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REGULATION OF GLUT4 TRAFFIC AND FUNCTION BY INSULIN AND CONTRACTION IN SKELETAL MUSCLE

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1. ABSTRACT

Glucose transport across the cell surface is a key regulatory step for glucose metabolism in skeletal muscle. Both insulin and exercise increase glucose transport into myofibers through glucose transporter (GLUT) proteins. Skeletal muscle expresses several members of the GLUT family but the GLUT4 glucose transporter is considered the main "regulatable" isoform that is modulated by insulin and contraction. Glucose transport rate can be stimulated either by recruitment of GLUT4 units from intracellular storage vesicles or through activation of cell surface transporters. Insulin activates GLUT4 translocation through a complex signaling cascade involving both the lipid kinase phosphatidylinositol 3-kinase and the proto-oncoprotein c-Cbl. Contraction, on the other hand, appears to trigger GLUT4 translocation at least in part through activation of the metabolite-sensing 5'-AMP-activated protein kinase. Furthermore, recent studies suggest that p38 MAP kinase activation represents a point of convergence of the signaling pathways utilized by insulin and contraction to increase GLUT4 activation at the cell surface. This review will summarize our current knowledge of these alternative pathways of GLUT4 regulation in skeletal muscle.

2. INTRODUCTION

Skeletal muscle is the main tissue responsible for whole-body glucose disposal when insulin levels raise after a meal and during physical exercise. It is now well established that the translocation or recruitment of the GLUT4 protein from one or more intracellular compartments to the cell surface represents an important mechanism by which both insulin and exercise stimulate glucose transport in muscle cells. More recent work also suggests that the activity of the recruited GLUT4 transporters can be regulated by insulin and contraction. Furthermore, there is now substantial evidence that the insulin-resistant glucose transport activity of skeletal muscle from type 2 diabetic individuals is not caused by a reduction in GLUT4 expression, but rather believed to be associated in large part with an impaired translocation and/or function of the transporter protein. It is therefore critical to fully understand the signaling mechanisms that control GLUT4 recruitment and activation in skeletal muscle cells in order to unravel the molecular defects behind the development of insulin resistance. In this article, we will review the current knowledge of the trafficking and functional regulation of muscle glucose transporters, focusing our attention on signaling mechanisms involved in the acute action of the two main stimuli of glucose transport in muscle, namely insulin and contraction.

3. GLUCOSE TRANSPORTERS IN SKELETAL MUSCLE:REGULATION BY INSULIN AND CONTRACTION

3.1. Expression of glucose transporters in skeletal muscle

The transport of glucose across the surface membrane is the rate-limiting step for glucose metabolism in skeletal muscle, both in the basal and stimulated states (1-3). Glucose transport in mammalian tissues, including

skeletal muscle, is mediated by facilitated diffusion through a family of membrane-embedded proteins known as the glucose transporters (GLUTs). So far, 15 different GLUT proteins, encoded by distinct genes, have been identified in mammalian tissues (4). At least 5 isoforms (GLUT1, 4, 5, 8, and 11) of the GLUT family are expressed in adult skeletal muscle (4). The GLUT4 transporter is known as the "regulatable" glucose transporter in insulin target cells and it is the predominant isoform expressed in skeletal muscle (5). The expression of the GLUT1 glucose transporter in muscle is considerably lower than that of GLUT4 (~ 20 times less) and is believed to contribute to basal glucose uptake, as this transporter is localized solely to the plasma membrane in the basal state (5). The GLUT5 hexose transporter, originally identified in the jejunum brush border, is also expressed in skeletal muscle (6). However, GLUT5 operates primarily as a fructose transporter and does not appear to be modulated by either insulin or exercise (6).

Individual muscle fibers are specialized entities that differ in their functional properties and cellular responses to mechanical, hormonal or neural factors (7). Previous studies have shown that slow twitch oxidative (Type I) and fast twitch oxidative-glycolytic (Type IIa) muscle fibers have a higher capacity for basal and insulin-mediated glucose transport because they express more GLUT4 and GLUT1 glucose transporters than fast twitch glycolytic (Type IIb) fibers (8-12). Furthermore, GLUT4 expression correlates with the metabolic nature (oxidative vs. glycolytic) of skeletal muscle fibers, rather than with their contractile properties (slow twitch vs. fast twitch) (12).

3.2. Translocation of glucose transporters to the muscle cell surface : plasma membrane vs T-tubules

When muscle is exercised or stimulated by insulin, GLUT4 is translocated from intracellular storage compartments to the cell surface so that extracellular glucose is transported into the cell at a much higher rate. In skeletal muscle, the cell surface membrane is composed of two distinct components, namely the plasma membrane and the transverse tubules (T-tubules). The T-tubules are deep inward extensions of the sarcolemma that interact with the terminal cisternae of the sarcoplasmic reticulum. Their roles are to provide deep access to the extracellular milieu as well as to propagate membrane potentials that lead to myofiber contraction following calcium release by the sarcoplasmic reticulum. Using a new subcellular fractionation procedure, we have previously shown that insulin and contraction increase glucose transport by mobilizing GLUT4, but not GLUT1, to both the plasma membrane and the T-tubules (13-15). Translocation of GLUT4 to muscle T-tubules in response to insulin and contraction has been further confirmed by immuno-electron/confocal microscopic approaches (16-18).

The subcellular distribution and translocation of glucose transporters is also subjected to a fiber-type specific regulation. Thus, Type I and Type IIa muscle fibers contain a higher amount of GLUT1 and GLUT4 transporters at the plasma membrane than white muscle in the basal and insulin-stimulated states (5). On the other hand, electrical stimulation induces GLUT4 translocation more importantly in T-tubules of glycolytic muscles than in muscles mostly enriched in oxidative fibers (15). This may explain the greater responsiveness of glycolytic muscle fibers to contraction-induced glucose transport despite their lower expression of GLUT4 and GLUT1 transporters as compared to oxidative fibers.

