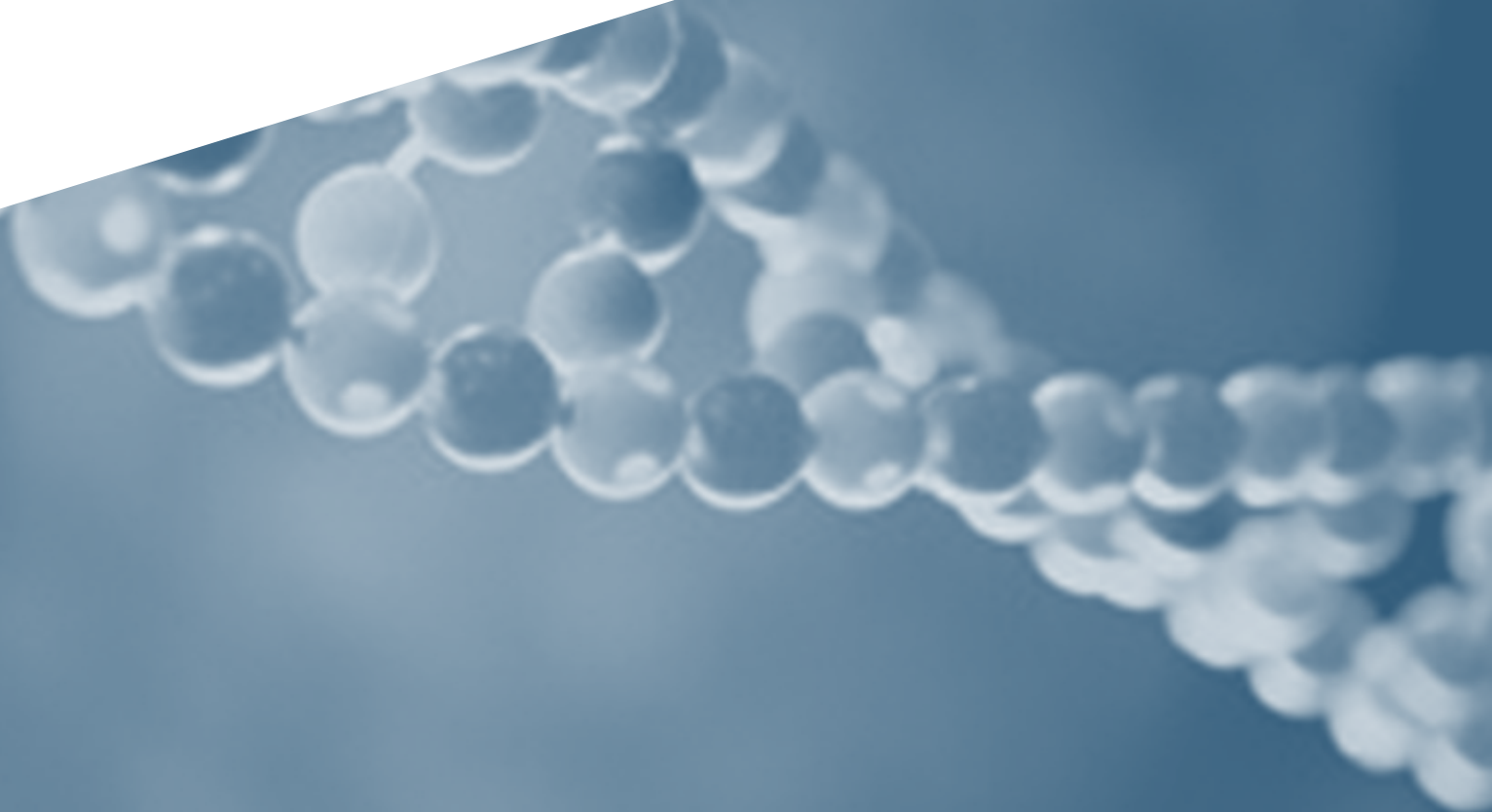# GOAL I

IDENTIFY THE GENETIC AND ENVIRONMENTAL CAUSES OF TYPE 1 DIABETES

Individuals with a type 1 diabetic relative are at substantially greater risk of developing the disease than the general population. However, more than 80 percent of cases occur in individuals with no family history of the disease. The incidence of type 1 diabetes appears to be influenced by geographic region, ethnicity, and socioeconomic status, among other factors. Collectively, these data suggest that type 1 diabetes has a strong, but not absolute, genetic basis that is modified by environmental factors.

