



*Insulin, shown here in crystalline form, is a key signaling molecule in blood glucose regulation.  
Photo: Dr. Donald F. Steiner, University of Chicago.*

# Extraordinary Opportunity:

## Cell Signaling and Cell Regulation

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Cells throughout the body signal to each other to coordinate vital functions, such as maintaining blood glucose concentrations within a narrow range and body weight at steady levels. A breakdown in this highly integrated communication network, or in the signaling and response pathways within a cell, can lead to diabetes, obesity, and diabetes-associated complications.

A key signaling molecule is the hormone insulin. Secreted by the beta cells of the pancreas, insulin travels throughout the body to other organs and tissues to regulate blood glucose levels and to influence a variety of other cellular processes. Insulin induces muscle and fat cells to take up glucose from the blood and halts production of glucose by the liver. It also regulates metabolism of fats and protein and stimulates cell growth and development. In addition to insulin, other signaling molecules are critical to regulating metabolism and body weight, including molecules that exert control over appetite in the brain and hormone-like molecules that are secreted by fat cells to communicate with other organs.

Some signaling molecules, like insulin, transmit information between cells. Others, such as those that mediate a cell's response to insulin, transmit the signal throughout an individual cell.

The DRWG report defined as a major opportunity the continued dissection of signaling pathways relevant to diabetes, especially the pathways of insulin action. Not only does insulin resistance generally precede the development of type 2 diabetes, it is also associated with altered lipid metabolism and increased risk of cardiovascular disease. Although many challenges remain, progress in understanding insulin action has been rapid. Since the DRWG report was issued, researchers have identified key new signaling molecules in cells of the liver, pancreas, fat, muscle, and brain, with important implications for understanding the molecular basis of diabetes and

developing new therapies. They have shown how these signals affect glucose uptake, disposal, storage, or production; alter other processes key to insulin action, such as fat metabolism; regulate growth, proliferation or movement of cells; initiate or protect against programmed cell death; and influence other vital processes. These recent scientific advances, touched on in the following highlights—coupled with the application of new technologies—have fostered a greater understanding of cell signaling and cell regulation relevant to diabetes and have identified potential targets for development of new therapies.

## Understanding Insulin Action

In order for a signaling molecule released by one cell to exert an effect on a target cell, the target cell must express a receptor (often a protein on the cell surface) that detects the presence of the hormone and transmits its signal. Cells that lack such receptors are not directly affected by the hormone because they do not “see” it.

When a cell, like a muscle or a fat cell, responds to a signaling molecule, such as the hormone insulin, a receptor on the cell surface first binds the signaling molecule. This event prompts a series of changes in proteins throughout the cell. The result can be immediate changes in cell function or later changes that occur only after activation of genes and subsequent synthesis of new proteins to carry out the function dictated by the signal.

The insulin signaling cascade has been shown to involve multiple interacting pathways rather than a single linear pathway. Moreover, many components of the insulin signaling network exist in multiple

forms. Different forms or amounts of these signaling components are expressed in various tissues, such as muscle or fat. This complexity makes it a daunting task to attain complete understanding of insulin action and to define the primary basis for insulin resistance. Nonetheless, rapid progress in understanding insulin action has been achieved by the ability of researchers to remove or “knock out” genes coding for specific proteins of the insulin signaling pathways in specific cell types that are important for insulin action. Knockout technology has enabled the creation of mouse models designed specifically to answer questions about the function of particular proteins in specific tissues, such as

muscle, fat, liver, or brain, that regulate glucose metabolism. The challenge now is to take what we have learned about mechanisms of insulin action from cell and animal models and apply it to the study of human disease. The goal is to elucidate at the molecular level the pathogenesis of type 2 diabetes and the mechanisms underlying increased risk of cardiovascular disease associated with insulin resistance. New tools, such as proteomics and genomics, provide opportunities to examine how signaling pathways are altered in well-characterized human populations, such as those participating in large clinical trials and epidemiologic studies. Repositories of human biologic samples are being established to facilitate this effort. The DRWG emphasized the importance of metabolic staging of diabetes to identify the evolving set of metabolic abnormalities that develops over time. Monitoring the participants in the Diabetes Prevention Program, who are now continuing to be carefully followed in the DPP Outcomes Study, will provide unique opportunities in that regard. As important signaling molecules and pathways are identified in basic research, there are increasing opportunities to explore their implications for predicting disease risk and response to therapy in this and other well characterized human populations.

