

Minireview: Recent Developments in the Regulation of Glucose Transporter-4 Traffic: New Signals, Locations, and Partners

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Glucose transporter (GLUT) 4 is the major glucose transporter of muscle and adipose cells, exquisitely regulated by insulin through posttranslational events. Twenty years after the seminal observations that GLUT4 levels rapidly rise at the plasma membrane (PM) and drop in endomembranes in response to an acute insulin challenge, we are still mapping the intracellular traffic of the transporter and the regulatory events that insulin unleashes. Newly synthesized GLUT4 enters an insulin-responsive compartment aided by GGA2 (an Arf-binding protein). In cultured adipocytes and myocytes, GLUT4 concentrates in a perinuclear pole through participation of microtubules and the EHD1 Eps15 homology domain-containing protein 1. In the absence of stimuli, GLUT4 distributes between recycling endosomes and the insulin-responsive compartment. A handful of proteins that bind to GLUT4 appear to regulate its half-life (*e.g.* Ubc9) and tethering within endomembranes (*e.g.* TUG). Insulin-derived signals

promote not only GLUT4 mobilization toward the PM but also its traffic between endosomal compartments and internalization from the PM. Class IA phosphatidylinositol (PI) 3-kinase plays a pivotal role at several steps of GLUT4 mobilization. The PI 3-kinase $\rightarrow$ atypical PKC and $\rightarrow$ Akt/PKB $\rightarrow$ AS160 signaling cascades are major regulators of GLUT4 exocytosis aided by small GTPases. At the cell periphery, GLUT4-containing vesicles tether, dock, and fuse with the PM assisted by the exocyst complex followed by engagement of a soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex [with vesicle-associated membrane protein (VAMP)2 as the vesicular (v)-SNARE and soluble NSF-attachment protein (SNAP)23 and syntaxin4 as target (t)-SNAREs] regulated by the accessory proteins Munc18c, Synip and Tomosyn. Vesicle tethering and fusion are regulated by insulin through input from class IA PI 3-kinase. (*Endocrinology* 146: 5071–5078, 2005)

GLUT4 IS A MEMBER of the facilitative glucose transporter (GLUT) family, characterized by preferential expression in muscle and fat tissues, where it is responsible for insulin-stimulated glucose uptake, and for the entry of glucose to muscle during contraction/exercise. GLUT4 is unique among other members of the GLUT family in its dynamic cycling within the muscle and adipose cell. In unstimulated cells, GLUT4 is rapidly removed from the plasma membrane (PM) to which it recycles only slowly, leading to a steady-state accumulation in intracellular organelles including the *trans*-Golgi network (TGN), recycling endosome (RE), and diverse tubulo-vesicular bodies. The intensive research on signals regulating GLUT4 traffic of the past decade

has been summarized in excellent reviews (1–3). Here we focus on recent discoveries of regulatory events downstream of Akt/protein kinase B (PKB), on new aspects of GLUT4 vesicle biogenesis, and novel developments on the regulation of GLUT4 at the PM.

Cellular Models Used to Study GLUT4 Regulation

Much of our knowledge and new developments on GLUT4 traffic stem from work in muscle and adipocyte cell lines, which approximate but may not fully represent events occurring in mature muscle and adipose cells. In particular, 3T3-L1 adipocytes have been extensively used as the gold standard, with insulin-stimulating glucose uptake about 10-fold above basal levels. By comparison, isolated rat adipocytes mount a greater than 20-fold response to the hormone, whereas human adipose cells response a mere 2- to 3-fold. Moreover, 3T3-L1 adipocytes express elevated levels of the housekeeping glucose transporter GLUT1, which contributes to basal as well as insulin-stimulated glucose uptake along with GLUT4. In these cells, GLUT4 is located preferentially in perinuclear depots and to a lesser extent in cytosolic vesicles in these cells, whereas the latter predominate in isolated rat adipocytes. Unlike the mature muscle fiber, primary muscle cell cultures express minimal levels of GLUT4, glucose uptake occurring largely through GLUT1 instead. Of the skeletal muscle cells available, only rat L6 myotubes express GLUT4, and this at levels 5- to 10-fold lower than those in mature skeletal muscle. In L6 myotubes, GLUT4 is largely expressed in perinuclear regions and dispersed in

