In mouse embryos, NIDDK researchers have demonstrated that the Edg1 gene is vital to maintaining the integrity of developing blood vessels. The mouse embryo on the left has normal Edg1 gene expression. The mouse embryo on the right lacks Edg1 and has leaky blood vessels, causing hemorrhages in the placenta and the embryo. By using knockout mice to gain an understanding of basic developmental processes, scientists will shed light on developmental problems that lead to diseases and disease complications. Photo: Dr. Richard Proia, NIDDK.

Cross-Cutting Science: Paving the Way to Discovery

Advances in medicine are largely dependent upon the accumulation of new knowledge about biologic processes, especially at the smallest levels of an organism—its genes, the proteins they encode, and the workings of cells. While the ultimate application of such basic research is not always obvious, 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, ranging from the development of cells to the development of organisms, and 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.

MOUSE MODELS OF METABOLIC DISEASES AND DEVELOPMENTAL PROCESSES

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 environmental factors—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. The study of genes has been revolutionized in recent years 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.

An equally important tool for studying gene function is the use of model organisms. Scientists can create a model of a disease caused by a mutation in a known gene, or study how a gene mutation that has been characterized in a single cell affects a whole organism, by generating an animal with an analogous mutation. From the animal model, they can learn how a disease or a developmental process progresses and what other genes may be involved. They can also use these animal models to test candidate disease therapies that are not yet ready for human trials. Mice have been particularly useful for such studies because of their small size, rapid reproduction, and large numbers of offspring per litter, allowing researchers to perform informative experiments quickly. More significantly, mice and humans share virtually the same set of genes and the DNA sequence of the mouse genome is therefore an essential tool to identify and study the function of human genes. In fact, the recently-obtained draft sequences of the human and mouse genomes indicate that the two are approximately the same size and are about 85 percent identical.

Moreover, the differences involve only a few hundred of the 35,000 or so genes in both organisms. The complete sequences for both are now being assembled. Because of this high degree of similarity, it is believed that much research into mouse models of human disease has application to humans.

Mice are relatively easy to modify genetically, either through direct genetic engineering or through selective breeding. Mouse embryonic stem cells can be manipulated in culture and then implanted in female mice to produce animals that lack a specific gene entirely—a traditional gene "knockout"—or that lack the gene only in certain tissues or only under certain conditions—a so-called "conditional knockout." (See section, "Further Developments in Stem Cell Biology," for more information about stem cells.) Both approaches contribute valuable information about the normal function of a gene by allowing scientists to observe the consequences of its absence.

A complementary approach to gene knockouts is the generation of "transgenic" mice through injection of foreign DNA into developing mouse embryos. This technique produces mice that possess a gene that they normally would not possess, or that turn on a gene at times or in tissues where it normally would be silent. Researchers can then study the impact of a gene where it is normally not present as a way to gain insight into the gene's function. Genes suitable for this kind of study are not limited to mouse genes, as genes isolated from a wide range of organisms are functional in mice.

Once derived, such knockout and transgenic animals may be simply interbred to produce mice with multiple genetic alterations. To date, literally hundreds of knockout and transgenic mouse lines have been derived, many possessing multiple genetic alterations. Such mouse models have provided important insights into the development and treatment of many diseases. As illustrated by the following examples, researchers continue to make interesting and sometimes unexpected discoveries about gene functions and their role in both normal and disease states using mouse models.

Cell Signaling Pathways and Blood Vessel Formation: As an embryo develops, blood vessels are generated, a heart begins to beat, and blood begins to flow. Each cell generated during development appears to have a set of instructions that tells it where to go and what to do. Cell signaling plays an essential role in facilitating the developmental process. Such signals are often transmitted between cells through small molecules, called ligands, that are released by one cell and "seen" by another cell. The second cell "sees" the signal because it has on its surface a molecule specifically designed to bind to the signaling molecule: a molecular "receptor." Binding of the ligand to its receptor launches a cascade of molecular events within the target cell.

NIDDK researchers, working in collaboration with other researchers, have studied the role played by ligand and receptor-mediated signaling in the development of the circulatory system, specifically the blood vessels. As blood vessels form during embryonic development, the central "tube" of a vessel forms first. Muscle cells then migrate to surround the immature vessels, providing the strength and support they require. These researchers created a knockout mouse that does not possess the cell surface receptor known as endothelial differentiation gene-1, or Edg-1. The ligand for this receptor is a fat-derived molecule, sphingosine-1-phosphate.

