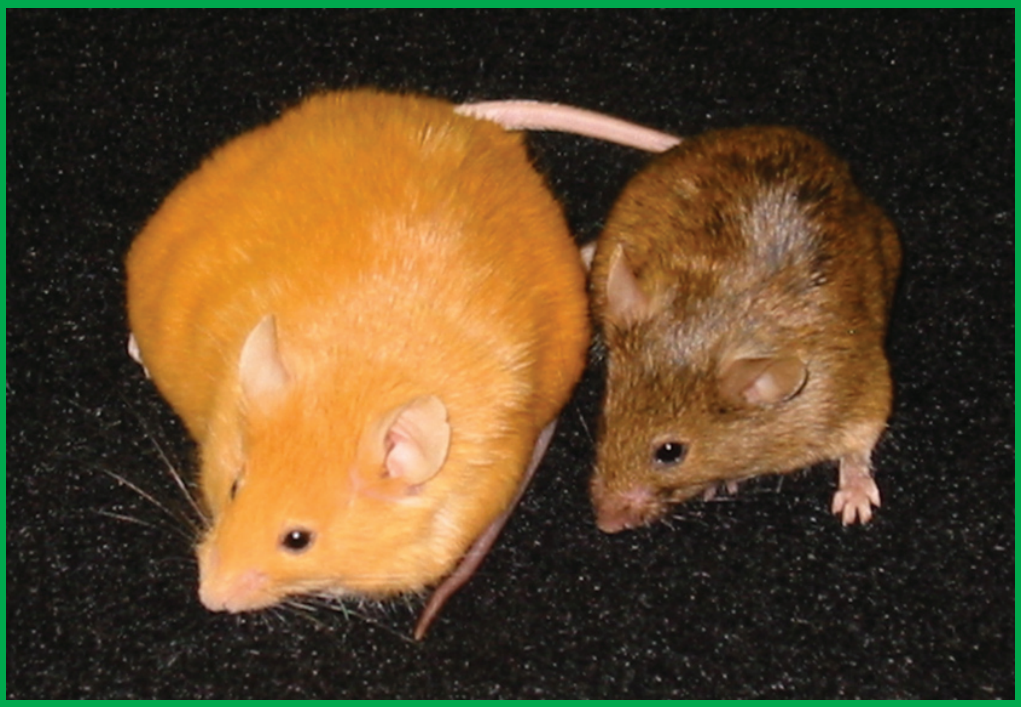


“Epigenetics” is the study of heritable changes in the regulation of gene activity and expression (whether genes are turned on or off) that are not dependent on the sequence of DNA. These changes may occur in several ways, including chemically marking the DNA itself. This image shows mice that have an identical DNA sequence in a gene that both determines the color of their fur and, when not properly regulated, also promotes obesity. Their different coat colors and body size arise from variation in the chemical modification of this gene. Therefore, even though the mice have the same DNA sequence, the epigenetic marks have a dramatic effect on their physical appearance. Regulation of gene activity by epigenetics plays a crucial role in human development, health, and disease. As described in this chapter, the NIDDK is spearheading new research in this emerging field of science.

Image provided by Dr. Robert A. Waterland and reprinted from Journal of Pediatrics, 149, Waterland RA, Epigenetic mechanisms and gastrointestinal development, S137-S142, Copyright 2006, with permission from Elsevier.

Cross-Cutting Science

Advances in medicine are largely dependent upon the accumulation of new knowledge about biologic processes, especially at the smallest levels of an organism—its genes, the proteins they encode, and the workings of cells. Major strides in fighting disease can be traced back to laboratory studies whose immediate relevance to health could not have been fully known or appreciated at the time they were conducted. Opportunities to make exciting new discoveries and advances are arising ever more rapidly with the development of new technologies, new approaches, and even new scientific disciplines. Described here are some recent studies that each span multiple areas within the NIDDK mission. These include research on fundamental biologic processes as well as development of new technologies that make such studies possible. The insights gained through this type of research can be expected to propel disease-oriented research, not only within the NIDDK mission, but also in many other fields. Today's cross-cutting advances may lead to tomorrow's health care strides.

BUILDING ON RECENT GENETICS STUDIES: PAVING THE WAY TOWARD IMPROVING PEOPLE'S HEALTH

New research tools, including the Human Genome Project and the International HapMap project, are making it easier for scientists to identify genes that influence a person's likelihood of developing common diseases. Taking advantage of these new research tools and technologies, scientists are conducting "genome-wide association studies" to identify genetic differences between people with specific illnesses and healthy individuals. Through this comparison, it becomes possible to identify even subtle genetic differences that may affect whether an individual develops a particular disease.

Recently, genome-wide association studies and other genetics studies have led to an explosion in the identification of genes important in diseases within the NIDDK mission. For example, genes associated with type 1 diabetes, type 2 diabetes, diabetic kidney disease, Crohn's disease, and focal segmental glomerulosclerosis have been identified. Often, the associated genetic region is unexpected—the function of the gene may be completely unknown or it may be involved in cellular processes that were not thought to be important in the particular disease. For example, as described elsewhere in this edition of *NIDDK Recent Advances & Emerging Opportunities*, a genetic region strongly associated with type 2 diabetes was also recently implicated in a very different condition: prostate cancer. In other cases, a gene may be involved in cellular processes already thought to be important in the disease. However, even if a gene's function has been previously studied, it is usually not precisely known how it plays a role in disease.

