2017

ddir innovation awards

National Institutes of Health
Office of Intramural Research

ddir innovation awards

2017 marked the inaugural year for the DDIR Innovation Awards program. The program provides seed money to stimulate innovative, high-impact research, and to foster collaborations. The program offers intramural investigators three types of award — a program project award for a team of 3 to 5 independent investigators, a center/facility award, and an award for collaborations with extramural investigators or industry. In 2017, we particularly welcomed proposals in the scientific fields that were identified as priorities as part of the intramural long-term planning process (inflammatory diseases, cell-based therapies, microbiome, drug resistance, neuroscience, RNA biology and therapeutics, vaccines, natural products, and animal modeling), but other topics were also considered. The program made 25 awards to intramural investigators, ranging from $48,000 to $750,000 each, with a total of $6.9 million dollars awarded.

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Peptide-conjugated PMOs to treat a common COL6 mutation via exon skipping

PI

Carsten Bonnemann (NINDS)

Project Summary

Collagen VI-related muscular dystrophies (COL6-RD) manifest with significant early childhood-onset muscle weakness, joint contractures, scoliosis and respiratory insufficiency. There is currently no specific disease-modifying treatment available. COL6-RD are caused by absence or dysfunction of the collagen VI protein in the connective tissue of skeletal muscles, leading to muscular dystrophy via multiple yet incompletely understood pathways. Recently our group, in a global collaborative effort, has uncovered a new and surprisingly common de novo deep-intronic splice-inducing mutation in one of the collagen 6 genes (COL6A1 c.930+189C>T, thereafter referred as +189C>T) promoting the insertion of an in-frame pseudoexon in the mature COL6A1 mRNA. The resulting mutant isoform is capable of interacting with wild-type collagen VI counterparts to exert a strong dominant-negative effect on collagen VI production. This newly described mutation bears high translational potential, as it can be rescued using a “pseudoexon-skipping” strategy, with the potential to suppress the splice defect to fully restore COL6A1 expression. Exon skipping, or pseudoexon-skipping in our case, can be achieved using antisense oligonucleotides, that can block access of the splicing machinery via targeted base pairing to key sequence elements present in the pre-mRNA, resulting in the specific exon to be ignored, or “skipped”. Importantly, splice modulation (including exon skipping) as a therapeutic principle is already in clinical use with recent FDA approval of two splice-modulating drugs (Spinraza and EXONDYS51), supporting the potential for rapid clinical development of an exon-skipping therapy for COL6-RD. Our preliminary data validate that the COL6A1 pseudoexon-inducing mutation is an excellent target for splice modulation, as we have already successfully repressed pseudoexon inclusion using phosphorodiamidate morpholino (PMO) antisense oligos (AOs) in patient-derived fibroblasts (NINDS patent on oligo target sequences). However, efficient *in vivo* targeting remains challenging. Amongst the most exciting recent developments in AO chemistries, peptide-conjugated PMOs (PPMOs) emerge as highly promising drugs, achieving enhanced potency by facilitating intracellular delivery, both in *in vitro* and *in vivo* applications. Here we propose to develop PPMOs to advance a pseudoexon-skipping therapeutic strategy for the COL6A1 +189C>T mutation, which also represents the first pseudoexon mutation creating a dominant protein isoform that is completely treatable by pseudoexon skipping. In complement to this application, we are generating a humanized model of the COL6A1 +189C>T mutation, that will allow the direct testing of the PPMOs developed here for the human sequence, to accelerate their clinical development.

Ultrasensitive detection of tau aggregates for tauopathy diagnostics and therapeutic trials

PI

Byron Caughey (NIAID)

Project Summary

Many neurodegenerative diseases such as Alzheimer's disease, chronic traumatic encephalopathy and other dementias involve the pathological accumulation of a protein called tau. The societal burden of these diseases, called tauopathies, is enormous and increasing with the aging of humankind. Alzheimer's disease alone accounts for ~6 million cases of dementia in the US, and is expected to increase to 15 million in the next 40 years. There is also increasing concern about tauopathies in younger people, notably chronic traumatic encephalopathy in both children and adults who participate in contact sports involving repeated head trauma. This project aims to develop ultrasensitive and specific tests for various disease-associated tau deposits in order to allow earlier and more accurate diagnosis of tauopathies. The strategy is to exploit the basic self-propagation mechanism of pathological tau deposits to amplify them to easily detectable levels *in vitro*. We have used this strategy to develop an ultrasensitive test called tau RT-QuIC for a tauopathy called Pick disease. Mechanistically related RT-QuIC tests are being broadly implemented for the clinical diagnosis of human prion diseases, which are caused by the pathological accumulation of prion protein, using cerebrospinal fluid or nasal brushings. In this project, we will adapt and optimize the tau RT-QuIC tests for other more common tauopathies that are not well-detected by our current Pick disease test. This will require the screening of different tau-based sensor molecules (substrates) and reaction conditions. Such tests should allow patients and clinicians to more appropriately focus care and treatment options on the specific underlying molecular causes of neurodegenerative disease. Clearer and earlier diagnosis of tauopathies should also facilitate therapeutic trials by improving selection of patient cohorts and allowing etiological biomarkers to be monitored longitudinally in treatment versus control groups.