When the researchers surveyed mouse litters in order to characterize the effect of knocking out Edg-1, they were unable to find any mice with two copies of the inactivated gene (each gene in each cell is present in two copies, or alleles: one from the father and one from the mother). Further examination revealed that the knockout mice were not viable: at about day 13 of development, Edg-1 knockout mice hemorrhage and die. The researchers determined that hemorrhaging is due to malformed blood vessels. Without Edg-1, the muscle cells do not completely envelop the vessels as they should, leaving them weak and vulnerable to hemorrhaging. These studies revealed that the Edg-1 receptor is required for blood vessel formation during embryonic development and that the signaling pathway is essential for mammalian development. Previous research suggests that this pathway may play important roles in adult blood vessel development and stability as well. If this is so, Edg/sphingosine-1-phosphate pathways may be involved in blood vessel development during wound healing and solid tumor growth. Thus, they present potential therapeutic targets for treating injury and disease, such as the blood vessel damage sustained as a complication of diabetes (see chapter on "Diabetes, Endocrinology, and Metabolic Diseases").

Cell Signaling and Insulin Resistance: All cells have a membrane that serves as a barrier between the cell and its surroundings. The cell membrane is composed of a fluid lipid (fat) bi-layer containing molecules and structures that facilitate interactions and communication between the cell and its environment. Lipid and protein molecules "swim" along the surface of this bilayer, forming raft-like clusters of molecules and receptors that constitute specialized "microdomains." The microdomains, in turn, can help direct cellular activity by regulating the transmission of signals to the cell's interior so that the cell can respond properly.

Gangliosides are molecules composed of lipid and sugars that are present on the surface of mammalian cell membranes. These molecules play a variety of roles in cell signaling, both as modulators of receptor cell signaling and as ligands—molecules that bind receptors. One molecule in particular, the GM3 ganglioside, can influence the responsiveness of the insulin receptor to its ligand, the hormone insulin. NIDDK researchers and their collaborators have studied the role of GM3 in insulin signaling by generating mutant mice in which the gene that synthesizes this ganglioside is knocked out. Mice that lack the gene necessary to synthesize ganglioside GM3—and, consequently, that lack GM3—are generally healthy. However, the mutant mice are more responsive to insulin than normal mice and respond to insulin by reducing their blood glucose levels more rapidly. In the mice lacking GM3, insulin receptor activity in skeletal muscle is elevated, suggesting that one function of GM3 gangliosides is to regulate insulin receptor activity.

When placed on a high fat diet, normal mice become overweight and develop glucose intolerance as evidenced by their decreased ability to respond to insulin. The researchers found that GM3 knockout mice were better at lowering their blood glucose levels and were more sensitive to insulin, even though they gained as much weight on the high fat diet as the normal mice did. Thus, GM3 may play a role in the insulin resistance resulting from high-fat diets that is seen in normal mice. Based on these findings, it is possible that inhibiting expression of the GM3 molecule could be a potential treatment for insulin resistance in type 2 diabetes.

Tay-Sachs and Sandhoff Diseases: Tay-Sachs disease and a related disorder, Sandhoff disease, belong to a large group of inherited disorders known as lysosomal storage diseases. Storage disorders are caused by a defect in one of the enzymes in the lysosome, the intracellular "recycling center" in which old or damaged molecules are broken down. If one of these disposal enzymes is missing or non-functional, intermediate breakdown products accumulate, and the disposal pathway becomes "backed up" at one particular point. The accumulation of partially degraded molecules within the lysosome ultimately causes damage to the cell. Tay-Sachs and Sandhoff diseases are members of a subcategory of lysosomal storage disorders known as GM2 gangliosidoses. These disorders are so named because they result from an inability to break down completely GM2 ganglioside, a molecule found in nerve cell membranes. Children with Tay-Sachs disease lack the enzyme beta-hexosaminidase A (Hex A), while children with Sandhoff disease lack Hex A and the related enzyme Hex B. In the absence of Hex A, partially degraded gangliosides accumulate in brain cells, resulting in brain damage and ultimately causing death.

