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“Acting” on GLUT4: Membrane & Cytoskeletal Components of Insulin Action

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Abstract

The dissection of mechanisms that regulate glucose transport by insulin has revealed an intricate network of signaling molecules scattered from the insulin receptor to the intracellular glucose transporter GLUT4. It is also appreciated that some insulin receptor signals jaunt in different directions to regulate events essential for the efficient redistribution of GLUT4 to the plasma membrane. Moreover key assists in the process appear to be arranged by membrane lipids and cytoskeletal proteins. Following current considerations of insulin signals regulating GLUT4, this review will focus on *in vitro* and *in vivo* evidence that supports an essential role for phosphoinositides and actin filaments in the control of glucose transport. The discussion will visit recent cell culture, whole animal, and human data highlighting membrane and cytoskeletal aspects of insulin resistance.

Keywords

Diabetes; glucose transport; insulin resistance; signaling; trafficking

INTRODUCTION

Of the plethora of biological responses elicited by insulin, perhaps the most important is the removal of excess circulatory glucose by skeletal muscle and adipose tissue. A key aspect of this cellular response to insulin entails the redistribution of the glucose transporter GLUT4 from a specialized intracellular insulin-sensitive storage compartment to the plasma membrane. This regulated redistribution or translocation of GLUT4 involves a complicated network of cellular interactions, many of which appear critically coupled to the actin cytoskeleton. In addition, membrane phospholipids appear to play a pertinent role in insulin signaling and cytoskeletal control of GLUT4. Accumulating *in vitro* and *in vivo* evidence highlights that membrane and cytoskeletal abnormalities may render the control of GLUT4 ineffective. Interestingly, blood cells (*neutrophils, mononuclear cells, erythrocytes*) isolated from insulin-resistant individuals exhibit alterations in cortical actin structure and membrane lipid composition [1–4]. These phospholipid and cytoskeletal irregularities in human blood cells raise the question whether similar changes are present in human muscle and fat cells and explain, at least in part, their insulin-resistant state.

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Insulin Signaling to GLUT4 Translocation

It is well established that activation of GLUT4 translocation by insulin requires a phosphatidylinositol 3-kinase (PI3K) signal involving the upstream insulin receptor (IR) and insulin receptor substrate (IRS) activators and the downstream protein kinase B (Akt) and C (PKC) target enzymes, however the identity of the downstream effector of this cascade remained elusive (Fig. 1). With the discovery of AS160 (Akt substrate of 160 kDa) in 2002 by Kane and colleagues, knowledge of the downstream signaling events to GLUT4 translocation after Akt activation became more complete [5]. Utilizing an antibody that recognizes the Akt phosphomotif RXXRXXpS/T, this group initially identified and characterized this protein, and since this initial report, AS160 protein expression has been confirmed in cultured adipocytes, rat and human skeletal muscle [Kane, 2002 #2006; Sano, 2003 #1731; Bruss, 2005 #2007; Karlsson, 2005 #2008], and L6 myotubes [6]. Additionally a key property of AS160 is that it contains a GTPase-activating domain (GAP) for Rabs, small G proteins involved in vesicle trafficking [7,8]. Intriguingly, Rabs 2A, 8A, 10, and 14 appear to be substrates of the AS160 GAP domain and are also associated with insulin-responsive GLUT4-containing vesicles [9, 10][11]. As Rab proteins have been shown to be necessary effectors in vesicle trafficking, docking and fusion, AS160 may represent a convergence between insulin signaling and vesicle trafficking. Two separate sets of studies utilizing AS160 knockdown and rescue have identified AS160 as a negative regulator of basal GLUT4 exocytosis [9,12]. In combination, these studies have created the following model of AS160 function in GLUT4 translocation: Basally, AS160 associates with GLUT4 vesicles and intrinsic GAP activity maintains Rab proteins in their inactive form, complexed to GDP. Insulin-stimulated phosphorylation of AS160 inhibits GAP activity towards Rabs, causing a shift towards active Rab-GTP complexes and allowing for Rab-dependent GLUT4 translocation to occur [9,12,13].

