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Endocytosis, Recycling, and Regulated Exocytosis of Glucose Transporter 4

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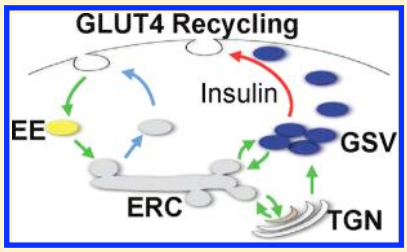
ABSTRACT: Glucose transporter 4 (GLUT4) is responsible for the uptake of glucose into muscle and adipose tissues. Under resting conditions, GLUT4 is dynamically retained through idle cycling among selective intracellular compartments, from whence it undergoes slow recycling to the plasma membrane (PM). This dynamic retention can be released by command from intracellular signals elicited by insulin and other stimuli, which result in 2−10-fold increases in the surface level of GLUT4. Insulin-derived signals promote translocation of GLUT4 to the PM from a specialized compartment termed GLUT4 storage vesicles (GSV). Much effort has been devoted to the characterization of the intracellular compartments and dynamics of GLUT4 cycling and to the signals by which GLUT4 is sorted into, and recruited from, GSV. This review summarizes our understanding of intracellular GLUT4 traffic during its internalization from the membrane, its slow, constitutive recycling, and its regulated exocytosis in response to insulin. In spite of specific differences in GLUT4 dynamic behavior in adipose and muscle cells, the generalities of its endocytic and exocytic itineraries are consistent and an array of regulatory proteins that regulate each vesicular traffic event emerges from these cell systems.



Maintaining a normal blood glucose level is essential for preventing hyperglycemia and its toxic effects. Skeletal muscle and adipose tissue serve as the major storage sites for glucose, and insulin is the major signal for the uptake of glucose into these tissues. Glucose transporter 4 (GLUT4) is a 12-transmembrane protein expressed in muscle and adipose tissues that catalyzes the transport of glucose across the plasma membrane (PM) via an ATP-independent, facilitative diffusion mechanism.[1] Under resting conditions, GLUT4 has mainly an intracellular distribution but is recruited to the PM in response to insulin and other stimuli.[2] Notably, sequestering GLUT4 within the cell is a dynamic process, which is intimately responsible for the subsequent release and mobilization of the transporter to the membrane. The aim of this review is to describe and discuss the evidence of the dynamic behavior of GLUT4.

GLUT4 continuously recycles between the PM and intracellular stores that are only scantly defined. Retention of GLUT4 in these stores is orchestrated by an array of regulatory and sorting proteins. Insulin induces the release of GLUT4 through signaling from the insulin receptor via the insulin receptor substrate-1 (IRS-1), phosphatidylinositol 3-kinase [PI(3)K], and Akt, along with additional parallel and downstream signals among which atypical protein kinase C isoforms and Rho family GTPases stand out. For comprehensive coverage of insulin signaling pathways, see refs 2−7. In ways that are beginning to unravel, these signals regulate every step of GLUT4 translocation, from mobilization of intracellular stores to fusion with the PM. On the other hand, muscle contraction, membrane depolarization, and mitochondrial uncoupling also increase the density of GLUT4 at the muscle PM, but mainly by decreasing the rate of endocytosis[8−10] via AMP-activated protein kinase and Ca^{2+}-dependent signals (for reviews, see refs 11−14).

Here we address the emerging molecular and cellular regulatory mechanisms of GLUT4 traffic and their integration with insulin signaling (to the exclusion of regulation elicited by other stimuli). We address recent discoveries and controversies with regard to the processes of GLUT4 endocytosis, transit of GLUT4 from endocytic vesicles to sorting and retention compartments, and GLUT4-regulated exocytosis in response to insulin. We compare and contrast results obtained with different adipose and muscle systems and comment on technical and fundamental issues that may reconcile diverse views on GLUT4 traffic.

INTERNALIZATION OF GLUT4 FROM THE PLASMA MEMBRANE

Mechanisms of GLUT4 Endocytosis. Endocytosis of membrane proteins is a fundamental process for the maintenance of cell size and the balance of exocytic functions. Selective retrieval of membrane proteins from the PM in the form of vesicular cargo occurs through two major types of processes: clathrin-mediated endocytosis (CME) and a number of clathrin-independent pathways that require organized lipid domains, including cholesterol, with or without the participation of caveolin (for caveolae-mediated endocytosis) or flotillin.[15] Whereas the transferrin receptor (TfR) typically internalizes via CME, interleukin-2 receptor β (IL-2Rβ)

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internalizes via a route that is independent of clathrin and caveolae but that requires cholesterol.[15] Dynamin, a large GTPase involved in the fission of vesicles from membranes, is necessary for CME and has also been implicated in caveolin-1-, IL-2Rβ-, and flotillin-dependent endocytotic processes.[15]

GLUT4, the major carrier of glucose into muscle and fat cells, is a recycling protein that is continuously removed from and recycled back to the PM.[5] Both arms of this process are subject to regulation by physiological demands. Most of our knowledge of the routes and rates of GLUT4 traffic derives from three cellular systems: primary rat adipocytes, cultures of 3T3-L1 mouse adipocytes, and cultures of rat L6 skeletal muscle cells (as myoblasts and myotubes). In the cultured cells, transient or stable expression of tagged GLUT4 has allowed tracking of the transporter, but in each case, one must consider idiosyncratic differences inherent to the species, cell type, or mechanism of study. Curiously, also, studies with the murine 3T3-L1 adipocytes have largely used *HA*-tagged human GLUT4 or *myc*-tagged rat GLUT4, whereas most studies with rat L6 muscle cells have used cognate rat GLUT4 (*myc*-tagged). With these caveats, it is interesting to note similarities in muscle and adipose cells, where GLUT4 internalizes through both CME and a cholesterol-dependent pathway.[8,12,16]

Several sequences in GLUT4 define its internalization, in particular the F^5QQI, LL^{490}, and $TE^{499}LE^{501}Y$ clusters (reviewed in ref 12), which are conserved in rodent and human GLUT4. The N-terminal F^5QQI^8 and C-terminal LL^{490} sequences bear similarities to motifs generally required for CME.[12] Accordingly, introducing an F^5A mutation increases by 3−5-fold the steady state level of surface expression of GLUT4 in adipocytes,[17,18] although this surprisingly resulted from altered intracellular retention rather than from a reduced level of endocytosis. In addition to these determinants of internalization or retention, other residues in the cytosolically exposed regions of GLUT4 potentially determine the susceptibility to regulation by different stimuli. Indeed, in adipocytes, GLUT4 endocytosis is predominantly dependent on cholesterol-dependent endocytosis[16] and appears to involve caveolae and VAMP8, as internalization is inhibited by dominant-negative caveolin-1[19] and knockdown of VAMP8.[20]

In contrast, in L6 muscle cells, approximately half of the transporter internalization occurs through CME and the other half through the IL-2Rβ receptor pathway.[8] Consistent with a role for VAMP8 in mediating GLUT4 endocytosis, VAMP8 null mice display an increased rate of glucose uptake and an increased level of surface GLUT4 in skeletal muscle.[21] Interestingly, not all species of GLUT4 bear the same internalization sequences or, consequently, routes of internalization. One case is brown trout GLUT4 (btGLUT4), which lacking the LL^{490} motif and expressing a modified N-terminal motif (FQHL) internalizes entirely via the IL-2Rβ route when expressed in rat L6 muscle cells.[8] This observation supports the hypothesis that the F^5QQI motif in mammalian GLUT4 encodes for CME. The differential internalization of mammalian and fish GLUT4 has physiological consequences, given that only the IL-2Rβ route is regulated by conditions of cellular energy demand[8,12] (see below). Thus, GLUT4 internalization occurs through both CME and cholesterol-dependent endocytosis in muscle and adipose cells, although a different mechanism of cholesterol-dependent endocytosis operates in each cell type.