#### IDENTIFYING THE PROTEINS THAT MEDIATE INSULIN ACTION

Using knockout models, researchers have studied the roles of members of a family of proteins involved in the first step of the pathway of insulin action. They have found that one family member (IRS-1) is important for stimulating glucose uptake by muscle and fat and for the development of fat cells, but its loss causes only mild increases in blood glucose levels. Another member of this

protein family (IRS-2) is important for relaying the insulin signal in additional tissues such as the liver and for growth of the insulin producing beta cells; its loss causes frank diabetes. A protein farther down an insulin signaling pathway has recently been shown to be essential for normal insulin activity. Mice lacking this protein, called Akt2, become insulin resistant, are unable to suppress liver glucose production and have elevated blood glucose levels.

A number of proteins have been identified that attenuate the insulin signal, generally by removing a chemical group (phosphate) from the proteins involved in insulin signaling. Increased activity of some of these “off switches” has been found in insulin resistant states. “Knock out” of one of these attenuators of insulin action (PTP1B) results in improved insulin sensitivity and resistance to obesity. Vanadium compounds, being investigated for possible therapeutic benefit in treating insulin resistance, may in part exert their effects on insulin sensitivity by inhibiting PTP1B or related proteins. Improved insulin sensitivity is also seen in mice in which other regulatory molecules in the insulin signaling pathway (p85 regulatory subunit of PI(3)K, SHIP2) are knocked out. While there is no conclusive evidence that alterations in these signaling molecules play a role in human diabetes, the findings from these mouse models suggest that an opportunity for treating diabetes may lie in the development of drugs designed to enhance or inhibit the expression or activity of these components of the insulin-signaling apparatus.

## BUILDING KNOWLEDGE OF HOW INSULIN ACTS IN DIFFERENT TISSUES

Studies of targeted knockouts of the insulin receptor in specific tissues such as muscle, fat, liver and brain underscore the complex nature of the communication between tissues in regulating glucose and fat metabolism. For example, when scientists genetically removed the insulin receptor in muscle or fat cells, mice did not develop diabetes even though these are the tissues which remove the most glucose from the blood in response to insulin. On the other hand, when insulin receptors were knocked out in liver cells only, animals developed insulin resistance with all the hallmarks of type 2 diabetes, including resistance to the action of insulin in fat and muscle. These important findings suggest that signals from the liver affect the response of fat and muscle cells to insulin. The challenge now is to identify what these signals are and how they are sensed by the various insulin-sensitive tissues of the body.

Sometimes the results of these knockout experiments have been surprising. Insulin was not expected to play a major role in the function of the beta cell, the “factory” that produces insulin for the entire body. The beta cell responds to glucose levels to effect regulated insulin release, but insulin was not thought to be a critical regulator of beta cell function. Tissue selective knockout of the insulin receptor in the beta cell demonstrated an important role for the insulin receptor in the beta cell itself. Researchers have also demonstrated that the insulin receptor in the brain plays an important role in suppressing appetite, perhaps explaining why excessive hunger can be a symptom of diabetes. Although insulin causes weight gain because it stimulates

storage of energy in fat and muscle, its effect on the brain promotes reduced food intake and weight loss. Mice lacking insulin receptors in the brain were found to eat more than normal mice and become obese. Already, this recent finding from academic investigators is being pursued by researchers from the pharmaceutical industry. Because relatively little insulin enters the brain, treatment with a small molecule that can enter the brain and activate the insulin receptor might be less likely to promote weight gain than insulin therapy. Such a small molecule that mimics the effects of insulin was tested and shown to reduce food intake and body weight in rats and adiposity and insulin resistance in mice.