Research using animal models has suggested that an intense inflammatory response may contribute significantly to the neurodegeneration seen in Tay-Sachs disease. The "Sandhoff mouse" lacks Hex A and serves as a model for both Tay-Sachs and Sandhoff diseases. Using a technique called DNA microarray analysis, researchers have previously found that a large number of genes that are expressed at elevated levels in the mouse model are related to an inflammatory response.

Researchers working at the NIDDK and their collaborators have now extended these findings to humans. Using tissue samples from a patient with Tay-Sachs disease, a patient with Sandhoff disease, and an unaffected child as a control, they generated a "profile" of gene expression patterns from all three patients using a technique known as serial analysis of gene expression (SAGE). The SAGE profiles show significant increases in expression of genes regulating pro-inflammatory responses, consistent with what was observed in the Sandhoff mouse. These results were confirmed by examining microscope slides of the tissue sections. Significantly, many of the genes identified in these studies have been proposed to play roles in Alzheimer's, Parkinson's, and other neurodegenerative diseases. This finding suggests that a common mechanism of brain cell damage may play a major role in these diverse diseases.

Mouse models continue to be an important approach to exploring gene function in health and disease. To facilitate development, validation, and sharing of information about mouse models of diseases within its mission, the NIDDK has initiated several collaborative research efforts. These include the "Mouse Models of Diabetic Complications" consortium, which seeks to refine or derive accurate mouse models of human diabetes complications for use by the research community for a variety of investigations, including the testing of therapeutic, prevention, early detection, or imaging strategies; and "Mouse Metabolic Phenotyping Centers for Models of Diabetes and Its Complications," to allow detailed characterization in mouse models of sometimes subtle metabolic changes associated with disease complications. Another current initiative will support individual projects for the development of mouse models to identify genetic modifiers of disease loci.

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ORPHAN NUCLEAR RECEPTORS IN HEALTH AND DISEASE

One critical aspect of cell signaling is the modification of gene expression. Molecules inside and outside the cell can change how genes are expressed—and hence the activities of a cell—by influencing the activities of a class of hormone receptors known as nuclear receptors (NRs).

Nuclear receptors are active in the nucleus at the center of the cell, as opposed to the cell surface membrane. They play an important role in endocrinology, contributing to regulation of development, metabolism, and disease. Nuclear receptors function in response to hormones, dietary lipids, xenobiotics (drugs), and other known and unknown metabolites. Most function primarily as transcription factors, proteins that affect how well a gene is expressed by modulating the transcription of a gene's DNA sequence into the messenger RNA that is eventually translated into a protein. The biology of NRs is complex, with cellular localization, tissue specificity, and gene target action dependent on a myriad of factors, including so-called "accessory proteins" in the nucleus and the DNA of the target gene(s). Nuclear receptors may bind to DNA singly (monomers) or in organized aggregates (dimers and heterodimers), with or without ligand. The presence or absence of ligands can define interactions with key regulatory proteins that determine whether a gene will be repressed or activated. Thus, over the long-term, the activities of NRs can have profound effects on cellular activities involved in obesity, development, metabolism, diabetes, heart disease, osteoporosis, and hormone-dependent cancers.

In particular, the Orphan Nuclear Receptor subfamily (ONR) of NRs has moved to the forefront in studies on lipid and drug metabolism. Although classified as orphans, i.e., without known hormone ligands (in contrast to the traditional nuclear receptors such as the estrogen, androgen, and thyroid hormone receptors), the ONRs respond to a variety of dietary lipids, xenobiotics, and natural metabolites. While considerable information exists on the structure and function of many of the nuclear receptors, far less is known about the ONRs. Not only can increased knowledge of the ONRs inform our understanding of basic metabolism, but it will also serve as the basis for the development of therapeutics to treat metabolic diseases, such as obesity and osteoporosis. For example, a major class of insulin-sensitizing drugs works through an ONR, PPAR-gamma. Greater knowledge of this cell signaling mechanism has the potential to lead to improved therapeutic agents for diabetes.

Through a series of grants and cooperative agreements, the NIDDK has fostered efforts designed to lead to a greater understanding of receptor specificity, ligand selectivity, interaction with cytoplasmic and/or nuclear accessory proteins, chromatin, and the transcriptional machinery, as well as identification of the downstream target genes regulated by the ONRs. Individual investigator-initiated efforts funded by the NIDDK have already led to numerous research advances that are elucidating the roles of ONRs.