Stimuli That Affect the Rate of GLUT4 Endocytosis. In addition to these differences in the pathways of GLUT4 endocytosis in muscle and adipose cells, its internalization is distinctly regulated in both cell types. In 3T3-L1 adipocytes, insulin reduces the rate of endocytosis of transiently expressed, HA-tagged GLUT4, although the relative contribution of this inhibition to the net gain in the level of surface GLUT4 is minor compared to the increase in the level of GLUT4 exocytosis.[7,19,22] In these cells, insulin specifically reduces the rate of cholesterol-dependent endocytosis in favor of CME.[16] In isolated rat adipocytes, the rate of endocytosis of the photolabeled transporter is also reduced by insulin, although the major effect of the hormone remains the stimulation of GLUT4 exocytosis.[23,24]

In contrast to 3T3-L1 or primary rat adipocytes, insulin does not reduce the rate of endocytosis of stably expressed, *myc*-tagged rat GLUT4 in rat L6 myoblasts or myotubes.[8,10,25] Similarly, in mature skeletal muscle[9] and isolated cardiomyocytes,[26] insulin regulates the rate of GLUT4 exocytosis and not its rate of internalization, assessed by following the traffic of the exofacially photolabeled transporter. Surprisingly, however, insulin reduced the internalization rate constant of transiently expressed *HA*-tagged human GLUT4 in another clone of rat L6 muscle cells generated by retroviral infection of myoblasts.[27] Of note, human GLUT4 has a glycine residue in position 255 of the large cytosolic loop, where the rat and mouse transporters express aspartic acid, and more subtle differences also occur at the C-terminal tail, particularly at position 482 (alanine in human vs threonine in rat). The question of whether the GLUT4 species or clonal or methodological differences account for the discrepant regulation of GLUT4 endocytosis could be answered by parallel comparisons in the cellular systems described.

The rate of retrieval of GLUT4 from the membrane can be regulated by other stimuli that, like insulin, cause a net increase in the level of surface GLUT4. Muscle cell depolarization,[10] hyperosmolarity,[28] and stimuli that alter oxidative metabolism [such as the mitochondrial uncoupler 2,4-dinitrophenol (DNP)][8] decrease the rate of GLUT4 endocytosis in L6 muscle cells stably expressing GLUT4*myc*. Interestingly, DNP treatment selectively reduces the rate of GLUT4 internalization through the IL-2Rβ route, while hyperosmolarity disrupts clathrin organization, inhibiting GLUT4 internalization through CME.[8] Similarly, direct activation of AMP-regulated kinase (AMPK), which participates in the DNP-mediated increase in the level of surface GLUT4,[29] can retard GLUT4 endocytosis.[8,9,26] Thus, distinct routes of GLUT4 endocytosis may function to allow stimulus-specific regulation of surface GLUT4.

SORTING OF GLUT4 INTO RECYCLING AND INSULIN-SENSITIVE COMPARTMENTS

GLUT4 is a very stable protein with a half-life of approximately 48 h.[30] A single GLUT4 molecule will undergo multiple rounds of recycling before being targeted for degradation. During these rounds of recycling, GLUT4 that internalizes from the cell surface must be sorted away from the continuously recycling pathway.

Steady State Distribution of GLUT4 in Intracellular Vesicles. GLUT4 accumulates in several intracellular compartments, and although it accumulates in perinuclear regions, it is also found in peripheral vesicles in both cells (muscle and adipocytes)[31] and mature skeletal muscle.[32,33] Functional studies indicate that GLUT4 is sorted into a specialized storage (GS) compartment that responds to insulin.[34,35] The terms GLUT4 storage vesicles (GSV) and insulin-responsive vesicles (IRV) have been used to identify both the GS and the vesicles that bud

