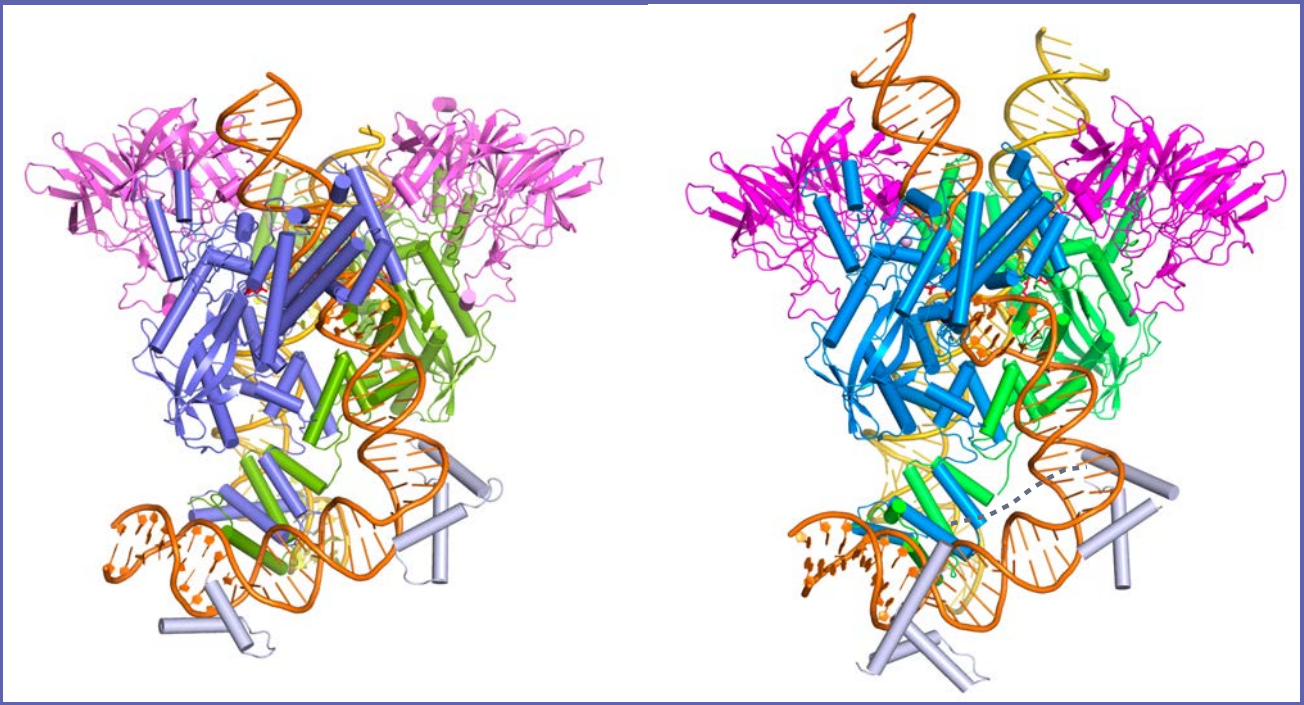

Determining the three-dimensional structure of a protein allows researchers to better understand its role in health and disease and identify the regions that are important for function. The proteins RAG1 and RAG2 are critical for generating a diverse population of antibodies that defend us against pathogens but could also be culprits in autoimmune diseases. This antibody diversity is generated by a process called "V(D)J recombination," in which segments of DNA are cut and rejoined by the RAG1/RAG2 protein complex to produce a variety of new combinations, a process that is essential for maturation of the immune system. Scientists in the NIDDK's Intramural Research Program have determined the three-dimensional structures of the RAG1/RAG2 protein complex before (left panel) and during (right panel) the process of cleaving its target DNA. Computer modeling revealed that the RAG1 (shown in blue and green) and RAG2 (shown in pink) proteins undergo significant rearrangements in three-dimensional space, cleaving the DNA in a nutcracker-like motion. The DNA molecule itself (shown in orange and yellow) also contorts significantly during the process. A better understanding of the dynamics of the RAG1/RAG2 complex during V(D)J recombination could yield important molecular insights into antibody production, as well as the diseases caused by immune system dysfunction.

