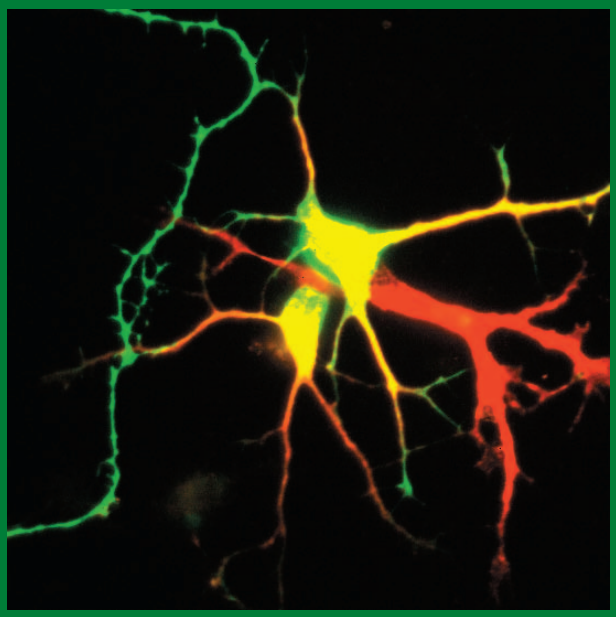

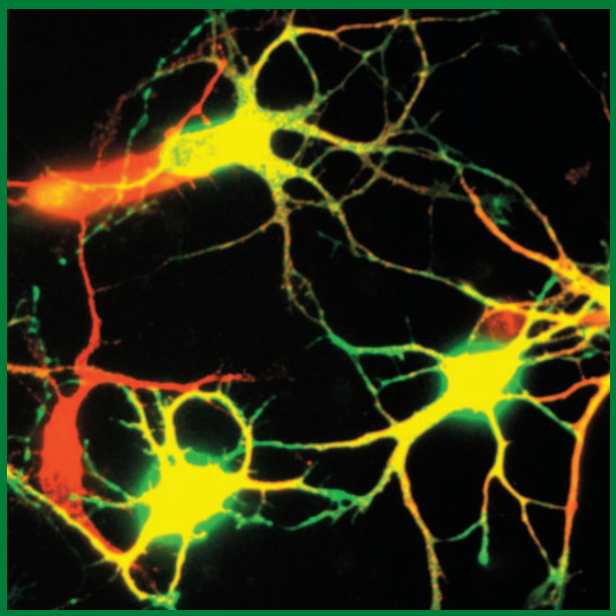

"MicroRNAs" are small inducible molecules that regulate a variety of biological processes. These images show the effect of higher-than-usual levels of one type of microRNA, *miR132*, on the development of cultured rat nerve cells. Nerve cells, which are stained yellow in both panels, send out projections, or neurites, to enable them to communicate with other nerve cells. Scientists engineered nerve cells to express elevated levels of *miR132*. These engineered nerve cells sprout more neurites (bottom) compared to cells with lower *miR132* levels (top). For more information about new insights into the role of small RNA molecules in cellular function, see the Scientific Presentation, "Role Reversal: RNA Control of Gene Expression," in this chapter.

Image courtesy of Dr. Richard Goodman. Photo credit: Soren Impey.

Cross-Cutting Science

Though advances sometimes happen in dramatic leaps and bounds, scientific progress more often occurs in incremental steps, with each new level of understanding building on previous discoveries. Insights into the nature of the fundamental and basic molecular components of an organism—its DNA, genes, proteins, and metabolites—and the exquisitely complex ways in which these elements are organized, regulated, and interact, provide a starting point for a wide range of inquiry. While ultimate application of this research may not always be immediately obvious, it forms the crucial foundation for future investigations, and knowledge gained from this research can be expected to facilitate disease-based research in a wide range of fields. A critically important aspect of cross-cutting research is its translation from research advances made in the laboratory into more effective therapies for patients. An equally important aspect of translational biology is the use of insights gained from clinical studies to spur novel research directions in the laboratory. The bi-directional flow of information, from "bench-to-bedside-and-back," allows scientists to address the widest range of research questions.