Central to efforts to unravel the roles of ONRs in health and disease is the "Functional Atlas of Orphan Nuclear Receptors," a trans-NIH cooperative agreement spearheaded by the NIDDK. The NIDDK, five academic institutions, the National Institute on Aging, and the National Cancer Institute have set a goal to develop this unique database to acquire, catalogue and integrate information on ONRs. The long-term goal is to delineate the role(s)

of ONRs in normal and pathophysiological conditions, including obesity, osteoporosis, hormone dependent cancers, processes of aging, and diabetes and its complications. The objective of the Atlas is to provide useful data to researchers in the scientific community in a timely fashion for use in their own investigations relevant to ONRs. The hope is that the Atlas will serve as a catalyst for progress in this area by leveraging ongoing efforts to elucidate the structure and function of ONRs. It is anticipated that the Atlas will enhance coordination with emerging information from the human (and other) genome effort(s) and catalyze progress toward development of greater understanding of ONR function and dysfunction, ultimately leading to better therapies for treatment of disorders of metabolism.

FURTHER DEVELOPMENTS IN STEM CELL BIOLOGY

As body cells wear out and die, stores of adult stem cells help to regenerate these functionally-compromised cells and the tissues they constitute. With the hope of one day treating diseases effectively through cell-based approaches, scientists are exploring the capability of undifferentiated cells, called "stem cells," to be coaxed into specialized cells of the body, including pancreatic, brain, liver and other types of cells. This line of research has also been called "reparative medicine."

Although promising, this field requires extensive fundamental studies to understand how stem cells function and react to their environment—an exciting pursuit which will enlighten scientists about the steps stem cells take as they differentiate, committing themselves to different developmental fates whereby they become the myriad tissues in the body.

There are several different types of stem cells under study. "Adult" stem cells are rare populations of undifferentiated cells found in the tissues of adult animals and humans. Studies have shown that adult bone marrow, which feeds the body's circulatory system, may be a good source of these cells. Another type of stem cells, called "embryonic" stem cells, may also be derived from either animal or human tissue. Consistent with the policy announced by President George W. Bush on August 9, 2001, the NIH only funds research involving human embryonic stem cells if it is in accordance with criteria established by the Administration.

Because research on all these types of cells is still in its earliest stages, scientists cannot predict in advance which of them may prove most effective and appropriate for therapeutic purposes. Thus, they are working diligently to characterize these cells and understand their workings. Clearly, many questions remain to be answered. For example, it is not fully known how stem cells accomplish their dual tasks of self-renewal (to generate more stem cells) and of differentiation into specialized cells—the two traits that define their "stemness."

From Stem Cells to Specialized Cells: In one recent study, NIDDK-supported scientists analyzed genes that are turned on (expressed) in mouse embryonic stem cells and adult neural and blood stem cells, but not in differentiated cells. Of all of the genes expressed in the stem cells, a core set of 216 genes was shared in common among all three stem cell varieties; this set of genes likely gives stem cells their "stemness." Scientists can now further investigate these genes.

As stem cells go through development to produce specialized cells, they first generate progenitor cells. Progenitor cells are intermediates in stem cell differentiation that retain varying degrees of "potential" to become various specialized cell types. The degree of potential depends upon their developmental stage. A team of NIDDK-supported scientists recently identified adult bone marrow progenitor cells from humans and rodents that appear to have differentiation potential rivaling that of embryonic stem cells. The scientists first identified these cells, called multipotent adult progenitor cells (MAPCs), in human bone marrow, demonstrating that these cells could be coaxed to differentiate into a variety of specialized cell types, including bone cells, fat cells, blood vessel cell types, and nervous system cells. Adapting the techniques they developed for human cells, the scientists then extricated MAPCs from the bone marrow of mice and rats to facilitate later experimentation in animal models.

To explore further the differentiation potential of MAPCs, the research team investigated whether these cells could morph into yet another cell type—liver cells. They isolated MAPCs from the bone marrow of human donors and from mice and rats, and tried growing the MAPCs in different ways to coax them to differentiate into liver cells. They then screened the differentiated cells for liver-specific biological markers and subjected the cells to a battery of functional assays; the tests revealed that the MAPCs could differentiate to acquire a distinct set of liver cell traits, adding another cell type to their repertoire.

