

How Cells Absorb Glucose

Glucose, a crucial nutrient, must enter cells with the aid of a special transporter. Recent research elucidates the structure and function of the transporter and how insulin regulates it

by Gustav E. Lienhard, Jan W. Slot, David E. James and Mike M. Mueckler

Glucose is the common currency of metabolism. Absorbed in the intestine or produced by the liver, the sugar travels in the blood to all the tissues of the body. There it serves as a source of energy and as a primordial precursor for other carbon-containing compounds. Yet the passage of glucose into a cell is quite intricate. It must be ferried inside by a special protein embedded in the membrane of the cell. Over the past decade, studies in several laboratories, including our own, have greatly clarified the structure of the protein and the remarkable way in which it works. Five molecular forms of the transporter have been discovered so far, each adapted to the metabolic needs of the tissue in which it is found.

The events that these vital molecules mediate begin when one eats carbohydrates. The intestine digests the carbohydrates into glucose, which it transfers to the bloodstream. The resulting rise in blood glucose stimulates the beta cells of the pancreas to release insulin. Insulin clears glucose from the

blood in two ways: it prevents the liver from releasing additional glucose, and it causes muscle and fat cells to absorb more of the sugar. Muscle cells convert the glucose into glycogen, a polymerized carbohydrate that can be quickly reconverted into glucose. Fat cells convert glucose into droplets of fat for long-term storage. As blood glucose levels drop, the beta cells stop secreting insulin, and the body's metabolism returns to the basal state.

Too much insulin lowers blood sugar, producing hypoglycemia. Because this condition starves the brain, an organ that lives chiefly on glucose, it can mean death. On the other hand, when there is too little insulin—or when muscle and fat cells resist its effect—blood sugar rises, producing hyperglycemia. The high concentration of sugar molecules creates an osmotic imbalance, so the blood draws water from the tissues and the kidneys excrete the water in the urine, along with an excess of salts. Dehydration and salt loss brought on by severe hyperglycemia can lead to coma and death. Milder hyperglycemia probably contributes to such long-term complications of diabetes mellitus as heart attacks, strokes, blindness, kidney failure and gangrene of the extremities.

A lack of insulin causes insulin-dependent diabetes mellitus (IDDM), or type I diabetes. It generally develops in children or adolescents when an autoimmune reaction destroys beta cells in the pancreas [see "What Causes Diabetes?" by Mark A. Atkinson and Noel K. Maclaren; *SCIENTIFIC AMERICAN*, July 1990]. Non-insulin-dependent diabetes (NIDDM), or type II, usually appears later in life and is by far the more prevalent form, afflicting about 5 percent of the U.S. population who are more than 40 years old. Early in the disease some NIDDM patients do not lack insulin, and so the disease may come from an insufficient hormonal effect in muscle, fat and liver cells.

The absorption of glucose is more complex than might be supposed. A

cell can survive only by preventing its interior from mixing with the watery environment outside. Such isolation is provided by the cell membrane, a double sheet of lipid molecules that repels water and those substances—such as glucose—that readily dissolve in water. (Lipids are thus said to be hydrophobic, whereas glucose is hydrophilic.) Because of this arrangement, the cell cannot absorb glucose by simple diffusion. It must instead employ a special protein: the transporter molecule.

The first glucose transporter was isolated in 1977 from human erythrocytes (red blood cells) by Michihiro Kasahara and Peter C. Hinkle of Cornell University. Eight years later a collaborative project, led by one of us (Mueckler) and Harvey F. Lodish of the Whitehead Institute for Biomedical Research, elucidated the transporter's amino acid sequence. The investigators worked backward, in this case the easiest strategy, by isolating the DNA that encodes the protein and determining the sequence of its nucleic acid bases. They then applied the genetic code to translate this sequence into a sequence of amino acids.

The protein described by this method consists of a chain of 492 amino acids that can be viewed as being organized into 25 segments. Thirteen segments are largely hydrophilic and so are likely to prefer the aqueous extracellular and intracellular environments. They alternate with 12 primarily hydrophobic segments that are likely to prefer the lipid environment of the cell membrane. This arrangement, together with some direct chemical information about those parts of the protein that face in and out of the erythrocyte, suggests that the protein weaves back and forth across the membrane 12 times [see *top illustration on page 88*].

