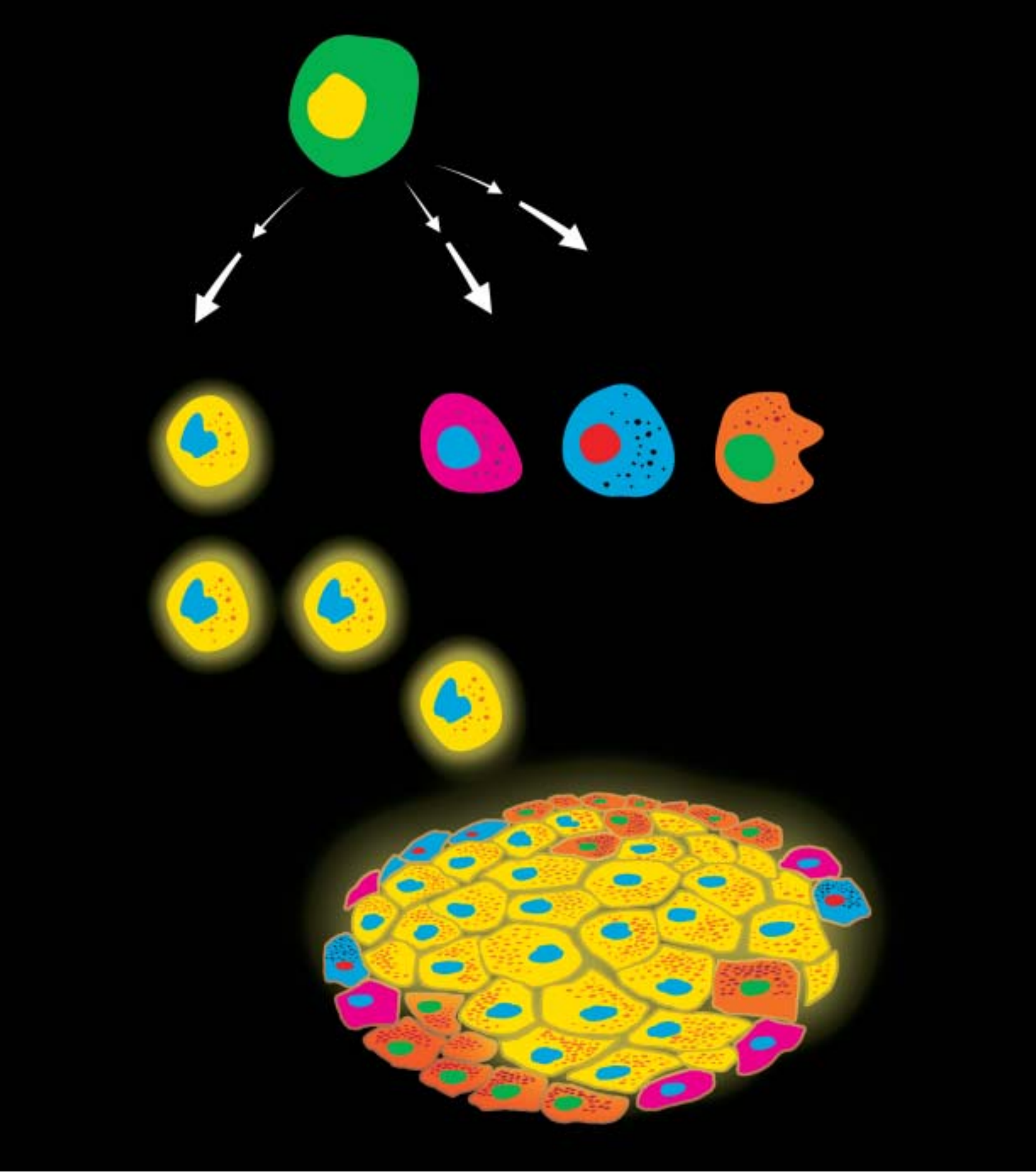

Stem cells are special cells in the body that have not yet committed themselves to a final function. As illustrated by this diagram, a stem cell can differentiate into a number of different cell types (represented by the different colors) that may then be part of the same organ or tissue, such as the pancreatic islet cell cluster represented at the bottom. As they learn how to guide the development of stem cells present in adult tissues, researchers may be able to develop therapies for a number of diseases in which these tissues are damaged or malfunctioning. Photo credit: Donald Bliss, Medical Arts and Photography Branch, National Institutes of Health.