Next, the scientists demonstrated that these different specialized cell types did in fact arise from a single "multipotent" cell, and were not simply descendants of several different less-potent progenitor cells—crucial evidence for the existence of MAPCs. By inserting special genetic tags into mouse MAPCs to uniquely mark the DNA of each MAPC and all cells derived from it, the scientists were able to trace the lineage of diverse specialized cell types back to the same original MAPC. Similar experiments with human MAPCs confirmed that they, too, are multipotent.

The scientists also showed that both human and rodent MAPCs can grow and divide extensively in the laboratory, repeatedly doubling their numbers while maintaining their differentiation potential. Further, despite these generations of cell divisions, the MAPCs did not show molecular signs of "aging." Finally, the scientists investigated the differentiation potential of MAPCs in an animal. When put into mice, mouse MAPCs developed the characteristics of specialized cells from a variety of tissues and organs, but did not form tumors in animals—an occurrence that has been associated with some embryonic stem cells.

In theory, clinicians could eventually develop therapies that use MAPCs retrieved from a patient as back-up cells, ready to adapt and replace damaged tissue anywhere in the body. The use of a patient's own cells would also eliminate risks associated with transplants from donors. By bringing to light the remarkable differentiation potential of MAPCs, these studies open new opportunities for stem and progenitor cell research and research on cell-based therapies.

Understanding the Physiology of Hematopoietic Stem Cells: Blood cells are regenerated by adult hematopoietic stem cells (HSCs), which reside primarily in the bone marrow. The generation of blood cells—both red and white—is termed hematopoiesis. Several recent studies have suggested that some HSCs can also differentiate into non-hematopoietic cell types—findings that are still under investigation. Importantly, the crucial role of HSCs in regenerating the blood supply in bone marrow transplant patients and their potential for use in gene therapy in genetic diseases of the blood (see "Kidney, Urologic, and Hematologic Diseases" chapter) have propelled research to understand the physiology of HSCs.

In bone marrow transplantation, suspensions of cells extracted from donor marrow are injected into the bloodstream of a patient whose own defective or diseased marrow cells have been ablated through radiation or chemotherapy. To be successful, a small subset of the donor cells, the most "primitive" HSCs—called multipotent long-term HSCs—must repopulate or "engraft" into the recipient's bone marrow and establish a new, renewable source of blood cells. The question is, how do these cells get from the bloodstream to the marrow—by random chance or through physiologically relevant mechanisms for cell migration?

To answer this question, researchers examined whether they could find evidence for HSC migration in normal, healthy mice. They physically joined the circulatory systems of pairs of healthy mice whose blood cells could be distinguished by a subtle difference in one cellular marker. The mice were paired this way for various lengths of time. After separating the pairs, the researchers assayed for the functional cross-engraftment of long-term HSCs as evidenced by their sustained contribution to each mouse's blood supply. They found that the cells successfully cross-engrafted in at least one partner from each pair, generating up to 3.7 percent of peripheral blood cells in mice joined for 7 weeks. Longer periods of joining increased the percentage of engrafted cells directly detectable in bone marrow. These findings indicate that long-term HSCs from the circulation can engraft into normal, healthy bone marrow, and not just replace absent bone marrow cells. Furthermore, the research team found that the engrafting activity of an enriched fraction of HSCs and progenitor cells can be reduced if the cells are first "filtered" through the circulatory system of another mouse—suggesting that a mechanism(s) does indeed exist for rapid removal of HSCs from the circulation.

In related work, researchers from the same laboratory defined another marker for HSCs, one that distinguishes between long-term HSCs and short-term HSCs (which maintain self-renewal for only 8-to-10 weeks). Through cell-sorting and bone marrow reconstitution assays, they determined that, in addition to previously known markers, the absence of a receptor tyrosine kinase on the cell surface (Flk-2) distinguishes long-term from short-term HSCs. They also found that the concomitant loss and gain of two cell surface markers coincides with the loss of self-renewal in HSC maturation.

