

Insulin Resistance, Diabetes, and the Insulin-regulated Trafficking of GLUT-4

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DIABETES affects ~5% of people in the United States of America and the incidence is much higher in sporadic populations such as Pima Indians in North America and Australian Aborigines. There are two major forms of diabetes, referred to as insulin-dependent diabetes (IDD)¹ and non-insulin-dependent diabetes (NIDD). The etiology of these diseases is quite different despite similarities in their pathophysiologies. IDD often arises in early life and is due to autoimmune destruction of pancreatic β cells resulting in partial or complete loss of β cell function. NIDD is more common in later life (>40 yr) and ~85% of all diabetics have this form. The increased prevalence of NIDD in developed countries together with its association with heart disease and stroke makes this one of the most devastating diseases in the western world.

NIDD appears to be due to a defect in glucose disposal (DeFronzo et al., 1992). Both glucose per se and insulin, play important roles in facilitating glucose uptake into target organs (for review see DeFronzo et al., 1992). Following ingestion of a carbohydrate meal, glucose uptake into many organs is increased simply due to the elevated blood glucose. A more pronounced increase which is evoked by insulin, is observed specifically in muscle and adipose tissue. The net result is that in the postprandial state, glucose is rapidly and efficiently cleared from the blood and stored primarily in muscle and adipose. Each of these processes is defective in NIDD making it extremely difficult to identify the primary lesion. Studies in first degree relatives of NIDDs indicate that one of the earliest detectable defects is impaired insulin action in muscle and adipose tissue or insulin resistance (Martin et al., 1992). Thus, identifying the physiological and molecular biological processes that control insulin-stimulated glucose utilization in these organs is of major significance.

Insulin causes a large increase in glucose transport into muscle and adipose tissue but not in other cell types (Elbrink and Bihler, 1975). Transport is thought to be rate de-

termining for glucose consumption in myocytes and adipocytes, at least under certain conditions, and it is defective in NIDD (Kahn, 1992). Two facilitative glucose transporter isoforms, GLUT-1 and GLUT-4, have been identified in muscle and fat (reviewed in Bell et al., 1990; James et al., 1993). Differences in their subcellular distribution appear to dictate the major functions of these proteins. GLUT-1 is targeted primarily to the plasma membrane in unstimulated cells and presumably maintains basal glucose requirements (reviewed in James et al., 1993). GLUT-4, the more abundant isoform, is excluded from the plasma membrane in the absence of insulin residing in intracellular tubulovesicular elements associated with the *trans*-Golgi reticulum (Slot et al., 1991a,b). Both exercise, in the case of muscle (Douen et al., 1990), and insulin, in the case of muscle and fat (Rodnick et al., 1992; Slot et al., 1991a,b), cause a rapid and pronounced increase in cell surface levels of GLUT-4 following recruitment of the transporter from intracellular stores.

Recruitment of GLUT-4 to the cell surface is likely impaired in insulin resistant muscle and adipose tissue because the overall levels of GLUT-4 are normal in patients with NIDD (Kahn, 1992). Thus, any strategy that potentiates the insulin-stimulated recruitment of GLUT-4 would have enormous therapeutic benefit. Overexpression of GLUT-1 in muscle of transgenic mice emphasizes the importance of transport in overall regulation of muscle glucose utilization because muscle glycogen levels are markedly increased in these mice (Marshall et al., 1993b). Transgenic animals overexpressing GLUT-4, in either adipose tissue or muscle, exhibit a marked improvement in whole body insulin sensitivity implying that this may well be a sound approach toward achieving successful management of this disease (Liu et al., 1993; Tozzo et al., 1993). However, insulin-independent glucose transport was also increased in these mice, presumably due to increased expression of GLUT-1 or GLUT-4 at the plasma membrane. This may have deleterious consequences to the whole animal in the long term and so more useful strategies will require modifying the cell surface levels of the transporter only in the insulin-stimulated condition.

