF NEW RNA-TARGETING CRISPR SYSTEMS FOR TRANSCRIPTOME ENGINEERING

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Early Independence Award, 2015

Class 2 CRISPR-Cas systems endow microbes with diverse mechanisms for adaptive immunity. We analyzed prokaryotic genome and metagenome sequences to identify an uncharacterized family of RNA-guided, RNA-targeting CRISPR systems which we classify as Type VI-D. Biochemical characterization and protein engineering of seven distinct orthologs generated a ribonuclease effector derived from Ruminococcus flavefaciens XPD3002 (CasRx) with robust activity in human cells. CasRx-mediated knockdown exhibits high efficiency and specificity relative to RNA interference across diverse endogenous transcripts. As one of the most compact single effector Cas enzymes, CasRx can also be flexibly packaged into adeno-associated virus. We target virally encoded, catalytically inactive CasRx to cis-elements of pre-mRNA to manipulate alternative splicing, alleviating dysregulated tau isoform ratios in a neuronal model of frontotemporal dementia. Our results present CasRx as a programmable RNA-binding module for efficient targeting of cellular RNA, enabling a general platform for transcriptome engineering and future therapeutic development.

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HOW ADIPOCYTES DRIVE TUMOR PROGRESSION

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New Innovator Award, 2012

Tumors exist as ecosystems composed of both cancer cells as well as a variety of stromal cell types that make up the tumor microenvironment. Interrogating the interactions between these various cell types in vivo has proven challenging, but is essential in deciphering the mechanisms of metastasis, the major cause of mortality in cancer. To address this, we have developed the zebrafish as a new system for metastasis due to its capacity for high-throughput genetic manipulation coupled with the availability of optically translucent strains (e.g. casper) which afford detailed single-cell imaging resolution. Using a zebrafish model of melanoma, we have uncovered an unexpected interaction between tumor cells and adipocytes, the fat-containing cells that are ubiquitous throughout the body. We find that the tumor cells can induce lipolysis in the nearby adipocytes, releasing fats into the extracellular environment. Using a fluorescent pulse chase-assay, we show that these lipids can then be directly taken up into the cytosol of the melanoma cell. Once inside the tumor cell, these lipids can be utilized as a source of cellular fuel through mitochondrial beta-oxidation, which yields high levels of ATP. The melanoma cells take up these lipids via expression of a fatty acid transport protein (FATP1/SLC27A1) that is aberrantly expressed on the melanoma cell surface. Both genetic as well as pharmacologic inhibition of FATP proteins abrogates lipid transfer from the adipocyte to the melanoma cell, and impairs melanoma growth and invasion. Our ongoing studies suggest that adipocyte-derived lipids can directly affect histone acetylation in the melanoma cell and alter gene expression, providing a direct connection between the tumor microenvironment and the epigenetic cell state of the cancer cell. These effects are conserved in human melanomas as well, highlighting how a poorly studied member of the microenvironment can directly drive tumor cell identity.

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TO MOLE OR TO MELANOMA: THE TRANSCRIPTIONAL AND GENETIC DETERMINANTS OF HUMAN MELANOCYTE TRANSFORMATION

Robert Judson-Torres

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Early Independence Award, 2014

Activating mutations in the gene *BRAF* drive half of cutaneous melanomas. Yet, when a human melanocyte acquires this mutation, the cell does not immediately transition into malignancy. Rather, it undergoes a transient period of rapid proliferation followed by growth arrest resulting in benign lesions called melanocytic nevi or moles. Few moles progress to melanoma, suggesting these cells harbor robust intrinsic defenses against further transformation. To dissect the molecular barriers that separate benign moles from malignant melanomas, we performed multi-omic analyses on microdissected clinical lesions, each presenting both benign precursor and malignant descendent regions. The transition from proliferative pre-malignant melanocytes to invasive melanomas was marked by genetic loss of the tumor suppressor *CDKN2A*. In contrast, no common genetic changes distinguished growth-arrested nevus melanocytes from proliferative melanocytes. Instead, transcriptional loss of a signature of nevus-

associated microRNAs accompanied this transition. To assess the functional consequence of these genetic and transcriptional changes during human nevus formation and melanoma initiation, we engineered *BRAF* activating mutations and *CDKN2A* loss into primary human melanocytes. Consistent with our clinical cohort, we discovered that *CDKN2A* loss drove a lineage-restricted invasive program through transcriptional activation of *BRN2*. Similarly, the nevus-associated microRNA signature was downstream of $BRAF^{V600E}$ and drove cell cycle arrest. Using independent cohorts of clinical cases, we validated that *CDKN2A* loss is associated with the initiation of invasive melanoma and that loss of the nevus-associated microRNA signature is a robust early marker of melanoma initiation.