How might such a structure convey glucose into the cell? It must somehow create a pore through the membrane. The architecture of such a pore is sug-

GUSTAV E. LIENHARD, JAN W. SLOT, DAVID E. JAMES and MIKE M. MUECKLER share an interest in the regulation of glucose transport by insulin and have worked together on several projects. Lienhard has been professor of biochemistry at Dartmouth Medical School since 1975. His current research focuses on the pathways by which the insulin receptor regulates cellular processes. Slot is in the department of cell biology at the Medical School of the University of Utrecht, the Netherlands. He locates individual protein molecules in the cell by electron microscopy and uses this technique to investigate the trafficking of membrane proteins. James and Mueckler hold appointments in the department of cell biology and physiology at Washington University School of Medicine. Together they were the first to clone the complementary DNA encoding the glucose transporter GluT4, which figures prominently in this article.

gested by the folding arrangement of the polypeptide chain and by the pattern of amino acids in the transmembrane segments. Spectroscopic evidence implies that each segment is coiled into a helix—indeed, up to 80 percent of the entire polypeptide chain appears to be helical. Because a helix takes the overall shape of a cylinder, the chemically reactive groups of the amino acids will be arrayed periodically along the surface. It turns out that in five transmembrane segments—numbers 3, 5, 7, 8 and 11—the groups are hydrophilic on one side

of the cylinder and hydrophobic on the other. Bound together with their hydrophobic sides facing away from their common axis—toward the remaining transmembrane segments and the lipid environment of the membrane—the five segments would form a pore whose inner surface could bind glucose [see *bottom illustration on next page*].

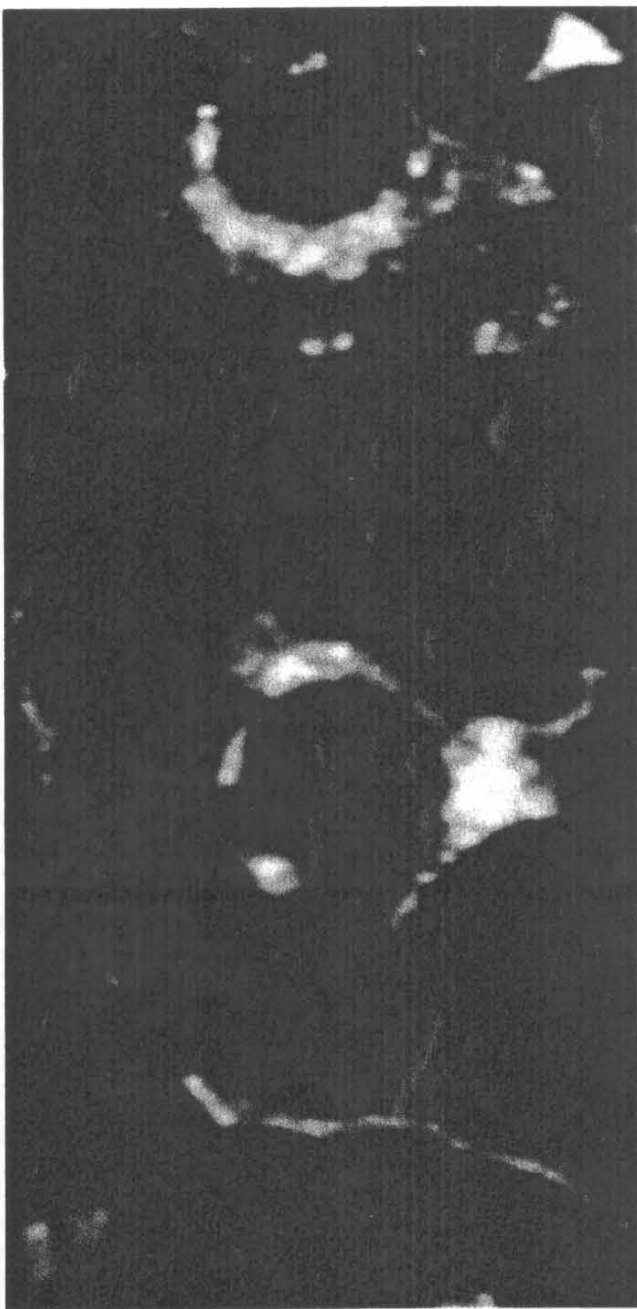
We emphasize that this model for the three-dimensional structure of the glucose transporter is speculative. To determine the real structure, X-ray crystallographers will have to learn

how to make the transporters into well-ordered crystals. So far, however, the lipid-loving nature of these proteins has made them resist all such efforts.

