The concerted regulation of glucose uptake, utilization, and storage by tissues is critical to maintaining blood glucose homeostasis. Insulin increases glucose uptake into peripheral tissues, primarily skeletal muscle and adipose tissues, which express the GLUT4 isoform of the glucose transporter. Under basal conditions, GLUT4 is efficiently sequestered in intracellular compartments. Upon insulin stimulation, targeted exocytosis of GLUT4 to the plasma membrane (PM) and possibly restrained transporter endocytosis participate to increase glucose uptake. The existence of a specialized GLUT4 compartment that is exclusively insulin responsive has been postulated, but such an organelle has not been fully biochemically characterized. The excursion of GLUT4-containing vesicles to and from the PM, the dynamic traffic of GLUT4 between multiple intracellular compartments, and vesicle–membrane fusion are exquisite. These processes are controlled through the coordinated temporal and spatial activation of phosphoinositide 3-kinase (PI3K), the serine/threonine kinases Akt and atypical protein kinase C (aPKC)-H9256/H9261, and possibly the adaptors c-Cbl-associated protein (CAP)/Cbl and small G protein TC10. In addition to GLUT4 translocation, insulin may also increase the intrinsic activity of GLUT4 at the PM to achieve maximal glucose uptake (4). Negative input also occurs at multiple levels of the insulin-signaling pathway to fine-tune the acute action of the hormone and to ensure appropriate biological responses. Defects in one or more postreceptor insulin-signaling components accompany the insulin resistance observed in type 2 diabetes mellitus and may be primary to the disease or further exacerbate it. Consequently, elucidating the key molecular components that mediate insulin action is required to fully understand insulin resistance. Here we highlight the key molecular signals that are turned on and off by insulin to accomplish this task.

GLUT4 Traffic Through Intracellular Compartments

In unstimulated fat or muscle cells (i.e., the basal state), ~3–10% of GLUT4 is located at the cell surface and >90% is in intracellular compartments (FIGURE 1A). Insulin shifts the steady-state distribution of GLUT4 toward the PM by elevating the exocytic rate of GLUT4 and minimally reducing its endocytic rate (82, 122). Concomitant with this shift, the perinuclear distribution of GLUT4 is remodelled from an initial conical cluster to a tighter ring (130) (FIGURE 1A). Within 10 min, the level of surface GLUT4 increases 2- to 3-fold in skeletal muscle tissue and muscle cells (130) (FIGURE 1B) and human adipocytes, but the increase is >10-fold in rodent adipocytes. There is ample evidence of partial segregation of GLUT4 from the endosomal recycling compartment (ERC) (86, 90, 156). Thus, as determined by velocity gradient centrifugation of adipose cells, GLUT4 is found in two endomembrane fractions, only one containing transferring receptor (TR, a marker of the ERC) (64). By chemical ablation of the TR-containing compartment in intact 3T3-L1 adipocytes, only 40% of GLUT4 is affected (86, 156). Upon immunoprecipitation of GLUT4-rich endomembranes from L6 muscle cells, recycling proteins are partly excluded (138). These and related studies have given strong support for the existence of two pools of GLUT4, differentiated by their inclusion or exclusion of ERC markers. Here we assign the term “specialized compartment” (SC) to the non-ERC pool of GLUT4. It has been debated whether there is a static pool of GLUT4 (43) or whether GLUT4
endocytic compartments, ERC, and SC. The possibility that the SC merges/interfaces with the trans-Golgi network (TGN) remains controversial (69, 106, 125). Hence the definition of SC to date is "an insulin-responsive GLUT4 compartment devoid of ERC markers." Although all of the GLUT4 complement cycles to the PM in unstimulated muscle cells (37, 111), GLUT4 vesicles arriving at the PM differ from those drawn by insulin, as only the latter require the v-SNARE vesicle-associated membrane protein (VAMP2) for fusion (20, 35, 89, 100, 109, 110). These results raise the possibility that GLUT4 arrives at the PM in vesicles distinct from those emanating from the ERC. Indeed, in insulin-stimulated Chinese hamster ovary cells, GLUT4 and TfR arrive at the PM in distinct vesicles despite the fact that in these fibroblasts the two proteins coexist within the ERC (80). Functionally, neither basal-state recycling nor insulin-stimulated GLUT4 vesicles depend on VAMP3 (110); instead, recent results suggest that VAMP7 participates in GLUT4 recycling in the basal state but not in response to insulin (111). Consistent with these observations, a significant proportion of intracellular GLUT4 colocalizes with VAMP2 by indirect immunofluorescence microscopy detection (88, 90) and a fraction of immunopurified GLUT4 compartment is enriched in VAMP2 (Refs. 49 and 109; also see Ref.

slowly recycles either to the PM or back to the ERC (37, 43, 69). We have observed that all of the intracellular GLUT4 of L6 muscle cells recycles to the PM within 6 h (37), whereas the existence of such behavior is in dispute in 3T3-L1 adipocytes (43, 69). In muscle cells, GLUT4 molecules from the PM reach the ERC within 20 min and rapidly exit this compartment 20 min later (37). Because recycling back to the PM is very slow, these results suggest that GLUT4 exits the ERC en route to the SC. Insulin accelerates the transit of GLUT4 through the ERC (37), suggesting that refueling of the SC from ERC is insulin dependent.

