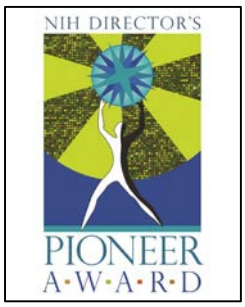

2015 NIH COMMON FUND HIGH-RISK, HIGH-REWARD RESEARCH PROGRAM SYMPOSIUM
NATCHER CONFERENCE CENTER, NIH CAMPUS
BETHESDA, MARYLAND
DECEMBER 7–9, 2015

Abstract Book

1.2

Illuminating Bacterial Signaling with RNA-Based Biosensors

Award Type: New Innovator Award

Award Year: 2011

Awardees: Ming C. Hammond, University of California, Berkeley

Presenter: Ming C. Hammond

Bacteria are both our friend and foe. They reside on our bodies and are ubiquitous in natural and manmade environments. To thrive in these different niches, bacteria adapt their physiology and lifestyle in response to other members of the microbial fauna, to host cells, and to abiotic surfaces. These changes underlie important interactions such as commensal and competitive relationships in microbial communities, pathogenesis leading to infectious disease, and beneficial effects of bacteria on animals, plants, and the environment. However, the signaling pathways that link environmental and chemical cues to the regulation of bacterial physiology are still incompletely understood. In the case of signaling mediated by second messengers, a major roadblock has been the difficulty in observing changes in the levels of these signaling molecules in live cells. As supported by the New Innovator Award, we invented a new class of fluorescent biosensors based on riboswitch scaffolds, which are among the first imaging tools for studying the temporal and spatial dynamics of cyclic dinucleotide signaling. We applied these biosensors to make several biological discoveries, including identifying components of a newfound signaling pathway that regulates surface attachment, which is required for bacteria to occupy and colonize different niches.

1.3

Cryo-EM Structure of the BK Ion Channel in a Lipid Membrane

Award Type: Transformative Research Award

Award Year: 2010

Awardees: Liguo Wang, University of Washington

Presenter: Liguo Wang

The large-conductance voltage- and Ca2+-activated potassium (BK) channel has many physiological roles including the control of firing patterns in neurons, the modulation of the tone of blood vessels and the regulation of heart rate. Among ion channels, it has served as a model system because of its remarkable ion-permeation properties and its accessibility for studies of allosteric control of gating.

As shown in both structural and functional studies, the lipid membrane environment has played an essential role for membrane proteins. To restore the lipid membrane environment, BK channels were reconstituted into liposomes (lipid vesicles) for both functional and structural studies. In 2009, we have reported the full-length BK structure in lipid environment at a resolution of 1.7nm using "random spherically constrained" (RSC) single-particle Cryo-EM method. Recent breakthrough in hardware and the optimization of image collection and processing techniques make it possible to achieve a higher resolution. Here, we present the BK structure in a lipid membrane in the closed state with a greatly improved resolution. The helices in the transmembrane region can be clearly identified, and the helix S0 is identified to be next to helices S2 and S3.

1.4

MOZART: High-Resolution Optical Molecular Imaging System

Award Type: Early Independence Award

Award Year: 2012

Awardees: Adam de la Zerda, PhD, Stanford University

Abstract Author(s): Adam de la Zerda, PhD, Stanford University

Presenter: Adam de la Zerda, PhD

We have developed a new high-resolution optical molecular imaging system, we call MOZART. It is the only imaging technique that is capable of providing real-time imaging of living tissue with single-cell spatial resolution over a large 3D imaging area of 2 mm3. This is achieved through innovations in nanoparticle synthesis, nanoparticle biofunctionalization, and image reconstruction algorithms. We have synthesized large gold nanorods (LGNR) with a 30-fold greater intensity per particle than conventional GNRs, and functionalized them for biological applications. We have demonstrated an imaging sensitivity of 40 nanoparticles per imaging voxel in living mice – approximately 1000-fold greater than other imaging modalities, such as photoacoustic contrast or fluorescence imaging. In this presentation, we describe the fundamental operation and application of MOZART. We show the ability to image small capillaries in tumor xenografts in living mice, highlighting the differences in vascular morphology between healthy and tumor tissue. We also show MOZART's functional abilities by multiplexed imaging of two spectrally-distinct LGNRs and use this to show lymph vessel drainage, including observing the functional states of individual lymphangions and valves in a lymphatic network. MOZART provides a promising platform for in vivo imaging of cellular expression of cancer biomarkers, visualizing intercellular signaling among 100 million cells to assess drug response or disease progression, such as may be associated with cancer metastasis. Following the presentation, attendees can view MOZART images on a 3D holographic display.