These findings suggest that there are normal physiological mechanisms for HSC migration between the circulation and the bone marrow. They also provide a tool to enable researchers to better isolate particular populations of HSCs, including long-term HSCs. As indicated, bone marrow transplantation is a serious procedure for both donor and patient. These research results may have long-term implications for improved therapy, facilitating less-risky isolation of long-term HSCs from the circulation rather than from bone marrow for use in transplantation. They also have important short-term implications for the enrichment and study of long-term HSCs and other HSCs.

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ONGOING AND NEWLY LAUNCHED NIDDK EFFORTS

The NIDDK supports cross-cutting, basic research endeavors through funding for investigator-initiated projects and ideas and for multiple Institute-led initiatives aimed at accelerating research in particular areas. The NIDDK is also responding to the enormous opportunities provided by advances in information technology, as well as biotechnology, to establish scientific resources that can be accessed by the research community at large. This response includes plans to establish an "NIDDK Data and Bisosample Repository"—a central facility for archival storage of biosamples and data collected in large, multi-site studies, and for processing of genetic samples. This repository will enhance the value of large studies by increasing access to the biosamples and data that have been collected and by facilitating efficient sharing of these resources. This resource will be enormously useful for both basic and clinical research scientists.

Furthermore, large-scale genome anatomy projects (GAPs) for multiple tissues and organs—including the pancreas, gut, and hematopoietic cells—have been or are being created by the NIDDK to develop and apply genomic approaches to basic and disease-related research areas. The GAPs will support the use of advanced technologies and bioinformatics techniques to describe gene expression in stem cells during development, and in adult stem cells during tissue maintenance and tissue repair following disease. To build on these projects, a program is planned to link the GAPs with individual investigator-initiated projects.

A number of diseases are caused primarily by a single gene. Yet, disease symptoms and severity often differ between individuals, likely as a result of "modifier" genes whose activities may vary from person to person. Some of these modifier genes may exert broad control over basic cellular processes (these are often referred to as "housekeeping genes"). Others may be specific to the particular organ or tissue that is affected in the disease. The NIDDK is launching a research initiative, "Genetic Modifiers of Mendelian Diseases of Interest to NIDDK," in order to solicit research to identify genes that may influence the course of a number of diseases, including cystic fibrosis and hemochromatosis (discussed in later chapters). The hope is that by understanding modifying elements in different diseases, new therapeutic targets may be identified to mitigate the damage and suffering caused by a disease.

Through investment in these and other efforts, the NIDDK can continue to foster advances and opportunities by supporting basic research that will propel our understanding of and ability to treat disease.

DR. JAMES ROTHMAN

"Traffic Report"—Lasker Award Honors Scientists for Fundamental Discoveries About the Transport of Molecules Through Cells

One of the two winners of the 2002 Albert Lasker Award for Basic Medical Research is Dr. James E. Rothman. This award is very prestigious—many of its former recipients have subsequently won a Nobel Prize. Dr. Rothman is a long-time grantee of the National Institute of Diabetes and Digestive and Kidney Diseases, and he has also been supported by the National Cancer Institute (NCI) and the National Institute of General Medical Sciences (NIGMS). Now Chairman and Paul A. Marks Chair of the Cellular Biochemistry and Biophysics Program and Vice Chairman of the renowned Sloan-Kettering Institute, Dr. Rothman was honored for his discoveries of the machinery that regulates traffic in a cell. The exquisitely-organized trafficking of molecules within a cell and between a cell and its external surroundings underlies extraordinarily diverse biological processes. These include, for example, insulin secretion, neurotransmitter release, the transport of proteins that bring glucose and other nutrients into a cell, and the transport of proteins that serve as channels for salt ions to flow into or out of cells. Defects in the transport of proteins to their proper locations are associated with diseases such as cystic fibrosis and diabetes.

Different activities within a cell take place in specially-designated compartments, which, like the entire cell itself, are surrounded by membranes. Small membrane-enclosed shuttles called vesicles transport cargo from one cellular compartment to another, from within a cell to the cell surface membrane, and between a cell and its environment. Dr. Rothman's experiments unraveled the mystery of how vesicles form and transport their contents. His approach was to break open cells and mix various cellular materials back together to try to reconstitute membrane trafficking in a test tube. This would allow him to purify the components of this mixture down to the individual molecules necessary for trafficking and to discover their identity. While this type of approach had revealed clues to other cellular processes, at the time Dr. Rothman began his experiments, many scientists believed that protein trafficking would not work outside the orderly confines of a cell. It seemed unlikely that vesicles, set adrift in a test tube, would find the appropriate target membrane-bound compartments and deliver their cargo. But they did.

