Insulin Signaling and the Regulation of Glucose Transport

LOUISE CHANG, SHIAN-HUEY CHIANG, AND ALAN R SALTIEL

Gaps remain in our understanding of the precise molecular mechanisms by which insulin regulates glucose uptake in fat and muscle cells. Recent evidence suggests that insulin action involves multiple pathways, each compartmentalized in discrete domains. Upon activation, the receptor catalyzes the tyrosine phosphorylation of a number of substrates. One family of these, the insulin receptor substrate (IRS) proteins, initiates activation of the phosphatidylinositol 3-kinase pathway, resulting in stimulation of protein kinases such as Akt and atypical protein kinase C. The receptor also phosphorylates the adapter protein APS, resulting in the activation of the G protein TC10, which resides in lipid rafts. TC10 can influence a number of cellular processes, including changes in the actin cytoskeleton, recruitment of effectors such as the adapter protein CIP4, and assembly of the exocyst complex. These pathways converge to control the recycling of the facilitative glucose transporter Glut4.
Online address: http://www.molmed.org
doi: 10.2119/2005-00029.Saltiel

INTRODUCTION

Insulin is the most potent physiological anabolic agent known, promoting the storage and synthesis of lipids, protein, and carbohydrates and inhibiting their breakdown and release into the circulation (1). The first step by which insulin increases energy storage or utilization involves the regulated transport of glucose into the cell, mediated by the facilitative glucose transporter Glut4. Insulin increases glucose uptake mainly by enriching the concentration of Glut4 proteins at the plasma membrane, rather than by increasing the intrinsic activity of the transporter (2,3). The cellular location of Glut4 is governed by a process of regulated recycling, in which endocytosis, sorting into specialized vesicles, exocytosis, tethering, docking, and fusion of the protein are tightly regulated. We discuss here the molecular basis for these events and the signaling pathways by which they are controlled.

GLUT4 TRANSLOCATION OCCURS IN MULTIPLE STAGES

In the absence of insulin, Glut4 slowly recycles between the plasma membrane and vesicular compartments within the cell, where most of the Glut4 resides. Insulin stimulates the translocation of a pool of Glut4 to the plasma membrane, through a process of targeted exocytosis (4,5) (Figure 1). At the same time, Glut4 endocytosis is attenuated (6,7). Thus, the rate of glucose transport into fat and muscle cells is governed by the concentration of Glut4 at the cell surface and the duration for which the protein is maintained at this site.

There is substantial evidence that Glut4 exists in specialized vesicles sequestered within the cell, but the precise intracellular location and trafficking pathways of these vesicles are unclear. Following internalization, Glut4 is localized into tubulovesicular and vesicular structures that are biochemically distinct from but possibly interacting with the recycling endosomal network (8). In adipocytes, these vesicles are retained in a perinuclear region in the cell via an unknown mechanism that might involve a tethering protein (9) or continuous futile recycling (10). The Glut4 compartment is enriched in the v-SNARE (soluble *N*-ethylmaleimide sensitive factor attachment protein) protein VAMP2 (vesicle-associated membrane protein 2) but not the related VAMP3/cellubrevin isoform that is present in recycling endosome (11). Consistent with these data, ablation of transferrin receptor containing endosomes does not impair insulin-stimulated Glut4 translocation (12).

The microtubule network and actin cytoskeleton play a role in Glut4 trafficking, either by linking signaling components or by directing movement of vesicles from the perinuclear region to the plasma membrane in response to insulin. Cortical actin is required for Glut4 translocation to the plasma membrane in response to insulin (13-15), which is regulated by TC10 (see Insulin Signaling From Lipid Rafts, below) (14,16). In addition, microtubule motor proteins kinesin KIF5b and KIF3 have been shown to facilitate insulin-stimulated Glut4 transit to the plasma membrane (17,18). Thus, it is likely that molecular motors move the Glut4 vesicles along tracks involving the microtubule and actin cytoskeletons, which may undergo dynamic remodeling in response to insulin.

As mentioned above, Glut4-containing vesicles contain the v-SNARE protein VAMP2. During Glut4 vesicle docking, VAMP2 interacts directly with its t-SNARE counterpart syntaxin 4 in the plasma membrane (3). Although these SNARE interactions are essential, none of the core proteins appear to be direct targets of insulin action. On the other hand, several important SNARE accessory proteins such as Munc18c, Synip, and NSF (*N*-ethylmaleimide sensitive factor) may be involved in the control of Glut4 docking and fusion events and might be targets of insulin action (19-22). In fact, Munc18c heterozygous knockout mice are less insulin sensitive than wild-type mice, with reduced insulin-stimulated Glut4 translocation in skeletal muscle (23). However, opposite results were seen with isolated adipocytes from homozygous Munc18c knockout mice (24).

Life Sciences Institute, Departments of Internal Medicine and Physiology, University of Michigan, Ann Arbor, Michigan, USA.