There are no known biochemical markers of the SC. The insulin-regulated aminopeptidase (IRAP) mimics GLUT4 in its itinerary and hence is not a marker of any specific compartment populated by GLUT4, yet it is often used as a surrogate marker when examining GLUT4 traffic in response to insulin. The role of IRAP in insulin action is unknown (72), but its NH2 terminus may serve to sequester/tether insulin-responsive GLUT4 vesicles in an intracellular site(s), possibly via binding a regulatory traffic protein (52, 148).

Although GLUT4-containing endomembranes have been purified using antibodies to the GLUT4 COOH-terminal end (and erroneously named GLUT4 vesicles), they include components of endocytic compartments, ERC, and SC. The possibility that the SC merges/interfaces with the trans-Golgi network (TGN) remains controversial (69, 106, 125). Hence the definition of SC to date is "an insulin-responsive GLUT4 compartment devoid of ERC markers." Although all of the GLUT4 complement cycles to the PM in unstimulated muscle cells (37, 111), GLUT4 vesicles arriving at the PM differ from those drawn by insulin, as only the latter require the v-SNARE vesicle-associated membrane protein (VAMP2) for fusion (20, 35, 89, 100, 109, 110). These results raise the possibility that GLUT4 arrives at the PM in vesicles distinct from those emanating from the ERC. Indeed, in insulin-stimulated Chinese hamster ovary cells, GLUT4 and TfR arrive at the PM in distinct vesicles despite the fact that in these fibroblasts the two proteins coexist within the ERC (80). Functionally, neither basal-state recycling nor insulin-stimulated GLUT4 vesicles depend on VAMP3 (110); instead, recent results suggest that VAMP7 participates in GLUT4 recycling in the basal state but not in response to insulin (111). Consistent with these observations, a significant proportion of intracellular GLUT4 colocalizes with VAMP2 by indirect immunofluorescence microscopy detection (88, 90) and a fraction of immunopurified GLUT4 compartment is enriched in VAMP2 (Refs. 49 and 109; also see Ref.
The PH domain of IRS-1 interacts with a protein called PH domain-interacting protein, and interfering with its binding to IRS-1 reduces insulin-dependent GLUT4 translocation (32) through a mechanism that requires further investigation. Better understood is the role of SH2 domain-containing proteins that bind to tyrosine-phosphorylation motifs in IRS, notably the regulatory subunit of class IA PI3K.

PI3K. Class IA PI3K is a heterodimer consisting of an 85-kDa regulatory subunit (p85) and a 110-kDa catalytic subunit (p110). Interaction of tyrosine-phosphorylated IRS-1/-2 with the SH2 domain of p85 activates the p110 subunit (28, 97). The major lipid substrate of class IA PI3K is phosphatidylinositol-4,5-bisphosphate [PI(4,5)P_2], which is phosphorylated to produce phosphatidylinositol-3,4,5-trisphosphate [PI(3,4,5)P_3]. A rise in PI(3,4,5)P_3 at the PM and endomembranes, in turn, provides a lipid-based platform that attracts downstream PH domain-containing signaling molecules (135) (FIGURE 2).

Stimulation of muscle and fat cells with insulin primarily increases the level of PI(3,4,5)P_3 but also of phosphatidylinositol-3,4-bisphosphate [PI(3,4)P_2] and phosphatidylinositol-3-phosphate [PI(3)P] (87). It is not clear if the latter two lipids arise from PI(3,4,5)P_3 or can also be engendered through other pathways. Indeed, insulin also causes a rapid increase in the activity of class IIa and class IIb PI3K (14, 15), which produce PI(3)P and PI(3,4)P_2 of PIKfyve, a phosphatidylinositol-5-kinase that generates phosphatidylinositol-3,5-bisphosphate (56, 123); and of type I phosphatidylinositol 4-phosphate 5-kinase, which generates PI(4,5)P_2 (66). Because activation of these lipid kinases by insulin produces an array of lipid products, the particular phosphoinositides involved and the distinct step(s) in insulin action at which they exert regulatory input requires thorough investigation.

An essential role for class IA PI3K in insulin regulation of GLUT4 translocation and glucose uptake was first revealed by using two inhibitors of PI3K, wortmannin and LY294002 (25), and was subsequently verified by using mutant forms of its p85 and p110 subunits (70, 77, 132) and microinjection of p85 fusion proteins (48). These studies scored the net gain of GLUT4 at the PM. More recent studies have focused on the specific steps in GLUT4 traffic that may be regulated via PI3K. In muscle cells, either pharmacological or molecular inhibition of PI3K reduced the interendosomal transit of GLUT4 (37), the actin remodeling that is required for GLUT4 translocation (146), and the insertion of GLUT4 at the PM (58). Likewise, in adipocytes, the PI3K inhibitor LY294002 prevented fusion of GLUT4 vesicles, rendering a population of vesicles apparently docked at the PM (12). Complementing these

**Turning the Signals On: Positive Regulators of Insulin Actions**

Although the insulin-responsive GLUT4 compartment has yet to be localized, purified, and fully characterized, insulin stimulation of GLUT4 translocation must entail GLUT4 vesicle budding out of the ERC and/or SC as well as its procession to, and fusion with, the PM. This intrinsically regulated sequence of events is initiated by a cascade of signaling molecules that are turned on upon insulin stimulation, and, in principle, these signals must impact these compartments to initiate GLUT4 translocation (FIGURE 2).