Dr. Rothman and his first postdoctoral fellow developed a clever way to detect this trafficking, using extracts from two different types of mammalian cells. One of these contained a viral protein called VSV G, but was deficient in an enzyme that normally attaches a particular sugar molecule onto the VSV G protein within an intracellular membrane-bound compartment. The other type of cell had this enzyme but not the viral protein. When the scientists mixed the two extracts, they found that the VSV G protein had acquired its sugar molecule. This indicated that the VSV G protein had been successfully transported—in a test tube—from a membrane-bound compartment of its original cell to one from the other type of cell, where the enzyme resided.

As they refined and varied their cell-free membrane trafficking system, Dr. Rothman and the members of his laboratory gained insight into how transport occurs. When cargo is to be sent out of a cellular compartment, it becomes wrapped in a stretch of membrane that then pinches off from the rest

DR. JAMES ROTHMAN

of the compartment to form a small, sealed, membrane-enclosed structure—the vesicle. This process is called "budding." Dr. Rothman's laboratory found that, as these vesicles form, they acquire a protein "coat." The coated vesicle then travels to its destination.

As a vesicle reaches its target location, it sheds its coat, and its membrane merges with the membrane of the target compartment in a process called "fusion." The stretch of membrane originally from the vesicle opens up to release its contents. In their investigation of membrane fusion, Dr. Rothman's laboratory identified proteins they called NSF and SNAP as important for this process. The discovery of NSF not only shed light on the fusion of membranes, but it also marked the converging of two different lines of inquiry into membrane trafficking. Working independently, another scientist, Dr. Randy W. Schekman, had also discovered NSF, but rather than reconstituting membrane transport from mammalian cell components in a test tube, Dr. Schekman's strategy was to analyze yeast cells harboring genetic mutations that disrupted membrane trafficking. The fact that these different experimental approaches led to the identification of the same protein validated the results. The subsequent findings from each laboratory built upon and enhanced the research of the other, and Dr. Schekman, an NIGMS grantee, was honored with a Lasker award along with Dr. Rothman.

Dr. Rothman's laboratory soon discovered another set of proteins integral to membrane fusion; they called these proteins SNAREs. SNAREs protruding from the membranes of vesicles (v-SNAREs) latch onto SNAREs residing in the target membranes (t-SNAREs) to form "SNAREpins." This interaction initiates fusion between the vesicle and target membranes. SNAREs also confer specificity on this process. It is critical that each of the many vesicles traveling about a cell dock at the correct target destination, because unloading cargo in the wrong place could wreak havoc in the cell. Dr. Rothman's laboratory demonstrated that, among the variety of different v-SNAREs and t-SNAREs, only certain combinations interact productively. Thus, a vesicle carrying a particular v-SNARE will only fuse with a target membrane that displays the appropriate t-SNARE. Once the membranes have fused, the earlier-identified proteins NSF and SNAP break apart the SNARE complex so that the SNARE proteins can be recycled.

Dr. Rothman's research continues to shed light on different aspects of cellular trafficking. Additionally, many scientists are investigating the association between defects in this process and disease. For example, insulin normally stimulates vesicle-mediated translocation of a protein called GLUT4 from the inside of cells to the cell surface membrane, where it functions to transport glucose into the cells. A defect in the insulin-induced trafficking of GLUT4 contributes to insulin resistance in muscle and fat cells in type 2 diabetes. The disease cystic fibrosis most commonly results from a mutant CFTR protein that cannot be exported to its proper location in the cell surface membrane. Left inside the cell instead, it does not function properly and is ultimately destroyed. One of the many strategies scientists are investigating towards treating cystic fibrosis involves tweaking the system that retains CFTR in an intracellular compartment so that the mutant CFTR proteins can escape to the cell surface membrane. Future research on vesicle transport will undoubtedly provide new insights into human disease.

For additional information on Dr. Rothman's research, see:

1. Rothman JE. Commentary—The machinery and principles of vesicle transport in the cell. *Nat Med* 8(10): 1059-1062, 2002, and references within.
2. The Lasker Foundation, http://www.laskerfoundation.org/awards/library/2002basic.html