Novel camel single domain antibody-based therapeutics

PIs

Mitchell Ho (NCI), Ira Pastan (NCI)

Project Summary

Antibody-based therapeutics are now a major component in the cancer treatment landscape. However, conventional antibodies are not effective in targeting many important antigens such as receptor/ligand signaling complexes, ion channels and peptide-MHC complexes. These protein groups contain functional sites that are unreachable by conventional antibodies because these antibodies usually have a flat surface as well as due to steric hindrence. Camel antibodies are small and made up of a single domain, so that they can fit into small cavities and buried functional

sites on proteins. Therefore, camel single domain antibodies have the potential to emerge as an important class of therapeutic antibodies that would have applications in precision cancer treatments, as well as in the treatment of other diseases. In this proposal, we aim to generate a very large antibody phage display library based on camel single domains and to screen this library for binders with therapeutic applications. We will use the library to develop a pipeline of novel therapeutic antibodies for the treatment and diagnosis of cancer, infectious diseases and neurological diseases.

High-resolution zoom-in CT for the diagnosis of lung disease

PIs

Han Wen (NHLBI), Marcus Chen (NHLBI), Joel Moss (NHLBI)

Project Summary

Computed tomography (CT) is a diagnostic imaging exam that uses penetrating x-rays to see the internal structures of the body in 3D. It has been proven effective in screening for lung cancer and in detailed evaluation of various types of lung diseases such as emphysema and chronic obstructive pulmonary disease (COPD). As lung CT becomes used more widely, there is a rising need to improve the level of detail of the images while maintaining a low radiation dose to the patient, since more detail of the lung tissue provides more accurate assessment of the type and severity of lung disease. Our project aims to implement a novel technology to substantially improve the level of detail of the CT scan while keeping the radiation dose and the scan time at the levels of a low-dose chest CT exam. It is a collaboration between NIH and industry partners that specialize in medical x-ray technologies.

Robotic electrical potential measurement to treat epithelial edema

PI

Kapil Bharti (NEI)

Project Summary

Epithelial tissues are formed by polarized cells that can segregate apical and basal compartments of epithelial tissue. This polarization feature pf epithelial cells is critical for them to maintain anti-inflammatory properties in that tissue. For example, within a healthy human eye, the retinal pigment

epithelium (RPE) transports water from the apical (retina) side to the basal (blood side), supported by cells' polarity, maintaining minimal inflammation in the eye. This polarity and water transport ability is disrupted by several well used anti-cancer drugs and antibiotics. However, the mechanism of action of these drugs on RPE cells in not known. We propose to develop a robot that can measure polarity of RPE cells in fully-automated manner. We have collaborated with an extramural co-investigator, Dr. Craig Forest, Associate Professor, Georgia Institute of Technology (GT), Atlanta GA, with expertise in miniaturized robotics and a track record of developing and implementing high-throughput single cell electrophysiology instrumentation. This robot will enable large-scale discovery of drug toxicity in RPE as well as other epithelia that suppress inflammation using barrier permeability similar to RPE.

Center on compulsive behaviors

PIs

Veronica Alvarez (NIAAA), Susan Amara (NIMH), Bruno Averbeck (NIMH), Antonello Bonci (NIDA), Andres Buonanno (NICHD), Guohong Cui (NIEHS), Zayd Khaliq (NINDS), George Koob (NIAAA), Michael Krashes (NIDDK), David Lovinger (NIAAA), Katherine Roche (NINDS), Marc Reitman (NIDDK), Yavin Shaham (NIDA), Constantine Stratakis (NICHD), Jerrel Yakel (NIEHS)

Project Summary

Compulsive behaviors are repeated, perseverative actions that are difficult to inhibit despite clear intentions and harmful consequences for the patients. While their expression is diverse – from ticks to compulsive eating and addiction — compulsive behaviors are driven by shared neuro-circuitry that must be studied at multiple levels, from genes and molecules to systems and behavior. Understanding the processes that promote or inhibit the development of compulsive behaviors is critical not only for optimizing existing treatments, but also for designing new pharmacological and behavioral interventions.