It has also been suggested that, a second pathway occurs as a consequence of Cbl tyrosine phosphorylation (Fig. 1) [14,15]. Cbl and the adaptor protein CAP (c-Cbl-associated protein; [16]) are recruited to the insulin receptor by APS (adaptor molecules containing pleckstrin homology (PH) and Src homology 2 (SH2) domains; [17]). Once tyrosine phosphorylated by the receptor, Cbl can recruit the adaptor protein CrkII to lipid rafts, along with the guanyl nucleotide exchange factor C3G [18]. C3G, in turn, can activate the GTP-binding protein TC10, which resides in lipid rafts [19]. The correct spatial compartmentalization of these signaling molecules in the lipid raft microdomain appears to be essential for insulin-stimulated GLUT4 translocation and glucose transport, as these insulin-mediated events are abolished by dominant-interfering mutants of CAP that prevent the localization of Cbl to lipid rafts [20]. Recent investigation suggests a role of TC10 in the regulation of actin dynamics [21–26] and phosphoinositides [27]. In particular, the actin-regulatory neural Wiskott-Aldrich syndrome protein (N-WASP) [23], the actin related protein-3 (Arp3) [23], and the exocyst protein complex [22] appear to be downstream targets of TC10. Whereas N-WASP/Arp3 apparently regulates actin polymerization [23], the exocyst protein complex is postulated to mediate the tethering/docking of GLUT4 vesicles at the plasma membrane [22]. This interaction is necessary for GLUT4 vesicle and plasma membrane fusion mediated by SNARE proteins (SNAP-23, syntaxin 4, Synip, Munc18c and VAMP2).

The burgeoning number of these GLUT4 effectors highlights a remarkably complex signaling architecture that includes key assists from membrane phospholipids and cytoskeletal proteins. Recent insights into the importance of membrane phospholipids such as phosphoinositol 4,5-bisphosphate (PIP₂) and cytoskeletal filaments such as filamentous actin (F-actin) in GLUT4 regulation are the focus of this review. The discussion visits the importance of these components in insulin signaling and GLUT4 vesicular trafficking.

Phosphoinositides & GLUT4

Phosphoinositides are a large group of inositol containing phospholipids, of which the major precursor is phosphatidylinositol (PI). This glycerolipid is comprised of an inositol ring attached to a glycerol backbone by a phosphate at position 1. Other polyphosphoinositides are derived from PI by phosphorylation at various positions of the inositol ring by a variety of lipid kinases and phosphatases (Reviewed in [28–32]). PIs represent a very small portion of the total membrane lipid content in the plasma membrane [31]. These amounts vary from between 5 and 10% for phosphatidylinositol 4,5-bisphosphate (PIP₂) and PI, respectively to as little as 0.25% for the 3' phosphorylated phosphoinositides [32]. These lipids are known to play important roles as precursors to second messengers and as signaling and scaffold targeting molecules [33]. Several PIs are known to bind to and regulate the actions of proteins that bind to actin filaments to regulate actin dynamics. Early experimentation in this field utilizing permeabilized platelets demonstrated that PIs regulate the actin cytoskeleton [34], and recent experiments have resulted in additional evidence supporting this conclusion [35–51]. Actin dynamics, and their interaction with phosphoinositides, which are also factors in GLUT4 translocation, will be reviewed later in this paper. (Reviewed in [33].) Here we focus directly on the participation of PIs in the key steps of GLUT4 translocation.

Signaling—Involvement of PIs in signal transduction is well-characterized. With respect to signaling, PIs are most well-known for their ability to serve as substrates for phospholipase C (PLC) and PI kinases and phosphatases [37,52,53]. Of the nine characterized PIs, PIP₂ is the most studied, most likely due to the fact that it is a versatile signaling molecule known to be a regulator of the actin cytoskeleton [38], membrane trafficking events, and plasma membrane ion channels and transporters (reviewed in [33]). It serves as a precursor to the second messengers IP₃ and diacylglycerol (DAG) and may in and of itself be a second messenger. Specific to insulin signaling, PIP₂ plays a major role as a substrate for PI3K in the synthesis of phosphatidylinositol 3,4,5-trisphosphate (PIP₃), which is a crucial second messenger propagating the insulin signal from the IR to GLUT4 [54]. In an effort to delineate the dynamics of 3-phosphoinositides (PI 3-P, PI 3,4-P₂ and PIP₃) *in vivo*, Dormann *et al.* analyzed the binding specificities for various PI species during *Dictyostelium* phagocytosis and chemotaxis [52]. These authors concluded that different PIP₃ binding PH domains gave distinct spatial and temporal readouts of the same underlying PIP₃ signal, demonstrating that distinct biological responses could be elicited from the same PIP₃ signal. This lends support to the hypothesis that PIP₃ might be able to participate in more than one of the cellular events involved in insulin-activated GLUT4 translocation [52].