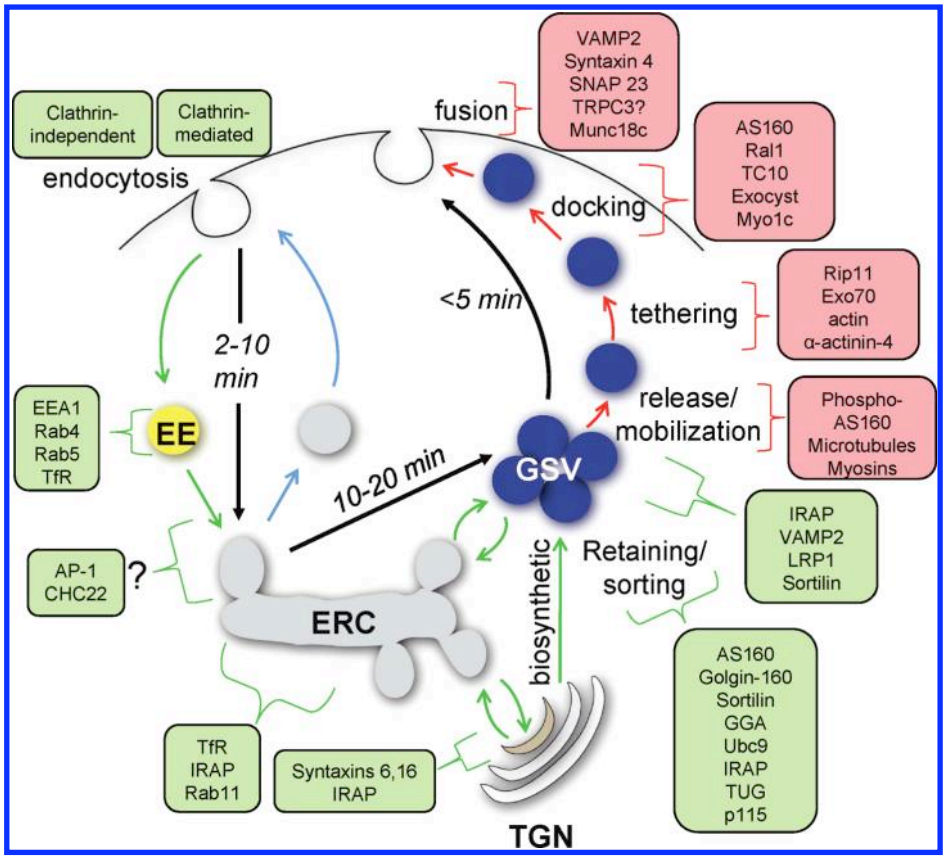

Figure 1. Model of the transit of GLUT4 through intracellular compartments during endocytosis and exocytosis (see the text for details). GLUT4 is internalized via clathrin-mediated endocytosis (CME) or cholesterol-dependent but clathrin-independent endocytosis. Within 2 min, GLUT4 traverses early endosomes (EE) en route to the endosomal recycling compartment (ERC). The ERC is marked by the presence of the transferrin receptor (TfR) and contains Rab11. GLUT4 accumulates in the ERC from where it is sorted back to the plasma membrane (PM) (<10%) or into GLUT4 storage vesicles (GSV) and a subcompartment of the trans-Golgi network (TGN) (50−60%). Twenty minutes after internalization, GLUT4 accumulates in GSV, marked by the presence of both VAMP2 (vesicle-associated membrane protein 2) and IRAP (insulin-responsive aminopeptidase). This pool of GLUT4 engages in idle cycling with the ERC and TGN. Within GSV, AS160 acts as a "brake" on the translocation of GLUT4 toward the PM. Other proteins involved in GLUT4 intracellular retention are indicated in the relevant box. In the presence of insulin, the brake is relieved and GLUT4 vesicles bud from GSV and fuse with the PM within 5 min. This process of GLUT4 vesicle mobilization, tethering, docking, and fusion in response to insulin is intricately regulated by various effector proteins, actin dynamics, and actin- and microtubule-based motors. Small GTPases of the Rho and Rab families are excluded from the model because the exact steps that they regulate in the insulin-induced transit of GLUT4 to the PM remain unknown. Newly synthesized GLUT4 molecules are sorted directly into GSV from the TGN. Green and blue arrows indicate sorting and constitutive exocytosis, respectively. Red arrows mark insulin-regulated exocytosis. Time courses for sorting and exocytosis steps are marked by black arrows. Proteins that localize to each identified compartment or regulate a specific sorting step are listed in adjacent boxes.

from the GS. However, to date, the subcellular localization of any of these predicted bodies is debated, a major question being whether the GS or GSV are located in the perinuclear region or make up a functional subset of the cytoplasmically dispersed vesicles. A second important question is whether GLUT4 retrieved from the plasma membrane sorts into GSV in a similar fashion in the absence and presence of insulin.

Biochemically, two proteins have been used as markers of the GSV: vesicle-associated membrane protein 2 (VAMP2) and insulin-regulated aminopeptidase (IRAP)[36,37] (Figure 1). A further determinant of GSV is its lack of TfR.[2,34,37] Other proteins that localize to GSV include sortilin,[38,39] TUG (tether containing a UBX domain for GLUT4),[40] and LRP1 (low-density lipoprotein receptor-related protein 1).[39] The functions of these proteins in regulating GLUT4 traffic will be addressed below. By subcellular fractionation and immunofluorescence analysis, GLUT4 is also found within the endosomal recycling compartment (ERC), marked by the TfR, and in a subcompartment of the trans-Golgi network (TGN), marked by Syntaxins 6 and 16 but not by other TGN markers such as furin and TGN38.[22,34,41,42] Approximately 40−50% of GLUT4 is retained in the ERC, while the remaining 50−60% is distributed in the GSV and TGN.[5,34,37]

Models of Retention of GLUT4 in Intracellular Compartments. The retention of GLUT4 in intracellular compartments is not static. GLUT4 can idly cycle between compartments, with a small percentage recycling to the PM at any point in time. Insulin increases the rate of recycling of GLUT4 to the PM by releasing retention from one or more of these compartments (reviewed in refs 2, 3, 5, and 43).

Two models for describing how GLUT4 distribution is maintained have been proposed: "static retention" and "dynamic recycling". In spite of extensive analysis, described next, the relative participation of each mechanism of GLUT4 retention is still being debated. The static retention model states that GSV remain segregated from the PM under resting conditions.[35,44,45] The TGN may act as a recycling compartment through which

GLUT4 transits en route to GSV, as the C-terminal TELEY motif of GLUT4 has been shown to regulate the transit of GLUT4 from endosomes to a subcompartment of the TGN.[42] Thus, although GLUT4 is mostly retained in GSV, idle cycling may be possible with the TGN.[2] The static retention model, largely proposed for 3T3-L1 adipocytes, is further supported by single-particle tracking of GLUT4 molecules.[46] One caveat of this method is that GLUT4 molecules are sparsely labeled and imaged for only 10 s,[46] such that the slow recycling of GLUT4 may not be readily observed. The static behavior of GLUT4 was also proposed for the clone of L6 muscle cells transiently expressing human *HA*-tagged GLUT4 by retroviral infection based on the observation that only 61% of GLUT4 molecules exchanged with the PM at the steady state.[27] However, this measure was based on GLUT4 recycling after only 180 min, when recycling is still incomplete.[25]

The dynamic recycling model states that GSV are in slow exchange with recycling endosomes (through the ERC) such that all GLUT4 is slowly accessible to the PM under resting conditions.[5,17] The dynamic recycling model has been experimentally supported by measurements that show the complete complement of GLUT4 molecules gains exposure to the cell surface over time in 3T3-L1 adipocytes[22] and in L6 muscle cells stably expressing *myc*-tagged GLUT4.[25] The participation of the TGN in this model, until recently, had been discounted by evidence that recycling GLUT4 does not share any significant intralumenal space with furin (TGN marker) in adipocytes.[22] However, recent work suggests that contributions of a non-GSV, non-ERC compartment could perhaps be a subcompartment of the TGN.[17,47]

An interesting explanation for the opposite models was provided by Muretta et al.[48] who ascribed them to differences in the confluence of the 3T3-L1 adipocytes to yield the contrasting behavior. Potentially, a similar difference may explain the different observations made in the clones of L6 muscle cells expressing GLUT4*myc* and GLUT4-*HA*.

Rab Family Proteins Involved in GLUT4 Sorting. Rab family GTPases are universal determinants of vesicular sorting,[49] and as expected, they have fundamental input in GLUT4 vesicle sorting.[50] Rab proteins act as molecular switches that, when bound to GTP, recruit effector proteins involved in all steps of vesicle movement from budding and motility to tethering and fusion.[49] Rab4, Rab5, Rab8a, Rab10, Rab11, Rab13, and Rab14 have all been implicated in the regulation of GLUT4 traffic (reviewed in refs 50 and 51). Insulin causes activation (GTP loading) of Rab4a,[52] Rab11,[53] Rab8a,[54] and Rab13[54] and inhibits GTP loading of Rab5a.[55] On the basis of their function in other cells, Rab5 and Rab11 are likely involved in GLUT4 endocytosis and sorting out of the recycling endosome, respectively, while Rab8a, Rab10, Rab13, and Rab14, all targets of the Rab-GAP AS160 (TBC1D4), may participate in selective sorting leading to regulated GLUT4 exocytosis (see below).[51,54]