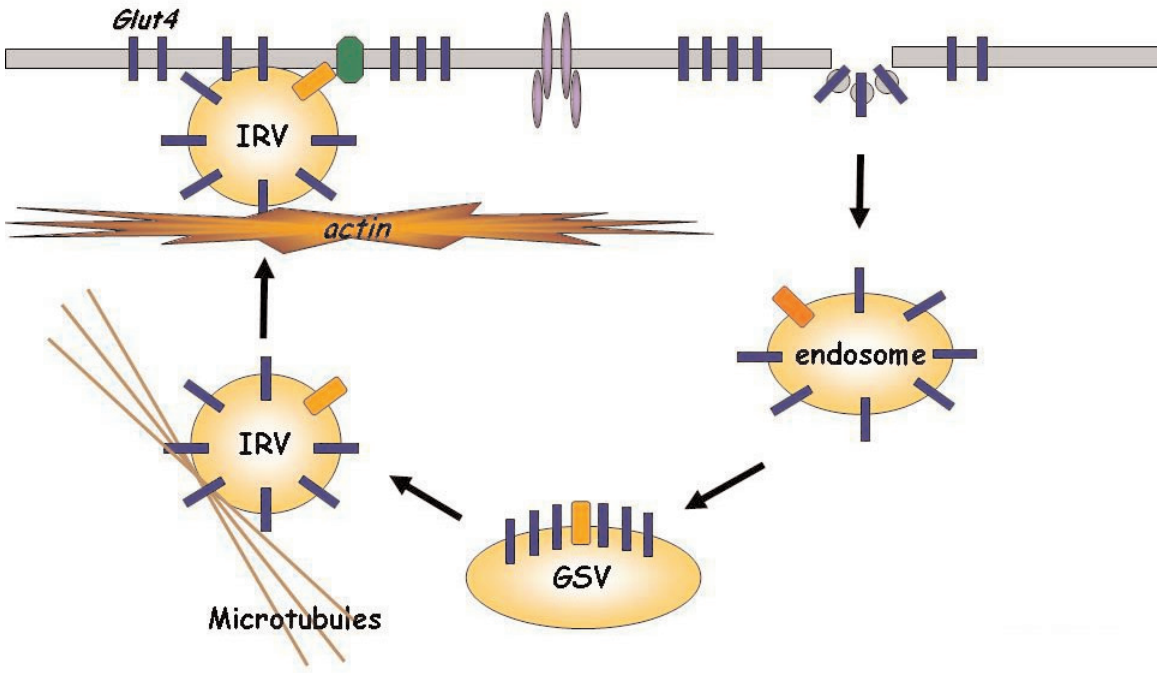

Figure 1. Recycling of Glut4. The cellular location of Glut4 is governed by a process of regulated recycling, in which the endocytosis, sorting into specialized vesicles, exocytosis, tethering, docking, and fusion of the protein are tightly regulated. In the absence of insulin, Glut4 slowly recycles between the plasma membrane and vesicular compartments within the cell, where most of the Glut4 resides. Insulin stimulates the translocation of a pool of Glut4 to the plasma membrane, through a process of targeted exocytosis. The microtubule network and actin cytoskeleton play a role in Glut4 trafficking, either by linking signaling components or by directing movement of vesicles from the perinuclear region to the plasma membrane in response to insulin. Once at the plasma membrane, the Glut4 vesicles dock and fuse, allowing for extracellular exposure of the transporter.

SIGNALING FROM THE INSULIN RECEPTOR

The insulin receptor (IR) is a heterotetrameric bifunctional complex, consisting of 2 extracellular α subunits that bind insulin and 2 transmembrane β subunits with tyrosine kinase activity. Insulin binding to the α subunit induces the transphosphorylation of one β subunit by another on specific tyrosine residues in an activation loop, resulting in the increased catalytic activity of the kinase (3,25). The receptor also undergoes autophosphorylation at other tyrosine residues in the juxtamembrane regions and intracellular tail (Figure 2). The activated IR then phosphorylates tyrosine residues on intracellular substrates that include the insulin receptor substrate family (IRS1 through 4), IRS5/DOK4, IRS/DOK5, Gab-1, Cbl, APS and Shc isoforms, and signal regulatory protein (SIRP) family members (3,25). Some of these proteins, including IRS and Shc, are recruited to a juxtamembrane region in the receptor containing an NPXY motif, while others, such as APS, bind directly to the activation loop. Upon phosphorylation, these substrates interact with a series of effector or adapter molecules containing Src homology 2 (SH2) domains that specifically recognize different phosphotyrosine motifs. Among these substrates, the best characterized are the IRS family of proteins.

Even though the IRS proteins share a high degree of homology, studies in knockout mice and cell lines indicate specific roles in insulin/insulin-like growth factor (IGF)-1 action (3,25). IRS-1 knockout mice are growth retarded and do not appear to develop diabetes, but are insulin resistant in peripheral tissues, with defective glucose tolerance (26). IRS-2 knockout mice are insulin resistant in both peripheral tissues and liver and develop type 2 diabetes due to insulin resistance along with decreased β-cell function (27).

THE PHOSPHATIDYLINOSITOL 3-KINASE SIGNALING PATHWAY

The stimulation of glucose uptake by insulin is mediated by phosphatidylinositol (PI) 3-kinase–dependent and –independent pathways (3,25). Upon tyrosine phosphorylation, IRS proteins interact with the p85 regulatory subunit of PI 3-kinase, leading to the activation of the enzyme and its targeting to the plasma membrane. The enzyme generates the lipid product phosphatidylinositol 3,4,5-trisphosphate (PIP_3), which regulates the localization and activity of numerous proteins (28). PI 3-kinase activation is attenuated by PIP_3 dephosphorylation via 3′ phosphatases such as PTEN (29) or 5′ phosphatases such as SHIP2 (30,31).

PI 3-kinase plays an essential role in glucose uptake and Glut4 translocation. Inhibition of the enzyme with pharmacological inhibitors such as wortmannin completely blocks the stimulation of glucose uptake by insulin (32). Additionally, overexpression of dominant-interfering forms of PI 3-kinase can block glucose uptake and Glut4 translocation, and overexpression of constitutively