NIDDK TRANSLATIONAL RESEARCH: OPPORTUNITIES TO IDENTIFY AND VALIDATE BIOMARKERS

Biomarkers represent valuable surrogate endpoints for diseases and can be used in clinical trials and with diagnostic tools. Good biomarkers correlate well with disease state or progression, allowing physicians and researchers to readily gauge a patient's status at various disease stages and monitor the effectiveness of treatments. The NIDDK has a long track record of successfully promoting the development of biomarkers that have transformed patient care for a number of diseases within its research mission. For example, the hemoglobin A1c (HbA1c) blood test has been shown to be a good surrogate measure of long-term blood sugar control in diabetes. HbA1c has been validated in a large NIDDK-funded clinical trial, and subsequently has served as the basis for approval of multiple drugs for therapy of diabetes. Similarly, methods for estimating glomerular filtration rate (GFR) using circulating levels of the protein creatinine and earlier ascertainment of kidney disease by measuring the level of the protein albumin in the urine have become important surrogate markers for kidney function and disease. However, additional biomarkers are urgently needed to speed development of potential new treatments.

Studies designed either to validate candidate biomarkers or to develop new technologies to monitor disease progression are particularly valuable and of special interest. For diseases for which no validated biomarkers are currently available, or for which measurement of well-characterized biomarkers is prohibitively invasive or expensive, the development of new biomarkers is particularly critical. To aid in this endeavor, the NIDDK has created a central repository with biological samples from individuals with a variety of the Institute's mission-specific diseases (*https://www.niddkrepository.org/niddk/home.do*). These samples are an extremely valuable resource made available to qualifying investigators who are pursuing research into biomarker discovery or validation.

Recent research has suggested a variety of specific biomarkers that may be valuable as surrogate endpoints for clinical trials of interventions for NIDDK mission-specific diseases. These may be particularly fertile areas for rapid translation, because they represent situations where a broad field of possibilities has been narrowed to a few promising candidates.

Examples of ongoing NIDDK-sponsored studies of potential biomarkers include:

C-peptide as a Biomarker of Beta Cell Function: In type 1 diabetes, residual beta cell function is associated with better glycemic control, less risk of hypoglycemia, and lower risk of long-term diabetes complications such as diabetic eye disease. In clinical trials designed to preserve beta cell function in individuals with new onset type 1 diabetes, it is critically important to be able to measure that function quantitatively. To achieve this, the NIDDK is working with the Centers for Disease Control and Prevention to improve sensitivity and standardization of assays for C-peptide—a byproduct of endogenous insulin secretion—and is conducting a clinical study to determine which of two methods is better to stimulate C-peptide for use as a surrogate outcome measure in clinical trials.

Antiproliferative Factor as a Biomarker for Interstitial Cystitis: Interstitial cystitis is a chronic inflammatory bladder disease of unknown etiology that primarily affects women. Symptoms include difficulty urinating, pain on urination, urinary urgency and increased frequency of urination. Antiproliferative factor is a protein found in the urine of patients with interstitial cystitis, and is therefore potentially valuable as a diagnostic biomarker, and as a surrogate endpoint for clinical trials.

Cystatin as a Biomarker for Kidney Function: Elevated levels of serum creatinine indicate lower glomerular filtration rate (GFR) and impaired kidney function. The development of an equation for estimating GFR from creatinine in the blood was a major advance for assessment of kidney health. While measurement of creatinine remains the best available means of estimating GFR, new research suggests that serum cystatin may provide a more accurate measure.

Other diseases and conditions for which the NIDDK is actively seeking to fund biomarker research include:

- Kidney and liver fibrosis
- Early diabetic microvascular complications
- Inflammation of the kidney
- Beta cell mass and inflammation of the pancreas
- Inflammation of adipose tissue
- Inflammatory bowel disease
- Hepatitis
- Insulin resistance
- Angiogenesis

All of the above are explicit goals of the "Development of Disease Biomarkers" research solicitation, released April 27, 2005, and active through September 1, 2008.