Session 2

2.1

Regulatory Protein Translation in the Human Nucleus

Award Type: New Innovator Award

Award Year: 2011

Awardees: Steven T. Kosak, Department of Cell and Molecular BiologyFeinberg School of Medicine, Northwestern University

Presenter: Steven T. Kosak

Abstract Author(s): Cogswell A.1, Fanslow D.1, Garza-Gongora A.1, Smith E.D.1, and Kosak S.T.1

1 Department of Cell and Molecular Biology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA.

Unlike the compartmentalization of cytoplasmic functions into membrane-bound organelles, activities within the nucleus are contained in non-membranous assemblies, or nuclear bodies (NBs). Protein synthesis in the nucleus was first reported over fifty years ago, and recent studies present strong and convincing evidence that nuclear translation does indeed occur. Although compelling, these studies have not addressed the functional significance of nuclear translation. Our investigation supports the idea of nuclear translation and indicates that protein synthesis in the nucleus is re-distributed to sites outside of the nucleolus under conditions of stress. These sites co-localize with the much studied but enigmatic promyelocytic leukemia (PML) nuclear bodies. Prior research suggests that PML bodies are involved in the stress response, protein homeostasis, and apoptosis. Interestingly, several components of the translation machinery including ribosomal proteins, eIF3, eIF4E, and elongation factor 1 are found in PML bodies, and eIF4E is required for their proper assembly. Our research suggests that PML bodies function as a site of protein quality control in the nucleus, particularly under stress. Moreover, we demonstrate that the nuclear aggregates characteristic of neurodegenerative disease is the result of this novel regulatory strategy being overwhelmed by aberrant mRNAs and misfolded proteins. We suggest that a variety of neurodegenerative diseases may be treated through the manipulation of PML body function.

2.2

Ether-Linked Phospholipids Modulate Stress Response in C. elegans

Award Type: Early Independence Award

Award Year: 2015

Awardees: Carissa Perez Olsen, Fred Hutchinson Cancer Research Center

Presenter: Carissa Perez Olsen

Ether-linked phospholipids including plasmalogens are a unique class of membrane lipids that are prevalent in eukaryotes but whose biological function is not yet understood. The vinyl-ether bond in plasmalogens is proposed to serve an important antioxidant function, and, here, we use Caenorhabditis elegans to explore the role of these ether-lipids in responding to oxidative stress. Using high performance liquid chromatography and tandem mass spectrometry (HPLC-MS/MS), we mapped the distribution of ether-linked phospholipids in the nematode and find that they represent nearly 10% of the membrane lipids. In fact, over half of the phosphatidylethanolamine population contains ether bonds, similar to the content of many mammalian tissues. Next, we characterized the ether-linked lipid synthesis pathway through homology with the mammalian enzymes and find that we can eliminate the production of these lipids with RNAi against three major synthesis genes including fard-1, a fatty acyl-CoA reductase. After establishing C. elegans as a model for studying plasmalogens, we looked for changes in the abundance of ether-linked phospholipids in animals exposed to oxidative stress and found an active upregulation of their production in control animals. Adult-only RNAi of fard-1 results in abrogation of the stress-induced ether lipid increase. Not only are fard-1 RNAi animals are sensitive to stress, but they also fail to remodel both ether-linked and typical ester-linked membrane lipids in response to oxidative insults. Taken together, we find that ether-linked lipids are a critical component of membrane remodeling in response to oxidative stress and that their depletion may contribute to membrane dysfunction and disease.

2.3

Conservation of a Fundamental Pathway of Stress Resistance from Worms to Man

Award Type: Pioneer Award

Award Year: 2010

Awardees: Bruce Yankner, Harvard Medical School,

Presenter: Bruce A. Yankner

Human neurons are functional over an entire lifetime, yet the mechanisms that preserve function and protect against neurodegeneration during aging are unknown. We recently showed that induction of the repressor element 1-silencing transcription/neuron-restrictive silencer factor (REST/NRSF) is a universal feature of normal aging in human cortical and hippocampal neurons. REST function is lost, however, in mild cognitive impairment (MCI) and Alzheimer's disease (AD). Chromatin immunoprecipitation with deep sequencing (ChIP-seq) and expression analysis show that REST represses genes that promote cell death and AD pathology, and induces the expression of stress response genes. Moreover, REST potently protects neurons from oxidative stress and amyloid β-protein (Aβ) toxicity, and conditional deletion of REST in the mouse brain leads to age-related neurodegeneration. To explore the evolutionary conservation of the REST stress resistance pathway, we investigated C. elegans orthologs of the REST co-repressor complex, the suppressor of presenilin signaling (spr) family of genes. Worms bearing mutations in these genes exhibit increased vulnerability to oxidative, thermal, genotoxic, and proteotoxic stress. Furthermore, loss of function mutations in the C. elegans REST ortholog spr-4 increase neuronal cell death and pathologic protein aggregation in worm models of AD and Huntington's disease. Finally, by utilizing a recently derived CRISPR-CAS9 transcriptional activator system, we show that activation of the endogenous spr-4 gene significantly increases lifespan. These results suggest that REST is a component of a conserved stress resistance pathway that protects against neurodegeneration and contributes to the regulation of organismal aging. This work was supported by an NIH Director's Pioneer Award (DP1OD006849).