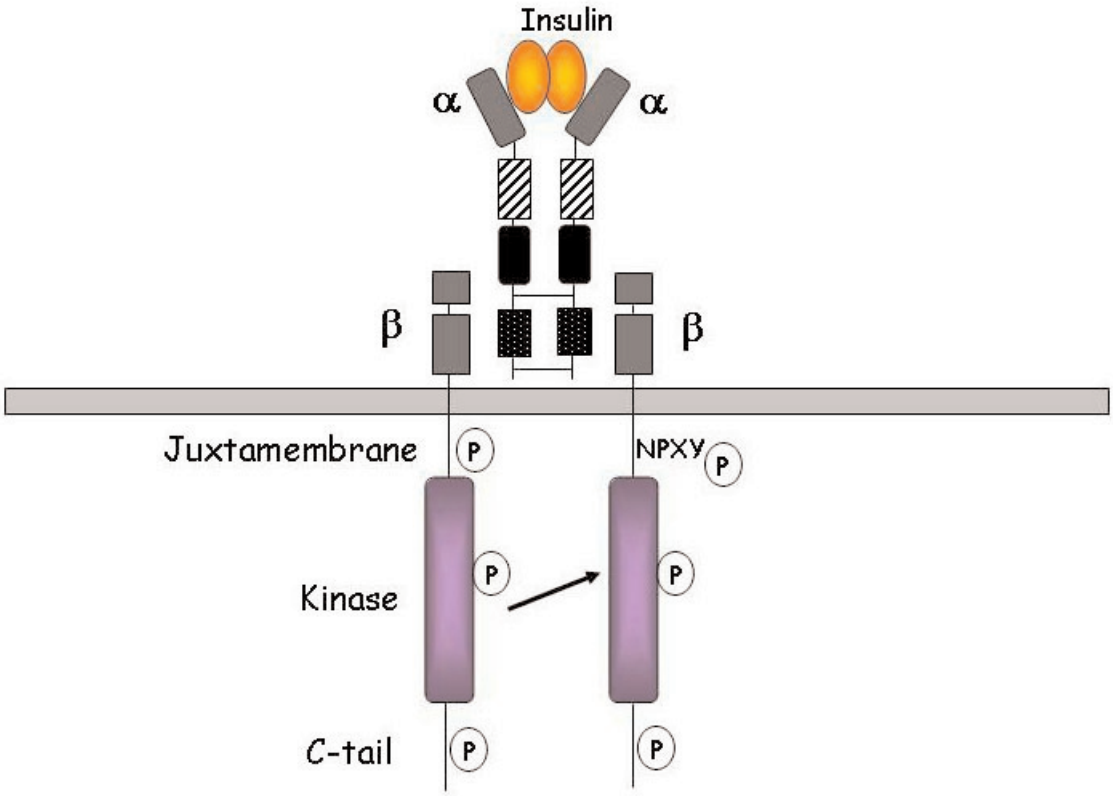

Figure 2. Activation of insulin receptor. The insulin receptor consists of 2 extracellular α subunits that bind insulin and 2 transmembrane β subunits with tyrosine kinase activity. Insulin binding to the α subunit induces the transphosphorylation of one β subunit by another on specific tyrosine residues in an activation loop, resulting in the increased catalytic activity of the kinase. The receptor also undergoes autophosphorylation at other tyrosine residues in the juxtamembrane regions and intracellular tail The activated IR then phosphorylates tyrosine residues on intracellular substrates.

active forms can partially mimic insulin action (33,34). Targeted deletion of the p85 PI 3-kinase regulatory subunit in mice results in increased insulin sensitivity (35-37). Conversely, gene knockout of the catalytic subunit results in insulin resistance and glucose intolerance (38). Collectively, these studies demonstrate that PI 3-kinase is required for insulin-stimulated glucose transport.

Insulin-stimulated increases in PIP_3 result in the recruitment and/or activation of pleckstrin homology (PH) domain–containing proteins, including various enzymes, their substrates, adapter molecules, and cytoskeletal proteins. Among these is the Ser/Thr kinase PDK1, which phosphorylates and activates several downstream kinases, including Akt1 through 3, protein kinase C (PKC)ζ/Λ, and serum and glucocorticoid-inducible kinase (SGK) (39). PIP_3 appears to mediate the translocation of Akt to the plasma membrane, via its PH domain (40). Additionally, the protein kinase mTOR (mammalian target of rapamycin), complexed to the regulatory protein rictor, has been recently identified as PDK2 (phosphoinositide-dependent kinase 2), which phosphorylates Ser^{473} on Akt (41). Overexpression of a membrane-bound form of Akt in 3T3L1 adipocytes resulted in increased glucose transport and localization of Glut4 to the plasma membrane (42,43). Insulin-stimulated Glut4 translocation was also inhibited by expression of a dominant-interfering Akt mutant (44,45).

Interestingly, the isoforms of Akt, Akt1, and Akt2 are not functionally redundant. Mouse gene knockout studies, as well as knockdown studies in 3T3L1 adipocytes using small-interfering RNA (siRNA), have shown that reducing only Akt1 does not alter insulin sensitivity, whereas reducing Akt2 levels decreases insulin sensitivity and reduces glucose disposal. In addition, the metabolic phenotype was more profound when both Akt1 and Akt2 protein levels were reduced (46-48), suggesting that both Akt1 and Akt2 are required for insulin signaling.

Although several putative substrates of Akt potentially involved in the regulation of glucose transport have been identified, only a few have been studied extensively. The Rab-GTPase activating protein (Rab-GAP), AS160, undergoes phosphorylation in response to insulin and contains multiple Akt phosphorylation sites (49). Overexpression of a mutant AS160 that cannot undergo phosphorylation blocks insulin-stimulated Glut4 translocation. This inhibition requires an intact GTPase activating domain (GAP) (49,50). Moreover, insulin-stimulated AS160 phosphorylation was reduced in patients with type 2 diabetes (51). Although the GAP activity of AS160 appears to be specific for Rabs 2A, 8A, and 14 in vitro (52), the precise target remains unclear.