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BUILDING THE MICROTUBULE CYTOSKELETON: XMAP215 IS A MICROTUBULE NUCLEATION FACTOR THAT FUNCTIONS SYNERGISTICALLY WITH THE GAMMA-TUBULIN RING COMPLEX

Sabine Petry

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New Innovator Award, 2016

How microtubules (MT) are generated in the cell is a major question in understanding how the cytoskeleton is assembled. For several decades, γ-tubulin has been accepted as the cell's universal MT nucleator. Although there is evidence that γ-tubulin complexes are not the sole MT nucleators, identification of other nucleation factors has proven difficult. Here, we report that the well-characterized MT polymerase XMAP215 (chTOG/Msps/Stu2p/Alp14/Dis1 homologue) is essential for MT nucleation in Xenopus egg extracts. The concentration of XMAP215 determines the extent of MT nucleation. Even though XMAP215 and γ-tubulin ring complex (γ-TuRC) possess minimal nucleation activity individually, together these factors synergistically stimulate MT nucleation in vitro. The N-terminal TOG domains 1-5 of XMAP215 bind γ-tubulin and promote MT polymerization, while the conserved C-terminus is required for efficient MT nucleation and directly binds γ-tubulin. In sum, XMAP215 and γ-TuRC together function as the principal nucleation module that generates MTs in cells.

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THREE-DIMENSIONAL VISUALIZATION OF THE EXTRACELLULAR MATRIX IN THE DEVELOPING MOUSE

Sarah Calve

Purdue University

New Innovator Award, 2017

Recent findings indicate that the extracellular matrix (ECM) of developing tissues may better promote regeneration than the adult ECM, suggesting that the material properties of embryonic tissues are more suitable for guiding engineered scaffold design. However, little is known about the spatiotemporal expression patterns and 3D structure of the ECM during embryonic development. This is primarily due

to light scattering lipids and refractive index mismatches that limit the visualization of the architecture of intact tissues. To better resolve the interactions of different ECM networks, we developed a novel decellularization method that removes signal interference from cellular components, enhances antibody penetration and maintains the geometry of fragile tissues. Optimization of decellularization enabled a significant increase in visibility of the internal structure of developing forelimbs, where different ECM maintained independent, interpenetrating networks in 3D. We found that distribution of ECM networks in various developing tissues, including the eye and spinal cord, can be visualized in E12.5 embryos at multiple scales. Comparative analysis of the forelimb revealed continuous proteoglycan-rich fibrils extended between the epidermis and cartilage at E12.5 and remained present after the formation of the extensor tendons between these two tissues at E14.5, suggesting that the matrix plays a role in regulating extensor tendon architecture and localization. By combining our new method with more finely resolved time points, a clearer picture will emerge regarding the role the ECM plays during forelimb musculoskeletal assembly. Knowledge regarding how the expression, structure, and localization of ECM proteins change over the course of development will be utilized to guide the design of regenerative scaffolds to repair damaged tissues of the musculoskeletal system.

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DECONSTRUCTING CONTROL OF LEUKOCYTE MIGRATION

Sean Collins

University of California, Davis

New Innovator Award, 2017

Leukocytes defend the body against infections and tumors. Critical for their job, they are highly motile and follow chemical gradients to reach sites of injury and infection where they unleash cytotoxic and inflammatory responses. Leukocyte responses are critical for fighting infections, but over-activity can cause tissue and organ damage and is associated with inflammatory diseases. Additionally, leukocytes are central players in emerging cell-based immunotherapies to treat cancer, including efforts to engineer leukocytes with synthetic receptors specifically targeting tumor cells. The ability to synthetically control and modulate the recruitment of leukocytes could be broadly therapeutically useful. However, current efforts to do so are limited by important outstanding questions about how attractant receptors direct leukocyte migration. We still lack molecular mechanisms explaining how directionality, response sensitivity, and prioritization among chemoattractants are achieved. To address these questions, we are using a combination of systematic genetic perturbations and an automated live-cell imaging assay to distinguish factors controlling different aspects of the migration response. Furthermore, we have developed tools to directly measure spatial propagation of intracellular signals downstream of activated receptors, including the integration of receptor inputs with pre-existing cell polarity. By understanding pathway specialization and crosstalk in this signaling network, we aim to identify strategies for fine-tuned control of leukocyte recruitment.