Image courtesy of Drs. Wei Yang and Martin Gellert, NIDDK. Kim M-S, Chuenchor W, Chen X,... Yang W. Cracking the DNA code for V(D)J recombination. Mol Cell 70: 358-370, doi: 10.1016/j.molcel.2018.03.008, 2018. Reprinted by permission from Elsevier, copyright 2018.

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 join together to tackle ever more complex challenges, new opportunities to make exciting discoveries arise each day. Described in this chapter are several recent studies, each of which spans multiple areas within the NIDDK research mission, and a workshop on understanding how the nervous system regulates metabolism. The insights gained through this research can be expected to further scientific progress in many research areas, for today's discoveries may hold the seeds of tomorrow's cures.

IMAGING AN IMPORTANT CLASS OF MEMBRANE PROTEINS

New Mouse Model Illuminates Timing and Location of Activation of a "G protein-coupled Receptor" Important in Inflammation: Scientists have developed a novel mouse model that enables visualization of G protein-coupled receptor (GPCR) activation in a living animal in real time. GPCRs are one of the largest and most diverse families of proteins. They are involved in most physiological functions, detecting a variety of signals outside the cell that activate GPCRs, leading to cellular responses. They are implicated in many diseases, and it is estimated that nearly a third of approved pharmaceutical drugs target GPCRs. Therefore, characterizing the dynamics of their activation is critical to understanding the important role of this protein family in health and disease. In this study, scientists in the NIDDK's Intramural Research Program created a genetic mouse model that enables imaging of a member of the GPCR family that is involved in inflammation and other responses, called $S1P_1$, in real time in a living animal.

To create this model, the researchers took advantage of the ability to split a protein, called luciferase, that is derived from fireflies and can produce fluorescent light. They tethered one part of luciferase to $S1P_1$ and linked its complement to a protein that binds activated $S1P_1$ (β-arrestin2). Thus, when $S1P_1$ was activated, β-arrestin2 was recruited to and bound the activated receptor, bringing the two luciferase pieces together and producing a faint glowing signal that could be detected with a special microscope. To test the model, the researchers sought to establish the timing and anatomical location of $S1P_1$'s activation during inflammation triggered by the bacterial toxin lipopolysaccharide (LPS). They found that injection with LPS activated $S1P_1$ systemically and with distinctive timing for different parts of the mouse's body. For example, after 24 hours, the signal was strongest in the head and chest of the mouse, including in the brain. At 72 hours, it was strongest in the abdomen. This example demonstrated that this model can reveal the locations and dynamics of $S1P_1$ activation. This strategy could be used to create a library of other GPCR models to gain new knowledge of the biological role of GPCRs in normal and

disease contexts toward new drug development for these important targets.

Kono M, Conlon EG, Lux SY, Yanagida K, Hla T, and Proia RL. Bioluminescence imaging of G protein-coupled receptor activation in living mice. Nat Commun 8: 1163, doi: 10.1038/s41467-017-01340-7, 2017.

IMMUNE SYSTEM REGULATION

Clues to the Maturation of the Immune System from Three-dimensional Structures of Proteins RAG1 and RAG2: Scientists in the NIDDK's Intramural Research Program have determined the three-dimensional structures of proteins, known as RAG1 and RAG2, in complex with the DNA they target and cut in a process critical to immune system function. Because animals encounter a wide variety of potential infectious agents, the immune system must be able to generate a large and diverse population of antibodies to recognize these various invaders. This diversity is generated by a process called "V(D)J recombination," in which segments of DNA are cut and rejoined to produce a variety of new combinations, a process that is essential for maturation of the immune system. The proteins RAG1 and RAG2 function as the "cleavers" of DNA during V(D)J recombination and have been shown to be critical to the function of the immune system, as demonstrated by the over 60 mutations in the genes encoding RAG1 and RAG2 that lead to severe combined immunodeficiency (SCID) in humans or a milder type of immunodeficiency called Omenn syndrome. Understanding their three-dimensional structures, therefore, could provide insight not only into the critical process of V(D)J recombination, but into human disease as well.