## CONTROL OF GLUCOSE PRODUCTION IN THE LIVER

Normally, the body maintains exquisite control over levels of glucose in the blood. During periods of fasting, the liver manufactures glucose for cells, especially brain cells, to use as an energy source. After a meal, insulin signals the liver to turn down its glucose production. Thus, normal liver function helps the body maintain blood glucose levels within the very narrow range critical for health. Investigators recently made significant discoveries about the cell signaling machinery that controls glucose production by the liver. In response to low blood glucose, liver cells turn on the production of a protein, PGC-1, which, in turn, interacts with other molecules to activate a series of genes required to produce glucose. Researchers have now found that PGC-1 is present in the liver cells of normal mice only when glucose production is needed, whereas diabetic mice appear to make PGC-1 continuously and the liver continues to

pump glucose into the bloodstream. These results are the first demonstration that PGC-1 is important for glucose production by the liver, and present major opportunities for developing new strategies to treat diabetes. Future research based on the findings about PGC-1 may lead to opportunities to develop new and more effective drugs for use in the treatment of type 2 diabetes.

### ▶ **ROLE OF THE FAT CELL GLUCOSE TRANSPORTER IN INSULIN RESISTANCE**

Insulin prompts fat and muscle cells to take up glucose so that the level of glucose in the blood does not become too high. To explore the molecular details of this process, scientists knocked out the gene encoding the protein, GLUT4, that transports glucose into cells in response to insulin. Recent technology enabled the scientists to remove GLUT4 only from fat cells, rendering them unresponsive to insulin-induced glucose uptake but leaving the insulin signaling apparatus intact in other tissues such as muscle and liver. Surprisingly, the lack of GLUT4 in fat cells not only made these cells insulin resistant but also made the muscle and liver insulin resistant. This finding suggests that fat cells normally secrete a factor that travels in the blood to the muscles and liver and that the absence of GLUT4 changes the amount of this factor circulating in the blood. After further experiments, researchers concluded that fat cells must use messengers to “talk” to liver and muscle cells. There may be a number of as yet undiscovered signaling molecules involved in insulin-induced glucose uptake that could prove to be useful drug targets for the treatment of type 2 diabetes and obesity.

### ▶ **SIGNALING PATHWAYS YIELD MANY POSSIBLE THERAPEUTIC TARGETS**

Changes in the activity of pathways that mediate inflammation have been associated with insulin resistance and defects in primary insulin secretion. Very high doses of the anti-inflammatory salicylate (aspirin) have been shown to improve glucose metabolism. Scientists recently learned from experiments with animal models that IKK—a protein which is part of the NF- $\kappa$ B signaling pathway that mediates inflammation—may be an effective target in therapies designed to reverse insulin resistance. A reduction in IKK levels or activity leads to improved insulin responses. Based on these results, scientists hypothesize that drugs designed to interfere with IKK, such as salicylates, might reverse insulin resistance. Salicylates are also reported to partially correct defects in insulin secretion. While aspirin itself would not be useful as a prospective drug therapy because the dose required to affect insulin response or secretion has too many side effects, it may be possible to develop related, less toxic drugs to correct critical defects that lead to type 2 diabetes.

A protein called CAP has been shown to play a major role in another signaling pathway involved in insulin-stimulated uptake of glucose by fat and muscle cells. High levels of CAP are found in muscle and fat, and the presence of CAP increases in fat cells in response to medications that make cells more sensitive to the effects of insulin. Opportunities exist to pursue the therapeutic relevance of this signaling pathway.

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A small protein made in the intestine called glucagon-like peptide-1 (GLP-1) has been shown to enhance insulin secretion; lessen the production of the hormone glucagon, which counteracts many effects of insulin; reduce appetite; and slow emptying of the stomach and thus, the absorption of glucose from meals. Several pharmaceutical companies are testing GLP-1 and related peptides in clinical trials. While the GLP-1 peptide is short-acting and must be given by infusion, opportunities are being pursued to develop modified agents that could be administered more easily.