The regulation of GLUT-4 offers interesting challenges to cell biologists. Understanding the nature of the intracellular compartment that houses GLUT-4, the membrane trafficking events that sequester GLUT-4 from the cell surface, and how insulin stimulation causes such a dramatic change in the

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1. *Abbreviations used in this paper:* IDD, insulin-dependent diabetes; MPR, mannose 6-phosphate/IFG II receptor; NIDD, non-insulin-dependent diabetes.

steady state distribution of GLUT-4 will provide important insight into insulin action and to the development of therapeutic strategies that may override the development of insulin resistance and NIDD.

The Intracellular GLUT-4 Compartment

One of the problems in precisely defining the nature of the intracellular GLUT-4 compartment is that insulin stimulates the translocation of other proteins to the cell surface in adipocytes, besides GLUT-4. These include the transferrin receptor, the mannose 6-phosphate/IGF II receptor (MPR), and the α 2 macroglobulin receptor, all proteins that recycle through the endocytic system (reviewed in James et al., 1994). GLUT-4 is also localized, at least in part, to the endocytic system in adipocytes as it constantly recycles via clathrin-coated pits (Robinson et al., 1992; Slot et al., 1991b). Thus, adipocytes have the capacity to alter the kinetics of recycling through the endocytic pathway resulting in a net accumulation of many constitutively recycling proteins at the cell surface. Other cell types, such as fibroblasts, can also acutely augment plasma membrane levels of nutrient receptors and transporters in response to growth factors (Davis et al., 1987). Whereas the magnitude of the effect observed in fibroblasts is somewhat lower than that observed in adipocytes, the existence of this regulatory pathway in both cell types points to a common mechanism of regulated recycling through the endocytic system in all cells. Thus, the insulin-dependent movement of GLUT-4 in adipocytes may simply represent an efficient adaptation of this pathway.

How have adipocytes adapted the regulated recycling pathway to effect such a tremendous increase in glucose transport following insulin stimulation? The use of the GLUT-4 glucose transporter isoform, that has targeting properties distinct from all other isoforms, is an important contributing factor. GLUT-4 is excluded from the plasma membrane in non-stimulated adipocytes and myocytes (Slot et al., 1991a,b). What little GLUT-4 is present at the surface of nonstimulated cells is associated with clathrin lattices (Robinson et al., 1992). Intracellularly, GLUT-4 colocalizes with

other recycling proteins such as the MPR and the transferrin receptor (Tanner and Lienhard, 1989; and D. Hanpeter and D. James, unpublished data). As is the case for many other endosomal proteins, GLUT-4 constantly recycles in the presence and absence of insulin (Yang and Holman, 1993). Furthermore, an assessment of the unique targeting information in GLUT-4 reveals two motifs that resemble internalization targeting domains found in a number of other recycling proteins (reviewed in James et al., 1994). Thus, there is considerable overlap between the intracellular GLUT-4 compartment and the endosomal system. The sequestration of GLUT-4 within this intracellular location in nonstimulated cells maintains a low steady state distribution of GLUT-4 at the plasma membrane, which in turn affords the capacity for a greater magnitude of translocation upon insulin stimulation.

Generalized targeting of GLUT-4 to the endosomal system does not however, adequately explain its insulin regulation because other endosomal proteins, such as the transferrin receptor, that are very efficiently internalized only exhibit a threefold insulin-dependent increase at the cell surface (Tanner and Lienhard, 1987). Despite the low cell surface levels of GLUT-4 in nonstimulated cells, its internalization rate is slower (0.1 min^{-1}) than that of the transferrin receptor (0.5 min^{-1}) in adipocytes (Tanner and Lienhard, 1987; Yang and Holman, 1993). Hence, GLUT-4 must be retained within the endosomal system, perhaps in a specialized subcompartment. This notion is supported by the ability to isolate a subpopulation of intracellular GLUT-4 vesicles that contain a high proportion of GLUT-4 and low amounts of other endocytic proteins such as the transferrin receptor and the MPR (Robinson and James, 1992; Zorzano et al., 1989; D. Hanpeter and D. James, unpublished data). The identification of another unique protein, vp165, that is located in GLUT-4-containing vesicles isolated from adipocytes and that translocates to the cell surface to the same extent as GLUT-4 lends further support to the presence of a specialized compartment in these cells (Mastick et al., 1994). Identification of other constituents of the authentic GLUT-4 compartment will provide a better understanding of the nature and specific

