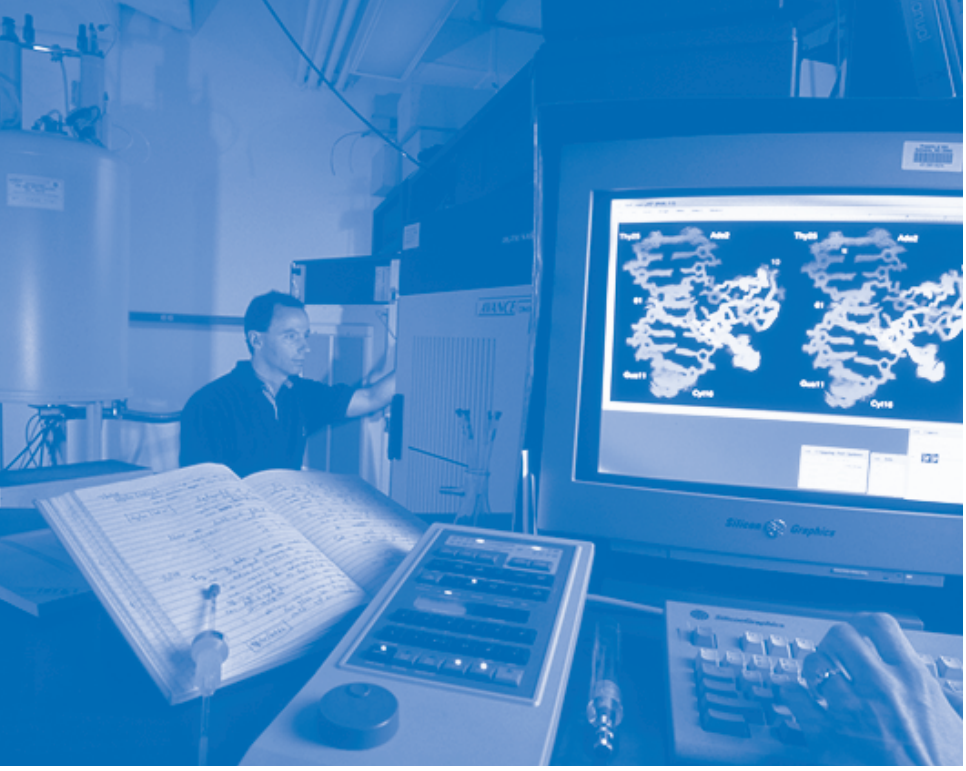


GOAL VI:

ATTRACT NEW TALENT AND APPLY NEW TECHNOLOGIES TO RESEARCH ON TYPE 1 DIABETES

Recent Scientific Advances

Novel Imaging Technologies To Monitor Type 1 Diabetes Disease Progression and Islet Transplantation

Systems Biology Approaches Reveal Identity of Genes Involved in Pathophysiology of Diabetes

miRNA Involved in Regulation of Insulin Secretion

Brain Imaging in Hypoglycemia Unawareness

Engineering an Endless Source of Beta Cells for Therapy

Research Objectives and Strategies To Achieve Goals

Engaging Talented Scientists

- Recruit Expertise from Diverse Fields
- Design Incentives That Reward Research Innovation
- Train New Scientists in Clinical Type 1 Diabetes Research

Development and Application of New Technologies

- Develop Noninvasive Imaging Technologies To Monitor Type 1 Diabetes
- Promote Application of Advances in Bioengineering to Type 1 Diabetes
- Foster Application of Gene Delivery and Gene Silencing Technology To Develop New Therapies for Type 1 Diabetes and Its Complications
- Apply New and Emerging Technologies in Functional Genomics, Proteomics, and Metabolomics to Type 1 Diabetes Research
- Improve the Power of Diabetes Research by Utilizing Computational Biology and Bioinformatics
- Apply New Technology to the Development of Improved Animal Models for the Study of Type 1 Diabetes

STRATEGIES TO ATTRACT NEW TALENT AND APPLY NEW TECHNOLOGIES TO RESEARCH

INTRODUCTION AND BACKGROUND

In the last 100 years, doctors and scientists have made remarkable progress in the understanding and management of type 1 diabetes. As a result, people with the disease are now living longer and healthier lives. As the quest for a cure continues, progress will increasingly require collaborations among clinical and basic scientists with diverse skills and expertise. To understand the complicated interplay of hereditary and environmental factors that cause the disease and the progression of its complications (Goals I and V), geneticists and epidemiologists are beginning to collaborate with biostatisticians and informational biologists to generate computer models that will allow them to understand and test these complex interactions in the biological system. Preventing and reversing the chain of events in autoimmunity and achieving immune tolerance for organ transplants (Goals II and III) will require cooperation between immunologists and clinicians, as well as the biotechnology industry that develops and tests therapeutic agents. Similarly, knowledge of the basic biology of the insulin-producing pancreatic beta cell (Goal III) has expanded because of efforts to recruit cell and developmental biologists, who may have been focused on other systems and diseases, to the study of diabetes. These talented scientists are now directing their skills toward understanding how the beta cell develops. The research challenge of hypoglycemia unawareness (Goal IV) will require recruiting more neurobiologists and endocrinologists to understand the brain circuitry and body interactions. Continuing the progress that has been made in early detection and slowing progression of diabetes complications (Goal V) will involve not only experts in heart, nerve, kidney and eye disease, but also experts in proteomics, imaging, and other skills needed to develop biomarkers and surrogate endpoints that can speed translation of new therapeutic concepts from the bench to the bedside. For each of the Strategic Plan's scientific goals, a crucial prerequisite is to recruit and retain talented scientists and to foster collaborations, in order to propel further scientific advances.