Session 3

3.1

piRNA Biogenesis and Trans-Generational Epigenetic Inheritance

Award Type: New Innovator Award

Award Year: 2010

Awardees: Alexei Aravin, California Institute of Technology

Abstract Author(s): Alexei Aravin, California Institute of Technology

Presenter: Alexei Aravin

Small non-coding RNAs called piRNAs serve as the sequence-specific guides for an adaptable immune system that represses transposable elements in germ cells of Metazoa. In Drosophila the adaptation of the piRNA pathway to novel transposons is believed to occur when active transposons integrate into piRNA clusters, special genomic regions, which encode piRNA precursors. However, transposons carry termination signals that have a potential to disrupt transcription of piRNA clusters calling for the mechanism to prevent premature termination. The RDC complex is enriched on chromatin of dual-strand piRNA clusters and required for transcription of piRNA precursors. Here we dissected the function of RDC complex and show that its effector component, Cuff protein, prevents premature termination of RNA polymerase II by two distinct mechanisms. First, Cuff prevents cleavage of nascent RNA at poly(A) sites. Second, if processing does occur, Cuff stabilizes the 5'-monophosphorylated transcripts that are formed downstream of the cleavage site, by protecting them from degradation by the exonuclease Rat1. Our findings identify Rat1 as a new player in the piRNA pathway that works as a suppressor of transposon silencing and reveal a conceptually novel mechanism of transcriptional control through inhibition of termination.

3.2

Uniting Major Constituents of the Genome: A Novel Function of the PIwi-piRNA Pathway in the Germline

Award Type: Pioneer Award

Award Year: 2010

Awardees: Haifan Lin, Yale University

Abstract Author(s): Toshiaki Watanabe, Yale University

Presenter: Haifan Lin

The eukaryotic genome has vast intergenic regions containing transposons, pseudogenes, repetitive sequences, and noncoding genes that produce numerous long non-coding RNAs (lncRNAs) and PIWI-interacting RNAs (piRNAs). Yet the functions of the intergenic regions remain largely unknown. In mammals, a unique set of piRNAs, pachytene piRNAs, is abundantly expressed in the germline in late spermatocytes and early spermatids. Recently, we showed that piRNAs derived from transposons and pseudogenes mediate the degradation of a large number of mRNAs and lncRNAs in mouse late spermatocytes. In particular, they have a large impact on the lncRNA transcriptome, as a quarter of lncRNAs expressed in late spermatocytes are upregulated in mice deficient in piRNA pathway. Furthermore, our genomic and in vivo functional analyses revealed that retrotransposon sequences are frequently found in the 3' UTR of mRNAs that are targeted by piRNAs for degradation. Similarly, the degradation of spermatogenic cell-specific lncRNAs by piRNAs is mediated by retrotransposon sequences. Moreover, we have shown that pseudogenes regulate mRNA stability via the piRNA pathway. The degradation of mRNAs and lncRNAs by piRNAs requires MIWI and, at least in part, depends on its slicer activity. Together, these findings reveal a highly complex and global RNA regulatory network through which transposons and pseudogenes regulate target mRNA and lncRNA stability via the piRNA pathway to promote meiosis-spermiogenesis transition.