Since the T-tubules cover 60-70% of the muscle cell surface (19,20) they are thought to mediate the bulk of insulin-stimulated glucose transport into muscle fibers. This is supported by several observations. Earlier work has shown that disruption of the T-tubules by glycerol shock abolished insulin-stimulated glucose transport in isolated muscle (18). Furthermore, GLUT4 translocation to the T-tubules represents the main determinant that predicts the extent of insulin-stimulated glucose transport in muscle fibers in various experimental conditions. Indeed, it was observed that streptozotocin-induced diabetic rats displayed impaired GLUT4 translocation to the T-tubules, but not to the plasma membrane and that whole-body insulin action in these animals was significantly correlated with GLUT4 levels in the T-tubules, but not with the transporter abundance in the plasma membrane (21). More recently, we also found that feeding fish proteins increases insulin-induced glucose uptake in muscles of obese high-fat fed rats by selectively improving recruitment to the T-tubules, but not to the plasma membrane (22,23). The determinant role of the T-tubules in whole-body insulin action on glucose homeostasis was also recently highlighted in mice overexpressing Munc18c, a protein that binds and inhibits the function of the t-SNARE (soluble N-ethylmaleimide-sensitive factor attached protein receptor) syntaxin 4 (24). Munc18 overexpression in muscle of these mice causes insulin resistance and abrogated insulin-induced GLUT4 recruitment to the T-tubules, but not to the plasma membrane (16). Collectively, these studies strongly argue for an important role of the T-tubules in the regulation of insulin-stimulated glucose transport and, possibly, in the development of insulin resistance.

3.3. Insulin- and contraction-responsive intracellular GLUT4 compartments

The nature and number of intracellular GLUT4 storage compartment(s) from which GLUT4 is recruited by insulin and contraction is still the subject of intense investigation. Earlier studies indicate that insulin and contraction recruit distinct GLUT4 storage pools in muscle (25-29) but the possible dynamic overlap between these GLUT4-containing compartments and the functional significance of multiple GLUT4 compartments in muscle cells still remain elusive. GLUT4 is mainly found in tubulo-vesicular organelles clustered in the cytoplasm, but a significant proportion can also be observed in several structures thought to represent elements of the endocytic pathway (30-32). In skeletal muscle, GLUT4 immunoreactivity has been detected near or at the transverse tubule area including the sarcoplasmic reticulum cisternae, as well as in the sub-plasma membrane region and vesicular structures located between cortical mitochondria (18,30,33,34). Studies in adipocytes suggest

that the bulk of GLUT4 appearing at the cell surface upon insulin stimulation are recruited from storage vesicles that are segregated from the conventional endosomal system (reviewed in (35)). However, there is a significant overlap between specialized GLUT4 vesicles and proteins found in endosomes such as the transferrin receptor (TfR) (36). Studies using various molecular approaches have confirmed that a significant proportion of GLUT4 transporters are mobilized from TfR-containing endosome-like vesicles by insulin (37-40). Members of the Rab GTPase family, proteins that mediate vesicular trafficking, were also shown to colocalize with GLUT4-enriched intracellular compartments. Among these, Rab4 and Rab11 are present in GLUT4 vesicles isolated from rat skeletal muscle (41) and cardiomyocytes (42), respectively. Rab11 is present both in the endosomal and non-endosomal GLUT4 pools (42), whereas Rab4 co-sediments with GLUT4 from a pool devoid of transferrin receptor (41). More recently, AS160, a newly discovered 160 kDa protein that is phosphorylated by Akt in response to insulin, was shown to possess a GTPase-activating domain for the Rab proteins (43). Mutational analysis further revealed that phosphorylation of AS160 was required for GLUT4 translocation in 3T3-L1 adipocytes (43).

A two-step model was recently proposed for GLUT4 trafficking in which the endosomal recycling compartment is required for the transport of GLUT4 from endosomes to a specialized compartment and for the insulin-induced translocation of GLUT4 to the cell surface of adipocytes (44). Whether the endosomal system plays a similar role in GLUT4 translocation to the cell surface of muscle fibers is still unclear. GLUT4 and the TfR partly share the same vesicular compartment in muscle cells but, unlike in fat cells, insulin induces the translocation of GLUT4 but not of the TfR in skeletal muscle (17,45). On the other hand, contraction, unlike insulin, stimulates both TfR and GLUT4 translocation from a distinct "contraction-responsive" GLUT4 pool (17,45). These data suggest that contraction induces the recruitment of GLUT4-containing recycling endosomes in muscle. Whether GLUT4 trafficking through the endosomal system in contracted muscle fibers may also be described by a two-step model is not known but recent studies do suggest that muscle cells also developed a specialized, postendosomal GLUT4 retention compartment (46,47).