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AN INTRINSIC FAST CELL CYCLE QUALIFIES THE CELL-OF-ORIGIN FOR MLL-AF9 MEDIATED TRANSFORMATION

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New Innovator Award, 2016

Active proliferation and impaired differentiation are two key features of cancer. However, whether these features are acquired consequent to oncogene activity, or inherited from the cancer cell-of-originremains elusive. Here, we report that an intrinsically fast-proliferating progenitor population initiates transformation by preserving its progenitor-like state in MLL-AF9 mediated leukemic transformation. Using a novel doxycycline (Dox) inducible MLL-AF9 knock-in mouse model yielding acute myeloid leukemia (AML), we uncoupled the normal intrinsic cell cycle kinetics from the proliferative response to prolonged oncogene expression. Combined with a single cell assay relating transformation efficiency to the cell cycle kinetics, we determined that the probability of a cell undergoing transformation is dictated by their intrinsic cell cycle kinetics. Overall, the faster a cell divides, the more likely it transforms. The fastest cycling subset of myeloid progenitors, those that divide 3 times or more within 24 hours, significantly enrich for transformed colony formation *in vitro* and induce lethal AML earlier *in vivo*. Importantly, transient cell cycle deceleration by a CDK4/6 inhibitor, Palbociclib, at the precise time window of oncogene induction, significantly reduced transformation, both *in vitro* and *in vivo*. These data indicate that cells with an intrinsically fast cell cycle provide the oncogene with a permissive cellular context to exert its function, qualifying them as the cell-of-origin for MLL-AF9 mediated transformation. We investigated the molecular mechanism of this phenomenon and discovered that MLL-AF9 functions to sustain the pre-existing gene expression program to initiate malignancy. Thus, the malignant cell fate induced by MLL-AF9 reflects the preservation of a molecular and cellular state normally occupied by the fast cycling myeloid progenitors. The quiescent/slow cycling hematopoietic stem cells are unfit to be the direct cell-of-origin, but rather are the likely source for sustaining the rapidly cycling progenitor compartment.

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REPAIRING RECESSIVE COMPOUND HETEROZYGOUS MUTATIONS IN VIVO VIA CAS9-MEDIATED ALLELIC EXCHANGE

Wen Xue

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New Innovator Award, 2016

Cas9-mediated gene correction by homology-directed repair (HDR) is a promising therapeutic strategy. However, monogenic genetic diseases typically can be caused by multiple, distinct mutations in the disease gene; treating a population of patients would require a different single-guide RNA (sgRNA) and donor template pair to repair each distinct lesion. Unlike animal models, whose mutations are usually homozygous, compound heterozygous mutations prevail in patients. Here, we report a genome-editing

strategy that recombines the pre-existing, correct genetic information present in the two heterozygous alleles into one functional allele, without the use of an exogenous DNA repair template. Recombinant adeno-associated viral (rAAV) vector delivery of Cas9 and a single sgRNA induced allelic exchange and rescued the disease phenotype in a mouse model of hereditary tyrosinemia type I (HT1) that carries compound heterozygous Fah gene mutations. We further showed phenotypic correction in heart for a lysosomal disease mouse model, suggesting that our strategy can target post-mitotic tissues where HDR is generally ineffective. Our strategy can be generalized to repair other compound heterozygous mutations, a genotype category that comprises a large population of patients with recessive genetic disorders.

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HIBERNATION IN A NOVEL RODENT MODEL: TOWARD THE GENETIC AND MOLECULAR BASIS OF TORPOR IN MAMMALS

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Early Independence Award, 2015

Hibernating mammals provide a natural example of torpor, a state of significantly depressed metabolism with potential applications in medicine. Despite longstanding historical interest in hibernation, a detailed understanding of its genetic and molecular basis is lacking. The meadow jumping mouse (Zapus hudsonius) is a small North American rodent that hibernates in response to shortened day length. We have developed these animals as a convenient model of hibernation because they can be maintained in a laboratory setting and induced to hibernate. Our work to de novo assemble and annotate the meadow jumping mouse genome has allowed comparative genomic analysis with other hibernating and non-hibernating species and provided the ability to study gene expression during torpor. To understand the cell-autonomous response to cold, meadow jumping mouse cell lines were exposed to temperatures typical of active and hibernating animals and subjected to RNA sequencing and biochemical analysis. The observed changes in gene expression and other cellular functions in isolated cells serve as a baseline for understanding the changes in cells and tissues seen during hibernation in intact animals. Preliminary results suggest that hibernation in mammals does not require unique genes that are lacking in non-hibernators, but that hibernation is instead based on differential regulation of conserved mechanisms. The meadow jumping mouse model has greatly enabled mechanistic hibernation research and will facilitate future discoveries.