With the recruitment of appropriate talent to the study of type 1 diabetes, cutting-edge technologies can be applied or developed for use in basic and clinical research. Certain technology themes cut across all the research goals and objectives outlined in this Strategic Plan. For example, the application of biophysical tools, such as labeled tracers, has opened the door for the use of new and improved methods of noninvasive imaging in both patients and animal models. These techniques will allow clinicians to assess the onset and progress of disease and the success of various therapeutic interventions. In recent years, biomedical research has witnessed an explosion of innovative tools that have paved the way for new fields of research, such as proteomics, functional genomics, metabolomics, bioinformatics, gene therapy, and gene silencing (siRNA). Scientists are rapidly applying these new technologies to type 1 diabetes research. However, it would also be beneficial to design new technologies in the context of type 1 diabetes research, so as to address the unique challenges of this disease. Additionally, new technologies may facilitate identification and validation of improved biomarkers for disease progression. Such biomarkers would make it less expensive and more efficient to conduct clinical trials and would thereby encourage industry investment in new therapies, from which patients might benefit more quickly.

RECENT SCIENTIFIC ADVANCES

Novel Imaging Technologies To Monitor Type 1 Diabetes Disease Progression and Islet Transplantation: Type 1 diabetes is usually diagnosed very late in disease progression, when most of the insulin-producing beta cells of the pancreas have already been destroyed. The development of a "toolbox" of imaging technologies capable of detecting the first signs of beta cell destruction would help to monitor therapy against immune attack or look for possible regeneration of beta cells. The first steps have been taken—scientists have recently developed a new, noninvasive imaging technology to monitor infiltration of inflammatory cells into the pancreas in an animal model of type 1 diabetes. This approach is now being tested in people. If successful, it could dramatically improve the ability of researchers to perform type 1 diabetes clinical trials.

Another important advance is the successful labeling of isolated human islets, mouse islets, and mouse T cells with nontoxic imaging probes that can be detected with magnetic resonance imaging (MRI), fluorescence, or nuclear imaging. The islets have been imaged quantitatively over time after implantation in the liver or under the kidney capsule in mice. T cells have been seen as they infiltrate the pancreas of a non-obese diabetic (NOD) mouse. Although such molecular imaging approaches are still very new, their application is now being introduced into human patients. It is hoped that they will soon be used in studies of type 1 diabetes.

Scientists are also exploring the use of positron emission tomography (PET) imaging to see radiolabeled ligands targeted to the pancreas. If such an approach proves successful, it would allow physicians to estimate the number or mass of a patient's own endogenous beta cells and to monitor the fate of transplanted islets.

Systems Biology Approaches Reveal Identity of Genes Involved in Pathophysiology of Diabetes:

Some diseases are caused by changes in a single gene that lead to a defective or missing protein, but complex diseases may involve subtle changes in the concentrations of a whole network of proteins working in concert. These changes can often be detected as a function of the concentration of the mRNA molecules that arise from DNA and code for proteins. The DNA microarray is a powerful tool that permits geneticists to simultaneously monitor the changes in gene expression (mRNA) of an entire genome to compare healthy and diseased tissues. Computational scientists are now working with biologists to develop adequate tools to analyze the vast amounts of data produced in each experiment, in order to more fully understand its value. Bioinformaticists recently introduced just such an analytical strategy that enabled them to compare gene expression in muscle biopsies from diabetic and non-diabetic individuals. Their analysis allowed them to identify a set of genes for which the expression is coordinately decreased in diabetic muscle. This group of genes carries out energy production in mitochondria. The affected mitochondrial protein genes are controlled by two transcription factors. Therefore, the few genes that code for these special transcription factor proteins regulate the expression of many other genes. Rare forms of monogenic diabetes, such as Maturity Onset Diabetes of the Young (MODY), provide another example of the key role of selected transcription factors in the pathogenesis of diabetes. These disorders originate from mutations in key transcription factors affecting entire networks of genes that regulate function in organs, such as the liver and pancreas. Using antibodies raised against a transcription factor known to be involved in pancreas development and liver metabolism, scientists can isolate all of the regions of the genome that bind to the transcription factor (chromatin immunoprecipitation or ChIP). The sequence of the DNA regions binding to the transcription factor of interest can then be identified using either large-scale sequencing strategies (Serial Analysis of Chromatin Occupancy or SACO) or hybridization on promoter microarrays containing the promoter regions of all known genes (ChIP-on-chip). These genome-wide analyses can result in fast, complex, and accurate modeling of transcriptional regulatory networks involved in the control of energy homeostasis, pancreatic beta cell function, or pancreatic islet mass.

miRNA Involved in Regulation of Insulin Secretion:

A novel class of natural molecules that controls translation of specific proteins was recently discovered and characterized. These are microRNAs (miRNA)—small single stranded chains of nucleic acid derived from non-coding portions of the genome. Building on this new discovery, researchers have recently found an miRNA in mice that suppresses insulin secretion. Using an miRNA-related technology called RNA interference (see sidebar on page 86), the researchers were able to mimic the effects of the miRNA to regulate insulin secretion. Furthermore, *in vivo* studies of chemically engineered oligonucleotide inhibitors of miRNA, called antagomirs, indicate that a single miRNA is likely to have not one but many gene targets. Therefore, antagomirs are powerful tools that can silence *in vivo* miRNA-controlled regulatory pathways and could become a therapeutic strategy for pathologies in which miRNAs participate in disease etiology.