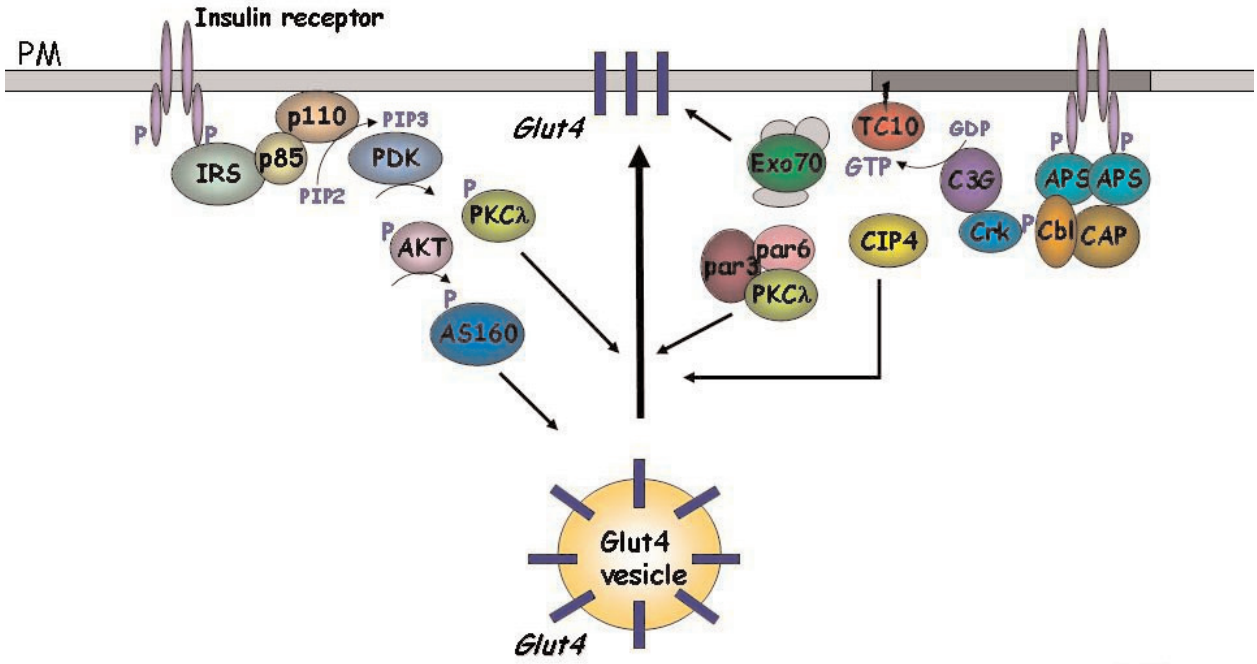

Figure 3. A model for diverse signaling pathways in insulin action. Two signaling pathways are required for the translocation of the glucose transporter Glut4 by insulin in fat and muscle cells. Tyrosine phosphorylation of the IRS proteins after insulin stimulation leads to an interaction with and subsequent activation of PI 3-kinase, producing PIP_3, which in turn activates and localizes protein kinases such as PDK1. These kinases then initiate a cascade of phosphorylation events, resulting in the activation of Akt and/or atypical PKC. AS160, a substrate of Akt, plays an as yet undefined role in Glut4 translocation through its Rab-GTPase activating domain. A separate pool of the insulin receptor can also phosphorylate the substrates Cbl and APS. Cbl interacts with CAP, which can bind to the lipid raft protein flotillin. This interaction recruits phosphorylated Cbl into the lipid raft, resulting in the recruitment of CrkII. CrkII binds constitutively to the exchange factor C3G, which can catalyze the exchange of GDP for GTP on the lipid-raft–associated protein TC10. Upon its activation, TC10 interacts with a number of potential effector molecules, including CIP4, Exo70, and Par6/Par3/PKCλ, in a GTP-dependent manner.

Despite the evidence supporting an important role for the PI 3-kinase pathway, activation of this enzyme is not sufficient for increased glucose transport observed in response to insulin. Stimulation of PI 3-kinase activity by platelet-derived growth factor (PDGF) or interleukin-4 does not increase glucose uptake (53,54). Overexpression of the IRS1 phosphotyrosine binding (PTB) or Shc and IRS1 NPXY binding (SAIN) domains decreased IRS1-associated PI 3-kinase activity, but was without effect on insulin-stimulated glucose uptake (34). Moreover, addition of a membrane-permeable analog of PIP_3, the product of PI 3-kinase, did not stimulate glucose uptake in the absence of insulin (55). Consistent with this, overexpression of constitutively active PI 3-kinase mutants did not fully mimic insulin-stimulated Glut4 translocation to the plasma membrane (33). Together these data suggest that PI 3-kinase activation is not sufficient to stimulate glucose uptake, suggesting that more than one signaling pathway is required.

INSULIN SIGNALING FROM LIPID RAFTS

Several studies have shown that a separate insulin signaling pathway is localized in lipid raft microdomains, specialized regions of the plasma membrane enriched in cholesterol, sphingolipids, lipid-modified signaling proteins, glycosylphosphotidylinositol (GPI)-anchored proteins, and glycolipids (56). At least some of the insulin receptor has been shown to reside in these microdomains (57-59), perhaps through its interaction with the raft protein caveolin (57-59). Activation of the insulin receptor in these plasma membrane subdomains stimulates the tyrosine phosphorylation of the proto-oncogenes c-Cbl and Cbl-b. This phosphorylation step requires recruitment of Cbl to the adapter protein APS, which contains SH2 and PH domains (59-61). APS exists as a preformed homodimer and interacts with the 3 phosphotyrosines in the activation loop of the insulin receptor upon its activation via its SH2 domain (62). Each partner in the APS homodimer binds to a β-subunit of the receptor, so that 1 receptor recruits 2 APS molecules (62). Upon binding to the receptor, APS is phosphorylated on a C-terminal tyrosine, resulting in the recruitment of Cbl via the SH2 domain of the latter protein. Cbl subsequently undergoes phosphorylation on 3 tyrosines (60).

