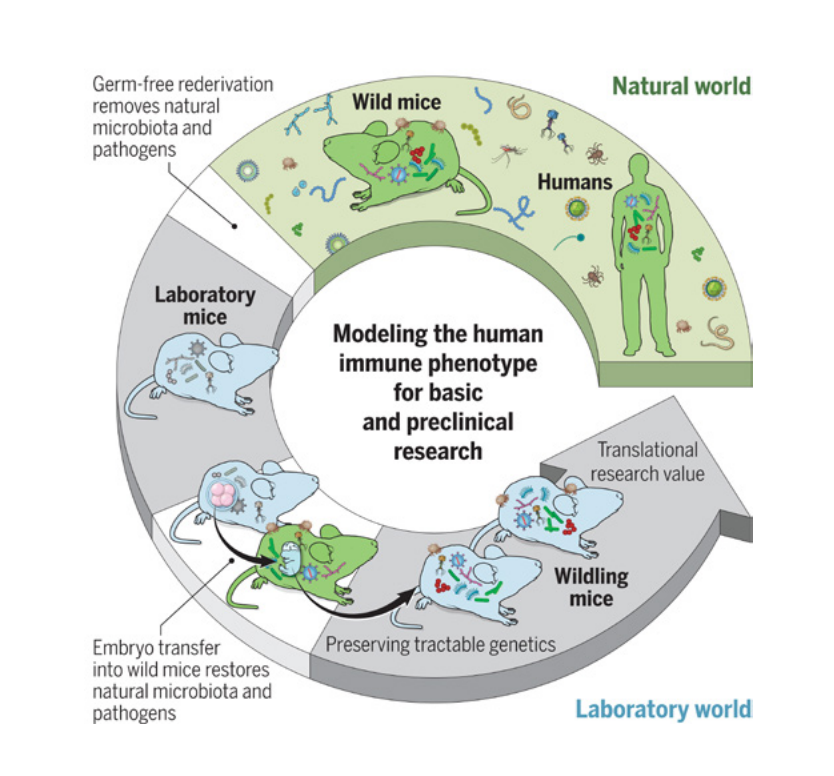


Mice are often studied in the lab as stand-ins for humans in basic research and early-stage studies of potential treatments. However, traditional lab mice vary greatly across different labs and in comparison to their free-living, wild cousins in terms of the microbes they carry, which can have profound effects on immune responses. A group of NIDDK intramural researchers and scientists from other NIH Institutes and universities have pioneered the development of a new mouse model, called the "wildling mouse." This mouse is the genetic offspring of conventional lab mice, but carries the microbes of its surrogate mother, a wild mouse, at all body sites. Compared to standard lab mouse microbiota, the wild mouse microbiota have increased resilience towards environmental challenges. With an immune state and response to immune-modulatory drugs more similar to that of humans than conventional laboratory mice, the wildling mouse may be a better translational model for multiple human diseases and for pre-clinical testing of drugs.

Image courtesy of Dr. Barbara Rehermann, NIDDK, and Ms. Nathalie Cary, AAAS. From Rosshart SP, Herz J, Vassallo BG,...Rehermann B. Laboratory mice born to wild mice have natural microbiota and model human immune responses. Science 365: pii: eaaw4361, 2019. Reprinted with permission from AAAS.

Cross-Cutting Science

Medical advances are not usually achieved in great, intuitive leaps. More often, new prevention strategies, treatments, and cures result from a long, gradual accumulation of knowledge from years of scientific research. Insights into the fundamental biologic building blocks and processes of an organism—its genes, the proteins they encode, the inner workings of cells, and the ways cells communicate with each other—can have broad and far-reaching implications. Indeed, many significant advances in our knowledge of disease and disease treatment can be traced to laboratory studies whose relevance to health could not have been fully known or appreciated at the time they were conducted.

With the development of innovative scientific technologies and the emergence of new scientific disciplines as talented and creative research teams tackle ever more complex challenges, new opportunities to make exciting discoveries arise each day. Described in this chapter are several recent studies as well as features on the Institute's 70th Anniversary and on some up-and-coming scientific investigators who have received special presidential recognition. The insights gained through research described here can be expected to further scientific progress in many research areas; for today's discoveries may hold the seeds of tomorrow's cures.