Brain Imaging in Hypoglycemia Unawareness:

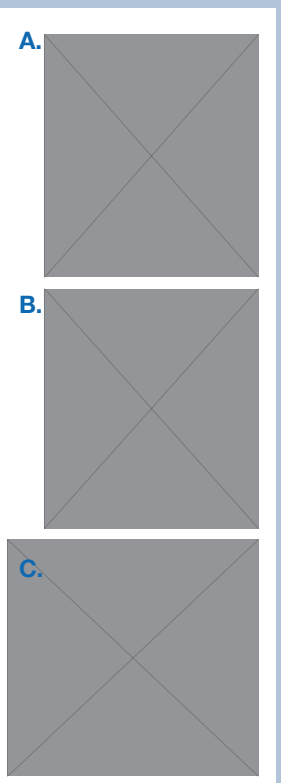
The brain is dependent on glucose for fuel, and it also contains glucose-responsive neurons in specialized regions that sense blood glucose concentration and then coordinate the hormonal, neurological, and behavioral responses that rescue a person when his or her blood glucose levels sink too low. Diabetes can be accompanied by a failure of the brain to both recognize hypoglycemia and generate signals to prevent blood glucose from falling to a dangerously low level. This hypoglycemia unawareness often develops after repeated episodes of hypoglycemia and is very frightening to diabetes patients who may lose consciousness without warning. Now, scientists are beginning to understand how the brain loses its ability to respond and warn of impending hypoglycemia. New imaging technologies have revealed differences in glucose use in different areas of the brain. Imaging has also shown that the brain increases blood flow when blood glucose falls and that its cells store glucose, which can be used when it cannot get enough from the blood. Combined, these mechanisms may help maintain glucose availability to the brain, and

thus cognitive function, when the body experiences hypoglycemia. Most people with diabetes have the same brain glucose concentrations as healthy people, but brain glucose is elevated in those who develop hypoglycemia unawareness; thus, glucose levels may stay higher in their brain cells even as blood glucose levels fall, masking the normal warning to respond to low blood glucose and, therefore, failing to prevent it. Considerable progress remains to be made in this area before achieving a true understanding of how the brain reacts to glucose concentrations and what goes awry in hypoglycemia unawareness in diabetes. However, the new imaging technologies of MRI and PET have opened the window on the brain and its metabolism.

Engineering an Endless Source of Beta Cells for Therapy: The limited supply of human islets available for transplantation dramatically reduces patient access to this potentially life-enhancing therapy. In laboratory studies, efforts to produce an increased quantity of glucose-regulated, insulin-producing cells for transplant have employed cell culture, tissue engineering, and gene therapy technologies to promote beta cell development from adult or embryonic stem cells. In addition, mature cells from tissues such as liver, spleen, intestine, or pancreas, or from cultured cell lines, have been tested for their ability to serve as donors in cell-based therapies. To enhance their therapeutic potential, these cells have been incubated with growth- and transcription-activating factors or hormones, co-cultured with additional cell types, or manipulated to express genes that code for proteins found in the beta cell. Insulin-secreting beta cell-like immortalized cell lines have been created by careful selection for beta cell specific traits. These efforts have yielded cells that, in some cases, are able to reverse diabetes in animal models, but have not yet been fully successful in reproducing the exquisite sensitivity to small changes in glucose levels that is characteristic of mature beta cells. This research has also provided considerable insight into the characteristics that make up a beta cell and a foundation for further progress toward improving the supply of beta cells or islets.

Potential Therapeutic Applications of RNA Interference (RNAi) in Type 1 Diabetes

The recent discovery of the natural molecules known as microRNA (miRNA) has challenged the prevailing scientific thinking regarding the role of ribonucleic acid (RNA) in gene regulation. The miRNA has much shorter chains of nucleic acids than messenger RNA (mRNA), which contains the coding sequences of proteins. These miRNAs can specifically silence the expression of a gene or a family of genes by blocking translation of the proteins they encode, and they are involved in the regulation of a wide variety of cellular functions, ranging from cell fate determination to suppression of glucose-induced insulin secretion in the beta cell. Mammalian genomes contain a large and diverse family of miRNAs; it is now believed that miRNAs, conserved across evolution from plants to animals, may affect one-third of all human gene expression. However, because scientists have spent decades focusing on protein-based mechanisms of gene regulation, the research tools for studying miRNA regulation are just now being developed.



Researchers are designing sequence-specific RNA interfering molecules (siRNAs) to silence expression of disease genes, such as genes involved in destroying beta cells in type 1 diabetes. The mechanism by which this technology works is: (A) A cell expresses a protein by transcribing genetic information stored in the DNA into messenger RNA (mRNA). The mRNA is then translated into a protein. (B) Researchers have identified a new type of RNA—microRNA—that comes from DNA, but does not code for proteins. Instead, the sequences of microRNA complement and bind to the sequences of specific "targeted" mRNA, thereby preventing the mRNA from being translated into a protein. (C) Small interfering RNA, or siRNA, is synthesized in the laboratory, but uses a similar mechanism to interrupt mRNA translation.