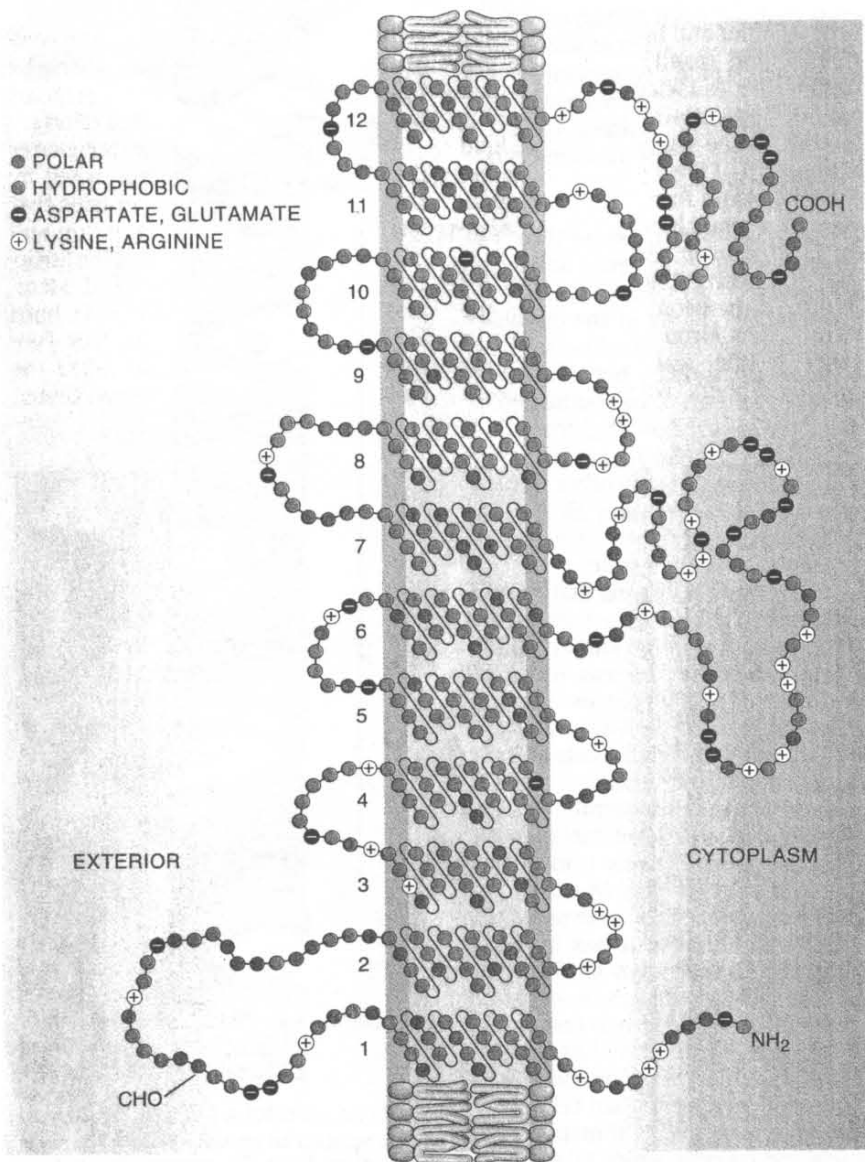
We therefore modeled the transporter in part on the examples furnished by the handful of membrane proteins that lend themselves to crystallization and have thus been imaged [see "The Structure of Proteins in Biological Membranes," by Nigel Unwin and Richard Henderson; *SCIENTIFIC AMERICAN*, February 1984]. One of them, called the bacterial photosynthetic reaction center,



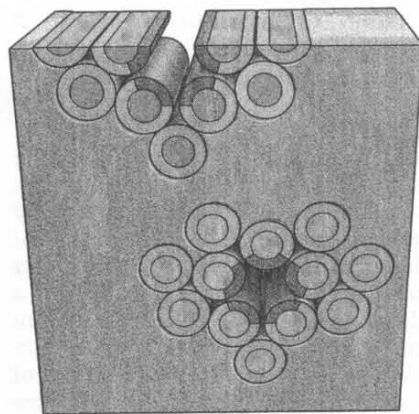
GREENISH GLOW tracks the deployment of glucose transporters from the interior of a fat cell to its surface. The transporters, called GluT4s, were tagged with an antibody linked to



a fluorescent chemical. Before exposure to insulin, they form clusters near the cell's nucleus (*left*). After exposure, some move to the surface—now defined by a halo of light (*right*).