**Insulin receptor and insulin receptor tyrosine kinase**

The insulin receptor (IR) is a heterotetrameric member of a large transmembrane tyrosine kinase receptor family. Insulin binding causes conformational changes in the receptor and stimulates its intrinsic tyrosine kinase activity in the β-subunit region (103). Several proteins with phosphotyrosine-binding (PTB) and Src homology 2 (SH2) domains have been identified as substrates of the IR, of which the IR substrate (IRS) family, CAP, and adaptor proteins associated with pleckstrin homology (PH) and SH2 (APS) domains, have been implicated in insulin’s metabolic actions (FIGURE 2).

**The proximal signaling intermediates: the IRS and PI3K families**

IRS. IRS proteins are the targets of the IR involved in the majority of the metabolic effects of insulin (FIGURE 2). Four isoforms (IRS-1 through -4) have been identified in rodents and three (IRS-1, -2, and -4) in humans, with differential tissue expression pattern, subcellular localization, and time course of response to the hormone (42). The PH domain at the NH2 terminus localizes IRS proteins in close proximity to the IR, and the PTB domain facilitates its binding to tyrosine-phosphorylated IR (143).

That IRS-1 is required for insulin-dependent GLUT4 translocation in muscle is supported by studies in which IRS-1 expression is eliminated via selective gene knockout or small interfering (si) RNA (see Ref. 54 and references therein). However, in rodent adipocytes, IRS-2 and possibly IRS-3 and -4 may also contribute to GLUT4 translocation (42).

In summary, GLUT4 cycles through diverse endosomal entities. In muscle and adipose cells, about half of the GLUT4 resides in a pool distinct from the ERC that may be distinguished by the presence of VAMP2. A viable model is that insulin increases surface GLUT4 by drawing from this pool.
to insulin. The molecular mechanism(s) involved in such regulation are so far elusive but might include dissociation of an inhibitory protein and/or a conformational change in GLUT4. In support of this notion, exogenous PI(3,4,5)P3 caused GLUT4 insertion in the membrane, but the COOH terminus of the transporter was unavailable for antibody detection in permeabilized cells or membrane-adhered lawns (58). Moreover, several studies have reported that the GLUT4 COOH terminus becomes more prone to antigenic recognition following insulin stimulation (147). These findings observations, delivery of exogenous PI(3,4,5)P3, caused GLUT4 translocation (58, 87, 130) and fusion with the membrane (58, 130), whereas delivery of PI(3)P promoted arrival (58, 63, 87) without insertion of GLUT4 into the PM (58, 63).

Possible regulation of GLUT4 activity

Intriguingly, GLUT4 translocation by PI(3,4,5)P3 (58, 87, 130) or by a PI(4,5)P2-binding peptide (40) was insufficient to promote glucose transport, suggesting that additional signals or events must occur to bring about the full effect observed in response to insulin. The molecular mechanism(s) involved in such regulation are so far elusive but might include dissociation of an inhibitory protein and/or a conformational change in GLUT4. In support of this notion, exogenous PI(3,4,5)P3 caused GLUT4 insertion in the membrane, but the COOH terminus of the transporter was unavailable for antibody detection in permeabilized cells or membrane-adhered lawns (58). Moreover, several studies have reported that the GLUT4 COOH terminus becomes more prone to antigenic recognition following insulin stimulation (147). These findings
raise the hypothesis that in addition to GLUT4 mobilization and fusion with the PM, insulin stimulation may unmask the GLUT4 COOH terminus to render a fully functional transporter. This scenario requires further experimental verification.

Since the initial studies reporting GLUT4 translocation by insulin, discrepancies have been reported between the gain in GLUT4 at the PM and the stimulation of glucose uptake. This has led to the idea that insulin might increase the intrinsic activity of GLUT4. The reader is referred to a full analysis of this concept in a recent review (4). The insulin-derived signals that might regulate GLUT4 activity remain elusive, because our previous implication of p38 MAPK in this process has not been supported by selective gene silencing of the α- and β-isofoms of the enzyme (3). Further studies are required to understand the higher susceptibility of insulin-stimulated glucose uptake to chemical inhibitors of p38 MAPK compared with glucose uptake in unstimulated cells, including a higher susceptibility of insulin-stimulated GLUT4 to these agents (113).