Researchers have learned how to manipulate the pathways used by miRNA by using synthetic double-stranded RNA molecules called small interfering RNA (siRNA). These are employed in RNA silencing or interference studies (RNAi) to suppress translation of the gene of interest and provide insight into its function. Successful *in vivo* delivery of siRNA has been demonstrated in a wide variety of organs, including the pancreas. This technology is quickly moving into the clinic, as several biotechnology companies have received FDA approval for conducting phase I clinical trials using RNAi-based therapies. In one such phase I trial, siRNA technology is used to target the vascular endothelial growth factor (VEGF) pathway to treat age-related macular degeneration; visual acuity improvement has already been reported in the treatment group. If successful, such an approach may be used for the treatment of diabetic retinopathy resulting from abnormal, VEGF-dependent angiogenesis in the retina. Other RNAi treatments in development are targeting specific immune and inflammatory responses. For example, a program using siRNA targeting key Th2 cytokines is entering a phase I trial for the treatment of asthma. RNAi-based therapeutic strategies targeting specific components of the immune response and/or the apoptotic pathways involved in beta cell destruction, combined with early detection of disease, could result in the restoration and maintenance of normal beta cell mass in patients with type 1 diabetes.

Engaging Talented Scientists

Pursuit of the full range of opportunities for prevention and improved therapy of type 1 diabetes and its complications requires a wide range of scientific expertise and the participation of investigators from diverse fields. These talented researchers must be recruited to the field, and promising new investigators must be trained in diabetes and enlisted in the research enterprise. High-yield, multidisciplinary approaches can only be fostered with appropriate new infrastructure. Finally, resources must be provided to promote an environment that values high-risk, high-impact projects. Tomorrow's ability to prevent and cure type 1 diabetes depends on the quality of today's research community and environment.

Research Objective—Recruit Expertise from Diverse Fields:

▸ *Encourage interdisciplinary collaborations.*

Type 1 diabetes affects many different organ systems and requires expertise in fields as diverse as genetics, neurobiology, immunology, biophysics, endocrinology, imaging, bioengineering, and biostatistics. The NIH has pioneered novel approaches to establish and empower new scientific teams for type 1 diabetes research.

Collaboration among scientists with complementary expertise has been forged via "innovative partnerships" (see sidebar) that encourage experts in type 1 diabetes research to recruit and work together with scientists from outside the field. In "bench-to-bedside" research partnerships, a team of clinical and basic scientists conducts collaborative research that, if successful, will bring basic research advances from the laboratory to a point where a potential new therapy can be tested in patients or in pre-clinical studies in animal models. A major obstacle to research is being alleviated by programs that provide scientists with access to collections of biological samples from well-characterized, consenting patients. The repositories that store and distribute such samples are an important resource that allows the creative research community to conduct mechanistic studies in virtual collaboration with the clinicians as they pursue patient-oriented research.

Scientific workshops bring together experts from different fields to stimulate discussions and cooperative endeavors in a particular field, and they have resulted in important new collaborations. For example, one such key workshop brought

Innovative Partnerships in Type 1 Diabetes: A Novel "Co-Principal Investigator" Support Mechanism

Because research on type 1 diabetes spans a broad range of scientific disciplines, propelling research progress requires a cadre of scientists with diverse research training and expertise. To attract new research talent to study type 1 diabetes and its complications, the NIH has supported an initiative on "Innovative Partnerships in Type 1 Diabetes Research." The overall objective of the initiative was to support collaborations between investigators who focus their research efforts on type 1 diabetes or its complications and investigators from other research areas with expertise relevant to type 1 diabetes. Type 1 diabetes researchers therefore acted as "talent scouts" by identifying and recruiting leading scientists with expertise relevant to the field of type 1 diabetes research. Using this mechanism, researchers with expertise in areas such as cell-based screening, imaging, genomics, and systems engineering are now pursuing research on type 1 diabetes.

The intent of the initiative was to encourage true partnerships in which two or more investigators with complementary expertise tackled a common problem. However, the standard policy at the NIH was to award a grant to only one principal investigator, while the partner was listed as a co-investigator—an arrangement that did not recognize both partners as being equal and thus posed a barrier to collaboration. Based on feedback received from the external scientific community, the NIH pioneered a novel solicitation so that both partners were named as co-equal principal investigators. This arrangement was first used under the *Special Statutory Funding Program for Type 1 Diabetes Research*. It provided an important incentive to collaboration, and attracted expertise from diverse fields. For example, one project brought together diabetes complications investigators with experts in angiogenesis (small blood vessel formation), thereby helping to move therapeutics currently used for cancer toward applications for diabetes complications. The new awards benefited both partners, who have now received equal recognition for their contributions to the research study. This recognition can be beneficial to investigators, who may be evaluated by their home institution in terms of the number of grant awards they have received. The "co-principal investigator" mechanism—first employed by the NIDDK with the "Innovative Partnerships in Type 1 Diabetes Research" initiative—is now being considered for broader implementation by the NIH as a whole, under the NIH Roadmap for Medical Research.

together clinical researchers from the landmark Diabetes Control and Complications Trial with cell biologists to explore the mechanisms underlying "metabolic memory" (see Goal V). Another workshop drew both neuroscientists and diabetes experts to address the problem of hypoglycemia unawareness that limits therapy for type 1 diabetes (see Goal IV). Attendees outlined collaborative strategies to elucidate the mechanisms underlying this condition and explored avenues to reverse it. A third workshop brought together proteomics experts and researchers in the fundamental and clinical aspects of diabetes research, yielding valuable suggestions for future efforts to develop much needed biomarkers and surrogate outcomes.