The NIDDK scientists used two different methods to "visualize" at high resolution the RAG1 and RAG2 proteins in complex with DNA: cryo-electron microscopy and X-ray crystallography. By modifying DNA pieces that bound to the protein complexes, the scientists were able to "freeze" the complex just before the process begins and at a critical point in time during the process. They found that the RAG1 and RAG2 proteins undergo significant movements in three-dimensional space during the process, cleaving the DNA in a nutcracker-like motion. In addition, the recombination signal sequences (RSS) in the DNA molecule itself also contort dramatically, which could help explain why the RAG1/RAG2-targeted RSS tend to be rich in sequences that are physically able to bend and deform. The findings from this study provide important insights into how RAG1 and RAG2 participate in the maturation of the immune system and may lead to a better understanding of the molecular basis of SCID and Omenn syndrome.

Kim M-S, Chuenchor W, Chen X,... Yang W. Cracking the DNA code for V(D)J recombination. Mol Cell 70: 358-370, doi: 10.1016/j.molcel.2018.03.008, 2018.

Friend, Not Foe: Good Bacteria That Promote Skin Immunity and Tissue Repair: Researchers have discovered that non-disease-causing bacteria that live on mammalian skin are sensed by the immune system to promote protection from environmental pathogens and induce tissue repair. As the body's most exposed surface, the skin is in constant communication with a multitude of microbes, including bacteria, viruses, and fungi, and it is the first line of immunological defense. The researchers previously showed in mice that skin association with certain microbes leads to the accumulation of immune cells, specifically $CD8^+$ T cells—but not inflammation. Further, wild-caught mice that are exposed to myriad microorganisms contain a significantly larger number of $CD8^+$ T cells in the skin compared to pathogen-free, laboratory-raised mice.

In this study, the researchers isolated non-harmful strains of the bacteria *Staphylococcus epidermidis (S. epidermidis)* from the skin of healthy human volunteers, and found that some of these strains, when put on mice, had the ability to promote the accumulation of $CD8^+$ T cells in the skin. This result suggests that $CD8^+$ T cell buildup is a health-promoting response that occurs in the absence of inflammation or other disease state. Similar results were seen in the skin of non-human primates. Next, the researchers explored potential mechanisms whereby certain non-harmful microbes are capable of initiating this beneficial immune response in skin. They isolated $CD8^+$ T cells from the skin of *S. epidermidis*-associated mice and determined that a molecule called MHC1b H2-M3 was required for alerting the immune system to the presence of bacteria, leading to an accumulation of $CD8^+$ T cells at the skin surface. Having determined a mechanism for this immune response in a healthy state, they next compared gene profiles of *S. epidermidis*-induced $CD8^+$ T cells to cells isolated from the skin in the context of infection. Strikingly, they found that,

compared to cells induced by infection, *S. epidermidis*-elicited $CD8^+$ T cells had higher levels of genes associated with tissue repair and a range of molecules involved in wound healing. In a mouse model of skin-wounding, the researchers showed a remarkable accumulation of $CD8^+$ T cells at the wound edge post-injury. After measuring the progression of healing, they found that cells induced by the beneficial *S. epidermidis* promoted accelerated tissue repair compared to cells not associated with these bacteria.

Taken together, these results identify an important role for non-harmful bacteria in driving a $CD8^+$ T cell immune response to enhance tissue repair. These findings could have important clinical implications for wound healing following trauma, illness, or disease.

Linehan JL, Harrison OJ, Han SJ,...Belkaid Y. Non-classical immunity controls microbiota impact on skin immunity and tissue repair. Cell 172: 784-796, doi: 10.1016/j.cell.2017.12.033, 2018.

Novel Technique To Edit Immune Cell DNA Could Make New Treatments Possible: Scientists have discovered a new method for editing the DNA of T cells (a type of immune cell involved in pathogenesis of type 1 diabetes and other autoimmune disorders) that has significant advantages over existing techniques. The researchers demonstrated how this new tool could offer ways to treat certain autoimmune or genetic diseases or to target T cells to attack tumors, among other possible applications. This new genetic editing technique is based upon the existing CRISPR-Cas9 genetic targeting system. That system uses a protein "scissor" called Cas9, customized DNA templates that include instructions for the desired edits, and the cells' own DNA repair machinery to latch onto, cut, and "edit" a cell's DNA. Previously, genetic editing in T cells required specially designed viruses to deliver the editing system to the cells. Viral delivery systems, however, are costly and time-consuming to produce, and they can make genetic changes in unwanted places in the target DNA. Viral delivery systems were also the only option for applications requiring delivery of large DNA templates, as those large fragments were toxic to cells when delivered using previous non-viral delivery methods. To find an alternative that would avoid these limitations, researchers tested different ways to deliver the genetic editing system to T cells via electroporation, a process that uses an electrical field to make cell membranes more permeable temporarily. They found that if a certain electrical field was used with specific ratios of T cells, DNA, and proteins, the genetic editing system efficiently entered the cells. This new technique resulted in precise genetic edits at the intended DNA target sites without the need for a virus. What DNA is targeted could also be easily changed by altering the DNA templates used, making this system flexible and suitable for a variety of possible uses, including those requiring large DNA templates.