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GATEKEEPERS OF MITOCHONDRIAL NAD+

Xiaolu Cambronne

University of Texas at Austin

New Innovator Award, 2017

Nicotinamide adenine dinucleotide (NAD) is a key intermediary metabolite compartmentalized in mitochondria as a distinct subcellular pool. Our previous work using a sensor for oxidized free NAD^+ demonstrated that mitochondrial concentrations are relatively higher than the nucleocytoplasm and independently regulated. In addition to driving flux through the citric acid cycle, free mitochondrial NAD^+ is turned over as substrate by NAD^+-consuming enzymes, such as SIRT3. Currently, however, we do not fully understand how free NAD^+ concentrations are sustained in mammalian mitochondria to counteract local consumption. NAD^+ cannot passively diffuse through double membranes and only a few specialized cell types can locally synthesize mitochondrial NAD^+. The challenge is that while mitochondrial NAD^+ transporters have been identified in yeast and plants, a direct mammalian homologue has been thus far elusive. Understanding how mammals sustain mitochondrial NAD^+ is critical for understanding how mitochondrial health can contribute to multiple human disorders.

Following demonstration that much of the mitochondrial NAD^+ in mammalian cells was sourced from the cytoplasm, I used the NAD^+ sensor in a focused RNAi screen to identify candidates that influenced mitochondrial NAD^+ concentrations. A top hit was a human cDNA corresponding to a six-transmembrane protein, which localized to the inner-mitochondrial membrane. Ectopic expression of this human cDNA complemented the fermentative growth phenotype of a yeast strain deleted for its mitochondrial NAD^+ transporters. To biochemically determine whether this candidate directly transports NAD^+, my group is currently pursuing the purification of this protein with Dr. Isabelle Baconguis. We are also investigating the candidate's role in deacylation of the mammalian mitochondrial proteome. Identification of a mammalian transporter would not only address the unanswered question of how mitochondria refuel NAD^+, but because mitochondrial heath and bioenergetics should depend on this mechanism, it may represent a target whose activities could contribute to disease onset.

Neuroscience

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SPINAL CORD NEURAL INTERFACE FOR NEUROPROSTHETICS IN A PRIMATE MODEL

Abhishek Prasad

University of Miami

New Innovator Award, 2015

There are currently 282,000 cases of spinal cord injury (SCI) in the United States (US) alone, with about 17,000 new cases occurring each year. During SCI, the descending fibers in the spinal cord are damaged and therefore, they lose their connections with the lower motor neurons that control the skeletal muscles. If the injury is at the cervical level, it results in quadriplegia with little or no function remaining in the four limbs. This patient population can benefit significantly from neural prosthetics to restore movement. Here, I will describe efforts to construct a neural interface by utilizing descending signals in

the intact regions of the spinal cord above the point of injury. The common marmoset (Callithrix jacchus) has been proposed as a suitable bridge between rodents and larger primates. They have been used in several types of research including auditory, vocal, visual, pharmacological and genetics studies. However, they have not been used for behavioral neuroscience studies. I will present new results on the feasibility, long-term stability, and decoding of signals recorded from marmoset cortex and spinal cord. The ability to chronically record cortical and spinal signals for neural prosthetics applications in the common marmoset extends the potential of this small non-human primate model in neural interface research.

