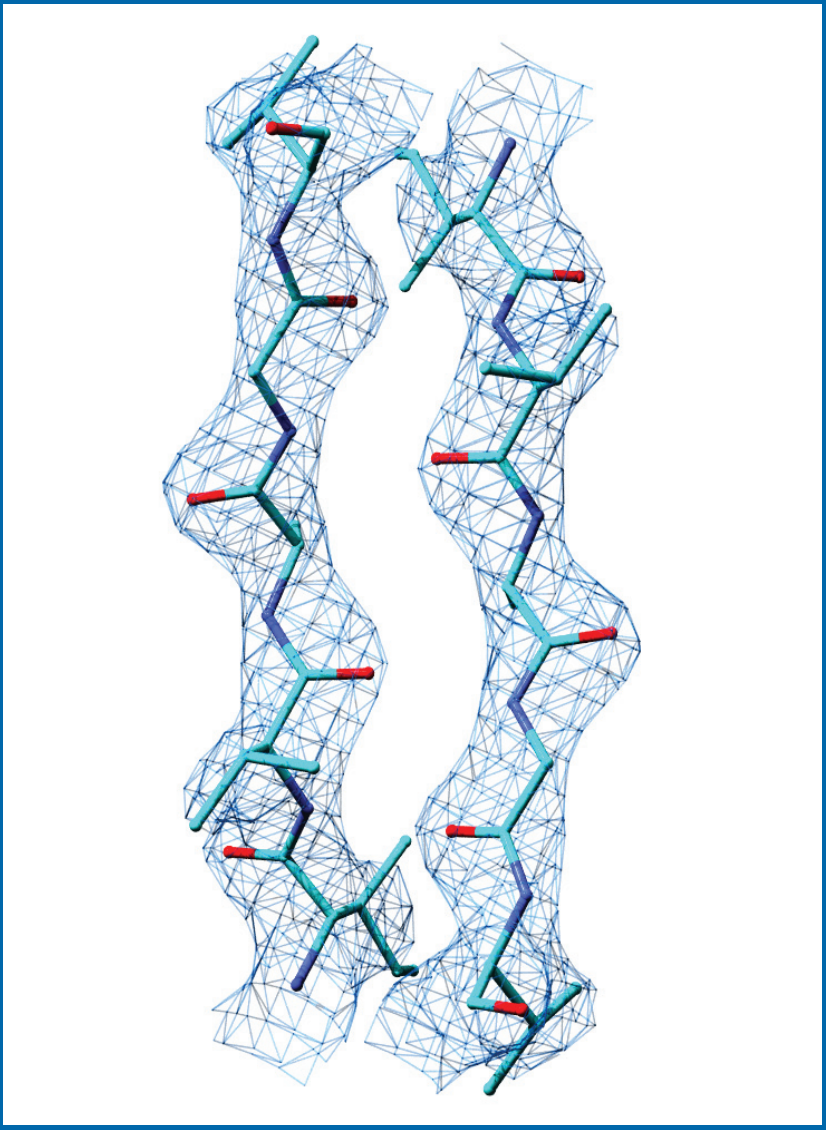

3-D model of a portion of human cystatin C—a serum protein being studied as a potential "biomarker" of impaired renal function. NIDDK-supported efforts to identify and qualify biomarkers for specific diseases within its mission are described in this chapter.

Image courtesy of Dr. Mariusz Jaskolski. Adapted by permission from Macmillan Publishers Ltd: Nature Structural Biology 8: 316-320, copyright (2001).

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, RNA, 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. Translational research also includes efforts to identify and use molecules found in the body, such as gene variants and proteins, as biological markers ("biomarkers") of specific disease states.

