RESEARCH PROGRAM

Medical Research Abstracts
for Grants Awarded in December 2020

Fred Hutchinson Cancer Research Center
Seattle, WA
Christopher D. Johnston, Susan Bullman, Angela H. Ting
$1,000,000
December 2020

Bacterial restriction-modification (RM) systems function as a defense mechanism against invasive DNA molecules. RM systems are ubiquitous in bacterial strains within the human microbiota and target specific DNA sequences for methylation or digestion. RM systems are known to be governed by their sequence specificity, and in the laboratory, RM enzymes will indiscriminately act on human DNA at their respective motifs. Yet, in humans, DNA methylation is primarily associated with much shorter CpG motifs. Although an essential epigenetic modification in normal human development, methylation is grossly disrupted in all human cancers. How such abnormality is established during cancer formation remains an unsolved mystery. Investigators at the Fred Hutchinson Cancer Research Center and the Lerner Research Institute at the Cleveland Clinic suggest that bacterial RM systems of the microbiota can directly induce aberrant methylation states within the human genome, thus, initiating prevalent epigenetic aberrancies observed in a subset of cancers. The invasive and intracellular pathogen *Fusobacterium nucleatum* is intimately associated with colorectal cancer (CRC), enriched in both tumors and associated metastases. Using CRC as a model, the investigators seek to determine if there is clear *in vivo* evidence of microbial RM enzymes interacting with human DNA, and if so, experimentally delineate the underlying molecular mechanism of these host-microbiota interactions.

The George Washington University
Washington, D.C.
Rong Li, Brett Shook, Yanfen Hu, Nu Zhang
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Cellular memory refers to the ability of multiple cell types in animals and plants to "remember" a previous stress event and respond to future episodes with more rapid gene activation. This self-educating ability based on past experience endows organisms with enhanced survival and fitness under fluctuating and adverse environmental conditions. How cells store records of past experience remains incompletely understood, but the current paradigm centers around chromatin-based epigenetic mechanisms. A team from George Washington University and the University of Texas Health San Antonio, will test the hypothesis that paused RNA polymerase II (Pol II) at genes that are activated by a past stress signal can prime future gene activation, thus potentially endowing multiple cell types with enhanced responsiveness to repeated stress attacks. The team's model for cellular memory is a departure from the current chromatin-centered paradigm. To establish proof-of-principle, the investigators will focus on two cell types with cellular memory: memory T cells and skin epithelial stem cells. Using mouse genetic models, they will interrogate the impact of Pol II pausing on the ability of these cells to preserve records of past experience of infection and injury. Furthermore, the researchers will use cutting-edge genomic tools to survey the dynamics of Pol II movement and transcriptional activation in response to repeated rounds of external insults, with and without Pol II pausing.

Stanford University
Palo Alto, CA
Helen Blau, Sarah Heilshorn
$1,000,000
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Cells are constantly receiving and responding to diverse signals from their microenvironment. Aberrant environmental cues, such as increased tissue stiffness from disease-related fibrosis, can drive disease progression. Genetic disorders that alter how cells interact with the extracellular environment make cells particularly sensitive to changes in environmental cues. One such disease, Duchenne muscular dystrophy (DMD), is caused by defective dystrophin, a protein that links the cytoskeleton and the extracellular matrix. DMD manifests as severe muscle wasting followed by dilated cardiomyopathy, leading to patient death around 20-30 years of age.
A Stanford University team has recently identified telomere shortening as a hallmark of DMD cardiomyopathy, as well as of other heritable cardiomyopathies, leading the investigators to postulate that telomere shortening plays a causal role in heart failure in many genetic diseases. However, this hypothesis is controversial, given that telomere shortening has historically been

associated with cell division, and cardiomyocytes do not divide. The investigators will determine whether mechanical stress drives telomere shortening and subsequent pathogenic signaling, leading to cardiomyocyte death. They have developed a novel hydrogel platform that can be stiffened and softened on demand to tune mechanical load, which will be used in conjunction with human induced pluripotent stem cell derived cardiomyocytes from DMD patients and live cell imaging to answer the fundamental question: How can telomeres shorten without cell division?

University of California, San Francisco
San Francisco, CA
James Fraser, Danica Galonić Fujimori
$1,000,000
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The ability to use a small molecule to selectively modulate a protein has incredible therapeutic and discovery consequences. However, most proteins lack the type of pockets that can bind a small molecule, making them "undruggable" by conventional approaches. These proteins are often understudied not because of a lack of importance, but simply because researchers lack molecules that can bind to them. Modern approaches like CRISPR provide a sequence-specific genetic handle on these proteins, but even when compelling new targets are uncovered investigators still lack chemical agents to modulate them specifically. A team at the University of California, San Francisco will develop a new platform to inhibit proteins: by selectively stopping them from ever being produced in the first place. Using a combination of small molecule synthesis, CryoEM, and ribosome profiling, they will design a new class of small molecules—Specific Tunable Obstructors of Protein Synthesis (STOPS)—that stall ribosome at specific peptide sequences. If successful, the project would deliver a platform to modulate any target in the proteome irrespective of its catalytic activity, ability to bind ligands, or mutation status. Additionally, by targeting a core mechanism in translation, STOPS could be used across the tree of life.

University of Texas Southwestern Medical Center
Dallas, TX
Vincent S. Tagliabracci, Krzysztof Pawlowski, John Schoggins, Diana Tomchick
$1,000,000
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The human genome contains nearly 3,000 enzymes, biological machines that perform critical jobs in the cell by speeding up, or catalyzing, chemical reactions. The genomes of all organisms also contain the instructions for making many pseudoenzymes, which are proteins that resemble other enzymes but appear to be inactive when tested using conventional methods for determining enzymatic activity. An alternative explanation for the apparent lack of activity is that pseudoenzymes are being asked to perform the wrong reaction. A team at the University of Texas Southwestern Medical Center discovered that the uncharacterized proteins, SelO and SidJ, which were predicted to be inactive "pseudokinases," adopt the same shape as a kinase, but instead of transferring a phosphate to another protein, as kinases do, they instead transfer AMP and glutamate, respectively. These discoveries were the first examples of kinase-like enzymes that can perform a different catalytic reaction. The investigators suggest that some pseudoenzymes are active but performing different functions than the active enzymes they resemble. Notably, an entire order of viruses, encompassing all coronaviruses, contains a SelO-like pseudokinase domain of unknown function, which is essential for viral replication. Thus, the goal of this grant is to discover new catalytic functions for pseudoenzymes, beginning with pseudokinases in mammals, bacteria, and viruses.