Table 1. The Facilitative Glucose Transporter Family

Isoform	Tissue distribution	Targeting	Function
GLUT-1	Ubiquitous. High expression in cultured cells, brain endothelial cells, human erythrocytes	Plasma membrane: basolateral, cell body	Basal glucose transport
GLUT-2	Kidney and small intestinal epithelial cells, liver, pancreatic β cells	Plasma membrane: basolateral	Low affinity transporter Glucose release into the blood stream. Part of the glucose sensor in islets and liver
GLUT-3	Neurons, placenta	Plasma membrane: apical, axonal	High affinity transporter Unabated transport into central nervous system
GLUT-4	Skeletal muscle, brown and white adipose tissue, heart.	Intracellular vesicles	Mediate insulin-regulated glucose transport
GLUT-5	Small intestine, kidney, brain endothelial cells, sperm	Plasma membrane	Fructose transporter
GLUT-7	Liver	ER	Part of the glucose-6-phosphatase complex-gluconeogenesis

For extensive review of the tissue distribution of the facilitative glucose transporters see (Bell et al., 1990). GLUT-6 is not indicated because it encodes a pseudogene. In some cases the targeting of transporter isoforms expressed in polarized epithelial cells (denoted as basolateral versus apical) or in neurons (apical versus cell body) has been reported.

function of this compartment. Adipocytes and myocytes also express proteins that have been implicated in the control of specific membrane trafficking events. These include a Rab3 homologue, Rab3D (Baldini et al., 1992), and synaptobrevin or VAMP (Cain et al., 1992), both of which are involved in the regulated exocytosis of small synaptic vesicles in neurons. Thus, adipocytes and myocytes appear to have borrowed certain regulatory features from more specialized cells in order to effect a more efficient secretory response, in this case, of a nutrient transporter.

Molecular Regulation of GLUT-4 Targeting

Many investigators have sought the targeting domains in GLUT-4 that confer intracellular sequestration. These studies have been restricted to cell types (eg., CHO, COS, and NIH 3T3's) in which there is no significant insulin-dependent movement of GLUT-4 to the cell surface. However, the differential targeting of GLUT-1 and GLUT-4 is preserved in fibroblasts (reviewed in James et al., 1993). Most of the GLUT-4 (~85–90%) is targeted to intracellular tubulovesicular structures, similar to that observed in adipocytes, with the remainder at the cell surface (Asano et al., 1992; Piper et al., 1992). In contrast, ~70–80% of GLUT-1 is present at the plasma membrane in fibroblasts (Asano et al., 1992; Piper et al., 1992). Analysis of chimeric transporters and amino acid substitution mutants has identified two targeting motifs in GLUT-4 that control intracellular sequestration and that are not found in other transporter isoforms. One of these motifs is found in the NH₂-terminal cytoplasmic tail and another in the COOH-terminal cytoplasmic tail.

The GLUT-4 NH₂-terminal targeting motif (FQQI) is structurally and functionally similar to the family of tyrosine-based internalization signals present in the cytoplasmic tails of a number of recycling receptors (reviewed in James et al., 1993). Mutating the phenylalanine to alanine causes a marked increase in cell surface levels of GLUT-4 and a decreased association of GLUT-4 with cell surface clathrin-coated pits (Piper et al., 1993). The GLUT-4 NH₂-terminus confers intracellular targeting when substituted for the cytoplasmic tail of either the H1 subunit of the asialoglycoprotein receptor (Piper et al., 1993) or the transferrin receptor (Garippa et al., 1994) and in both cases mutating the phenylalanine at position 5 to alanine causes accumulation of the protein at the cell surface. Kinetic analysis of the transferrin receptor/GLUT-4 hybrids in CHO cells reveals that the GLUT-4 NH₂ terminus regulates efficient internalization but not intracellular retention (Garippa et al., 1994).