4. INSULIN SIGNALING AND GLUT4 TRAFFIC

4.1. “Classical” signaling pathways involved in GLUT4 translocation

The movement of GLUT4 from its intracellular storage site to the muscle cell surface is initiated by the binding of insulin to its receptor extracellular alpha-subunits leading to the autophosphorylation of the transmembrane beta-subunits (see Figure 1 for a schematic representation of insulin signaling pathways). This in turn activates the intrinsic kinase activity of the beta-subunits towards a variety of intracellular substrates most notably the insulin receptor substrate (IRS) family (48). Of interest is the observation that the internalized receptor in skeletal muscle exhibits a sustained activation at the level of the intracellular GLUT4 vesicles, in close vicinity to the IRS proteins (49). In skeletal muscle, IRS-1 and IRS-2 appear to be the main IRS isoforms expressed, of which, IRS-1 seems the most prominent isoform responsible for insulin-stimulated glucose transport (50,51). Tyrosine-phosphorylated IRS proteins serve as docking proteins for Src homology 2 (SH2)-containing molecules. The best examplified such protein is the 85 kDa regulatory subunit (p85) of the phosphatidylinositol (PI) 3-kinase which, once attached to IRS-1 or IRS-2, activates the 110 kDa catalytic (p110) subunit of PI 3-kinase, a lipid kinase that mediates the formation of 3’ phosphoinositides, such as $PI(3,4)P_2$ and $PI(3,4,5)P_3$ (52). These membraneous lipids are required for the activation of 3’-phosphoinositide-dependent protein kinase-1 (PDK-1) which relays the signal to serine/threonine kinases, including Akt (also termed protein kinase B [PKB]) and atypical protein kinase C-zeta/lamda (aPKC). This is achieved via the phosphorylation of Akt on threonine 308 and aPKC-zeta on threonine 410 (threonine 403 for aPKC-lamda) (53-57). Full activation of Akt further requires phosphorylation on serine 473 by the putative PDK-2 (58) whereas aPKC activity appears to be dependent on autophosphorylation and/or transphosphorylation by a yet unknown mechanism (59). Both of these kinases have been proposed as downstream mediators of PI 3-kinase responsible for insulin-stimulated GLUT4 translocation in muscle cells. On one hand, expression of a kinase-inactive and phosphorylation-deficient Akt construct in L6 myocytes inhibits insulin-stimulated GLUT4 translocation (60) while a line of L6 cells expressing a constitutively active form of Akt obtained via membrane targeting showed elevated glucose transport and plasma membrane GLUT4 level similar to what is observed in parental L6 muscle cells treated with insulin (61). On the other hand, it was shown that adenoviral expression of a kinase-inactive form of aPKC inhibits insulin-stimulated GLUT4 translocation in L6 muscle cells whereas a constitutively active form of the enzyme increases glucose transport to the same extent as that observed with insulin stimulation (62). While the participation of PI 3-kinase in the control of GLUT4 translocation stimulated by insulin has been shown using multiple approaches that include pharmacological inhibition (wortmannin and LY294002) and overexpression of a mutated p85 regulatory subunit lacking the ability to bind the p110 catalytic subunit (52), the exact contribution of either Akt or aPKC in this process still needs to be elucidated.

The mechanism linking PI 3-kinase activation and GLUT4 translocation in muscle cell might involve the reorganization of certain elements of the cytoskeleton. Indeed, insulin-induced cortical actin remodeling was shown to promote the colocalization of the p85 subunit of PI 3-kinase with actin structures thereby facilitating the association of PI 3-kinase with GLUT4 vesicles in L6 myotubes (63). As a consequence, disruption of the actin cytoskeleton was associated with a decrease GLUT4 arrival at the plasma membrane of muscle cell. An important question in the understanding of the actual movement of GLUT4 from intracellular compartments to the plasma