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Abbreviations: EE, Early endosome; EHD1, Eps15 homology (EH) domain-containing protein 1; EHBP1, EHD2-binding protein 1; GFP, green fluorescent protein; eGFP, enhanced GFP; GLUT, glucose transporter; GSV, GLUT4-storage vesicles; HA, hemagglutinin; IRAP, insulin regulated amino-peptidase; PI 3-kinase; phosphatidylinositol 3-kinase, PI(3,4,5)P₃; phosphatidylinositol 3,4,5-trisphosphate; PI(3,4)P₂, phosphatidylinositol 3,4-bisphosphate; PI3P, phosphatidylinositol 3-phosphate; PKB, protein kinase B; PKC, protein kinase C; PM, plasma membrane; RE, recycling endosomes; SC, specialized compartment; siRNA, small interfering RNA; SNARE, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors; t, target; TfR, transferrin receptor; TGN, *trans*-Golgi network; TUG, tethering protein containing a UBX domain for GLUT4; v, vesicular; VAMP, vesicle-associated membrane protein.

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discrete bodies along the cytosol, which is unobstructed by sarcomeric actin-myosin characteristic of mature skeletal muscle. Insulin induces a 2-fold increase in glucose uptake in L6 myotubes that fits closely the effect of the hormone in isolated muscles from rodents or humans. Stable expression of *myc*-tagged GLUT4 in L6 myoblasts that differentiate into myotubes is a useful system to study GLUT4 dynamics in muscle cells.

Intracellular GLUT4 Compartments: How Specialized Are They?

In unstimulated muscle or adipose cells, more than 90% of GLUT4 is intracellular and a mere 4–10% resides at the PM, the result of slow exocytosis and fast endocytosis (4, 5). Electron microscopy or immunofluorescence studies in primary or cultured adipose cells detect the majority of GLUT4 in tubulo-vesicular structures in the perinuclear region and cytosol (6–8). Upon subcellular fractionation, GLUT4 colocalizes in part with markers of the TGN, Golgi complex, and RE. In the basal state, chemical ablation of the transferrin receptor (TfR)-rich compartments of 3T3-L1 adipocytes spares about half of the GLUT4 complement, leading to the concept that a specialized compartment harbors GLUT4 that may or may not be able to recycle to the PM in the basal state (9, 10). In L6 myoblasts stably expressing *myc*-tagged GLUT4 and in 3T3-L1 adipocytes transiently expressing hemagglutinin (HA)-tagged GLUT4-eGFP [enhanced green fluorescent protein (GFP)], all the transporters recycle in the basal state, and insulin markedly accelerates such recycling (5, 10). However, in 3T3-L1 adipocytes stably overexpressing HA-tagged GLUT4 (achieved by retrovirus gene transfer), a proportion of transporters fail to recycle (11). Hence, the existence of a static pool of GLUT4 in adipose cells is a matter of current debate, and solving this may require careful analysis of the behavior of the transfected GLUT4 in each study.

Whether static or dynamic, half of the GLUT4 molecules in 3T3-L1 adipocytes and L6-GLUT4*myc* myoblasts escape colocalization with TfR or GLUT1 and instead colocalize with the v-SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors) vesicle-associated membrane protein (VAMP)2 (12–14). This latter protein segregates away from the TfR (15) and is required for fusion of the GLUT4 vesicles mobilized by insulin with the PM. Interestingly, VAMP2 is not involved in the fusion of the continuously recycling GLUT4 vesicles with the PM. Moreover, GLUT4 recruitment to the cell surface by platelet-derived growth factor or hyperosmolarity is also independent of VAMP2 and instead appears to involve the vesicular (v)-SNARE TI-VAMP/VAMP7 (16, 17). These observations suggest the existence of a GLUT4 specialized compartment that can be regulated by insulin and is characterized by the presence and functional requirement of VAMP2 for final fusion with the PM. Names assigned to such a compartment include specialized compartment (SC) and GLUT4-storage vesicles (GSV). Furthermore, these findings suggest that the GLUT4 vesicles reaching the PM in response to insulin may differ from those arriving in the basal state. Such a conclusion is also supported by studies comparing the segregation and recycling of GLUT4 and the TfR in 3T3-L1 adipocytes, suggesting that insulin stimulates the transfer of GLUT4 from

the RE to SC/GSV as well as translocation of GLUT4 from SC/GSV to the PM (10, 18). However, a contrasting model proposes that GLUT4 molecules stored in elements of the TGN recycle back to the RE in response to insulin from where they are stimulated to exit to the PM in response to the hormone (19, 20). Further studies are required to reconcile these models. In both models, GLUT4 is dynamically regulated by insulin, not only its mobilization toward PM but also its traffic between endosomal compartments (Fig. 1).