PROPOSED STRUCTURE of the transporter's 492 amino acids is depicted as a folded chain that traverses the lipid membrane in 12 segments. Amino acids having charged groups appear as (+) or (-). Because such groups are more stable in water than in lipid, most have been located outside the membrane rather than in it.



PENTAGONAL PORE is postulated to consist of five helical segments of the transporter molecule, such as segment number 8 (left). If the helices were to face their lipid-loving sides (blue) toward the membrane and their glucose-binding sides (red) inward, they would create a channel that could accommodate a glucose molecule.

incorporates three separate protein chains, two crossing the membrane five times and another crossing it once. Each of the 11 transmembrane segments has a helical structure resembling the one we propose for the glucose transporter.

The detailed molecular events that bring glucose into the cell are even more complicated than our structural description suggests. The transporter is believed to manipulate glucose by holding it in weak, and therefore transient, hydrogen bonds. Such a bond stretches a hydrogen atom between an atom of nitrogen or oxygen in one compound and the unpaired electrons on an oxygen or nitrogen atom of another compound. Transmembrane segments 3, 5, 7, 8 and 11 contain many amino acids having hydroxyl (OH) and carbamido (CONH₂) groups that can participate in hydrogen bonding with the many hydroxyl groups on glucose. Moreover, the transporter protein takes two shapes: one binds glucose on the extracellular side of the membrane; the other binds it on the intracellular side.

Considerable experimental evidence suggests that a glucose molecule enters the cell in four steps. First, it occupies the outward-facing binding site. Second, the complex of transporter and glucose changes conformation, so that the glucose now occupies the binding site that faces into the cell. Third, the transporter releases the glucose into the cytoplasm of the cell. Fourth, the unoccupied transporter changes to the conformation in which the binding site for glucose faces outward. This final step returns the transporter to its initial shape, enabling it to transport another molecule of glucose.

The structures of the two conformations are not known, but it seems likely that in each conformation the pore is open at one end and constricted at the other and that glucose binds in the pocket at the open end. Glucose would then move as the open end closes behind it and the constricted end opens in front of it.

We thus envision the transporter as a conformational oscillator that shifts the binding pocket for glucose between opposite sides of the membrane. Kinetic studies, including several performed at Dartmouth Medical School by James R. Appleman and one of us (Lienhard), indicate that such oscillation is extraordinarily rapid. When glucose is not present, each transporter molecule in the membrane of an erythrocyte converts between the two states about 100 times per second at 20 degrees Celsius. When

glucose is bound to the transporter, the rate is even greater, about 900 times per second. Glucose speeds the oscillation by lowering the energy barrier between the two conformations.

The question arises why the glucose transporter works in such a complicated way. One can imagine simpler strategies. The transporter protein might have maintained a permanently open pore, or it might have fluctuated between such a conformation and another in which the pore is closed.

The explanation probably lies in the cell's need to maintain chemical imbalances with respect to the extracellular medium. Sodium ions, for example, are typically 10 times more concentrated outside than inside a cell. Because a glucose molecule is of comparable size, a permanent pore for glucose might allow sodium ions to leak in. Of course, a sodium ion may occasionally hitch a ride into the cell on the transporter. But such leakage would be insignificant: cells have special transmembrane channels that, when open, pass about 10 million sodium ions a second, 100,000 times faster than the glucose transporter operates.

Because each tissue has its own glucose requirements, one might suppose that each would also have a different glucose transporter. Considerable evidence for this hypothesis accumulated by the early 1980s. Transporters reacted differently to chemical inhibitors, and they were more or less efficient at transporting glucose. That is, although they all transported glucose at increasing rates as the concentration rose from zero, their rates topped out, or saturated, at different glucose concentrations. Workers characterize transport systems by the glucose concentration at which the transport rate equals half the saturation rate. The half-saturation concentration for the transporter in liver cells is high, for example, whereas that for the transporter in brain cells is low.

Proof that transporters differed came after 1985, when researchers found the DNA sequence encoding the human erythrocyte glucose transporter and used it as a probe to isolate DNA encoding transporters from other tissues. This approach has been pursued vigorously by many investigators, most notably Graeme I. Bell of the University of Chicago, Morris J. Birnbaum of Harvard Medical School, Lodish and two of us (Mueckler and James). A total of five glucose transporters have been discovered so far.