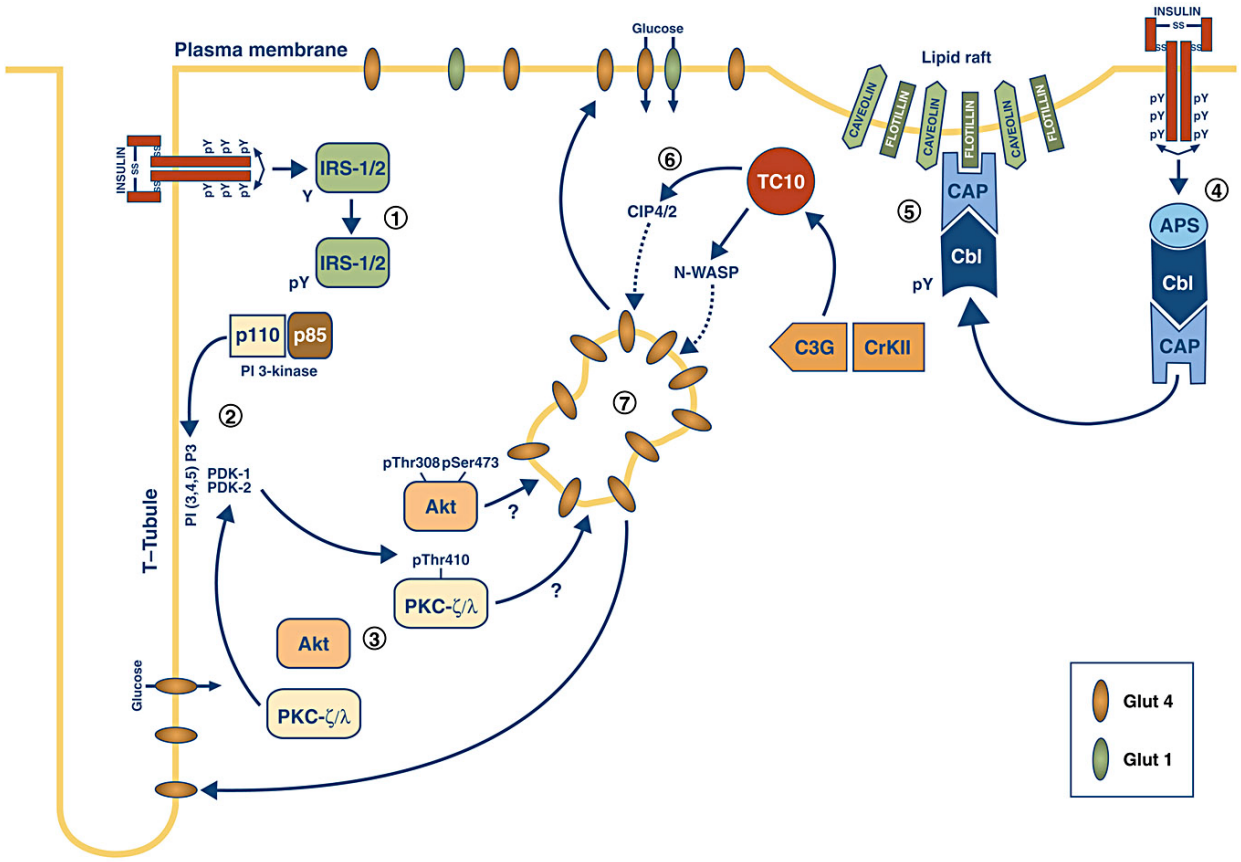

Figure 1. Signaling pathways involved in GLUT4 translocation. 1) Once insulin binds to its receptor extracellular alpha-subunits, it leads to the autophosphorylation of the transmembrane beta-subunits which in turn activates the intrinsic kinase activity of the beta-subunits towards IRS-1/2. 2) Tyrosine-phosphorylated IRS proteins serve as docking proteins for the 85 kDa regulatory subunit (p85) of the phosphatidylinositol (PI) 3-kinase which, once bound to IRS-1 or IRS-2, activates the 110 kDa catalytic (p110) subunit of PI 3-kinase. 3) PI 3-kinase mediated production of PI(3,4,5)P3 activates the 3'-phosphoinositide-dependent protein kinases (PDK1/2) which phosphorylate Akt (Thr308 and Ser473) and PKC-zeta/lamda (Thr410). Both kinases have been proposed as downstream mediators of PI 3-kinase responsible for insulin-stimulated GLUT4 translocation in muscle cells. 4) Insulin receptor signaling also increases tyrosine phosphorylation of the proto-oncogene Cbl, a process that requires the presence of the adapter protein APS. Cbl is then recruited to the insulin receptor along with a second adapter protein, CAP. 5) The CAP-Cbl complex dissociates from the insulin receptor and moves to caveolin- and flotillin-enriched compartments, also termed lipid rafts, of the plasma membrane where flotillin forms a ternary complex with CAP and Cbl. This allows the recruitment of CrkII to lipid raft microdomains along with the guanine nucleotide exchange factor C3G, specifically activating TC10, a small GTP-binding protein of the Rho family. 6) TC10 has been proposed to promote GLUT4 translocation through CIP4/2 (cdc42-interacting protein 4/2) and N-WASP (neural Wiskott-Aldrich syndrome protein) signaling mechanisms. 7) The convergence of the two signaling pathways is necessary for optimal GLUT4 translocation to the cell surface.

membrane is still unanswered: how insulin signaling directly regulates the interactions between GLUT4 containing membranes and cytoskeletal elements implicated in GLUT4 exocytotic recruitment ?

4.2. "Novel" signaling pathways involved in GLUT4 translocation

It is noteworthy that activation of PI 3-kinase by insulin is not sufficient to stimulate glucose transport since other receptors that activate the enzyme such as the platelet-derived growth factor (PDGF) and interleukin-4 receptors do not increase glucose transport in adipose or muscle cells (64-66). Further evidence for the involvement of PI 3-kinase-independent pathway(s) for insulin-stimulated glucose transport came with studies using acetoxymethyl esters of PIP_3 (PIP_3/AM), a membrane permeant form of PIP_3 (67). The addition of PIP_3/AM to 3T3-L1 adipocytes had no effect on basal glucose uptake (67). However, pretreatment with the PI 3-kinase inhibitor wortmannin, plus addition of both PIP_3/AM and insulin resulted in nearly complete restoration of insulin-mediated glucose uptake (67). This suggests the presence of one or more pathways that are also activated by insulin, but are independent of PI 3-kinase and not inhibited by wortmannin. Evidence for the existence of a second signaling pathway was recently provided by Pessin and

Saltiel's groups (68,69). This signaling cascade is initiated by the tyrosine phosphorylation of the proto-oncogene Cbl by the activated insulin receptor, a process that requires the presence of the adapter protein containing PH and SH2 domain (APS) (70-72). Cbl is then recruited to the IR along with a second adapter protein, CAP (Cbl-associated protein) (72). The CAP-Cbl complex dissociates from the IR and moves to caveolin- and flotillin-enriched compartments, also termed lipid rafts, of the plasma membrane where flotillin forms a ternary complex with CAP and Cbl (68). Cbl was also shown to recruit the SH2 domain-containing adapter protein CrkII to lipid raft microdomains along with the guanine nucleotide exchange factor C3G, where this latter specifically activates a small GTP-binding protein of the Rho family, TC10 (69). Recently, a TC10-interacting protein has been identified as a downstream effector of TC10 upon insulin stimulation. CIP4/2 (Cdc42-interacting protein 4/2) translocates from an intracellular compartment to the plasma membrane upon insulin stimulation and interacts with TC10 in a GTP-dependent manner (73). This protein might function as an adapter to recruit different additional proteins to the plasma membrane in response to insulin in the process leading to GLUT4 translocation and glucose uptake. Additionally, it was proposed that N-WASP (neural Wiskott-Aldrich syndrome protein) functions downstream of TC10 in response to insulin to mobilize cortical F-actin, which in turn promotes GLUT4 responsiveness to insulin in 3T3-L1 adipocytes (74). TC10 could also function in adipocytes as an important regulator of perinuclear actin polymerization that is necessary for efficient membrane protein trafficking (75). Our knowledge of the Cbl-CAP-TC10 pathway involved in insulin stimulated GLUT4 translocation and glucose transport is derived from experiments performed in cultured adipocytes. Although TC10 is also expressed in skeletal muscle (76), further investigations are necessary to determine whether or not this pathway is operative in mature skeletal muscle.