The cellular localization of the SC/GSV remains to be elucidated, but given the large proportion of GLUT4 in a perinuclear region it is speculated that the insulin-responsive compartment may reside within this cohort. The tight, polarized perinuclear distribution of GLUT4 is disturbed by a variety of cellular manipulations that include treatment with brefeldin A, with microtubule and microfilament disrupting agents, or by interfering with the expression of proteins among which EHD1 [Eps15 homology (EH) domain-containing protein 1] stands out. Whereas neither brefeldin A nor some microtubule disruptors prevent the insulin-induced GLUT4 mobilization to the PM, agents that inhibit actin remodeling halt GLUT4 translocation, presumably through events additional to the dispersion of the perinuclear GLUT4 cluster. EHD1 regulates recycling of a variety of membrane proteins (21, 22) including GLUT4, as expression of a mutant EHD1 missing the EH domain that links it to the EHD2-binding protein 1 (EHBP1), or small interfering RNA (siRNA) to EHD1 dispersed GLUT4 throughout the cytosol, and concomitantly impaired insulin-induced GLUT4 gain at the PM (23).

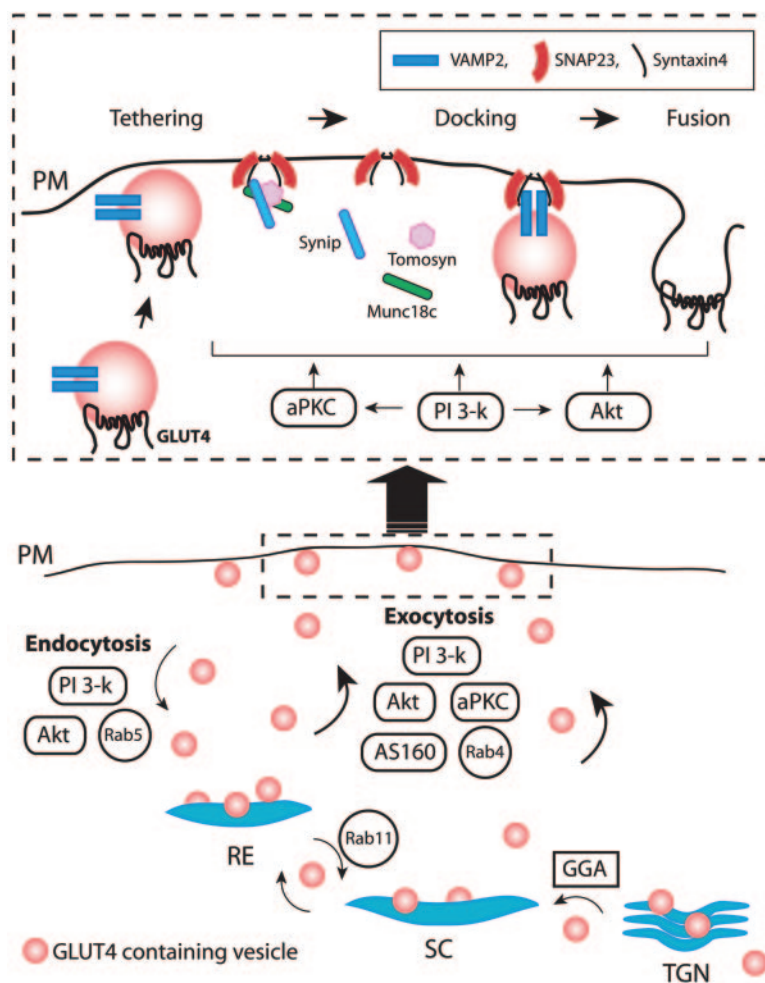
In mature adipocytes and muscle cells, GLUT4 is a long-lived protein ($t_{1/2}$ of about 40 h), so each polypeptide chain is likely to cycle to the PM many times during its lifetime. An emerging question is then whether newly synthesized GLUT4 polypeptides must reach the PM before entering the endosomal sorting cycle. Recent studies indicate that newly synthesized GLUT4 enters the insulin-sensitive compartment directly from the Golgi/TGN, aided by GGA (Golgi-localized, γ -ear-containing, Arf-binding protein) (24). Although the N terminus tail and the large cytosolic loop of GLUT4 contain amino acid motifs important for its transit from the Golgi/TGN to the SC/GSV (25), GGA does not directly interact with GLUT4 but rather with sortilin and the cation-dependent and -independent mannose-6-phosphate receptors, proteins found in isolated GLUT4-containing endomembranes (26).