Cross-Cutting Science: Paving the Way to Discovery

Advances in medicine are largely dependent on the accumulation of new knowledge about biologic processes, especially at the smallest levels of an organism–its genes, the proteins they control, and the workings of cells. While the ultimate application of such basic research is not always obvious to the public, major strides in fighting disease can be traced back to laboratory studies whose immediate relevance to health could not have been fully known or appreciated at the time they were conducted. Described here are some recent studies of fundamental processes, as well as the technologies that make such studies possible. The insights gained through this type of research can be expected to propel disease-oriented research, not only within the NIDDK mission, but also in many other fields. Investment in such cross-cutting scientific research today will have future applications that we cannot now describe with certainty, but which we know will surely be realized.

Functional Genomics: Tools for Discovering the Functions of Genes Relevant to Disease

In the quest for new and better treatments for disease, biomedical scientists are creating and using exciting advances in modern technology to discover important genes and learn their functions at an ever-increasing rate. This knowledge can propel new advances in diagnostics and drug development. Because different diseases and medical conditions arise from disparate causes—mutations in genes, infectious agents, and even adverse reactions to certain medical therapies—scientists delve into the inner workings of living cells with a great diversity of approaches. In genetic research, genes are often identified, and clues to their function obtained, by investigating what goes wrong when a gene is mutated, as is the case in many diseases. The study of genes has been revolutionized by modern functional genomics, the use of large-scale, high-throughput techniques to discover the function of genes and how all the genes in the genome of an organism work together. For example, scientists can now scan hundreds or thousands of genes at a time to see which may be active in a certain type of cell. For cases in which a disease is caused by a mutation in a gene that is already known from previous research, new technologies are also expediting experiments in which scientists generate an animal with an analogous mutation. From the animal model of the disease, they can learn how the disease progresses and what other genes may be involved, and they can use these animal models to test candidate therapies that are not yet ready for human trials. Because most genes are blueprints for the construction of specific proteins, scientists also gain critical insights into disease by studying how proteins function—or malfunction—when genes are mutated. In parallel with investigations into the causes of particular diseases, researchers also seek to build upon fundamental knowledge of genes and their functions to perpetuate the cycle of scientific discovery: critical insights and breakthroughs in medicine are often predicated upon the accumulation of such knowledge and upon the development of new technologies. Thus, investment in genetic and genomic research can be expected to have cross-cutting implications, advancing medical research within the NIDDK mission and in other fields as well.

Modeling the Control of Cell Growth Through Mice: Cancer is the result of uncontrolled cell growth. Normally, the body regulates when a cell should grow and divide to produce new cells where they are needed, and when the growth should stop. A cell must also carefully monitor its genome as it grows to ensure that if mutations arise, they are not propagated into new cells. How this elaborate regulatory system breaks down to allow mutant cells to grow into tumors has been the subject of much research. Mouse models of cancer are particularly useful to researchers precisely because they provide insights into the development of cancer in the context of a whole

organism, where all of the regulatory systems should normally be in place.

Cell Growth and DNA Damage in Breast Cancer: Breast cancer strikes approximately one in nine women. It can be caused by new mutations or by mutations that were inherited. Many of the hereditary cases of breast cancer are caused by mutations in the gene *BRCA1*. *BRCA1* appears to be a central player in many biological pathways, including regulating cell growth and maintaining the integrity of the genome through repair of DNA damage. Thus, mutations in this gene are particularly insidious because without *BRCA1*, cells are left vulnerable to acquiring even more mutations.

While some mutations are caused by environmental factors, other mutations just occur spontaneously in cells. One of the body's defenses against DNA damage is to have cells die rather than perpetuate potentially harmful mutations; this programmed cell death is called "apoptosis." How do some *BRCA1* mutant cells escape death and instead grow uncontrollably into tumors? Scientists recently deduced that some of these cells do so by mutating the protein that imposes the death sentence: this protein is called p53.

In studies in mice, NIDDK-funded scientists found that *BRCA1* mutations resulted in massive numbers of dead cells as a result of apoptosis. This was likely in response to DNA damage, the result of the accumulation of many spontaneously arising mutations left unchecked in the absence of *BRCA1*. Surprisingly, this massive cell death was not seen in mice that both lacked *BRCA1* and additionally had a mutation that impaired p53 function—and most of these mice eventually developed breast tumors. These results are particularly significant to human cancer because *BRCA1*-associated tumors have a relatively high frequency of p53 mutations.

