

The facilitative glucose transporter and insulin action

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Abbreviations: GLUT, glucose transporter; GTBT, glucose transporter binding protein; GST, glutathione S-transferase; HAD, L-3-hydroxyacyl CoA dehydrogenase; TMH, transmembrane helix

Abstract

Cellular uptake of glucose is a tightly controlled process. It is mediated by a family of intrinsic membrane proteins (facilitated glucose transporters or GLUTs), and further regulated by metabolites and hormones. By far the most important GLUT regulation in human physiology is that by insulin, dysfunction of which may produce insulin resistance, a hallmark of diabetes mellitus and obesity. Six isoforms are now known in GLUT family. They differ in tissue-specific expression and regulation, yet share a common transmembrane topology showing a highly conserved transmembrane domain, and less conserved cytoplasmic and exoplasmic domains. Evidence indicates that the transmembrane domain accommodates a water-filled glucose pathway (the catalytic domain). Little is known about the role of the exoplasmic domain. As for the cytoplasmic domain, a large body of evidence indicates its importance in tissue-specific regulation of GLUT function (the regulatory domain). This domain includes the N-terminal segment, the large, central loop, and the C-terminal segment. Exactly how this domain participates in GLUT regulation is not known. An interesting possibility is that the regulation involves a specific cellular protein, which interacts with GLUT at the cytoplasmic domain and modulates the function. This putative, glucose transporter binding protein (GTBP) may be an enzyme, or a nonenzymatic adaptor or docking protein. Indeed, we have identified several cytosolic proteins that bind to the cytoplasmic domain of GLUT proteins; these include glyceraldehyde-3-phosphate dehydrogenase, glucokinase, GTBP70, GTBP85, GTBP28, and L-3-hydroxyacyl

CoA dehydrogenase. Interaction of GLUT protein with some of these GTPBs are functionally coupled. Whether any of these interactions is actually responsible for the insulin-induced GLUT regulation is yet to be determined.

Introduction

Glucose transport in animal cells

Virtually all living cells use glucose as a major energy and carbon source. The very first critical step of this glucose utilization is the entry (transport) of glucose into the cell across the plasma membrane diffusion barrier with a speed and selectivity compatible with metabolic requirement. Three distinct mechanisms are known to mediate this glucose entry (Silverman, 1991). The simplest of these is the mechanism known as facilitated diffusion or carrier-mediation (Jung, 1974). This mechanism is subserved by a family of intrinsic membrane proteins (the facilitated glucose transporter or GLUT, in short) (Pessin and Bell, 1992), and simply catalyses the transmembrane movement of glucose and other sugars with an enzyme-like stereoselectivity. It is an equilibrating system; it does not produce any glucose accumulation by itself. This particular glucose transport mechanism is found in all eukaryotic cells. Second mechanism is the so-called, sodium-glucose cotransport (recently reviewed by Koepsell and Spangenberg, 1994), which is subserved by another intrinsic membrane protein family known as SGLT family. This mechanism is different from the facilitated diffusion in that it catalyses an up-hill movement of glucose driven by a down-hill movement of sodium ion. This mechanism is operative in the apical membrane of epithelial cells as an essential step of transcellular uphill transport (absorption) of glucose. The third mechanism is known as group-translocation (Meadow *et al.*, 1990). It is found only in bacteria, and subserved by the phosphoenolpyruvate phosphotransferase system (PTS). It catalyzes transmembrane movement of glucose and, at the same time, its phosphorylation, producing an intracellular accumulation of glucose phosphate. This review deals with the facilitated diffusion subserved by GLUT family only.