As previously mentioned, insulin stimulated PI3K activity, generation of PIP₃, and the subsequent activation of the downstream kinases Akt and PKC ζ/λ has been shown to be crucial for activation of glucose transport in both muscle and fat cells [55–61]. In addition, overexpression of various PI lipid phosphatases, such as the SH2-containing inositol phosphatase (SHIP) or skeletal muscle and kidney-enriched inositol phosphatase (SKIP), both 5' phosphatases that act on PIP₃, inhibits insulin-induced GLUT4 translocation [62–64]. In addition, similar data has been reported using overexpression of phosphatase and tensin homolog (PTEN), a 3' phosphatase that produces PIP₂ from PIP₃ [65]. Although there is good evidence that PIP₃ production is crucial for insulin-stimulated glucose transport, some controversy exists as to the nature of the specificity of insulin generated PIP₃ for this process versus the PIP₃ generated by other growth factors (EGF, IGF or PDGF). Data has suggested that this signal specificity of insulin lies in the threshold of duration and/or intensity of the PIP₃ signal produced [66,67]. Further, Sweeney *et al.* showed that intracellular delivery of PIP₃ augmented plasma membrane GLUT4 content [68]. However, delivery of exogenous PIP₃ was unable to stimulate the same increase in plasma membrane GLUT4 content like insulin, and it did not increase glucose uptake. Thus, while PIP₃ may be necessary for

stimulation of glucose transport, it does not completely recapitulate the signaling cascade for this process, indicating a further level of complexity.

Indeed, several studies have shown that in addition to the role PIP₃ plays in GLUT4 regulation by insulin, direct roles also exist for PIP₂ and various other PIs in insulin-regulated GLUT4 translocation. There is some evidence that insulin may activate phospholipase C (PLC) in a PI3K-dependent manner, which generates the second messengers diacylglycerol (DAG) and inositol trisphosphate (IP₃) from PIP₂. Several previous studies have suggested that PLC may play a role in insulin-stimulated GLUT4 translocation potentially via generation of phosphatidic acid from DAG and subsequent activation of PKC ζ [69–71]. Additionally, PI 3-P also has also been experimentally demonstrated to play a role in insulin-induced GLUT4 translocation. Insulin increases the plasma membrane/lipid raft levels of PI 3-P in L6 cells and 3T3-L1 adipocytes, and this pool of PI 3-P is relatively resistant to PI3K inhibitors. Further, exogenously supplied PI 3-P induces the plasma membrane translocation of both over-expressed and endogenous GLUT4. It has also been shown that a dominant-negative mutant of TC10 inhibits the insulin-stimulated formation of PI 3-P, while a constitutively active mutant mimics the effect of insulin. Taken together, these data suggest that PI 3-P is produced downstream from insulin-mediated activation of TC10 to promote GLUT4 translocation [27].

Additional evidence that PI 5-P, is involved in GLUT4 translocation was suggested by Sbrissa *et al.* [72]. These investigators found that microinjected PI 5-P stimulated GLUT4 translocation in 3T3-L1 adipocytes, and that expression of protein-3xPHD, which selectively binds PI 5-P, blocked insulin-induced GLUT4 vesicle dynamics. These authors postulated that insulin could generate PI 5-P via recruitment of PIKfyve (an enzyme that produces PI 5-P from PI) to the plasma membrane, or via breakdown of PIP₂ by an unidentified 4-phosphatase. Thus, PI 5-P may represent an additional PI signaling lipid involved in PI3K-independent insulin-signaling leading to GLUT4 translocation. The role of other PI lipids in insulin signal propagation remains an area for further exploration.

Trafficking, Docking, & Fusion—An extensive role of PIs in vesicle exocytosis is well documented in other vesicle trafficking systems, such as in nerve terminals, however new studies are needed aimed at dissecting the functionality of distinct PIs [73]. Recently, Ishiki *et al.* reported that PIP₃ and PI 3-P directly regulate GLUT4 vesicle trafficking [27,74]. These PIs seem to coordinate membrane arrival and fusion, and GLUT4 unmasking events critical in plasma membrane accumulation of GLUT4. Other cell systems also shed light on the importance of PI metabolism in membrane trafficking. For example, in chromaffin granules, PIP₂ and other PIs participate in the priming stage of exocytosis [75,76]. Specifically, PIP₂ binds to the granule protein synaptotagmin, thought to function as a calcium sensor in neurotransmitter release. Also, PIP₂ binds to rabphilin 3a, a peripheral membrane protein that may also bind calcium and the docking and fusion regulators Rab3A and Rab3C. Since PIs also regulate actin binding proteins and actin dynamics, it is believed that PIs on the membrane of secretory granules may serve to coordinate secretion of several secretory granule proteins, or they may modulate the necessary dynamic changes that occur in the actin cytoskeleton during exocytosis [77]. In agreement with this hypothesis, recent data from Bittner and Holz recently demonstrated that secretion in primary neuroendocrine cells relies upon the coordinate interactions of PIP₂, actin, and other molecules involved in the secretory response [78]. As introduced above, similar to neuroendocrine cells, insulin responsive tissues express Rabs 2A, 8A, 10, and 14 that appear to be substrates of the AS160 GAP. In addition, it has also been suggested that Rab4 co-localizes with the GLUT4-enriched microsome fraction and may be involved in GLUT4 translocation [79–83].