3.3

Apoptosis during Fetal Development Eliminates Clonally-Related Germ Cells

Award Type: New Innovator Award

Award Year: 2010

Awardees: Diana Laird, UCSF

Abstract Author(s): Daniel H. Nguyen, UCSF

Presenter: Diana Laird

The ability to pass on genetic information to the next generation requires that a germ cell successfully navigate a developmental gauntlet from specification through maturation into a gamete-producing cell. In mice, a significant portion of germ cells fails during this process and is eliminated through developmentally programmed waves of apoptosis during the fetal period and then again postnatally. The basis for this apoptosis, as well as its effects on the composition of the developing germline, remains poorly understood. We investigated the spatial distribution of male germ cell apoptosis in mice during the fetal wave (e12.5-e15.5) using wholemount imaging and showed mathematically that it occurs nonrandomly in highly localized clusters. We found no evidence for localized environmental factors contributing to this distribution, and the persistence of clustered germ cell apoptosis in a mutant that lacks specialized intercellular bridges formed by incomplete cytokinesis of fetal germ cells indicates that apoptosis operates independently on the basis of inherent properties of each cell. To determine if apoptotic clusters were clonally-related, we undertook random multicolor labeling with a lineage-specific drug-inducible Cre and the Rainbow and Confetti reporter alleles. After inducing labeling at the conclusion of germ cell migration (e10.5), we observed at e13.5 that clusters of apoptotic germ cells always shared the same color and hence derived from the same clonal ancestor. Multicolor labeling also facilitates a comparative perspective on clonal development and how clonal differences alter germ cell composition; variance in clone size suggests that clonal development is individualistic and uncoordinated across compartments. Our results suggest that germ cell clones have distinct developmental fates and are consistent with the function of scheduled apoptosis as a quality control mechanism in targeting specific clones for elimination.

3.4

Identification of Molecular Signature in Cystic Fibrosis Using Serum-Based Functional Genomics

Award Type: New Innovator Award

Award Year: 2010

Awardees: Hara Levy, Human Molecular Genetics Stanley Manne Children's Research Institute Ann and Robert H Lurie Children's Hospital of Chicago Department of Pediatrics, Section of Pediatric Pulmonary Medicine

Presenter: Hara Levy

Genomic technologies, including transcriptomics, offer unprecedented opportunities to advance our understanding of how environmental, genetic, and epigenetic factors modify disease progression. Importantly, there is no consistent phenotype-genotype correlation in cystic fibrosis (CF), perhaps due to protein-activity thresholds, modifier genes, and/or system dynamics. We have therefore validated an integrative genomics approach to assess the extracellular milieu associated with CF, an inherited, multisystem disease. In the present work supported by grant DP2 OD007031 (Integration of Genomics with Genetics—Molecular Phenotypes for CF Lung Disease), we analyzed plasma-induced transcriptional profiles in CF patients and correlated these data with common outcome measures including CFTR genotype, mutation class pancreatic function, pulmonary function, and infection status; we performed similar analyses in age-matched healthy controls. Our methodology, which utilizes serum-induced transcription in peripheral blood mononuclear cells functioning as reporter cells, consistently identified a small number of genes that are unique to CF patients and that correlate with disease severity. These genes, many of which are involved in immune and transcriptional regulation, are promising candidates for future therapeutic targeting.

This molecular signature has the potential to serve as a non-invasive clinical method for monitoring disease progression, clinical phenotypes, and response to treatment in CF. Our study's overall goal was to determine whether such a patient-based model system better captured disease complexity in CF than other approaches and could extended to other chronic lung diseases. Our gene-expression data discriminated between CF cases and controls, correlated with lung-function phenotypes, and identified new CF molecular phenotypes. Continued involvement with CF newborn screening programs will ensure the critical longitudinal follow-up of young patients necessary for determining whether profiles are established early in life and are correlated with the molecular signatures uncovered by our mononuclear cell-based protocol and validated with NanoString, RNAseq, and miRNA analyses. Our findings therefore lay the foundation for the inclusion of gene-expression arrays in real-time clinical settings, perhaps leading to the development of novel prognostic tools for CF and other lung disorders.

Tuesday, December 8, 2015

Session 4

4.1

Seek, Destroy and Heal: Disease-Responsive Nanoparticles as In Vivo Targeted Delivery Systems

Award Type: New Innovator Award

Award Year: 2011

Awardees: Nathan Gianneschi, University of California, San Diego

Presenter: Nathan Gianneschi

Nanoparticle targeting strategies have largely relied on the use of surface conjugated ligands designed to bind overexpressed cell-membrane receptors associated with a given cell-type. We envisioned a targeting strategy that would lead to an active accumulation of nanoparticles by virtue of a supramolecular assembly event specific to tumor tissue, occurring in response to a specific signal. For this purpose, we utilize enzymes as stimuli, rather than other recognition events, because they are uniquely capable of propagating a signal via catalytic amplification. We will describe the preparation of highly functionalized polymer scaffolds, their development as in vivo probes and their utility as a multimodal imaging platform and as drug carriers capable of targeting selectively to tumor tissue having efficacy in treating cancer. This approach represent an entirely novel direction in tissue targeting with promise in the treatment of cancer where current formulations have limited therapeutic effect due to toxicity and off-target accumulation.