IMPROVING ANIMAL MODELS FOR RESEARCH ON HUMAN HEALTH AND DISEASE

Where the "Wildlings" Are—Lab Mice with Wild Microbes Better Model Human Immune Responses: NIDDK's intramural scientists, together with researchers from other NIH Institutes and universities, have developed laboratory mice that better reflect the natural complexity of mammals and the microbes that co-evolved within them, and serve as an improved model of human physiology. Scientists using mice as stand-ins for humans in early-stage drug development studies have come to appreciate the importance of having a more complex system that better reflects how mammals exist in their "natural" state, colonized by a unique mix of co-evolved microbes, including bacteria, viruses, fungi, protozoans, and other organisms that make up their "microbiome." Traditional lab mice allow researchers to control for genetic background and other factors in their studies, but carry microbes that vary greatly across labs and when compared to wild mammals. This variation may have contributed to past failures to translate successful pre-clinical drug studies into humans.

To address this, a group of NIDDK intramural researchers and scientists from other NIH Institutes and universities created the so-called "wildling" mouse. They started with conventional female lab mice raised in a sterile environment, and transferred fertilized embryos from these mice into wild mice. The offspring—the wilding mice—are therefore the genetic offspring of conventional lab mice but have been exposed to the natural microbes of their surrogate (wild) mothers. The scientists catalogued bacteria, fungi, and viruses present in wildling, wild, and conventional lab mice in parts of the body where microbes are usually found, including the gut. The microbe communities were more similar in the wildling and wild mice than in conventional lab mice. A similar result was seen when looking at the immune landscape—the types and numbers of different immune cells—at various sites in the body important to an immune defense. The wildling mice microbiomes were also more stable than laboratory mice microbiomes and showed greater resilience to challenges such as antibiotic treatment and a high-fat diet. In a final coup de grâce, the group demonstrated how wildling mice may better model human disease and treatment response by testing two drugs with a history of beneficial effects on the immune system in conventional laboratory mice, but adverse immune effects when tested in humans.

In the wildling mice, these drugs provoked a similar immune response as in humans. Thus, wildling mice may be an improved animal model for research that is reproducible and predictive of human response, saving time and money, and sparing clinical trial participants from adverse effects of potentially harmful drugs.

Rosshart SP, Herz J, Vassallo BG,...Rehermann B. Laboratory mice born to wild mice have natural microbiota and model human immune responses. Science 365: pii: eaaw4361, 2019.

RESEARCH ON NEURAL SLEEP SIGNATURES

New Technique Illuminates Sleep Similarities Between Humans and Zebrafish, with Implications for Better Understanding Human Sleep: Researchers recently identified several neural activity signatures in the zebrafish brain that suggest certain sleep states in these fish are similar to human sleep and that they could thus be used for studying the role of sleep in health and disease. In humans, appropriate sleep patterns support emotional and physical well-being and quality of life, and chronic sleep deficiency has been linked to obesity, diabetes, kidney disease, and other health problems. How exactly sleep contributes to good health, however, is still unclear, and more research in this area could yield important insights. Zebrafish share neural and biochemical similarities with other vertebrates, including humans, that make them a potentially good model for studying sleep and sleep-related disorders. Zebrafish are also uniquely useful in biological research, partially because their larvae are transparent, allowing use of advanced visualization techniques to answer scientific questions in living animals. However, measuring the neural signatures of sleep in zebrafish has been technically challenging, and it was unknown whether or not they organize their sleep cycle similar to humans and other vertebrate animals.

To explore this question, researchers sought to determine whether the zebrafish brain undergoes sleep states found in people and other vertebrates, such as the slow-wave and rapid eye movement (REM) states. To do this, the scientists developed a technique they called fluorescence polysomnography to visualize the brain and muscle activity, eye movement, and heart rate of living fish during sleep. This technique allowed the researchers to analyze not just the behavioral characteristics of sleep such as muscle relaxation and decrease in heart rate, but also the neural signatures of sleep in the zebrafish brain. One state they observed, which they named "slow bursting sleep," shared features with slow-wave sleep, while another state, named "propagating wave sleep," was similar to REM sleep. This new visualization technique also enabled the researchers to study and identify cellular and molecular regulators of zebrafish sleep, such as a hormone that controls the onset of propagating wave sleep.