The Cbl associated protein (CAP) is recruited with Cbl to the insulin receptor:APS complex (60). CAP is a bifunctional adapter protein with 3 SH3 domains in its COOH-terminus, and an NH_2-terminal region of similarity to the gut peptide sorbin, called the sorbin homology (SoHo) domain (63). CAP expression correlates well with insulin sensitivity. The protein is found predominantly in insulin-sensitive tissues, and expression is increased upon acti-

vation of the nuclear receptor PPARγ (peroxisome proliferator-activated receptor γ), the receptor for the thiazolidinedione class of insulin-sensitizing drugs (64). CAP also binds directly to the cytoskeletal protein vinculin and is found in focal adhesions in other cell types (65).

The COOH-terminal SH3 domain of CAP associates with a PXXP motif in Cbl, such that these proteins are constitutively associated. Upon recruitment to the insulin receptor, CAP interacts with the lipid raft domain protein flotillin, via the SoHo domain of the former protein (63,66). Overexpression of dominant-interfering CAP mutants that do not bind to Cbl (CAPΔSH3) or flotillin (CAPΔSoHo) blocked translocation of phosphorylated Cbl to lipid rafts, and also prevented insulin-stimulated glucose uptake and Glut4 translocation (61,63). Interestingly, CAPΔSH3 expression did not affect PI 3-kinase activity, suggesting that the CAP/Cbl complex plays a critical role in insulin-stimulated glucose utilization through a PI 3-kinase–independent pathway (61). Consistent with these studies, siRNA knockdown of APS or c-Cbl plus Cbl-b results in inhibition of insulin-stimulated glucose uptake (67), although Czech and colleagues showed that siRNA knockdown of c-Cbl and Cbl-b or CAP was without effect on insulin-stimulated glucose transport (68,69). The discrepancy between these two studies is not understood.

Upon tyrosine phosphorylation, Cbl interacts with the protein CrkII, an SH2/SH3-containing adapter protein (70). CrkII binds to specific phosphorylation sites on Cbl via its SH2 domain (70) and is constitutively associated with the nucleotide exchange factor C3G via its SH3 domain (71). Thus, insulin stimulates the translocation of both CrkII and C3G to lipid rafts, an effect of the hormone that can be blocked by transfection of cells with CAPΔSH3 or CAPΔSoHo (61,63).

Upon its translocation to lipid rafts, C3G can catalyze the activation of the small G proteins TC10α and TC10β (72,73). This effect of insulin requires the CAP/Cbl complex, because overexpression of CAPΔSH3 blocked TC10 activation by insulin. Together these data indicate that the CAP/Cbl/TC10 pathway is required for insulin-stimulated glucose uptake in parallel with and independent of the PI 3-kinase signaling cascade (72,73).

Overexpression of a dominant-interfering TC10 mutant inhibits insulin-stimulated glucose uptake and Glut4 translocation (72,73). Surprisingly, the overexpression of the wild-type TC10 protein also blocked insulin action, reminiscent of effects observed with the Rab family of small GTP-binding proteins that are also involved in vesicular trafficking (74). The Rab proteins cycle between GTP- and GDP-bound states to affect vesicle budding from donor membranes and fusion with acceptor membranes. Overexpression of wild-type Rab titrates out key exchange factors, thereby preventing Rab GTP/GDP exchange. Upon overexpression, a large fraction of TC10 might saturate the endogenous exchange factor, without producing a downstream signal in the appropriate cellular compartment. Alternatively, activated TC10 may play a scaffolding role by simultaneously bridging several effectors together. Overexpression of wild-type TC10 may alter this binding stoichiometry, attenuating the downstream signaling pathway.

Upon its activation, TC10 interacts with a number of potential effector molecules. One of these is a splice variant of the adapter protein, CIP4, first identified as an effector of Cdc42. The multidomain structure of CIP4 isoforms suggests that this family of proteins may serve an adapter function. CIP4 contains 1 FCH domain, 2 coiled-coil domains, and 1 SH3 domain. The FCH domain interacts with microtubules, and the 2nd coiled-coil domain interacts with TC10 in a GTP-dependent manner. CIP4 localizes to an intracellular compartment under basal conditions, and translocates to the plasma membrane upon insulin stimulation (75). Overexpression of mutant forms of CIP4/2 containing an NH_2-terminal deletion or with diminished TC10 binding inhibits insulin-stimulated Glut4 translocation, suggesting that CIP4/2 may play an important role in insulin-stimulated glucose transport as a downstream effector of TC10 (75).

Upon its activation, TC10 also interacts with Exo70, a component of the exocyst complex (76). The exocyst complex has been implicated in the tethering or docking of secretory vesicles. A mutant form of Exo70 inhibited insulin-stimulated glucose uptake by preventing fusion of the Glut4 vesicle with the plasma membrane; however, overexpression of wild-type Exo70 increased insulin-stimulated glucose transport (76). Additionally, overexpression of sec8 and sec6, components of the exocyst complex, increased glucose transport following insulin stimulation in 3T3-L1 adipocytes (77). Together, these studies indicate that the exocyst complex is involved in insulin-regulated Glut4 exocytosis.

INTERSECTING INSULIN SIGNALING PATHWAYS

Consistent with the notion of a two-step regulation of Glut4 trafficking in response to insulin, it is now clear that previously considered independent and parallel pathways such as PI 3-kinase, atypical protein kinase C (PKCζ/λ), and the CAP/Cbl/Crk/C3G/TC10 signaling cascades intersect (Figure 3). Atypical PKCs belong to the AGC subfamily of protein kinases that can be phosphorylated by PDK1 downstream of PI 3-kinase. PKCζ/λ is then recruited to lipid rafts in a TC10-dependent manner, via Par3 and Par6 proteins (78). Thus, atypical PKC may represent a point of convergence for the PI 3-kinase and TC10 signaling pathways. It is likely that other effectors of TC10 and related proteins will be targets of PI 3-kinase signaling, perhaps explaining the network of signaling pathways that govern the complex series of events involved in the regulation of Glut4 recycling by insulin.