Finally, evidence is emerging that PIs play roles in membrane docking, fusion and retrieval events. Adipocytes from mice null for the syntaxin 4 binding protein Munc18c have been

reported to show increased sensitivity to insulin-stimulated GLUT4 vesicle fusion. Although in normal cells PI 3-P induces GLUT4 trafficking to the plasma membrane without causing vesicle fusion [74], in Munc18c-null mice, this lipid induced both GLUT4 trafficking to and fusion with the plasma membrane [84]. Thus, Munc18c may function as a negative regulator of PI-regulated GLUT4 vesicle docking and fusion, and disruption of the interaction between syntaxin 4 and Munc18c might result in enhancement of insulin-stimulated GLUT4 vesicle and plasma membrane fusion. In contrast, the internalization of GLUT4, which occurs through a dynamin-dependent mechanism [85–88], may entail PIP₂ activation [89–93].

Actin & GLUT4

An abundance of cellular functions have been ascribed to cytoskeletal actin including maintenance of cell polarity [94], activation of osteoclasts during bone remodeling [95], maturation of granulocytes [96], platelet function [34,97,98], and sperm capacitation and acrosomal reaction [99]. With regard to insulin action and glucose transport, an increasing body of evidence supports a critical role for actin in GLUT4 translocation (Fig. 2) [100–105]. One of the best documented actin events engaged by insulin is “membrane ruffling”, which is an energy-dependent cytoskeleton-driven process controlled by PI3K. Further, the PI3K products PI 3,4-P₂ and PIP₃—as well as PIP₂—can bind to the cytoskeletal protein profilin, which serves as an organizational site for actin remodeling [106]. It has been reported that insulin elicits the formation of F-actin, and that pharmaceutical disruption of cortical actin filament formation with latrunculin B inhibits insulin-stimulated GLUT4 translocation [25, 100,107]. Just as the number of signaling proteins coupling the IR to GLUT4 action has escalated, several actin binding proteins seem to be decorating the GLUT4-regulatory network. For example, as presented above under the *Insulin Signaling & GLUT4* section, *in vitro* study suggests a role for the actin regulatory protein N-WASP in mediating the effects of insulin on the actin filament network. The subsections below detail findings suggestive of a role of actin in insulin-regulated GLUT4 function.

Signaling—As previously mentioned, data exists indicating that the actin cytoskeleton forms a scaffold for signaling molecules in a variety of cellular processes [34], [50], [96], [108], [109], [110], [111]. Analogously, it is hypothesized that actin may play a similar role in the insulin signaling pathway [104]. Additionally, evidence exists that there are interactions between the actin cytoskeleton and PI metabolism [39] discussed below under *Phosphoinositides & Actin*.

With regards to insulin, this hormone elicits a rapid, dynamic remodeling of actin filaments into a cortical mesh, and in a variety of insulin-sensitive cell types, such as differentiated L6 muscle cells, 3T3-L1 adipocytes, primary fat cells, and primary muscle cells, this mesh is a necessity for GLUT4 translocation [23,24,112–115]. Since insulin signaling involves activation of PI3K, it is believed that the insulin-induced formation of a cortical F-actin mesh serves to spatially localize the generated PIP₃ and/or downstream signaling components in order to elicit the appropriate downstream effects. Thus, the actin cytoskeleton may comprise a matrix in which the enzymes involved in PI metabolism can associate, and segregate the responses in the insulin signaling pathway (Fig. 2). In agreement with this idea, Patel *et al.* demonstrated that when insulin-stimulated actin remodeling is disrupted using excess PIP₃, this resulted in the inhibition of GLUT4 translocation [112].

