



The bacteria *Neisseria* can cause serious diseases in humans. These disease-causing bacteria require iron for their survival and use a surface receptor to extract iron directly from a human iron-binding protein abundant in the bloodstream. Shown here is the structure of the bacterial receptor protein (shown in gold, blue and magenta) extracting an iron atom (red sphere) from the human protein (green). Details about molecular interactions between complex proteins may identify new targets for treatments.

Image courtesy of Dr. Nicholas Noinaj, NIDDK.

Cross-Cutting Science

Advances in medicine sometimes are achieved in great leaps, but more often result from the gradual accumulation of new knowledge over years. Insights into fundamental biologic processes at the smallest levels 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 understanding of disease and in the development of novel treatments can be traced to laboratory studies whose immediate relevance to health could not have been fully known or appreciated at the time they were conducted. With the development of innovative technologies, novel scientific approaches, and the emergence of new scientific disciplines as teams of talented, creative researchers join together to pursue increasingly complex challenges, new opportunities to make exciting discoveries arise each day. Described in this chapter are several recent studies, whose themes span multiple areas within the NIDDK research mission. Also featured are the NIDDK efforts at public outreach to better disseminate important health information to the public using both traditional and social media.

The efforts outlined here illustrate the Institute's commitment to basic and applied research that is relevant across a broad spectrum of science. The insights gained through this research can be expected to aid progress in many scientific endeavors, for today's discoveries may hold the seeds of tomorrow's cures.

NEW INSIGHTS INTO STEM CELLS

Induced Pluripotent Stem Cells Hold on to Past Identity: Researchers have found genomic marks in human induced pluripotent stem (iPS) cells that currently limit their scientific and therapeutic potential, but also suggest opportunities to improve the development of these cells.

Researchers initially developed iPS cells with the hope of overcoming challenges posed by other types of stem cells. Human embryonic stem (ES) cells, for example, hold promise in the treatment of disease because they are “pluripotent,” meaning that unlike most other cells, they have the ability to form virtually any cell type and thus could generate cells for repair of human tissues and organs. The use of human ES cells, however, is controversial because their isolation entails the destruction of early-stage human embryos; ES cells have other limitations as well. In recent years, scientists developed strategies to reprogram cells, such as blood or skin cells, to revert from their specific cell

types back to an ES cell-like state, with the potential to form not only new cells of their original type, but also stem cells and a multitude of different cell types. These pluripotent, reprogrammed cells, called iPS cells, could potentially be used to study diseases and to generate cells to treat specific diseases, potentially with a tissue match for the recipient (avoiding transplant rejection).

For reasons that have been poorly understood, iPS cells generated to date are significantly less pluripotent than ES cells; they are more easily able to form the cell type from which they were originally derived than to form cells of other types. To understand why this might be the case in human iPS cells, researchers analyzed the cells' DNA, building on previous findings in mouse cells. Mouse iPS cells retain a pattern of chemical modifications on their DNA characteristic of their past cell type, rather than a pattern characteristic of ES cells. Although this modification does not alter the sequence of the genetic code, it can affect the cell's ability to turn genes on or off. The combination of genes that are active and inactive characterizes a cell type; therefore

this important modification has the effect of helping a cell to “remember” its identity. In this new research, scientists sought to determine whether human iPS cells retained the chemical modifications of their past cell type, like the mouse iPS cells did. They produced iPS cells from both blood and skin cells. The researchers found, as expected, that blood-derived iPS cells were more likely to form blood cells and skin-derived iPS cells were more likely to become skin cells. By comparing the patterns of chemical modifications of iPS cells to ES cells, the researchers determined that the iPS cells retained patterns characteristic of their original cell types. Current techniques to generate iPS cells, therefore, do not fully erase the cell’s memory, limiting its potential to become another cell type.

Scientists will continue research to develop new techniques that may be able to erase the residual patterns more fully. In the meantime, scientists may be able to take advantage of the bias of iPS cells toward their original cell type in the study of and development of therapies for diseases associated with those cell types. Cautious optimism continues for the eventual, wider use of iPS cells.

Kim K, Zhao R, Doi A, et al. Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells. Nat Biotech 29: 1117-1119, 2011.

Genetic Mutations in Experimentally Derived (Induced Pluripotent) Stem Cells: Researchers have recently determined the source of genetic mutations found in induced pluripotent stem (iPS) cells. In recent years, scientists have developed ways of reprogramming cells, such as those derived from blood or skin, to revert back to an embryonic stem cell-like state. These stem cells have the potential to give rise not only to new cells of the original type but also to more stem cells and to cells of many different types of tissues. One technique involves the introduction of three to four genes into adult cells that direct the reprogramming of these cells into iPS cells. Recent studies assessing the genetic integrity of iPS cells have found mutations that could limit the therapeutic potential of these cells, but the origins of these mutations have not been clear.

Scientists determined the complete sequence of all the DNA—the “genome”—from 10 different mouse iPS cell lines derived from three different original, or parental, cells. The entire genomes were sequenced to determine the number and location of any mutations. Hundreds of mutations were detected in each iPS cell line genome. By comparing the genome sequences of the parental cells to those of derivative iPS cells, the researchers concluded that most of the genetic mutations in iPS cell lines were not caused during the reprogramming process but rather were derived from mutations that pre-existed in the parental cells.

This study illustrates the importance of selecting the most appropriate adult cell to undergo the reprogramming process to form iPS cells, because any mutations that exist in the parental cell will be passed on to the iPS cells. Knowledge gained from sequencing iPS cell genomes may help to improve the selection and derivation of iPS cells such that these cells can be more safely used for regenerative therapies.