Other NIDDK initiatives relevant to biomarkers include:

Proteomics: Diabetes, Obesity, and Endocrine, Digestive, Kidney, Urologic, and Hematologic Diseases—This initiative takes a broad approach to finding new candidate biomarkers by examining protein expression in bio-fluids (e.g., plasma, serum, urine, bile, pancreatic and gastrointestinal fluids). One specific goal is to find a less burdensome test for diagnosis of diabetes that does not require fasting.

Non-Invasive Methods for Diagnosis and Progression of Diabetes, Kidney, Urological, Hematological and Digestive Diseases—In addition to non-invasive (e.g., imaging) methods, this initiative explicitly includes development of "minimally-invasive" methods, (e.g., through assays of bio-fluids).

Small Business Innovation Research (SBIR) and Small Business Technology Transfer (STTR) to Develop New Therapeutics and Monitoring Technologies for Type 1 Diabetes (T1D) and its Complications—This initiative seeks to promote the development of methodologies or biomarkers to help understand the physiology or improve the diagnosis and treatment of type 1 diabetes and its complications (e.g., applications of proteomic technologies, and development of better predictors of the patients with diabetes most prone to accelerated development and progression of macrovascular complications).

Research Grants for Studies of Hepatitis C in the Setting of Renal Disease—This initiative seeks to develop biomarkers or non-invasive means to assess the activity and stage of liver disease to avoid the need to perform liver biopsy in patients with kidney disease on dialysis or after kidney transplantation.

Calcium Oxalate Stone Disease—This initiative seeks to identify biomarkers that signal susceptibility to development of calcium oxalate stone disease or specific complications of this disease.

Mechanisms of Alcoholic and Nonalcoholic Fatty Liver (Steatosis)—This initiative seeks proposals

designed to develop non-invasive biomarkers for fatty liver, using genomic, proteomic, and metabolomic technologies.

Mechanisms of Alcoholic Pancreatitis—A specific goal of this initiative is the identification and characterization of biomarkers of early cell or tissue perturbation that can be used for diagnosis of alcoholic pancreatitis.

In addition to these ongoing initiatives, additional opportunities in biomarker research will also be addressed in several NIDDK initiatives in fiscal year 2006:

- Toward Imaging the Pancreatic Beta Cell in People
- Biomarkers of Autoimmunity in Type 1 Diabetes
- Collaborative Research in Proteomics of Obesity: A Search for Co-Morbidity Biomarkers

Current NIDDK-funded biomarker research includes, but is not limited to:

- Proteomic and metabolomic approaches to finding new diabetes diagnostics
- Proteomics and metabolomics in type 1 diabetes and its complications
- Initiatives to promote imaging of pancreatic inflammation and beta cell mass

NIH ROADMAP FOR MEDICAL RESEARCH IN THE 21ST CENTURY

Developed with input from meetings with more than 300 nationally recognized leaders in academia, industry, government, and the public, the NIH Roadmap provides a framework of the priorities NIH as a whole must address in order to optimize its entire research portfolio. It lays out a vision for a more efficient and productive system of medical research. It identifies particularly compelling opportunities in three main areas: New Pathways to Discovery, Research Teams of the Future, and Re-engineering the Clinical Research Enterprise.

The NIDDK has played a major leadership role in the Building Blocks, Biological Pathways, and Networks (BBPN) Roadmap Implementation Working Group, and the NIDDK has made significant contributions to implementation of BBPN initiatives on proteomics and metabolomics technology development. These efforts have included international scientific workshops to develop and promote information standards and resource sharing in these rapidly growing fields. Improved tools in proteomics—the study of all proteins within cells—and metabolomics—the study of all metabolites, such as salts, sugars, and fats—can directly benefit the study of diseases within the NIDDK mission. For example, metabolomics could lead to the identification and validation of surrogate markers that correlate with stage or rate of progression of diabetes and its complications.