It has recently been reported by Martin *et al.* that activated PI3K was itself sufficient to mediate actin rearrangement and GLUT4 translocation in 3T3-L1 adipocytes, suggesting that insulin’s ability to produce these two effects must depend upon its ability to trigger a PI3K-induced signaling cascade [116]. However, at least one other PI3K independent pathway has been hypothesized to be involved in actin remodeling and GLUT4 translocation, which involves the

insulin stimulated activation of TC10, as described under *Insulin Signaling & GLUT4*. Although the significance of this pathway remains controversial [18,19,21,23] its connection to the actin network via N-WASP is intriguing. Moreover, insulin mimetic stimulation of GLUT4 translocation by osmotic shock, which does not require PI3K activation, does require TC10 and presumably N-WASP [117]. Interestingly, expression of a dominant negative N-WASP lacking the Arp and actin binding domains attenuated cortical F-actin rearrangements by insulin and inhibited insulin-induced GLUT4 translocation in cultured adipocytes [23]. Additionally, expression of an inhibitory TC10 mutant inhibited cortical localization of N-WASP and F-actin formation in response to insulin. Taken together, these data reveal a pathway in which N-WASP likely functions downstream of both PI3K and TC10 to mobilize cortical F-actin, thereby promoting GLUT4 translocation to the plasma membrane. Nonetheless, while these studies demonstrate a necessary role for TC10 activation in actin remodeling events required for GLUT4 translocation in adipocytes, there is evidence that this TC10 pathway may not function in muscle cells [118,119].

Trafficking, Docking, & Fusion—Although it has been shown that the cell cytoskeleton plays important roles in membrane trafficking events, it is still unclear as to whether GLUT4 vesicle trafficking involves both the microtubule and the actin cytoskeleton [100,101,120–127]. Since the majority of the evidence in this area concerns the actin cytoskeleton, this review will focus on the involvement of this structure. As previously indicated, there is considerable evidence implicating the cortical actin cytoskeleton in formation of GLUT4 vesicle actin comet tail formation and translocation in both adipocytes and muscle cells [25,105,128]. One of the major components of GLUT4-containing vesicles is the zinc-dependent protease designated insulin-responsive aminopeptidase (IRAP) that traffics in a similar or identical fashion to GLUT4 [129–134]. There is some evidence that the N terminus of IRAP functions in the regulation of the intracellular retention and trafficking of GLUT4 vesicles via interaction with the cytoskeleton in an insulin dependent manner [135]. Indeed, adenoviral overexpression of the IRAP interacting protein FHOS (formin homolog overexpressed in spleen) enhances glucose uptake in L6 muscle cells. Since FHOS has been shown to have a skeletal muscle isoform with a binding affinity for profilin, this may suggest that FHOS acts as a linker protein between GLUT4-containing vesicles and the actin cytoskeleton [136].

It has recently been reported that the calpain family of cysteine proteases have a potential role in insulin-induced glucose uptake. Intriguingly, a SNP in this protease has been shown to be associated with development of diabetes. Paul *et al.* reported that inhibition of calpain activity in 3T3-L1 adipocytes reduced insulin-induced GLUT4 translocation [137]. Significant to the point of this review, the inhibition of calpain activity was associated with an inhibition of insulin-stimulated actin reorganization, with no effect on the immediate steps of insulin signaling. Therefore, these data would lead to the hypothesis that calpain-10 may facilitate GLUT4 translocation via reorganization of the cortical actin cytoskeleton [138]. Hence, in addition to identifying calpain as a potential novel factor involved in the trafficking of GLUT4 vesicles, this study also provided more evidence of the necessary role of actin dynamics in the overall process of GLUT4 translocation.

In addition to the exocytosis of GLUT4 in adipocytes, PIP₂-regulated cortical F-actin structure appears essential for the release of adiponectin and may explain why the levels of this adipokine are reduced in insulin resistant states [139]. In other secretory cells, the actin cytoskeleton has also been observed to have profound influence on exocytosis. In a study of pancreatic acinar cells undergoing regulated exocytosis, it was observed that in response to the secretagogue carbamylcholine, zymogen granules participating in exocytosis became coated with F-actin and released rab3d. These observations suggest that rab3d is involved in the regulation of actin polymerization around secretory granules and that the subsequent coating of the vesicles with actin facilitates the movement of granules across the subapical actin network and toward their

fusion site [140]. Other studies of secretory cells have suggested that cortical F-actin instead functions as a physical barrier to vesicle docking based upon its transient depolymerization during exocytosis, and that secretion preferentially occurs at sites where the actin cortex is relatively thin. Moreover, to add to the complexity of this area in some cell systems, disruption of the actin cytoskeleton markedly potentiates agonist-stimulated secretion, while in others, depletion of F-actin structures either by sequestering actin monomers or by stimulation of actin severing results in inhibition of agonist-induced secretion [141–147].