Mutations in p53 have long been associated with many types of human cancers. One possible reason for this is that p53 can suppress uncontrolled cell growth in several ways, including by triggering cell death. To further investigate the interactions between p53 and *BRCA1*, the researchers induced extensive DNA damage in mice by exposing them to radiation. The cells of normal mice bolstered their levels of p53 protein to help protect against the damaging effects of the radiation, but in mice with *BRCA1* mutations, the p53 response was impaired. These results help show that in normal cells, *BRCA1* and p53 must coordinate to protect against conditions that can lead to cancer.

These experiments suggest a mechanism for *BRCA1*-mediated tumor formation. In the absence of normal *BRCA1* function, mutations accumulate in the DNA. In some cells, this DNA damage eventually strikes the gene encoding the p53 protein, causing a mutation that destroys it. Once p53 function is lost, these mutant cells can escape death, continue to grow and divide, and eventually form tumors. The mice developed in this study, with *BRCA1* and p53 mutations, will continue to be of value to increase our understanding of cancer progression; these mice may also serve as useful models to test new treatments for breast cancer.

Multiple Endocrine Neoplasia: Multiple endocrine neoplasia is a cancer syndrome characterized by multiple tumors in the parathyroid glands, pancreas, anterior pituitary, and other parts of the body. It is caused by mutations in a gene called *MEN1* (for multiple endocrine neoplasia type 1), which was discovered by the collaborative efforts of NIH scientists from the NIDDK, the National Cancer Institute, and the National Human Genome Research Institute. To gain further insight into this cancer syndrome, scientists recently generated a mouse model of the disease. Like humans, mice have two copies of the *MEN1* gene. Using genetic engineering, the researchers mutated one of these copies. The mice developed symptoms remarkably similar to human multiple endocrine neoplasia, including tumors in the pancreas, parathyroid, pituitary, and other tissues. The scientists observed that the tumor cells had spontaneously lost the remaining normal copy of the *MEN1* gene. Future research on these mice may reveal whether other genetic events accompany tumor formation. Ultimately, as current therapies for this syndrome are often unsatisfactory, the availability of a mouse model should be an asset for the testing of possible new therapeutic approaches.

Unfolding Protein Folding: Just as gears and wires must be perfectly crafted to make a machine work, the proteins of the human body must assume very distinct shapes to perform the functions necessary for life. A gear of the wrong shape or a mass of wires tangled randomly together will disrupt a machine's function. Likewise, an improperly-shaped protein, or certain aggregates of proteins interacting abnormally, can lead to devastating diseases such as Alzheimer's and "mad cow" disease. Proteins are chains of amino acid building blocks; the

nature and order of amino acids in each protein are dictated by the sequence of the gene encoding the protein. Each amino acid chain must "fold" into a particular intricate shape so that the protein can function. A mutation in a gene that changes the nature of even just one of the amino acids can make it impossible for the protein to fold properly. A few types of proteins seem to be able to change their structure spontaneously—more alarmingly, some of these proteins sabotage the folding of other proteins, even in the absence of genetic mutations. Proteins that can do this after infecting a living organism are called prions. Research on prions and on abnormal aggregates of misshaped proteins, called amyloids, continues to shed light on protein-folding diseases and will lead to new ideas for treatment approaches.

Prions and Amyloids in a Yeast Model System: Many diseases that ravage the brain, such as the much-feared mad cow disease and other transmissible spongiform encephalopathies, are caused by infectious proteins called prions. In recent years, creative experiments by NIDDK-funded scientists in a seemingly unlikely model system—baker's yeast—have provided insights not only into prion formation, but also into amyloids, which are abnormal forms of protein observed in many diseases.

Yeast are ideal model organisms for investigating many biological processes because they are readily amenable to highly sophisticated genetic manipulation and other experimental techniques; they require little storage and growth space; and they are relatively inexpensive to grow. (Such features would contrast sharply, for example, with a herd of "mad cows" that one might wish to study.) Yeast also have their own set of prions which, like those of animals and people, often start out as normal proteins but then spontaneously change into a sinister form. Prions propagate by converting other proteins into this abnormal form. One such yeast prion is called [URE3], which is an altered form of a normal yeast protein called Ure2p.