4.2

Shrink Induced Manufacturing Platform for Low-Cost Diagnostics (SIMPL-CD)

Award Type: New Innovator Award

Award Year: 2010

Awardees: Michelle Khine, UC Irvine

Presenter: Michelle Khine

The challenge of traditional 'top down' micro- and nano-fabrication lies in the difficulties and costs associated with patterning at such high resolution. To make such promising technology – which could enable pervasive health monitoring and disease detection/surveillance – more pervasive, there is a critical need to develop a manufacturing approach such that prototypes as well as complete devices cost only pennies. Instead of relying on traditional fabrication techniques largely inherited from the semiconductor industry, my lab leverages the inherent heat-induced relaxation of pre-stressed thermoplastic sheets: commodity shrink-wrap film. We pattern at the large scale and achieve our desired structures by controlled shrinkage down to 5% of the original, patterned sizes. This enables us to beat the limit of resolution inherent to traditional 'top-down' manufacturing approaches.

With these tunable shape memory polymers, compatible with roll-to-roll as well as with standard lithographic processing, we can robustly integrate extremely high resolution, high surface area, and high aspect ratio nanostructures directly into our microsystems. Importantly, when the underlying polymer substrate relaxes and 'shrinks', a stiffer deposited thin film cannot and therefore buckles. We can control the buckling and therefore create nanostructures of deterministic sizes and patterns. Interestingly, our metallic nanostructures that self-assemble due to the stiffness mismatch between the thin metal film deposited on the retracting plastic sheet have demonstrated unprecedented electromagnetic field enhancements.

Our ultra-rapid fabrication approach therefore results in field-compatible plastic based microfluidic systems with integrated nanostructures for robust signal amplification. This design-on-demand approach to create a suite of custom biomedical tools for low cost diagnostics including sample prep with magnetic nanotraps, embedded on-chip electrodes, microlens arrays, surface enhanced sensing substrates, and flexible electronics. We have developed a process to lift-off the unique nanostructured patterns from the shrink plastic and to transfer them into other materials. This allows us to create truly conformal, high resolution epidermal electronics that move with the skin.

Our suite of tools has allowed us to create a platform to engage and to teach modern science and engineering concepts to the next generation of inventors through our outreach program, A Hundred Tiny Hands. Motivated by the facts that: > 67% of all engineers received their PhDs in the US are not US citizens, only 18% of working engineers are female, and that the US is ranked 52nd in STEM education, we clearly recognized that we need to come up with a better way to attract and retain our young scientists and engineers. Using the technology we have developed in our lab, we have developed Inventors Toolboxes for children as young as 5 years old to learn fundamental engineering concepts.

4.3

Nanoscale Tools to Advance Biomedical Frontiers

Award Type: Pioneer Award

Award Year: 2015

Awardees: Michael Roukes, Caltech

Presenter: Michael Roukes

I will describe two main projects carried out with support from my Pioneer Award:

1. Integrated Neurophotonics. In 2011, I banded together with with five other U.S. scientists from different disciplines, outlined a vision [1], and we then managed to convince the Obama administration of the unprecedented opportunity for launching a coordinated, large-scale effort to map brain activity. This culminated in the U.S. BRAIN Initiative (Brain Research through Advancing Innovative Neurotechnologies), which was launched in 2013. Our perspective was predicated, in part, on the current level of maturity of diverse fields of nanotechnology that can now be coalesced to realize powerful new tools for neuroscience. I will highlight our own development of the new field of integrated neurophotonics for realizing this vision. It leverages advances in integrated nanophotonics, optical reporters and effectors for neural recording and stimulation, and our recent developments in multisite neural nanoprobes for electrophysiology based on silicon large-scale integration.

2. Native Single-Molecule Analysis. We have employed NEMS (nanoelectromechanical systems) to realize a new form of mass spectrometry (MS) with single molecule sensitivity [2], and have demonstrated the analysis of individual large-mass biomolecular complexes, one-by-one, in real-time [3]. I will describe its prospects in the field of native MS, which focuses upon the topological investigation of intact protein complexes with a theoretically unrestricted mass range. Recently, we have developed an enhanced approach that greatly extends the capabilities of NEMS-MS by enabling imaging the spatial mass distribution of individual analytes – in real time, and with molecular-scale resolution – when they adsorb onto a NEMS resonator [4]. This new approach is somewhat akin to Ion Mobility Spectrometry, which provides rotationally-averaged molecular collision cross sections (CCS) – however, it is superior in providing single-molecule resolution, without rotationally averaging the CCS. NEMS-based single molecule analysis offers the prospect of direct stratification of generically-heterogeneous mixtures of protein complexes without arduous up-front sample preparation protocols, which will find important applications in analyzing complex protein assemblies, individual organelles and viruses, and membrane proteins.