Six isoforms of the facilitative glucose transporter family

So far six distinct functional isoforms of GLUT family (GLUT1-5, and 7) have been identified by molecular cloning (Table I). They show a high amino acid

Table I. The facilitated glucose transporter isoforms

Isoforms	Primary expression sites and other characteristics	References
GLUT1	Human erythrocytes, brain endothelial cells, and transformed cells	(Mueckler <i>et al.</i> , 1985)
2	Liver, pancreatic beta cells, kidney and intestinal epithelia	(Asano <i>et al.</i> , 1989)
3	Neurons and placenta	(Kayano <i>et al.</i> , 1988)
4	Skeletal and heart muscles, adipocytes. Regulated by insulin.	(James <i>et al.</i> , 1989)
5	Small intestine and sperm cells. High affinity fructose transporter	(Kayano <i>et al.</i> , 1990)
6	No gene product	(Kayano <i>et al.</i> , 1990)
7	Liver. Mediates glucose exit from the endoplasmic reticulum	(Waddell <i>et al.</i> , 1992)

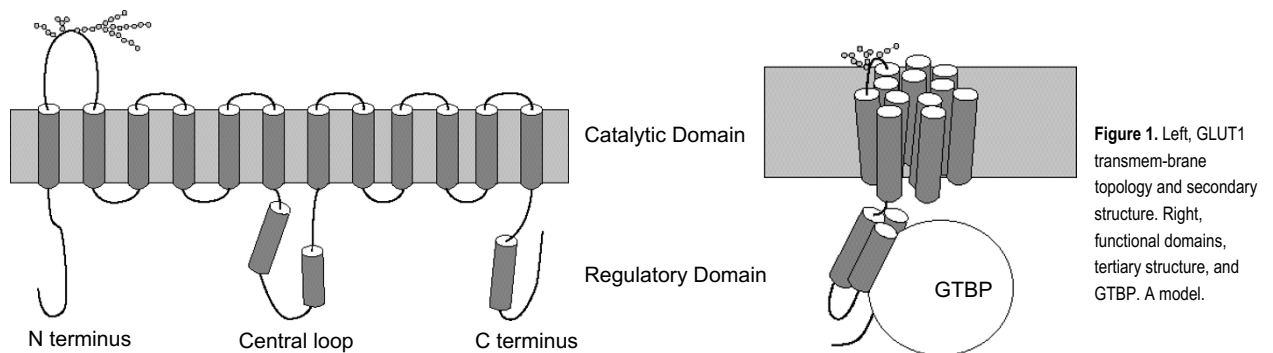


Figure 1. Left, GLUT1 transmembrane topology and secondary structure. Right, functional domains, tertiary structure, and GTBP. A model.

sequence homology, yet differ significantly in tissue-specific expression, transport kinetics, substrate specificity, and, most importantly, in response to metabolic and hormonal regulation (Stephens and Pilch, 1995). Thus, insulin regulates GLUT4 function in muscles and adipocytes, a key control point for glucose homeostasis in humans and mammals (Birnbaum, 1992). The dysfunction of this regulation is known to produce the insulin resistance seen in diabetes and obesity (Garvey, 1992; James and Piper, 1994).

Transmembrane topology common to all GLUT isoforms

The cDNA-deduced amino acid sequences of six GLUT isoforms (Mueckler *et al.*, 1985; Kayano *et al.*, 1988; Asano *et al.*, 1989; James *et al.*, 1989; Kayano *et al.*, 1990), together with the biochemical data obtained from purified GLUT1 protein (Kasahara and Hinkle, 1976; Baldwin *et al.*, 1982; Cairns *et al.*, 1987; Alvarez *et al.*, 1987) indicate that all of these isoforms share a common transmembrane topology, having a large (about 50% of protein mass) transmembrane domain, with two grossly asymmetric nonmembrane domains, the cytoplasmic domain (about 35% of protein mass) and the exoplasmic domain (Figure 1). The trans-