Scientists recently learned that yeast prions such as [URE3] do not propagate by themselves: they use other proteins, called chaperones, as unwitting accomplices to help them convert more Ure2p proteins into [URE3] prions. Further experiments showed that [URE3] prions aggregate together to form networks of amyloid filaments, which resemble amyloid found in a number of human diseases, including Alzheimer's, late-onset diabetes, multiple myeloma, and transmissible spongiform encephalopathies. Continued research on yeast prions will generate further insights into both prion disease and amyloid formation and propagation.

Protein Folding in Amyloid Disease—Implications for Therapy: A recent study of the disease familial amyloid polyneuropathy explained an intriguing case of two genetic wrongs making a right. The disease is caused by a mutant version of the protein transthyretin. People have two copies of the gene for transthyretin, one from each parent. If one copy is normal, but the other copy codes for a protein with a mutation called "V30M," then disease occurs. (V30M is a shorthand designation that scientists use to note that the 30th amino acid building block of the protein is mutant. The chemical nature of the defect is abbreviated by the letters V and M.) Curiously, individuals are protected from disease if the second copy of the transthyretin gene—rather than being normal—has a different mutation, called "T119M."

NIDDK-funded scientists recently discovered how the T119M mutation overcomes the adverse effects of the V30M mutant. Transthyretin proteins usually snap together in groups of four, but the V30M mutation renders them unable to maintain this normal configuration. Separated from the group, the individual proteins with the V30M mutation begin to unfold, lose their characteristic shape, and then aggregate in a harmful mass that interferes with nerve and muscle function. By contrast, scientists found that proteins with the T119M mutation exert an especially stabilizing influence on the transthyretin group, even if the foursome includes some of the V30M mutants. (Not all mutations, then, are bad.) The extra stability of the T119M version of transthyretin counteracts the unfolding propensity of the proteins carrying the V30M mutation. The implications of this finding are that this amyloid disease—and potentially others like it, such as Alzheimer's—may be amenable to treatment strategies designed to stabilize the proper groups of proteins to prevent misfolding.

"Insights" into the Digestive System—Feeding Fluorescent Fats to Zebrafish: In a clever new approach to identify genes involved in fat processing, scientists combined modern genetic techniques with glow-in-the-dark fats. The model organisms they used, zebrafish larvae, process fats in the intestine and liver and respond to cholesterol-blocking drugs in a manner similar to humans. Thus, genes identified as important in zebra-

fish for fat processing are likely to be important in humans also.

Zebrafish are common pets throughout the world. In recent years, however, they have acquired a new purpose: they have become established as a powerful model organism for biological research. Zebrafish are readily amenable to genetic manipulation, facilitating the identification and characterization of genes. They are vertebrates, with organ systems similar to those of people and other mammals. Because zebrafish are relatively small, scientists can maintain large numbers of them in the lab. Zebrafish also have a striking characteristic that makes them particularly valuable for studying how internal organs and tissues are formed: during their rapid development, the fish embryos and larvae are transparent, permitting their insides to be viewed easily. Thus, the zebrafish can be a virtual window to enable researchers to see how cells differentiate and organs develop.

Exploiting the ability to synthesize fluorescent fats and the ability to perform sophisticated experiments on zebrafish, NIDDK-funded scientists screened for and identified zebrafish larvae with mutations that disrupt proper fat processing. After these mutant larvae were fed fluorescent fats, their gall bladders did not glow as brightly as those of normal larvae. Thus, the scientists can deduce that the genes that were mutated must be important for fat processing. By tracking down the location of the mutations within the genome, the researchers will eventually be able to find and study these important genes.

One of the mutants, nick-named "fat-free," had a digestive tract that otherwise appeared normal. Thus, without the use of fluorescent fat technology to detect a defect, this mutant would have been overlooked, and potentially valuable insights into the genetics of the digestive system would have been missed. This research predicts that genetic screens in zebrafish, along with sensitive fluorescent fat technology, may identify genes important for diseases of fat metabolism, biliary disorders, and even certain types of cancer in which fat signaling plays a role.

With support from multiple NIH components under the leadership of the NIDDK and the National Institute of Child Health and Human Development, the community of scientists who study zebrafish have been developing sophisticated genomics tools to facilitate the mapping and identification of important genes and to determine their functions. An effort to sequence the entire zebrafish genome is now beginning.