Although these genetic findings are extremely exciting in and of themselves, there is still much work to be done to understand how the genes function in healthy conditions and what goes wrong in disease. Research in this area sets the stage for even more scientific breakthroughs. For example, a newly-associated gene may produce a protein that interacts with numerous other proteins. Therefore, discovering the disease association not only implicates that protein in the disease, but also the proteins with which it interacts. This knowledge could illuminate several new therapeutic targets for disease prevention or treatment. Studying genes that were not thought to be involved in a disease can lead to brand new avenues for research that would likely not have been pursued otherwise. Identifying the functions of genes may not only enhance understanding of molecular mechanisms that underlie disease, but may also reveal new targets for therapy.

To propel research progress, the NIDDK is spearheading new efforts to elucidate the functions of genes associated with diseases within its mission. For example, the NIDDK sponsored a scientific conference in April 2008, entitled "Diabetes Genes and Beta Cell Function: How Can We Assemble the Puzzle?" The

workshop included presentations on genes newly-discovered to be associated with type 2 diabetes, and specific beta cell functions and pathways potentially influenced by those genes. These presentations served as a springboard for trying to put together the pieces of the type 2 diabetes genetics puzzle and to understand how the genes may influence beta cell function and contribute to disease. To foster collaboration and new research directions, the workshop brought together scientists from multiple disciplines, including investigators researching the genetics of diabetes and scientists studying the development and function of the insulin-producing beta cell. Furthermore, the NIDDK announced a new initiative to support research toward understanding the functions of genes newly-discovered to be associated with type 1 diabetes.

In addition to fostering research to understand the function of new genes, the NIDDK is spearheading research on translating genetics findings to the clinic and examining the social and ethical implications of this research. In March 2008, the NIDDK sponsored a meeting on behalf of the NIH-wide Genes, Environment, and Health Initiative (GEI) entitled, "Translating Whole Genome Association Data into Clinical Research and Practice." The meeting included presentations from scientists on important new genetic findings on certain diseases, such as inflammatory bowel diseases and type 2 diabetes. It also included discussion about approaches to use those genetic findings for therapeutic or diagnostic purposes, and the ethical and social issues pertinent to this type of research. The meeting was an opportunity for participants to discuss emerging themes and questions about translating genetic data from genome-wide association studies into clinical research and applications and to identify key questions for future research.

The NIDDK leads two GEI initiatives related to topics discussed at the March 2008 meeting. One initiative focuses on the translation of significant genetic findings into clinical or public health use. The other initiative will support planning grants for major clinical studies to increase understanding of how the public responds to genetic findings. This research will measure the responses of patients and providers to information about genetic determinants of common diseases and to determine how to effectively educate the public to use the information appropriately for clinical care and disease prevention.

These new and emerging research areas have been made possible by breakthroughs in the fields of genetics and biotechnology during the last few years. The NIDDK will continue to build on these exciting genetics discoveries, paving the way toward capitalizing on new knowledge and improving people's health.

NIH ROADMAP FOR MEDICAL RESEARCH

The NIH Roadmap for Medical Research is an "incubator space" for nascent programs that cut across the NIH in terms of relevance or complexity. In order to be considered for Roadmap funding, initiatives must be truly transforming, outcomes should synergize with the missions of individual NIH Institutes and Centers to promote health, and trans-NIH participation must be required to address a scientific area in which no single NIH entity alone is likely to engage. The initiatives are developed under the auspices of the Division of Program Coordination, Planning, and Strategic Initiatives (DPCPSI), a trans-NIH coordinating and planning structure within the NIH.

Roadmap initiatives emerge from input gleaned from a wide range of stakeholders in the intramural and extramural scientific community, the patient advocacy community, and the general public. DPCPSI coordinates a review of the submitted scientific concepts and the relevant NIH portfolios—applying criteria that potential Roadmap initiatives should meet. Directors of the NIH Institutes and Centers then select the general areas and the specific initiative concepts that could be pursued. NIDDK representatives have participated in several of the Working Groups charged with developing these concepts. Two concepts approved as major Roadmap initiatives that began in Fiscal Year 2008 as 5-year programs were the Epigenomics Program and the Human Microbiome Project.

Epigenetics is an emerging frontier of science that involves the study of heritable changes in gene function that cannot be explained by changes in DNA sequence. The Epigenomics Program aims to accelerate the promise of this field into applications that affect human health and a wide range of common complex human diseases by fostering the development of novel resources for research in this field. Research supported by the Epigenomics Program will characterize the

"epigenome" (a catalog of the stable epigenetic modifications or "marks" that occur in the genome) and its impact on health and disease. Initiatives include the creation of mapping centers to develop reference epigenomes, the establishment of a publicly available epigenetic database, as well as other research resources. In addition, support will be provided for fundamental discoveries of novel epigenetic marks and of the roles of epigenetic marks in specific diseases, conditions of development or aging, and responses to environmental exposures. Epigenetics may provide a mechanism by which environmental factors, such as diet, contribute to diseases such as type 2 diabetes and gastrointestinal cancers. As such, the NIDDK has taken an active role in the Epigenomics Working Group, and serves as the lead Institute for the novel marks discovery initiative. Awards for the Epigenomics initiatives were made in summer 2008, with the exception of the initiative focused on the Epigenomics of Human Health and Disease. The first Request for Applications under this initiative was released in summer 2008; it is expected that additional requests will be released annually for 5 years.

The Human Microbiome Project is focused on defining the variability in the microbiome across the population as a first step to assessing its role in both health and disease. The NIDDK Director serves as a co-leader of this Project, which will develop new tools and reference sequence data needed to study the human microbiome. Because the gastrointestinal tract is the site of the body's microbial population, the Human Microbiome Project could greatly aid efforts to understand microbial effects on digestive health and disease.