ACCELERATING BIOMARKER RESEARCH

A biomarker can be defined as a physical, functional, or biochemical indicator of a physiological or disease process that has diagnostic or prognostic utility. 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. Some biomarkers are extremely valuable surrogate endpoints for diseases, and can be used as outcome measures in clinical trials. The capacity to study and treat disease is hampered when unique, reliable, quantifiable, easily measured, and verified biomarkers that correlate well with disease progression are lacking. Therefore, the NIDDK Translational Research Working Group, charged with identifying opportunities for accelerating the transition of basic biomedical research advances from the laboratory to the common clinical application, has identified biomarker research as a particularly critical area for study.

The NIDDK has a long track record of successfully promoting the development of biomarkers for a number of diseases within its research mission that have transformed patient care. For example, the hemoglobin A1c (HbA1c) blood test has been shown to be a good surrogate measure of long-term blood glucose control in diabetes. HbA1c has been validated in a large NIDDK-funded clinical trial, and subsequently has served as the basis for the Food and Drug Administration's approval of multiple drugs for therapy of diabetes. Similarly, methods for assessing kidney function by estimating glomerular filtration rate (GFR) using circulating levels of the metabolite protein creatinine and earlier ascertainment of kidney disease by measuring the level of the protein albumin in the urine, have become important biomarkers for kidney function and disease. However, additional biomarkers are urgently needed to speed development of potential new treatments. For example, cystatin C is a different serum protein now being investigated as a possible biomarker for estimating GFR.

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.

This year, the Foundation for the National Institutes of Health (FNIH), the National Institutes of Health (NIH), the Food and Drug Administration (FDA), and the Pharmaceutical Research and Manufacturers of America (PhRMA) announced a major public-private biomedical research partnership, *The Biomarkers Consortium*, to search for and validate new biomarkers to accelerate dramatically biomarker usage for early detection, diagnosis, and treatment of disease. Among the first projects to be undertaken by the *Consortium* will be one on diabetes and pre-diabetes biomarkers. This project would build upon an existing NIDDK pilot study, and would seek to discover new biomarkers related to type 2 diabetes and pre-diabetes that could be used in developing a new assay and speed up and improve the translation of this assay from the bench to the bedside. The hope is that this project will lead to a more reliable, economical, and faster diagnostic test for diabetes by identifying biomarkers from before the disease really begins to the time of full-blown diabetes. This would allow earlier treatment, better monitoring, and ultimately reduce morbidity and mortality from diabetes and provide the potential for dramatic savings in health care costs.

Additional new NIDDK-sponsored initiatives to pursue potential biomarkers include:

- *Development of Disease Biomarkers:* Previous studies have identified candidate biomarkers. Other studies test biomarkers in clinical trials. This initiative will fill the gap between these steps by providing resources to demonstrate that candidate biomarkers meaningfully reflect actual disease processes. The initiative's scope includes well-defined human diseases of liver, kidney, genitourinary tract, digestive and hematologic systems, and endocrine and metabolic disorders, diabetes and its complications, and obesity, for which there are no or very few biomarkers, or for which standard biomarkers are currently prohibitively invasive or expensive.

- *Noninvasive Methods for Diagnosis and Progression of Diabetes, Kidney, Urological, Hematological, and Digestive Diseases:* This initiative is similar in scope to the one described previously, but rather than focusing on validation, it emphasizes development of new technologies or new applications of existing technologies, including molecular imaging and functional imaging approaches; imaging methods with high spatial, chemical, or time resolution; and new spectroscopic or sensor array technologies for monitoring metabolic or physiological events.

- *Biomarker Development for Diabetic Complications:* Clinical studies, such as the Diabetes Control and Complications Trial, have demonstrated that tight glycemic control can dramatically reduce the micro- and macrovascular complications of diabetes. However, intensive glycemic control is difficult to obtain in all patients and is associated with an increased risk of hypoglycemia. This initiative seeks biomarkers that can help identify

patients with diabetes who are at particular risk for various comorbidities. Better assessment of the progression of complications may also help to facilitate the development of therapeutics for these devastating conditions.