Using a Genetic Database to Prevent Blood Transfusion Reactions: Blood transfusions save the lives of accident victims, surgery patients, and people suffering from blood disorders such as dialysis-induced anemia, sickle cell anemia, and Cooley's anemia. However, patients can develop an adverse reaction to transfused blood if certain molecules displayed on the surface of the donor red blood cells differ from those on the patient's own cells. There are many groups of such surface molecules, including the group used to classify blood into the commonly-known A, B, or O types. Among the many other blood groups is one called the Dombrock group, named after a blood donor named Dombrock in 1965. A reaction against a surface molecule of the Dombrock group can cause the destruction of transfused blood cells as well as fever, chills, and other symptoms. However, reliable products have not been available to screen blood for the Dombrock type.

Recently, with modern large-scale genomic techniques, NIDDK-funded investigators working at the NIH discovered the gene coding for the Dombrock molecules. They began with two clues from prior research. First, genetic studies had linked the Dombrock gene to chromosome 12. Second, research on red blood cells suggested that the Dombrock molecules are anchored to the cell membrane in a specific way. The scientists prepared DNA from developing red blood cells (because mature red blood cells lack chromosomes) and generated a database of 5,000 genes that are active in these cells. They then screened this database to look for a gene that both localized to chromosome 12 and that also had a sequence characteristic of molecules that are anchored to cell membranes in the same way as the Dombrock molecules.

The strategy worked. Over thirty years after the initial identification of Dombrock blood types, the gene that encodes the Dombrock molecules has now been cloned. The scientists were even able to find versions of the gene with slight sequence variations that correlate with the different types of Dombrock molecules. With this breakthrough, gene-based methods can now be developed to screen patients, and also donor blood, for Dombrock type. This type of screen would allow doctors to match a patient with donor blood of the same Dombrock type to reduce painful transfusion reactions.

This research also illustrates the value of a database of genes that function in red blood cells. Such a database will undoubtedly propel further discoveries in red blood

cell biology in order to better understand and treat human diseases involving these cells. With this as a goal, NIDDK is supporting the development of a large database, called "Hembase," to provide worldwide access to genetic-based studies of red blood cells performed by scientists working at the NIH.

Taking Tools into the Future—Building Knowledge of Disease Genes: The NIDDK is continuing to advance biomedical research by identifying and pursuing cross-cutting areas of research within its mission that can be addressed with recent and developing technologies.

Modifier genes: The symptoms and severity of all diseases vary from one person to the next due to differences in genetic makeup and environmental exposure. For many medical disorders, a mutation in a single gene plays the predominant role in the development of disease. However, even among those who have identical mutations in such a gene, the severity of the disease can vary. To better understand this phenomenon, it is necessary to find other genes, called "modifier genes," that contribute to this variability. Identifying these modifier genes would improve our ability to predict the symptoms and severity of a disease in a particular individual, and may lead to improved treatments. With the primary genes for many diseases now known, the NIDDK will strive to build upon our understanding of the genetics of these diseases by stimulating research to identify modifier genes. A planned symposium on the search for modifier genes will encompass such diseases as cystic fibrosis, polycystic kidney disease, Gaucher disease, and other disorders within the NIDDK mission.

Increasing Understanding of Membrane Transport: Cell membranes are like the walls of a house, with windows and doors that can open to the outside and to interior rooms. On a much smaller but far more elaborate scale, the membranes of living cells are designed to let nutrients and other molecules pass through in a highly-regulated fashion. Many diseases, such as cystic fibrosis, diabetes, renal tubular acidosis, congestive heart failure, and several intestinal disorders, arise from defects in the transport of substances across membranes. Membrane transport processes in humans and other mammals are very similar to those in lower organisms, such as bacteria and yeast, among others. These similarities can be exploited to build upon our knowledge of membrane transport because these non-mammalian organisms are also easily experimentally-manipulated (more so than mammals), and the genome sequences for many of these are also known. The NIDDK plans to support innovative approaches to studying membrane transport in bacteria, yeast, zebrafish, and other non-mammalian organisms, and to search for novel genes and discover how they function. This knowledge will lead to important insights into the workings of human cells and membrane transport-associated diseases.