Type 1 diabetes is known to be a polygenic disease that arises from the interaction of mutations in multiple genes. Variants in one gene locus—the major histocompatibility HLA class II region—have been found that confer either susceptibility (i.e., HLA-DR3 and HLA-DR4 sequences) or resistance (i.e., HLA-DR2 sequences) to type 1 diabetes. Although the HLA proteins are important components of the immune system, the precise role of these factors in diabetic autoimmunity has not been defined. Approximately 20 other gene loci—large stretches of DNA—are suspected of contributing to the development of type 1 diabetes, but few specific genes have been pinpointed. Environmental triggers of type 1 diabetes are postulated to include viral infections, dietary factors, environmental toxins, psychological stress, and season of the year; however, no single trigger has been conclusively identified.

Epidemiological research to adequately investigate the underlying genetic and environmental factors that trigger type 1 diabetes in susceptible individuals requires a large-scale, well-coordinated research effort. The Special Statutory Funding Program for Type 1 Diabetes Research has enabled the establishment of genetics and epidemiologic research consortia and the assembly of appropriate populations for study that will facilitate investigations by the broad diabetes scientific community. Long-term investment in these research programs will provide the opportunity to follow at-risk individuals for sufficient lengths of time to observe progression to type 1 diabetes and to correlate the onset of disease with suspected risk. The special funds have also stimulated the development of research tools—such as core laboratories, bioinformatics support resources, and a central repository of mouse models—that are invaluable to the conduct of genetics and epidemiology research.

Graphic
DNA double helix.

With the marked increase in special statutory funds that became available in FY 2001, major research consortia, trial networks, and resources for the diabetes research community were launched in FY 2001 and FY 2002. Brief descriptions of the research efforts and expected outcomes of initiatives supported in whole or in part by the special funds are presented below. More detailed scientific plans are available in Appendix 3.

International Type 1 Diabetes Genetics Consortium

Identifying genes that confer susceptibility or resistance to type 1 diabetes will propel the search for novel therapeutic targets and new assays to pinpoint those at risk who can benefit from interventions to prevent type 1 diabetes. This type of genetics research requires a large collection and multifaceted analysis of patient samples. The International Type 1 Diabetes Genetics Consortium, which was formed in 2001, will develop a renewable source of DNA on 7,500 families with a type 1 diabetic child. A consortium database containing clinical, genetic, and medical history information will facilitate the search for type 1 diabetes susceptibility genes. This genetics consortium is a collaborative effort of the NIDDK, NIAID, NHGRI, JDRF, and Diabetes UK, and its research efforts are expected to extend for more than 5 years.

International Histocompatibility Working Group (IHWG)

Type 1 diabetes is an autoimmune disorder and, thus, can benefit from and contribute to the broader research effort to identify the genetic basis of autoimmune diseases. These diseases result not from mutations in a single gene, but rather, they are polygenic diseases with multiple gene changes. The special statutory funds have enabled expansion of an ongoing effort through the International Histocompatibility Working Group to identify single nucleotide polymorphisms (SNPs)—single DNA base pair changes—in candidate type 1 diabetes susceptibility genes. To ensure discovery of the common SNPs and determination of their frequency in a population, high-density SNP maps for 100-200 candidate genes will be generated from DNA sequencing of twenty individuals. This research effort, which is co-sponsored by the NIAID, NIDDK, NCI, NHGRI, and JDRF, began in 2001 and is expected to extend 5 years.
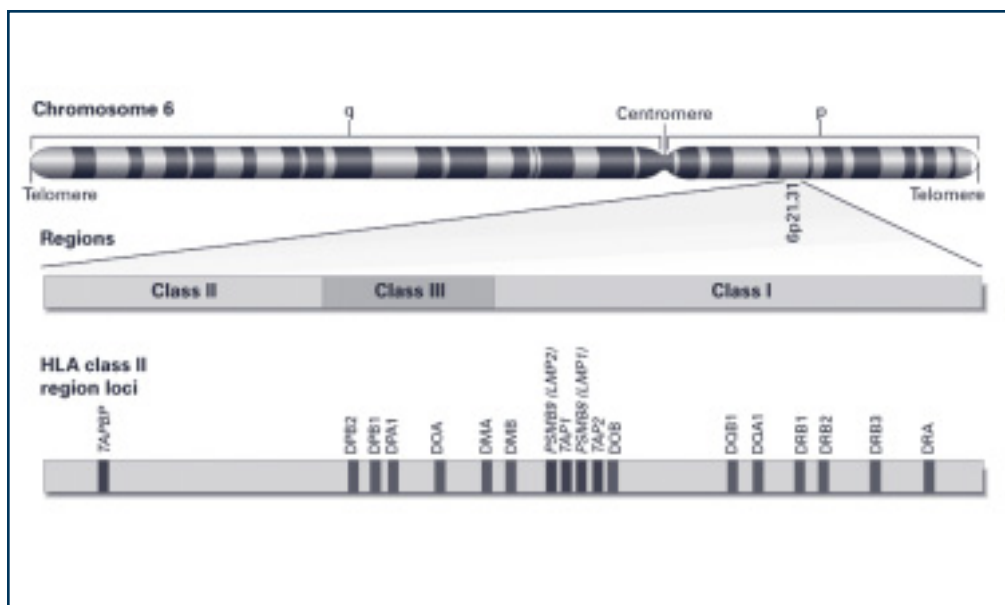
Location and organization of the Histocompatibility Leukocyte Antigen (HLA) gene locus on chromosome 6. The HLA locus is one of the most polymorphic loci in the genome. Specific variants in class II HLA genes are known to be associated with increased susceptibility or resistance to the development of type 1 diabetes. Investigators of the International Type 1 Diabetes Genetics Consortium (T1DGC) and the International Histocompatibility Working Group are pooling their resources and access to patient groups to understand how HLA contributes to type 1 diabetes. In addition, the T1DGC will search for additional genes that influence disease susceptibility. Identifying such genes will help researchers define the underlying molecular triggers of type 1 diabetes.