Sorting of GLUT4 into Distinct Intracellular Compartments. The transit of GLUT4 through internal membranes has been characterized vis-à-vis that of the TfR (for a review, see ref 56). The TfR internalizes via CME into clathrin-coated vesicles (CCV) that then fuse with early endosomes (EE), also termed sorting endosomes (SE). From EE, vesicle cargo may be sorted to the ERC and TGN, or it may proceed to late endosomes (LE) and lysosomes for degradation. The TfR is sorted from EE into the ERC, from which it slowly returns to the PM. Rab5 (EE-localized) and Rab11 (ERC-localized) are involved in directing TfR to and from the ERC, respectively.[56]

Although GLUT4 is retrieved from the PM through CCV and through a cholesterol-sensitive route, it is not known if each route determines subsequent differences in the GLUT4 endocytic itinerary. Subcellular fractionation and immunofluorescence data have established that internalizing GLUT4 vesicles fuse with EE.[25,41] GLUT4 colocalizes with the EE marker EEA1 (early endosome antigen 1) within 2 min of internalization.[25] Furthermore, inhibiting Rab5 (which mediates CCV fusion with EE and EE homotypic fusion during EE maturation) increased the level of cell surface GLUT4 without affecting insulin-stimulated GLUT4 translocation.[55] This suggests that Rab5 is involved in GLUT4 sorting through EE (see Figure 1).

Current evidence suggests that, like the TfR, GLUT4 is sorted from EE to the ERC, because GLUT4 internalized from the PM accumulates in the ERC from which it is sorted into GSV.[18,22,34] Inhibiting Rab11 activity[34] or stable knockdown of IRAP[47] traps GLUT4 in the ERC and depletes it from GSV in adipocytes. The FQQI and TELEY motifs of GLUT4 are involved in its sorting through the ERC, as mutating these sequences (as in $F^{5}A$ or $EE^{499,501}AA$) additively decreases the rate of basal retention of GLUT4 and causes the redistribution of GLUT4 from GSV to recycling endosomes.[17,18] It has been suggested that these sequences regulate different steps in GLUT4 traffic because retention of GLUT4 by AS160 (discussed below) is additive for FQQI but not TELEY.[17] Interestingly, retention of GLUT4 by IRAP is additive with respect to that of AS160, FQQI, and TELEY.[47] McGraw and colleagues postulate that the FQQI motif is necessary for GLUT4 retention in a non-TfR-containing, non-GSV compartment that contains IRAP,[17] the TELEY motif is necessary for the sorting of GLUT4 into GSV,[17] and IRAP is required for the retention of GLUT4 in one or more of these compartments.[47] This hypothesis supersedes previous work that described the TELEY motif as being essential for the transit of GLUT4 to a subdomain of the TGN enriched in Syntaxins 6 and 16.[42] Thus, GLUT4 is thought to move to the ERC, from which it recycles back to the PM (small fraction) or sorts into either GSV (Rab11-dependent step) or another retention compartment (TGN subcompartment that contains IRAP) (see Figure 1). Exactly how IRAP and GLUT4 sequence motifs regulate this transit remains to be determined.

The regulated traffic of GLUT4 upstream of GSV sorting (between EE and/or the ERC and TGN) is poorly understood. Adaptor protein complex AP-1 has received attention in this process. AP-1 is involved in the movement of protein between endosomes and TGN,[57] localizes to GLUT4 vesicles,[58] and binds GLUT4 at the FQQI motif.[59] Clathrin heavy chain (CHC) isoform 22 (CHC22), which is a homologue of CHC17 involved in CME,[60] also binds AP-1 and is reported to act downstream of EE in sorting multiple cargo proteins toward the TGN in HeLa cells and GLUT4 toward GSV in human muscle.[61,62] One proposal is that AP-1 acts on the LL^{490} motif during GLUT4 sorting, as mutations in the LL^{490} motif prevent its transfer from the fast recycling pathway (EE to PM) to the retention pathway (EE to ERC), an effect mirrored by AP-1 knockdown.[17] However, this notion has been challenged by a report showing that AP-1 knockdown in 3T3-L1 adipocytes actually increases the level of cell surface GLUT4, like mutations in the FQQI motif.[57] Thus, it remains to be determined how AP-1 regulates GLUT4 sorting and the motifs within GLUT4 that are involved.

Although GLUT4 accumulates with TfR in the ERC, some evidence suggests that the two proteins transit within it through

different peripheral vesicles with unique temperature sensitivity to perinuclear accumulation,[63,64] at least in the CHO cells used. In those cells, recycling of GLUT4 from the ERC was slower than that of TfR, and these two proteins exited the ERC on different vesicles.[63] Thus, although GLUT4 and TfR transited through the same internal membranes, they remained physically segregated from each other.

Unlike the case of GLUT4 recycling, it is well established that the TGN partakes in sorting newly synthesized GLUT4 into GSV.[7,43] Newly synthesized GLUT4 does not sort to the PM; rather, it enters GSV directly via the TGN through a mechanism regulated by the proteins golgin-160, sortilin, and GGA (Golgi-localized, γ-ear-containing ARF binding proteins).[38,65,66] The FQQI and cytoplasmic loop motifs of GLUT4 are essential for this regulated sorting through the TGN.[67] Interestingly, GLUT4 internalized from the PM cannot access GSV in the absence of golgin-160,[65] suggesting that the TGN is necessary for the sorting of GLUT4 into GSV and that GLUT4 may recycle between the TGN and GSV, directly or indirectly.

■ PROTEINS INVOLVED IN THE INTRACELLULAR RETENTION OF GLUT4

In addition to the FQQI and TELEY sequence motifs (see above), GLUT4 intracellular retention is regulated by a number of proteins. Overexpression of proteins involved in GLUT4 intracellular retention would be expected to enhance GLUT4's response to insulin from the GSV to the PM, and depletion of these proteins would be expected to redirect GLUT4 to the ERC and hence promote its spillover to the PM. By this criterion, the following proteins have been suggested to partake in GLUT4 intracellular retention (see Figure 1).

TUG. The tether, containing a ubiquitin-like UBX domain, for GLUT4 (TUG)[40,43,68] binds GLUT4 directly, and expression of a dominant-negative TUG fragment or TUG knockdown shifts the distribution of GLUT4 from GSV to TfR-containing endosomes and the PM and further accelerates its degradation.[40,68] Conversely, overexpression of a long spliced variant of TUG causes the exclusion of GLUT4 from TfR-containing endosomes.[40] This major function of TUG in intracellular retention does not rule out, however, the possibility that this protein may have additional input in the insulin-stimulated movement of GLUT4.