Sortilin is also required for the formation and maintenance of SC/GSV, as demonstrated by the generation of small vesicles containing ectopic *myc*-GLUT4 upon coexpression of sortilin in preadipocytes, and by the loss of vesicles upon reduction of endogenous sortilin via siRNA in mature adipocytes. The sortilin-induced formation of SC/GSV enhances the half-life of GLUT4 and its response to insulin, strongly suggesting that SC/GSV protects GLUT4 from degradation and presents it to insulin-derived signals (27).

Regulatory Signals Governing GLUT4 Traffic

Insulin stimulation of muscle and adipose cells causes a net gain in surface GLUT4 that peaks within 10–15 min of

FIG. 1. Schematic representation of GLUT4 traffic to and from the plasma membrane. Newly synthesized GLUT4 enters the insulin-responsive SC (also called GSV) from the TGN, regulated by GGA. Insulin accelerates GLUT4 exocytosis from SC/GSV and possibly also from the RE resulting in accumulation of GLUT4 at the PM. Involvement of exocyst components is predicted to allow GLUT4-containing vesicle movement to appropriate sites for docking and fusion. SNARE mechanisms accomplish these processes along with accessory proteins (see text). The involvement of small GTPases at distinct steps is indicated. At the PM, class IA PI 3-kinase (PI3-k) input regulates vesicle fusion with the PM. Proteins involved in GLUT4 vesicle tethering and fusion with the PM is illustrated within the dotted-line square.



stimulation, brought about by a robust increase in the rate of GLUT4 exocytosis and a smaller reduction in its endocytosis. The insulin-derived signaling pathways regulating the net gain in surface GLUT4 are depicted in Fig. 2 and include phosphorylation of insulin receptor substrate-1 but not -2, activation of class IA phosphatidylinositol 3-kinase (PI 3-kinase) and of the serine/threonine kinases atypical protein kinase C (PKC) and Akt/PKB. The participation of each of these signals has been demonstrated by a concerted use of chemical inhibitors when available, expression of dominant-negative mutants, gene silencing, and gene knockout strategies. The reader is referred to recent reviews on the topic for further detail (28, 29). As well, PI 3-kinase-independent signals involving the sequence Cbl-CAP-APS-Crk II-C3G-TC10 contribute to GLUT4 gain at the surface of adipocytes (30) but not muscle cells (31). In the latter cells, a PI 3-kinase input independent of Akt contributes to cortical actin remodeling that is critical for the gain in surface GLUT4. The role of actin remodeling likely involves the generation of hot spots of signals and vesicles destined for fusion with the PM (32).

Importantly, the input of signaling molecules on individual steps in GLUT4 sorting, exocytosis, endocytosis, and fusion with the membrane remains to be precisely mapped. Recent developments have begun to shed light on the molecules that regulate GLUT4 at the level of its mobilization to

the surface, fusion with the membrane and retrieval through endocytosis, as reviewed next.

Insulin-Derived Signals and Proteins Regulating GLUT4 Sorting and Exocytosis

It is well accepted that class IA PI 3-kinase is required for the insulin-dependent gain in surface GLUT4, but until recently it was not known whether PI 3-kinase participates at the level of GLUT4 sorting to a compartment, at the level of GLUT4 mobilization to the membrane or at the level of the internalization of the transporter. Foster *et al.* (33) showed that PI 3-kinase activation is required for the rapid interendosomal transit of GLUT4, and Yang *et al.* (34) demonstrated that the net action of the enzyme is on the exocytic rather than the endocytic route of the transporter. Accordingly, carrier delivery into adipocyte or muscle cells of PI(3,4,5)P₃, the major phosphoinositide generated by class IA PI 3-kinase in response to insulin, promotes GLUT4 mobilization to the surface (35, 36).