membrane domain is highly (97%) conserved among the isoforms. Evidence indicates that this domain, made of twelve membrane-spanning alpha helices (transmembrane helices or TMHs 1-12) some of which line a water-filled glucose pathway (Mueckler *et al.*, 1985; Jung *et al.*, 1986), is similar for all isoforms (the catalytic domain). The cytoplasmic domain includes the amino and carboxyl termini, and the large central loop between TMHs 6 and 7. The exoplasmic domain includes a fairly large, glycosylated loop between TMHs 1 and 2. These nonmembrane domains show a significant amino acid sequence divergence, which suggests that they play a role in tissue-specific regulation of transporter function (the regulatory domain). Little data is available for the functional significance, if any, of the exoplasmic domain. For the cytoplasmic domain, an ample body of evidence indeed points to its importance in tissue-specific regulation of GLUT function, as will be discussed (*vide infra*). Exactly how, at the molecular level, the cytoplasmic domain participates in the regulation is not known. It is quite likely that specific cytosolic protein or proteins interact with GLUT protein at the cytoplasmic domain, thus modulates GLUT function in response to hormonal or metabolic signals (James *et al.*, 1994).

How GLUT proteins transport glucose?

Transmembrane domain structure and glucose channel

In contrast to the rapid progress witnessed in cloning these isoforms, the progress in understanding the structural basis of GLUT function and regulation has been disappointingly slow. GLUT1 of human erythrocytes is the only isoform available as a pure and functional protein (Rampal *et al.*, 1986). Thus, the biochemical, biophysical, and structural informations available for this protein family are almost exclusively on this isoform. Circular dichroism measurements (Chin *et al.*, 1987) have revealed that GLUT1 contains 70–80% alpha helices and some random coil and beta-turns (10% each), with no beta-structures. These data not only support the transmembrane helical structure predicted by hydropathy analysis (Mueckler *et al.*, 1985), but also suggest the presence of a significant amount of alpha-helices in the nonmembrane domains, particularly in the cytoplasmic domain. Linear dichroism and Fourier transform infrared (FTIR) spectral measurements on oriented film (Chin *et al.*, 1986) further indicate that all the twelve TMHs in GLUT1 are nearly perpendicular to the plane of the membrane, while nonmembrane (cytoplasmic) domain helices are quite flexible. Furthermore, hydrogen-tritium and hydrogen-deuterium exchange studies (Jung *et al.*, 1986) offered a strong indication for the presence of an aqueous pore for glucose passage.

Based on these and other findings, we (Zheng *et al.*, 1996) have recently proposed a working model for the structure of GLUT1 transmembrane domain, including the putative glucose channel. In this model, we emphasized the physical dimensional requirement of substrates and helical packing energy and macrodipole moments of the putative 12 TMHs, and predicted that TMHs 2, 5, 7, 8, and 11 form glucose channel. The model predicts discrete TMH-lipid bilayer interface, TMH-transporter substrate interaction, as well as an interhelical proximity pattern for each TMH. This model is significantly different from the one previously proposed by Holman and his coworkers (Gould and Holman, 1993): They have proposed, primarily based on inhibitor binding studies, that only the C-terminal half of the transmembrane domain (namely, TMHs 7–12) participates in glucose transport function. More recently, we (Lachaal *et al.*, 1996) have shown that 3-deoxy-3,3-azido-D-glucopyranose (3DAG), a photolytically-reactive GLUT1 substrate, labels the 30-kDa trypsin cleavage product containing TMHs 4 and 5. This is a clear indication that both, in support of our model, N- and C-terminal halves of the transmembrane domain line aqueous glucose channel.

Substrate transport is known to accompany

significant conformational dynamics in GLUT1 protein (Rampal *et al.*, 1987; Appleman and Lienhard, 1989). By studying the intrinsic fluorescence quenching of purified GLUT1 protein, we (Chin *et al.*, 1992) have demonstrated dynamic interactions between Trp 388 and His 337, Trp 412 and Cys 347, and Trp 412 and Glu 380, depending on liganded state of GLUT1. We suggested that the kinky, bulky, and proline-rich cytoplasmic end of TMH-10 may form a cam structure, and that this structure may undergo oscillational rotation upon binding and release of substrate, while cytochalasin B and forskolin block transport in part by preventing this oscillation.