The transporters form a family whose members are broadly alike in structure and function. Each consists of a poly-

peptide chain of about 500 amino acids. The amino acid residues at about half the positions in the five sequences are the same or very similar. Moreover, the predicted pattern of folding for each transporter consists of 12 membrane-spanning segments.

Each glucose transporter (GluT) is numbered in the order of its discovery. The first, GluT1, is expressed in high levels in the endothelial cells that line the blood vessels and that form the barrier between brain and blood. It seems to be specialized to provide the steady flow of glucose that the brain requires. Smaller amounts of GluT1 are found in many other tissues, suggesting that it supplies all the glucose they need when their cells are in a comparatively inactive state.

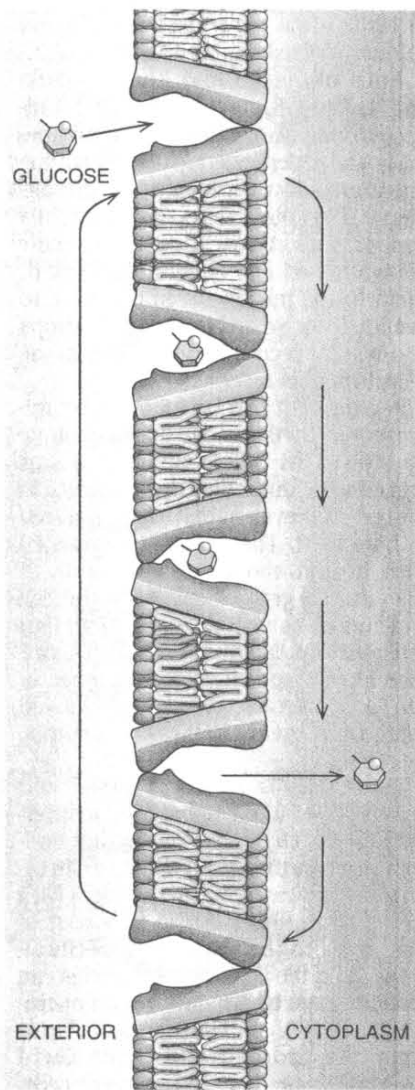
GluT2 appears in organs that release glucose into the blood, such as the intestine, the liver and the kidney, and in the beta cells of the pancreas, which secrete insulin. The high glucose concentration required for half-saturation of GluT2 means that it transports glucose in proportion to the sugar's concentration in the blood. Consequently, changes in the blood glucose level during meals or exercise are effectively transmitted to the liver and beta cells by GluT2.

GluT3 is found in the neuronal cells of the brain. Because it has a higher affinity for glucose than GluT1 does, this transporter ensures a constant movement of the sugar into these cells. One glucose transporter thus cooperates with another, assuring the flow of this vital nutrient into brain cells.

GluT4 is the major transporter in muscle and fat cells, which take up glucose in great spurts and convert it to other energy-yielding compounds. This transporter is distinguished by its extraordinary ability to move back and forth between internal reservoirs and the surface of the cell.

GluT5 is found mainly in the small intestine and kidney. Its function has not yet been described in detail.

These five GluTs all move glucose across the membrane along its concentration gradient, that is, from the higher to the lower glucose concentration. The family is distinct from another transporter that pulls glucose against this gradient. It is called a cotransporter because it couples the transport of a glucose molecule with that of a sodium ion. The energy needed to pull the glucose comes from the movement of sodium along its own gradient. The cotransporter enables cells lining the lumen of the intestine and kidney to absorb even quite small traces of glucose from food and urine, respectively. GluT2



GLUCOSE ENTERS A CELL by binding to a transporter in an outward-facing conformation. Next the transporter reconfirms, closing the pore to the outside and opening it to the cytoplasm. The glucose then dissociates. (This representation is not drawn to scale.)

then releases that sugar into the blood.

The transporters also vary in the way they respond to insulin. Such specializations are what one would expect to find in tissues having markedly different metabolic needs. The most marked response is found in GluT4, an isotype first identified in 1988 by James, then at the Boston University School of Medicine, and co-worker Paul F. Pilch.

The effect of insulin on glucose transport is dramatic. We commonly work, for example, with a mouse fat cell in culture, called the 3T3-L1 adipocyte. The addition of insulin to such a culture, held at 37 degrees C, increases glucose uptake 15-fold. The peak rate is attained within 10 minutes.