Signals downstream of PI3K: Akt and aPKC

Downstream of IRS/PI3K, two major serine/threonine protein kinases, Akt and aPKC-α/γ, are activated and contribute to insulin regulation of GLUT4 traffic and glucose uptake (Figure 2).

Akt. Three mammalian isoforms of Akt (1–3) have been identified, and all are activated in response to insulin in a PI3K-dependent manner (153). Akt1 and Akt2 are expressed and activated in adipose and skeletal muscle tissue and cells lines, and Akt3 is also activated by the hormone in cell cultures (153). The insulin-dependent rise in PI(3,4,5)P3, recruits Akt1/2 to the PM and possibly to endomembranes, where the enzyme is phosphorylated on the Thr473 residue of its activation loop by 3-phosphoinositide-dependent kinase (PDK1) (34). The role of Ser473 phosphorylation in the COOH-terminal loop of Akt1/2 is controversial, but the emerging view is that Ser473 phosphorylation precedes and is required for Thr473 phosphorylation by PDK1 (34). Dual phosphorylation of Akt is required for its full activation. The identity of the kinase (referred to as either PDK2 or hydrophobic motif kinase) that phosphorylates Akt at Ser473/474 has remained elusive, although numerous candidates have been proposed. PDK2 was recently characterized as a protein kinase distinct from PDK1 (33, 53) that associates with cytoskeletal elements in the PM (53). Interestingly, the rictor-containing mammalian target of rapamycin (mTOR) complex, which is insensitive to rapamycin, phosphorylates Akt on Ser472 and facilitates Thr389 phosphorylation by PDK1 (120). It would be interesting to learn if the rictor-mTOR complex is indeed the Ser473 kinase that phosphorylates Akt following insulin stimulation.

The observation that overexpression of a PM-targeted, constitutively active (CA) Akt mutant promoted (76) but overexpression of kinase dead (KD) and inactivatable, dominant-negative (DN) Akt mutant inhibited (146) GLUT4 translocation implicates Akt as an essential component in this pathway. Introduction of peptides competing with endogenous Akt targets also reduced GLUT4 translocation in response to insulin (51). Although a requirement for Akt activation in insulin actions has been disputed (6, 74), more recent studies with Akt-null animals and cells derived thereof (5, 23), as well as Akt gene silencing via siRNA in 3T3-L1 adipocytes (61, 71), cement a role for the enzyme in GLUT4 translocation. In all of those studies, Akt2 was predominantly involved in this process, whereas Akt1 had a relatively modest contribution (5, 61, 71).

aPKC-α/γ. Of the three classes of PKC isoforms—conventional (α, β, γ), novel (δ, ε, η, ζ) and atypical (λ, η)—insulin activates only PKC-α/γ. Consistent with this finding, transient overexpression of mutants of conventional and novel PKC isoforms failed to alter basal or insulin-stimulated GLUT4 translocation (7). The mechanism for activation of PKC-α/γ requires PI3K input and involves phosphorylation of Thr410 in the activation loop of the enzyme via PDK1 (8), followed by autophosphorylation of Thr560 in the turn motif and a phosphorylation-independent conformational change that relieves autoinhibition (128).

That activation of PKC-α/γ contributes significantly to insulin-stimulated GLUT4 translocation and glucose uptake was suggested from the following observations. First, transient overexpression of either wild-type or CA PKC-α/γ in muscle and fat cells in culture mimicked, whereas KD PKC-α/γ attenuated, the stimulatory effects of insulin on GLUT4 translocation and glucose uptake (7, 78). Second, expression of PKC-α rescued the impaired insulin-stimulated glucose transport in mouse embryonic stem cells and adipocytes deficient in PKC-α (9). Third, in IRS-2-deficient brown adipocytes that also exhibit impaired insulin-stimulated glucose transport, PKC-γ was not activated (127). In contrast to the above findings, siRNA-mediated silencing of PKC-α/γ failed to alter insulin-stimulated glucose uptake in 3T3-L1 adipocytes, suggesting that either activation of aPKC is unnecessary or that activation of Akt is sufficient to maintain insulin-stimulated glucose uptake (158). It is important to explore further the discrepancies observed upon overexpression of diverse mutants vs. elimination of the expression of the endogenous protein in insulin signal transduction.
The specific aspects of GLUT4 traffic that are regulated by either Akt or aPKC have been the subject of recent studies. Akt has been proposed to act both at the level of the SC and the PM, as follows. First, insulin promotes the recovery of Akt2 in intracellular compartments enriched in GLUT4, biochemically isolated from rat adipocytes (79). Second, DN Akt prevented the insulin-induced accelerated movement of GLUT4 through the ERC in L6 myoblasts (37). Such interendosomal acceleration may be linked to the transit of GLUT4 from ERC to SC, and hence the molecular detail of such input must be further analyzed. In this regard, PIKfyve was proposed to increase GLUT4 sorting from internalizing endosomes through the TGN and into the insulin-sensitive GLUT4 pool, and the Ser\(^{318}\) located in the PI(3)P-binding FYVE domain of PIKfyve is phosphorylated by Akt in an insulin- and PI3K-dependent manner (10). Third, further implication of Akt action on intracellular compartments stems from studies with Akt and GLUT4 chimeras. Expression of either kinase-inactive Akt1 (29) or Akt2 (21) fused to GLUT4 prevented GLUT4/IRAP translocation in response to insulin. However, although expression of wild-type Akt2 fused to the COOH terminus of GLUT4 (21) promoted insulin-stimulated GLUT4 translocation, expression of a CA Akt1 fused to the NH\(_2\) terminus of GLUT4 was without effect on IRAP translocation (29). The disparity between these results may have resulted from the different constructs used in the studies. Nonetheless, all of the findings summarized above provide evidence that activation of Akt1/2 either at or in close proximity to GLUT4 vesicles is necessary for insulin to promote GLUT4 translocation (21, 29).