How GLUT proteins are regulated?

The three different levels of GLUT regulation

By far the most important and well established mode of GLUT regulation is the stimulation of GLUT4 function by insulin in muscle and adipose cells (James and Piper, 1994). In the absence of insulin, the glucose transport activity (flux) in these two classical insulin target cells is very slow (by 100 fold) compared to those in noninsulin target cells such as brain, liver, or human erythrocytes, and it increases greatly upon insulin treatment, approaching to the speed that is seen in metabolically insulin-insensitive group of the cells (Silverman, 1991; Carruthers, 1990). Consistent with these long known facts is the finding that more than 95% of GLUT4 and 30–40% of GLUT1 in rat adipocytes are stored intracellularly, thus nonfunctional, and that insulin recruits these GLUTs from the storage pool to the plasma membrane (recruitment theory) (Cushman and Wardzala, 1980; Suzuki and Kono, 1980). In the former group of cells whose glucose transport function is not affected by insulin, practically all GLUTs are at the cell surface even in the absence of insulin.

The insulin-induced GLUT recruitment discussed above, however, does not always account for the entire insulin-induced glucose transport stimulation quantitatively, suggesting that insulin also increases the intrinsic activity of glucose transporters (Obermair-Kusser *et al.*, 1990; Harrison *et al.*, 1989). Recently, it also became clear that the intrinsic activity of GLUT1 is regulated in muscles, avian erythrocytes, and a cultured hepatocyte line in response to metabolic alteration such as reduced oxidative phosphorylation and hypoxia (Lawrence *et al.*, 1990; Shetty *et al.*, 1992).

Like other biologically important proteins, GLUT proteins are also regulated by alterations in the protein content available in cell. This cellular GLUT protein content, in turn, is determined by the rate of biosynthesis and the rate of degradation. Evidence indicates that

both of these rates are regulated in muscle and adipocytes. Significant reduction in cellular GLUT4 or GLUT1 content occurs in experimentally induced diabetes in rats (Berger *et al.*, 1989; Garvey *et al.*, 1989; Moller and Flier, 1991). Reduction in cellular GLUT4 content in adipocytes of diabetic rats is accompanied by a compensatory reduction in the rate of GLUT4 degradation (Kim *et al.*, 1994). In normal rat adipocytes, GLUT4 biosynthetically turns over with a half time of approximately 12 h, which increases to 15 h or greater (each GLUT protein lives longer) when its synthesis was reduced in streptozotocin treated rats.

The cytoplasmic domain may participate in GLUT regulation

Evidence indicates that the cytoplasmic domain modulates GLUT protein catalytic activities: Lawrence Jr. *et al.* (1990) have shown that the phosphorylation of a C-terminal serine residue appears responsible for isoproterenol-induced inactivation of GLUT4 transport activity. Oka *et al.* (1990) have shown that a partial truncation of GLUT1 C-terminal sequence results in complete inactivation of GLUT1 transport activity. These findings suggest that the C-terminal cytoplasmic domain may modify the glucose pathway structure of the transporter. This modulation is likely to be regulated by a specific cellular protein through an interaction at this domain. Consistent with this notion is the experimentally supported suggestion that the cytoplasmic nonmembrane domain of GLUT1 contains a large amount of alpha-helices of flexible disposition (Chin *et al.*, 1987).

Evidence also indicates that the cytoplasmic domain contains the putative molecular determinants for the constitutive and insulin-regulated GLUT targeting to the plasma membrane. Results of transfection of GLUT mutants and chimera in cultured cell lines (Mueckler, 1994; James *et al.*, 1994; Verhey and Birnbaum, 1994; Verhey *et al.*, 1993) support this contention. These studies show that primarily the C-terminus, and in part the central loop, determine constitutive propensity of GLUT1 and GLUT4 targeting to the plasma membrane. The N-terminus, on the other hand, may be important for the retention of GLUT4 in its microsomal storage pool (James *et al.*, 1994). These gene transfection experiments, however, failed to answer one important question, namely, which of these cytoplasmic subdomains is important in insulin-induced recruitment. This question can be answered most clearly if one can introduce each of mutated isoforms with a modified cytoplasmic domain as a purified protein into rat adipocytes, the best understood insulin target cell in terms of GLUT regulation, and study their behavior in response to insulin. We have shown that PEG-induced cell fusion protocol using rabbit erythrocyte ghosts

makes such a reconstitution possible (unpublished data by Lee and Jung).