Research Objective—Design Incentives That Reward Research Innovation:

▸ *Promote high-risk, high-impact research.*

Scientists have identified certain barriers as critical bottlenecks that impede progress in type 1 diabetes. Particularly noteworthy is the need for biomarkers and surrogate endpoints to conduct clinical research to evaluate potential new therapeutics in small pilot trials. However, this type of discovery research is inherently risky, with no assurance of a positive outcome. Moreover, applicants find it difficult to obtain funding for high-risk research even if the work has potential for high, positive impact. Although not all high-risk research makes it to the clinic, investment in pioneering research may eventually stimulate the next major breakthrough. Therefore, it is imperative to provide incentives for talented scientists to undertake such research. These can take the form of limiting the requirements for preliminary data for pilot studies and of providing quick turn-around for continued or expanded funding when pilot studies meet defined milestones for achievement.

▸ *Create an environment conducive to innovation and collaboration.*

Although science has traditionally been fueled by competition, recent efforts focused on aspects of type 1 diabetes research have attempted to alter that paradigm and foster a community-oriented approach through the establishment of large research consortia, clinical trial networks, research centers, and team science. Such cooperation has enabled researchers to undertake large interdisciplinary projects that could not be pursued independently by any single investigator or small research group. Within a consortium, team members can share data, samples, protocols, research resources, and even cost-intensive patient recruitment in an efficient and effective way. The *Special Statutory Funding Program for Type 1 Diabetes Research* has not only created many effective consortia, but it has also recently provided support for infrastructure to promote cooperation among consortia. A consortia coordinating committee has been formed to resolve issues such as interoperability of databases and standardization of patient informed consent procedures. Furthermore, the NIH supports a website to announce the availability of research resources and funding opportunities to the research community (accessed at: www.T1Diabetes.nih.gov/investigator).

Research Objective—Train New Scientists in Clinical Type 1 Diabetes Research:

▸ *Attract and train new diabetes investigators.*

New scientists and engineers often bring energy and creativity to a field; future progress depends on the research training and mentoring of new students, postdoctoral fellows, and independent investigators. Currently, the *Special Funding Program* provides competitive institutional research career and training awards for pediatric endocrinologists involved in type 1 diabetes research. While development of pediatric endocrinologists as diabetes researchers was considered the highest priority for the limited resources available, this program could be productively expanded to promote research career development and training for investigators in other areas of importance to type 1 diabetes research. Programs that provide funding for exploratory projects could be very influential at the critical time when junior investigators are making the choices that will determine their long-term career paths.

Development and Application of New Technologies

The past decade has seen major advances in biotechnology with direct relevance to type 1 diabetes research. It is imperative to put cutting-edge technology into the hands of type 1 diabetes researchers and to foster future development of these tools in the context of their application to type 1 diabetes. Highlighted below are some of the most promising new technologies with important applications for type 1 diabetes.

Research Objective—Develop Noninvasive Imaging Technologies To Monitor Type 1 Diabetes:

▸ *Develop imaging for pancreatic beta cell mass, function, and inflammation.*

As discussed in the "Recent Scientific Advances" section of this chapter, there has been impressive progress in this area despite substantial challenges inherent in visualizing a tiny population of cells that reside deep inside the abdomen and

share many defining characteristics with neighboring cell types. Success in imaging will provide insights into the natural history of islets in diabetes; facilitate clinical trials to test therapies to slow or reverse beta cell loss; and allow physicians to monitor engraftment following islet transplantation. An islet imaging program funded by the NIH and JDRF has supported promising projects in animals and in humans looking at original surviving islets, as well as transplanted ones. Parallel projects are ongoing to provide the important reagents for imaging. These include the identification of unique cell surface proteins and production of monoclonal antibodies and other specific ligands that can be tagged for molecular imaging. The next steps are to create an environment in which various imaging approaches and reagents can be translated from the laboratory to clinical application in a well characterized patient population. This transition will require a balanced team of cutting-edge physicians, imaging experts, chemists, and biologists who have access to state-of-the-art equipment for imaging in both animal models and patients. Finally, entirely new approaches are being designed for imaging live tissues. These include:

- New optical tools that are more sensitive to small differences among tissues, can image more deeply into the body, or can take advantage of optical fibers and miniaturized detectors that can be introduced into the body in a mildly invasive manner;
- New x-ray imaging that can enable researchers to see soft tissues with very high resolution and reduced radiation exposure;
- New highly sensitive ultrasound and MRI contrast agents; and
- Enhanced resolution through powerful image reconstruction paradigms.

The diabetes research community must be positioned to take advantage of the best of these technologies as they appear, so that they can immediately be brought to bear on the challenging problem of imaging the pancreatic islet beta cell.