(Credit: Reproduced from Klein, J. and Sato, A. New Engl. J Med (2002) 343 (10): 702-709. Copyright © 2000 Massachusetts Medical Society. All rights reserved. Adapted with permission.)

Population Based Registries for Children with Type 1 Diabetes (SEARCH)

Lack of uniform national information on the rates of childhood diabetes and whether these are changing over time has been a major impediment to diabetes research and efforts to improve the public health. The SEARCH epidemiological study is developing a uniform, population-based approach to case ascertainment, typology, surveillance, and epidemiologic research on childhood diabetes in the U.S. Six centers around the nation will identify prevalent and incident cases of diabetes in youth younger than 20 years of age. The SEARCH study will document the burden of disease associated with type 1, type 2, and other types of diabetes in children and will develop knowledge to facilitate the identification and management of diabetes in children. SEARCH, which is a joint initiative of the CDC and the NIDDK, is being conducted over a 5-year period from 2000 to 2005.

Epidemiology of Diabetes Interventions and Complications (EDIC) Genetics Study

An important finding of the Diabetes Control and Complications Trial (DCCT) was that the development of diabetic complications—specifically, severe eye and kidney disease—is influenced by genetic factors. EDIC, the long-term follow-up of the DCCT, is an ideal platform for correlating genetic markers with the development of diabetic complications. DNA and cell lines from over 1,400 DCCT/EDIC participants and their diabetic and non-diabetic relatives are being collected and analyzed to aid in the search for susceptibility genes for diabetes complications. This NIDDK-sponsored study, which began at 32 clinical centers in 2002, will require 2 years for sample collection and at least an additional 5 years for analysis of genetic associations.

(Photo Credit: Richard Nowitz for NIDDK)

Type 1 Diabetes TrialNet Epidemiology Study

TrialNet—a nationwide network of clinical trial centers for type 1 diabetes (*see Goal II*)—and the Diabetes Prevention Trial for Type 1 Diabetes (DPT-1) have screened over 100,000 relatives of individuals with type 1 diabetes in the process of conducting immunoprevention studies for this disease. Because the vast majority of the screened relatives do not have type 1 diabetes, yet are at increased genetic risk, this screening effort offers an excellent opportunity for epidemiology studies to assess environmental triggers, genetic interactions, and the natural history of disease onset and progression. The identification of individuals who have evidence of autoimmunity, but have not yet developed type 1 diabetes, will be useful for finding environmental antecedents to diabetes. The epidemiology study began in parallel with the overall TrialNet initiative in 2001 and is anticipated to continue for more than 5 years to follow the onset of disease in this at-risk population. TrialNet is supported by the NIDDK, NIAID, NICHD, JDRF, and ADA.

Triggers and Environmental Determinants of Diabetes in Youth (TEDDY) (RFA DK02-029)

A thorough understanding of the infectious agents, dietary factors, or other environmental conditions that trigger type 1 diabetes in genetically susceptible individuals is essential to developing strategies to prevent this disease. An international consortium has been established to provide a coordinated, multidisciplinary approach to this complex problem with collection of information and samples in a standardized manner, access to state-of-the-art laboratory tests, and greater statistical power than can be achieved in smaller, independent studies. Seven cooperative agreements to fund TEDDY were awarded in September 2002 by the NIDDK in partnership with the NIAID, NICHD, NIEHS, CDC, JDRF, and ADA. In this long-term project, high risk infants will be identified at birth and followed through adolescence to identify environmental factors associated with subsequent development of type 1 diabetes.