## ▶ PROGRESS TOWARD UNDERSTANDING AND COMBATING INSULIN RESISTANCE

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As described previously, a first step toward the goal of combating insulin resistance is understanding how insulin normally transmits its signal. Scientists are uncovering a variety of different molecular pathways that can transmit the insulin signal within a cell. As the intracellular signaling proteins in these pathways are identified and their roles and interactions clarified, scientists will pursue those that are the most promising targets for development of new therapeutic agents. Opportunities to develop therapeutic strategies are emerging not only from insights about the pathways that transmit the insulin signal within a cell, but also from new knowledge about the signaling proteins generated in response to insulin that travel between cells. While we have gained an increased appreciation for the complexity of insulin signaling pathways and their perturbations in insulin resistance and diabetes, the challenge of unraveling this complex system is continually underscored by the recognition that combating insulin resistance is central to the prevention and treatment of type 2 diabetes.

## Beyond Insulin: Other Key Signaling Pathways That Influence Development of Diabetes and Obesity

Because obesity is a major risk factor for type 2 diabetes and is itself an important health problem, it is essential to understand the signaling pathways that affect body weight. Since the discovery of the fat cell hormone leptin, researchers have elucidated its role in sensing energy balance and communicating this information to the brain as well as signaling other tissues to regulate glucose and fat metabolism.

Moreover, spurred by the seminal discovery of leptin, scientists have proceeded to discover other fat cell hormones with key metabolic roles at an accelerating pace. Identification of the receptor for leptin in the brain has stimulated the identification of the signaling molecules and pathways involved in regulating appetite; these are potential targets for therapeutic development.

### CELLS IN THE BRAIN THAT SIGNAL TO US THAT WE HAVE HAD ENOUGH TO EAT

The discovery of leptin and its receptor has served as a beacon to guide scientists to a critical region of the brain that links the body's need for energy with the motivation to eat. Leptin receptors are located on only a small number of cells at the base of the brain in a region called the hypothalamus. There, leptin acts directly to inhibit cells that stimulate appetite, and it also stimulates the neighboring cells that signal satiety. One of the most important discoveries in the last several years has been the identification of specific cells in the brain that cause one to feel satiated or full. These cells (called POMC cells because they make the protein POMC) integrate information from many sources: glucose levels and signals

from the gut provide information about recent food intake; metabolic signals from fat cells, such as leptin, provide information about energy stores; and connections from higher levels of the brain also signal the need for food. All of these signals converge on the POMC cells, which are believed to be the master cells regulating appetite. The POMC cells signal satiety with hormones called melanocortins (derived from breakdown of the POMC protein), which bind to very specialized receptors located on only a few cells. These melanocortin receptors are particularly promising targets for drugs for treating obesity. It is hoped that, because their distribution is limited to so few cells, their effects may be specific to signaling satiety and mediating reduced food intake. A number of pharmaceutical companies have compounds based on these receptors in development as weight loss drugs.

Scientists are also studying the signaling pathways in the brain that regulate the POMC cells. Researchers recently demonstrated in mice that deletion of the gene for the M3 muscarinic receptor—a receptor for one of the major signaling molecules in the brain, acetylcholine—

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caused the mice to eat less; as a result, they were leaner than normal mice. In these animals, there are much lower than normal levels of melanin-concentrating hormone (MCH), a brain neuropeptide that appears to be involved in stimulating eating, and which is usually elevated in the fasting state. Consistent with this finding, mice genetically modified to overproduce MCH are obese and insulin resistant. Both acetylcholine and MCH are distributed widely throughout the brain and provide interconnections to the hypothalamus. Tremendous new opportunities to design treatment strategies for obesity and also for diabetes are now emerging from research that builds upon these insights into molecules in the brain that control eating.

### ▶ A HORMONE THAT PLAYS AN IMPORTANT ROLE IN LONG-TERM BODY WEIGHT REGULATION

Ghrelin is a hormone secreted by the stomach and small intestine. A recent study determined that the amount of ghrelin secreted into the blood increases shortly before and decreases shortly after every meal. Diet-induced weight loss produced increased levels of circulating ghrelin. Patients who had a portion of their stomach removed via gastric bypass surgery produced decreased levels of ghrelin even though they lost weight. These results suggest that ghrelin may play a role in stimulating appetite and, when the amount of secreted ghrelin increases after weight loss, in stimulating weight regain by further enhancing the appetite. The development of drugs that may block the action of ghrelin offers a promising therapeutic opportunity for preventing obesity and weight regain.