- *Develop brain imaging techniques to use in understanding hypoglycemia.*

How do minutes or hours of hypoglycemia affect structure, function, and metabolism of the brain? What are the short- and long-term consequences of multiple hypoglycemic episodes? What leads to hypoglycemia unawareness? To answer these questions, improved brain imaging with high spatial resolution is needed to elucidate the relationship between the specific neurons and their supporting cells involved in the detection and response to low blood sugar. It will likely be necessary to introduce artificial molecular imaging tags that bind to specific surface proteins in order to distinguish cell types from one another in glucose-sensing regions of the brain (see sidebar on page 90). The ability to visualize neural function is needed to understand hypoglycemia unawareness. There are several novel functional imaging technologies being used to study the brain, such as BOLD fMRI, arterial spin labeling techniques, diffusion tensor imaging, and magnetoencephalography. Because of the relatively small brain regions involved, the ability to study hypoglycemia may benefit from an investment in additional novel functional imaging tests. In addition to technology, experimental paradigms are needed that couple physiological responses measured by imaging with reliable measures of behavior in response to hypoglycemia.

Research Objective—Promote Application of Advances in Bioengineering to Type 1 Diabetes:

- *Develop novel drug delivery methods.*

Effective drug delivery depends on applying the proper dosage in the proper location over the proper time course, while overcoming issues of target specificity and drug degradation. Bioengineered drug-eluting polymers can be implanted to slowly release a drug over time directly at the site where it is needed, such as in the eye to treat diabetic retinopathy or directly in a foot ulcer. Cardiac stents can be coated with drugs that locally suppress immune reactions or prevent reocclusion of the vessel. Scientists are also embedding immune suppression drugs or compounds that promote angiogenesis or islet replication into materials used to encapsulate and protect islets for transplant.

- *Develop noninvasive glucose monitoring technologies.*

An artificial pancreas would require a continuous glucose sensor whose output could be used to regulate an insulin pump in a feedback loop. To achieve such a closed-loop system, glucose monitors are needed that are faster, more accurate, and easier to use. Flexible algorithms are needed to link the changes in blood or interstitial fluid glucose to insulin delivery. Although the artificial pancreas is not yet available, the first steps have been taken. The NIH supports basic sensor research in universities and industry, as well as clinical assessment of devices arising from these projects by independent academic investigators.

- *Integrate tissue engineering and regenerative medicine to develop tissues and organs to replace those destroyed by diabetes and its complications.*

Tissue engineering is an exciting emerging field in which biocompatible synthetic polymers, cells, and tissues, as

Imaging: An Inside Look

Seeing is believing. Imaging scientists are working to find ways to visualize the processes that lead to diabetes and how the body responds to therapy. These new tools will further a better understanding about how the disease starts and progresses. Imaging techniques will provide insights into why, how, and when diabetes occurs, as well as point to new ways for treating the disease.

The secret to imaging diabetes is the use of drug-like imaging agents that selectively "light up" the cells or biological processes involved in disease. For instance, the metals iron and gadolinium change the signal in magnetic resonance imaging (MRI). Compounds that contain these metals can be designed to home in specifically on the insulin-producing beta cells in the pancreas, thereby permitting them to be counted. Similar compounds have been used to light up the inflammation in the pancreas that accompanies the autoimmune destruction of the beta cells and causes type 1 diabetes. Other imaging agents mimic nutrients or hormones and, when taken up by cells, reveal clues to their function and metabolism. These types of agents are commonly labeled with minute levels of radioactivity and detected by positron emission tomography (PET). Thus, they might allow researchers to distinguish among active and distressed beta cells. Currently, considerable effort is focused on putting imaging labels on the isolated pancreatic islets used for transplantation into diabetic patients. This approach would enable doctors to actually watch the locations to which the transplanted tissues migrate once they are infused into patients and to determine their fate—that is, to know how many survive to produce insulin, find out whether they grow in their new environment, and see what happens to those that die. Imaging might also disclose the formation of new blood vessels and nerves around the islets, as well as reveal the importance of these processes for insulin secretion.

Scientists have learned to incorporate into mice a family of proteins that either emit light (such as the luciferase/luciferin system from the firefly) or fluoresce (such as green fluorescent protein). These constitute a very powerful set of imaging tools that are used in basic animal research. For instance, fluorescently labeled insulin can be tracked by the microscope to uncover defects in insulin secretion that might be involved in diabetes. It is hoped that these tools will help researchers identify and monitor a precursor cell that can become a new insulin-producing beta cell.

Imaging may one day help manage diabetes or identify patients prone to diabetic complications before they become clinically obvious. For instance, new glucose-sensitive imaging agents may make possible the continuous monitoring of plasma glucose without finger sticks. Such an advance would be enormously beneficial for patients. Therefore, scientists are working to bring emerging imaging tools to bear on all aspects of diabetes and its treatment.

well as gene manipulation technology, drugs, and natural biological molecules, are all brought to bear. Bioengineered tissues could improve therapy for diabetes complications: artificial skin for the repair of diabetic foot ulcers, heart muscle patches, and improved vascular access for dialysis. Glucose-responsive, insulin-secreting cells engineered from a readily available cell source may replace the human cadaver islets that are currently being used for transplantation and are in short supply. If a patient's own cells could be used as the precursor, this may alleviate the need for immunosuppressive drugs and greatly increase the number of patients who could be treated.