Young MA, Larson DE, Sun C-W, et al. Background mutations in parental cells account for most of the genetic heterogeneity of induced pluripotent stem cells. Cell Stem Cell 10: 570-582, 2012.

Aging of Blood Stem Cells: Recent research has linked blood stem cell aging with increased activity of the cell division cycle 42 (Cdc42) protein. All blood cell types are derived from a population of self-renewing hematopoietic (blood) stem cells (HSCs) that first appear during embryonic development. However, there are changes in HSC structure and a decline in function over time that leads to reduced production of both red and white blood cells by the HSC. The molecular mechanisms underlying HSC aging are not known, but previous research has shown that Cdc42 activity is significantly increased in both HSCs and other cell types of old mice when compared with those from young mice. Whether enhanced activity of Cdc42 is responsible, in part, for the aging phenotype is not known.

Using a mouse model that exhibits increased Cdc42 activity due to genetic deletion of an inhibitor of the

protein, scientists found that HSCs from young animals with this mutation exhibit a decline in function similar to that observed in normal older animals. HSCs from young mutant mice showed diminished organization of intracellular structures similar to that seen in aged mice. HSCs from the mutant mice that were grown in culture in the presence of a drug that inhibits Cdc42 activity reverted to a “younger” appearance, with intracellular organization restored. This observation provides evidence that Cdc42 activity plays a role in structural degradation seen in the aging of HSC, and that this can be reversed through inhibition of this protein.

Additional studies suggested that Cdc42 plays a role in the decline in HSC function observed during aging. Using an experiment that measures how well HSCs are able to “home” or localize to the bone marrow and begin to grow there, the scientists found that HSCs from a mutant mouse with elevated Cdc42 activity no longer had the ability to repopulate the hematopoietic system of a mouse whose HSCs have been eliminated by prior radiation treatment. In contrast, cells from a mutant mouse that had been treated with a Cdc42 inhibitor in culture prior to being injected into the recipient mouse had an increased ability to repopulate the bone marrow, though not as robustly as normal HSCs from young animals. This finding shows that even a brief exposure to the Cdc42 inhibitor was sufficient to partially restore function lost during aging of HSCs.

Future studies will determine whether drugs that target Cdc42 can restore function in aged human HSCs. As elevated Cdc42 activity has been observed in multiple tissues in aged mice, this finding may have implications that extend beyond the hematopoietic system and blood cell production.

Florian MC, Dörr K, Niebel A, et al. Cdc42 activity regulates hematopoietic stem cell aging and rejuvenation. Cell Stem Cell 10: 520-530, 2012.

Studies Shed Light on Resident Stem Cells that Repopulate Normal and Injured Intestine:

Two research teams have illuminated unique roles in intestinal regeneration for two distinct stem cell populations found on the inner surface of the intestine.

The inner lining of the intestine plays an essential role in absorption of nutrients and balancing absorption and secretion of water and electrolytes, as well as providing a barrier against entry of bacteria. The lining is only one cell thick and cells slough off the surface continuously, lasting only about 1 week, requiring a process of continuous regeneration. Cells are also replaced following any injury. Recently, two populations of intestinal stem cells that are necessary for regeneration were identified by their different locations in the intestinal surface, as well as the unique proteins marking their outer membranes—either Lgr5 or Bmi1. Two research teams took a closer look at these stem cells to characterize their roles in intestinal regeneration.

One of the teams conducted research as part of the NIDDK’s Intestinal Stem Cell Consortium. Using a microscopic technique that highlights intestinal stem cells with fluorescent markers for Lgr5 and Bmi1 in genetically modified mice, the researchers measured proliferation of the cells under normal, healthy conditions, as well as after injury caused by radiation. They found that the stem cells with Lgr5 on their surface actively proliferated under normal conditions but were destroyed by radiation. The stem cells marked by Bmi1 were relatively inactive under normal conditions but resisted radiation and proliferated dramatically following the injury. The Bmi1-marked stem cells in culture could also form some Lgr5-marked stem cells, showing their capacity to repopulate the intestine with both stem cell populations following injury.

Another team examined whether the Paneth cell, another type of intestinal cell located near Lgr5 stem cells, was essential for the ability of Lgr5 stem cells to repopulate the intestinal lining as part of normal cell turnover and tissue renewal. Paneth cells are known for secreting substances, such as proteins with antimicrobial properties, enzymes, and growth factors. Considering their proximity to intestinal stem cells, Paneth cells were thought to be involved in stem cell functions. This study used genetically modified mice and fluorescent markers to identify the Lgr5-producing stem cells and Paneth cells during early intestinal development. They found that the appearance of the intestinal stem cells preceded

Paneth cells in the developmental process, and that the stem cells appeared to function normally. They then created a genetically modified mouse that lacked Paneth cells. The stem cells functioned normally in this mouse model as well, proliferating to renew the intestinal surface as usual. These experiments indicate that Paneth cells are not essential for many aspects of Lgr5-marked stem cell function, though Paneth cells may still have other beneficial effects on their neighboring stem cells.

Taken together, the work of these two research teams paints a more nuanced picture of the complementary stem cell types that renew the intestinal surface throughout life, in terms of continuing cell turnover as well as regeneration following injury. This knowledge could be applied to optimizing recovery from different forms of intestinal injury.

Kim T-H, Escudero S, and Shivdasani RA. Intact function of Lgr5 receptor-expressing intestinal stem cells in the absence of Paneth cells. Proc Natl Acad Sci USA 109: 3932-3937, 2012.