Another new NIH Roadmap initiative is the Transformative R01 (T-R01) Program. This program is designed to stimulate research to challenge existing paradigms or create new paradigms where none currently exist. It will support highly creative, "outside-the-box" projects. This program also is a High Risk/High Reward Demonstration Project in which novel approaches to peer review and program management will be piloted. Several "highlighted needs" topics are articulated as being in particular need of transformative research. However, the program is open to transformative ideas from any field relevant to biomedical or behavioral research. The first T-R01 awards are expected to be announced in Fiscal Year 2009. As with all Roadmap initiatives, investigators with proposals relevant to the NIDDK research mission are strongly encouraged to apply.

While these new Roadmap initiatives and pilot studies go forward, Roadmap Coordination Working Groups will continue to assess current efforts and future opportunities for cross-cutting collaborations and will continue to seek ideas for Roadmap initiatives from NIH stakeholders. More information on the NIH Roadmap programs can be found at: *http://nihroadmap.nih.gov/*

STEM CELLS, PROGENITOR CELLS, AND DISEASE APPROACHES

Stem cells have the potential to develop into many different cell types in the body. To better understand these cells and "progenitor cells," which have a more limited developmental potential, scientists continue to characterize their properties and seek potential new ways of using them to benefit patients.

Reprogramming Human Skin Cells to Embryonic Stem Cell-like Pluripotency: In a groundbreaking study, scientists have shown that, by introducing just four genes into adult human skin cells, cells resembling embryonic stem cells could be produced. This approach also worked in human cells from various developmental stages.

A key characteristic of embryonic stem cells is that, unlike most cells in the body, they remain "pluripotent." That is, a single embryonic stem cell is able not only to multiply and produce more stem cells, but, by definition, can develop, or differentiate, into a wide variety of mature cell types and tissues. However, the use of cultured stem cells derived from human embryos poses ethical dilemmas, and any potential cell-based therapies derived from stem cells would need to address issues related to patient compatibility. Scientists are therefore seeking ways to "reprogram" differentiated cells, such as skin cells, so they revert back to a pluripotent state.

The genes used in these studies—*Oct4*, *Sox2*, *Klf4*, and *Myc*—encode transcription factors, cellular components that regulate whether other genes are turned on or off. When introduced into certain types of human cells, such

as those derived in the laboratory from differentiated embryonic stem cells, these four factors caused the cells to regain characteristics very closely resembling those of the original embryonic stem cells. The scientists refer to these as induced pluripotent stem (iPS) cells. Remarkably, researchers were also able to generate iPS cells from more developmentally advanced cells—human neonatal and adult skin cells—by introducing these genes, together with two additional genes, *hTERT* and *SV40 large T antigen*. These two additional factors enhanced the efficiency of reprogramming in these more mature cells.

These findings show that it is possible to reprogram differentiated human cells into pluripotent stem cells. This approach may facilitate the establishment of human iPS cell lines from patients with specific diseases that could be used as research tools. Such cells would provide a rich source of pluripotent embryonic stem cell-like material. Further studies of these cells will thus complement ongoing research on other types of stem cells. This technique, or variations of it, may also, one day, allow patient-specific stem cells to be generated for use in stem cell-based therapies. One caution: the genes used for reprogramming were introduced into the cells with a method involving viruses, which could have adverse health effects. If, however, safe alternate methods based on this research were developed for reprogramming cells, then iPS cells may lead to novel, personalized therapies.

Park I-H, Zhao R, West JA, Yabuuchi A, Huo H, Ince TA, Lerou PH, Lensch MW, and Daley GQ: Reprogramming of human somatic cells to pluripotency with defined factors. Nature 451: 141-146, 2008.

New Stem Cell Resource To Advance Understanding of Human Diseases and Disorders: Scientists have generated stem cell lines from patients with 10 different genetic diseases and disorders, providing a valuable resource for the scientific research community. Significant understanding of human biology, including healthy and diseased states, has come from studies of human cell lines grown in the laboratory. Since human tissue has a limited life span in the laboratory, scientists most commonly study cells that have been "immortalized" by their tumoral origin or by techniques that modify the cells in the laboratory. These and other limitations, until now, have resulted in a lack of models for normal and pathogenic tissue growth and formation.

To generate the new disorder-specific cell lines, investigators first obtained cells from patients with different genetic (inherited) conditions. These included Parkinson's disease, type 1 diabetes, Huntington's disease, Down syndrome, "bubble boy disease" (severe combined immunodeficiency), Gaucher's disease, forms of muscular dystrophy, and others. The scientists had previously discovered a small set of genes that, when introduced into adult cells, could reprogram the cells to turn them into embryonic-like cells with the capacity to give rise to a variety of different cell types (pluripotency). By introducing these reprogramming factors into the cells obtained from patients, the scientists have now been able to transform them into cells that looked like embryonic stem cells and had many of the markers of stem cells. These cells are known as induced pluripotent stem (iPS) cells—cells that originate from adult body cells, but upon introduction of reprogramming factors, change into cells that have the properties of stem cells.

To ensure that the resulting iPS cells truly resembled disorder-specific stem cells, several tests were conducted of the iPS cell lines. First, for disorders with a clearly defined genetic basis, it was confirmed that the iPS cells still maintained the genetic defect that caused the human disorder. Next, it was confirmed that the observed stem cell markers came from the genome of the iPS cells, not from the technique the scientists used to introduce the reprogramming factors into the cells. Also, by analyzing the DNA of the cell lines, it was determined that the iPS cell lines were pure—that the cells had not been contaminated by other stem cell lines the scientists were studying in the laboratory. Finally, the investigators showed that the iPS cells were able to become several different types of tissue—one of the hallmarks of stem cells. The results of these tests confirmed that the investigators had generated disorder-specific iPS cell lines.