How insulin works was a mystery un-

til 1980, when Lawrence Wardzala and Samuel W. Cushman of the National Institutes of Health and Kazuo Suzuki and Tetsuro Kono of Vanderbilt University School of Medicine simultaneously and independently discovered the phenomenon of transporter recruitment. They found that cells maintain a pool of extra glucose transporters (now known to be GluT4) and move some of them to the membrane in response to insulin. Later, when blood glucose drops and insulin secretion drops with it, the recruitment is reversed.

Recruitment can be followed by microscopic methods. In one technique the GluT4 in 3T3-L1 adipocytes was tagged with antibodies that fluoresce in the green wavelengths when exposed to blue light. The cells not stimulated with insulin showed many points of the emitted green light inside the cell and almost none at the cell surface. But after insulin treatment, the cell's surface glowed green: a large number of GluT4 molecules must have moved there from the interior [see illustration on page 87].

These results were confirmed and quantified by a study that used a different tag. In thin slices of tissue, antibodies were specifically attached to GluT4. The workers then treated the slices with tiny gold particles linked to a protein that binds to the antibodies, specifically marking the GluT4. The particles can easily be seen under the electron microscope. One of us (Slot) has used this technique to locate and count GluT4 molecules in insulin-sensitive cells. Before insulin stimulation, only 1 percent of the tagged GluT4 molecules was on the cell surface; after stimulation, the proportion had risen to about 40 percent.

How does the cell shuttle GluT4 to and from the cell membrane in response to insulin? The answers are still sketchy, and many researchers are trying to fill in the details. Because the glucose transporter is a protein embedded in the membrane both inside the cell and at the cell surface, it surely migrates as part of a membranous vesicle. Insulin probably induces intracellular vesicles containing GluT4 to move to the inner surface of the cell membrane and fuse with it.

The reverse process appears to be more complicated. In a sequence of events, the details of which are not yet understood, small vesicles containing GluT4 pinch off from the inner side of the membrane and then fuse with larger membranous sacs, called endosomes. Within the endosomes the GluT4 molecules somehow segregate into tubular

extensions, which repackage themselves into vesicles. This removal of GluT4 to the cell's interior occurs all the time, but when insulin is present, the vesicles immediately re-fuse with the cell surface. Withdrawal of insulin breaks the cycle, causing vesicles to accumulate.

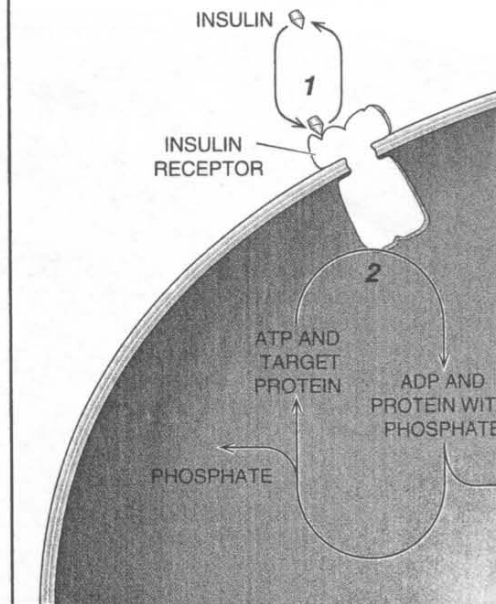
Because GluT4 is the main isotype that moves, and because it normally redeploys from the interior of the cell to the surface (and vice versa, depending on the insulin level), we suspect that it has a guidance mechanism possessed by no other isotype. The key feature of this mechanism would be the placement of GluT4 in intracellular vesicles when insulin is absent; the GluTs in the other tissues reside mainly at the cell surface whether or not insulin is present.

Such a mechanism must depend on a unique segment of amino acids in GluT4, one that might direct the transporter to vesicles by interacting with other proteins. The segment would thus function as a ticket stamped with a destination. Workers are examining this hypothesis by using molecular biological techniques to produce mutant forms of GluT4 that can be expressed in cells. If such a mutant can be found residing at the surface of a cell in the basal state, then the site where the mutation occurred will probably coincide with the ticket.

When insulin binds to the cell, it triggers a cascade of molecular events that ultimately redistributes the glucose transporters to the cell membrane. Only the beginning and the end of this cascade are known. It starts when insulin from the blood binds to a specific protein embedded in the cell membrane. The protein projects from either side of the membrane. When insulin binds to the outer projection, the receptor reconfirms, enabling the inner projection to put a phosphoryl group on the amino acid tyrosine at specific locations within specific target proteins. No target protein known to be involved in signaling the recruitment of the glucose transporter has yet been found, although one obvious candidate—the glucose transporter itself—has been ruled out.