*Yan KS, Chia LA, Li X, et al. The intestinal stem cell markers *Bmi1* and *Lgr5* identify two functionally distinct populations. Proc Natl Acad Sci USA 109: 466-471, 2012.*

PROTECTING THE GENOME AND REGULATING GENE EXPRESSION

Long-range Regulation of How Genes Are Turned “Off” and “On”: Two studies have advanced our understanding of the transcriptional regulation of a set of red blood cell genes called globin genes, and of how these genes are turned on and off. Transcription is a biologic process that involves the transcribing or copying of genetic information from DNA into RNA. Immature red blood cells produce globin proteins, key components of hemoglobin, which carry oxygen in red blood cells from the lungs to the rest of the body. Production of globin proteins is a highly regulated process to ensure that these genes are turned on (“expressed”) or off at appropriate times during the development of red blood cells from their precursors in the bone marrow.

The mammalian *β-globin* gene locus was among the first gene clusters to provide insight into how gene regulation is influenced by long-range chromosomal interactions between DNA sequences far from and near to the protein-coding segment of a gene. Specifically, these interactions occur between a powerful element called an enhancer that helps turn on the *β-globin* gene, also referred to as the gene’s locus control region (LCR), and a DNA element called a promoter, which is immediately adjacent to the gene and helps regulate whether it is on or off. Scientists continue to study enhancers such as the LCR in order to more fully understand their role in the regulation of gene expression generally.

In the first study, scientists devised a strategy to delineate whether loops of chromosomal DNA, created by the interaction between the *β-globin* gene promoter and LCR, are a cause or an effect of gene transcription. Using erythroid precursor cells (cells that develop into normal red blood cells) to study the *β-globin* gene locus transcription process, researchers showed for the first time that the protein Ldb1 is a key looping factor involved in long-range regulation of gene transcription. Furthermore, their experimental manipulations that forced or impaired the creation of the DNA loop which allows the LCR to interact with the *β-globin* gene confirmed that loop formation plays an important role in initiating *β-globin* gene transcription and is not simply a consequence of the gene being “turned on.”

In the second study, investigators sought to determine the relative contribution of four segments of the LCR to its function in regulating *β-globin* gene transcription and also to determine the location of the *β-globin* locus within the cell’s nucleus. Nuclear location influences whether or not a gene is expressed. Scientists refer to these LCR segments as DNase I hypersensitive sites (HSs) because they are regions of the enhancer that are extremely sensitive to breakdown into smaller pieces (digestion) by the enzyme DNase I. Sensitivity to DNase I is a measure of the transcriptional status of a gene and indicates that this region of DNA is “open” or exposed, so that factors that promote transcription can bind to it. Mice were engineered that had various combinations of HSs deleted, and the effects of these

deletions on gene transcription were measured in relationship to the position of the β -globin gene locus in the nucleus. The results showed that while the effects of the HSs are additive, only two were needed for the β -globin locus to be repositioned towards the center of the nucleus where gene transcription can become active. However, all four of the HSs of the LCR were shown to be needed for β -globin transcription to be completed efficiently.

These studies add considerable knowledge to our understanding of the regulation of gene transcription. Identification of looping factors such as Ldb1 and delineation of the various components required for proper β -globin transcription may help the development of new ways to treat hematologic diseases, such as sickle cell disease, by reactivating dormant hemoglobin genes.

Bender MA, Ragoczy T, Lee J, et al. The hypersensitive sites of the murine β -globin locus control region act independently to affect nuclear localization and transcriptional elongation. Blood 119: 3820-3827, 2012.

Deng W, Lee J, Wang H, et al. Controlling long-range genomic interactions at a native locus by targeted tethering of a looping factor. Cell 149: 1233-1244, 2012.

Guiding Genetic Rearrangement To Protect the Genome: Scientists discovered that genetic rearrangement—the process by which cells intentionally break, shuffle, and repair their DNA to create new combinations of genes—is directed away from functional genomic regions. Genetic rearrangement occurs naturally in cells destined to become sperm and eggs to generate genetic diversity, ensuring that each new organism will be unique. This process can be beneficial in that new, advantageous traits can arise from these genetic rearrangements; but, if it goes awry, this process can also generate abnormalities that result in miscarriages, congenital birth defects, and mental retardation. Researchers in the NIDDK’s Intramural Research Program and colleagues previously demonstrated that locations in the genome where genetic rearrangements occur more frequently—“hotspots”—were associated with

the activity of a protein called PRDM9, but they did not know if the hotspot location was determined by PRDM9.

To evaluate PRDM9’s role, the researchers mapped hotspots in two mouse strains that were nearly genetically identical, but encoded different versions of PRDM9. They found a similar number of hotspots in the two different strains, but almost no overlap in the locations of the hotspots. Hotspots in the two lines centered on distinct DNA sequences that aligned with the predicted DNA-binding sites for the different PRDM9 proteins. This indicated that the location of rearrangement hotspots was dependent on PRDM9. When the scientists looked at mice genetically engineered to lack PRDM9, they found that rearrangements still occurred in hotspots, but that these hotspots were not in the same locations as those in normal mice. Rather, the hotspots in mice without PRDM9 were re-routed to sites in the genome associated with gene activity. The researchers propose that PRDM9 directs the rearrangement machinery to preferred sites in the genome and away from functional genomic regions. This re-routing away from important genomic elements may protect against potential harmful effects of genetic rearrangement.