[1] Alivisatos AP, et al., The Brain Activity Map project and the challenge of functional connectomics. Neuron 74, 970-4 (2012).

[2] Naik, AK, et al., Nature Nanotechnology 4, 445–450 (2009).

[3] Hanay, MS, et al., Nature Nanotechnology, 7, 602-608 (2012).

[4] Hanay, MS, et al., Nature Nanotechnology 10, 339-344 (2015).

4.4

An Accommodative Contact Lens for Presbyopic Correction

Award Type: New Innovator Award

Award Year: 2011

Awardees: Hongrui Jiang, University of Wisconsin-Madison

Presenter: Hongrui Jiang

Presbyopia is the most common ocular affliction and presents an extraordinary public health issue. Our goal is to correct presbyopia by developing a new type of contact lens called an accommodative contact lens (ACL) that incorporates a tunable lens for accommodation and devices to convert light energy to electricity and store it in situ for the operation. We first demonstrate different types of flexible lenses based on electrowetting on dielectrics, dielectrophoretic force, and Fresnel zone plates. These lenses are fabricated onto soft polymers for ultimate integration and embedment into contact lenses. We then report on light energy harvesting devices that can simultaneously achieve storage within the same single device structure. Our approach is to combine capacitive storage with dye-sensitized solar cells (DSSCs). To improve the charge storage capacity, we developed a novel hydrothermal process to prepare porous hierarchically nanostructured tungsten trioxide (WO3), and then applied WO3 to fabricate flexible supercapacitors as a storage device. Compared with traditional carbon electrodes, WO3 nanomaterials significantly enhanced energy storage capability. In order to improve the light-harvesting efficiency of our device, we introduced a light-trapping structure in the photoelectrode via a femtosecond laser ablation technique. The processed photoelectrode was then used to fabricate DSSCs to enhance the photon-harvesting efficiency (η) by up to 13.5%. We also developed an all-optical approach to enhance the photosensitivity of the overall imaging device by realizing micro-scale light concentrators. Lastly, we report on a fabrication platform to integrate the accommodative liquid lens, control electronics, and energy harvesting and storage device into the soft contact lens for presbyopic correction.

Session 5

5.1

Mapping the Human Toxome by Systems Toxicology

Award Type: Transformative Research Award

Award Year: 2011

Awardees: Thomas Hartung, Johns Hopkins University

Presenter: Thomas Hartung

Technological advances allow high-resolution biological phenotyping of the responses of cells and organisms for the elucidation of mechanisms of toxicity; these include the various omics, high-throughput and high-content technologies. Some of these information-rich tools have the potential to provide a molecular understanding of toxicological mechanisms and are the focal point of our transformative research project. The NIH Project "Mapping the Human Toxome by Systems Toxicology" (NIEHS grant R01ES020750) is a collaboration of Johns Hopkins Bloomberg School of Public Health, Brown University, The Hamner Institute, Georgetown University, U.S. EPA National Center for Computational Toxicology and Agilent; it aims to establish a workflow for the systematic identification and annotation of pathways of toxicity (PoT). A Human Toxome knowledge database and its governance shall be established. This shall represent a point of reference for the toxicology for the 21st century. The project combines untargeted mass spectrum-based metabolomics and gene array-based transcriptomics with bioinformatics. The pilot model is estrogenic response of MCF-7 cells, a well-established test for endocrine disruption.

In this presentation, we cover the recent developments in systems biology and toxicology in terms of computational tools necessary to cope with the flow of information-rich omics datasets. In particular, the bioinformatics tools for pathways of toxicity deduction represent a core deliverable and a real challenge. A number of contributions to quality and standardization of cell systems, omics technologies and bioinformatics will also be addressed. In addition, we developed frameworks and concepts for pathway annotation, validation and sharing. Generally, the presentation will present the general framework on how to use the new toxicity testing paradigm to create a Human Toxome and how this can change the paradigm of establishing safety and risk of substances. This demonstrates the value of systems approaches in toxicology and risk assessment.