Type 1 Diabetes Mouse Repository

Mouse models of type 1 diabetes, such as the non-obese diabetic (NOD) mouse, are an essential resource for a diversity of research programs aimed at understanding the genetic and pathophysiologic bases of this disease and developing new therapeutic strategies. To enhance access and ensure the continued availability of these mouse models to the research community, the NCRR and NIDDK created a Type 1 Diabetes Mouse Repository at the Jackson Laboratory in FY 2001. This central repository will acquire, preserve, and disseminate 150 mouse strains that are vital for research on type 1 diabetes for more than 5 years.

Bioinformatics Integration Support Contract (BISC)

Advanced technologies, as well as the increased sample collection and analysis capabilities of the newly established genetics consortia, will generate large amounts of data that must be captured, analyzed, and stored. To facilitate the management of scientific data within the immunology community and to provide information technology support, the NIAID awarded contracts for BISC in September 2002. The planning, implementation, and operation of a bioinformatics integration support system are expected to take at least 6 years.

NOD (non-obese diabetic) mice serve as a useful model of type 1 diabetes. Although these mice are genetically identical, the development of diabetes in an individual animal is influenced by a variety of environmental factors, including the location of the mouse colony, exposure to viruses, and diet. Similarly, environmental factors are thought to trigger type 1 diabetes in genetically-susceptible human patients. The Triggers and Environmental Determinants of Diabetes in Youth (TEDDY) consortium is leading the search for such factors.

*(Credit: Dr. C. Ronald Kahn, Joslin Diabetes Center)*
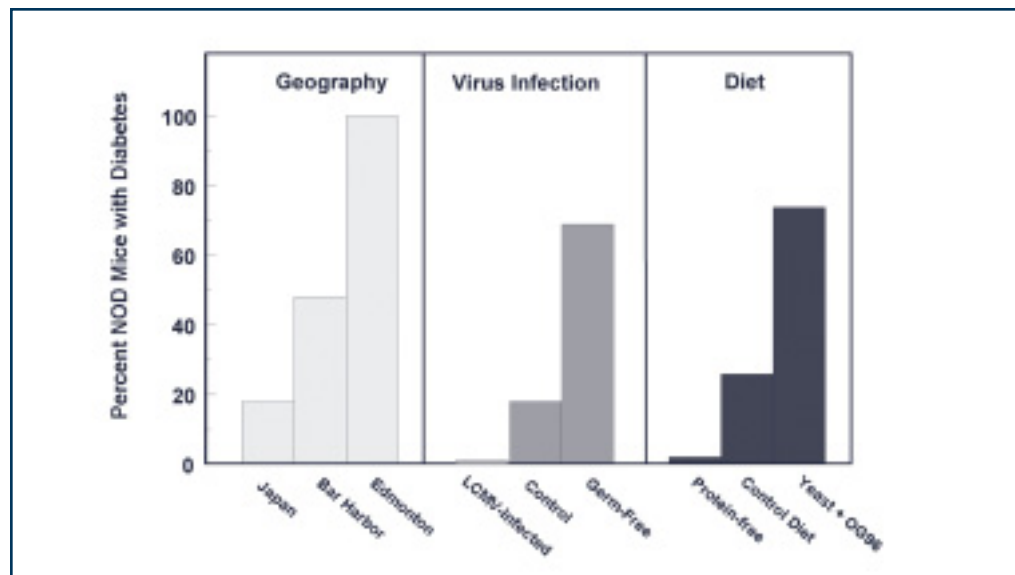
Many significant scientific advances have emerged from investigator-initiated research that began in the early years of the special statutory funding program. Highlights of these discoveries are provided here. More extensive discussion of initiatives and their research progress can be found in Appendix 3. Some programs supported by the early initiatives are still in progress and the full impact of these projects on uncovering the genetic and environmental causes of type 1 diabetes may not be realized for several years. The establishment of large-scale, collaborative research consortia and resources is a significant accomplishment in itself; however, it is premature to identify scientific advances from those initiatives that were begun in FY 2001 or FY 2002.

Genetics of Type 1 Diabetes

▶ Researchers in the IHWG have detected over 700 SNPs—single nucleotide polymorphisms— in candidate diabetes susceptibility genes. These SNPs have been deposited in a public database, facilitating access for independent studies.