Given the zebrafish's place in the vertebrate family tree, these results suggest that common mechanisms of sleep are shared among animals as disparate as zebrafish and humans. These findings suggest that zebrafish, and the novel imaging techniques possible in this model organism, could be powerful new tools for research to increase understanding of human sleep and its impacts on health and disease.

Leung LC, Wang GX, Madelaine R,...Mourrain P. Neural signatures of sleep in zebrafish. Nature 571: 198-204, 2019.

VISUALIZING DNA REPLICATION MACHINERY

Determining the Architecture of DNA Replication Machinery: Scientists in NIDDK's Intramural Research Program have determined the three-dimensional structure of a protein complex that copies DNA—a fundamental molecular process required by all biological organisms. In order for a cell to divide, its DNA must be replicated to form two identical copies—one copy for each of the two cells resulting from the division. The process of DNA replication is a highly ordered, complex orchestration of many proteins serving different functions to achieve complete fidelity. The two intertwined strands that make up each DNA molecule must be unwound, and the sequence of each strand read as a template for synthesizing a new partner strand, to generate two double-stranded copies of the original DNA. However, researchers had not previously been able to visualize the complete structure of the genome replication protein machine, also known as the "replisome," as it fully engages all critical portions of its DNA target. Therefore, important questions about the inner workings of the protein machinery have remained unanswered.

In recent research, NIDDK scientists used a method called cryo-electron microscopy to visualize the structure of a relatively simple replisome complex, originating from a virus called bacteriophage T7. They mixed isolated proteins, DNA pieces, and other molecules necessary for DNA replication together in conditions that promoted the formation of replisomes.

By quickly freezing the assembled replisomes, thus capturing the complexes in their natural states, the scientists could use a powerful electron microscope to visualize their three-dimensional structures at atomic resolution. They found that six "helicase" proteins encircled one separated DNA strand and moved along the strand sequentially in a "hand-over-hand" motion to continue unwinding the DNA. They showed that the helicase proteins served as a central organizer of the replisome, providing a focal point for the positioning of "polymerase"—the protein that actually synthesizes new DNA strands based on the original DNA sequence. The structures also revealed that the DNA molecule bends in a specific manner as the replisome advances. These findings define core replisome operating principles that are found in all organisms even though specific components of the replication machinery differ, shedding important light on a critical, foundational molecular process.

Gao Y, Cui Y, Fox T,...Yang W. Structures and operating principles of the replisome. Science 363: pii: eaav7003, 2019.

RESEARCH ON DNA EDITING TECHNOLOGIES

Determining the Fidelity of DNA Editors: Scientists calculated the rate of anticipated and unanticipated mutations generated by use of an emerging DNA editing technology. Many human diseases result from the change of the smallest element of the genetic code—a "single base"—and therefore considerable effort has been made to develop tools for altering single bases as a means of treating disease. Recently, several new technologies have emerged, including "base editing" which directly converts a target base into a different base. As with any technology that alters DNA, however, it is critical to understand how accurate (*i.e.*, was the targeted base edited correctly?) and precise (*i.e.*, were other edits introduced?) the changes are.

Researchers in this study examined how well several base editors worked for changing two different kinds of DNA bases, cytosine and adenine, at specific places in the mouse genome. For this, they generated their own test DNA edits and also analyzed DNA editing done by others, for a total of 430 base edits examined. They found that the adenine base editor tested was both accurate and precise, but that the cytosine base editors, in general, were less faithful. However, two of the five cytosine base editors tested resulted in higher frequencies of the intended genomic changes and caused fewer unintended changes than did the other three. This study furthers the potential of base editors over other genetic editing technologies that routinely introduce unintended edits. These results provide scientists with important information to consider when deciding which technology to choose for research and, potentially, for future clinical applications.

Lee HK, Willi M, Miller SM,...Hennighausen L. Targeting fidelity of adenine and cytosine base editors in mouse embryos. Nat Commun 9: 4804, 2018.

UNDERSTANDING GENETIC UNDERPINNINGS OF A RARE DISEASE

An Unusual Cause of a Rare Disease—Finding a Needle in an Expanded Haystack: Researchers have discovered that a rare disease can be caused by an unusual genetic mechanism. Roughly 2 percent of human DNA contains genes that code for proteins, which are critical to all cellular structure and function. Thus, much disease research, especially for rare genetic diseases, has focused on sequencing only this subset of a person's genome—collectively called the "exome"—as many diseases are rooted in variants found in the protein-encoding genes, and narrowing the search this way can save a great deal of time. However, the remaining 98 percent of the genome does include some vital stretches of DNA. For example, certain regions that do not encode proteins themselves can have a large impact on how much protein gets produced by nearby genes or may determine which specific organs and cell types produce them. Exome sequencing alone can therefore miss critical variations in such regulatory DNA regions.