The scientists noted that the disorder-specific iPS cell lines produced in this study will be maintained and made available to the scientific community. These cell lines can be used in numerous ways to advance the understanding of these disorders. Comparing the disorder-specific cell lines to normal cell lines

may provide insights into the development and progression of these disorders. The cell lines may also be used in the laboratory to screen new therapies. Finally, research on these cell lines could inform the development of cell therapies. With modifications of the technique used to introduce the reprogramming factors, patient-specific iPS cells could one day be generated for cell replacement therapies that would avoid immune rejection and gene therapies to correct genetic defects. This exciting new resource has the potential to transform the study, understanding, and treatment of human disease.

Park I-H, Arora N, Huo H, Maherali N, Ahfeldt T, Shimamura A, Lensch MW, Cowan C, Hochedlinger K, and Daley GQ: Disease-specific induced pluripotent stem cells. Cell 134: 877-886, 2008.

Identification of Progenitor Cells in the Adult Pancreas that Form Insulin-Producing Beta Cells: Scientists have discovered a novel group of adult pancreatic progenitor cells that generate insulin-producing beta cells. Researchers previously demonstrated that new beta cells could be regenerated from existing beta cells in the pancreas. However, it remained unknown as to whether embryonic-like progenitor cells exist in the adult pancreas that have the ability to form beta cells. To gain further understanding about pancreatic progenitor cells, scientists, including members of the NIDDK-supported Beta Cell Biology Consortium, surgically induced a specific type of wound to the adult mouse pancreas, which caused the number of beta cells to double. The scientists took advantage of this doubling in number to test for the presence of a well-established marker of embryonic pancreatic progenitor cells, called Ngn3. Ngn3 is essential for development of the endocrine pancreas, but is not normally found in the adult pancreas. They observed that levels of *Ngn3* increased in the pancreas in response to the injury. When the scientists first inhibited the production of Ngn3 and then damaged the pancreas, the beta cells did not double in number like before. This observation indicated that Ngn3 plays a role in increasing beta cell numbers following the injury.

The scientists needed to determine, however, whether the cells expressing *Ngn3* produced the new beta cells seen in response to the pancreatic injury, or if the new beta cells were arising from different cells. To do this, they first purified the *Ngn3*-expressing cells from an adult mouse pancreas that had been damaged in the way that would increase beta cell numbers. They then placed the cells into an embryonic pancreas that had been isolated from a mouse that did not have its own *Ngn3*, due to a genetic mutation, and would thus not normally produce beta cells. However, when the purified *Ngn3*-expressing cells were added, beta cells developed within this embryonic pancreas. In addition, these newly-formed beta cells responded to glucose by releasing insulin. These results demonstrated that the cells expressing *Ngn3* in response to the injury were truly progenitor cells capable of producing beta cells. By further examining the *Ngn3*-expressing cells within the injured mouse pancreas, the scientists demonstrated that these cells showed many of the same characteristics as embryonic progenitor cells.

This research suggests that the adult mouse pancreas contains progenitor cells that are able to regenerate beta cells. If, with further research, these embryonic-like progenitor cells are identified in the human pancreas, then this discovery may foster the development of therapies for type 1 and type 2 diabetes—diseases in which the number and/or function of beta cells has been adversely affected. These progenitor cells could potentially be isolated from human pancreata to grow new beta cells in the laboratory that could be transplanted into a patient with diabetes. It may also be possible to develop new therapies to stimulate these cells within the patient to form new beta cells.

Xu X, D'Hoker J, Stangé G, Bonné S, De Leu N, Xiao X, Van de Casteele M, Mellitzer G, Ling Z, Pipeleers D, Bouwens L, Scharfmann R, Gradwohl G, and Heimberg H: Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell 132: 197-207, 2008.

Purified Stem Cell Transplants Capable of Repopulating the Liver: Researchers have demonstrated that purified rat fetal stem cells transplanted into animals missing two-thirds of their livers are capable of fully repopulating these organs, lending support for the consideration of stem cell transplantation as an alternative to whole or partial liver transplantation. Currently, liver transplantation

is the only successful treatment option available to patients with end-stage liver disease. But the number of livers available for transplant is limited, and many patients die while awaiting a transplant. Although the adult liver contains its own stem cells capable of some degree of regeneration, they are unable to fully replace the functional liver as is possible with liver transplantation. However, stem cells from fetal livers possess unique properties that could enable them to repopulate the organ and restore its function, provided that these cells could be delivered in sufficient quantity and purity to the recipient. Researchers developed a strategy to purify fetal liver stem cells by separating them from other cell types that might have unwanted effects. To accomplish this purification of the stem cells, they mixed cells isolated from rat fetal liver with special beads under conditions that allowed the beads to attach to the cells via a protein on the cells' surface called Dlk-1. This protein is found on stem cells in the fetal liver of rodents and humans, but not on other liver cell types present. They subsequently retrieved the stem cells that had attached to the beads. The purified stem cells were then tested in culture to ensure that they displayed all the hallmarks of liver stem cells, including production of unique proteins and robust proliferation. After a few days in culture, they also started to produce proteins associated with functions of mature liver and bile duct cells. The researchers then tested the "behavior" of a transplanted population of purified fetal stem cells in an animal model of liver loss—adult rats in which two-thirds of the liver had been surgically removed. Transplantation of the purified fetal stem cells into these rats led to an impressive repopulation of their liver and bile duct cells after 6 months. The scientists were able to visualize where the transplanted cells had repopulated the liver using a marker for an enzyme unique to these cells as they matured. The purified stem cells specifically homed in on the liver and did not lodge in other organs where their robust proliferation could have adverse effects. This series of groundbreaking experiments provides the methods necessary to prepare purified liver stem cells suitable for transplantation, laying the foundation for possible therapeutic applications in the future.