IRAP. This protein is both a marker of compartments populated by GLUT4 and a functional element in its intracellular retention. Newly synthesized IRAP is recruited to GSV along with GLUT4, mediated by sortilin.[69] It has been argued that IRAP regulates GLUT4 intracellular retention,[70] because microinjection of the small cytosolic portion of IRAP resulted in the translocation of GLUT4 to the cell surface. An essential role of IRAP in GLUT4 retention and sorting of GLUT4 from endosomes to GSV was further confirmed by the relocalization of GLUT4 to endosomes upon IRAP knockdown.[47]

Ubc9. Sequestration of GLUT4 in GSV not only populates a compartment that will respond to insulin but also spares GLUT4 from degradation. GLUT4 stabilization is achieved by direct binding to Ubc9, the small ubiquitin-related modifier (SUMO)-conjugating enzyme.[71,72] Ubc9 expression is necessary to target GLUT4 to the insulin-responsive storage compartment in 3T3-L1 adipocytes[72] and L6 myoblasts,[71] and overexpression of Ubc9 in 3T3-L1 adipocytes promoted accumulation of GLUT4 in GSV.[72] These findings suggest that Ubc9 shepherds GLUT4 to GSV, thereby sparing its degradation. This is consistent with the shorter half-life of GLUT4 exogenously expressed in 3T3-L1 fibroblasts (lacking GSV), compared to a much longer half-life in 3T3-L1 adipocytes (that contain GSV).[38] It is less clear if this function is directly linked to GLUT4 SUMOylation, because overexpressing inactive Ubc9 also promotes GLUT4 storage in GSV. In contrast to 3T3-L1 fibroblasts (adipocyte precursors), L6 myoblasts already present a GLUT4 compartment that excludes TfR (i.e., GSV), and in these cells, GLUT4 has a half-life as long as that in differentiated L6 myotubes (S. Ishikura and A. Klip, unpublished observation).

Sortilin. Sortilin interacts with both GLUT4 and IRAP[38] and promotes GLUT4 storage in GSV in 3T3-L1 adipocytes, thereby allowing its recruitment to the PM by insulin.[38,73] Furthermore, sortilin depletion leads to GLUT4 degradation.[38] In contrast, sortilin overexpression or deletion is inconsequential to the ability of insulin to recruit GLUT4 to the membrane of L6 myoblasts (S. Ishikura and A. Klip, unpublished observation). In C2C12 muscle cells, sortilin is also required for maturation of the entire myogenic program, including the development of insulin regulation of GLUT4.[73]

Golgin-160 and p115. Golgi proteins Golgin-160 and p115[65,74] are also involved in intracellular retention of GLUT4 and IRAP, because siRNA-mediated depletion of Golgin-160 caused a gain in the level of surface GLUT4 and IRAP in unstimulated cells.[65]

Synapsins. Synapsins are peripheral membrane proteins that coat synaptic vesicles in a "reserve pool" and cluster vesicles within an actin-rich filamentous matrix.[75] Their wider function in non-neuronal cells is unknown, but synapsin II is expressed in adipocytes, colocalizes with perinuclear GLUT4, and participates in the intracellular retention of GLUT4 in unstimulated cells.[76]

Key questions that arise are whether any of the proteins mentioned above interact functionally or hierarchically and whether insulin directly regulates their activity. TUG is released from GLUT4 in response to the hormone in 3T3-L1 adipocytes, skeletal muscle, and L6 myotubes,[33,68] preceding the movement of GLUT4 out of GSV-containing light microsomes to the PM.[68] Regulation of the other proteins, however, remains to be tested.

In this regard, it is interesting that a bona fide insulin signal, the Akt substrate of 160 AS160/TBC1D4 (reviewed in ref 4), is found in GSV in the basal state and is released into the cytosol in response to insulin (ref 77 and see below). AS160 encodes a TBC domain with GAP activity that targets a number of Rab GTPases in vitro (reviewed in ref 4). Mutations in the TBC domain, and mutation of the residues targeted by Akt and other kinases (AS160-4A, also called AS160-4P), suggest that insulin-mediated AS160 phosphorylation inactivates its GAP activity, allowing the active GTP-bound form of its target Rabs to prevail.[51,78] AS160 can bind to the cytosolic N-terminus of IRAP, contributing to the intracellular retention of GLUT4 in the same vesicles.[77,79,80] However, both the presence of AS160 in GSV and the AS160 regulation of GLUT4 traffic are unaffected by IRAP knockdown,[47] indicating that IRAP−AS160 interaction is not the only determinant for the localization of AS160 to GSV.[47,80] AS160 knockdown increases the level of surface GLUT4 in unstimulated adipocytes and muscle cells,[77,80] and AS160 GAP activity is required for basal state GLUT4 retention.[79,80] The protein may not be as much a physical retainer of GLUT4 in GSV as a signal for its availability to respond to stimuli (see below).

TRAFFIC OF GLUT4 FROM INTRACELLULAR COMPARTMENTS TO THE PM

Insulin causes a rapid increase in the level of surface GLUT4 in muscle and fat cells, and there is general consensus that such GLUT4 emanates from GSV, eventually arriving at the cell surface in the form of vesicles (IRV as described above) that in turn tether, dock, and fuse with the plasma membrane. Although the entire process has been classically called GLUT4 translocation, we here break it down into transport to the periphery and peripheral events. Most studies also agree that insulin-derived signals likely act on different intracellular traffic steps to promote this increase in the level of surface GLUT4.[46]

TUG and AS160. According to the static retention model, signals must command the release of GLUT4 from the sequestered pool (GSV) into the continuously recycling system (ERC) for subsequent exit toward the PM. In this context, a key step may be the dissociation of TUG from GLUT4, as discussed above. On the other hand, dissociation of the TUG−GLUT4 protein could also be envisaged as necessary for the release of GLUT4 from GSV for traffic directly to the PM, as predicted by the dynamic retention model.

As mentioned above, AS160 is found on GLUT4 vesicles under basal conditions[77,81] and is released into the cytosol upon stimulation with insulin. These findings suggested that AS160 phosphorylation causes its dissociation from GLUT4-containing vesicles. Consistently, AS160-4A does not translocate to the cytosol in response to insulin in 3T3-L1 adipocytes or L6 myoblasts.[82] It is the phosphorylation of AS160, rather than its dissociation from membranes, that is required for insulin-stimulated GLUT4 translocation.[82] This was corroborated by the fact that a GLUT4−AS160 chimeric protein could undergo insulin-dependent translocation to the PM.[82]

Not only is AS160 phosphorylated by a number of kinases, but ancillary proteins bind to and regulate such phosphorylation, such as RuvB-like protein 2 (RUVBL2)[83] and transcriptional coregulator RIP140.[84] Depletion of RUVBL2 in adipocytes inhibits insulin-stimulated GLUT4 translocation and insulin-stimulated AS160 phosphorylation.[83] In contrast, RIP140 negatively regulates GLUT4 traffic by competing with Akt for AS160 binding.[84]

The precise steps in GLUT4 traffic regulated by AS160 phosphorylation are still controversial. In myoblasts, expression of nonphosphorylatable AS160-4A not only abrogated membrane insertion of GLUT4 after insulin stimulation but also partially reduced its level of cortical buildup.[85] Consistently, using fluorescence quenching of transferrin-containing compartments, Zeigerer et al. showed that AS160-4A inhibits the insulin-induced shift of GLUT4 from the SC or GSV to the ERC or the PM, indicating that a step prior to membrane fusion is regulated by AS160.[86] Surprisingly, the AS160-4A mutant did not affect the vesicle density within the TIRF zone under basal or insulin-stimulated conditions but significantly reduced the rate of docking of GLUT4 vesicles with the PM.[87] AS160 also participates in the regulation of intracellular GLUT4 "vesicle behavior", as revealed by analysis of intracellular GLUT4 dynamics at the single-molecule level using quantum dot technology combined with TIRF.[46] Interestingly, overexpression of the T642A AS160 dominant-negative mutant abolished insulin-induced acceleration of GLUT4 vesicles, although its underlying mechanisms involving AS160 remain to be further clarified. Therefore, this evidence suggests that AS160 may regulate movement, speed, and docking, but not the fusion step of GLUT4 traffic. This is compatible with its ability to target diverse Rab molecules and suggests that the specificity of each action may in fact be determined by the subcellular location of the AS160-targeted Rabs.