The purpose of the CCB is to advance scientific discovery in the field of compulsive behaviors and to develop expertise on the topic within the IRP. The center will bring together basic scientists and clinical researchers across the neuroscience community from seven different Institutes and will provide unique opportunities for collaborations and interaction.

The goals of the CCB are to understand the neurobiology of complex behaviors that result in compulsive and repetitive actions, and to develop and test new therapeutics aimed at alleviating or reversing these behaviors. CCB will foster collaborations across the IRP neuroscience community and train future experts in the field of compulsive behaviors.

Natural product library acquisition for Klebsiella screening

PIs

Matthew Hall (NCATS), Susan Gottesman (NCI), Susan Buchanan (NIDDK)

Project Summary

Compounds from nature provide have been the source of a stunning proportion of anti-infectives, including antibiotics and antiparasitics, and provide the advantage of dramatic chemical diversity.

The National Center for Advancing Translational Sciences (NCATS) conducts collaborative high-throughput screening, with libraries composed of small molecules generated through synthetic chemistry. While they are highly diverse, NCATS does not currently possess a significant natural products (NP) library. The laboratories of Susan Gottesman (NCI) and Susan Buchanan (NIDDK) have jointly developed an assay for identifying agents active against capsule synthesis in the clinically-important Klebsiella pneumoniae, and this has been optimized for high-throughput screening at NCATS. This award will create a natural product extracts screening capability for the NCATS Chemical Genomics Center. It a genuine opportunity to identify novel antibiotics against Klebsiella pneumoniae and other gram-negative bacteria. In addition, it will also generate a research capability available to other intramural investigators working in the discovery space around infectious diseases, many of which are rare and/or neglected and currently in development at NCATS (such as Zika virus, Rabies virus, and malarial targets). This is an area of unmet global need, and a capability not currently available to intramural investigators through NCATS.

Establishment of a CRISPR-based trans-NIH functional genomics HTS platform

PIs

Marc Ferrer (NCATS), Madhu Lal-Nag (NCATS), Ji Luo (NCI), Kapil Bharti (NEI), Michael Ward (NINDS), Natasha Caplen (NCI)

Project Summary

The Trans-NIH RNAi screening facility (TNRF) is a shared intramural resource with the mission of conducting large-scale RNAi screens to explore the function of genes in cell biology and diseases. Recently, CRISPR/Cas9 technology has emerged as a powerful genome editing tool that provides an orthogonal/complementary approach to RNAi for large scale functional genomics screens. This award will bring together the expertise of several NIH investigators to enable CRISPR-based screening at the TNRF, by validating different CRISPR/Cas9 screening approaches and acquiring libraries that will expand the scope of the functional genomics screening capabilities at the TNRF. The outcome of this collaborative DDIR project will be the development of flexible CRISPR-based functional genomic platforms to complement the existing RNAi screening platform at the TNRF. Our goal will be to offer Intramural Investigators the opportunity to select the most appropriate functional genomic screening platform available to address their biology of interest.

Center for Advanced Tissue Imaging (CAT-I)

PI

Ron Germain (NIAID)

Project Summary

The immune system is not only crucial for protection against infectious agents, but plays a key role in responses to tumors and in various chronic diseases. The combination of monoclonal antibodies and flow cytometry has had an enormous impact on immune system studies but it does not provide information on the spatial distribution of the cells of interest within a tissue. Immunohistochemistry (IHC) provides information about the spatial organization of cells in a tissue, but most IHC methods are of low complexity (2-4 colors) insufficient for identifying the many known immune cell types in tissues. The Lymphocyte Biology Section (LBS) of the Laboratory of Systems Biology (LSB) in NIAID has developed a new IHC approach called Histo-cytometry that involves immunofluorescent staining of tissues in up to 14 colors, imaging at high resolution across large areas, obtaining quantitative information about staining intensity on a per cell basis, and locating identified cell subsets in a tissue section. Recent improvements include sequential staining to achieve > 30 parameters, addition of in situ mRNA FISH, and development of a new method (Ce3D) that allows applying the Histo-cytometry technique to 3D tissue volumes. To make these advanced research tools available to the broader NIH community, NIAID and NCI have partnered to develop a Center for Advanced Tissue Imaging (CAT-I). The CAT-I is comprised of confocal instruments together with the ancillary devices needed for conducting Histo-cytometry and Ce3D tissue analysis, run by staff scientists in a collaborative venture with PIs. CAT-I plans to undertake several large clinical studies per year, with the most obvious near-term efforts likely to involve analysis of tissue samples from cancer patients receiving immunotherapies. CAT-I studies can provide previously unobtainable insight into the number, positioning, states, and functions of cells in biopsy samples from such patients as well as those from subjects with autoimmune or allergic diseases or infections.