Oertel M, Menthena A, Chen Y-Q, Teisner B, Jensen CH, and Shafritz DA: Purification of fetal liver stem/progenitor cells containing all the repopulation potential for normal adult rat liver. Gastroenterology 134: 823-832, 2008.

Bacterial Interaction with Gastric Stem Cells Related to Stomach Cancer Development:

NIDDK-sponsored research has yielded insights into how the bacterial species *Helicobacter pylori* evolves and interacts with stem cells in the human stomach, contributing to disease progression to conditions such as cancer. *H. pylori* is the major cause of peptic ulcers, which affect a large number of individuals in the U.S. Most of those who become infected with this bacterium develop an inflammation of the stomach known as gastritis. However, in a small subset of individuals, gastritis progresses to a more severe form called chronic atrophic gastritis, in which some stomach cell types are destroyed. This condition may progress further to a type of stomach cancer known as gastric adenocarcinoma. Little is known about the role that *H. pylori* plays in these disease progressions; however, the bacteria are known to interact with the outer surface of epithelial cells that line the stomach and to establish themselves within gastric stem cells.

To explore the impact of interactions between *H. pylori* and gastric cells on disease progression, researchers engaged in a multi-species series of studies based largely on genomic techniques. Starting in the clinical realm, they analyzed samples taken originally for a population-based endoscopy study in Sweden. They focused their attention on samples collected from the stomach of one participant who developed gastric adenocarcinoma over the course of 4 years after an initial diagnosis of chronic atrophic gastritis. From these samples, they isolated *H. pylori* present before and after cancer development, and compared their genomes. They found that a single strain of *H. pylori* persisted throughout disease progression, but that several characteristics of the bacteria had changed over time. To study this disease-related *H. pylori* strain further, they switched to two experimental models: a mouse model of chronic atrophic gastritis, and a gastric stem cell culture model in which they could compare the direct effects of infection by the *H. pylori* specimens. Using these models, they were able to identify unique characteristics of the *H. pylori* present before and after stomach cancer development, including a gene turned on only in the cancer-associated bacteria that could serve as a biomarker for bacterial adaptation and stomach cancer. The cancer-associated *H. pylori* was also found to be better adapted to life inside the gastric stem cells, where it could influence cancer development. At the same time, the experiments also revealed changes in the

mouse stomach stem cells. For example, the cancer-associated form of the bacteria affected mouse genes related to tumor development, among other genes. This study provides insights into how *H. pylori* interacts with stomach stem cells to influence disease progression that can culminate in stomach cancer. This new knowledge enhances understanding of the host-microbe interactions that contribute to these gastric diseases, and how this information might be used to predict risk of disease development and progression.

Giannakis M, Chen SL, Karam SM, Engstrand L, and Gordon JI: Helicobacter pylori evolution during progression from chronic atrophic gastritis to gastric cancer and its impact on gastric stem cells. Proc Natl Acad Sci USA 105: 4358-4363, 2008.

Stem Cell Gene Therapy with a HOXB4-expressing Retroviral Vector Causes Leukemia in Large Animals: Researchers have recently reported the development of leukemia in large animals approximately 2 years after transplantation with retroviral-infected stem cells that overexpressed a gene called *HOXB4*. The researchers sought to investigate the effects of retroviral gene transfer into stem cells because of earlier reports of adverse effects in humans. Several patients with severe combined immunodeficiency disease (SCID) who were enrolled in French- and British-supported gene therapy clinical trials developed leukemia 2 to 3 years after transplantation of stem cells containing a retrovirus vector. The researchers suspected that some cells with the retrovirus may have had a growth advantage compared to other cells, which could lead to excess proliferation and cancer. For their experiments, they studied the effects of retroviruses that carried the *HOXB4* gene, because overexpression of this gene (excess production of the protein it encodes) was previously shown to confer a growth advantage in blood cells. Although blood cell abnormalities have not been detected in mouse studies using transplanted stem cells overexpressing *HOXB4*, experimental studies in mice do not always translate to outcomes in humans. Thus, the scientists evaluated the safety of transplanting retroviral-infected stem cells expressing *HOXB4* in larger animals, such as monkeys and dogs. Three of the four animals studied developed leukemia approximately 2 years after transplantation with retroviral-infected stem cells overexpressing *HOXB4*. In contrast, retroviral-infected stem cells not expressing *HOXB4* did not drive the development of leukemia. The researchers also suggest that in the clinical trials with patients with SCID, the gene used in the gene therapy may also have conferred a growth advantage to the transplanted cells. This study underscores the value of including large animal models in risk assessment analyses when developing gene therapies, and that for such therapies, overexpression of a gene in stem cells that confers a particular growth advantage to the cells, such as *HOXB4*, may carry an unacceptably high risk of cancer or have other serious unintended consequences.

Zhang X-B, Beard BC, Trobridge GD, Wood BL, Sale GE, Sud R, Humphries RK, and Kiem H-P: High incidence of leukemia in large animals after stem cell gene therapy with a HOXB4-expressing retroviral vector. J Clin Invest 118: 1502-1510, 2008.