It is still unclear as to whether GLUT4 vesicles have properties more consistent with secretory granules or synaptic vesicles, although recent evidence points to them having properties unique from both. In muscle cells the insulin-dependent gain in surface GLUT4 functionally requires the fusogenic v-SNARE VAMP2 [148]. Additionally, in response to insulin, VAMP2-containing vesicles partially localize with the actin structures induced by the hormone. Thus, insulin-induced actin remodeling may be required to specifically segregate VAMP2-containing GLUT4 vesicles and allow their recruitment to the plasma membrane [112,119,148,149]. VAMP2 would then act as a fusogen either at the plasma membrane, or through additional intermediate fusion (and fission) steps [150]. An interesting report suggests that insulin-stimulated activation of PKC ζ , in a PI3K-dependent manner, induces serine phosphorylation of VAMP2 in the GLUT4 compartment, which was hypothesized to lead to GLUT4 translocation. It is unclear, however, how phosphorylation of this protein could initiate this process or trigger the association of VAMP2 containing vesicles with the actin cytoskeleton, suggesting there are yet unknown interactions between GLUT4 vesicles and the actin cytoskeleton that modulate exocytosis and fusion [151,152].

Phosphoinositides & Actin

The coordination of rearrangements of the actin cytoskeleton depends on its tight connection to the plasma membrane, and is thought to occur through signals transmitted to the underlying actin cytoskeleton via PIP₂. This lipid messenger binds to, and influences the activity of, several actin-associated proteins in vitro that regulate the architecture of the actin cytoskeleton, such as the Rho GTPase Cdc42 [33,153,154]. Conflicting data exists, however, that suggests Cdc42 in fact does not bind to PIP₃, which implies that it may not be a key player in the PI3K signaling pathway involved in insulin-induced GLUT4 translocation. Moreover, other data exists showing that the Rho GTPases Rac1 and RhoA are capable of binding to PIP₃, which lends support to the possibility that these molecules may have a role in this process [155]. Complicating this issue is the fact that ARF6, another well-known small GTP binding protein, appears to be involved in insulin-induced actin cytoskeletal rearrangement, but does not seem to affect GLUT4 translocation [156,157]. This may point to the importance of cellular compartmentalization in regulating intracellular trafficking.

Recent work by Kanzaki *et al.* supports this hypothesis and illustrates the potential role of membrane cholesterol content in this process. These authors produced elevated levels of PIP₂ in both 3T3-L1 fibroblasts and adipocytes via overexpression of PIP5K. In fibroblasts PIP5K expression yielded motile actin comet tails, whereas in differentiated adipocytes the formation of vacuoles coated with F-actin, cortactin, dynamin, and N-WASP, and an inhibition GLUT4 endocytosis was noted. Further, these authors demonstrated that cholesterol loading of fibroblasts expressing PIP5K promoted formation of similar vacuoles, leading these authors to hypothesize that the effect of cholesterol on membrane thickness and curvature or its influence on PIP₂ levels and distribution—both of which affect actin cytoskeletal organization and membrane trafficking events such as GLUT4 endocytosis—is involved [128].

Concordant with the insulin-sensitizing and cholesterol-lowering effects of CrPic [158–160], this dietary supplement improves insulin sensitivity by lowering membrane cholesterol from insulin-resistant, cholesterol-laden PMs [161]. These beneficial actions of CrPic are consistent

with newly recognized effects of statins on improving insulin resistance in dyslipidemic patients [162–164]. The mechanism by which CrPic decreases cholesterol is speculative but may occur by means similar to that used by the anti-diabetic drug metformin. For example, CrPic [161,165] and metformin [166,167] have recently been reported to activate 5'-AMP-activated kinase AMPK. Metformin stimulated AMPK activity has been shown to suppress expression of a sterol regulatory element binding protein, SREBP-1 [167] SREBP-1 belongs to a family of key lipogenic transcription factors directly involved in the expression of more than 30 genes dedicated to the synthesis and uptake of cholesterol, fatty acids, triglycerides, and phospholipids, as well as the NADPH cofactor required to synthesize these molecules [168] More recent work also shows that acute contraction restores insulin-stimulated glucose uptake in muscle of obese insulin-resistant Zucker rats to levels seen in lean controls [169]. As predicted, contraction decreased intramuscular triacylglycerol content; however, diacylglycerol and long chain acyl-CoAs, lipid intermediates presumed to trigger insulin resistance, were either unchanged or increased [169]. Also, it was observed that only a partial improvement in insulin signaling to AS160 occurred, thus implicating another target of the beneficial effects of exercise. Together, these findings raise the question whether metformin and/or acute exercise decreases PM cholesterol.