Workers are following two approaches to understand the chain of events initiated by the insulin receptor. One approach is to find the target by isolating and characterizing proteins that have tyrosines that are phosphorylated in response to insulin. A complementary approach focuses on other proteins present in the GluT4 storage vesicles. Insulin may change one of these proteins in a way that accelerates the movement of the vesicle to the cell surface or that induces it to fuse with the surface. Other proteins may also be

An outwardly projecting part of the insulin receptor binds insulin (1), causing the receptor's inwardly projecting part to transfer a phosphoryl group from adenosine triphosphate (ATP) to one or more target proteins, creating



removed by the vesicle to subserve functions that are not yet imagined. Such possibilities are suggested by the vesicular mechanism itself. Although it seems a cumbersome way to regulate the rate of glucose transport into the cell, it would be quite efficient for moving several different proteins to and from the cell surface.

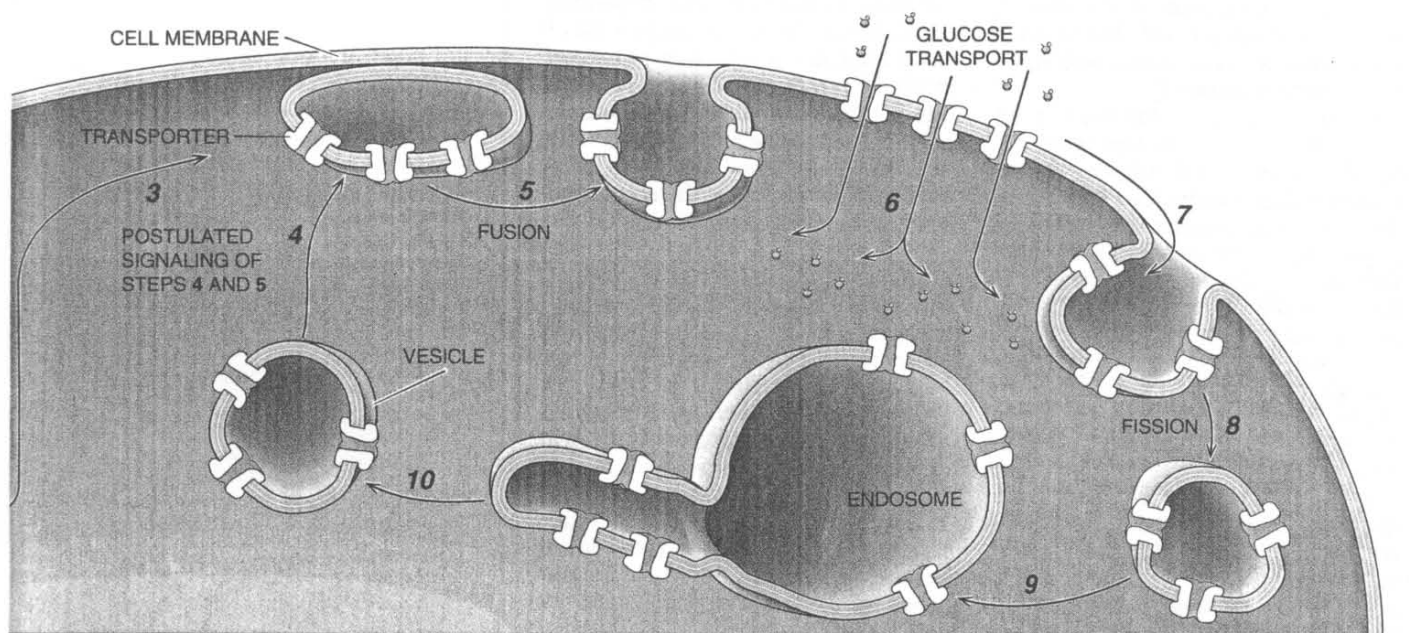
These findings imply no cause for the insulin-dependent form of diabetes mellitus, but they are relevant for the second form: NIDDM. Because the propensity to acquire NIDDM is certainly inherited, at least one gene must predispose individuals to the disease. One possibility is that a defective gene affects the way insulin regulates GluT4.