Kinetic basis of insulin-induced GLUT4 recruitment

Steady state kinetic measurements using an impermeable, photoreactive glucose analog have revealed that GLUT4 in rat adipocytes constantly recycles between the plasma membrane pool and its intracellular storage pool, with two discrete first-order rate constants, one for internalization (k_{in}), and the other for externalization (k_{ex}) (Jhun *et al.*, 1992). The study also revealed that insulin affects both rate constants, it reduced k_{in} by 2.8 fold and increased k_{ex} by 3.3 fold. These findings indicate that insulin-induced GLUT4 recruitment results from modulation of at least two, rate-limiting steps, namely, endocytosis and exocytosis, the biochemical basis of which may also be distinct, thus should be studied individually.

Okadaic acid, a well-known specific inhibitor of protein phosphatases 1 and 2A (PP1 and PP2A), stimulates glucose transport in adipocytes by inducing insulin-like recruitment of GLUT4 to the plasma membrane. By steady-state kinetic measurements, Jhun *et al.* (1995) have demonstrated that okadaic acid selectively increases GLUT4 externalization rate constant (k_{ex}) in rat adipocytes. Okadaic acid and insulin are not additive in the effect on GLUT4 recruitment; okadaic acid at higher concentrations interferes with the insulin effect. Based on these findings, they have suggested that insulin may also stimulate GLUT4 externalization by increasing the phosphorylation state of a specific serine/threonine phosphoprotein, possibly by a specific PP1 or PP2A. It is an interesting possibility that a phosphatase, or its associated protein, may interact with thus modulate GLUT4 externalization.

How GLUTs receive insulin and metabolic signals

Exactly how the cytoplasmic domain of a GLUT protein may modulate GLUT catalytic activity or participate in GLUT redistribution suggested above is an important open question. Protein-protein interaction is known to play a key role in many important cellular functions and regulations (Srere, 1987; Phizicky and Field, 1995; Myers Jr. and White, 1995), thus it is quite likely that a specific protein or proteins mediates the insulin signal by interacting with GLUT4 at its cytoplasmic domain. When insulin binds to its receptor, it activates the receptor tyrosine kinase activity by autophosphorylation (Kasuga *et al.*, 1982). The activated tyrosine kinase of the insulin receptor causes tyrosyl-phosphorylation of IRS-1 (Rothenberg *et al.*, 1991). The IRS-1 in turn interacts with and activates PI3-kinase (Backer *et al.*,

1992). This IRS-1-PI3-kinase interaction has been suggested to be part of the signal transduction pathway for insulin-induced GLUT4 redistribution (Kanai *et al.*, 1993; Katagiri *et al.*, 1996). The downstream elements in this signal pathway that link PI3 kinase and the related signal to GLUT4, however, are yet to be identified (Marcusohn *et al.*, 1995). It is quite likely that this downstream pathway also involves a series of protein-protein interactions, including one that directly interacts with GLUT4 protein and modulates its subcellular redistribution or catalytic activity. The protein could be an enzyme such as a specific phosphokinase or phosphatase that covalently modifies GLUT4. It may be a non-enzymatic adaptor protein. To identify this putative GLUT4 regulatory protein, we have used several different assay methods and identified a number of glucose transporter binding proteins (GTBPs), as summarized below.