Heterotrimeric G protein alpha subunits, such as Galphaq and Galphai2, also appear to play a role in the control of glucose transport by insulin. The involvement of Galphaq has been evidenced by the observation that adenoviral overexpression of a constitutively active form (Q209L-Galphaq) stimulates glucose transport and GLUT4 translocation in 3T3-L1 adipocytes, thus mimicking the effect of insulin (77,78). Reciprocally, microinjection of anti-Galphaq/11 antibody blocked insulin-stimulated GLUT4 translocation (77,78). While one study indicates that Q209L-Galphaq stimulates GLUT4 translocation in a PI 3-kinase-dependent fashion as shown by its sensitivity to wortmannin (77), another group reported that both wortmannin or the expression of a dominant-interfering p85 regulatory subunit were without effect on Q209L-Galphaq-induced GLUT4 translocation in cultured adipocytes (78). In addition, expression of a constitutively activated, GTPase-deficient mutant form of Galphai2 in transgenic mice increased GLUT4 localization at the plasma membrane in skeletal muscle and adipose tissue in the absence of insulin (79).

4.3. Signaling pathways involved in GLUT4 targeting and insertion at the cell surface

The mobilization of GLUT4 vesicles to the plasma membrane in muscle and adipose cells involves the interaction between proteins present both on vesicles (v-SNAREs) and target membranes (t-SNAREs), thereby promoting the fusion of the two membranes. It has been shown that synaptobrevin/VAMPs (vesicle-associated membrane protein) proteins were located in GLUT4-containing vesicles in rat adipocytes and translocated to the plasma membrane in response to insulin. The v-SNARE proteins, VAMP2 and VAMP3/cellubrevin have been identified as part of the GLUT4-containing vesicles in muscle and fat cells (reviewed in (35,80)). The v-SNAREs have been shown to form a complex with the t-SNAREs syntaxin 4 and SNAP-23 which are enriched at the cell surface membrane of muscle and fat cells (35). Recent studies indicate that only VAMP2 is detected in cortical actin mesh following insulin stimulation in muscle cells, whereas VAMP3/cellubrevin does not seem to be involved in insulin-stimulated translocation of GLUT4 in skeletal muscle (81,82). Some factors have been proposed to specifically couple insulin signaling to SNARE complex formation in the movement of GLUT4 vesicles. A putative mechanism linking insulin signaling to membranes fusion is through the interaction of atypical PKC-zeta with VAMP2. Indeed, overexpression of this PKC isoform in skeletal muscle cells induced serine phosphorylation of the GLUT4 compartment-associated VAMP2 and this was found to elevate glucose uptake (83). Insulin also modulates the formation of SNARE complexes by reducing the binding of pantophysin and VAP33 (VAMP-associated protein of 33 kDa) to VAMP2 thus favoring VAMP2 interaction with syntaxin 4 (84,85). Insulin also increases VAMP2 binding to syntaxin 4 by decreasing the association of the protein Synip (syntaxin 4-interacting protein) to syntaxin 4 (86). However, the signaling components that integrate these effects of insulin remain unknown.

4.4. Signaling pathways involved in GLUT4 endocytosis and inter-endosomal trafficking

Aside from its role in GLUT4 exocytosis, the insulin signaling cascade was also shown to acutely participate in other aspects of GLUT4 movement, namely its endocytosis and inter-endosomal traffic. Dynamin, a key protein involved in GLUT4 endocytosis has been shown to be tyrosine phosphorylated in response to insulin (87,88). It was further shown to interact with Grb2 allowing the association with tyrosine-phosphorylated Shc and IRS-1 (88,89). The formation of such complex results in the modulation of dynamin GTPase activity and, as a consequence, the formation of endocytotic coated vesicles. The important contribution of dynamin in GLUT4 endocytosis was evidenced by the observation that expression of a dominant-negative form of dynamin inhibits GLUT4 endocytosis without affecting insulin-induced GLUT4 arrival at the plasma membrane resulting in the retention of the glucose transporter at the cell surface (90). More recently, insulin was shown to affect the interaction of Rab5, a GTP-binding protein, and the motor protein dynein in relationship with GLUT4 endocytosis