- *Toward Imaging the Pancreatic Beta Cell in People:* Pancreatic beta cells secrete insulin, which signals other cells in the body to take up glucose from the blood. It is the beta cells that are destroyed during type 1 diabetes. Imaging of those beta cells could allow physicians to follow disease progression, and assess treatments, as well as keep track of new beta cells introduced via transplant. This initiative solicits research applications focused on detection (within the body) of beta cell mass, function, inflammation, or transplanted islet engraftment, especially using imaging technologies. It is intended to support the development of novel imaging technologies that will provide new opportunities for evaluating and quantifying beta cell mass and function with potential to lead to the development of a clinically useful exam.

In addition, the NIDDK is the lead Institute for the *Using Metabolomics to Investigate Biological Pathways and Networks* Request for Applications under the NIH Roadmap for Medical Research. Metabolomics—the study of all metabolites (e.g., salts, sugars, and fats) in order to understand physiological and disease processes—is a relatively new and fertile area of research. This initiative seeks to encourage the use of innovative metabolomics technologies to establish methods and model systems for advancing the understanding of biological pathways and networks, their temporal and spatial resolution, and their regulation in health and disease states. Many of the applications are expected to be relevant to NIDDK mission-specific diseases.

Kidney Disease of Diabetes Project Selected for Whole Genome Association Study

The Genetic Association Information Network (GAIN) is a new public-private partnership of the Foundation for the National Institutes of Health (FNIH) and includes partnerships with the NIH and the private sector to encourage whole genome association studies of common diseases. Using biological samples already collected in clinical studies, GAIN will evaluate the subtle differences between the genomes of people with and without six common diseases that affect the public health. After vigorous peer, technical, and ethical reviews, the Genetics of Kidneys in Diabetes (GoKinD) study was selected as one of the six studies for analysis by GAIN. The NIDDK participates in GoKinD, which is led by the Centers for Disease Control and Prevention and the Juvenile Diabetes Research Foundation International. GoKinD has the largest single collection of biosamples and data for research on the genetic causes of kidney disease in type 1 diabetes. The collection consists of samples from people with both type 1 diabetes and kidney disease, people with type 1 diabetes but no kidney disease, and samples from some of the parents of affected patients. Using the GoKinD collection, investigators will search for genes that predispose patients to, or protect them from, developing this devastating complication of diabetes. This knowledge could accelerate the development of new methods for prevention, diagnosis, and treatment. Data from this effort will be deposited into a central database and made available for broad research use. The GAIN study and the public release of data are helping to ensure the broadest possible access to genetic data from the valuable GoKinD collection.

NUCLEAR RECEPTOR SIGNALING ATLAS (NURSA)

The Nuclear Receptor Signaling Atlas (NURSA) is a trans-NIH initiative, led by the NIDDK, designed to develop a comprehensive understanding of the structure, function, and role in disease of nuclear hormone receptors. Nuclear receptors are a superfamily of transcription factors—proteins that regulate the expression of genes. This superfamily includes receptors for steroid hormones, thyroid hormones, and fat-soluble molecules, such as Vitamins A and D. Nuclear receptors regulate the expression of many genes involved in a broad range of metabolic, reproductive, developmental, and immune response programs. 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. Researchers in the NURSA consortium have recently made key discoveries that are increasing knowledge about the role of nuclear receptors in physiology and underlying mechanisms of disease. The long-term goal of NURSA is to translate fundamental observations on the role of nuclear receptors in metabolism into applications that can be used in interventions to treat or prevent disease.