Advancing NIH super-resolution optical microscopy capabilities

PIs

Joshua Zimmerberg (NICHD), Sergey Bezrukov (NICHD), Leonid Chernomordik (NICHD), Patrick Duffy (NIAID), Michal Fried (NIAID), Richard Leapman (NIBIB), Thomas Leto (NIAID), Victor Lobanenkov (NIAID), Leonid Margolis (NICHD), Thomas Reese (NINDS), Alexander Sodt (NICHD), Constantine Stratakis (NICHD)

Project Summary

We propose to create a Super-Resolution Optical Microscopy core facility located in the newly renovated Building 29 research complex where the combined expertise of NICHD and NIAID member labs will be focused on developing techniques, procedures, and analyses that will allow routine application of the recently developed techniques of super-resolution optical microscopy and correlative electron microscopy to mission critical NIH biomedical research. The super-resolution optical microscopy core will complement the existing Building 29 Electron Microscopy core providing users with a seamless capability of performing both super-resolution optical

microscopy and correlative electron microscopy. This combined modality represents the most recent and advanced techniques for bridging the spatial scales required for single molecule investigations at the electron microscopy scale (~nm) in the context of cellular and tissue physiology at the optical microscopy scale (> 10's nm - mm). While individual labs and a few core facilities have acquired super-resolution microscopes, applications of the technique to the broader NIH community has been slow, often the result of the steep learning curve required for the uninitiated. The project leaders and collaborating PIs have a long history of developing novel microscopy techniques, including super-resolution microscopy, and applying this technique to many important physiological and pathophysiological problems spanning spatial scales relevant to single molecule interactions to intact, macroscopic tissue. Towards the goal of routine application of super-resolution microscopy and the newly developed optical correlative electron microscopy, we request funding for the purchase of a commercial super-resolution optical microscope to serve as the primary instrument in a multi-center, multi-user, microscopy suite that is being developed as a result of the Building 29 renovation project. The microscopy suite was developed with the necessary infrastructure (vibration, electrical, and HVAC support) required by the optical microscope system and the project leader has an additional laser system that will expand the capability of the microscope system. The proposed system will allow routine acquisition of super-resolution data utilizing either the STED (Stimulated Emission Depletion) principle as implemented, for example, in the Leica SP 8 system or SML (Single Molecule Localization) nanoscopy as implemented, for example, in the Bruker Vutara 352. In addition, our expertise in microscopy analysis and data interpretation will benefit all users of the system as this aspect is often the most difficult phase in any super-resolution project.

Multi-institute cryo-EM facility, specimen preparation

PIs

Jenny Hinshaw (NIDDK), Christopher Bleck (NHLBI), Kenton Swartz (NINDS)

Project Summary

With recent advances in the field of cryo-electron microscopy (cryo-EM), there has been an exponential increase in structures solved by this method at resolutions similar to X-ray crystallography. One key advantage of cryo-EM is that samples are prepared in solution, and imaged in more native conditions than in crystallography, which requires high concentrations of salt or solvent-crowding molecules to unnaturally pack proteins into crystalline symmetry. Furthermore, cryo-EM is optimal for resolving large, >300 kilodalton complexes and their potential movements, which are not amenable to X-ray crystallography or NMR. Thus, cryo-EM is poised to transform structural biology from the study of individual protein domains and small complexes to the structures of functional molecular machines in motion. This structural revolution is due to the development of direct electron detectors in combination with new cryo-EM microscopes and image processing methods. To ensure structural biology at the NIH can take advantage of these revolutionary developments, NHLBI initiated a plan in 2015 to establish a cryo-EM facility with state-of-the-art equipment to be shared by four institutes, NHLBI, NIDDK, NIAMS and NINDS. For this Multi-Institute cryo-EM Facility (MICEF), a Titan Krios (FEI) microscope with an energy filter

and direct electron detector (Gatan BioQuantum 967) has been purchased. In support of the Krios microscope, MICEF will provide sample preparation equipment that will greatly facilitate overall productivity and accessibility for researchers lacking access to specialized cryo-EM methods. To streamline sample preparation and allow easy user access to the required equipment, our goal is to consolidate instruments and create a dedicated sample prep room close to the new microscope(s). Furthermore, a dedicated sample prep room will provide an ideal training facility for the growing number of new cryo-EM users.