In the current study, researchers studying rare diseases wished to determine why three unrelated young children from different parts of Europe and Canada had experienced similar developmental delay and neurological problems. The first clue came with the discovery that each child had much higher than normal blood levels of glutamine, one of the amino acid building blocks of proteins. Exome sequencing did not provide a clear explanation, but one finding of the analysis did stand out: two of the children were found to have a rare variant in one of their two copies of the gene for the protein glutaminase, an enzyme that is needed to break glutamine down into other amino acids. However, each of these children also had an apparently normal version of the gene as well, and in the third child, both copies of the gene were normal. Intriguingly, the scientists then discovered

that, despite having at least one normal glutaminase gene, all three children had extremely low levels of glutaminase activity. Reasoning that non-coding variation might be playing a role, they sequenced the whole area around the glutaminase gene. In the great majority of people, an area adjacent to the gene has between 8 and 20 repeats of 3 of the 4 chemical bases that make up DNA—designated by the letters G, C, and A—in that order. In dramatic contrast, the normal glutaminase genes in the three children were accompanied by a much larger than normal number of the GCA repeats, ranging from 680 in 1 child to about 1,500 in another. These additional repeats had the effect of preventing cells from properly producing glutaminase. Unfortunately, there is currently no way to treat glutaminase deficiency, but this research provides a compelling case for expanding the search when exome sequencing alone is not enough to identify genetic causes of disease. Thus, this research may spur discovery in a range of other genetic diseases.

van Kuilenburg ABP, Tarailo-Graovac M, Richmond PA,...van Karnebeek CDM. Glutaminase deficiency caused by short tandem repeat expansion in GLS. *N Engl J Med 380: 1433-1441, 2019.*

NIDDK Celebrates Its 70th Anniversary

In 2020, the NIDDK celebrates 70 years since its founding in August 1950 (see "History of the NIDDK" inset). Over the course of its history, the Institute that is known today as the National Institute of Diabetes and Digestive and Kidney Diseases is proud to have supported and conducted research on many of the Nation's most serious chronic diseases. Affecting people of all ages and racial and ethnic groups, the diseases within the NIDDK research mission encompass some of the most common, costly, and disabling conditions, as well as less prevalent but nonetheless debilitating diseases, affecting Americans today: endocrine and metabolic diseases and disorders such as diabetes and obesity, digestive diseases such as nonalcoholic fatty liver disease and inflammatory bowel disease, chronic kidney disease and kidney failure, urologic diseases and conditions such as interstitial cystitis/bladder pain syndrome and benign prostate enlargement, and blood diseases such as anemias.

The research advances made possible through 70 years of NIDDK support have saved lives, improved quality of life, and laid the foundation for future progress. The Institute has supported a number of winners of the world's greatest scientific honors. Many have won the Nobel Prize in Physiology or Medicine, and others have received the Nobel Prize in Chemistry. These include extramural scientists at universities and other research institutions across the country who have been supported by the NIDDK (Institute grantees), as well as scientists within the Institute's Intramural Research Program.

As part of activities to mark its 70th anniversary, starting in the summer of 2020, the NIDDK will highlight the research accomplishments supported over the past seven decades, and how they inform the Institute's current activities and vision for the future. These communications will be highlighted on the NIDDK website and in social media, and additional communications will take place through such venues as the "Healthy Moments" radio broadcast featuring the NIDDK Director. During the 70th anniversary year, the NIDDK will also undertake the development of its first-ever, Institute-wide strategic plan, which will complement disease-specific planning efforts and help guide research planning across its mission.