On the other hand, it has also been proposed that Akt regulates the docking/fusion of GLUT4 vesicles with the PM, based on two intriguing observations: First, at 19°C insulin promotes GLUT4 translocation but neither fusion with the PM nor Akt activation (141). Subsequent rewarming to 37°C allows Akt activation and GLUT4 insertion into the PM (141). Second, Akt phosphorylates Synip, a syntaxin-4 interacting protein presumed to mediate vesicle fusion with the PM (154), although a recent report finds that phosphorylation of Synip on Ser\(^{39}\) is not required for insulin-stimulated GLUT4 translocation (118). Clearly, more studies are required to unravel the full extent of Akt action, and it is quite possible that the enzyme may impinge on more than one intracellular locus to exert its regulatory action on GLUT4.

As with Akt, aPKC appears to have effects on both intracellular and surface target events of GLUT4 traffic. The intracellular events involve PKC-\(\lambda\)-dependent interaction between the small GTPase Rab4, the motor protein kinesin KIF3, and microtubules (57). The cell-surface events involve regulation of the vesicle-fusion machinery, specifically of proteins of the SNARE complex (VAMP2, syntaxin-4, and SNAP23) and its regulators munc18c and Synip. Indeed, DN PKC-\(\zeta\) prevented the insulin-induced serine phosphorylation of VAMP2 (13), and insulin increased PKC-\(\zeta\) communoprecipitation with munc18c (43, 63). Both munc18c and Synip bind to syntaxin-4 to mediate insulin-stimulated GLUT4-vesicle fusion with the PM (36). Although syntaxin-4 and SNAP23 can be phosphorylated in vitro by conventional PKCs and syntaxin-4 is also phosphorylated by PKA (38), phosphorylation of these proteins in response to insulin has not been demonstrated (36).

In summary, aPKC and Akt each have regulatory inputs on intracellular pools and fusion events at the PM. The latter events might include regulation of the SNARE complex. The extent to which activation of aPKC and Akt might reflect reciprocal and/or redundant inputs in insulin regulation of GLUT4 traffic and hence might share target substrates requires further examination.

### The APS-CAP-Cbl-TC10 link

In recent years, a PI3K-independent pathway involving CAP and APS interaction with c-Cbl was proposed to participate in insulin-stimulated GLUT4 translocation and glucose uptake in adipose cells in culture (FIGURE 2). In this paradigm, APS and CAP recruitment to the vicinity of the IR engages Cbl that becomes tyrosine phosphorylated, thereafter binding the complex of CrkII and Crk SH3-binding guanine nucleotide-releasing factor (C3G), a proposed exchange factor for the small GTP-binding protein TC10 (2, 22). Activation of TC10 via CAP-Cbl-CrkII was proposed to ultimately impinge on actin dynamics and GLUT4 translocation, as overexpression of mutants of CAP and TC10 interfered with one or both of these outcomes (22). An alternative possibility was proposed whereby TC10 activation was linked to PI(3)P production (87), potentially via activation of class II PI3K downstream of TC10. As stated above, PI(3)P may contribute to GLUT4 mobilization toward the PM. However, the PI3K and TC10 pathways may not be as independent from each other as originally proposed, as Cbl can lead to aPKC via PI3K in

"...\(\alpha\)PKC and Akt each have regulatory inputs on intracellular pools and fusion events at the PM. The latter events might include regulation of the SNARE complex."
parallel to aPKC activation by the conventional IRS → PI3K axis (93, 129). Curiously, TC10 may affect GLUT4 translocation irrespective of its Cbl-CrkII-activated GTPase activity (24).

The participation of elements of the APS-CAP-Cbl-TC10 pathway in insulin-dependent GLUT4 translocation to the PM has largely been supported by studies using overexpression of diverse mutants of these proteins. However, as in the case of the aPKC signal, elimination of the endogenous proteins via siRNA has challenged those studies. Indeed, siRNA-targeted silencing of CAP, Cbl, and CrkII in 3T3-L1 adipocytes was innocuous to insulin-stimulated GLUT4 translocation or glucose uptake (92). Conversely, however, APS gene silencing attenuated insulin-mediated glucose transport (21). Knockout of APS (91) and Cbl (95) genes in mice failed to reduce insulin action and in fact produced an insulin-hypersensitive phenotype. These observations may be linked to the apparent lack of participation of the CAP-Cbl-TC10 link in skeletal muscle. Indeed, Cbl is not phosphorylated in this tissue in response to an in vivo insulin challenge (134). Similarly, L6 myoblasts that do not express CAP mount an insulin-dependent GLUT4 translocation, and overexpression of TC10 mutants in these cells fails to prevent such translocation (60), unlike the situation in 3T3-L1 adipocytes.