Nuclear Receptor Expression Network Illuminates Control of Metabolism and Other Physiologic Processes: Studying the function of a single nuclear receptor in a single tissue yields information about the receptor's biological function. However, just like looking at only one piece of a puzzle, it does not always give the overall picture with respect to how the receptor may be working with others to govern higher-order biological processes. To begin to address this more complex question, NURSA researchers used a systems biology approach to examine the expression of all 49 mouse nuclear receptors in 39 mouse tissues. When the data were clustered for receptor expression by tissue, the researchers uncovered a hierarchical, integrated transcriptional network tying nuclear receptor function to physiology. At the top of this hierarchy, the researchers unexpectedly observed that the network branched into two major clusters of receptors involved in: (1) reproduction, and (2) nutrient metabolism. Although individual receptor function is important, these results suggest the existence of a higher-order transcriptional network, which extends beyond individual tissues and governs physiology of the entire organism. These experiments examined nuclear receptor expression at a single point in time. In a related study, NURSA researchers measured expression at different time points during the day and night in certain key metabolic tissues. They discovered that 25 of the nuclear receptors were expressed in a rhythmic cycle (i.e., their expression changed at different times during the day and night) in these metabolically-active tissues. While it is known that organisms have a circadian clock that dictates different behaviors at different times, the workings of this clock are unknown. The new data suggest that the nuclear receptors are a link between the circadian clock and metabolism. Together, these studies provide tools for additional research on the biological role of both individual nuclear receptors and nuclear receptors as a superfamily.

Bookout AL, Jeong Y, Downes M, Yu RT, Evans RM, and Mangelsdorf DJ: Anatomical profiling of nuclear receptor expression reveals a hierarchical transcriptional network. Cell 126: 789-799, 2006.

Yang X, Downes M, Yu RT, Bookout AL, He W, Straume M, Mangelsdorf DJ, and Evans RM: Nuclear receptor expression links the circadian clock to metabolism. Cell 126: 801-810, 2006.

Key Role for Nuclear Receptors in Liver Regeneration from Bile Acid Signals: The liver is one of few internal organs that is capable of regenerating itself if part of it is damaged or removed. Up until now, the mechanism by which the body "senses" liver size and the elements that initiate liver regeneration have been elusive. Now, recent studies using mice that had undergone partial liver removal ("partial hepatectomy") have found that an

important role in liver growth and regeneration is played by bile acids. These factors are produced in the liver, stored in the gallbladder, and released into the small intestine to aid in the digestion of fats, after which they are returned to the liver where they may be reused. Bile acids also exert biological effects on liver cells through signaling pathways activated by the nuclear receptor FXR. Researchers hypothesized that, when part of the liver is removed or damaged, remaining liver cells are exposed to relatively higher levels of bile acids, because there are fewer cells to process the same levels of bile acids returning from the intestine. Furthermore, they wondered whether this might be a signal to the body of diminished functional capacity and trigger proliferation of the remaining cells through the FXR pathway. Starting with experiments in mice with intact livers given dietary bile acids, researchers showed that bile acids have a growth-promoting effect on the liver. Then, in partially hepatectomized mice, they demonstrated that bile acids are needed for growth processes during liver regeneration. This effect required bile acid signaling through FXR, because mice lacking this nuclear receptor showed reduced liver regeneration. This set of experiments identifies bile acids as key players responsible for signaling the regulation of liver growth and regeneration, and positions FXR as a key mediator of these processes. It also suggests a dynamic regulatory mechanism influencing liver size based on bile acid flux and functional liver capacity.

Huang W, Ma K, Zhang J, Qatanani M, Cuvillier J, Liu J, Dong B, Huang X, and Moore DD: Nuclear receptor-dependent bile acid signaling is required for normal liver regeneration. Science 312: 233-236, 2006.