History of the NIDDK: On August 15, 1950, President Harry S. Truman signed into law the Omnibus Medical Research Act, establishing the National Institute of Arthritis and Metabolic Diseases (NIAMD)—which would become today's NIDDK. The new Institute incorporated the laboratories of the Experimental Biology and Medicine Institute and expanded to include clinical investigation in rheumatic diseases, diabetes, and a number of metabolic, endocrine, and gastrointestinal diseases. That same year, the NIAMD Council held its first meeting and recommended approval of NIAMD's first grants. Over the years, the NIAMD evolved into the National Institute of Arthritis, Metabolism, and Digestive Diseases (in 1972) and the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (in 1981). In 1986, the Institute's Division of Arthritis, Musculoskeletal and Skin Diseases became the core of a new, independent Institute. The NIDDK then acquired its current name—the National Institute of Diabetes and Digestive and Kidney Diseases.

NIDDK-supported Scientists Receive Presidential Award

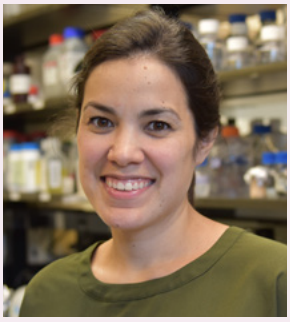
Scientists supported by the NIDDK were among the recipients of the Presidential Early Career Award for Scientists and Engineers (PECASE) in 2019. The PECASE is awarded to scientists and engineers who, while early in their research careers, have pursued innovative research and shown outstanding scientific leadership. Current NIDDK extramural grantees who received the PECASE include Melena Bellin, M.D.; Zachary Knight, Ph.D.; Sandeep Mallipattu, M.D.; and Jason Wertheim, M.D., Ph.D. NIDDK Intramural Research Program scientist Katherine McJunkin, Ph.D., was also a PECASE recipient.

Dr. Bellin, an Associate Professor in the Departments of Pediatrics and Surgery at the University of Minnesota Medical School, received a PECASE award in recognition of her work on optimizing a surgical treatment for severe chronic pancreatitis, called total pancreatectomy-islet autotransplantation, in which the pancreas is removed but patients are given back their insulin-producing pancreatic islets to reduce risk of diabetes. Determining which patient and disease characteristics are associated with better resolution of pain and health-related quality of life with this procedure, optimal timing for performing it, and improved methods for preserving the islets may lead to new strategies to address severe chronic pancreatitis. (See the Diabetes, Endocrinology, and Metabolic Diseases chapter for a profile of an individual who underwent this procedure.)

Dr. Knight, an Associate Professor in the Department of Physiology at the University of California, San Francisco, received a PECASE award in recognition of his work on neural mechanisms in the brain that control hunger, thirst, and thermoregulation. One facet of his research indicates that certain pathways regulating food intake are affected by the mere presence of food even before it is eaten; new knowledge about how the brain regulates behavior in response to food and other cues can help pave the way to new approaches to prevent or treat obesity.

Dr. Mallipattu, Chief of the Division of Nephrology and Hypertension and the DCI-Martin R. Liebowitz Associate Professor of Medicine at Stony Brook University, received a PECASE award in recognition of his work on mechanisms that regulate the proliferation and differentiation of certain cells in the kidney, especially as they relate to the progression of chronic kidney disease in people with diabetes. These efforts, combined with the ability to grow kidney cells in the laboratory into three-dimensional structures, could someday lead to being able to build a functional kidney—a potential treatment approach for kidney injury and disease.

Dr. Wertheim, Vice Chair for Research, Department of Surgery, and an Associate Professor in the Feinberg School of Medicine and the McCormick School of Engineering, Northwestern University, received a PECASE award in recognition of his work on how injured tissues and organs heal, regenerate, and repair, with a focus on bioengineering approaches tailored to treat liver and kidney damage and disease. This research could help to develop new tissues and regenerative approaches as future treatments for chronic organ failure.



Dr. McJunkin, Acting Chief, Section on Regulatory RNAs, Laboratory of Cellular and Developmental Biology at NIDDK, received a PECASE award in recognition of her work on the role of microRNAs in embryonic development and how these critical molecules—and hence, the biological pathways they help govern in cells—are themselves regulated over time. This research could ultimately lead to a better understanding of normal human development and of how various diseases arise.

In addition to these investigators, other scientists supported by the NIH and other federal agencies also received the PECASE for their scientific achievements. The PECASE is the most prestigious award given by the U.S. government to scientists at the outset of their independent research careers. These awards support the continued professional development of awardees, promote careers, foster innovation in science and technology, and recognize the scientific missions of participating agencies.