Brick K, Smagulova F, Khil P, Camerini-Otero RD, and Petukhova GV. Genetic recombination is directed away from functional genomic elements in mice. Nature 485: 642-645, 2012.

Multi-tasking UTX Protein Plays Key Role Early in Development: NIDDK intramural scientists studying mice have discovered that a protein capable of unleashing genes important to tissue formation has a second, independent job regulating early embryonic development. As a fertilized egg develops into an embryo, it must first grow and divide to organize into a ball of cells containing three distinct cellular layers, called germ layers. Each germ layer will go on to generate a specific subset of the organs, tissues, and cells that make up an organism. Embryonic stem (ES) cells grown in the laboratory can be coaxed to differentiate into the three germ layers, thus offering a model to understand factors influencing this critical

aspect of early development. The UTX protein has already been shown to regulate processes important later in development, such as those governing generation of the heart and muscle cells. In some cases, this occurs when UTX exerts an enzymatic function that enables quiescent developmental genes to become activated. Now, researchers studying UTX in mouse ES cells and mouse embryos have discovered that this protein is also critical to an early step in development—formation of one of the three germ layers—and that this function is independent of its enzymatic activity.

Working in mouse ES cells, the researchers generated three experimental strains: a control strain with an intact *UTX* gene, a strain in which the *UTX* gene was deleted, and a strain in which the *UTX* gene was intact but mutated so that the protein produced no longer had enzymatic activity. These different strains were treated to encourage formation of germ layers. While apparently able to support formation of other germ layers, ES cells lacking UTX could not form a layer called the mesoderm. In contrast, ES cells with the mutated UTX developed similarly to the control cells—indicating that UTX, but not its enzymatic activity, was important to mesoderm formation. Further experiments revealed a possible mechanism: UTX protein binds to a DNA region controlling activation of the gene for *Brachyury*, a factor essential to mesoderm formation. In the absence of UTX, *Brachyury* gene activation in ES cells was reduced significantly, and could not be artificially induced—indicating that UTX is an essential component of the molecular machinery that activates *Brachyury*.

The *UTX* gene is found on the X chromosome and is thus normally present in both male and female mice. Male cells possess a gene on the Y chromosome, called *UTY*, that encodes a protein similar to UTX but which lacks detectable enzymatic activity. Suspecting that UTY may act like UTX, the researchers conducted a series of molecular experiments and found evidence suggesting that UTY can also activate *Brachyury*. To test whether UTX and UTY are important in actual embryonic development, the researchers mated mice to produce female embryos lacking UTX, and male embryos lacking UTX but retaining UTY. Female embryos lacking

UTX had significantly reduced *Brachyury* activation, severe developmental defects similar to those seen in mouse embryos lacking the *Brachyury* gene, and died before birth. In contrast, male embryos lacking UTX, while suffering defects that made it impossible for them to survive much beyond birth, developed much more normally and activated *Brachyury*—further suggesting that UTX and UTY are functionally redundant early in development. Together, these findings shed further light on both early development and the multi-tasking of factors during development—information that could also be useful in recapitulating developmental processes important to regenerative medicine efforts.

Wang C, Lee J-E, Cho Y-W, et al. UTX regulates mesoderm differentiation of embryonic stem cells independent of H3K27 demethylase activity. *Proc Natl Acad Sci USA* 109: 15324-15329, 2012.

BASIC SCIENCE RESEARCH REVEALS NEW INFORMATION ABOUT CELLULAR PROCESSES AND METABOLISM

Biological Chemistry: How Is a Rare Bond Created? An ancient enzyme that can be traced back 500 million years forms the chemical “rivet” that reinforces connective tissue throughout the body. This “sulfilimine” chemical bond links a nitrogen and sulfur atom in a manner that had not been observed in a biological system until 3 years ago. The sulfilimine bond reinforces the collagen IV network, which in the kidney, provides the structural scaffolding for the glomerular basement membrane. Researchers explored the chemistry underlying the creation of this rare bond and found that it is created by the enzyme peroxidase.

The researchers used cultured mouse cells to study the how the sulfilimine bond that links one collagen segment to another is created. They characterized each of the components and steps involved in the formation of this bond by peroxidase, which include the generation of a highly reactive and potentially toxic intermediate acid that is similar to household bleach. The elucidation of the process that leads to the creation of this rare chemical bond is an important advance in

researchers' knowledge of collagen biochemistry at a fundamental level and provides broader insights into one way that these networks are stabilized.

Levels of peroxidase are increased in models of high blood pressure and atherosclerosis, suggesting that it may play a role in inflammation and disease. The researchers hypothesize that these findings could also provide insight into treatment of the autoimmune disease Goodpasture's syndrome, in which antibodies target collagen IV molecules. Future studies may elucidate additional roles of this enzyme in a variety of diseases and illuminate possible approaches to treatment.

Bhave G, Cummings CF, Vanacore RM, et al. Peroxidase forms sulfonamide chemical bonds using hypohalous acids in tissue genesis. Nat Chem Biol 8: 784-790, 2012.

Bacterial Strategy To Acquire Iron from Humans:

A three-dimensional reconstruction detailing the interactions between proteins from the bacteria *Neisseria meningitidis* and the human iron-binding protein transferrin has recently been determined. Two members of the *Neisseria* family of bacteria can cause disease in humans; one of these, *N. meningitidis*, is a leading cause of meningitis. It requires iron both for its survival and to cause disease. *N. meningitidis* collects iron by extracting it from the human transferrin receptor, a protein on the cell surface that binds to iron in the blood and transports it into cells.