Epidemiology of Type 1 Diabetes

▶ By demonstrating that risk of developing type 1 diabetes can accurately be predicted in relatives of people with this disease, the injected insulin arm of the Diabetes Prevention Trial for Type 1 Diabetes (DPT-1) has laid the groundwork for future prevention trials for type 1 diabetes. This study provided critical information on genetic and antibody markers that accurately predict an individual's risk of developing this disease. The second trial of the DPT-1 testing the efficacy of oral insulin, which targets a distinct immune mechanism, is under way within the TrialNet infra- structure and results are anticipated in June 2003.
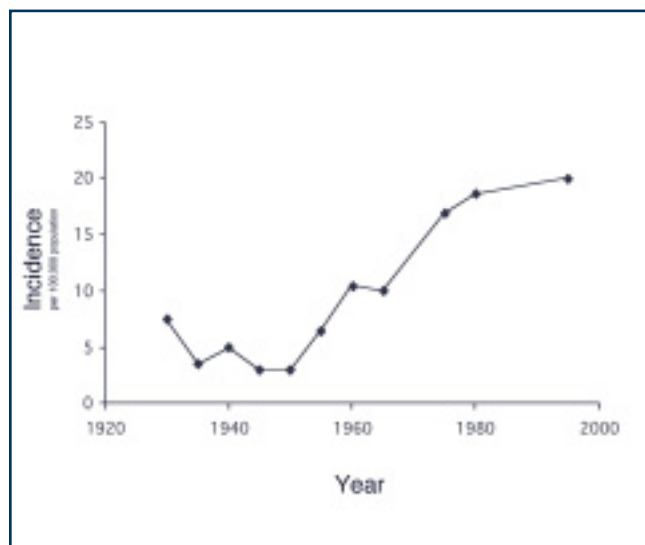
Incidence of diabetes in children under age 10 years in Norway, 1925-1995. The incidence of type 1 diabetes has risen in the last several decades in many countries around the world. The CDC-led SEARCH program will document the prevalence and incidence of diabetes in youth younger than 20 years of age in the U.S. These data will assist research on understanding the burden of disease associated with diabetes in children.

(Credit: Copyright© 2002 American Diabetes Association. From Diabetes, Vol. 51, 2002; 3353-3361. Reprinted with permission from The American Diabetes Association.)

This section provides commentary from leading scientific experts within the diabetes research community who assessed the accomplishments of the special statutory funding program and from researchers who participated in the use of the special funds. A complete description of the evaluation process and the use of evaluative data regarding the special funding program is available in the Assessment chapter and Appendix 2.

Advisory Panel

A panel of scientific and lay experts on type 1 diabetes research convened at the NIH in May 2002 to review the use of the special statutory funds. Comments from the advisory panel regarding genetics and epidemiology initiatives established by the special funding program include:

▶ The growth of the type 1 diabetes genetics research field that has been gained by establishment of the Type 1 Diabetes Genetics Consortium and other genetics initiatives made possible by the special statutory funding was considered very important by the advisory panel. By greatly expanding the size of the genetic sample collections, these projects will add much-needed power to the search for non-HLA susceptibility genes and shorten the time needed for analysis.

▶ The Bioinformatics Integration Support Contract (BISC) program was recognized as one means to address the critical need for bioinformatics initiatives. Bioinformatics support will ensure that databases established by the various genetics and epidemiology consortia are compatible with each other and with those associated with related projects, such as the Beta Cell Biology Consortium (*see Goal III*) and the Type 1 Diabetes Genetics Consortium.

▶ The advisory panel strongly supported the planned structure of the Triggers and Environmental Determinants of Diabetes in Youth (TEDDY) consortium, which will promote data sharing and assist investigators in powering their large-scale epidemiological studies.

▶ The advisors recognized the importance of the animal repository initiative established with the special statutory funds and encouraged the continued expansion of available models to include non-NOD mouse backgrounds, as well as other relevant strains.

Extramural Grantees

Principal investigators who received grants related to the genetics of type 1 diabetes responded to a survey asking, in part, about the value of this grant or funding source. Representative remarks include:

▶ "This grant allowed us to explore a new field of research in type 1 diabetes. The tools we developed will not only benefit my own career [for] a long time to come, but also many collaborators and young scientists in the field of type 1 diabetes."

▶ "The opportunity was almost lost in the DPT-1 study [to] have the measurement of anti-islet autoantibodies with modern "biochemical" assays, and in fact such measurements only began several years after the start of the Diabetes Prevention Trial, when funding for this grant was obtained. Fortunately, frozen samples could be utilized and a complete evaluation will now be possible to set the stage for future large-scale diabetes prediction/prevention trials."