Essential amino acids in the COOH terminus of GLUT-4 that regulate targeting are leucine/leucine at positions 489/490, although an extensive analysis of other residues in this domain has not been reported (Verhey and Birnbaum, 1994). Similar dileucine motifs have been shown to regulate internalization of the T cell antigen receptor γ and δ chains (Letourneur and Klausner, 1992) and the invariant chain (Pieters et al., 1993), and to regulate sorting of the MPR from the Golgi to endosomes/lysosomes (Chen et al., 1993). A functional analysis of the GLUT-4 COOH-terminal dileucine motif remains to be performed.

Both tyrosine and dileucine-based targeting motifs have been shown to regulate internalization and/or intracellular sorting in a variety of different proteins (Harter and Mellman, 1992; Johnson and Kornfeld, 1992). Thus, it will be

important to determine if the GLUT-4 targeting signals have dual sorting roles when GLUT-4 is expressed in a more appropriate cell type, such as adipocytes. Under these conditions it will be possible to examine the effects of insulin to modulate the specific function of these domains. These will be particularly informative as it has previously been suggested that insulin both stimulates GLUT-4 exocytosis and inhibits its internalization (Jhun et al., 1992).

There is controversy concerning the relative role of each of these targeting domains in GLUT-4. Piper et al. (1992) observed little effect of the COOH terminus while others have observed little effect of the NH₂ terminus (Asano et al., 1992; Czech et al., 1993; Marshall et al., 1993a). In the former study a recombinant Sindbis virus was used to rapidly express glucose transporters in CHO cells. This mode of expression may bias the analysis toward an early step in the trafficking itinerary of GLUT-4, thus accentuating the role of the NH₂ terminus. Failure to observe an effect of the GLUT-4 COOH terminus (Piper et al., 1992) may be related to the phosphorylation state of the transporter, as GLUT-4 is phosphorylated at Ser488, adjacent to the LL motif (Lawrence et al., 1990). Infection with Sindbis virus may modify phosphorylation within the GLUT-4 COOH terminus thus overriding the sorting function of this domain. In the studies of Czech et al. (1993) and Marshall et al. (1993a) the inability to observe an effect of the NH₂ terminus may lie in the inappropriateness of expression systems and the nonquantitative nature of the analytical methods employed. Studies by Asano et al. (1992) further underscore the complications involved in analyzing chimeric transporters as they have reported an involvement of *trans* membrane domains in GLUT-4 sorting possibly implying that the oligomeric state of the transporter may play a role in targeting.

Despite these discrepancies, minimal amino acid substitutions and the use of heterologous proteins suggests that GLUT-4 is distinguished from GLUT-1 by the presence of targeting motifs in its NH₂ and COOH termini. In the future it will be important to define the function of these motifs in GLUT-4 targeting in insulin-sensitive cells. More important will be to define domains that confer insulin-regulated movement as opposed to simply intracellular sequestration. This may prove challenging as both GLUT-1 and GLUT-4 have the ability to move efficiently from the intracellular compartment following insulin stimulation.

The complexity involved in unravelling the genetic basis for NIDD is underscored by an appreciation for the many proteins that are involved in mediating the regulation of just one of insulin's biological actions, namely glucose transport in muscle and adipose tissue. Our challenge is to understand these processes at both the cellular and molecular biological level and synthesize what is known about signal transduction pathways and membrane trafficking dynamics from a host of different biological paradigms. Grasping these basics will allow precise physiological studies in transgenic mice and perhaps provide a framework for developing sound therapeutic strategies that overcome some of the pathophysiological complications associated with this major disease.

In view of space limitations it was not possible to quote the GLUT of papers on this topic. For this reason key review papers rather than original articles have been quoted. Our apologies to those whose work was not directly cited.

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