In further support of an association between PIP₂ and zones of actin re-organization, Huang *et al.* showed that PIP₂-rich plasma membrane patches (PRMPs) organize active zones of endocytosis and membrane ruffling in cultured adipocytes [54,170]. Although their data provided a correlation between the location of PRMPs and zones of concentrated F-actin where endocytic activity was occurring, it did not provide evidence of a causative link between the two. To explore this relationship, these authors conducted additional experiments using the molecular motor protein Myo1c, a mediator of membrane ruffling which requires F-actin for proper function. Data from these experiments suggested that PRMPs may function to locally concentrate F-actin and Myo1c to enhance their ability to promote membrane ruffling [54, 170]. Previous evidence has also indicated that Myo1c may play some role in GLUT4 translocation and insulin-stimulated GLUT4 recycling [102]; thus it is also possible that PRMPs may promote this process as well [54]. (For a review of spatial control of actin-based motility through plasmalemmal PIP₂-rich raft assemblies, see [171])

Insulin Resistance: A Disease of the Actin Cytoskeleton?

It is clear that the molecular etiology of insulin resistance involves multiple genetic and non-genetic mechanisms contributing to the final phenotype [172]. Furthermore, the resultant hyperglycemia of the syndrome and the compensatory rise in insulin in turn exacerbate insulin resistance, contributing significantly to the pathogenesis of the disease [173]. Superimposed on that backdrop is the negative contribution of the obese state [174]. Although pinpointing the site of the abnormality induced by these conditions is still an intensive area of current research, evidence strongly suggests that the defects induced by each occur at different loci in the insulin-signaling network. Previous studies show that prolonged insulin treatment of primary rat adipocytes and 3T3-L1 adipocytes cause a state of insulin resistance as manifested by decreased insulin-stimulated glucose transport [176,177]. This defect is associated with an impaired net GLUT4 translocation to the cell surface of adipocytes [178, 179]. Examination of the protein and activity levels of the IR, PI3K and Akt demonstrate a clear progressive disturbance in the insulin stimulated sequential communication of these signaling molecules [180,181]. Interestingly, in support of this signaling defect being corrected by changing membrane fluidity, the decreased IR, PI3K and Akt activities were reversed when metformin was included in the chronic insulin culture [181].

In contrast to an insulin receptor abnormality as a basis of signal interference, multiple studies suggest that lipid oversupply contributes to the development of insulin resistance, perhaps by

promoting the accumulation of ceramide or a ceramide metabolite that in turn inhibits insulin stimulation of Akt without a decrease in the tyrosine phosphorylation of the IR or IRS1 or a decrease in the interaction of IRS1 with PI3K [182–189]. In addition, work suggests that defects in the IR, IRS1, PI3K, Akt, and Cbl do not readily account for decreased insulin responsiveness induced by increasing the flux of glucose through the hexosamine biosynthesis pathway [179,188,190–194]. Buse *et al.* [195] recently demonstrated that chronically increased glucose flux elevated muscle UDP-N-acetylglucosamine levels and this in turn induced O-linked glycosylation of membrane proteins *in vivo*.

In the same way that increased hexosamine biosynthesis contributes, at least in part, to the insulin resistant state without insulin signaling defects, new perspective shows a membrane/cytoskeletal basis of hyperinsulinemia-induced insulin resistance. This finding has significant merit as a preliminary component of insulin resistance is hyperinsulinemia [196]. This compensatory increase in insulin during the insulin resistant state initially offsets insulin's reduced ability to stimulate GLUT4 translocation and glucose uptake, which is essential for the maintenance of blood glucose homeostasis. However, *in vivo* [197,198] and *in vitro* [180, 199] studies demonstrate that the hyperinsulinemic state has a negative effect on insulin action. To date, extensive study has built a good understanding of how insulin itself can be involved in the staging of diabetes. For example, chronic exposure to pharmacological doses of insulin (≥ 100 nM) have been shown by several laboratories to markedly attenuate expression levels and/or activity states of the IR, IRS-1, PI3K, Akt and GLUT4 proteins in 3T3-L1 adipocytes and thus, produce a defect in the cell's ability to respond to subsequent acute insulin stimulation with an increase in GLUT4 translocation and glucose transport [181,200,201]. On the other hand, similar defects in the ability of insulin to acutely regulate the glucose transport system can be induced by lower, more pathophysiological concentrations of insulin (≤ 5 nM) that are not associated with disturbances in early insulin signaling [6,103,193,202]. Therefore, defects positioned more distal in the insulin signaling pathway may contribute to the insulin-mediated cellular insulin resistance.