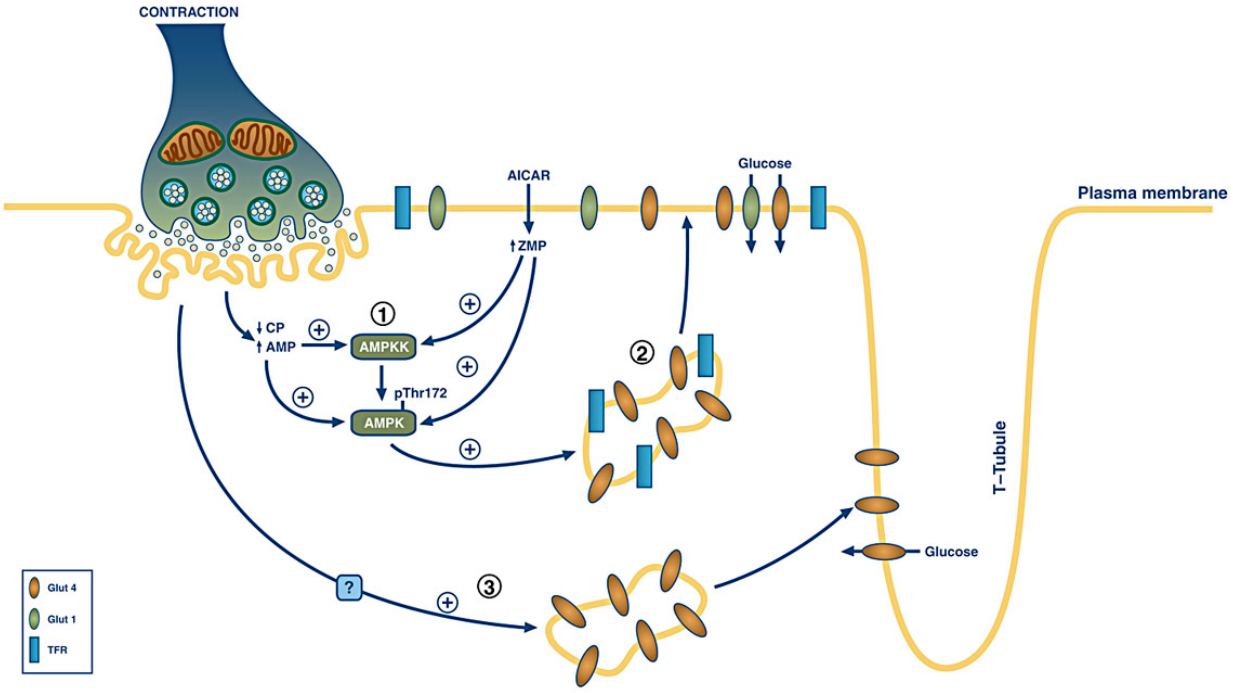

Figure 2. Signaling pathways involved in contraction-induced GLUT4 translocation. 1) Muscle contraction reduces creatine phosphate and increases AMP levels, leading to the activation of AMPK through allosteric activation. Furthermore, AMPK is covalently activated by AMPK kinase via phosphorylation on Thr172 of the alpha-subunit. AMPK kinase, like AMPK, is also allosterically activated by AMP. Similarly, treatment of muscle with AICAR activates both kinases via an elevation in the level of intracellular ZMP. 2) Activation of AMPK by either contraction or AICAR lead to the translocation of GLUT4 to the plasma membrane, but not to the T-tubules. 3) Contraction, but not AICAR treatment, further promotes GLUT4 translocation to the T-tubules by an unknown signaling mechanism.

(91). Indeed, it was shown that insulin inhibited Rab5 signaling and decreased the binding of dynein to microtubules, thereby inhibiting GLUT4 endocytosis (90). Furthermore, microinjection of anti-Rab5 or anti-dynein antibody prevented GLUT4 internalization without affecting its rate of exocytosis (90).

Studies performed in L6 skeletal muscle cells expressing GLUT4myc, indicate that insulin greatly influences the transporter inter-endosomal traffic. Indeed, insulin accelerates the transit of the internalized GLUT4myc from the early endosomes to the recycling endosomes, but also out of the recycling endosomes (47). This accelerated passage of GLUT4myc to and from the recycling endosomes was blocked by the PI 3-kinase inhibitor LY294002 and by expression of a dominant-negative form of the p85 subunit of PI 3-kinase or a kinase-dead and phosphorylation-deficient mutant form of Akt (47). The functional consequence of this GLUT4 recycling scheme in muscle cells awaits further investigation.

5. CONTRACTION-INDUCED SIGNALING AND GLUT4 TRAFFIC

The stimulatory effects of moderate exercise on muscle glucose disposal (92,93) and GLUT4 translocation (94) are preserved in insulin-resistant type 2 diabetic subjects. It is therefore critical to delineate the proximal signaling mechanisms leading to contraction-induced muscle glucose uptake. Classic candidates such as Ca^{2+}, conventional and novel PKC isoforms, and adenosine are still being debated, but new pathways are also emerging including 5'-AMP-activated protein kinase (AMPK), nitric oxide (NO), and mitogen-activated protein kinases (MAPK). There is evidence for and against each of the above candidates and an in dept discussion can be found in two recent reviews on the subject (see (95,96)). In this review article, discussion will be limited to the recently established role of AMPK and its potential downstream targets in contraction-induced glucose uptake (see Figure 2).

AMPK is a critical signaling molecule for the regulation of multiple metabolic processes and growth in contracting skeletal muscle (97,98). AMPK is a member of a metabolite-sensing protein kinase family that acts as a fuel gauge monitoring cellular energy levels (99). When AMPK "senses" decreased energy storage, it acts to switch off ATP-consuming pathways and switch on alternative pathways for ATP regeneration. AMPK is active as a heterotrimer consisting of one catalytic subunit (alpha) and two noncatalytic subunits (beta, gamma) (99,100). The noncatalytic subunits are essential for optimum enzyme activity and may participate in substrate targeting. Several isoforms for each subunit have been identified, and the

contribution of each isoform to the AMPK heterotrimer varies in different tissues. Exercise (101,102) and electrically-stimulated contraction (103-105) increase the activity of AMPK in muscle. Moreover, several studies have shown that the cell-permeable AMPK activator AICAR (5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside) stimulates glucose uptake in fast-twitch muscles and that its effect is not additive to that of contraction (103,106-108). The role of AMPK in contraction-induced glucose uptake was further demonstrated by the observation that expression of a dominant inhibitory mutant of AMPK in transgenic mouse muscle reduced contraction-induced glucose uptake by 30-40% whereas it fully abrogated the stimulatory effect of AICAR on glucose transport (109). This study not only supports a role for AMPK in contraction-induced glucose uptake but also indicates that other AMPK-independent mechanism(s) are likely to explain the full effect of contraction on glucose uptake.

AICAR has been previously reported to increase GLUT4 translocation to the plasma membrane in hindlimb muscles (110). However, it was still unknown if AICAR also triggers the recruitment of GLUT4 to the T-tubules, which represent the bulk of the total cell surface area in skeletal muscle fibers (19,20). We have recently shown that in fact, AICAR failed to induce GLUT4 translocation to T-tubules (111). Since exercise/contraction stimulates GLUT4 translocation to both the plasma membrane and the T-tubules (14,15), our data suggest that contraction induces GLUT4 translocation to the tubular elements throught an AMPK-independent mechanism, which is consistent with the finding that contraction-induced glucose uptake is only partially inhibited in muscle expressing a dominant-negative AMPK (109). The fact that AICAR selectively activated GLUT4 recruitment to the plasma membrane suggests that the transporters were mobilized from the contraction-responsive TfR-enriched endosomal compartment since this pool is only recruited to the plasma membrane in contracting muscle (45). This is supported by the observations that 1) AICAR also induced TfR protein translocation to the plasma membrane but not to the T-tubules, and 2) the AMPK activator increased recycling of the TfR, as revealed by enhanced [^{125}I]-transferrin uptake in isolated muscles (111). An important goal of future studies will be to clarify the relative contribution of this AMPK-dependent mobilization of GLUT4-containing recycling endosomes to the full stimulation of glucose transport by contraction.