GLUT binding proteins

The possible association of GLUT proteins with other cellular proteins *in vivo* was first suggested by large target size, observed for glucose-sensitive cytochalasin B-binding activity in rat adipocytes (Jacobs *et al.*, 1987), and subsequently supported by an *in vitro* demonstration of an ATP-modulated association of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with purified GLUT1 (Lachaal *et al.*, 1990). More recently, the apparent requirement of GLUT2 expression in pancreatic beta cells for the insulin secretory response to ambient glucose (Unger, 1991) has been shown to be best explained by assuming an isoform-specific interaction of GLUT2 with glucokinase (Lachaal *et al.*, 1993), again emphasizing the importance of a protein-protein interaction. Indeed, purified bacterial glucokinase (GK) binds to GLUT1 purified from human erythrocytes *in vitro* (Lachaal and Jung, 1993). This binding was abolished if GLUT1 was digested with trypsin, indicating that glucokinase binds to the cytoplasmic domain of GLUT1. The binding was stimulated by ADP and glucose 6-phosphate, but not by ATP and D-glucose. The binding caused no change in GLUT1 function, but it increased glucokinase enzymatic activity. A similar interaction may occur between GLUT2 and glucokinase and generate glucose signal for insulin secretion in pancreatic beta cells.

Using glutathione S-transferase (GST)-fusion protein technology, Liu *et al.* (1995) have identified a 70-kDa cytosolic protein (GTBP70) in rat adipocytes that binds to the cytoplasmic domains of GLUT1, GLUT2 and GLUT4. The binding showed little isoform-specificity, but was significantly subdomain-specific; it bound to the C-terminal domain and the central loop, but not to the N-terminal domain of GLUT4. The binding was inhibited

by ATP in a dose-dependent manner, but not by ADP and AMP-PCP. GTBP70 was not metabolically labeled with ³²P, suggesting that it is not a phosphoprotein. The binding, however, was little affected by insulin treatment. The role, if any, of GTBP70 in glucose transporter regulation in adipocytes is yet to be demonstrated.

Also using GST-fusion proteins, Shi *et al.* (1995) have identified two GTBPs (GTBPs 28, and 85) in Clone 9 cell cytosol, which may be involved in azide-induced GLUT1 modulation. A brief (1-2 h) exposure to azide is known to increase glucose uptake in Clone 9 cells, without increasing the plasma membrane level of GLUT1, the only GLUT isoform expressed in these cells (Shetty *et al.*, 1992). The glucose-displaceable, cytochalasin B-binding activity was barely detectable in Clone-9 cells, but increased greatly after a 2-h incubation with azide. Furthermore, the cytochalasin B binding activity of purified GLUT1 was inactivated by azide-treated Clone 9 cell cytosol, but not by untreated cytosol. These findings strongly suggest the presence of a cytosolic protein in Clone 9 cells that inactivates GLUT1, and an azide-induced metabolic alteration releases GLUT1 from this inhibitory interaction. Shi *et al.* (1995) showed that GTBP28 is a 28-kDa cytosolic protein, and binds to the cytoplasmic domain of GLUT1 and GLUT4. They also demonstrated that GTBP85 is a 85-kDa phosphoprotein, and binds to GLUT4, but not to GLUT1, and only in azide-treated Clone 9 cytosol, but not in control cytosol. The roles, if any, of these proteins in azide-induced modulation of GLUT1 substrate-binding activity are to be demonstrated.

More recently, we used a yeast-based, two hybrid system (Yang *et al.*, 1992; Guarente, 1993) to search for GTBPs. Screening a human skeletal muscle library with GLUT4 C-terminus as bait, revealed one clone (C-10) which encodes an interactive protein (unpublished observation by Shi, Y.W., Samuel, S.J., Lee, W., Yu, C.H., Zhang, W., Lachaal, M. and Jung, C.Y.). The interaction was not an artifact of two hybrid system, as C-10 by itself, GAL4bd alone, lamin, or Glut1C was incapable of activating beta-galactosidase activity. The deduced amino acid sequence of this protein (YP10) was found to be 89% identical, and 92% similar, to the well-known, pig heart mitochondrial L-3-hydroxyacyl CoA dehydrogenase (HAD). An *in vitro* binding assay using GST-YP10 fusion protein and synthetic peptides confirmed that YP10 binds to GLUT4C, but not to GLUT1C. Most significantly, recombinant YP10 *in vitro* showed the dehydrogenase activity of HAD, which was inhibited by GLUT4C peptide, but not by GLUT1C peptide. YP10 crossreacts with anti-HAD antisera in immunoblot. Subcellular distribution of anti-HAD antisera immunoreactivity revealed that YP10 and/or HAD are/is associated not only with mitochondria, but also with the plasma membrane, but is virtually absent