Recent study has found that plasma membrane phosphoinositide-regulated cortical F-actin structure is perturbed in muscle and fat cells made insulin resistant by hyperinsulinemia [6, 103]. Consistent with previous studies that induce insulin resistance via overnight treatment of cells with a more pathophysiological dose of insulin [193], the impaired ability of insulin to acutely regulate the glucose transport process did not correlate with reduced protein levels or activation of several known insulin signaling intermediates. In contrast, there was an associated loss of PIP₂ and cortical F-actin during the insulin-induced state of insulin resistance. Particularly striking was that correction of that via PIP₂ replenishment restored insulin sensitivity. Thus, these recent data define a novel diabetic-state alteration and reveal a counteractive tactic to normalize insulin sensitivity. Data from another set of studies demonstrates a strikingly similar importance of plasma membrane PIP₂ and cortical F-actin in the development of insulin resistance induced by endothelin-1 [203,204]. This vasoactive peptide is well-documented to diminish insulin sensitivity by a direct effect on one or more mechanisms involved in insulin-stimulated glucose transport and not from a vasoconstrictive decrease in skeletal muscle blood flow [205–208]. As this vasoactive peptide also displays insulin mimetic activity [207,209–211], it is tempting to speculate that both endothelin-1- and insulin-induced insulin resistance result from stimulated glucose transport and increased hexosamine biosynthesis, which as indicated above may have ties to cell surface abnormalities.

The cortical actin network also interacts with the extracellular cytoskeletal matrix proteins (dystrophin, α/β -dystroglycan, spectrin, etc.), which serves to stabilize the structure of the plasma membrane. Thus, it would be expected that disruption of either of these structures could influence insulin signaling and GLUT4 translocation. In agreement with this idea, it has been reported that *mdx* mice, an animal model of Duchene's Muscular Dystrophy which possess a

mutation of the dystrophin gene, exhibit impaired regulation of carbohydrate metabolism [212]. Conversely, and more pertinent to the topic of this review, it has recently been reported that the diabetic Goto-Kakizaki rat exhibits diminished levels of the dystrophin and α/β dystroglycan isoforms that form a link between the extracellular cytoskeletal matrix and the actin membrane cytoskeleton [213]. Thus, impaired anchoring of the actin cytoskeleton to the plasma membrane may contribute to the reduced insulin stimulated GLUT4 translocation observed in this model. This may be of relevance to humans, as muscular weakness has long been recognized as a phenomenon observed in human diabetics [214]. In light of this observation, it has recently been reported that endurance exercise, which has insulin sensitizing effects, is associated with an increase in the phosphorylation of filamin A [175], an additional protein that along with dystrophin, is involved in the linkage of the cortical actin cytoskeleton to the extracellular matrix. Although the importance of this event remains to be determined, it is tempting to speculate that muscular contraction may be positively modulating the interaction between the extracellular matrix and the actin cytoskeleton to facilitate improved insulin signaling and GLUT4 translocation. However, the true significance of this finding remains to be determined through future investigation.

CONCLUSIONS

Among the many facets of insulin signaling currently being studied, intense effort is being focused on insulin's ability to effect GLUT4 translocation. Although there is still much to learn about the complex regulation of GLUT4 translocation by insulin; as highlighted in this review, it is clear that regulatory events involving actin and phosphoinositides are major players in this process. What remains unclear, however, is how elevations in insulin, perhaps in combination with elevated plasma lipids, alter the plasma membrane and actin cytoskeleton to induce insulin resistance. This, for certain will be an area of concerted study in the years to come.

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Fig. 1. Model showing the various signaling events involved in GLUT4 translocation. TUG, tether containing a UBX domain for GLUT4; SNARE, soluble NSF-attachment factor receptor.

Fig. 2.
Proposed model of GLUT4 translocation incorporating signaling through phosphoinositides and integration with actin/cytoskeletal remodeling.