It will be important to identify the downstream targets of AMPK and their role in mediating AMPK-induced GLUT4 translocation and glucose transport. Among the known targets of AMPK, one has been recently suggested to be linked with glucose transport stimulation in muscle: the nitric oxide synthase (NOS). Indeed, recent studies have shown that AMPK can phosphorylate key serine residues in both the endothelial (eNOS) and neuronal (nNOSμ) forms of the NOS family, leading to increased NOS activity in muscle (112,113). This led to the hypothesis that NO production following AMPK activation could mediate contraction-induced glucose uptake. This hypothesis was linked to previous observations that NO donors increase muscle glucose uptake (114-117). It was reported that NOS activity in a muscle cell line (H-2Kb) was activated after stimulation of AMPK by AICAR and that NOS inhibition blocked AMPK-induced glucose transport (118). They further showed that inhibition of guanylate cyclase (a known mediator of NO action) also abrogates activation of glucose transport by AICAR. However, other groups have failed to establish a role for NO and cGMP in the stimulation of glucose uptake by contraction. Thus, various NOS inhibitors failed to inhibit contraction-induced glucose uptake (115,116) and cGMP levels did not raise in contracting muscle (115). Furthermore, we recently observed that AICAR-induced glucose uptake is not affected by the NOS inhibitor L-NAME (N-nitro-L-arginine methyl ester) and that AICAR stimulates glucose uptake even in cultured L6 myocytes despite the lack of expression of either eNOS or nNOSμ in these cells (Lemieux, Pilon and Marette, unpublished data). These studies do not support a role for NO as a direct mediator of contraction or AMPK-dependent glucose transport in muscle.

6. GLUT4 ACTIVATION BY INSULIN AND CONTRACTION

In recent years, it has become evident that the full effect of insulin to increase glucose transport could not be entirely explained by its ability to trigger GLUT4 translocation from storage sites to the cell surface. Several studies have shown a discrepancy between the extent of GLUT4 translocation and the stimulation of glucose uptake in response to insulin (5,8,119), contraction (8,15), or the combination of insulin plus contraction (45) in skeletal muscle. However, the existence of a GLUT4 activation component to the effect of insulin and/or contraction on glucose transport was questioned for several years because the determination of GLUT4 translocation relied on techniques that could not accurately quantified the number of cell surface GLUT4 transporters in living cells. The use of a cell-impermeant photolabels (e.g. ATB-BMPA [2-N-4-(1-azi2,2,2-trifluoroethyl)-benzoyl-1,3-bis-(D-mannose-4-yloxy)-2-propylamine]) coupled to selective GLUT4 immunoprecipitation is not giving an accurate determination of GLUT4 number at the cell surface since it reacts with the exofacial glucose binding site (120) and, therefore, gives an estimate of the amount of activated transporters rather than total transporters at the cell surface. To circumvent this limitation, an L6 muscle cell line (L6 GLUT4myc) expressing GLUT4 harboring a myc epitope in the first exofacial loop of the transporter was developed (121). It is therefore now possible to accurately determine GLUT4 at the cell surface of intact myocytes by immunofluorescent or immunochemical labeling of the myc epitope (122,123). Since the behaviour of GLUT4myc (e.g. transport capacity, subcellular traffic) is virtually identical to that of endogenous GLUT4 (124), this now allow to directly compare the effects of hormones/agents on GLUT4 translocation vs their effects on glucose transport and thus make predictions about the modulation of GLUT4 intrinsic activity by these hormones/agents. Using this L6 GLUT4myc muscle cell line, it was possible to demonstrate