- *Apply nanomedicine to drug delivery, islet encapsulation, noninvasive imaging, and glucose-sensing technologies.*

The Office of Science and Technology Policy in the Executive Office of the President has launched a government-wide initiative to invest in the burgeoning field of nanotechnology. Nanotechnology is the manufacture, study, and use of molecules with unique properties when observed at a nanoscale level—larger than atoms, but much smaller than cells. This is a fertile field in which to engage engineers and scientists to work on the technologic challenges described previously with respect to type 1 diabetes research, including: imaging, tissue engineering, drug delivery, immunoprotective coatings, and development of an artificial pancreas.

Research Objective—Foster Application of Gene Delivery and Gene Silencing Technology To Develop New Therapies for Type 1 Diabetes and Its Complications:

- *Develop technology for gene delivery to cells and tissues that are therapeutic targets for type 1 diabetes.*

Gene therapy is an experimental approach to introduce into cells a gene that either replaces a mutated, disease-causing gene or provides a new cellular function. Complications, such as diabetic retinopathy, neuropathy, and wound healing, are potential targets for gene therapy. Instead of introducing vectors globally into the whole body, vectors could be applied directly to the affected site, which should reduce toxicity. Gene therapy has been used in an animal model to deliver growth factor genes to skin ulcers and has been successful in accelerating wound healing. Based on these studies, a human trial has been initiated using this approach to improve wound healing. Gene therapy applications are also being tested in animal models to deliver genes to the retina or to nerve cells to prevent cell damage. Gene therapy using viral vectors in a dog model has been successful in treating another disease of the retina, retinitis pigmentosa. Despite the fact that this

technology is still at a very early stage of development, these applications to diabetic complications are being actively pursued.

- *Create siRNA vectors for gene silencing in target tissues.*

As described in a sidebar, siRNA is a new technology that allows researchers to efficiently and rapidly reduce the level of expression of proteins in cells and tissues. Used as a research tool, *in vitro* and *in vivo*, siRNAs enable researchers to better understand the contribution of specific proteins to regulatory or disease pathways. For example, using siRNAs to specifically silence disease-causing genes in NOD mouse models will help geneticists dissect the particular contributions of these genes to the development of a diabetes phenotype. Similar techniques will allow immunologists to understand costimulatory pathways that control the balancing of immune cell function (e.g., effector and regulatory T cells). In addition to being used in basic science, siRNAs are being combined with gene therapy vectors to silence a variety of genes involved in disease onset and complications in patients.

Research Objective—Apply New and Emerging Technologies in Functional Genomics, Proteomics, and Metabolomics to Type 1 Diabetes Research:

- *Use "omics" technologies to identify interactions among genes, proteins, and metabolites in type 1 diabetes and its complications.*

Functional genomics is the new field of science that employs DNA microarrays to measure those genes that are active in a tissue under a given set of conditions and identifies clusters of genes that work together. Human and mouse "PancChip" microarrays were developed by the Beta Cell Biology Consortium (BCBC; see Goal III) and contain thousands of genes expressed specifically in the pancreas. This resource is critical for identifying the pathways involved in the development and function of the beta cell and is being distributed widely to investigators seeking to develop an unlimited supply of beta cells for cure of diabetes. The PancChip has significant importance for other diseases as well, such as pancreatic cancer.

The function of a gene is fulfilled through the proteins whose formation it directs. The complete set of proteins and their interactions, or proteome, provide further opportunities for systematic analysis and exploration. Proteomics involves the use of several novel integrated technologies to identify and quantitate proteins and study their interactions, modifications, and dynamics. Proteomic technologies have been successfully used for the identification of cancer biomarkers, elucidation of biochemical pathways, and pinpointing of novel drug targets. Metabolomics studies the small molecules, such as amino acids, carbohydrates, and lipids in the cells, tissues, and biofluids of an organism. The metabolome responds quickly to disease, diurnal and nutritional variation, and can be a very sensitive indicator of a person's current metabolic state. Large-scale approaches, such as proteomics, genomics, and metabolomics, are promising technologies for understanding the complex molecular mechanisms that underlie type 1 diabetes and its complications.

- *Utilize proteomic and metabolomic technologies to identify and validate surrogate markers that predict risk, rate of progression, or response to therapy for type 1 diabetes and its complications.*

A current major barrier in conducting type 1 diabetes clinical trials is the need for easily measured biomarkers that adequately predict disease risk and progression well before a measurable clinical outcome, such as diabetes onset, or a serious complication, such as a heart attack. "Omics" technologies will provide "fingerprints" or patterns of molecules that are diagnostic of disease and may be more powerful as clinical biomarkers than a single molecule, such as glucose or glycosylated hemoglobin. Many events that clinicians would like to monitor do not have such a single marker, such as the autoimmune process of type 1 diabetes or the early manifestations of diabetes complications. If these events could be identified, those individuals most likely to benefit from immunomodulation or who are at highest risk to develop complications could be intensively treated. Some effort in this direction has already begun. For example, investigators are now collaborating on proteomic projects to identify beta cell proteins that might give rise to the immune attack. Appropriate surrogate markers could dramatically enhance researchers' ability to conduct clinical trials, as well as shorten the duration of the trials.