Beta Cell Biology Consortium: Central to the onset and progression of diabetes are abnormalities in cells called beta cells. Beta cells reside in the pancreas and produce insulin, a hormone that is essential for life. In type 1 diabetes, beta cells are destroyed, so no more insulin is produced. In type 2 diabetes, the body does not respond properly to insulin; the beta cell initially compensates by secreting extra insulin but eventually fails, leading to overt diabetes. It is believed that signaling defects involving beta cells are at the root of insufficient insulin secretion in type 2 diabetes. A comprehensive understanding of beta cells will therefore lead to new diagnostic and treatment approaches for both forms of this devastating disease. The NIDDK has launched a functional genomics initiative to identify the genes that are critical for beta cell development and to learn how they function. To build on this initiative, the NIDDK proposes to establish a Beta Cell Biology Consortium. Through the Consortium, individual Beta Cell Biology Programs would have access to information, resources, technologies, expertise, and reagents that are beyond the scope of any single research effort. The goals of the Consortium will be to advance our knowledge of beta cell biology and to develop technologies that will have implications for early detection of disease, for therapeutic transplantation of beta cells in type 1 diabetes, and for understanding derangements of cell signaling and regulation in type 2 diabetes.

Progenitor Cell Genome Anatomy Projects (GAPs): The successful treatment of many chronic and debilitating diseases afflicting Americans today will depend on the ability to replace organs or to stimulate regeneration and recovery of damaged organs. To build upon the achievements of the Human Genome Project, the NIDDK and other NIH institutes have established a range of Genome Anatomy Projects (GAPs) to map the complex network of cellular interactions in normal and diseased tissues. In the NIDDK, for example, one new initiative is the Diabetes Genome Anatomy Project, which will look at the

expression of genes in pancreatic and other tissues affected by diabetes. Now, complementary new research initiatives will apply this same comprehensive approach to focus on progenitor cells. Progenitor cells develop into different types of cells that form the organs and tissues of the body. It is important to understand how tissues and organs develop from progenitor cells, and how progenitor cells maintain and regenerate tissues and organs in health and disease. Approaches will include developing biomarkers to detect and classify stem cells and progenitor cells; profiling the cells to catalogue genes that are active; and creating tools for characterizing the functions of these genes. Such research would not only capitalize on the sequence data from the Human Genome Project, but could also take full advantage of human embryonic stem cell lines which meet established criteria for use in research supported by the NIH. It is also a goal that well-characterized cells, DNA, and specific tools for progenitor cell analysis developed by the GAPs will be distributed to the broader research community. Further, the development of bioinformatics systems, including databases, will ensure that data produced are available to researchers worldwide soon after being generated in the laboratories.

Crabtree JS, Scacheri PC, Ward JM, Garrett-Beal L, Emmert-Buck MR, Edgemon KA, Lorang D, Libutti SK, Chandrasekharappa SC, Marx SJ, Spiegel AM, and Collins FS. A mouse model of multiple endocrine neoplasia, type 1, develops multiple endocrine tumors. *Proc Natl Acad Sci USA* 98(3):1118-1123, 2001.

Farber SA, Pack M, Ho S-Y, Johnson ID, Wagner DS, Dosch R, Mullins MC, Hendrickson HS, Hendrickson EK, and Halpern ME. Genetic analysis of digestive physiology using fluorescent phospholipid reporters. *Science* 292(5520):1385-1388, 2001.

Gubin AN, Njoroge JM, Wojda U, Pack SD, Rios M, Reid ME, and Miller JL. Identification of the Dombrock blood group glycoprotein as a polymorphic member of the ADP-ribosyltransferase gene family. *Blood* 96(7):2621-2627, 2000.

Hammarström P, Schneider F, and Kelly JW: *Trans*-suppression of misfolding in an amyloid disease. *Science* 293(5539):2459-2462, 2001.

Wickner RB, Taylor KL, Edskes HK, Maddelein ML, Moriyama H, and Roberts BT. Yeast prions act as genes composed of self-propagating protein amyloids. *Adv Protein Chem* 57:313-334, 2001.

Xu X, Qiao W, Linke SP, Cao L, Li W-M, Furth PA, Harris CC, and Deng, C-X. Genetic interactions between tumor suppressors Brca1 and p53 in apoptosis, cell cycle and tumorigenesis. *Nat Genet* 28(3):266-271, 2001.