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CIRCUIT AND BEHAVIORAL MECHANISMS OF OBJECT LOCALIZATION IN MICE

Andrew Hires, Jon Cheung, Philip Maire

University of Southern California

New Innovator Award, 2017

Behavior is driven by activity patterns in neural circuits. Identifying the computations performed by neural circuits in animal models is supposed to reveal general mechanisms of perception. However, even tasks with identical constraints may be approached in different ways, leading to differences in what information is gathered, how it is weighed and acted upon. We investigate the impact of variation in approach on perceptual computations using single whisker-based object localization along the anteroposterior axis of head-fixed mice. We find that for identical tasks, mice deploy a range of exploration strategies. Choice of strategy produces different sets of sensorimotor features available to drive location perception. This gives rise to differences in optimal and observed decision criteria. We use supervised learning to identify the key sensorimotor features that predict psychometric performance. By manipulating the task difficulty, we find that more difficult tasks bias exploration and decision strategies towards those which integrate more sensorimotor features. The features used include number of touches, whisker angle and motion components at touch, but not radial distance to the object. Identification of these features suggests which neural correlates of sensorimotor features are meaningful for circuit computations that drive perception. We then use loose-seal juxtacellular recordings from excitatory neurons in layer 5B of primary somatosensory cortex to identify electrophysiological signatures of these features. We show that activity patterns in a large portion of these neurons encode object location in head-centered coordinates. We quantify the angular resolution of this population code using a naïve Bayes classifier.

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RATIONAL DESIGN OF SILICON STRUCTURES FOR OPTICALLY-CONTROLLED MULTISCALE BIOINTERFACES

Bozhi Tian

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New Innovator Award, 2016

Silicon-based materials have been widely used. However, remotely controlled and interconnect-free silicon configurations have been rarely explored, because of limited fundamental understanding of the complex physicochemical processes that occur at interfaces between silicon and biological materials. Here, we describe rational design principles, guided by biology, for establishing intracellular, intercellular and extracellular silicon-based interfaces, where the silicon and the biological targets have matched properties. We focused on light-induced processes at these interfaces, and developed a set of matrices to quantify and differentiate the capacitive, Faradaic and thermal outputs from about 30 different silicon materials in saline. We show that these interfaces are useful for the light-controlled non-genetic modulation of intracellular calcium dynamics, of cytoskeletal structures and transport, of cellular excitability, of neurotransmitter release from brain slices, and of brain activity in vivo.

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A HUMAN STEM CELL-DERIVED NEUROMUSCULAR JUNCTION MODEL FOR AMYOTROPHIC LATERAL SCLEROSIS

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New Innovator Award, 2017

Amyotrophic lateral sclerosis (ALS) is a devastating disease of the motor nervous system. While mouse models have been the basis for mechanism and drug evaluation for more than 20 years, only about 10% of ALS cases result from monogenetic mutations. Thus, the accuracy and direct relevance of the mouse models to the vast majority of ALS patients remain unclear. A key question in ALS research focuses on distinguishing between a “dying back” versus “dying forward” pathogenesis, whether the disease originates in the distal terminals of the lower motor neuron or in the cell body of the lower or even upper motor neuron. To help address these issues, we developed an iPSC-derived model that captures the lower motor neuron and neuromuscular junction (NMJ). Key features include (1) co-localization of motor neuron-specific presynaptic and skeletal muscle-specific postsynaptic NMJ markers, (2) physiological function with contraction of muscle that is abrogated by specific NMJ blockers, (3) generation of astrocytes, as astrocyte have been shown to contribute to ALS in a non-cell autonomous manner. The use of more complicated in vitro models in ALS may help assess the contributions of different cellular processes, such as axonal transport, for which a more complete biological context may reveal more robust phenotypes, and motor neuron excitability, for which in vitro studies of motor neurons alone have yielded different results after different culture periods.

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FINDING THE CELLS THAT TELL OUR EYES HOW TO FOCUS

Gregory Schwartz, Adam Mani, Jared Levine

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New Innovator Award, 2015

Your ability to read the words on this page depends on the precise focus of each letter's image onto the photoreceptors at the back of your retina. Humans have the ability to dynamically adjust focus over a limited range through accommodation of the eye's lens and pupil. More substantial errors in focus, called refractive errors, like myopia or hyperopia, are generally corrected with glasses, contact lenses, or surgery. Extreme cases lead to a variety of irreversible vision problems. Myopia (nearsightedness) occurs when the eye grows too long during development, so the image is focused in front of the retina. Environmental factors that remain incompletely understood have caused myopia to reach epidemic proportions. More than 2 billion people are currently myopic, and that number is expected to rise to 5 billion (50% of the world population) by 2050.

Despite extensive research on refractive disorders, no link has been established to the physiological responses of retinal neurons, which are known to be a key part of the disease mechanism. My work will provide this pivotal missing link. What has been established is that the retina itself, without input from the brain, knows whether the image is in or out of focus. The retina uses this "defocus" signal to regulate eye growth during development (emmetropization) and to drive pupil and lens accommodation to focus visual scenes in adulthood.