Systems approach to tackling drug resistance by exploiting natural products

PIs

Carole Bewley (NIDDK), Clifton Barry (NIAID), Eugene Koonin (NLM)

Project Summary

The discovery and development of antibiotics represent one of the most important breakthroughs in medicine. By and large our antibiotic arsenal has been built almost exclusively (circa 80%) from genome-encoded small molecules, commonly referred to as natural products (NPs) and generally produced by environmental bacteria. In recent years infections caused by bacterial strains that are resistant to one, or even many, clinically prescribed antibiotics has increased steadily and now account for around 2 million infections and 25,000 deaths per year in the U.S. alone. At the same time the development of new classes of antibiotics that can overcome resistance mechanisms has steadily declined with only a handful of new antibiotics being approved in the last decade. Motivated by this need, our program project brings together three independent investigators – Clifton Barry (NIAID), Carole Bewley (NIDDK) and Eugene Koonin (NCBI) – with expertise in natural products chemistry and antibiotic discovery, tuberculosis drug discovery and clinical trials, and state-of-the art genomics and bioinformatics. Together we aim to identify new classes of antibiotics effective against high-priority drug-resistant pathogens, including Gram-negative bacteria and Mycobacterium tuberculosis; build natural products libraries comprising microbial strains and natural products available for screening to the NIH community; and develop methods for genome mining of proven antibiotic-producing organisms by targeted analyses of genes that encode antibiotic production and resistance islands.

Investigating collective migration in the Zebrafish lateral line primordium

PIs

Ajay Chitnis (NICHD), Kandice Tanner (NCI), Jian Liu (NHLBI)

Project Summary

Small communities of cells within malignant tumors can become mobile and move together by a process called collective migration. This facilitates metastasis, where the cancer spreads to distant parts of the body and becomes very difficult to control. While this kind of collective migration in metastatic cancer cells is a spontaneous and unregulated process, collective migration has a much

more predictable and regulated role in the movement of cells during the development of an embryo. A remarkable example of regulated collective migration is seen in zebrafish, where the posterior Lateral Line (pLL) primordium, a group of about a hundred cells, migrates under the skin, from near the ear to the tip of the tail, to pioneer formation of the zebrafish Lateral Line sensory system. During its migration, cells along the length of the primordium undergo systematic changes in morphology and migratory ability. Starting from its trailing end, cells of the migrating primordium sequentially reorganize to form multicellular clusters or rosettes as they generate nascent neuromasts, the sensory organs of the Lateral Line. Trailing cells in the pLL primordium initially migrate together with the remaining quasi-mesenchymal leading cells, as a cohesive column of cells. However, the trailing rosettes eventually lose their capacity for collective migration, disengage from more leading cells, and are deposited periodically as neuromasts by the migrating primordium. The goal of this program project is to leverage the unique expertise of the research groups of developmental biologist Ajay Chitnis (NICHD), experimental biophysicist Kandice Tanner (NCI), and theoretical cellular physicist Jian Liu (NHLBI) to develop a comprehensive understanding of how interactions between cells determine collective migration and self-organization of the pLL primordium. The Chitnis lab will take advantage of transgenic fish with fluorescent primordium cells to characterize the dynamic behavior and shape of the cells during collective migration. They will also manipulate development with molecular, cellular and genetic tools to elucidate molecular mechanisms that contribute to collective migration. Kandice Tanners' group will help investigate questions related to mechanical coupling of cells: Does the pull of a leading cell determine polarized migration of a trailing cell or does attachment to a trailing cell polarize migration of a leading cell? Do these behaviors systematically change along the length of the primordium? Jian Liu's group will help integrate quantitative data related to intercellular and intracellular signaling systems, cell shape changes and the measurement of mechanical coupling to help develop a comprehensive computational model of cell signaling and collective migration in the pLL primordium. Our expectation is that a deep understanding of the mechanisms that regulate collective migration during healthy development of the zebrafish lateral line system will also help understand how collective migration spontaneously develops in communities of malignant cells. The project will help define what needs to be changed to prevent the emergence of this ultimately devastating collective cell behavior in cancer.