5.2

Metabolic Control of Early Mammalian Development

Award Type: Pioneer Award

Award Year: 2012

Awardees: Utpal Banerjee, UCLA

Abstract Author(s): Raghavendra Nagaraj, UCLA

Presenter: Utpal Banerjee

Recent studies point to an essential role for intermediary metabolism in transcriptional control of gene expression. The transcriptional input of metabolism occurs at the level of epigenetic modifications of histone and non-histone proteins in the nucleus and is directed by metabolites derived from the tricarboxylic acid (TCA) cycle. In the mouse pre-implantation embryo, the transcriptional activation of the genome of the zygote (zygotic genome activation (ZGA), has been shown to require global epigenetic changes during 2-cell stage. Interestingly, at this stage a majority of the mitochondria of the early embryo are inactive and glycolysis is blocked. This is surprising since metabolic quietness seems incompatible with genome-wide remodeling. Embryos are dependent on pyruvate for development beyond 2-cell stage. Our results show that pyruvate is essential for zygotic genome activation and its lack in the culture medium results in global changes in multiple epigenetic marks. We find that a number of enzymes belonging to or associated with the TCA cycle that generate essential metabolites, are transiently localized to the nucleus during ZGA, thus enabling the synthesis of essential metabolites such as acetyl-CoA and alpha-ketoglutarate for global epigenetic reprogramming. These enzymes return to the mitochondrion following ZGA. Our studies further show the nuclear translocation of TCA cycle enzymes in the early embryos is pyruvate dependent, and requires O-linked glycosylation and HSP90 chaperon. Inhibition of OGT, an enzyme which O-glycosylates proteins, recapitulates the phenotypes seen upon pyruvate withdrawal, and prevents the translocation TCA cycle proteins to the nucleus. Finally, nuclear localization of a mitochondrial enzyme associated with the activation of the TCA cycle also showed nuclear localization in human embryos. Instead of the 2-cell stage seen in mouse, this nuclear localization occurred in 4-cell and 8-cell stages. Amazingly, this later stage is coincident with embryonic genome activation in humans. This work has long-term implications for research on obesity related infertility, cancer and stem cell function.

5.3

Extending Caenorhabditis elegans Lifespan by Extending the Duration of Young Adulthood

Award Type: New Innovator Award

Award Year: 2011

Awardees: Michael Petrascheck, TSRI

Abstract Author(s): Sunitha Rangaraju, Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA, USA

Presenter: Michael Petrascheck

Interventions that extend lifespan are generally thought to slow the course of aging across the lifetime of an organism. An alternative would be that some longevity mechanisms act only during specific periods of life thus extending lifespan by extending the duration of a specific period. However, as a molecular description of physiological age remains elusive, it has not been possible to distinguish these possibilities. We found that aging causes a profound loss of synchronized gene expression that is evolutionarily conserved from worms to humans. This loss of transcriptional synchrony, which we have termed “transcriptional drift”, parallels the age-associated loss of homeostatic capacity and provides a quantifiable measure for physiological age. Measuring transcriptional drift in C. elegans allowed us to dissociate physiological age from chronological age, and revealed that pharmacological inhibition of serotonergic signals extends lifespan by exclusively prolonging the duration of young adulthood. Thus, our results show that aging mechanisms exist that exclusively act during certain periods of time and that lifespan can be extended by specifically extending a period in life.

2.1

Direct Observation of Force-Induced Conformational Transitions in F-Actin

Award Type: Early Independence Award

Award Year: 2013

Awardees: Gregory M. Alushin, NHLBI / NIH

Abstract Author(s): Pinar S. Gurel, NHLBI / NIH

Presenter: Gregory M. Alushin

The ability of a cell to sense and respond to the mechanical properties of its environment ("mechanosense") influences many core cellular processes, including division, migration, differentiation, and survival. Dysregulation of mechanosensory pathways has recently been implicated in malignant transformation, tumorigenesis, and metastasis, highlighting this process as a key element of cancer progression. At the foundation of this behavior lies an intricately coordinated contractile network consisting of the actin cytoskeleton, myosin motor proteins, and their myriad binding partners; however, we currently lack a basic understanding of the underlying molecular mechanisms connecting actin to cellular mechanosensation. Actin filaments are flexible polymers that can adopt multiple conformational states, and recent evidence suggests that forces applied to filaments can influence interactions with binding partners. Here, we explore how mechanical stimuli alter the actin filament structural landscape and how this may influence downstream interactions, potentially serving as an initial signal in mechanotransduction. We have developed a novel reconstitution system to place actin filaments under tension suitable for functional biophysical studies with fluorescence microscopy and high-resolution structural studies with cryo-electron microscopy (cryo-EM). Using a modified gliding assay, we find that the combined activity of two surface-immobilized myosin motor proteins which move in opposite directions induces mechanical strain in filaments, evidenced by increased straightening at the micron scale and breakage in the presence of ATP. In contrast, either myosin alone produces processive gliding of filaments. Cryo-EM micrographs demonstrate global straightening of filaments at the nanometer scale in the presence of ATP and reveal the appearance of a novel architectural feature at discrete sites along filaments. High-resolution studies are currently underway to investigate the conformational changes produced in actin in detail. Resolving these structures will provide unprecedented insight into the molecular mechanisms of mechanosensation and ultimately advance the development of targeted therapeutics against specific actin conformational states.