THE PANCCHIP: A POWERFUL TOOL FOR DIABETES RESEARCH

The Endocrine Pancreas Consortium is a collaborative research effort funded by the NIH and led by Dr. Klaus Kaestner of the University of Pennsylvania Medical School and Dr. Alan Permutt of Washington University Medical School. The overall goal of the Endocrine Pancreas Consortium is to more fully characterize the genes involved in the function of the endocrine pancreas—the portion of the pancreas made up of specialized cells involved in the production and secretion of hormones, including the insulin-producing beta cells. These research teams and their collaborators have developed a microarray specifically tailored to the endocrine pancreas—the "PancChip"—that can be used to study gene expression in this tissue and that may provide insights into diabetes. The story behind the creation of the PancChip illustrates how targeted research funding—like that provided by the Special Statutory Funding Program for Type 1 Diabetes Research—can catalyze the adaptation of cutting-edge technology into valuable tools targeted toward diabetes.

What is a Microarray?

With only a few exceptions, every cell of the body contains a full set of chromosomes and identical genes. Only a fraction of these genes is turned on, however, and it is the subset that is "expressed" that confers unique properties to each cell type. "Gene expression" is the term used to describe the multi-step process whereby information contained within the DNA is first transcribed into an intermediary molecule, messenger RNA (mRNA), and subsequently translated into the proteins that carry out important cellular tasks. Scientists study the kinds and amounts of mRNA in a cell to learn which genes are expressed—and how that expression might change—under certain conditions or at certain times. Gene expression is a highly complex and tightly regulated process that allows a cell to respond dynamically both to environmental stimuli and to its own changing needs. Importantly, gene expression is not just a simple "on/off" switch, but may also be thought of as a "volume control," increasing or decreasing levels of expression as necessary.

A microarray is a tool for analyzing gene expression that consists of a small membrane or glass slide containing small samples of many genes. A microarray works by exploiting the ability of a given mRNA molecule to bind specifically to the DNA template from which it was transcribed. By using an array containing many DNA samples, scientists can determine—in a single experiment—the expression levels of hundreds or thousands of genes within a cell by measuring the amount of genetic material bound to each site on the array. With the aid of a computer, the amount of labeled genetic material bound to the spots on the microarray is precisely measured, generating a profile of gene expression in the cell. Microarrays are therefore useful when one wants to survey a large number of genes quickly or when the sample to be studied is small. Microarrays may be used to assay gene expression within a single tissue or cell type as a function of some treatment or developmental change, to compare gene expression in two different cell types or tissue samples, or to monitor changes in gene expression that coincide with the onset of disease.

Over the past 5 years or so, microarrays have greatly facilitated large-scale analysis of gene expression in a wide range of tissues. Although commercially available arrays have not been specifically geared to represent the cells and organs known to be affected by diabetes, they have nevertheless been used in studies of both type 1 and type 2 diabetes. Perhaps not surprisingly, given their relative lack of specificity, many of these studies have shown few differences in gene expression in the disease state. It was this relative dearth of genomics tools geared specifically to diabetes that spurred the NIH to act.

Building the PancChip

Before the PancChip could be created, it was first necessary to generate a pancreas-specific "library" of genes expressed in this tissue. How does one go about defining which genes are specifically expressed in a particular tissue?

To do this, the researchers used a combination of gene expression analysis and database mining. Using a variety of pancreatic tissues, including whole pancreas from adult and fetal mice, mouse insulinoma cells (a tumor of pancreatic islet cells), and human islets, the researchers identified genes that were highly expressed in these cells. Second, they identified additional genes by examining previously prepared libraries generated from human islets and human or mouse whole pancreas. The scientists in the Consortium used this information to assemble the PancChip, a microarray containing a

Researchers of the Endocrine Pancreas Consortium at the University of Pennsylvania collect freshly printed PancChips, each containing over 10,000 genetic samples. These chips are used in experiments to determine the profile of gene expression in the pancreas and other tissues of interest to diabetes researchers. Support from the Special Statutory Funding Program for Type 1 Diabetes Research helped fund the Endocrine Pancreas Consortium, which created the PancChip and made it available to the wider diabetes research community.

(Photo Credit: Rana Gupta, University of Pennsylvania)

total of 3,400 genes. Of these, 3,139 represent genes whose expression is enriched in the pancreas, 231 represent genes expressed in cell signaling pathways important in diabetes, and 30 represent so-called "housekeeping" genes that are responsible for general cellular function and metabolism. Of the pancreas-specific genes, 2,369 had been previously identified while 310 represented novel, heretofore undescribed genes.