The phosphoinositide signal is likely transmitted through PDK1 and its downstream targets Akt/PKB and atypical PKC (37–40). That each of these enzymes is required for the net gain in surface GLUT4 elicited by insulin is supported by the use of dominant-negative mutants, and in the case of

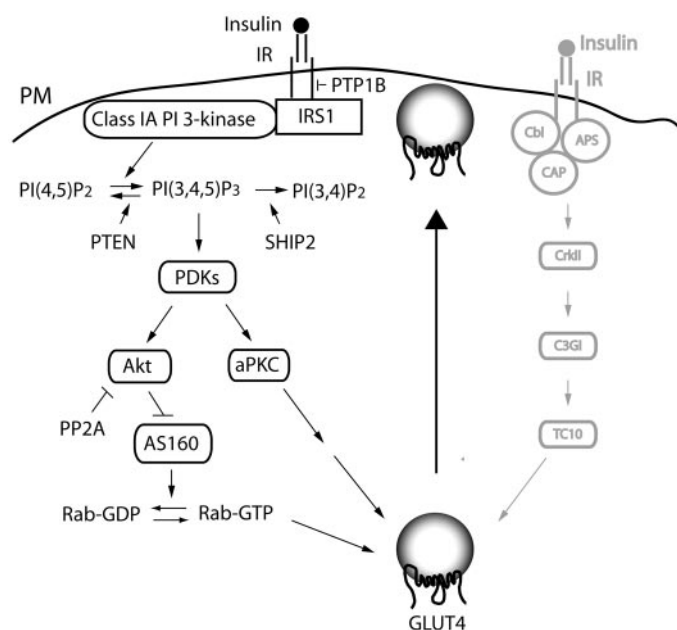


FIG. 2. Insulin signaling pathways regulating GLUT4 traffic. Insulin causes GLUT4 recruitment to the plasma membrane through two distinct major pathways. In the class IA PI 3-kinase-dependent pathway, PI(3,4,5)P₃, the major phosphoinositide generated by insulin, activates PDK followed by Akt and atypical PKC in muscle cells or adipocytes. Phosphorylation of AS160 allows GLUT4 traffic to proceed. In the adipose cell-specific, class IA PI 3-kinase-independent pathway, Cbl-CAP-APS complex formed by insulin causes recruitment of CrkII and C3G followed by TC10 activation facilitating GLUT4 traffic. Several phosphatases are predicted to negatively regulate these pathways.

Akt2, of specific gene elimination or gene silencing (41, 42). In the case of atypical PKC, its input appears to be at the level of exocytosis based on the inhibition by dominant-negative mutant of the enzyme of Rab4 binding to the motor protein KIF3B. The latter is thought to regulate GLUT4 mobilization to the membrane via microtubules (43).

Akt/PKB impinges on several steps of GLUT4 cycling. Evidence supporting input of Akt/PKB at the level of intracellular compartments includes: 1) migration of Akt/PKB to endomembranes containing GLUT4 in response to insulin (44); 2) prevention of GLUT4 interendosomal acceleration in cells expressing dominant-negative Akt/PKB (33); 3) sorting of a chimera of GLUT4 fused to Akt/PKB (45) and of IRAP (insulin-regulated amino-peptidase, a surrogate cargo of the SC/GSV) to the PM upon expression of constitutively activated Akt/PKB targeted to the GLUT4-containing compartment (46); and 4) prevention of insulin-induced GLUT4 translocation in cells expressing dominant-negative Akt/PKB fused to GLUT4 in 3T3-L1 adipocytes (46) although not in rat adipocytes (45).

AS160 is a *bona fide* Akt/PKB substrate with the molecular signature of a Rab-GAP. The concerted mutation of four of its Akt/PKB-target sites in a mutant named AS160-4P inhibits the insulin-induced gain in surface GLUT4 (47, 48). In cells expressing AS160-4P, insulin stimulation caused accumulation of GFP-GLUT4 away from the RE, and GFP-GLUT4-containing vesicles were not detected within the vicinity of the membrane, assessed by total internal reflection

fluorescence microscopy (48). These results suggest that AS160 may participate in the insulin-induced efflux of GLUT4 from the SC/GSV, and that AS160-4P halts GLUT4 exit, essentially building up the content of GLUT4 at a post-RE stage, presumably in the SC/GSV. Very recently, three possible Rab targets of AS160 (Rab 2A, 8A, 14) were described in immunopurified GLUT4-containing compartments (comprising SC/GSV but possibly also other GLUT4-endowed endomembranes) (49). Ongoing investigations should uncover the role of these Rabs and their targets, potentially completing the chain of events that is initiated by the insulin receptor and culminates at the insulin-sensitive GLUT4 compartments. Additionally, Rab11, which regulates membrane protein traffic from endosomes to the PM and from endosomes to the TGN, is implicated in GLUT4 traffic between endosomal compartments because expression of a Rab11-inhibitory peptide prevented segregation of GLUT4 away from the TfR-rich compartment (10) (see Figs. 1 and 2).