Research Objective—Improve the Power of Diabetes Research by Utilizing Computational Biology and Bioinformatics:

- *Enhance type 1 diabetes research efforts by incorporating bioinformatics at the inception of the research effort.*

Bioinformatics is a newly emerging field that combines data storage, organization, and analysis. Bioinformatics has the power to correlate genetic, biochemical, cell function, demographic, and clinical data from disparate data sets from all over the world to create a comprehensive picture of disease. It has critical applications for analyzing complex data sets generated by clinical trials of immunomodulation and their associated mechanistic ancillary studies, or for analyzing genetic samples from thousands of patients with type 1 diabetes

and their families. In both instances, huge sets containing data as disparate as a patient's genotype and the immune cell complement in his or her blood will be analyzed together with descriptions of the clinical and biochemical manifestations of the disease. The efforts of bioinformatics experts will be critical to isolating in these populations of research patients the significant variables that either cause or protect against type 1 diabetes and its complications. It is expected that new hypotheses regarding the pathology of disease will be generated by searching for novel correlations within such data sets. Because of this, future data collections could benefit greatly from involving bioinformatics experts early in the study design. The ultimate goal would be to work toward interoperability, so that data stored in all these databases could be freely accessed and combined by the research community—efficiently and productively.

- *Apply computational biology to the complex systems in type 1 diabetes.*

The ability to organize complex data sets is only the first step for bioinformatics. As biology becomes increasingly sophisticated, computer models can save time and maximize the use of resources for analysis of complex systems. For example, insulin secretion involves networks of genes and metabolites interacting among all the cell types in the pancreatic islet, which, in turn, receives signals from the rest of the body in the form of hormones, nutrient levels, cells, molecules of the immune system, and nerve impulses. Computer models could help biologists predict how all of these signals are integrated to control blood glucose levels. Similarly, the development of an artificial pancreas (see Goal IV) will depend on developing algorithms that can use data on physical activity, diet, insulin administration, and glucose levels to calculate and effect fine-tuned insulin delivery.

- *Integrate information technology into type 1 diabetes self-care and medical management.*

Studies of the care of patients with a variety of chronic health problems demonstrate that information technology may improve health care access and quality for individuals with type 1 diabetes. Randomized trials have found that structured e-mail communication with health educators, interactive DVDs, and automated calls with nurse follow-up can all improve important physiologic endpoints, and in some cases may even improve survival. Future work is needed to demonstrate the effectiveness of a variety of communication tools for patients with type 1 diabetes, particularly as patients have access to unprecedented amounts of data generated by continuous glucose monitors. The goal is to maximize the impact of these technologies by empowering patients and providers to synthesize the data and use it to inform management. Research is needed to understand the cost-effectiveness of integrating computer supports into traditional treatment models and the extent to which these services should either supplement or substitute for face-to-face clinical visits.

Research Objective—Apply New Technology to the Development of Improved Animal Models for the Study of Type 1 Diabetes:

- *Develop models needed to identify cellular and molecular pathways influencing beta cell formation and function.*

Cell-based therapies designed to replace the beta cells lost due to immune-mediated destruction require a firm understanding of their developmental paths and fates, as well as a ready source of expanded cells for treatment. In pursuit of this goal, the BCBC is producing mice bearing fluorescent tags (the "Rainbow Mouse") that illuminate the developmental path followed by beta cells as they arise and begin to populate the pancreas. Investigators are extending this approach to produce animal models in which it is possible to identify the cellular source of newly arising beta cells, as well as the molecular pathways responsible for regulating beta cell growth and function in the adult pancreas. Through studies in these and other new animal model systems, it may be possible to identify novel molecular targets for therapeutics development in type 1 diabetes.

- *Develop animal systems with greater fidelity to human disease to enhance pre-clinical testing and biomarker development.*

In the Type 1 Diabetes-Rapid Access to Intervention Development (T1D-RAID) program, promising new drugs are being produced and tested in existing animal models for their ability to reduce type 1 diabetes autoimmunity or diabetic complications. These pre-clinical studies are being facilitated by the activities of the Animal Models of Diabetic Complications Consortium (AMDCC), which is developing new mouse models of diabetic complications. Particularly promising are new AMDCC models of diabetic cardiovascular disease, nephropathy, and neuropathy that will be of tremendous value for testing new drugs for these conditions. How closely these new models reproduce the human condition, and their ultimate utility as models for pre-clinical testing of new drugs, will be determined through the joint phenotyping efforts of the AMDCC and the Mouse Metabolic Phenotyping Centers (MMPC).

New mouse strains bearing HLA or other human disease susceptibility genes are providing systems to study the genetic components of human type 1 diabetes in mice. The value of newly discovered genes will be dramatically enhanced by the coming availability of new immunocompromised mouse models that allow for efficient reconstitution of the human immune system in mice. This important advance will, for the first time, allow in-depth mechanistic studies in mice of human autoimmunity, transplantation, and tolerance in the context of human genetic susceptibility loci and the human immune system.

While rodent models have been and continue to be a valuable tool for dissecting mechanisms of disease and for testing new drugs, studies in fish, pigs, and non-human primates are also providing valuable insights, including for the study of islet development and transplantation. As these models are validated and come into widespread use, they will allow for improved and more predictive tests of new therapies. Moreover, models in pigs and non-human primates may be particularly valuable tools for identifying new biomarkers of disease progression that are needed to improve type 1 diabetes clinical trial design and medical care.