2.36

Next-Generation Drug Discovery

Award Type: New Innovator Award

Award Year: 2011

Awardees: Brian Paegel, The Scripps Research Institute

Presenter: Brian Paegel

The NIH Molecular Libraries Program (MLP) was founded to translate the discoveries of the Human Genome Project into therapeutics. With gene sequences (and thereby target identities) in hand, the only obstacle to discovery was access to high-throughout screening (HTS) technology. A decade of discovery produced hundreds of probes — highly selective small molecules that modulate cellular function — but the Genome Project's promise of proteome-wide drug discovery remains out of reach because centralized compound screening bears the same cost and infrastructure burdens of millennial DNA sequencing centers. We are building a next-generation drug discovery platform to eliminate the need for HTS centers. We have developed DNA-encoded solid-phase synthesis to generate ultra-miniaturized compound libraries and engineered microfluidic instrumentation for miniaturizing automated screening. An integrated circuit loads individual microscopic compound library beads into picoliter-scale droplets of assay reagent. Compound attached to the bead via photolabile linker is released into the droplet in a UV dose-dependent fashion. The dosed droplets are then incubated, evaluated for activity using laser-induced confocal fluorescence detection, and sorted for PCR amplification and high-throughput sequencing. To demonstrate the feasibility of the platform, we synthesized a modest (~100k compounds) DNA-encoded library of aspartic protease inhibitors, developed an HIV protease activity assay, and demonstrated high-throughput dose-response screening. Not only are the molecular libraries and screening technology deployable in any laboratory setting, but dose-response screening will generate whole-library structure activity relationship profiles. The unprecedented molecular detail of these data will yield portfolios of new leads and replenish the pipeline of therapeutics, especially those targeting rapidly-evolving bacterial and viral pathogens.

2.45

Cortical Hierarchy Underlies Preferential Connectivity Disturbances in Schizophrenia

Award Type: Early Independence Award

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Awardees: Alan Anticevic, Yale University

Abstract Author(s): Genevieve Yanh, Yale University

Presenter: Alan Anticevic

Schizophrenia (SCZ) is a neuropsychiatric illness associated with abnormal neural connectivity. In particular, patients show prefrontal cortex (PFC) hypo-connectivity, assessed by computing blood-oxygen level-dependent (BOLD) signal correlations. However, recent studies reveal elevated BOLD signal variance in SCZ, which may impact correlations, computed as covariance normalized by variance. We hypothesized that functional connectivity (using covariance) may be elevated in SCZ, but that this may occur in the context of elevated signal variance. Further, we hypothesized that preferential PFC effects may intrinsically arise from the information-processing hierarchy, with corresponding physiological consequences, which we tested via biophysically-grounded computational modeling.

We conducted resting-state fMRI in 161 SCZ and 164 matched healthy subjects, assessing group differences in connectivity and BOLD variance. Both voxel-wise and network-level analyses were performed. To mechanistically inform fMRI findings, we used a biophysically grounded neural network model to simulate a well-known synaptic hypothesis of SCZ pathology—namely excitation/inhibition (E/I) imbalance, and analyzed resulting in silico 'BOLD' signals.

Empirically, we observed hyper-connectivity in PFC and other associative regions in SCZ, with concurrent increases in BOLD variance. These effects were absent in a comparison group of bipolar patients (N=73). In initial simulations of increasing E/I imbalance, we observed global elevations in covariance and variance of model-generated BOLD signals. To investigate our empirical associative effects, we extended our model to reflect known differences in associative vs. sensory neuronal dynamics. This extended model reproduced preferential associative effects, and predicted that covariance and variance elevations would be positively correlated, which we confirmed empirically.

Collectively, we show that elevations in BOLD covariance and variance in chronic SCZ co-occur and are strongly related phenomena. Thus, some connectivity elevations may not be fully captured by correlation measures that normalize connectivity by variance. Hypo-connectivity seen in previous studies may be reconciled with our findings by considering the effect of elevated variance in reducing correlation-based measures. We also computationally demonstrate how a common cellular-level mechanism can produce elevations in covariance and variance of BOLD signals. Further, we show how this global perturbation can produce preferential effects in associative regions due to neural differences arising from the intrinsic functional cortical hierarchy.