### ▶ UNCOUPLING FOOD INTAKE AND ENERGY STORAGE

When food intake exceeds metabolic need, excess energy from food is stored and body weight increases. While one approach to prevention of obesity focuses on regulation of appetite and food intake, a second focuses on energy utilization. Exercise is one way to increase the metabolic rate but alternative approaches to influence metabolic pathways in cells are desirable. In fact, differences in metabolic rates may explain part of human variation in susceptibility to diet-induced obesity. Animal studies have suggested that muscle and fat are potential sites for increasing energy expenditure and causing excess energy to be released as heat. These studies have focused on a family of “uncoupling proteins” that exist in multiple forms and are expressed in many tissues. They appear to affect the linkage between fatty acid oxidation, a pathway of fuel metabolism; and generation of ATP, a biochemical form of energy. When a potent uncoupling protein (UCP3) was overexpressed in muscle through genetic engineering, mice became resistant to diet-induced obesity and more sensitive to insulin. However, when another form of uncoupling protein (UCP2) was knocked out in the beta cells of the pancreas, mice produced more insulin and had lower blood sugar. Thus, these studies found that increasing the amount of one form of uncoupling protein in muscle may protect against obesity and insulin resistance but reducing another form in the pancreatic beta cell may enhance insulin secretion. While much remains to be understood about the role of these uncoupling proteins in control of weight and susceptibility to diabetes in humans, dissecting the pathways that regulate their production and activity in specific tissues could provide new targets for therapy.

## FAT CELL HORMONES

Prior to the discovery of leptin, fat tissue was thought to serve as a static storage depot for calories, rather than as an endocrine tissue intricately involved in regulating the use and storage of fuel by itself and by other tissues. Recently, new fat hormones, such as resistin and adiponectin/ACRP30, have been shown to be secreted by fat cells and sensed by liver and muscle. These peptides appear to play an important role in regulating whole body glucose levels and sensitivity to insulin. The challenge now is to understand at the molecular level how fat cells talk to other tissues and organs in the body—such as the pancreatic beta cell, liver, brain, and muscle—and ultimately, to determine how to correct the altered or disrupted signaling events that occur in obesity and diabetes. As with the insulin signaling pathways, each intermediate in the signaling pathways mediated by these newly discovered fat hormones provides additional opportunities for drug development.

## REGULATION OF FAT CELL GENE EXPRESSION

In addition to receptors for signals at the cell surface, another key group of signaling molecules, called transcription factors, act directly in the nucleus of a cell to regulate gene expression. A key transcription factor that regulates fat cell gene expression is called PPAR gamma. The insulin-sensitizing thiazolidinedione (TZD) drugs, used for treatment of type 2 diabetes, act through PPAR gamma. The TZDs reduce the levels of several key mediators of insulin resistance, includ-

ing free fatty acids in the blood, the fat cell hormone TNF-alpha, and an enzyme (11betaHSD1) that catalyzes formation of cortisol. PPAR gamma, like the estrogen receptor, is a member of the nuclear receptor family of transcription factors. In an approach analogous to the development of estrogen analogs such as tamoxifen, with specific desirable effects in particular estrogen-sensitive tissues, scientists are trying to develop selective drugs with specific beneficial effects mediated through PPAR gamma. Although the mechanism of action of the TZDs was discovered well after their use for therapy of diabetes was established, our new understanding of the signaling pathway through which they work creates the opportunity to develop drugs with an improved risk/benefit profile that target PPAR gamma.

## LIPOTOXICITY— DIRECT ROLE OF FATS IN MEDIATING INSULIN RESISTANCE AND CELL DAMAGE

Elevations of fatty acids in the blood and fatty infiltration of muscle and liver are now recognized to contribute substantially to insulin resistance. Also, there is now evidence that increased lipid in the beta cells impairs insulin secretion. Thus, the signaling pathways that regulate fatty acid metabolism and fat accumulation in tissues are now potential targets for therapeutic intervention. One particularly promising key mediator, called AMPK, has been identified. In response to a signal of low cellular energy stores, AMPK is activated and causes fat to be burned rather than stored. An agent that activates AMPK has shown several other beneficial effects, including reduced glucose

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production in the liver and enhanced glucose uptake in muscle. There is evidence that the diabetes drug metformin may exert its effects in the liver through AMPK. Metformin is associated with reduced fatty infiltration of the liver, as well as reduced glucose production from liver. While highly useful for many patients with type 2 diabetes, it cannot be used in patients with evidence of impaired kidney function, and other patients stop its use due to gastrointestinal side effects. Currently, metformin is the only drug in its class; no alternative drug with the same mechanism of action is available. Researchers are particularly eager to define its mechanism of action because identification of the molecular target of a drug is useful for development of related agents with improved potency, reduced side effects and other benefits. Thus, the identification of AMPK may be an important clue to understanding how metformin works.