Liver cancer: biomarker discovery, pathogenesis and animal models

PIs

Jake Liang (NIDDK), Xin Wang (NCI), Tim Greten (NCI)

Project Summary

Hepatocellular carcinoma (HCC) is a leading cause of death from cancer in the US and worldwide. Suboptimal diagnosis and treatment contributes to its poor prognosis. Three collaborative projects are being initiated to address unmet needs in HCC. First, hiPSC-hepatic differentiation and genome-wide CRISPR/Cas technologies will be combined with mouse engraftment models of HCV-induced HCC to identify and characterize genes that are potential premalignant targets of chronic

HCV infection. Second, a high-throughput method (VirScan) that can facilitate the discovery of new virus-host associations by profiling all known human viruses, will be developed to evaluate the association of host-viral signatures in a large cohort of patients with chronic liver diseases and to determine their roles as biomarkers in early HCC detection. The third project aims to study the interactions of gut microbiome and host immune response in the pathogenesis of HCC by applying RNA-Seq and proteomic studies and bioinformatics in mouse models of HCC.

Harnessing and characterizing stem cell-like T cells for adoptive T cell therapy

PIs

Pamela Schwartzberg (NHGRI), Luca Gattinoni (NCI), John O'Shea (NIAMS)

Project Summary

During chronic viral infections and cancer, T cells frequently develop into a dysfunctional state, called T-cell exhaustion, which hinders the effective clearance of infected cells or tumor cells by the immune system. In recent years, immunotherapies, including adoptive T cell therapies, have achieved remarkable success in treating cancers that were refractory to traditional treatment. However, despite recent progress, it remains unclear which T cell populations are the main responders to these immunotherapies. We and other groups have recently identified a stem cell-like CD8 T cell population that is crucial for viral clearance and exhibits superior therapeutic potential in mice chronically infected by lymphocytic choriomeningitis virus (LCMV). We have also found a similar CD8 population in tumor infiltrating lymphocytes (TILs), which appears less exhausted than other CD8 TILs. Here, we propose to study the molecular determinants of the therapeutic effects mediated by stem cell-like CD8 T cells. This program project grant will leverage the strengths of three groups to advance our understanding of this important cell population, using cutting edge technologies. We will identify critical transcriptional and metabolic nodes that regulate the differentiation of stem cell-like CD8 T cells (Project 1), discover novel biological markers that correlates with the therapeutic effects of stem cell-like CD8 T cells (Project 2), and evaluate the therapeutic effects of stem cell-like CD8 T cells in newly developed mouse and humanized mouse models (Project 3). The overall goals of these projects are to identify CD8 subsets that exhibit the greatest potential for cellular-based immunotherapies, and to understand pathways that can be manipulated to enhance their therapeutic effects. Our ability to correlate markers of stem cells with functional outcomes for chronic infection and tumors *in vivo* provides an important strength for both understanding and evaluating effectiveness of CD8 cell therapies.

Deciphering the mechanisms linking neural stress and injury to persistent pain

PIs

Mark Hoon (NIDCR), Clare Le Pichon (NICHD), Alexander Chesler (NCCIH), Nick Ryba (NIDCR)

Project Summary

Neuropathic pain is a major type of chronic and intractable pain; previous studies have revealed altered patterns of gene expression in sensory neurons, effects on neuronal circuits and changes in behavioral sensitivity. However, it remains unclear how these lead to the development of chronic pain. Indeed, understanding how a variety of different nerve injuries all cause pain requires a coordinated multifaceted research program to transform our understanding of this condition. In this project, we propose to apply a range of cutting edge approaches to define the contributions of sensory neurons and central circuits in the development of neuropathic pain. We will examine neuropathic pain models with differing etiologies to determine whether common pathways or convergent processes result in the symptoms. By combining functional imaging of large populations of single neurons with cellular-level molecular characterization and directed methods for manipulating neural (and neural circuit) activity we will explore how different types of nerve damage result in changes both in sensation and in sensory processing. We will also test whether neurogenic pain can be prevented by blocking key components of the inflammatory cascade. Combining our diverse research interests and skills presents a great opportunity for synergy and a valuable extension of intramural pain research at a basic science level. Ultimately, defining the mechanisms responsible for neuropathic pain will be an important advance and should aid rational design of effective strategies to prevent and treat this type of chronic pain.

Characterization of the crosstalk between mitochondria and inflammation

PIs

Jennifer Martinez (NIEHS), Richard Youle (NINDS), Michael Fessler (NIEHS)