Address correspondence and reprint requests to *Alan R. Saltiel, Life Sciences Institute, University of Michigan, 210 Washtenaw Avenue, Ann Arbor, MI 48109-2216. Phone: 734-615-9787; fax: 734-763-6492; e-mail: saltiel@umich.edu.*

Submitted October 17, 2005; accepted for publication October 17, 2005.

REFERENCES

1. Saltiel AR, Kahn CR. (2001) Insulin signaling and the regulation of glucose and lipid metabolism. *Nature* 414:799-806.
2. Furtado LM, Somwar R, Sweeney G, Niu W, Klip A. (2002) Activation of the glucose transporter GLUT4 by insulin. *Biochem. Cell. Biol.* 80:569-78.
3. Watson RT, Kanzaki M, Pessin JE. (2004) Regulated membrane trafficking of the insulin-responsive glucose transporter 4 in adipocytes. *Endocr. Rev.* 25:177-204.
4. Rea S, James DE. (1997) Moving GLUT4: the biogenesis and trafficking of GLUT4 storage vesicles. *Diabetes* 46:1667-77.

5. Kandror KV, Pilch PF. (1996) Compartmentalization of protein traffic in insulin-sensitive cells. *Am. J. Physiol.* 271:E1-14.
6. Jhun BH, Rampal AL, Liu H, Lachaal M, Jung CY. (1992) Effects of insulin on steady state kinetics of GLUT4 subcellular distribution in rat adipocytes. Evidence of constitutive GLUT4 recycling. *J. Biol. Chem.* 267:17710-5.
7. Czech MP, Buxton JM. (1993) Insulin action on the internalization of the GLUT4 glucose transporter in isolated rat adipocytes. *J. Biol. Chem.* 268:9187-90.
8. Martin S et al. (2000) Effects of insulin on intracellular GLUT4 vesicles in adipocytes: evidence for a secretory mode of regulation. *J. Cell. Sci.* 113 Pt 19: 3427-38.
9. Bogan JS, Hendon N, McKee AE, Tsao TS, Lodish HF. (2003) Functional cloning of TUG as a regulator of GLUT4 glucose transporter trafficking. *Nature* 425:727-33.
10. Holman GD, Lo Leggio L, Cushman SW. (1994) Insulin-stimulated GLUT4 glucose transporter recycling: a problem in membrane protein subcellular trafficking through multiple pools. *J. Biol. Chem.* 269:17516-24.
11. Randhawa VK et al. (2000) VAMP2, but not VAMP3/cellubrevin, mediates insulin-dependent incorporation of GLUT4 into the plasma membrane of L6 myoblasts. *Mol. Biol. Cell.* 11:2403-17.
12. Livingstone C, James DE, Rice JE, Hanpeter D, Gould GW. (1996) Compartment ablation analysis of the insulin-responsive glucose transporter (GLUT4) in 3T3-L1 adipocytes. *Biochem. J.* 315 (Pt 2):487-95.
13. Brozinick JT Jr, Hawkins ED, Strawbridge AB, Elmendorf JS. (2004) Disruption of cortical actin in skeletal muscle demonstrates an essential role of the cytoskeleton in glucose transporter 4 translocation in insulin-sensitive tissues. *J. Biol. Chem.* 279:40699-706.
14. Kanzaki M, Pessin JE. (2001) Insulin-stimulated GLUT4 translocation in adipocytes is dependent upon cortical actin remodeling. *J. Biol. Chem.* 276:42436-44.
15. Tong P, Khayat ZA, Huang C, Patel N, Ueyama A, Klip A. (2001) Insulin-induced cortical actin remodeling promotes GLUT4 insertion at muscle cell membrane ruffles. *J. Clin. Invest.* 108:371-81.
16. Kanzaki M, Watson RT, Hou JC, Stamnes M, Saltiel AR, Pessin JE. (2002) Small GTP-binding protein TC10 differentially regulates two distinct populations of filamentous actin in 3T3L1 adipocytes. *Mol. Biol. Cell* 13:2334-46.
17. Imamura T, Huang J, Usui I, Satoh H, Bever J, Olefsky JM. (2003) Insulin-induced GLUT4 translocation involves protein kinase C-lambda-mediated functional coupling between Rab4 and the motor protein kinesin. *Mol. Cell. Biol.* 23:4892-900.
18. Semiz S et al. (2003) Conventional kinesin KIF5B mediates insulin-stimulated GLUT4 movements on microtubules. *EMBO J.* 22:2387-99.
19. Thurmond DC, Kanzaki M, Khan AH, Pessin JE. (2000) Munc18c function is required for insulin-stimulated plasma membrane fusion of GLUT4 and insulin-responsive amino peptidase storage vesicles. *Mol. Cell. Biol.* 20:379-88.
20. Mastick CC, Falick AL. (1997) Association of *N*-ethylmaleimide sensitive fusion (NSF) protein and soluble NSF attachment proteins-alpha and -gamma with glucose transporter-4-containing vesicles in primary rat adipocytes. *Endocrinology* 138:2391-7.
21. Chen X et al. (2005) Demonstration of differential quantitative requirements for NSF among multiple vesicle fusion pathways of GLUT4 using a dominant-negative ATPase-deficient NSF. *Biochem. Biophys. Res. Commun.* 333:28-34.
22. Min J et al. (1999) Synip: a novel insulin-regulated syntaxin 4-binding protein mediating GLUT4 translocation in adipocytes. *Mol. Cell* 3:751-60.
23. Oh E, Spurlin BA, Pessin JE, Thurmond DC. (2005) Munc18c heterozygous knockout mice display increased susceptibility for severe glucose intolerance. *Diabetes* 54:638-47.
24. Kanda H et al. (2005) Adipocytes from Munc18c-null mice show increased sensitivity to insulin-stimulated GLUT4 externalization. *J. Clin. Invest.* 115:291-301.
25. Saltiel AR, Pessin JE. (2003) Insulin signaling in microdomains of the plasma membrane. *Traffic* 4:711-16.
26. Tamemoto H et al. (1994) Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1. *Nature* 372:182-6.
27. Withers DJ et al. (1998) Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* 391:900-4.
28. Shepherd PR. (2005) Mechanisms regulating phosphoinositide 3-kinase signaling in insulin-sensitive tissues. *Acta Physiol. Scand.* 183:3-12.
29. Maehama T, Dixon JE. (1999) PTEN: a tumor suppressor that functions as a phospholipid phosphatase. *Trends Cell Biol.* 9:125-8.
30. Pesesse X, Deleu S, De Smedt F, Drayer L, Erneux C. (1997) Identification of a second SH2-domain-containing protein closely related to the phosphatidylinositol polyphosphate 5-phosphatase SHIP. *Biochem. Biophys. Res. Commun.* 239:697-700.
31. Habib T, Hejna JA, Moses RE, Decker SJ. (1998) Growth factors and insulin stimulate tyrosine phosphorylation of the 51C/SHIP2 protein. *J. Biol. Chem.* 273: 18605-9.
32. Okada T, Kawano Y, Sakakibara T, Hazeki O, Ui M. (1994) Essential role of phosphatidylinositol 3-kinase in insulin-induced glucose transport and antilipolysis in rat adipocytes: studies with a selective inhibitor wortmannin. *J. Biol. Chem.* 269:3568-73.