OMEGA-3 FATTY ACIDS AND INFLAMMATION

Understanding How Fish Oil Reduces Inflammation: Findings from a recent study of the anti-inflammatory properties of omega-3 fatty acids may help increase the therapeutic potential of these essential nutrients. Chronic inflammation damages body tissues and plays a critical role in many diseases and conditions, including diabetes, obesity, inflammatory bowel disease, heart disease, and rheumatoid arthritis. Omega-3 fatty acids have emerged as one therapeutic option for reducing inflammation, but the mechanism(s) by which they exert this effect is not well understood. The present study focused on an omega-3 fatty acid, docosahexaenoic acid, or DHA, found in fish oil and other foods. DHA is oxidized in the body to form a variety of bioactive molecules. One class of molecules produced by DHA oxidation, called A_4-NP, resembles a group of anti-inflammatory agents found naturally in the body. Researchers used this knowledge to determine whether A_4-NP acts on the same molecular pathway to suppress inflammation as the natural agents. They found that A_4-NP does indeed suppress activation of the same pro-inflammatory molecular pathway—the NF-kappaB pathway—in cells of the immune system called macrophages. When the researchers pre-treated laboratory-grown macrophages from mice with A_4-NP and then added bacterial compounds or other stimulants, inflammation was potently inhibited. Delving deeper, they discovered

that A_4-NP prevents inflammation by short-circuiting the pro-inflammatory molecular pathway, keeping the NF-kappaB protein from moving into the cell's nucleus, where it would promote production of cyclooxygenase-2 (COX-2) and other pro-inflammatory molecules. Migration of NF-kappaB normally depends upon the activity of a cellular enzyme that is activated by inflammatory signals. The investigators found evidence that at least part of the mechanism underlying A_4-NP's effect is through interference with this enzyme. Finally, to determine how much of DHA's anti-inflammatory properties are actually due to the activity of A_4-NP, the team tested whether DHA needed to be oxidized to inhibit inflammation. They found that only oxidized DHA inhibited inflammation and that the anti-inflammatory properties of oxidized DHA were strongly linked to the amount of A_4-NP and to a similar oxidation product, J_4-NP. While these experiments were performed using laboratory-grown mouse cells, the results suggest that DHA's anti-inflammatory effects in humans may be due, at least in part, to its oxidation and subsequent inhibition of a key pro-inflammatory pathway. A fundamental understanding of the anti-inflammatory properties of DHA and other omega-3 fatty acids may lead the way to new, targeted therapeutic approaches for a wide range of inflammatory conditions.

Musiek ES, Brooks JD, Joo M, Brunoldi E, Porta A, Zanoni G, Vidari G, Blackwell TS, Montine TJ, Milne GL, McLaughlin B, and Morrow JD: Electrophilic cyclopentenone neuroprostanes are anti-inflammatory mediators formed from the peroxidation of the omega-3 polyunsaturated fatty acid docosahexaenoic acid. J Biol Chem 283: 19927-19935, 2008.

HUMAN IMMUNODEFICIENCY VIRUS (HIV) RESEARCH

NIDDK Scientists Identify Key Steps in Processing of HIV Proteins: Researchers at the NIDDK's Laboratory of Chemical Physics have characterized a short-lived intermediary that appears during the maturation of a key protein involved in the replication of HIV. Following infection, one of the first proteins synthesized by the virus is "protease," which is capable of cleaving itself and other viral proteins to allow their assembly into new virus particles. Protease is essential for HIV infection—an entire class of drugs, protease inhibitors, is an important part of anti-AIDS therapy today. The NIDDK researchers were studying how HIV protease is processed from an inactive precursor into its final active form. This represents a seeming contradiction: if mature protease is responsible for activating molecules of inactive protease precursor, how is the first molecule of protease cleaved and activated? To study this question, the researchers used an inactive, shortened form of the precursor protein and a technique called paramagnetic relaxation enhancement to identify the structure of protein-protein complexes that form during protease processing. The scientists found that the mini-precursor formed a highly transient, very rare complex of two mini-precursor molecules. This was an important insight because mature protease acts as a dimer—two molecules of protease joined together to cleave other proteins. The fleeting mini-precursor complex had little enzymatic activity; however, it may be sufficient to allow self-processing of a few molecules of protease. The consequent mature, fully active protease enzymes can then go on to cleave other nascent proteins, including additional immature protease molecules. Because HIV protease plays an integral role in HIV infection, it is a prime target for the development of therapies aimed at preventing AIDS. This finding provides a detailed structural model for maturation of the largely inactive precursor into a fully active protein. An improved understanding of the steps involved in early HIV infection and progression may reveal new targets for therapy.

Tang C, Louis JM, Aniana A, Suh J-Y, and Clore GM: Visualizing transient events in amino-terminal autoprocessing of HIV-1 protease. Nature 455: 693-696, 2008.

Dr. Alexandra McPherron and Dr. Michelle Winn: NIDDK Scientists Receive Presidential Award

Two scientists supported by NIDDK were honored at a White House ceremony in November 2007 for their outstanding scientific leadership in diabetes and kidney disease research.

The Presidential award, known as the Presidential Early Career Award for Scientists and Engineers (PECASE), was bestowed upon Alexandra C. McPherron, Ph.D., a scientist in NIDDK's Division of Intramural Research, and Michelle P. Winn, M.D., an NIDDK extramural grant recipient, along with 10 other grantees from the National Institutes of Health (NIH). PECASE is the most prestigious award given to young scientists in the U.S.