Project Summary

For his discovery of the molecular mechanism of autophagy, the evolutionarily-conserved response to stress and starvation, Dr. Yoshinori Ohsumi was awarded the 2016 Nobel Prize in Medicine and Physiology. We now recognize that the reach of autophagy extends far beyond nutrient deprivation, into non-canonical roles of organelle quality control and host defense against internalized pathogens or dying cells. Despite seemingly disparate functions, it seems that the autophagy machinery can function broadly in serving as ancient mechanisms for containment and suppression of inflammation, and its unified ability to prevent autoinflammatory activation by mitochondrial components that mimic bacterial molecules has suggested an original immune

function for autophagy. Taken together, emerging evidence indicates that autophagy, through both canonical and non-canonical pathways, is critically required immunological self-tolerance to both for intracellular and extracellular threats. Defects in autophagic machinery have been linked to many inflammatory, autoimmune, and neurodegenerative disorders. However, the molecular mechanisms by non-canonical autophagy suppresses auto-inflammation, and the connections between mitochondrial maintenance and non-canonical autophagy, remain very poorly defined. The present proposal newly partners three NIH intramural investigators with diverse expertise in a collaboration that aims to define novel autophagic mechanisms of immunological self-tolerance and explore the role of mitochondria in maintenance of immunotolerance.

Transforming orphan drug research via translational science collaborations

PIs

Katherine Meilleur (NINR), Brandon Harvey (NIDA), Carsten Bonnemann (NINDS)

Project Summary

Among the variety of genetic skeletal muscle disorders, ryanodine receptor 1-related myopathies (RYR1-RM) are the most common in the United States, with an estimated prevalence of 1/90,000 (1). RYR-1 is a channel that aids in the regulation of intracellular calcium stores, serving as an integral component of muscle contraction. Compromised function and/or reduced amount of RYR-1 can lead to muscle weakness and dysfunction. Here we propose a highly collaborative approach, spanning multiple institutes and facets of research, to identify potential therapeutic compounds to stabilize intracellular calcium. Using novel biological sensors and biomarkers (2-4), we plan to screen therapeutic candidates in both primary cell cultures and immortalized cell lines isolated from healthy and RYR1-RM skeletal muscle. Compounds that successfully pass validation will be further evaluated in pre-clinical *in vivo* studies. RYR1-RM zebrafish is an established *in vivo* model of RYR1-RM and has previously been used in support of a RYR1-RM clinical trial. Functional studies that focus on zebrafish motility will be used to assess drug efficacy. This project seeks to establish translational science collaborations among the NIH intramural program as way to streamline orphan drug research in the context of RYR1-RM.

Regulatory chromatin dynamics in ‘inflamm-aging’ and neurodegeneration

PIs

Mia Sung (NIA), Mark Cookson (NIA), Michael Bustin (NCI)

Project Summary

Changes in the packaging of our DNA inside the cell nucleus and low-grade chronic inflammation are two major hallmarks of aging. Our project explores how these two phenomena are related in the natural course of aging and in Parkinson's disease. The Innovation Award brings together NIH intramural investigators with widely different expertise. We will examine the age-related changes with the biochemical packaging of genetic material and the consequent effects on gene expression in immune cells. Particular attention will be given to a class of chromosomal proteins called high mobility group (HMG) proteins and their role in maintaining the structural organization of the genome in immune cells. We hypothesize that HMG proteins may be important for the integrity of the genome and proper gene regulation in brain-resident immune cells. We will study whether a gene mutation associated with Parkinson's disease may have exacerbated effects in the absence of HMG proteins in an animal model of the disease. Our novel genomic and molecular analysis tools will reveal new insight into the inter-relationship of inflammatory signals, HMG proteins, aging, and a Parkinson's disease gene variant.

Leveraging human sequence variation to understand enhancer control of RNA

PIs

John (Jay) Chiorini (NIDCR), William Gahl (NHGRI), Brian Brooks (NEI)

Project Summary

Approximately 98% of the human genome is considered to be regulatory and control the activity of the 2% of the genome that encodes proteins. Expression is controlled by DNA motifs contained close to the beginning of each gene, and by enhancers that may exist millions of base pairs away. Of the more than 200,000 reported clinically significant sequence variants, only 0.2% are currently considered to be regulatory. As less than half of heritable human diseases are explained by protein-encoding genes, we hypothesize that regulatory sequence variants have a clinically important effect on gene expression that translates into a significant proportion of diseases. This proposal seeks to develop a fundamental understanding of the role of regulatory DNA on gene expression by identifying regulatory variants in human genetic diseases. Functional characterization of these regulatory variants will identify novel mechanisms for disruption of the regulatory program underlying human health.

A novel imaging method for activated T cells with PET

PIs

Peter Choyke (NCI), Joshua Farber (NIAID), Rolf Swenson (NHLBI)

Project Summary

Immunotherapy has become an important part of treatment for many diseases including cancer. Usually successful immunotherapy involves activation of lymphocytes that kill tumor cells, however, there is currently no method of monitoring for successful lymphocyte activation. CXCL9 is a cytokine produced exclusively by activated lymphocytes and is found in high amounts. It would therefore be an excellent potential imaging biomarker of immunotherapy if it could be targeted. The purpose of this project is therefore to develop imaging agents that target CXCL9 and thus, could be used for monitoring the effects of immunotherapy. The project will initially focus on antibodies that bind to CXCL9 and progress to antibody fragments and even smaller molecules that bind CXCL9. This project makes use of unique facilities at NIH including Dr. Josh Farber's lab in NIAID, Dr. Rolf Swenson's Image Probe Development Center in NHLBI and Dr. Peter Choyke's Molecular Imaging Program in NCI.