To acquire iron, the bacterium uses two proteins on its surface, TbpA and TbpB, that can bind to and remove iron from the transferrin receptor in the human host. The exact nature of this interaction is unknown. Determining the structure of these bacterial proteins and how they interact with host proteins is an important step in understanding how they function.

Researchers have, for the first time in the laboratory, generated complexes of TbpA and TbpB bound to human transferrin that permitted the assembly of a three-dimensional reconstruction defining important interaction sites between the bacterial proteins and transferrin. They examined complexes of TbpA and transferrin, TbpB and transferrin, and of all three

proteins together using three different methods. The reconstruction suggests a mechanism for bacterial uptake of iron, including how the bacteria latches on to transferrin, how a part of protein TbpA inserts itself into and displaces the iron from transferrin, and how the recently acquired iron is transported inside the bacteria.

This study provides critical information regarding the structural basis for iron piracy by disease-causing bacteria. Furthermore, as TbpA and TbpB are on the surface of the bacteria, this finding may have implications for the development of both structure-based vaccines and drug design.

Noinaj N, Easley NC, Oke M, et al. Structural basis for iron piracy by pathogenic Neisseria. Nature 483: 53-58, 2012.

A NOVEL APPROACH TO ALLEVIATE CHRONIC PAIN

New Insights into Chronic Pain: Researchers have shown that molecules that activate the A₃ subtype of the cellular receptor for the molecule adenosine have a potent anti-pain effect in two different rodent models of chronic pain. These results identify a novel class of drugs that could be used to alleviate chronic pain arising from various causes, including some forms of chemotherapy for cancer.

“Neuropathic pain” is caused by damage to the nervous system. It often manifests as abnormal sensations, such as numbness or tingling, or as pain produced by mild stimuli that are normally not painful, such as light touching. Using a mouse model of pain sensation, the scientists observed that treatment with any one of three different molecules that selectively activate the A₃ subtype of the adenosine receptor—receptor “agonists”—could alleviate neuropathic pain. Similar pain relief following treatment with A₃ adenosine receptor agonists was seen in rats that had been given drugs used in chemotherapy. While these agents were effective in alleviating neuropathic pain, they had no effect on so-called “nociceptive pain,” which is pain sensed in response to a potentially harmful stimulus such as heat.

Notably, the administration of any one of the three A₃ receptor agonists at very low doses could significantly increase the effectiveness of opiate pain relievers, such as morphine, in the mouse model. Studies of cells in culture showed that the A₃ receptor agonists did not limit the ability of chemotherapeutic drugs to inhibit the growth of cancer cells, an important consideration given that patients undergoing chemotherapy sometimes develop neuropathic pain that is so debilitating that it leads them to discontinue treatment.

Currently, the most effective treatments for chronic neuropathic pain involve opiate-derived drugs. However, their usefulness is limited because patients often develop tolerance for the drugs, necessitating higher and higher doses to achieve pain relief, and because these high doses can result in serious side effects, including the possibility of addiction. The identification of the A₃ adenosine receptor signaling pathway as a potential target for treatment of chronic neuropathic pain, and the existence of highly specific, potent activators of this pathway, represents a novel approach to treatment of chronic pain.

Chen Z, Janes K, Chen C, et al. Controlling murine and rat chronic pain through A₃ adenosine receptor activation. FASEB J 26: 1855-1865, 2012.

INSIGHTS INTO HIV TRANSMISSION AND TREATMENT

Drug Therapy To Prevent HIV Infection:

Researchers recently investigated whether two drugs that have been used to treat infection with HIV-1, the virus that causes AIDS, might also be used to prevent transmission of the virus. For years, a combination of drugs termed “highly active antiretroviral therapy” (HAART) has been used to treat people infected with the human immunodeficiency virus, HIV-1. The use of drugs that are part of HAART in individuals before they are exposed to HIV-1 has been considered as a possible strategy to prevent the transmission of this virus. Scientists have now examined the metabolism of two drugs used in HAART and have described their distribution in different tissues.

Because HIV-1 is often transmitted through sexual contact, it was important that the researchers accurately measure levels of the active forms of the drugs in genital and colorectal mucosal tissue. The investigators gave 15 healthy men and women a single oral dose of a combination of two antiretroviral drugs, tenofovir (TFV) disoproxil fumarate and emtricitabine (FTC), and subsequently measured the concentration of these drugs over the next 14 days in the volunteers’ blood and genital secretions, as well as in their vaginal, cervical, and rectal tissues. The drugs were detected in the blood and genital secretions for the full 14-day duration of the study and were present at higher concentration in the genital secretions, with a particularly high concentration of FTC in these samples. The biologically active metabolites of the drugs were detected in the vaginal, cervical, and rectal tissues for varying durations and at different levels. The active form of TFV was found at high levels for all 14 days of the study in rectal tissue, but was present at much lower levels in vaginal and cervical tissue. The active form of FTC was present at higher levels in vaginal and cervical tissues than in colorectal tissues, but could be detected for less than 2 days.

The wide range of tissue exposure to an orally-administered drug reported in this study illustrates the need for more detailed studies of the pharmacology of drugs currently used to treat HIV infection as possible agents to prevent transmission of the virus. Ultimately, the success of drug therapy to prevent the spread of HIV-1 will depend on selecting the proper combination of drugs and their doses.

Patterson KB, Prince HA, Kraft E, et al. Penetration of tenofovir and emtricitabine in mucosal tissues: implications for prevention of HIV-1 transmission. Sci Transl Med 3: 112re4, 2011.