Contribution of Microtubules. Once released into the recycling pool, as proposed by the static retention model, GLUT4 may require microtubules for long-range movement of vesicles across the cytoplasm before reaching the PM for docking and fusion. Indeed, a number of studies in 3T3-L1 adipocytes show bidirectional movement of GFP-GLUT4 vesicles along microtubules, motored by kinesin.[88] Closer to the membrane, in the TIRF (total internal reflection fluorescence) zone of 150−200 nm beneath the PM, microtubule depolymerizing (nocodazole and colchicine) or stabilizing (paclitaxel) agents attenuate insulin-stimulated PM docking[89] and fusion[90,91] of IRV. This would suggest that microtubules have an additional input distinct from long distance transit. A caveat of either interpretation is that nocodazole inhibits insulin-mediated GLUT4 traffic via microtubule-independent mechanisms.[92,93] Even though the requirement for microtubule integrity in insulin-stimulated GLUT4 PM translocation is thus controversial, microtubule-based motors appear to be required for insulin-mediated GLUT4 PM translocation. Expression of a dominant-negative mutant of kinesin KIF5B[88] or microinjection of an anti-KIF3 antibody into 3T3-L1 adipocytes significantly decreased the rate of insulin-induced GLUT4 translocation.[94]

In contrast to the static retention model, the dynamic retention model suggests that insulin shifts the basal traffic equilibrium, increasing the rate constants for traffic out of the GSV, tethering, docking, and/or fusion of vesicles with the PM. Several molecular events may facilitate this process, as follows.

Dynamic Changes in the Actin Cytoskeleton. Numerous studies support a requirement for actin filaments and their dynamic remodeling in insulin-stimulated translocation of GLUT4 to the PM.[5] Preventing actin remodeling with inhibitors of actin polymerization such as latrunculin B and cytochalasin D[95,96] or inhibiting actin depolymerization with jasplakinolide[97,98] abrogates the translocation of GLUT4 to the PM in adipocytes and muscle cells, whether in cell culture or primary tissues. The Arp2/3 complex, which is responsible for initiating the polymerization of newly branched actin filaments,[99] is required for insulin-stimulated actin remodelling at the cortex of L6 myoblasts.[100] The Arp3 subunit of the Arp2/3 complex colocalized with remodeled actin, and siRNA-mediated down-regulation of two different subunits of the complex abrogated actin remodeling and impaired GLUT4 translocation. Whether other types of actin nucleators that produce unbranched filaments (such as formins mDIA1−3, the mammalian Spire, or cordon-bleu)[101] also regulate GLUT4 traffic remains to be tested. Interestingly, along with inducing actin polymerization, insulin promotes actin filament depolymerization by enhancing dephosphorylation (activation) of the actin-severing protein cofilin.[100] This observation supports the hypothesis that dynamic cycles of actin polymerization and severing at the cell cortex are required for insulin-mediated GLUT4 translocation.

How such actin dynamics, induced by insulin, facilitates the transit of GLUT4 to the PM is still under investigation. Using TIRF microscopy in combination with probes that can distinguish between vesicle transport and fusion, Lopez et al.[95] showed that defective actin remodeling in 3T3-L1 adipocytes was accompanied by normal insulin-regulated accumulation of GLUT4 vesicles close to the PM, but impairment of the final

exocytotic fusion step. Further, latrunculin markedly altered the dynamic behavior of incoming GLUT4 vesicles beneath the plasma membrane,[46] suggesting that cortical actin remodeling may regulate events near the surface that precede docking and fusion. Consistent with this view, inhibition of cortical actin filament remodeling in L6 myoblasts precluded the enrichment of GLUT4 within 1−2 μm of the plasma membrane, in this case leading to the collapse of GLUT4 vesicles back to perinuclear regions.[85]

Input from Rho Family GTPases. The actin branching complex of Arp2/3 is regulated upstream by the nucleation promoting factors N-WASP, WAVE, and cortactin,[101] under the command of small GTPases of the Rho family.[102] Input from Rac1 to insulin-mediated GLUT4 translocation is well-substantiated. Endogenous Rac1 is activated (GTP-loaded) in response to insulin stimulation in myoblasts,[103] adipocytes,[103] and other cell types,[104] and this response is downstream of PI(3)K. Importantly, insulin-dependent Rac1 activation and its consequent reorganization of nonsarcomeric actin were recently confirmed in rodent skeletal muscles.[105,106] Expression of a dominant-negative Rac1 mutant or Rac1 downregulation via siRNA in muscle cells precluded both insulin-induced actin remodeling[107,108] and insulin-stimulated GLUT4 translocation.[107,108]

A related Rho family GTPase, Cdc42, is also rapidly GTP-loaded in response to insulin stimulation in muscle cells (A. Koshkina and A. Klip, unpublished observation) and adipocytes.[109] In the latter, microinjection of an anti-Cdc42 antibody or Cdc42 siRNA decreased the rate of insulin-induced GLUT4 translocation, and conversely, constitutively active Cdc42 promoted GLUT4 translocation in a PKCλ-dependent manner.[109] Downstream of Cdc42, the dominant-negative mutant of N-WASP (N-WASP-ΔWA) also inhibits the action of insulin on GLUT4 translocation, indicating a dependence of GLUT4 recycling on N-WASP-directed cortical F-actin assembly.[110] However, because in earlier studies neither constitutively active, wild-type nor dominant-negative Cdc42 had any effect on the cortical actin structures or insulin-stimulated GLUT4 translocation,[111,112] the significance of Cdc42 activation for GLUT4 traffic is not fully resolved. In adipocytes, but not in muscle cells, a Cdc42-related GTPase, TC10, promotes cortical actin polymerization and contributes to GLUT4 exocytosis in a manner independent of the canonical PI(3)K input, instead becoming activated via a cascade involving proteins Cap, Cbl, and C3G.[103,111,113,114] Downstream substrates of TC10 include Cdc42-interacting protein 4/2 (CIP4/2),[115] N-WASP,[110] and Exo70.[116] Insulin-mediated activation of TC10 may also lead to the formation of PI(3)P at the PM,[117] recruitment of the exocyst complex to the PM via Exo70,[116] and inactivation of Rab31.[118] However, the relevance of this pathway in insulin-stimulated GLUT4 translocation is controversial because of conflicting results from studies using siRNA,[114] or selective gene knockout in mice,[119] to deplete individual elements of the pathway.