Myostatin and Metabolism

Dr. Alexandra C. McPherron

Dr. McPherron, a tenure-track investigator with the NIDDK's Genetics of Development and Disease Branch, was chosen for discovering myostatin, a secreted protein produced by skeletal muscle that inhibits muscle growth. Inhibiting myostatin might be therapeutically useful for treating muscle wasting diseases, diabetes, or obesity, according to Dr. McPherron. She is trying to understand the role of myostatin in adult metabolism. "The myostatin protein circulates in the bloodstream, so it might act on other tissues, such as adipose tissue, in addition to skeletal muscle," said Dr. McPherron. Her research in mice showed that loss of this protein results in improved glucose metabolism and reduces fat accumulation. The mechanisms for these effects are not yet clear. "We don't yet know whether the improvement in glucose metabolism is due purely to the increase in skeletal muscle mass, the loss of circulating myostatin acting on other tissues, or metabolic changes in skeletal muscle, such as becoming more sensitive to insulin. We are also trying to understand how myostatin regulates the proliferation and differentiation of muscle precursor cells and their incorporation into muscle fibers."

The Intramural Research Program of the NIDDK conducts basic, translational, and clinical biomedical research related to: diabetes mellitus, endocrine, bone, and metabolic diseases; digestive diseases, including liver diseases and nutritional disorders; kidney diseases; and hematologic diseases.

Familial Kidney Disease

Dr. Michelle P. Winn

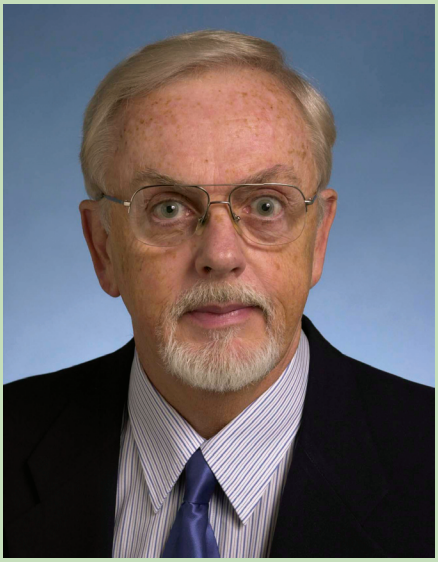
Dr. Winn, who is an Assistant Professor in the Division of Nephrology at the Duke University School of Medicine, was recognized for the discovery of the *TRPC6* gene as a cause of familial kidney disease. NIDDK-supported genetic studies aim to determine why focal segmental glomerulosclerosis (FSGS), which causes kidney failure, sometimes runs in families. FSGS is a common, irreversible process that can result in steroid-resistant

nephrotic syndrome, a condition marked by very high protein levels in the urine; low protein levels in the blood; swelling, especially around the eyes, feet, and hands; and high cholesterol.

Dr. Winn's research is important because it has opened a new angle to understanding a disease that is poorly understood and which disproportionately affects African Americans. Dr. Winn, who previously held an NIDDK K08 award, which provides physicians with up to 5 years of support to pursue research careers, is now in the third year of an R01 grant from the NIDDK. The R01 research project grant is awarded to eligible institutions on behalf of a principal investigator to support a discrete project related to the investigator's area of interest and competence.

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 previous NIH recipients of this prestigious award is available at *www.grants.nih.gov/grants/policy/pecase.htm*

Dr. Bert O'Malley Wins National Medal of Science: Long-term NIDDK Grantee Pioneered Molecular Endocrinology



Dr. Bert W. O'Malley

Dr. Bert W. O'Malley has won a National Medal of Science for his outstanding contributions to knowledge in the biological sciences. Dr. O'Malley is a long-term grantee of the NIDDK and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) and former section chief for the National Cancer Institute (NCI). On September 29, 2008, Dr. O'Malley was presented the prestigious award in recognition of "his pioneering work on the molecular mechanisms of steroid hormone action and hormone receptors and coactivators, which has had a profound impact on our knowledge of steroid hormones in normal development and in diseases, including cancer." Dr. O'Malley, chair of Baylor College of Medicine's Department of Molecular and Cellular Biology, is the first scientist in the field of molecular endocrinology to receive the Medal, considered the highest national honor in biological sciences.

"Dr. O'Malley's prodigious career is a tribute to the importance of basic research," says Dr. Griffin P. Rodgers, NIDDK Director. "His research revolutionized the understanding of hormone action and the molecular regulation of processes as basic as metabolism and reproduction. By studying the mechanisms of hormone action, he unexpectedly found molecular pathways that lead to a number of diseases. Some therapies that capitalize on his findings are already in clinical trials."

Much of Dr. O'Malley's early work focused on the steroid hormones—glucocorticoids, mineralocorticoids, androgens, estrogens, and progestagens—which regulate reproduction and basic metabolism. He used the tools of physiology and biochemistry to study the hormones' role in reproduction and developmental diseases and was one of the first to apply new methods as they were introduced. The challenges in those days were immense, because scientists had not yet found the receptors for these hormones nor had they yet discovered that they all belonged to a common family.

In the 1980s, evidence was growing that receptors for steroid hormones had unique structural properties and belonged to a common family of receptors. Instead of attaching to receptors on the cell surface, these hormones linked up with receptors in the cell and its nucleus and acted as transcription factors to change the expression of genes. After the first nuclear receptor was cloned, scientists went on to find 49 such receptors, including those for steroid hormones, thyroid hormones, certain vitamins, and receptors for hormones that were still unknown. These "orphan receptors" also turned out to have profound effects on cells.

Dr. O'Malley was one of the first to create an *in vitro* transcription assay, or a test tube system, that could recapitulate what happened inside a cell to study the changes in gene expression. His assay stimulated much research that led to an even greater understanding of hormone action because scientists could use the method to study their favorite hormone and receptor.

Dr. O'Malley's discoveries led to the development of selective steroid receptor modulators. This class of new drugs selectively targets one tissue while leaving other tissues unaffected. His findings also laid the groundwork

for the development of two important drugs: a progestin compound that prevents preterm birth in certain cases and raloxifene, which prevents osteoporosis.