RNA INTERFERENCE (RNAi) APPROACHES UNCOVER FUNDAMENTAL PROCESSES

NIDDK-funded investigators are building upon a scientific discovery that was recognized by the award of the 2006 Nobel Prize in Physiology or Medicine to two long-time NIH grantees, Andrew Z. Fire, Ph.D., of the Stanford University School of Medicine and Craig C. Mello, Ph.D., of the University of Massachusetts Medical School. The two researchers were honored for their discovery of RNAi, a mechanism for silencing genes that could lead to new disease treatments. In a seminal laboratory study, Drs. Fire and Mello showed that double stranded RNA can interfere with a gene's activity. RNAi is a technique in which small double stranded RNA, complementary to the mRNA of a given gene, is added to cells. The cells respond by degrading the mRNA, thereby preventing it from being translated into a protein. The technique has proved to be an excellent general method for figuring out the function of genes. Furthermore, RNAi offers the potential to design a new generation of drugs for human diseases that act by inhibiting the expression of certain genes. Both of the following advances use the RNAi approach to glean new understanding into biological processes.

Gene Expression Triggered by Protein Damage Protects Cells from Stress:

Researchers have gained new insights into the processes by which cells respond to environmental stress. Specifically, they examined the stress caused by high salt in the environment (also called hypertonic stress). Hypertonic stress harms cells by causing cellular water loss, cell shrinkage, and protein damage (such as protein unfolding). In a previous study, NIDDK-supported researchers showed that the roundworm, *Caenorhabditis elegans*, adapts to hypertonic stress by increasing expression of the glycerol 3-phosphate dehydrogenase gene. The result is an increase in the small molecule glycerol. More recently, the investigators provided evidence that the increase in glycerol production is essential and specific for *C. elegans* survival in hypertonic conditions. They further sought to identify key signaling pathways and gene expression associated with these conditions. Using RNAi, the researchers determined that a subset of genes is expressed during hypertonic conditions. Many of the genes in this subset code for proteins that function normally to regulate translation and protein folding and to prevent the accumulation of damaged

or denatured proteins in the cell cytoplasm. Thus, the investigators suggest that protein damage may be the signal that triggers the hypertonic stress response in *C. elegans*. Because misfolded proteins accumulate in various diseases, understanding the underlying molecular mechanisms of protein damage caused by hypertonic stress could provide new insights into other diseases having a protein damage component.

Lamitina T, Huang CG, and Strange K: Genome-wide RNAi screening identifies protein damage as a regulator of osmoprotective gene expression. Proc Natl Acad Sci USA 103: 12173-12178, 2006.

The Effects of Aging on "Proteotoxicity":

NIDDK-supported research is shedding light on a poorly understood aspect of the aging process: protein aggregation. As humans age, deposits of an aggregated protein fragment ($A\beta_{1-42}$) accumulate in the brain. In autopsies of people with Alzheimer's disease, these aggregates have been found to be larger and more numerous than in the brains of other individuals, although the link between the aggregates and disease symptoms remains a subject of scientific debate. Researchers have noted an important connection between the aging process and an important signaling pathway (the insulin/insulin-like growth factor-1 signaling pathway). When functioning normally, this pathway acts to reduce expression of a group of proteins called "chaperones," which have the job of preventing protein aggregation.

This observation has led researchers to hypothesize that protein aggregation leads to senescence in normal worms. RNAi used to interrupt this signaling pathway results in higher chaperone levels, and longer life. The aggregated protein fragment is not found naturally in worms, but when it is placed in them experimentally, it accumulates in aggregates as the worms age. Researchers recently found that RNAi interruption of the signaling pathway associated with aging in worms that express the aggregated protein fragment not only made them live longer, but it also substantially delayed and reduced formation of these aggregates. Their results showed that worms fared worse when they had large numbers of smaller aggregates than when they had smaller numbers of larger clumps. This finding suggests that the smaller aggregates are the more toxic ones. The researchers found that chaperones controlled by the signaling pathway detoxify the aggregates not only by eliminating them, but also by clumping smaller, more toxic aggregates into larger, less toxic forms. Similar processes may account for the importance of this signaling pathway in human aging, and it may be possible to modulate chaperones to prevent or delay some of the burdensome effects of growing older.

Cohen E, Bieschke J, Perciavalle RM, Kelly JW, and Dillin A: Opposing activities protect against age-onset proteotoxicity. Science 313: 1604-1610, 2006.