Actin-Based Molecular Motors. Vesicular transport along cortical actin filaments is achieved by actin-associated molecular motors, typically of the myosin family. Members of the myosin V (MyoV) family form homodimers that act as processive motors, advancing in 36 nm steps along actin filaments (corresponding to their helical periodicity). Interestingly, isoforms MyoVa and MyoVb have been implicated in the transit of GLUT4 to the PM.[120,121] Downregulation of myosin Va or expression of dominant-negative mutants attenuated the insulin-stimulated GLUT4 translocation in 3T3-L1 adipocytes.[120] MyoVa is regulated by insulin through Akt2-dependent serine phosphorylation and consequent binding to both GLUT4-containing vesicles and the actin cytoskeleton.[120] The potential participation of MyoVb or related proteins is suggested by its interaction with Rab8A, a target of AS160, and by the abrogation of insulin-dependent GLUT4 translocation in L6 myoblasts overexpressing the Rab8A-binding fragment of MyoVb.[121]

In contrast to members of the MyoV family, class I myosins are single-headed and nonprocessive.[122] Downregulation of Myo1c expression dampens the overall insulin-stimulated translocation of GLUT4 to the PM in adipose[90,123,124] and muscle cells.[125] The ATPase activity of Myo1c can be regulated through phosphorylation on S701 by Ca^{2+}- and calmodulin-dependent kinase II (CaMKII) in insulin-stimulated 3T3-L1 adipocytes.[126] Accordingly, expression of wild-type (WT) Myo1c, but not S701A or an ATPase-dead mutant, rescued the inhibition of GLUT4 translocation caused by siRNA-mediated Myo1c knockdown. However, CaMKII is not required for GLUT4 translocation in myoblasts or muscle tissue,[125,127] highlighting once again differences in the fine-tuning of this process in adipose and muscle cells. Myo1c also dictates recruitment of the exocyst complex to the PM through interaction with the small GTPase RalA[128] that binds the exocyst subunits Sec5 and Exo84;[129,130] however, whether the Myo1c ATPase activity is required for exocytic GLUT4 vesicle recruitment is unknown.

In addition to type I and V myosin motors, non-muscle myosin II (NM-MyoII) class motors, expressed by muscle and non-muscle cells,[131] may participate in GLUT4 traffic.[132,133] Whether NM-MyoII regulates GLUT4 vesicular transport remains to be elucidated. However, NM-MyoIIA depletion inhibited the insulin-stimulated binding of the GSV-resident SNARE protein VAMP2 to the PM SNARE Syntaxin4,[132] raising the interesting possibility that MyoIIA may act at the level of IRV docking with the PM.

Regulation of GLUT4 Vesicle Docking and Fusion. In addition to the regulation of IRV movement and availability near the PM, the membrane itself is an important target of regulatory events. As revealed by in vitro fusion assays, insulin-stimulated plasma membrane liposomes supported vesicle fusion in the presence of cytosol from unstimulated cells.[134] Recent studies using TIRF microscopy further supported the possibility that the PM is an important target of insulin action. In unstimulated primary rat and 3T3-L1 adipocytes, highly mobile GLUT4 vesicles are observed in the TIRF zone.[87,135] As reviewed recently in ref 13, insulin stimulation (a) increases the rate of redistribution of GLUT4 vesicles closer to the PM, (b) causes a reduction in the mobility of GLUT4-containing vesicles within the TIRF zone[136,137] and increases ~2-fold the rate of immobilization events (considered to be tethering or docking),[87] (c) decreases the vesicle tethering and/or docking duration (dwell time) prior to membrane fusion, and (d) significantly increases the rate of fusion with the PM (varying from 4- to 42-fold increases in different studies).[87,135,136,138] In contrast to previous studies[87,135] that reflected the behavior of vesicles successfully captured at the PM, the dwelling time of the GLUT4-containing vesicles, prior to their capture, is increased upon insulin stimulation.[46] The mechanisms underlying insulin-responsive regulation of this capturing step ahead of vesicle tethering and/or docking remain to be determined. In addition, the possibility that the stalled GFP-GLUT4 vesicles observed in the TIRF zone are clathrin-rich endocytic foci[90] requires further investigation.

Table 1. Components of GLUT4 Traffic Machinery in Which Defects Have Been Linked to Insulin Resistance

step in GLUT4 traffic	model of insulin resistance (IR)	expression level in IR	evidence of defect	ref
RETENTION				
sortilin	C2C12 myotubes, 16 h with 1 mM palmitate	decreased	PPARγ agonist prevented palmitate-induced reductions in sortilin expression and GLUT4 translocation, without restoring insulin signals; sortilin knockdown inhibited GLUT4 translocation without reducing pAkt	168
STEPS?				
components?	3T3-L1 adipocytes, overnight with 17 nM insulin	not available	reduced rate of insulin-stimulated GLUT4 accumulation beneath the PM and reduced rate of docking and fusion	18
Myo1c	Rodent high-fat feeding, 9 weeks, 60% fat	decreased	correlated with reduced rate of insulin-dependent uptake of glucose into muscle	167
DOCKING AND FUSION				
SNAP-23	HL-1 cardiomyocytes, 24 h with 360 μM oleate	unchanged	less SNAP-23 in PM and more SNAP-23 in lipid droplets; SNAP-23 overexpression restored insulin-dependent glucose uptake and GLUT4 translocation	170
	Skeletal muscle biopsies from type 2 diabetes patients	increased	less SNAP-23 in PM and more in microsomes and cytosol; SNAP-23 correlated positively with lipid accumulation	171
	Skeletal muscle of streptozotocin-diabetic rodents	decreased	lower protein levels associated with insulin resistance	172
Munc18c	Skeletal muscle biopsies from type 2 diabetes patients	increased	high Munc18c levels associated with insulin resistance	171
	3T3-L1 adipocytes, 18 h with 25 mM glucose and 0.6 nM insulin	unchanged	impaired insulin-stimulated Munc18c traffic to the PM	165
	3T3-L1 adipocytes, 14 h with 2 mM glucosamine	unchanged	reduced insulin-stimulated content of Munc18c in the PM; Munc18c was O-glycosylated	166
Stx-4 and VAMP2	Zucker Diabetic Fatty Rats	increased	high protein levels associated with insulin resistance in skeletal muscle; rosiglitazone restored normoglycemia, as well as Stx-4 and VAMP2 expression	169
	3T3-L1 adipocytes, 24 h with 500 nM insulin	increased	high protein levels associated with insulin resistance	169
	3T3-L1 adipocytes, 14 h with 2 mM glucosamine	unchanged	reduced insulin-stimulated content of both proteins in the PM; blocked insulin-stimulated interaction of VAMP2 and Stx-4	166
	Skeletal muscle of streptozotocin-diabetic rodents	increased	VAMP2 levels increased in the PM and reduced intracellularly; Stx-4 levels increased in both locations; high protein levels associated with insulin resistance	172