Metformin Is Shown To Be Effective in Treating HIV Patients at Risk for Cardiovascular Disease (CVD):

In a recent clinical trial, scientists studying the effects of the drug metformin and lifestyle modification (LSM) on HIV-infected patients with metabolic syndrome found that metformin significantly reduced the progression of coronary artery calcification (CAC), a risk for CVD.

Lifestyle modification therapy was not as effective in achieving this goal. HIV-infected patients have high rates of metabolic abnormalities, including a large waistline, high levels of circulating triglycerides, low levels of HDL (“good”) cholesterol, high blood pressure, and high fasting glucose. Having three or more of these contributing factors meets the criteria for metabolic syndrome and increases an individual’s risk of developing CVD and type 2 diabetes.

Because metformin has been shown to significantly reduce CVD events in individuals without HIV, and because LSM is considered important in the treatment of HIV-infected patients with metabolic syndrome, clinical researchers hypothesized that treatment with metformin and/or LSM would be beneficial for HIV-metabolic syndrome patients in reducing CAC and other risk factors for CVD. To test this hypothesis, the researchers conducted a clinical study in which HIV-metabolic syndrome patients were divided into four groups and treated for 1 year with placebo alone, LSM and placebo, metformin alone, or metformin and LSM. Treatment with metformin and/or LSM was assessed by changes in several criteria including CAC, calcified plaque volume, and other measures of subclinical CVD. In addition, changes in several metabolic indices including fasting blood sugar and HDL-cholesterol levels were

measured. Metformin was found to significantly reduce progression of CAC and calcified plaque volume in these patients and had a significantly greater effect on CAC progression than treatment with LSM. Also, metformin improved insulin resistance, a measure of prediabetes and type 2 diabetes. Although LSM had a lesser effect than metformin on CAC progression, it had significantly beneficial effects on HDL-cholesterol and cardio-respiratory fitness.

The results of this clinical study provide clinicians and researchers with valuable information on metformin as an effective treatment for preventing cardiovascular plaque progression in patients with HIV and metabolic syndrome. In addition, the study has shown LSM to be effective in treating some metabolic conditions in these patients. The metformin finding is particularly advantageous because, although additional studies are needed to assess whether metformin can prevent CVD events, metformin is an FDA-approved generic drug that could be used in the treatment of CVD risk factors in HIV-infected patients with metabolic syndrome.

Fitch K, Abbara S, Lee H, et al. Effects of lifestyle modification and metformin on atherosclerotic indices among HIV-infected patients with the metabolic syndrome. AIDS 26: 587-597, 2012.

Dr. Peter P. Reese and Dr. Georgios Skiniotis: NIDDK-Supported Scientists Receive Presidential Award

Two scientists supported by the NIDDK have received the 2011 Presidential Early Career Award for Scientists and Engineers (PECASE). PECASE is awarded annually to scientists and engineers who, while early in their research careers, have demonstrated the pursuit of innovative research and outstanding scientific leadership. Among the recipients were two NIDDK extramural grantees—Peter P. Reese, M.D., M.S.C.E., and Georgios Skiniotis, Ph.D. In addition to the NIDDK-supported recipients, 18 other scientists supported by the NIH received the award for their scientific achievements; the NIH has now funded 213 PECASE recipients since the award's inception in 1996. PECASE is the most prestigious award given in the United States to scientists at the outset of their independent research careers.

Developing Ethical Approaches to Expanding Access to Organ Transplantation



Peter P. Reese, M.D., M.S.C.E.

Dr. Reese, an Assistant Professor of Medicine and Epidemiology at the University of Pennsylvania in Philadelphia, received a 2011 PECASE award in recognition of his contributions to organ transplant research. His primary research focus is in the development of effective strategies to increase access to kidney transplantation. The growing and unmet need for kidney transplants is driven by the rising prevalence

of end-stage renal disease (ESRD) in the United States. ESRD impairs quality of life, decreases survival, and is increasingly common among older adults. A shortage of available organs for transplantation has driven strong interest in providing kidney transplants to the patients who benefit the most. Dr. Reese's novel approach will evaluate the use of the patient's functional status—a measure of the ability to complete important daily activities—as a tool to predict which patients derive the greatest survival benefit from a kidney transplant.

Understanding the Structure and Function of Signaling Cell Surface Receptors



Georgios Skiniotis, Ph.D.

Dr. Skiniotis, a Pew Scholar in the Biomedical Sciences and Research Assistant Professor at the University of Michigan in Ann Arbor, received a 2011 PECASE award for his innovative work using electron microscopy to study the structure of signaling cell surface receptors, such as the leptin hormone receptor and the β 2-adrenoceptor. To detect and respond to signaling molecules, cells often employ specialized proteins on their surface termed “receptors.” Once a signaling molecule binds, the receptor initiates a cascade of events that results in a cellular response. One of the main areas of research of Dr. Skiniotis's laboratory is on the leptin signaling pathway and its role in the regulation of mammalian energy balance

and body weight. The binding of leptin to the leptin receptor on the cell surface results in the intracellular activation of other signaling proteins, which, in turn, regulate a number of physiological signaling cascades. Dr. Skiniotis and his team have been using electron microscopy techniques to study the molecular architecture of the leptin/leptin receptor complex to understand how the leptin signal is transmitted to the intracellular space. A mechanistic understanding of this complex will inform the design of therapeutic strategies targeting the leptin receptor complex.