The NIDDK has taken a leadership role in a Roadmap pilot program to make available, on a competitive basis, certain governmental contractual resources for the preclinical development of small molecules. This program was first developed for cancer research, and was expanded by the NIDDK to cover type 1 diabetes. Now, the NIH-RAID (Rapid Access to Intervention Development) Pilot has been expanded under NIDDK leadership to cover all diseases. It is intended to reduce some of the common barriers that impede the translation of laboratory discoveries, and clinical trials of new therapeutic entities. During the pilot phase, proposals are limited to small molecule development, but it is anticipated that eventually the program may be expanded to include other therapeutic agents. At present, the program is managed by NIDDK staff. While the program is designed to benefit researchers in any NIH-supported research area, it should prove particularly helpful to NIDDK-supported investigators, many of whom are actively involved in the development of new treatment approaches.

The NIDDK serves as the lead institute in administering the NIH Roadmap *Short Programs for Interdisciplinary Research Training* initiative, which was intended to provide training for investigators at all levels of their careers. The NIDDK also participates in the administration of the Training for a New Interdisciplinary Research Workforce initiative, through which institutional training grants have been developed.

The NIDDK Director has served as a member of the Roadmap Implementation Coordinating Committee, providing leadership to the structuring of Roadmap initiatives; their funding, progress review, and evaluation; and means of staff recognition. The NIDDK has also furthered the Roadmap goal of "Re-engineering the Clinical Research Enterprise" within the Institute by: (1) developing central

repositories for patient biosamples and data from NIDDK-funded clinical studies and trials, and (2) developing and implementing policies for, and funding of, ancillary studies to specific large clinical trials.

RESEARCH TRAINING

Diabetes-Based Science Education in Tribal Schools: Type 2 diabetes is a serious, growing problem in minority groups, including American Indians. The *Diabetes-Based Science Education in Tribal Schools (DETS)* program is developing a national, science-based diabetes prevention education curriculum for American Indian students in grades K-12. One goal of the program is to enhance awareness and understanding of diabetes among students, families, community members, and teachers in order to prevent the disease and to help affected tribal members better manage their diabetes. A second goal of the program is to increase the numbers of American Indians entering the health research professions. The program is sponsored by the NIDDK in close collaboration with American Indian tribal schools, and the Indian Health Service, the Centers for Disease Control and Prevention, and the Office of Science Education of the NIH.

Programs To Increase Diversity in Biomedical Research in NIDDK Mission Areas: Many diseases and disorders that disproportionately affect the health of minority populations in the U.S. are NIDDK research areas, including diabetes, obesity, nutrition-related disorders, hepatitis C, gallbladder diseases, *H. pylori* infection, sickle cell disease, kidney diseases, and certain complications from infection with HIV. The NIDDK supports research to encourage specific efforts in these areas of health disparity, including noteworthy efforts to enhance translation of new or improved therapy into clinical practice.

Because racial and ethnic minorities are under-represented among investigators carrying out the NIDDK mission, the Institute has a number of efforts to engage minority scientists and foster their career development. These efforts are aimed at ensuring an adequate cadre of future minority researchers by supporting current investigators and encouraging junior scientists and students to pursue research careers. The *Network of Minority Research Investigators*, composed of current and potential biomedical research investigators, is designed to help minority investigators achieve career success while working on issues concerning health-related racial and ethnic disparities. *Pre-doctoral Fellowship Awards for Minority Students* (F31) provide support for research training leading to a Ph.D., M.D./Ph.D., or other professional degree in the biomedical sciences, behavioral sciences, or health services research. These fellowships are designed to enhance the racial and ethnic diversity of the research labor force in the U.S. Under the *Minority Supplements to Institutional National Research Service Awards* (T32) program, the NIDDK awards an extra position, designated specifically for a selected under-represented minority trainee—either pre-doctoral or post-doctoral—to an existing T32 award. That position then remains a part of the award for as long as the named individual is a member of the training program. Undergraduate students are eligible for a number of NIDDK programs designed to encourage minorities to pursue a research career. Awards through the *Short-Term Educational Program for Under-represented Persons (STEP UP)* offer research-education opportunities for minority students in an effort to encourage them to pursue a research career in an area of science relevant to the research mission of the NIDDK. The ten-week *Summer Internship Program* provides an opportunity for students to participate in research under the direction of preceptors in NIDDK laboratories. This program advances the state of biomedical knowledge and introduces the students to state-of-the-art laboratory methods.