Stem Cells: Developing Potential

Scientists are striving to understand the processes that occur during normal development, when a vast number of different cell types are generated from a single fertilized egg. If they can understand normal development, scientists will have a better chance of determining how to recapitulate development in an adult in order to replace cells damaged by disease. Even within adults, special cells known as stem cells retain the ability to divide, and the divisions can give rise either to more stem cells or to cells that will differentiate into specific cell types. This process is analogous to harvested wheat seeds: the farmer can use the seeds either to plant more wheat, as when stem cells divide to produce more stem cells, or to produce specific products such as bread, as when stem cells divide to produce cells that differentiate into specific cell types.

Currently, scientists are determining the usefulness of different types of stem cells for treating human disease. Until now, replacement of cells has only been possible via organ or cell transplantation. Doctors are unable to treat every needy patient with transplantation, however, because there are limited supplies of donated cells and organs. Stem cells are heralded as a possible means for overcoming this treatment barrier.

The various types of stem cells are believed to differ mainly in the limits of their "potential"—their ability to produce other cell types. Embryonic stem cells arise early in development. Because they must give rise to all the different cell types and tissues of the body, embryonic stem cells are thought to have almost unlimited potential. Adult stem cells, on the other hand, reside within a mature tissue or organ and are thought to be able to differentiate into a more limited number of cell types. Experiments are still being performed to test the validity of these assumptions.

In the past year, NIDDK-funded scientists studying stem cells have made a number of exciting discoveries. Investigators identified a population of adult stem cells present in both rat and human pancreas capable of generating all types of pancreatic cells in culture. Investigators studying adult stem cells in the blood were surprised to discover that they are capable of producing not only numerous types of blood cells, but also liver, lung, gut, and skin cells. Another team showed that stem cells of the adult mouse pancreas are capable of producing both

pancreas and liver cells. Still another group of investigators developed a new technique for expanding cultured umbilical cord stem cells in order to generate enough cells for a transplant. These studies have all advanced our progress towards developing alternative sources of cells for transplantation to treat human diseases.

The NIDDK is supporting several efforts designed to capitalize on and extend previous stem cell and developmental biology discoveries and to stimulate new discoveries. As previously mentioned, two Genome Anatomy Projects (GAPs) support the use of advanced technologies and bioinformatic techniques to describe gene expression patterns in stem cells both during development, and in adult stem cells during tissue maintenance and tissue repair following disease. The planned Progenitor Cell GAPs will support research to identify and describe stem cells located within specific tissues of the gastrointestinal lining, liver, exocrine pancreas, kidney, urinary tract, prostate, and bladder. Hematopoietic Cell Lineage GAPs will support work to describe gene expression in blood (hematopoietic) stem cells. Another NIDDK effort will sponsor studies to describe normal development and stem cells of the gastrointestinal tract, liver, and exocrine pancreas. The NIDDK hopes that knowledge gained from these studies will enable doctors to use stem cells and developmentally-regulated genes to repair and replace damaged and diseased tissue.

Cheung AT, Dayanandan B, Lewis JT, Korbutt GS, Rajotte RV, Bryer-Ash M, Boylan MO, Wolfe MM, and Kieffer TJ: Glucose-dependent insulin release from genetically engineered K cells. *Science* 290(5498):1959-1962, 2000.

Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW, Entman ML, Michael LH, Hirschi KK, and Goodell MA: Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest* 107(11):1395-1402, 2001.

Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, Neutzel S, and Sharkis SJ: Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* 105(3):369-377, 2001.

Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, Wang X, Finegold M, Weissman IL, and Grompe M: Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. *Nat Med* 6(11):1229-1234, 2000.

Lewis ID, Almeida-Porada G, Du J, Lemischka IR, Moore KA, Zanjani ED, and Verfaillie CM: Umbilical cord blood cells capable of engrafting in primary, secondary, and tertiary xenogeneic hosts are preserved after ex vivo culture in a noncontact system. *Blood* 97(11):3441-3449, 2001.

Wang X, Al-Dhalimy M, Lagasse E, Finegold M, and Grompe M: Liver repopulation and correction of metabolic liver disease by transplanted adult mouse pancreatic cells. *Am J Pathol* 158(2):571-579, 2001.

Zulewski H, Abraham EJ, Gerlach MJ, Daniel PB, Moritz W, Müller B, Vallejo M, Thomas MK, and Habener JF: Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine, exocrine, and hepatic phenotypes. *Diabetes* 50(3):521-533, 2001.