I will propose that a new cell type that my lab discovered is, in fact, the defocus detector used for accommodation and emmetropization. My interdisciplinary approach combines electrophysiology, genetics, pharmacology, circuit tracing, optical modeling, and behavior. This work promises enormous impact in our basic understanding of how defocus signals are used in the visual system as well as translational potential in developing new interventions for refractive disorders and reversing the epidemic of myopia.

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"SUBCELLULAR RNA-PROTEOME MAPPING" SUBTYPE-SPECIFIC GROWTH CONE CONTROL OVER CEREBRAL CORTEX CIRCUIT DEVELOPMENT, DIVERSITY, AND POTENTIALLY DISEASE, REGENERATION THROUGH ~AUTONOMOUS LOCAL RNA AND PROTEIN MACHINERY

Jeffrey Macklis

Harvard University

Pioneer Award, 2017

Formation and function of circuits throughout the nervous system, within and from cerebral cortex in particular, relies critically on molecular machinery localized in growth cones (GCs) at tips of growing axons. Previously unknown subsets of neurons, transcriptomes and proteomes localize to GCs to implement growth of axons toward specific targets, driving brain circuit development, function, likely maintenance, disease, failed regeneration. Previously, these subcellular and subtype-specific RNA/protein networks were not experimentally accessible directly from brain.

We developed subtype-specific GC purification and "subcellular RNA-proteome mapping" as a generalizable approach to investigate this molecular machinery. Applied to long-range axons of callosal projection neurons connecting the cortical hemispheres (also applied to other neurons), we identify that 1) native GCs possess remarkably rich molecular constituents for local protein synthesis, folding, turnover, suggesting function as "mini-cellular"/~autonomous units; 2) each subtype contains both distinct, subtype-specific machineray plus shared machinery; 3) hundreds of proteins and RNAs are enriched orders of magnitude in GCs compared to their own parent somata, indicating subcellular polarization of functions; 4) targeting motifs direct GC localization. We identify, e.g., that the hub mTOR pathway regulating cell growth is highly enriched in extending GCs compared with their own cell bodies, and that mRNA classes distribute within developing neurons based on a 5'TOP motif.

Beyond enabling identification of specific molecular substrates of circuit development, function, and mis-wiring causing neuronal circuit pathology, subcellular organization might also have important implications for degenerative diseases and regenerative strategies. It enables direct molecular investigation of subtype-specific GCs compared with GCs from mutant, regenerative, non-regenerative, or reprogrammed neurons to discover molecular mechanisms of circuit development, mis-wiring, lack of circuit/synaptic maintenance, and regeneration. The ability to directly compare multiple distinct GC-soma subtypes using multi-color sorting makes this approach broadly applicable to future studies in the fields of axon guidance, neurodevelopmental disorders, neurodegenerative disease, regeneration, and neuron reprogramming.

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CORTICAL CONTROL OF KINEMATIC PRIMITIVES IN MICE PERFORMING A CENTER-OUT REACH TASK

Jesse Goldberg

Cornell University

New Innovator Award, 2015

Motor sequences are constructed from motor primitives, hypothesized building blocks of movement, but neural circuits that pattern primitives into a trajectory remain unclear. Using automated homecage training and a novel forelimb sensor, we trained freely moving mice to initiate forelimb trajectories with tiny, decamicron-scale micromovements followed by large, millimeter-scale reaches to learned spatial targets. Hundreds of thousands of trajectories were decomposed into millions of kinematic primitives, while closed-loop, reach onset-triggered photoinhibition was used to test the roles of different motor cortical areas. Surprisingly, cortical inactivations did not affect reach direction nor the initiation, termination, or complexity of motor primitives. Instead inactivation of contralateral motor cortex reduced peak speeds and pathlengths of all primitives without changing their direction or timing, resulting in spatially contracted, or “shrunk,” trajectories that failed to reach rewarded targets. These findings implicate subcortical circuits in generating primitives and directing them to targets and, more generally, demonstrate the utility of an automated system for high-throughput dissection of neural circuits for motor control in mice.