in microsomes in rat adipocytes. This suggests that YP10 is an isoform of HAD that interacts with GLUT4 selectively at the plasma membrane, and is thereby down-regulated. The physiological significance of this interaction is not known. It may be a part of GLUT4 regulation mechanism involving protein acylation.

Other potential GTBPs which may participate in modulation of GLUT4 recycling

There are a few interesting GTBP candidates that may be involved in regulation of GLUT4 recycling. The GLUT4 recruitment in rat adipocytes in response to insulin is controlled at two discrete rate-limiting steps (represented by the first-order rate constants, k_{in} and k_{ex}). This suggests that separate mechanisms for endocytosis and exocytosis, where two distinct GTBPs, X and Y, modulate k_{in} and k_{ex} , respectively. X could be a clathrin or AP2-like protein if not clathrin or AP-2 itself (Moore *et al.*, 1987). Y could be NSF and/or SNAP like protein(s) if not NSF or SNAP itself (Warren, 1990). They may also be specific GTP-binding proteins (Keller *et al.*, 1991; Baldwin *et al.*, 1991; Douset and Tuana, 1991) or specific kinases (Del Vecchio and Pilch, 1991; Clancy and Czech, 1990). *N*-ethylmaleimide (0.05-1 mM) inhibits the insulin-stimulated (but not the basal) glucose transport, and arrests insulin-induced (but not basal) GLUT4 translocation (data to be published). This suggested us that a *N*-ethylmaleimide-sensitive factor (NSF) may be required for GLUT translocation. NSF and SNAP may be involved in GLUT4 externalization, most likely acting at the step of IRGTV fusion to plasma membrane. It is also known that *N*-ethylmaleimide at low concentrations (0.1 mM or above) interacts with a membrane protein and effectively inhibits LDL-receptor internalization in fibroblasts (Lin *et al.*, 1991).

Concluding remarks

Understanding at the molecular level how insulin causes GLUT4 recruitment in normal cells would give an insight to the identification of the nature of the defect underlying the insulin resistance. Although exact nature is not known, it becomes increasingly evident that the cytoplasmic domain of GLUT proteins is involved in this insulin-induced GLUT recruitment by interacting with a specific cellular protein or proteins. These putative regulatory proteins are expected to modulate one or both of the two rate-limiting steps in GLUT4 recycling. The identification, cloning, and characterization of this GLUT4 regulatory GTBP will contribute significantly to the elucidation of the molecular mechanisms by which insulin regulates glucose transport in normal muscles and adipose tissue.

Since the two hybrid screening and the *in vitro* binding assay using GST-fusion protein use GLUT4

cytoplasmic domain segments rather than whole protein, they may miss the GTBP-GLUT4 interaction responsible for the insulin action, if the cytoplasmic segments do not fold into a native conformation. Although not likely, the interaction of X with GLUT may not involve the cytoplasmic domain. Finally, the putative protein X may not exist; GLUT4 targeting may be regulated by a non-protein, secondary messenger such as lipid. Even in these unlikely situations, the systematic search as proposed would certainly uncover a number of GTBPs that may be important for other aspects of the GLUT function and regulation. These include regulation at the levels of catalytic activity, and of metabolic turnover. Alternatively, some GTBPs identified may be an important enzyme whose function is regulated via its interaction with GLUT4.

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