The genes contained on the PancChip encode proteins that are representative of a wide spectrum of varied cellular activities, and include enzymes, signaling molecules, transporters, and structural proteins. In a report published in the July 2002 issue of the journal *Diabetes*, the researchers described the generation of the PancChip and its use for the characterization of changes in gene expression patterns in the mouse pancreas from mid-embryonic development through adulthood. They reported that the profile of gene expression in the pancreas— as measured using the PancChip—changed markedly from the embryonic stage through adulthood, with proteins involved in binding DNA and RNA highly expressed during development and many enzymes highly expressed in adulthood. The ability to generate a profile of gene expression in this tissue at various time points during development demonstrates the value and utility of the PancChip as a research tool.

The PancChip and the Future of Diabetes Research

The generation and availability of the PancChip represent a major success of an initiative funded through the Special Funding Program for Type 1 Diabetes Research. Furthermore, the set of genes used to prepare the PancChip has been made available to the larger diabetes research community through certain NIH-funded biotechnology centers. The availability of this tool will be of great assistance as other researchers pursue new avenues for research. Areas in which the PancChip may provide important insights include:

▶ **Islet transplants:** Does increased (or decreased) expression of a particular gene or set of genes correlate with success of the transplant? If so, is it possible to manipulate gene expression in the islets prior to transplant in order to improve outcomes?

▶ **Stem cell therapy:** What genes give islet or pancreatic cells their unique nature? Is it possible to influence the differentiation of stem cells so that they can be efficiently coaxed into islets?

▶ **Profiles of gene expression:** Using the PancChip, it may be possible to generate "snapshots" of gene expression within the pancreas under various physiologic conditions. How might gene expression differ in people predisposed to developing diabetes? How does it change early in disease progression? Is it possible to influence pancreas gene expression—either through drugs or gene therapy—and alter the course of disease development?

▶ **Target discovery:** More comprehensive knowledge of gene expression patterns in the pancreas may identify novel genes important in normal function of the organ. What new targets for therapy might there be? Such research could increase treatment options for people with diabetes and those at risk.

The Endocrine Pancreas Consortium will continue its work in this critical area. The efforts of the Consortium to identify and characterize the genes expressed in the pancreas have allowed these researchers to identify over 160,000 individual sequence fragments. Analysis of these sequence fragments has identified close to 14,000 unique human gene sequences and over 9,400 mouse gene sequences. Furthermore, the researchers have identified roughly 2,000 sequences in both human and mouse that have never been previously described. These discoveries have allowed the members of the Consortium to expand and improve the PancChip. The most recent version of the PancChip, Version 4.0, now contains more than 10,000 unique elements that can be used to measure gene expression levels in a single assay.

The efforts of the researchers of the Endocrine Pancreas Consortium are an important complement to those of the Beta Cell Biology Consortium (BCBC) and will provide a valuable resource to this related effort. The BCBC *(see Goal III)* is a collaborative research effort funded by the NIH that seeks to coordinate interdisciplinary approaches to study the development, function, and regulation of pancreatic islets. In November 2002, the PancChip clone set was adopted as a standard platform by the BCBC, which will print microarrays for all its members. Overall, it is anticipated that a more complete understanding of the mechanisms involved in the development of the endocrine pancreas may allow researchers to better coax human stem cells into pancreatic endocrine cells for treatment of type 1 diabetes. Insights from these efforts may also provide new approaches to improve insulin secretion in people with type 2 diabetes.

Scearce LM, Brestelli JE, McWeeney SK, Lee CS, Mazzarelli J, Pinney DF, Pizarro A, Stoeckert CJ, Clifton SW, Permutt MA, Brown J, Melton DA, and Kaestner KH. Functional genomics of the endocrine pancreas: the pancreas clone set and PancChip, new resources for diabetes research. *Diabetes.* 2002; 51: 1997-2004.

ANIMAL MODELS OF TYPE 1 DIABETES

The Special Statutory Funding Program for Type 1 Diabetes Research has enhanced the study of animal models of this disease. The diabetes-prone BioBreeding rat (BBDP) and the Non-Obese Diabetic (NOD) mouse are of particular importance to research efforts in type 1 diabetes. Both models are being investigated to identify genes that influence the risk of developing autoimmune-induced diabetes. Because the DNA of both rats and mice is very similar to human DNA, identification of disease-susceptibility genes in the animals' DNA is likely to help point investigators to disease-susceptibility genes in humans as well. In addition to their use in genetic analysis, animal models of type 1 diabetes can be used to test strategies to prevent and treat the disease in humans.