One of the earliest manifestations of NIDDM is insulin resistance: the inability of muscle, fat or liver to respond appropriately to elevated blood insulin levels. The pancreas responds to such resistance by oversecreting insulin, so that some patients in the early stages of NIDDM exhibit both transient hyperglycemia after a carbohydrate meal and consistently elevated blood insulin levels. As the disease progresses, the pancreas often loses the ability to secrete enough insulin to compensate for insulin resistance. By the time that hap-

How Insulin Helps Cells Recruit Transporters

ADP (2). A current hypothesis is that a target protein bearing phosphate signals the redistribution of glucose transporters (3) through the movement of transporter-containing vesicles to the membrane (4). The vesicles fuse with the membrane (5), accelerating the transport of glucose (6). The transporters are retrieved to the interior when small vesicles formed through membrane

invagination (7) and fission (8) fuse with larger endosomes (9), where the transporter segregates into tubular extensions that pinch off to form new vesicles (10). As long as insulin remains, the vesicles will continue to fuse with the cell membrane, but a lowered level of insulin breaks the cycle, and the glucose transporters accumulate in intracellular vesicles.



pens, hyperglycemia persists between meals, and the patient may require the administration of insulin or other drugs to lower blood glucose levels.

A basis for this development is suggested by recent results by Lodish and Bernard Thorens of the Whitehead Institute and Roger H. Unger of the University of Texas Southwestern Medical School. Using two different animal models of NIDDM, these workers found that the beta cells of the pancreas have a reduced amount of GluT2, their isotype. The reduction correlates with the decreased secretion of insulin in response to elevated blood glucose. Because the transport of more glucose into the beta cells normally triggers insulin release, the reduction of GluT2 could be a cause of inadequate insulin secretion.

The key to insulin resistance may well reside in skeletal muscle, which accounts for 80 percent of the body's glucose use in the period after a carbohydrate meal. (At other times, when body metabolism is in the basal state, the brain consumes fully 60 percent of the sugar.) Muscle cells convert excess blood glucose into glycogen at a rate that is limited by glucose transport. Because NIDDM patients deposit glycogen in their muscles at about half the

normal rate, they probably have a defect in at least one of the proteins that regulate transport.

Another consideration narrows the scope of the quest. Insulin resistance cannot come from a lack in the total amount of GluT4 protein in muscle cells. Recent measurements of GluT4 in biopsies of muscle from normal and diabetic individuals indicate that those with NIDDM have normal or only slightly decreased amounts of this protein. But GluT4 might cause insulin resistance by another route, perhaps by being targeted to the wrong intracellular compartment—one from which it

cannot be recruited to the cell surface. Alternatively, there may be a lesion in some other part of the signaling pathway through which insulin promotes recruitment of GluT4. Such a lesion might involve the insulin receptor on the cell surface or one of the unknown proteins that transduce the signal from the receptor to the GluT4 vesicles.

The big question, then, is whether recruitment is impaired in the muscle cells of diabetic patients. If it is, then the elucidation of the events beginning at the insulin receptor and ending in recruitment will surely show how insulin's message is misaddressed or mislaid.

FURTHER READING

SEQUENCE AND STRUCTURE OF A HUMAN GLUCOSE TRANSPORTER. M. Mueckler et al. in *Science*, Vol. 229, pages 941-945; September 6, 1985.

KINETICS OF THE PURIFIED GLUCOSE TRANSPORTER: DIRECT MEASUREMENT OF THE RATES OF INTERCONVERSION OF TRANSPORTER CONFORMERS. J. R. Appleman and G. E. Lienhard in *Biochemistry*, Vol. 28, No. 20, pages 8221-8227; October 3, 1989.

FACILITATIVE GLUCOSE TRANSPORTERS: AN EXPANDING FAMILY. G. W. Gould and

G. I. Bell in *Trends in Biochemical Sciences*, Vol. 15, No. 1, pages 18-23; January 1990.

FAMILY OF GLUCOSE-TRANSPORTER GENES: IMPLICATIONS FOR GLUCOSE HOMEOSTASIS AND DIABETES. M. Mueckler in *Diabetes*, Vol. 39, No. 1, pages 6-11; January 1990.

IMMUNO-LOCALIZATION OF THE INSULIN REGULATABLE GLUCOSE TRANSPORTER IN BROWN ADIPOSE TISSUE OF THE RAT. J. W. Slot et al. in *Journal of Cell Biology*, Vol. 113, No. 1, pages 123-135; April 1991.