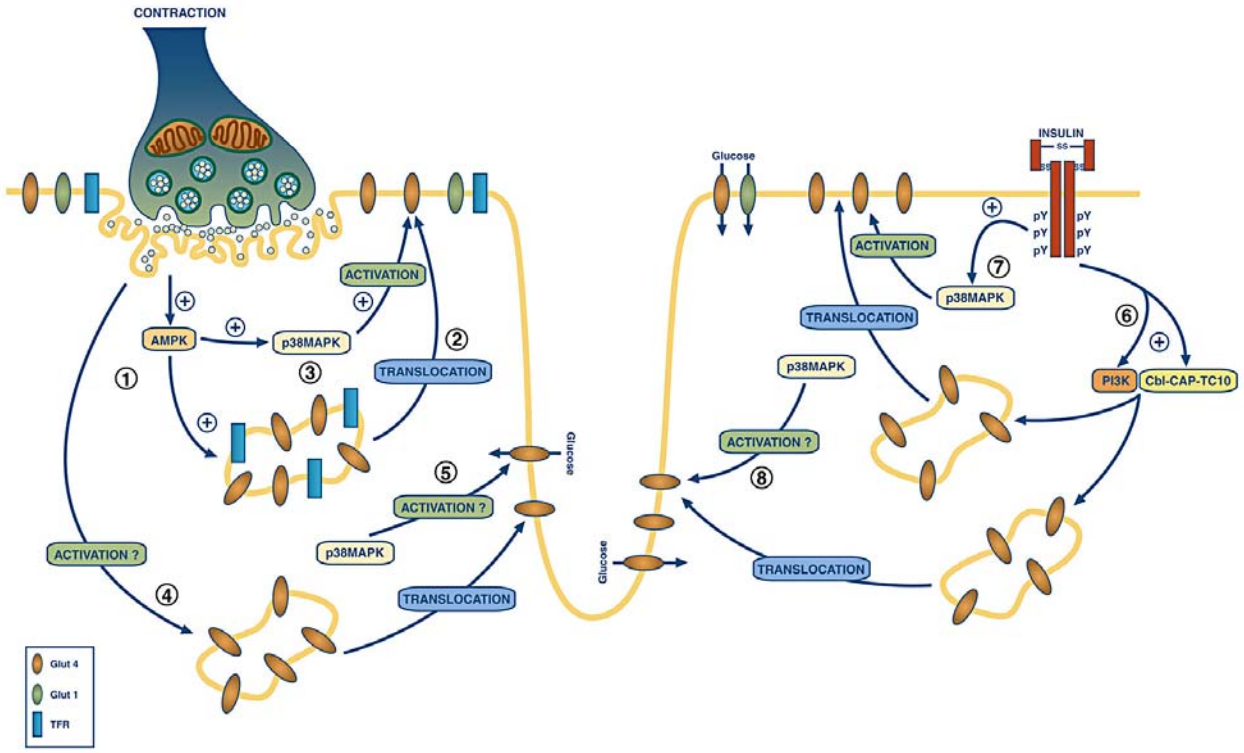

Figure 3. Integration of signaling pathways involved in GLUT4 translocation and activation. 1-2) Contraction activates AMPK leading to the the translocation of GLUT4 to the plasma membrane. 3) Contraction-mediated activation of AMPK is involved in the stimulation of p38 MAPK leading to the activation of cell surface glucose transporters. 4) Contraction also activates an AMPK-independent pathway that leads to the translocation of GLUT4 to the T-tubules. 5) The p38 MAPK might also participate in the activation of the translocated GLUT4 in the T-tubules. 6) Insulin receptor-mediated activation of PI 3-kinase and the Cbl-CAP-TC10 pathway is responsible for the translocation of GLUT4 to both the plasma membrane and the T-tubules. 7-8) The insulin receptor also activates p38 MAPK which is responsible for the activation of cell surface GLUT4 in the plasma membrane and the T-tubules.

that GLUT4myc translocation actually precedes the stimulation of glucose transport by insulin (125). Furthermore, pretreatment of cells with SB203580, an inhibitor of p38 mitogen-activated protein kinase (p38 MAPK), caused a reduction in insulin-dependent glucose uptake without preventing GLUT4 translocation in muscle and adipose cells in culture (122).

Using several different in vivo and in vitro assays to monitor enzyme phosphorylation and activity, we recently reported that insulin, both in vivo and in vitro, activates p38 MAPK alpha and p38 MAPK beta in various rat skeletal muscles (126). Electrically-stimulated contraction of the isolated extensor digitorum longus (EDL) muscle also increased the activity of both p38 MAPK isoforms (126). Furthermore, an inhibitor of p38 MAPK, SB203580, was found to inhibit the stimulation of glucose uptake by either insulin or contraction, suggesting that stimulation of p38 MAPK and intrinsic activation of GLUT4 may participate in the stimulation of glucose uptake by both stimuli in rat skeletal muscle (see Figure 3).

More recently, we have also shown that the AMPK activator AICAR increases the activity of both p38 MAPK alpha and beta with a time-course identical to that of stimulation of AMPK and glucose transport (111). The p38 MAPK inhibitor SB203580 abrogated the stimulatory effect of AICAR on glucose uptake suggesting that p38 MAPK acts downstream of AMPK to stimulate glucose uptake. These results indicate that AICAR increases muscle glucose uptake not only by mobilizing GLUT4 to the plasma membrane surface domain but also by activating p38 MAPK, which may be involved in the activation of cell surface GLUT4. One obvious methodological limitation to the demonstration of the concept of GLUT4 intrinsic activation by p38 MAPK in mature skeletal muscle is the lack of experimental models in which GLUT4 transporter levels at the cell surface can be accurately determined. Indeed, it is presently difficult to directly assess whether activation of p38 MAPK by insulin, contraction or AICAR increases GLUT4 catalytic activity since current methods to assess cell surface GLUT4 translocation in muscle cannot distinguish between 1) transporters which are fully inserted into the plasma membrane and T-tubules and those which are docked but not fused, or 2) cell surface transporters that are activated or not by these stimuli.

The above studies have rejuvenated the concept of intrinsic activation of cell surface GLUT4 transporters.

Accordingly, the possibility that a defective p38 MAPK-dependent activation of GLUT4 may contribute to insulin resistance has recently attracted a lot of attention. So far only a few studies in cultured cells have been published but they do suggest that insulin resistance induced either by chronic insulin treatment or by high leptin concentrations are linked to impaired activation of p38 MAPK (127,128) and reduced GLUT4 intrinsic activity (128). Conversely, it was recently reported that treatment of L6 GLUT4myc cells with combined high glucose and high insulin increases p38 MAPK and GLUT4 activity in the basal state presumably to compensate for the lack of insulin-induced GLUT4 translocation observed in these cells (129). However, there are yet no studies where the action of insulin on p38 MAPK and the predicted GLUT4 intrinsic activity have been evaluated in skeletal muscle of animal models of insulin resistance or diabetic subjects.

7. CONCLUDING REMARKS

Studies in the last decade have revealed the complex orchestration of signaling mechanisms leading to glucose transport stimulation by insulin and contraction. The understanding of intracellular GLUT4 traffic and the unique nature of this highly regulated vesicle transport mechanism allowing skeletal muscle to increase its uptake of glucose for either storage or to fulfill cellular energy demand during contraction has greatly progressed over the last few years. More recent work further suggests that insulin and contraction also stimulate glucose transport through modulation of GLUT4 intrinsic activity. The challenge for us in the future will be to better define the intersection points where signaling components converge to allow fine tuning of GLUT4 traffic and function in insulin-stimulated and contracting muscle.

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