The PECASE awards support the continued professional development of awardees, promote careers and foster innovation in science and technology, and recognize the scientific missions of participating agencies. A list of NIH scientists who have received this prestigious award is available at www.grants.nih.gov/grants/policy/pecase.htm

Healthy Moments



In addition to supporting biomedical research, the NIDDK engages in education and outreach activities to disseminate science-based health information to patients and their families, health care professionals, and the public. These efforts include targeted outreach to populations disproportionately affected by the diseases and conditions under its purview, including African Americans, Hispanic Americans, and Asian and Pacific Islander Americans. *Healthy Moments* is an example of one such activity.

Featuring NIDDK Director Dr. Griffin Rodgers, *Healthy Moments* is a series of 60-second weekly radio reports that include health information as well as tips on how to prevent and control diseases that are of interest to the Institute. It is broadcast on radio stations that target African American and other minority listeners. The goals of *Healthy Moments* include providing the results and recommendations of NIDDK-supported studies to the public and translating these results for public benefit. Another goal of *Healthy Moments* is to raise awareness locally of patient recruitment efforts for ongoing clinical studies at the NIH. *Healthy Moments* complements the NIDDK's other efforts to disseminate health information to the public, such as the NIDDK Information Clearinghouses and Education Programs, the NIDDK's web-site, and press releases.

Launched in May 2008, *Healthy Moments* began as a weekly radio feature in Washington, DC. Today, more than one and a half million listeners nationwide tune in to these broadcast spots. In addition to the broadcast of weekly episodes, seasonal episodes are produced during back-to-school time, holidays, National Diabetes Month, and National Kidney Month.

Healthy Moments features can also be found on the NIDDK web-site at www2.niddk.nih.gov/HealthEducation/HealthyMoments. Visitors can listen to past broadcasts and download audio files or transcripts. The *Healthy Moments* web-site can also be accessed by scanning this QR code:



In addition to regular radio spots, *Healthy Moments* is using social media and email to disseminate health information. *Healthy Moments* can be followed on Twitter, @HealthyMoments. The NIDDK Facebook page updates when new *Healthy Moments* episodes are available and key tweets are sent. Through the use of a variety of forms of communication, the NIDDK is working to engage the widest possible audience.

NIDDK Training Programs

NIDDK Programs Cultivate the Next Generation of Scientists

Training and developing young researchers is an important goal of the NIH and the NIDDK. NIDDK Director Dr. Griffin Rodgers highlights the fostering of exceptional research training and mentoring opportunities as one of the overarching principles that guide his leadership and vision for the NIDDK. He states, “Maintaining an NIDDK-focused pipeline of outstanding investigators is critically important to our research progress. We will continue to support significant opportunities at the graduate student and postdoctoral levels, as well as through research career development awards, and undergraduate research educational opportunities.”

The NIDDK supports research training and career development at a wide range of institutions, both intramural and extramural. NIDDK extramural programs support research training and career development at academic institutions throughout the United States, primarily through funding to the institutions. Through its Intramural Research Program, the NIDDK provides opportunities ranging from summer programs for high school students through employment of postdoctoral researchers on the NIH campus in Bethesda, Maryland, as well as at a Diabetes Epidemiology and Clinical Research Branch in Phoenix, Arizona.

NIDDK-funded research training programs work to maintain a “pipeline” of new investigators at every career stage. Summer training programs provide opportunities for high school and undergraduate students to obtain research experience. The NIH provides support for M.D./Ph.D. and Ph.D. students during the predoctoral phase of their research training and support for research fellows who have received their M.D., Ph.D., or other doctoral-level degree. The NIDDK also supports opportunities for medical students to engage in research. For example, the NIDDK Medical Student Summer Research Program in Diabetes aims to encourage medical students to consider research in diabetes and its complications as a career and to educate students about diabetes.

The NIH has several types of awards to assist career development for physicians engaged in patient-based or basic research and for Ph.D. scientists transitioning to independent positions. Clinical demands make it challenging for physicians to also pursue research careers. Furthermore, there is typically a long process of training and career development before a new independent investigator obtains grant support and leads a research laboratory. Therefore, NIH supports Mentored Patient-oriented Research Career Development Awards, aimed at clinical investigators engaged in patient-based research, and Investigator Awards in Patient-oriented Research to support mid-career physicians who have funded clinical investigations in patient-oriented research and who are mentoring young clinicians. Mentored Research Scientist Development Awards, Mentored Clinical Scientists Development Awards, and the NIH Pathways to Independence awards support scientists transitioning to independent positions. The NIH also provides support to individuals with a quantitative background (*e.g.*, engineering, mathematics, computer science) who wish to pursue biomedical research through the Mentored Quantitative Research Career Development Awards.

Beyond support focused on training and career development, the NIDDK also creates opportunities to help new investigators advance scientific discovery through research project grants for their own laboratories. Each year, the NIDDK sets a percentile “payline” for R01 research project grant applications based on available funds and the volume of applications. The payline is essentially a target threshold for funding based on the percentile score applications receive in the first level of peer review. For “Early Stage Investigators” (a new investigator who has completed his or her terminal research degree or medical residency within the past 10 years), the payline is more generous than the regular payline for established investigators. For example, for fiscal year 2012, the payline for early stage investigator applications was five percentile points higher than the regular payline. In addition, all new investigator R01

applications within 10 percentile points of the payline received individual consideration during the second-level review process by the NIDDK Advisory Council. The NIDDK can also choose to award a 1- or 2-year grant to an R01 application that appears promising but scored outside the payline. These provide support for an investigator to collect preliminary data in order to submit an improved revised R01 application. During second-level review, new investigators are given special consideration for these awards. The NIDDK also regularly holds workshops for recently funded new investigators to provide them with the information they will need to be successful in securing continued support for their research programs.