In 1995, Dr. O'Malley and others discovered a group of nuclear receptor coregulators, molecules in the nucleus that control how nuclear receptors work. Coregulators, he found, helped turn on and off transcription factors, such as nuclear receptors, which in turn helped to orchestrate the expression of many other genes. Probing deeper, he identified a subset of coregulators, called coactivators, which are required for hormone action. Other researchers soon found corepressors, which silence transcription.

"Hormones control almost all cellular physiology," Dr. O'Malley explains. "Receptors for steroid hormones, the most important class of hormones, are activated by the hormone, then they go into the cell's DNA and search out and find the target genes to be turned on or off. In the final step, they recruit complexes of coregulators, including coactivators, that perform all the functions to turn the genes on. In a sense, these coactivators are master genes because they can activate different transcription factors at the same time, so you get a coordinated physiologic outcome."

Forging ahead, Dr. O'Malley and colleagues came to a stunning conclusion: nuclear receptor coregulators control physiologic processes as basic as cell growth, metabolism, inflammation, and reproduction. And if defective, these "little molecules with big goals" can lead to disease. "When the activities of these master genes are compromised, cellular processes can quickly deteriorate," says Dr. O'Malley. In overdrive, some can spur the uncontrolled growth of cancer cells. For example, the steroid receptor coactivator SRC-3 fuels the growth of most prostate tumors and 65 percent of breast tumors. Another coactivator, SRC-2, controls glucose production by the liver. When it is defective, a form of glycogen storage disease develops. Other SRCs influence fat cells, energy balance, and carbohydrate metabolism.

"Basic research is our fountain of knowledge," says Dr. O'Malley. "I always felt that if I knew how things work in normal cells, I'd have much better insight how to fix them when they go wrong in disease. If you open the hood of a car, you don't know how to fix it if you don't know how the motor works."

Dr. O'Malley is principal investigator of the Nuclear Receptor Signaling Atlas (NURSA). NURSA is a trans-NIH effort, led by the NIDDK, designed to develop a comprehensive understanding of the structure, function, and role in disease of nuclear hormone receptors. NURSA has a particular focus on metabolism and the development of a number of metabolic disorders, including type 2 diabetes, obesity, lipid dysregulation, and others, as well as on processes of aging and hormone-dependent cancers. For more information on NURSA, see *http://www.nursa.org/*

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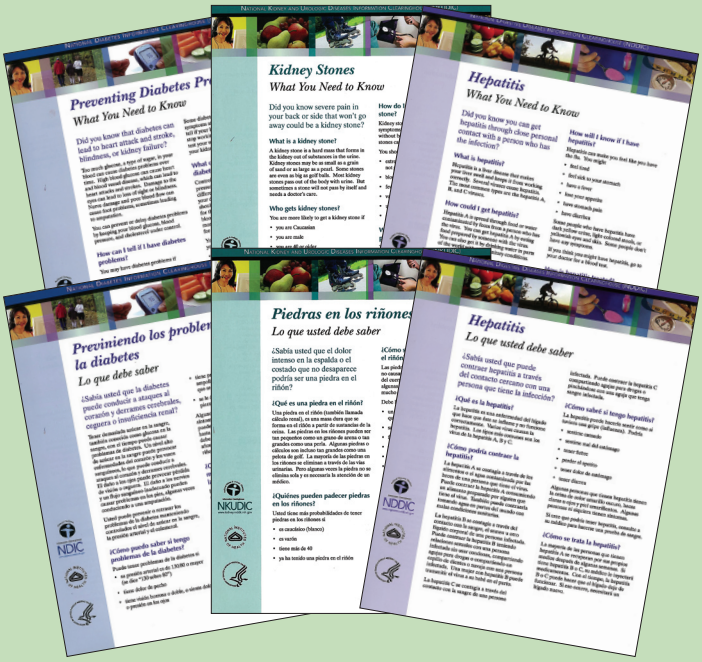
NIDDK Releases New Awareness and Prevention Series for Community Health Events

The NIDDK has developed a new health information series to raise awareness about diabetes, digestive diseases, and kidney and urologic diseases among people not yet diagnosed with these illnesses. The NIDDK developed the Awareness and Prevention Series for community health fairs, workplace health forums, family reunions, and other similar events.

The Awareness and Prevention Series publications are each two-page fact sheets—one side in English and the other in Spanish—on a wide range of health topics, including bladder control, celiac disease, foodborne illness, irritable bowel syndrome, pre-diabetes, preventing diabetes complications, urinary tract infections, and many others. Each fact sheet gives readers a snapshot of an illness, highlighting risk factors, symptoms, prevention tips, and where to go for more information.

The new series is designed to encourage readers to consider whether these illnesses could be affecting them or a loved one. By raising awareness of these illnesses, their causes, and symptoms, the NIDDK is providing necessary information to the public to promote prevention and early diagnosis of many common conditions.

Copyright-free full texts of the Awareness and Prevention Series publications—and all other publications from the NIDDK Information Clearinghouses—are online at *www.niddk.nih.gov*. To order copies of the Awareness and Prevention Series fact sheets, click on "NIDDK Awareness and Prevention Series" and then on "*catalog.niddk.nih.gov*"



The NIDDK's Awareness and Prevention Series was developed to raise awareness about diabetes, digestive diseases, and kidney and urologic diseases. The fact sheets are two-pages, with one side in English (top row) and the other side in Spanish (bottom row). In addition to the fact sheets shown here, the Series includes fact sheets on a wide range of other health topics within the NIDDK's mission.