## ▶ GLUCOTOXICITY

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How does chronically increased blood glucose (hyperglycemia) result in the characteristic damage to the beta cells, eyes, kidneys, nerves, and blood vessels associated with diabetes? Hyperglycemia has been shown to alter multiple signaling pathways. A vicious cycle arises when chronic hyperglycemia resulting from failing beta cells in turn causes oxidative stress that further damages the already failing beta cells. Studies with a rat model of type 2 diabetes demonstrate that treatment with antioxidants can prevent the development of the severe hyperglycemia typical of this model. Researchers have demonstrated attachment of excess glucose to proteins involved in signaling pathways within cells and to cell-surface proteins and nearby (matrix) proteins involved in transmitting signals between cells. Receptors for aggregated proteins with excessive attached sugars have also been found in the endothelial cells that line the blood vessels, which may trigger signaling pathways that damage these cells. Increased glucose levels within cells trigger movement of nutrients through cellular pathways that may generate toxic byproducts that damage cells. Understanding how increased levels of glucose affect metabolic and signaling pathways is critical for the development of therapies to prevent or reverse these complications. Identification of the key intermediaries that mediate this damage is an area of intense interest, as described in the section of this report dealing with complications of diabetes.

# Opportunities for Applying New Tools to Cell Signaling and Cell Regulation in Diabetes

Since issuance of the DRWG's Strategic Plan, the NIH has moved rapidly to apply new technology to understand cellular processes that are important for the development of diabetes and its complications and to determine how cell signaling and cell regulation are altered by diabetes.

This is a tremendous undertaking given the many cells and tissues affected by diabetes. Nonetheless, the chances for success have been revolutionized by the development of new technologies. Functional genomics provides tools for discovering the functions of genes in specific cells. 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 under specific conditions. Proteomics is a relatively new concept that refers to approaches, often in very large scale, for identifying and obtaining a comprehensive understanding of the proteins in a cell and how they function together. Complementary genomics and proteomics approaches will likely accelerate the understanding of the molecular pathways that are altered in insulin resistance, diabetes, and the devastating complications of diabetes. The development of Internet-based bioinformatics tools will facilitate rapid dissemination of data and resources generated by different laboratories to help speed the flow of new information and the testing of new ideas. Finally, using a systems-based approach to understanding all of these interactions will help researchers to grasp the “big picture” of how molecules, cells, tissues, and systems function in the living organism in both normal and disease states.

## ▶ ESTABLISHING CONSORTIA TO ELUCIDATE BETA CELL SIGNALING

Working to understand cell signaling in the beta cell has been a top priority because altered function of this cell is central to the pathogenesis of both type 1 and type 2 diabetes. Research momentum was spurred through a series of workshops to define the current state-of-the-art in signaling in the beta cell. These workshops were an important means of assembling key investigators, recruiting new investigators to the field, identifying new technologies and approaches to be applied, and defining goals for future work. Initiatives have been designed to promote the development of multi-disciplinary consortia among laboratories to bring resources to bear more effectively on cell signaling and cell regulation in the beta cell. The Beta Cell Biology Consortium will work to apply the tools of functional genomics to the endocrine pancreas, to identify the genes expressed in mouse and human pancreas at all stages of development, and to

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generate DNA arrays and other tools to facilitate the identification of important signaling components. This consortium will also attempt to delineate pathways of islet and beta cell signaling in development, and potentially, in beta cell renewal. A second initiative, Comprehensive Programs in Beta Cell Biology, will bolster investigator-initiated collaborative research focused on signaling networks within the adult beta cell and the integration of signaling networks within the pancreatic islet. It will seek to identify each step in the molecular pathway by which glucose stimulates insulin secretion, mechanisms by which other signals modify beta cell function, and the factors that regulate beta cell growth.