In summary, PI3K activation is essential for GLUT4 translocation in response to insulin, and its downstream effectors Akt and aPKC control GLUT4 traffic within/from intracellular compartments and at the PM. This signaling axis was substantiated in all muscle and adipose cell systems studied thus far. In adipose cells, there may be additional contribution of the CAP-Cbl-TC10 link, but more studies are required to elucidate specific roles of each of these proteins in GLUT4 mobilization.

**Specificity of insulin signaling**

The signaling cascades that mediate the majority of the metabolic actions of insulin are not unique to this hormone. Activation of PI3K/Akt is common to numerous stimuli, yet they do not elicit the same metabolic responses. An obvious question arising is what confers biological specificity to insulin regulation of intracellular GLUT4 traffic and glucose uptake? A first level of discernment of the signal concerns the IRS proteins, which are unique to signal transduction by insulin and insulin-like growth factor receptor I. IRS may activate a subset of PI3K or may direct this enzyme to key locations in the cell, resulting in GLUT4 translocation. A second level of discernment may lie in the time course and amplitude of the PI(3,4,5)P3 signal elicited by each stimulus. It has been proposed that a threshold level of PI(3,4,5)P3 (as in the case of insulin stimulation) is required for GLUT4 insertion into the PM, whereas a persistent but low-amplitude rise in this lipid (as in the case of PDGF stimulation) is not conducive to this outcome (133). However, overexpression of PDGF receptors in cells expressing GLUT4 or of GLUT4 in cells expressing the receptors can lead to PDGF-induced GLUT4 translocation (137, 152). In the latter case, GLUT4 appears to be recruited from a compartment distinct from that engaged by insulin as it does not involve VAMP2 fusion with the PM (137). Yet another level of distinction between insulin- and PDGF-induced signals concerns their subcellular distribution. Following insulin stimulation, the IRS/PI3K complex (50), PKC-ζ (39), and Akt2 (19) co-purify with subcellular fractions enriched in GLUT4. Conversely, following PDGF stimulation, PI3K is more likely to co-purify with PM fractions (115). Collectively, these findings suggest that activation of signaling proteins in distinct spatial locations with differential temporal control might confer specificity to insulin action.

**Actin remodeling in insulin signaling**

Following insulin stimulation, dynamic remodeling of the actin cytoskeleton is observed in L6 muscle cells (136) and 3T3-L1 adipocytes (67), as well as in mature skeletal muscle (16) and adipose cells (101). A dynamic actin cytoskeleton is required for insulin-induced GLUT4 translocation and glucose uptake (136) and, in muscle cells, actin filament remodeling requires PI3K but not Akt activation (146). Hence, several outcomes derive from PI3K activation, some involving Akt and some independent of this enzymatic activity. However, the full activation of Akt requires actin dynamics (107), suggesting that Akt activation lies downstream of actin remodeling. It has been postulated that actin remodeling may generate a scaffold that coordinates the localized production of PI(3,4,5)P3, and spatially segregates a subpopulation of signaling molecules (IRS-1, p110α catalytic subunit of PI3K, and Akt) along with GLUT4 vesicles near the PM (31, 104). Alternatively, the insulin-induced actin remodeling may provide a physical platform for GLUT4 vesicles to travel to the PM, aided by motor proteins (11, 67).

**Turning Signals Off: Negative-Feedback Mechanisms**

The protein kinases activated in response to insulin are subjected to the opposing actions of protein phosphatases and negative-feedback loops. The fine balance between activation and deactivation is important to maintain appropriate insulin sensitivity. Conversely, alterations in such a balance may contribute to insulin resistance. We briefly review next those negative regulatory events that reduce
insulin-dependent GLUT4 translocation and its consequent elevation of glucose uptake (FIGURE 2). Finally, we identify a mechanism whereby insulin removes a “brake” to allow mobilization of GLUT4.

Serine phosphorylation of IRS-1

Concomitant with insulin-induced tyrosine phosphorylation of IRS-1, there is also a rise in phosphorylation of diverse serine residues (46). Whereas Akt phosphorylation of serine residues located within the PTB (Ser^{323} and Ser^{336}) and Shc and IRS-1 NPXY-binding (SAIN) (Ser^{325} and Ser^{358}) domains of IRS-1 prevents tyrosine-residue dephosphorylation by phosphatases (105), phosphorylation of other serine residues within the PTB domain (see Table 1) attenuates signaling, possibly by hindering the interaction of IRS with IR and/or the p85 regulatory subunit of PI3K (131). Also, phosphorylation of Ser^{312} following insulin stimulation promotes IRS-1 degradation (44). Finally, phosphorylation of Ser^{307} residue in IRS diminishes signaling toward PI3K (108), but interestingly phosphorylation of this residue can also elicit a positive effect on insulin signaling (59). Table 1 lists the diverse IRS-1 residues involved in downregulation of insulin signaling and the serine/threonine kinases that phosphorylate them. The reader is referred to more extensive reviews on the regulation of IRS-1 (42, 46, 62, 151).