33. Martin SS, Haruta T, Morris AJ, Klippel A, Williams LT, Olefsky JM. (1996) Activated phosphatidylinositol 3-kinase is sufficient to mediate actin rearrangement and GLUT4 translocation in 3T3-L1 adipocytes. *J. Biol. Chem.* 271:17605-8.
34. Sharma PM et al. (1998) Inhibition of phosphatidylinositol 3-kinase activity by adenovirus-mediated gene transfer and its effect on insulin action. *J. Biol. Chem.* 273:18528-37.
35. Ueki K et al. (2003) Positive and negative roles of p85 alpha and p85 beta regulatory subunits of phosphoinositide 3-kinase in insulin signaling. *J. Biol. Chem.* 278:48453-66.
36. Mauvais-Jarvis F et al. (2002) Reduced expression of the murine p85alpha subunit of phosphoinositide 3-kinase improves insulin signaling and ameliorates diabetes. *J. Clin. Invest.* 109:141-9.
37. Terauchi Y et al. (1999) Increased insulin sensitivity and hypoglycaemia in mice lacking the p85 alpha subunit of phosphoinositide 3-kinase. *Nat. Genet.* 21: 230-5.
38. Brachmann SM, Ueki K, Engelman JA, Kahn RC, Cantley LC. (2005) Phosphoinositide 3-kinase catalytic subunit deletion and regulatory subunit deletion have opposite effects on insulin sensitivity in mice. *Mol. Cell. Biol.* 25: 1596-607.
39. Mora A, Komander D, van Aalten DM, Alessi DR. (2004) PDK1, the master regulator of AGC kinase signal transduction. *Semin. Cell Dev. Biol.* 15:161-70.
40. Corvera S, Czech MP. (1998) Direct targets of phosphoinositide 3-kinase products in membrane traffic and signal transduction. *Trends Cell Biol.* 8: 442-6.
41. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. (2005) Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 307:1098-101.
42. Kohn AD et al. (1998) Construction and characterization of a conditionally active version of the serine/threonine kinase Akt. *J. Biol. Chem* 273:11937-43.
43. Kohn AD, Summers SA, Birnbaum MJ, Roth RA. (1996) Expression of a constitutively active Akt Ser/Thr kinase in 3T3-L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation. *J. Biol. Chem.* 271:31372-8.
44. Wang Q et al. (1999) Protein kinase B/Akt participates in GLUT4 translocation by insulin in L6 myoblasts. *Mol. Cell. Biol.* 19:4008-18.
45. Cong LN et al. (1997) Physiological role of Akt in insulin-stimulated translocation of GLUT4 in transfected rat adipose cells. *Mol. Endocrinol.* 11:1881-90.
46. Jiang ZY, Zhou QL, Coleman KA, Chouinard M, Boese Q, Czech MP. (2003) Insulin signaling through Akt/protein kinase B analyzed by small interfering RNA-mediated gene silencing. *Proc. Natl. Acad. Sci. U. S. A.* 100:7569-74.
47. Cho H, Thorvaldsen JL, Chu Q, Feng F, Birnbaum MJ. (2001) Akt1/PKBalpha is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. *J. Biol. Chem.* 276:38349-52.
48. Cho H et al. (2001) Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). *Science* 292:1728-31.
49. Sano H et al. (2003) Insulin-stimulated phosphorylation of a Rab GTPase-activating protein regulates GLUT4 translocation. *J. Biol. Chem.* 278: 14599-602.
50. Zeigerer A, McBrayer MK, McGraw TE. (2004) Insulin stimulation of GLUT4 exocytosis, but not its inhibition of endocytosis, is dependent on RabGAP AS160. *Mol. Biol. Cell.* 15:4406-15.
51. Karlsson HK, Zierath JR, Kane S, Krook A, Lienhard GE, Wallberg-Henriksson H. (2005) Insulin-stimulated phosphorylation of the Akt substrate AS160 is impaired in skeletal muscle of type 2 diabetic subjects. *Diabetes* 54:1692-7.
52. Miinea CP et al. (2005) AS160, the Akt substrate regulating GLUT4 translocation, has a functional Rab GTPase-activating protein domain. *Biochem. J.* 391 Pt 1:87-93.
53. Wiese RJ, Mastick CC, Lazar DF, Saltiel AR. (1995) Activation of mitogen-activated protein kinase and phosphatidylinositol 3'-kinase is not sufficient for the hormonal stimulation of glucose uptake, lipogenesis, or glycogen synthesis in 3T3-L1 adipocytes. *J. Biol. Chem.* 270:3442-6.
54. Isakoff SJ, Taha C, Rose E, Marcusohn J, Klip A, Skolnik EY. (1995) The inability of phosphatidylinositol 3-kinase activation to stimulate GLUT4 translocation indicates additional signaling pathways are required for insulin-stimulated glucose uptake. *Proc. Natl. Acad. Sci. U. S. A.* 92:10247-51.
55. Jiang T, Sweeney G, Rudolf MT, Klip A, Traynor-Kaplan A, Tsien RY. (1998) Membrane-permeant esters of phosphatidylinositol 3,4,5-trisphosphate. *J. Biol. Chem.* 273:11017-24.
56. Smart EJ et al. (1999) Caveolins, liquid-ordered domains, and signal transduction. *Mol. Cell. Biol.* 19:7289-304.
57. Parpal S, Karlsson M, Thorn H, Stralfors P. (2001) Cholesterol depletion disrupts caveolae and insulin receptor signaling for metabolic control via insulin receptor substrate-1, but not for mitogen-activated protein kinase control. *J. Biol. Chem.* 276:9670-8.
58. Gustavsson J et al. (1999) Localization of the insulin receptor in caveolae of adipocyte plasma membrane. *FASEB J.* 13:1961-71.
59. Kimura A, Mora S, Shigematsu S, Pessin JE, Saltiel AR. (2002) The insulin receptor catalyzes the tyrosine phosphorylation of caveolin-1. *J. Biol. Chem.* 277:30153-8.
60. Liu J, Kimura A, Baumann CA, Saltiel AR. (2002) APS facilitates c-Cbl tyrosine phosphorylation and GLUT4 translocation in response to insulin in 3T3-L1 adipocytes. *Mol. Cell. Biol.* 22:3599-609.
61. Baumann CA et al. (2000) CAP defines a second signaling pathway required for insulin-stimulated glucose transport [see comments]. *Nature* 407:202-7.
62. Hu J, Liu J, Ghirlando R, Saltiel AR, Hubbard SR. (2003) Structural basis for recruitment of the adaptor protein APS to the activated insulin receptor. *Mol. Cell* 12:1379-89.
63. Kimura A, Baumann CA, Chiang SH, Saltiel AR. (2001) The sorbin homology domain: a motif for the targeting of proteins to lipid rafts. *Proc. Natl. Acad. Sci. U. S. A.* 98: 9098-103.
64. Ribon V, Printen JA, Hoffman NG, Kay BK, Saltiel AR. (1998) A novel, multifunctional c-Cbl binding protein in insulin receptor signaling in 3T3-L1 adipocytes. *Mol. Cell. Biol.* 18:872-9.
65. Kioka N et al. (1999) Vinexin: a novel vinculin-binding protein with multiple SH3 domains enhances actin cytoskeletal organization. *J. Cell. Biol.* 144:59-69.