Role Reversal: RNA Control of Gene Expression

Dr. Richard H. Goodman

Dr. Richard H. Goodman is the Director of the Vollum Institute, Oregon Health and Science University, and professor of Cell and Developmental Biology and Biochemistry and Molecular Biology. The main research focus of Dr. Goodman and his colleagues is to determine how extracellular and intracellular signals are integrated, and how they control the onset and level of gene expression. Dr. Goodman described studies from his laboratory and related developments by other scientists at the May 2005 meeting of NIDDK's Advisory Council. The following scientific highlights are based on Dr. Goodman's presentation.

The human genome is described as our book of life—a book whose "stories" consist of genetic messages that are encoded within DNA molecules and spelled by means of four chemicals represented by the letters A, T, G, and C. Strings of these letters specify the thousands of different kinds of proteins that perform essential tasks in cells, tissues, and organs. But only 1.5 percent of the DNA in the human genome codes for such proteins. Some of the non-protein-coding DNA helps to regulate whether or not a particular gene is turned on ("expressed"), and to what extent. As scientific exploration of the genome continues, sequences within the rest of the non-coding 98.5 percent of the genome—once referred to as "junk DNA"—are also being found to regulate gene expression, in ways only recently discovered. Dr. Goodman has contributed significantly to current knowledge of the regulation of gene expression.

The processes that control gene expression are critical to enabling cells to perform their functions—for example, insulin secretion by cells in the pancreas, or activities of cells in the central nervous system. Moreover, they permit the formation of these cells in the first place—along with the myriad of other different types of cells in the body. Increased understanding of the regulation of gene expression, integral to life processes, may spur ideas for new therapeutics for many health conditions.

Basics of Controlling Gene Expression

The overall process of selectively turning some genes on and others off across hundreds of different types of cells involves a variety of intricate molecular mechanisms. Some gene control mechanisms can be likened to switches: when a gene is switched "on," cells make the specific protein that it encodes. But when that gene is "off," its particular protein is not made. In each cell throughout the body, the amounts and types of proteins being made are carefully controlled. This control is necessary because the aggregate levels and activities of proteins determine how specific cell types function in different parts of the body—for example, the pancreas, brain, heart, or muscle. This tight control is critical at all times, including during embryonic development, when from a fertilized egg, hundreds of different types of cells take shape, forming specialized tissues and organs. Each different cell type turns on only particular genes while suppressing others, so as to make a characteristic suite of proteins.

Early in their research, Dr. Goodman and his colleagues focused their attention on the gene encoding the protein called somatostatin, by studying when and how this gene is turned on, or expressed. They reasoned that, by investigating in depth how this particular gene is controlled, they would elucidate mechanisms that control the expression of

various genes. Many researchers had previously found that, as an early step in expressing the somatostatin gene or any other protein-encoding gene, certain information must be transcribed from DNA—the master genetic blueprint—to create a similar but distinctive molecule called messenger RNA or mRNA. In this copying process, proteins called transcription factors first "read" signals generally located upstream from the protein-coding portion of the gene. This sequence information in the protein-coding portion is incorporated into RNA molecules, which then move to another part of the cell, where the encoded message they carry is "read" to make a specific protein—in this case, somatostatin. Essentially, the first step in "turning on" or "expressing" a gene is the cell's recruitment to the master DNA of the necessary transcription factors needed to make RNA copies essential for the translation of key genetic information into proteins.

While studying the somatostatin gene, Dr. Goodman and his colleagues identified a discrete and important regulatory segment, located next to the protein-coding sequence of this gene. This site turned out to be the place where a subsequently-identified factor called CREB binds, to help turn on the gene under certain conditions. (CREB itself is a protein.) They later found another factor, termed CREB-binding protein (CBP), a "co-activator" that assists CREB in turning on genes. These findings have led to new insights into the control of numerous genes.