Tyrosine and serine phosphatases

Insulin signaling is diminished by the action of the tyrosine phosphatases leukocyte common antigen-related phosphatase (LAR) and protein tyrosine phosphatase 1B (PTP1B) (FIGURE 2), although this is not universally demonstrated (41, 124). Overexpression of PTP1B attenuated insulin-stimulated GLUT4 translocation by ~20% in primary rat adipocytes (124), possibly by promoting dephosphorylation of IRS-1 (41). However, the phosphatase activity of PTP1B alone may be insufficient to modulate insulin action, since overexpression of this protein in 3T3-L1 adipocytes did not impair glucose transport despite a substantial reduction in insulin-stimulated IRS/PI3K activity (142). An additional negative regulatory input on insulin signaling toward GLUT4 occurs at the level of dephosphorylation and inactivation of Akt and PKC-\(\alpha\) by serine/threonine protein phosphatase 2A (139) (FIGURE 2), and such negative input participates in the insulin resistance generated by hypertonic shock or exposure to ceramide.

Lipid phosphatases: phosphatase and tensin homolog deleted on chromosome 10 and SH2-containing inositol phosphate 2

The tumor suppressor phosphatase and tensin homolog deleted on chromosome 10 (PTEN) hydrolyzes PI(3,4,5)P\(_3\) to PI(3,4)P\(_2\) and SH2-containing inositol phosphate 2 (SHIP2) hydrolyzes PI(3,4,5)P\(_3\) to PI(3,4)P\(_2\) (FIGURE 2). Both phosphatases are expressed in insulin-responsive tissues and are thought to negatively modulate insulin signaling. Overexpression of PTEN reduces insulin-induced GLUT4 translocation and glucose uptake in 3T3-L1 adipocytes (98), possibly through a decrease in PI(3,4,5)P\(_3\) levels, Akt2 and PKC-\(\alpha\) activation (102). The contribution of PTEN as a negative input in insulin action was confirmed by ablation of this protein via siRNA in 3T3-L1 adipocytes (158). In contrast to PTEN, the contribution of SHIP2 as a negative regulator of insulin action is controversial. Overexpression of SHIP2 in 3T3-L1 adipocytes inhibits the insulin-induced increase in PI(3,4,5)P\(_3\) levels, Akt2 and PKC-\(\alpha\) activation, membrane ruffling, GLUT4 translocation, and glucose uptake (121, 144, 145). However, elimination of the protein via siRNA did not alter insulin-stimulated glucose transport (158).

In summary, phosphorylation of diverse serine residues in IRS-1 and tyrosine phosphatases acting on the IR and IRS-1 can contribute to taper downstream signaling toward PI3K. In addition, lipid phosphatases control the duration of signaling through PI(3,4,5)P\(_3\). Although not discussed here,

Table 1. Serine residues on IRS that are phosphorylated by downstream kinases to attenuate insulin signal transduction

<table>
<thead>
<tr>
<th>Ser residues</th>
<th>Phosphorylated Kinase</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>302</td>
<td>PI3K-dependent</td>
<td>1</td>
</tr>
<tr>
<td>307 (312)*</td>
<td>PI3K-dependent</td>
<td>44, 116</td>
</tr>
<tr>
<td>307</td>
<td>mTOR/JNK</td>
<td>46, 150</td>
</tr>
<tr>
<td>318 (323)*</td>
<td>PKC(\xi)</td>
<td>94</td>
</tr>
<tr>
<td>332, 336</td>
<td>GSK3</td>
<td>84</td>
</tr>
<tr>
<td>408</td>
<td>PKC</td>
<td>85</td>
</tr>
<tr>
<td>570</td>
<td>PKC(\xi)</td>
<td>126</td>
</tr>
<tr>
<td>612 (616)*</td>
<td>PKC</td>
<td>150</td>
</tr>
<tr>
<td>612</td>
<td>ERK/mTOR/S6K1</td>
<td>46</td>
</tr>
<tr>
<td>612, 632, 662, 731</td>
<td>ND</td>
<td>96</td>
</tr>
<tr>
<td>632</td>
<td>ERK/mTOR/S6K1</td>
<td>46</td>
</tr>
<tr>
<td>632, 662, 731</td>
<td>Akt</td>
<td>83</td>
</tr>
<tr>
<td>789 (794)*</td>
<td>GSK3</td>
<td>108</td>
</tr>
<tr>
<td>9</td>
<td>GSK3</td>
<td>30</td>
</tr>
<tr>
<td>ND</td>
<td>PKC(\xi)</td>
<td>112</td>
</tr>
</tbody>
</table>

The sequence numbers shown are rat or mouse IRS-1. GSK3, glycogen synthase kinase 3; mTOR, mammalian target of rapamycin; ND, not determined; PI3K, phosphatidylinositol 3-kinase; S6K1, 70-kDa ribosomal S6 kinase 1.
additional long-term regulation can and does often occur at the level of the expression of the signaling molecules. Hence a more in-depth understanding of the balance of these events will be key to our ability to modulate insulin signaling and to understand the molecular basis of insulin resistance.