Insulin-Derived Signals and Proteins Regulating GLUT4 Endocytosis

Endocytosis comprises the initial internalization step and the transit of the internalized cargo through the endocytic pathway. GLUT4 internalization is mediated in large part via clathrin-coated pits (50), presumably through interaction of GLUT4 with the μ 2 subunit of adapter protein 2 (51, 52) and aided by the GTPase dynamin (53, 54) believed to pinch-off GLUT4-containing vesicles from the membrane. Contribution of a caveolin-based endocytosis has also been reported (55). EHD binding protein 2 (EHD2) and its partner EHBP1, which colocalize with actin filaments, appear to be involved in GLUT4 endocytosis because silencing of these genes reduces this process (56). However, because clathrin and its associated adapter proteins as well as EHBP1 are involved in endocytosis of diverse cargo molecules, specific mechanisms for GLUT4 internalization are yet to be described.

Insulin reduces the rate of GLUT4 internalization (57, 58) and accelerates its transit through endocytic compartments (33). The signals that delay GLUT4 internalization remain to be mapped, but AS160 may contribute to such procession because overexpression of AS160-4P slightly enhanced the endocytosis of membrane-tagged GLUT4 (48). The nascent endocytic vesicles transit rapidly (within 2 min) to the early endosome (EE) and subsequently to the RE. Insulin reduces the activation (GTP loading) of the small GTPase Rab5, a resident of EE, as well as the interaction of the motor protein dynein with microtubules in a PI 3-kinase-dependent manner. These events are thought to retard progression of GLUT4 from the membrane to the EE (59). Subsequent to this step, insulin accelerates GLUT4 transit through the RE, presumably toward the SC/GSV, and this step involves input from class IA PI 3-kinase and Akt/PKB (33). GLUT4 endosomal traffic may also involve the participation of the PI 5-kinase PIKfyve, but although this protein can be phosphorylated by Akt/PKB, its activation in response to insulin remains to be demonstrated (60, 61).

Insulin-Derived Signals and Proteins Regulating GLUT4-Vesicle Fusion with the PM

In addition to the participation of class IA PI 3-kinase in the endosomal transit of GLUT4 described above, it was recently reported by several groups that inhibition of the enzyme still allows for significant gain of GLUT4 at the cell periphery, without achieving its insertion in the membrane. Such realization was possible by the simultaneous detection of exofacial- and cytosolic-facing epitopes on GLUT4. Indeed, 100 nM wortmannin, which abolishes class IA PI 3-kinase activity at this concentration, allows significant, insulin-dependent accumulation of GLUT4 at the periphery of myoblasts and adipocytes (62, 63) but prevents detection from the extracellular milieu of *myc* or HA epitopes engineered in the first exofacial loop of GLUT4. GLUT4-containing vesicles perched at the PM can also be visualized by electron microscopy on membrane lawns derived from wortmannin-pretreated, insulin-stimulated cells (62) (Fig. 3). Because vesicles are not observed in lawns from unstimulated nor insulin-stimulated cells, these results suggest that only the insulin-dependent fusion of GLUT4 vesicles with the membrane requires input from class IA PI 3-kinase. Supporting these results, carrier delivery of PI(3,4,5)P₃, the main product of this PI 3-kinase, causes GLUT4-vesicle fusion in L6 muscle cells (36). Buttressing this concept, activation of Akt/PKB downstream of PI 3-kinase correlates with the vesicle fusion step upon shifting the temperature from 19 C to