CRISPRi-scRNA-seq screening to dissect retrotransposon silencing

PIs

Guang Hu (NIEHS), Todd Macfarlan (NICHD), Kai Ge (NIDDK), Paul Wade (NIEHS)

Project Summary

Retrotransposons are mobile elements that spread by a copy-and-paste mechanism, and they comprise ~40% of the mammalian genome. Retrotransposons increase genome complexity and regulate gene activity. But they can also lead to genome instability and erroneous transcription. In this application, we propose to carry out CRISPR-interference followed by single RNA-sequencing (CRISPRi-scRNA-seq) screens to systematically study retrotransposon repression in mouse embryonic stem cells (ESCs). Our proposal joins the efforts of four groups from NIEHS, NICHD, and NIDDK with expertise in high-throughput genetic screens, stem cell biology, retrotransposon silencing, chromatin remodeling, and histone modifications. We aim to uncover and define the components and molecular pathways in the defense against retrotransposons. Furthermore, we hope to stablish the CRISPRi-scRNA-seq technology and analysis pipelines for future functional genetic studies.

Creating animal models with natural microbiota to study disease resistance

PIs

Barbara Rehermann (NIDDK), Yasmine Belkaid (NIAID), Jonathan Yewdell (NIAID)

Project Summary

Mouse models are paramount for understanding of basic immunological mechanisms but are limited in recapitulating human diseases. This proposal will explore how a microbiota that has co-evolved with its host in the natural world can offer novel understanding of host immunological protection compared to standard laboratory microbiotas. This is based on the hypothesis that laboratory mice are missing important. symbiotic host-microbe interactions crucial for host physiology, in particular immunity, that can be found in free-living organisms like wild mice since they (like humans) evolved under greater evolutionary pressure with regards to infectious diseases.

Next-generation studies of RNA structure and dynamics enabled by the X-ray

PIs

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Project Summary

The X-ray free electron laser (XFEL) has revolutionized protein structural biology because it is a billion times brighter than synchrotrons and its X-rays are delivered in femtosecond pulses. These properties allow the study of nanocrystals while outrunning radiation damage, and open the way to unprecedented time-resolved studies. We propose a program project to bring to bear the XFEL to RNA structural biology in NIH.

Development of primate brain circuits for vocal/visual social communication

PIs

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Project Summary

Our research project investigates the neurodevelopment of brain circuits involved in social communication. In primates, social communication is achieved primarily through visual and acoustic signals, such as facial expressions and vocalizations. The production and perception of these communications are mediated by evolved regions that occupy significant fractions of the primate brain. The underlying circuits are shaped during development by a combination of genetic predetermination and social experience. In this project, we bring together expertise from five NIH laboratories to study the development of areas related to facial and vocal communication in the marmoset, which is a small New World monkey that is quickly becoming an important primate model for neuroscience. The project has two broad components. The first component of the project undertakes a multiscale investigation of the anatomical development of communication circuits. Here we will use modern viral anatomical tracing methods to track the development of cortical and subcortical projections related vocal projection, as well as the efferent and afferent connections to the cortical “face patch” system. In both systems, we will investigate the development and maturation of inhibitory interneurons. In parallel, we will use longitudinal magnetic resonance imaging (MRI) to establish an integrated developmental atlas and track the macroscopic course of growing gray matter and fiber tracts. The second component of the project will examine functional changes in social circuits and socio-communicative behaviors during development, with an eye toward critical windows of behavioral and neural plasticity. In behaving marmosets, we will use genetically encoded calcium indicators (e.g. GCaMP) to study the activity of cortical projection neurons and inhibitory interneurons involved in face perception and vocal production. These indicators will be delivered locally with a virus or expressed ubiquitously in transgenic animals. At a macroscopic scale, we will also examine the evolution of whole-brain networks using resting state fMRI functional connectivity, focusing on regions related to vocalization and face perception. This two-phased approach will set the foundation for understanding the development of neural circuits underlying social communication in primates. This topic is very important for understanding a range of psychiatric brain disorders, which are thought to be developmental in nature, and in which social cognition is nearly always affected.