66. Liu J, Deyoung SM, Zhang M, Dold LH, Saltiel AR. (2005) The stomatin/prohibitin/flotillin/HflK/C domain of flotillin-1 contains distinct sequences that direct plasma membrane localization and protein interactions in 3T3-L1 adipocytes. *J. Biol. Chem.* 280:16125-34.
67. Ahn MY, Katsanakis KD, Bheda F, Pillay TS. (2004) Primary and essential role of the adaptor protein APS for recruitment of both c-Cbl and its associated protein CAP in insulin signaling. *J. Biol. Chem.* 279:21526-32.
68. Mitra P, Zheng X, Czech MP. (2004) RNAi-based analysis of CAP, Cbl, and CrkII function in the regulation of GLUT4 by insulin. *J. Biol. Chem.* 279:37431-5.
69. Zhou QL et al. (2004) Analysis of insulin signaling by RNAi-based gene silencing. *Biochem. Soc. Trans.* 32:817-21.
70. Ribon V, Hubbell S, Herrera R, Saltiel AR. (1996) The product of the cbl oncogene forms stable complexes in vivo with endogenous Crk in a tyrosine phosphorylation-dependent manner. *Mol. Cell. Biol.* 16:45-52.
71. Knudsen BS, Feller SM, Hanafusa H. (1994) Four proline-rich sequences of the guanine-nucleotide exchange factor C3G bind with unique specificity to the first Src homology 3 domain of Crk. *J. Biol. Chem.* 269:32781-7.
72. Chiang SH, Hou JC, Hwang J, Pessin JE, Saltiel AR. (2002) Cloning and functional characterization of related TC10 isoforms, a subfamily of Rho proteins involved in insulin-stimulated glucose transport. *J. Biol. Chem.* 277:13067-73.
73. Chiang SH et al. (2001) Insulin-stimulated GLUT4 translocation requires the CAP-dependent activation of TC10. *Nature* 410:944-8.
74. Novick P, Zerial M. (1997) The diversity of Rab proteins in vesicle transport. *Curr. Opin. Cell Biol.* 9:496-504.
75. Chang L, Adams RD, Saltiel AR. (2002) The TC10-interacting protein CIP4/2 is required for insulin-stimulated Glut4 translocation in 3T3L1 adipocytes. *Proc. Natl. Acad. Sci. U. S. A.* 99:12835-40.
76. Inoue M, Chang L, Hwang J, Chiang SH, Saltiel AR. (2003) The exocyst complex is required for targeting of Glut4 to the plasma membrane by insulin. *Nature* 422:629-33.
77. Ewart MA, Clarke M, Kane S, Chamberlain LH, Gould GW. (2005) Evidence for a role of the exocyst in insulin-stimulated Glut4 trafficking in 3T3-L1 adipocytes. *J. Biol. Chem.* 280:3812-6.
78. Kanzaki M, Mora S, Hwang JB, Saltiel AR, Pessin JE. (2004) Atypical protein kinase C (PKCzeta/lambda) is a convergent downstream target of the insulin-stimulated phosphatidylinositol 3-kinase and TC10 signaling pathways. *J. Cell Biol.* 164: 279-90.