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NETWORK CONTROLLABILITY IN THE INFERIOR FRONTAL GYRUS RELATES TO CONTROLLED LANGUAGE VARIABILITY AND SUSCEPTIBILITY TO NEUROMODULATION

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Early Independence Award, 2015

In natural language production, humans are confronted with considerable selection demands. We must select words from among alternative words to construct sentences that convey our intended meaning. In recent years, the left inferior frontal gyrus (LIFG) has been identified as critical to this ability. However, the mechanism(s) by which the LIFG interacts with other nodes (brain regions) in the language network remains poorly understood despite an increasing emphasis on "network-based" approaches to understanding the neurobiology of language. Here, we examined selection demands as a process relying on the LIFG's role in controlling activity in the brain. We posited that distinct network control roles relate to different language selection demands: whether several responses are acceptable (open-ended demands) vs. a single acceptable response (closed-ended demands). To test this hypothesis, we collected high resolution anatomical network data from 28 healthy adults. Then, we computed network controllability statistics in these networks. Out of the scanner, subjects performed two production tasks with open-ended responses (i.e., verb generation and sentence completion) and one with closed-ended responses (i.e., number naming) before and after transcranial magnetic stimulation (TMS). We found a double dissociation. Specifically, boundary controllability, a theoretical measure of a node's ability to integrate or segregate network activity, related to TMS effects on the open-ended tasks. Modal controllability, a theoretical measure of a node's ability to easily drive a network into specific hard-to-reach states, related to TMS effects on the closed-ended task. These findings suggest that selection under open-ended response demands depends on the LIFG's role in coordinating intermodular activity across the brain, whereas selection under closed-ended response demands depends on the LIFG's specialized role in driving the brain toward difficult-to-reach states. More broadly, these findings establish a mechanistic link between network controllability and controlled language processing that could guide interventions with brain stimulation.

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CELLULAR AND MOLECULAR IDENTIFICATION OF BREATHING PACEMAKER NEURONS

Kevin Yackle, Paul Wei, Matthew Collie

UCSF

Early Independence Award, 2016

Breathing is a seemingly simple, essential, automatic behavior that is generated by a cluster of several thousand neurons in the brainstem. On their own, this collection of neurons, called the preBötzinger

Complex, has the intrinsic ability to create a basic breathing rhythm which is then constantly modulated, for example, by sensory signals reflecting the body's carbon dioxide and oxygen levels or higher order brain centers that coordinate breathing with other behaviors, like vocalization. Despite the obvious, vital role of breathing and the prevalence and devastation of breathing pathologies like sleep apnea and sudden infant death, the key cell types and ion channels used to generate the breathing rhythm remain unknown. We have identified a novel, small subset of preBötC neurons that remain synchronously, rhythmically active after elimination of fast synaptic neurotransmission from the preBötC brain slice *in vitro*. Characterization of these neurons by single cell transcriptional profiling and patch-clamp electrophysiology has shown, surprisingly, that these neurons are heterogeneous, for example both excitatory and inhibitory, and connected by gap junctions which are required for their synchronous rhythmic activity. When synaptic signaling is intact, the autonomous rhythmic activity of these special neurons anticipates and is phase-locked to the rhythmic bursting of the entire preBötC, suggesting that they play a critical role in pacing the breathing rhythm. When they are excluded from the preBötC slice, the entire rhythm significantly slows and currently, we are using optogenetic approaches to tune the intrinsic frequency that these rhythmic neurons oscillate and to determine if this then entrains the breathing rate in the slice preparation and *in vivo*.

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FUNCTIONAL STRIATAL IMAGING DURING THE PROGRESSION OF PARKINSONISM

Nicolas Tritsch, Marta Maltese, Jeffrey March

New York University

New Innovator Award, 2017

Parkinson's disease (PD) is a common neurodegenerative disorder defined clinically by motor impairments known as parkinsonism (tremor, rigidity, slowness of movement and postural imbalance), and pathologically by the loss of dopamine (DA)-producing neurons in the substantia nigra pars compacta (SNc). Our current understanding of PD espouses a relatively static view of the nervous system, centered on the notion that parkinsonism arises from gross imbalances in the activity of striatal neuron subpopulations upon loss of DA. However, this view remains to be empirically tested. We developed a two-photon calcium imaging method to chronically monitor the activity of striatal projection neurons belonging to the direct and indirect pathways simultaneously in awake behaving mice with subcellular resolution. This approach enables, for the first time, longitudinal studies of the activity of genetically-identified neurons as the disease progresses. Our preliminary results reveal that current models incompletely account for neural activity changes observed following chronic loss of DA neurons, prompting a re-evaluation of the pathophysiology of PD.

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