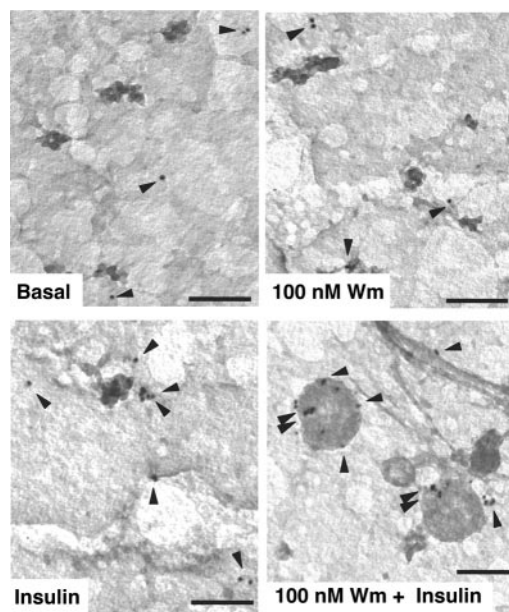


FIG. 3. Immunoelectron microscopy reveals GLUT4 vesicles on plasma membrane lawns from L6-myotubes treated with wortmannin (Wm) before insulin stimulation. Myotubes were treated with vehicle (dimethylsulfoxide), 100 nM wortmannin for 30 min, 100 nM insulin for 10 min, or wortmannin for 20 min before and along the 10 min insulin stimulation. Plasma membrane lawns were generated, labeled with polyclonal anti-C terminus antibody followed by goat polyclonal antirabbit antibody conjugated with 6 nm-gold particles and processed for transmission electron microscopy. Gold particles are indicated by arrowheads. Scale bar, 100 nm. [Adapted with permission from Ishiki *et al.* *J Biol Chem* 280:28792–28802, 2005 (62). © American Society for Biochemistry and Molecular Biology.]

37 C (64). Additional regulation of fusion events is achieved by another PI 3-kinase effector, the atypical PKC, through complexation with 80K-H and munc18c, promoting VAMP2 binding to syntaxin4 (65). Finally, phospholipase D1 also appears to regulate GLUT4 vesicle fusion with the PM based on the use of siRNA to reduce expression of the enzyme (66). Collectively, these studies support the involvement of class IA PI 3-kinase and additional proteins in insulin-induced GLUT4 vesicle fusion with the PM.

Fusion of vesicles with target membranes is mediated by SNARE proteins (67). The gain in surface GLUT4 caused by insulin is prevented by strategies that interfere with the SNAREs VAMP2 (a v-SNARE), syntaxin4 and SNAP23 [two target (t)-SNAREs] (68–71) but not with the v-SNAREs VAMP3 or VAMP7 (16, 72). Interestingly, interfering with VAMP2 does not alter the steady-state level of surface GLUT4 in unstimulated cells. Because VAMP2 is found in immunopurified GLUT4 compartments, likely SC/GSV, it is possible that the continuously recycling GLUT4 vesicle and the insulin-mobilized GLUT4 vesicle differ in their v-SNARE complement to promote vesicle docking and fusion. Recent use of total internal reflection fluorescence microscopy in rat adipocytes reveals that insulin slows down the constitutive movement of GLUT4-GFP within 100 nm of the PM, presumably through tethering or docking events. These results suggest that, in the basal state, GLUT4 vesicles arrive at the PM but do not dock, and that docking/fusion is an insulin-regulated event (73). Indeed, components of the exocyst complex, in particular Exo70, contribute to directing the vesicle to the precise site of fusion (74) followed by SNARE complex formation between VAMP2 of the insulin-mobilized vesicle and the PM t-SNAREs syntaxin4 and SNAP23. Additional accessory proteins regulate this process. munc18c, Synip, and Tomosyn bind syntaxin4 and inhibit insulin-stimulated GLUT4 fusion with PM (75–78). These proteins may be subject to regulatory input from insulin, and indeed Synip is phosphorylated by Akt/PKB in response to the hormone, freeing up syntaxin4 (77). However, the relevance of Synip phosphorylation has been contested because substitution of the Akt/PKB phosphorylated residue to alanine does not prevent insulin-dependent GLUT4 accumulation at the cell surface (79). Nonetheless, emerging evidence supports an active regulation of the SNARE complex by insulin-derived signals and the mechanisms involved deserve further scrutiny. The regulation of GLUT4 at the PM is illustrated in Fig. 1.