**Akt substrate of 160 kDa: removal of a “brake”**

A new paradigm in insulin action was recently revealed, with the identification of the protein Akt substrate of 160 kDa (AS160) as a direct target of Akt (45, 65) (FIGURE 2). AS160 contains six putative Akt substrate of 160 kDa (AS160) as a direct target of Akt (45, 65) (FIGURE 2). AS160 contains six putative Akt (45, 65) (FIGURE 2). When four of them were mutated to alanine residues in the mutant termed “4P” (FIGURE 3A), insulin-induced GLUT4 translocation was significantly reduced in both 3T3-L1 adipocytes (119, 155) and in L6 GLUT4 myotubalts (Thong F. S. L. and Klip A., unpublished data) (FIGURE 3B). The effect of 4P is specific to insulin mobilization of GLUT4 from the SC as GLUT4 content is increased in this compartment, whereas traffic of TIR from the ERC is not affected (155).

A prominent feature of AS160 is the presence of a GTPase-activating protein (GAP) domain toward an as-yet unidentified Rab protein (65) (FIGURE 3A). Hence AS160 would be expected to maintain its target Rab in the GDP-bound form. Expression of a 4P mutant also harboring a mutated (inactive) GAP domain or expression of the GAP domain mutant alone were each without effect on insulin-induced GLUT4 translocation (119). These results are consistent with a scenario illustrated in FIGURE 3C, whereby insulin action through Akt leads to inactivation of the AS160 GAP activity. Mutant 4P would preclude the inactivation of the GAP required for GLUT4 translocation, whereas an inactive GAP would not be a “brake,” and therefore, phosphorylated or not, it would not be of consequence to insulin action. A corollary of this interpretation is that phosphorylation of AS160 (i.e., removal of the brake) is required but not sufficient to cause GLUT4 translocation. Rab proteins are small molecular G proteins that coordinate diverse vesicular traffic processes, including vesicle budding, tethering, and fusion (157). Interestingly, Rab 4 (26, 55, 57, 75), Rab 5 (156), and Rab 11 (73, 140, 156) participate in insulin regulation of GLUT4 traffic (27). Clearly, establishing whether Akt phosphorylation of AS160 affects its GAP activity in vivo and identification of the Rab protein(s) targeted by AS160 are critical to fully understand its role in GLUT4 translocation to the PM.

The selectivity of AS160 toward insulin signaling is still to be determined. Indeed, muscle contrac-
series of dynamically related intracellular compartments dictates that insulin-derived signals must recognize specific target loci. Insulin-dependent input may occur on distinct events such as the dynamic recycling of GLUT4 between the ERC/TGN and SC, targeted exocytosis of GLUT4 vesicles to the PM, and docking/fusion of GLUT4 vesicles at the PM and possibly directly on GLUT4 to modulate its transport activity. The recent identification of AS160 and PIKfyve as Akt effectors has provided new insight into the molecular mechanism that couples insulin-targeted signaling to GLUT4 traffic. Characterization of the insulin-responsive GLUT4 compartment and identification of events downstream of Akt and aPKC will be critical to complete our understanding of the molecular mechanisms that regulate intracellular GLUT4 traffic in the insulin-signaling highway. The GLUT4 translocation elicited by insulin, but not by exercise, is defective in insulin-resistant skeletal muscle. Moreover, insulin signaling at the level of IRS1/PI3K is impaired, whereas defects in Akt are less apparent in insulin-resistant skeletal muscle (81), although insulin stimulation of AS160 phosphorylation is also impaired in skeletal muscle of type 2 diabetic patients (68). Because exercise is a viable mechanism to improve insulin action, it will be important to clarify if AS160 is unique to insulin action or contributes to GLUT4 mobilization by muscle contraction. Knowledge of the insulin- and exercise-derived signaling events proximal to
GLUT4 may reveal interesting strategies to bypass insulin resistance associated with type 2 diabetes mellitus and obesity.

We thank Dr. Phil J. Bilan for careful reading of the manuscirpt and Dr. Gustav E. Lienhard for providing the manuscript and Dr. Gustav E. Lienhard for providing the

The original work reviewed herein from our laboratory was supported by grants to A. Klip from the Canadian Institutes of Health Research (CIHR) and the Canadian Diabetes Association (CDA). E. S. L. Thong is supported by a Graduate Student Scholarship from NSERC.

References


135. Wang W, Hansen PA, Marshall BA, Hoflosso JO, and Muscalier M. Insulin upregulates a COOH-termini