To demonstrate the promise of this genetic editing system, researchers performed two demonstrations where editing genes in T cells could treat disease. In the first demonstration, they used their non-viral genetic editing system to correct a specific genetic defect (mutation) in the DNA of T cells that causes a rare, inherited autoimmune disease that is resistant to treatment. The scientists collected T cells donated by people with the disease and used their electroporation-based gene editing technique to correct the disease-causing mutation, thus restoring the T cell functions that were disrupted by the mutation. In a second demonstration, researchers used large DNA templates to reprogram T cells, which are usually tasked with attacking infections, to instead target a specific type of tumor cell. The reprogrammed T cells homed in on the target tumor cells both in a laboratory dish and in male mice carrying the tumors, attacked the tumor cells, and reduced tumor size.

Overall, these experiments showed that this novel non-viral genetic editing system allows rapid and economical production of genetically tailored T cells that could potentially be used for a variety of applications, including cancer immunotherapy, genetic disease therapies, and potentially for combating other diseases in which T cells play a role, like type 1 diabetes. Future research is required to determine whether this technology is safe for clinical applications, but this system provides a new tool that greatly expands the possibilities for using genetic editing to treat human disease.

Roth TL, Puig-Saus C, Yu R,...Marson A. Reprogramming human T cell function and specificity with non-viral genome targeting. Nature 559: 405-409, doi: 10.1038/s41586-018-0326-5, 2018.

Workshop Explores Role of the Autonomic Nervous System in Metabolic Health and Disease

On September 20–21, 2018, the NIDDK sponsored a workshop in Bethesda, Maryland, focusing on the role of the autonomic nervous system (ANS)—nerves that transmit signals among the brain, spinal cord, and internal organs—in regulating healthy metabolism and how changes in nerve function may influence metabolic diseases such as obesity, diabetes, and fatty liver disease.

The ANS plays a key role in regulating physiological responses. Nerves running from peripheral organs to the central nervous system (spinal cord and brain) relay sensory and metabolic information, while nerves running in the opposite direction regulate metabolic processes, including the body's metabolism of glucose (sugar) and fat, as well as hormonal secretion in organs such as the liver, adipose (fat) tissue, intestine, and pancreas. New techniques in the fields of neuroscience and molecular genetics are being used to elucidate the structures, connections, and function of the ANS. The NIH Common Fund program called Stimulating Peripheral Activity to Relieve Conditions, or "SPARC," is supporting research to transform understanding of nerve-organ interactions controlling organ functions throughout the body and advance research geared toward more precisely treating diseases and conditions through neuromodulation. However, much remains unknown about ANS contributions to regulating glucose and fat metabolism, as well as metabolic disease processes.

This workshop brought together speakers and other participants with the goals of: 1) increasing foundational knowledge of the role of the ANS in regulating metabolism and metabolic disease; 2) fostering interactions between basic and clinical scientists with expertise in metabolism and neuroscience; 3) addressing limitations of current technologies and methods for measuring tissue-specific ANS activity and function in humans; 4) building on the NIH SPARC program to expand its focus to metabolic disease; and 5) identifying research gaps in basic and clinical science. Speakers presented on a range of topics related to the role of the ANS in regulating processes in the gastrointestinal tract, glucose metabolism and balance, liver metabolism and disease, metabolic diseases such as obesity and diabetes, and adipose tissue and fat metabolism.

The meeting organizers plan to develop a summary for publication in the scientific literature describing the workshop proceedings and highlighting current gaps in knowledge, which will inform future efforts to advance research on the ANS and its impacts on metabolic health and disease.