The docking process likely involves the association of GLUT4 vesicles with proteins localized to the PM. One possibility is that changes in phospholipid content in the PM, mediated by insulin, attract tethering proteins to the PM. The levels of phosphoinositides are precisely regulated by kinases (such as PIKfyve) and phosphatases (such as PTEN, SHIP2, SKIP, and 72-5ptase) in the insulin-regulated process of GLUT4 translocation (for reviews, see refs 5 and 139). By this reasoning, α-actinin-4, a protein that binds to phospholipids[140] and actin filaments, as well as GLUT4 itself,[141] is a candidate GLUT4 tether at the PM. Other possible tethers are Rab11 and AS160-interacting protein Rip11, which binds phosphoinositide PIP_3 and phosphatidic acid (PA).[142] In adipocytes, Rip11 is required for the net translocation of GLUT4 to the PM in response to insulin. Finally, the exocyst protein complex, consisting of Exo70, Sec6, Sec8, and SAP97, functions in the tethering of GLUT4 vesicles to the PM in 3T3-L1 adipocytes.[116,143] Insulin increases the amount of Exo70 in the vicinity of the PM,[137] and Exo70 binds phosphatidylinositol 4,5-bisphosphate.[144] An alternative scenario is that Exo70 binds TC10 and Snapin,[145] thereby associating with the SNARE machinery. However, fusion of GLUT4 with the PM of primary rat adipocytes was not affected by overexpression of Exo70,[137] highlighting cell type differences and calling for more in-depth analysis of the function of Exo70 in this step.

Fusion of GLUT4 vesicles with the muscle or fat cell PM is mediated by SNAP-associated receptor (SNARE) proteins VAMP2, Syntaxin 4, and SNAP23 and their regulatory partners, munc18c, synip, and possibly synaptotagmin (reviewed in ref 13). Although VAMP2 is by far the most validated v-SNARE involved in this process, a recent study presented evidence that VAMP3 and VAMP8 may be somewhat functionally redundant for insulin-induced docking of GLUT4 vesicles at the PM.[146]

A novel input of insulin on the PM was discovered recently by Stenkula and colleagues.[136] In 3T3-L1 adipocytes, GLUT4 exists in the PM as freely diffusing molecules and as relatively stationary clusters.[136] After fusion, GLUT4 molecules are either dispersed along the PM (fusion with release) or retained at the site of fusion (fusion with retention). Insulin stimulation caused an ~2-fold acceleration of the fusion-with-retention[136] and more than 60-fold increases of the fusion-with-release step,[136] as revealed by monitoring the fusion of single GSV doubly labeled with HA-GLUT4-mCherry and pH-sensitive IRAP-pHluorin. Interestingly, in the skeletal muscle of a living animal, GLUT4-EGFP "storage structures" are also depleted during insulin-induced GLUT4−EGFP translocation.[147] Whether these two steps of insulin-stimulated fusion exist in rat primary adipocytes and muscle cells as well the underlying molecular mechanisms remain to be determined.

Input of Ca^{2+} to GLUT4 Translocation. Ca^{2+} is involved in a number of exocytic processes, both as a signal and as a regulator of docking complexes, yet its participation in insulin-mediated GLUT4 traffic is still controversial. Insulin does not change the total cytosolic Ca^{2+} levels in skeletal muscle cells or fibers,[148−150] but it elevates the concentration of free Ca^{2+} beneath the plasma membrane in isolated mouse skeletal muscle fibers[150] and elicits a cytosolic Ca^{2+} transient in primary cardiomyocytes.[151] Chelating cytosolic Ca^{2+} decreases the rate of insulin-stimulated uptake of glucose in adipocytes,[152,153] skeletal muscle fibers,[154] and cardiomyocytes.[151] However, this procedure also impairs insulin-stimulated Akt phosphorylation[151,152] and may depolymerize microtubules,[155] precluding assignment of the input of the action to a traffic or docking step. It is possible that insulin triggers calcium influx through TRPC3 channels into skeletal muscle, as TRPC3 knockdown decreased the rate of insulin-mediated uptake of glucose without affecting Akt activation or the resting intracellular Ca^{2+} concentration.[154] However, the effect of TRPC3 knockdown on the insulin-mediated increase in the submembrane Ca^{2+} concentration was not tested. Conversely, in cardiomyocytes, the insulin-induced cytosolic Ca^{2+} transient was prevented by knockdown of the endoplasmic reticulum IP_3 receptor-gated channel concomitant with prevention of GLUT4 translocation.[151] It will be important to dissect whether steps in docking and fusion of GLUT4 with the PM are sensitive to Ca^{2+}.

On the other hand, cytosolic Ca^{2+} has a fundamental role in contraction-mediated glucose uptake in skeletal muscle[156] and glucose uptake and GLUT4 translocation in contracting C2C12 myotubes[157] likely associated with triggering tropomyosin activation and sarcomere contraction. Whether the cation has additional input in vesicle traffic or docking per se is difficult to discern against its background as a contraction signal.

NEW LEADS INTO INSULIN RESISTANCE

Although it is outside of the scope of this review, it is important to highlight the fact that the insulin-dependent increase in the level of surface GLUT4 is defective in insulin resistance and type 2 diabetes. This was first demonstrated by subcellular fractionation of rodent adipose[158] and muscle[159,160] tissues and later by fractionation[161] and photolabeling[162] of human muscle. The defect may arise from impaired insulin signaling, and indeed, a body of literature documents altered phosphorylation of IRS-1, Akt, atypical PKC, and more recently AS160, as well as altered activation of Rac1.[107,163,164] Not all of these alterations necessarily occur in all instances of insulin resistance, and moreover, their quantitative contribution to the reduction in the level of GLUT4 traffic is unknown. The defect may also arise from impairments in the translocation machinery as defined by molecules directly involved in GLUT4 sorting, retention, movement, docking and tethering, and fusion. Recent exciting studies using diverse conditions to provoke insulin resistance have shown changes in the expression and localization of sortilin, Myo1c, and the SNARE complex components SNAP-23, VAMP2, syntaxin 4, and their regulator Munc18c (Table 1).[165−172] While most of these changes are so far only correlative to defective GLUT4 translocation and glucose uptake, future work may reveal if they are suitable targets for pharmacological intervention to combat type 2 diabetes.

CONCLUDING REMARKS

Recent discoveries have mapped the intricate yet organized routes and kinetics of GLUT4 traffic within muscle and adipose cells. The regulatory proteins identified will now offer the possibility of exploring whether there are defects in specific steps of GLUT4 traffic under conditions of insulin resistance. It will also be possible in the future to establish whether each step is regulated in concert or independently of others. We are hopeful that strategies will thereby emerge to accelerate or slow the permanence of GLUT4 at the cell surface and consequently to control or fine-tune the rate of uptake of glucose into muscle and fat cells.

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ABBREVIATIONS

AMP, adenosine monophosphate; AMPK, AMP-regulated kinase; AS160, Akt substrate of 160 kDa; CME, clathrin-mediated endocytosis; EE, early endosomes; ERC, endosomal recycling compartment; GLUT4, glucose transporter 4; GSV, GLUT4 storage vesicles; IRAP, insulin-regulated aminopeptidase; IRS-1, insulin receptor substrate-1; IRV, insulin-responsive vesicles; PI(3)K, phosphatidylinositol 3-kinase; PM, plasma membrane; TfR, transferrin receptor; TGN, trans-Golgi network; TUG, tether containing a UBX domain for GLUT4; VAMP, vesicle-associated membrane protein.

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