Because CREB is involved in controlling expression of many genes in addition to somatostatin, Dr. Goodman and his collaborators very recently developed an innovative molecular search strategy to identify the entire set of sites within a mammalian genome where the CREB protein binds. This search strategy, they reasoned, would lead them to all the various genes that CREB regulates. Using this search strategy in a rat cell model, the scientists identified a very large number of CREB binding sites throughout the genome. Many of these sites were near genes not previously known to be CREB-regulated. Strikingly, some of these turned out to be genes that encode types of RNA molecules, called "microRNAs," which have critically important regulatory functions. MicroRNAs are very short RNA molecules that don't get read to make proteins, like messenger RNAs do. Instead, microRNAs have a regulatory function: they bind to messenger RNA molecules and either block protein synthesis directly or cause those mRNA molecules to disintegrate.

Thus, CREB not only influences expression of many protein-coding genes, but, based on the surprising results of Dr. Goodman's genome-wide search, CREB also appears to be involved in the expression of genes for a number of microRNAs. This is also notable because so little had been known about how cells turn on genes that encode microRNAs.

MicroRNA Molecules Offer Another Means for Regulating Genes in Cells

Dr. Goodman recounted that the first microRNA had been discovered by other researchers working on worms as a model system in the early 1990s. MicroRNAs have since been found in many other organisms, including mammals.

MicroRNAs regulate a variety of cellular functions, including processes during development to help early-stage cells turn into nerve or other types of cells. One surprising and potentially important finding for researchers working on diabetes is that a particular microRNA molecule, designated *miR375*, appears to regulate the secretion of insulin, a hormone produced in the pancreas. Insulin is required for regulating glucose levels in the blood. When people eat a meal, the pancreas releases insulin to redirect the surge of sugar in the blood, thereby maintaining appropriate blood glucose levels and redistributing this chemical fuel to other tissues and organs in the body where it is needed. However, disrupting insulin functions can lead to diabetes. Type 1 diabetes results when specialized insulin-producing cells in the pancreas are mistakenly attacked and destroyed by the

body's immune system. Even when insulin is being properly produced, individuals may develop type 2 diabetes when the body no longer responds appropriately to this hormone.

Dr. Goodman highlighted research by Dr. Markus Stoffel of Rockefeller University and his colleagues, who found that the *miR375* microRNA appears to inhibit glucose-stimulated insulin secretion in mouse pancreatic cells. It does this by interacting with the messenger RNA from another gene, which the scientists identified. The scientists also showed that experimentally inhibiting *miR375* molecules enhances insulin secretion. Thus, the discovery of the *miR375* molecule and its function may lead to a novel therapeutic approach for treating diabetes.

Dr. Goodman also pointed to exciting but preliminary research results from Dr. Gail Mandel's lab at SUNY Stony Brook showing that other microRNA molecules, along with CREB and another regulatory protein called REST, are involved in determining whether or not some cells become neural cells (neurons)—that is, part of the nervous system. They do this by turning some genes on, and others off. Dr. Goodman and his collaborators found that CREB helps turn on a microRNA that is involved in the development and specialization of neurons.

In contrast to CREB, which helps turn genes on, REST keeps genes turned off, including, for example, yet another microRNA gene, called *miR124*, that is needed in neurons. REST is present in both non-neural cells and in those precursor cells that are destined to become neural. In the latter cells, such as those in the brain, REST disappears during development but CREB persists; as a result, *miR124* and other factors can work to permit cells to express the genes needed to be neurons. However, in other cells—for instance, liver cells—REST persists and thus helps to prevent expression of neural-type genes. The *miR124* microRNA can shift cells towards becoming neural-like, apparently by destroying transcripts, or mRNA molecules, that ordinarily confer a non-neural character on those cells. If these findings are further supported by additional analysis, they will help to explain how highly specialized cells, tissues, and organs such as the brain and liver can arise from precursor cells and then maintain their new specialized identities. These findings also could prove instrumental as scientists study stem cells and learn how to modulate their functions to repair damaged tissues and organs.

By further elucidating how cells regulate gene expression and protein synthesis, Dr. Goodman's research is shedding light on critical biological processes relevant to development and health.