GLUT4 Binding Proteins—Possible Regulators of GLUT4 Traffic

An insulin-dependent increase in antigenicity of the GLUT4 C terminus has been reported using immunofluorescence and electron microscopy (62, 80, 81), raising the hypothesis that this arises from dissociation of a ligand protein or from a conformational change in GLUT4. To date, several GLUT4-binding proteins have identified, using the C terminus of the transporter as bait in either two-hybrid screens or recombinant protein pull-down experiments probing adipocyte cytosol. Among these the following are noteworthy: Aldolase (82) and α -actinin 4 (Foster, L., A. Rudich, I. Talior, X. Huang, P. Bilan, and A. Klip, unpub-

TABLE 1. GLUT 4 binding proteins

Binding proteins	GLUT	Cell type	Methods	Proposed function	Ref.
28 and 70 kDa	1, 4	Clone 9	C terminus	Unknown	87
85 kDa	4		Pull down		
GTB70 (glucose transporter-binding protein of 70 kDa)	1, 2, 4	Rat adipocytes	C terminus and cytosolic loop	Unknown	88
C109 peptide	4	Rat adipocytes	Pull down		
			C terminus	GLUT4 sorting	89
			Pull down ^a		
L-3-hydroxyacyl-coenzyme A dehydrogenase	4 (not 1)		C terminus	Primary fatty acids for protein acylation	90
Aldolase	4	3T3-L1 adipocytes	Pull down ^a		
			C terminus	Scaffold linking GLUT4 to actin cytoskeleton	82
			Pull down		
Ubc9	1, 4 (not 3)	L6 cells	C terminus	Regulation of transporter turnover	83
			Pull down ^a		
Daxx	4 (not 1)	3T3-L1 adipocytes	C terminus	Regulation of GLUT4 SUMOlation	84
			Pull down and co-i.p. ^a		
TUG	4	3T3-L1 adipocytes	Pull down and co-i.p.	Tethering GLUT4 to intracellular compartments	85
		L6 cells	Cytosolic loop		
			Pull down and co-i.p.		86
α Actinin4	1, 4	L6 cells	co-i.p. ^a	Scaffold linking GLUT4 to actin cytoskeleton	

The listed binding proteins were identified by one of two methods, as indicated:

1) By pull-down experiments using glutathione-S-transferase-linked constructs of the indicated binding region of the indicated GLUTs to probe cytosol of the indicated cell type.

2) By coimmunoprecipitation (co-i.p.) with full-length, endogenous GLUT4 immunoprecipitated from lysates of the indicated cell type. GLUT indicates the isoforms of the transporter that were positive in those assays.

^a Indicates studies where the results were confirmed using yeast two-hybrid analysis.

lished observation), which bind to actin filaments and may work as scaffolds linking GLUT4 to actin cytoskeleton; Ubc 9, the sentrin-conjugated enzyme, predicted to regulate GLUT4 and GLUT1 protein turnover (83); Daxx, the adapter protein associated with Fas and the type II TGF- β receptors, binds GLUT4 (but not GLUT1) and may lead to GLUT4 SUMOlation (84). Although these proteins are predicted to bind to the GLUT4 C-terminus, insulin does not change such interactions. In contrast, TUG (tethering protein containing a UBX domain for GLUT4) was proposed to be an intracellular tether for GLUT4 because its overexpression slows down insulin-induced GLUT4 translocation (85). Recombinant TUG interacts directly with the large cytosolic loop of GLUT4 (86) potentially linking it to a specific intracellular anchor. The aminopeptidase IRAP may also link GLUT4/IRAP-containing compartments to specific tethers. Indeed, IRAP interacts with p115 and overexpression of this protein reduces insulin-induced GLUT4 translocation to the PM, presumably by increasing its endosomal retention (91). The existence of tethering proteins may underpin the slow exocytosis of GLUT4 in the basal state, either through static or dynamic retention within endosomes. Table 1 lists these and other proteins identified to date to interact with GLUT4. Clearly, molecular detail is needed to understand the impact of GLUT4 binding to these proteins, and it is especially important to elucidate if and how insulin regulates such interactions. Potentially, specific manipulation of such GLUT4-ligand interactions could result in strategies to improve GLUT4 availability at the cell surface in insulin-resistant states.

Conclusion

Regulation of glucose uptake into muscle and fat cells via GLUT4 is a fundamental action of insulin, and this process

is impaired in type 2 diabetes. Because the disease is expanding with epidemic proportions, understanding the molecular basis of GLUT4 regulation is paramount. Here we highlighted the complexities of GLUT4 traffic and its regulation. In particular, we reviewed recent findings on regulatory events down-stream of Akt/PKB and at the cell surface leading to vesicle fusion, as well as the interaction of GLUT4 with partner proteins. Elucidation of each of the regulatory inputs on GLUT4 compartment biogenesis, storage, cycling, and membrane retention will be essential to pinpoint defects in insulin resistance and to create approaches to improve insulin action and glucose uptake.

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