Investigations of the BBDP rat model of type 1 diabetes have provided new insights into diabetes susceptibility genes. Studies have determined that the DNA of this rat contains many loci that make it susceptible to the development of type 1 diabetes. In order to study a particular locus which is essential for disease development, investigators produced "congenic" rat strains. Such strains are produced by isolating that particular locus from one strain, in this case the BBDP rat, and causing it to be expressed in another strain, in this case the diabetes-resistant BBDR. In the animal line that is resistant to diabetes, diagnosis of diabetes is attributed to the locus introduced into the strain. Using this technique, investigators identified a mutant gene in the diabetes-prone rat that results in both diabetes and a blood disorder known as lymphopenia. This gene is a new member of the immune-associated-nucleotide (IAN)-related gene family. Although the lymphopenia gene's precise function is unknown, other members of the same gene family are expressed in immune
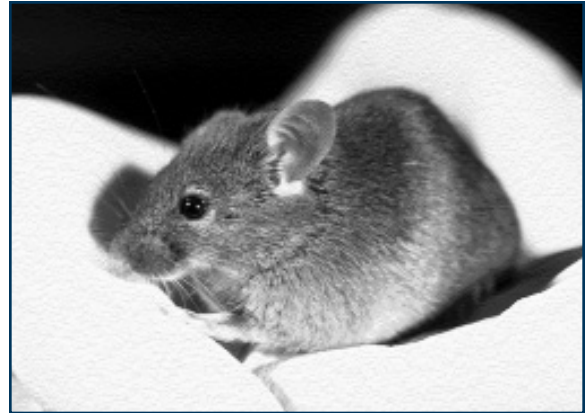
Mouse and other animal models are important tools for type 1 diabetes research. Mice that are genetically predisposed to type 1 diabetes can help researchers identify the genes or environmental factors that trigger disease. Research animals are also essential for developing new therapeutic strategies to ameliorate both type 1 diabetes and its complications.

(Photo Credit: Richard T. Nowitz for NIDDK)

system T cells and are thought to play a role in their development. The next challenge facing investigators will be to determine how this gene family functions in both normal and disease states.

Ongoing studies of NOD mice may increase our under-standing of how human beings develop type 1 diabetes. Using the special type 1 diabetes research funds, NIH has solicited applications to sequence the regions of the NOD mouse genome that are involved in the development of this disease. Investigators hope to complete the sequences in FY 2003. By taking advantage of the similarity between mouse and human DNA, they hope to use these mouse disease gene sequences to identify type 1 diabetes susceptibility genes in humans.

ANIMAL MODELS OF TYPE 1 DIABETES (CONTINUED)

Animal models are also a valuable tool for testing the safety and efficacy of emerging diabetes treatments. In response to advisors' recommendations, NIH has established a Type 1 Diabetes Mouse Repository at the Jackson Laboratory in Bar Harbor, Maine. Supported through the Special Statutory Funding Program for Type 1 Diabetes Research, this repository will serve as a central resource for maintaining and distributing at least 150 mouse strains important to research on type 1 diabetes. Some of the strains, selected with the advice of an external scientific advisory committee, will also be frozen to ensure that the genetic material from the mice continues to be available for future research.

Existing mouse and rat models of type 1 diabetes are helping to shed light on the underlying causes of disease and to reveal potential new approaches to prevent or delay disease onset. Development of new and improved animal models of type 1 diabetes is critically important if researchers are to continue their progress towards treating the disease more effectively and identifying approaches for preventing or curing type 1 diabetes in humans.

The Use of Animals in Biomedical Research

To ensure the appropriate care and use of animals involved in scientific research, the U.S. Government requires that individuals and institutions utilizing live animals adhere to a set of rules designed to ensure the safe and humane treatment of animals and to minimize discomfort, distress, and pain during experimental procedures. The NIH maintains an Office of Laboratory Animal Welfare within the Office of the Director to ensure compliance with the rules and regulations regarding animal research.

MacMurray AJ, Moralejo DH, Kwitek AE, Rutledge EA, Yserloo BV, Gohlke P, Speros SJ, Snyder B, Schaefer J, Bieg S, Jiang J, Ettinger RA, Fuller J, Daniels TL, Pettersson A, Orlebeke K, Birren B, Jacob HJ, Lander ES, and Lernmark A. Lymphopenia in the BB rat model of type 1 diabetes is due to a mutation in a novel immune-associated nucleotide (IAN)-related gene. *Genome Research*. 2002; 12: 1029-1039.