#### ▶ **DIABETES GENOME ANATOMY PROJECTS**

To build upon the achievements of the Human Genome Project, the NIH has established a range of Genome Anatomy Projects (GAPs) to map the complex network of cellular interactions in normal and diseased tissues. The beta cell consortia described previously comprise one such GAP. Other related large-scale projects will foster multi-disciplinary team approaches applying genomic and proteomic technology to understanding, at the molecular level, the signaling events in tissues involved in the pathogenesis of diabetes and its complications. Efforts will focus on developing a comprehensive understanding of tissues that are major sites of insulin action—such as liver, muscle and fat—and how signaling and cell regulation are perturbed in diabetes. Understanding how diabetes affects signaling in endothelial cells—which line blood vessels and play a key role in the development of diabetes complications—is also vital for developing new targets for therapy of complications of diabetes.

#### ▶ **OVERCOMING INSULIN RESISTANCE**

As described previously, the insulin-sensitizing thiazolidinedione (TZD) drugs, used for treatment of type 2 diabetes, act through PPAR gamma, a member of a family of nuclear receptors that regulates fat cell gene expression. The currently available TZD drugs are useful for improving responsiveness to insulin but have the drawback of stimulating the development of fat cells and promoting weight gain. Researchers hope to develop more selective drugs that act through PPAR gamma to enhance insulin sensitivity without the associated weight gain. Accrual of more information to define the signaling processes through PPAR gamma is important for achieving this goal. Although researchers have identified drugs that activate PPAR gamma, they are still not certain of the normal cell signals that regulate its activity. Thus, PPAR gamma has been considered an “orphan nuclear receptor,” because its signaling partners have not been fully defined. A number of orphan nuclear receptors are implicated as playing important roles in lipid metabolism and fat cell development and they may influence insulin resistance, obesity and diabetes. A Functional Atlas of Orphan Nuclear Receptors is being created to develop an integrated understanding of the structure, function and physiologic roles of orphan nuclear receptors in insulin resistance, obesity, and diabetes. We know that aggregates of multiple signaling proteins are involved in gene regulation through these receptors. The Functional Atlas will catalog the various forms of these proteins and their interactions as well as the three-dimensional structures of these signal components, separately and in functional aggregates. These molecular tools will provide critical new opportunities for exploring approaches to the development of drugs targeting the orphan nuclear receptors and their associated proteins.

## ▶ METABOLIC PHENOTYPING

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Type 2 diabetes is a heterogeneous disease. Our increasing knowledge of the pathways and proteins that contribute to this multifactorial syndrome will enable us to characterize subtypes of the disease. Identification of such phenotypes is important for identifying diabetes genes and for defining optimal therapies for individuals with specific subtypes. This knowledge is also important for identifying very preliminary clinical signs so physicians can intervene early to prevent diabetes and its complications. For these reasons, the DRWG identified a tremendous need to develop simple and accurate markers of the insulin-resistant state that researchers can use in their studies and that will help physicians monitor the clinical status of patients. These markers should provide information at the level of the whole body and also at the level of individual tissues—because insulin resistance in different tissues may have different consequences.

As noted previously, genetic engineering techniques have permitted the development of powerful mouse models in which genes for specific signaling molecules can be over-expressed or knocked out in specific tissues. These transgenic and gene knockout mouse models provide a particularly powerful approach to dissecting the steps in the insulin signaling network. By altering the expression of key genes in specific tissues and by creating mice with defects in multiple genes through cross-breeding particular mouse models, researchers are defining much more precisely the key pathophysiologic steps leading to insulin resistance and type 2 diabetes. To enhance what we can learn from these models and as a first step toward developing techniques for human phenotyping, the NIH has created Mouse Metabolic Phenotyping Centers. These Centers provide shared resources for careful analysis of engineered mouse models with sophisticated technology such as NMR measurement of glucose utilization. They will foster approaches to new drug development by facilitating analyses of drug effects on carefully defined parameters and surrogate markers in animals with well-characterized phenotypes.