The NIDDK Office of Minority Health Research Coordination (OMHRC) oversees the NIDDK's efforts to recruit and retain minorities in biomedical research. Several programs provide opportunities for minority students to obtain research experience. For example, through the NIDDK/OMHRC Summer Internship Program for Underrepresented Groups, undergraduate African American, Hispanic and Latino American, American Indian and Alaska Natives, Native Hawaiians, and other Pacific Islanders can participate in a 10 week summer program conducting research at an NIDDK intramural research laboratory. The Alaska Native Undergraduate Summer Internship Program provides research education and training for college students, including Alaska Natives, who have a demonstrated interest in health disparities affecting Alaska Native communities and intend to pursue a career in biomedical research. The program provides 10 weeks of basic or clinical research education and training at the University of Alaska in Anchorage or Fairbanks. In addition, the NIDDK's Short-Term Education Program for Underrepresented Persons (STEP-UP) provides research education grants to seven institutions to coordinate four high school STEP-UP programs and three undergraduate STEP-UP programs that provide students with summer research experience and training opportunities. Further information on opportunities through OMHRC can be found at www2.niddk.nih.gov/OMHRC/OMHRCResearchTrainingForStudents/OMHRCStudentTraining.htm

OMHRC has established a communication network of current and potential biomedical research investigators and technical personnel interested in minority health research, including individuals from traditionally under-served communities. The major objective of the Network of Minority Health Research Investigators (NMRI) is to encourage and facilitate participation of members of underrepresented population groups and others interested in minority health in the conduct of biomedical research in the fields of diabetes, endocrinology, and metabolism; digestive diseases and nutrition; and kidney, urologic, and hematologic diseases. A second objective is to encourage and enhance the potential of the investigators in choosing a biomedical research career in these fields. An important component of this network is promotion of two-way communications between network members and the NIDDK. Through the NMRI, the NIDDK elicits recommendations for strategies to enhance the opportunities and implement mechanisms for support of underrepresented population groups and others in biomedical research. The NMRI will advance scientific knowledge and contribute to the reduction and eventual elimination of racial and ethnic health disparities. The annual NMRI Workshop is currently in its 11th year. More information about NMRI is available at: <http://nmri.niddk.nih.gov/>

Research breakthroughs happen only through the efforts of a creative, diverse, and well trained workforce. Thus, the NIDDK will continue programs to train and support researchers at all stages of their careers. The NIDDK will continue to encourage and facilitate participation of members of underrepresented racial and ethnic minority groups in the conduct of biomedical research in NIDDK-relevant fields. This next generation of scientists will then be poised to take advantage of a wealth of research opportunities to improve the health of Americans.

Additional information on NIDDK-supported opportunities in training and career development can be found on the NIDDK web-site at www2.niddk.nih.gov/Funding/TrainingCareerDev

DIVERSE STUDENTS EXPLORE RESEARCH THROUGH NIDDK SUMMER PROGRAMS

On the surface, Camille Miller and Yvonne Johnny appear to have little in common. Camille is an American Indian, lives in the dusty deserts of Arizona, and is enrolled in college to be a registered nurse. Yvonne hails from the tropical paradise of the Federated States of Micronesia (FSM), a U.S.-affiliated territory in the western Pacific Ocean. She hopes to pursue science education when she graduates from high school. But both young women share important similarities: each of their communities suffers from unusually high rates of diabetes and its complications. And each student spent the summer looking for a way to change that.

In the summer of 2012, Camille and Yvonne both participated in NIDDK programs designed to diversify the biomedical research field. Camille studied type 2 diabetes through the NIDDK Summer Internship Program (SIP) at the NIDDK's Phoenix Epidemiology and Clinical Research Branch (PECRB). Yvonne researched type 2 diabetes through the Short-Term Education Program for Underrepresented Persons (STEP-UP) in her native Micronesia. Both presented their research at conferences on the NIH campus in August 2012.

The two programs are designed to provide research opportunities for students from groups underrepresented in biomedical research—including certain racial and ethnic minorities. NIDDK SIP students conduct research related to the NIDDK's mission either at a lab on the main NIH campus or at PECRB, and STEP-UP students work at one of several NIDDK-funded labs in the United States and its territories. In 2012, STEP-UP welcomed students from the Marshall Islands, the FSM, and the U.S. Virgin Islands for the first time, and the NIDDK established a new molecular biology lab in the Marshall Islands.

Dr. Lawrence Agodoa directs the NIDDK's Office of Minority Health Research Coordination, which manages both programs. He said having a diverse pool of researchers to tackle some of science's most pressing issues is crucial.

"People of all walks of life need to come together and think about how to solve these problems," Dr. Agodoa said. "Many chronic diseases such as diabetes affect minority communities disproportionately. Having friends and family who are affected by a disease often gives people extra motivation to pursue biomedical research."

Camille and Yvonne concur. Part of Camille's passion for her research on type 2 diabetes in Pima Indians comes from having experienced the disease within her own American Indian community, the Cocopah Tribe, and even closer to home.

"Diabetes runs in my family, and my grandparents were amputees from diabetes complications. They ended up dying from diabetes. I really think that my family and tribal members could benefit from my research," she said.

The research that Camille conducted this summer at PECRB was her first foray into the world of biomedical research, and she hopes to apply for an NIH grant one day to resume her investigations.

Yvonne also hopes to continue her research.

"I want to find a cure for diabetes to help my family and friends back home," she said. "I see myself as the future of diabetes research."