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GLUT4's itinerary in health & disease

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Following the discovery of insulin 85 yr ago and the realization thereafter that in some individuals, tissues lose their responsiveness to this hormone, an enormous world-wide effort began to dissect the cellular mechanisms of insulin action and define abnormalities in the insulin-resistant state. A clear goal through the years has been to unravel the insulin signal transduction network regulating glucose transport. This line of investigation has provided tremendous insight into the physiology and pathophysiology surrounding the cellular processes controlled by insulin. Between the plasma membrane insulin receptor and the intracellularly sequestered insulin-responsive glucose transporter GLUT4, many events participate in the transduction of the insulin signal. In this review, we detail our current state of knowledge on the intricate insulin signaling network responsible for glucose transport in peripheral adipose and skeletal muscle tissues. In particular, we identify signaling connections spanning the insulin receptor and GLUT4. In addition, we discuss cytoskeletal mechanics and membrane docking and fusion mechanisms pertinently involved in the cellular redistribution of GLUT4 to the plasma membrane. On the whole, this review highlights the considerable progress in our understanding of insulin signaling in health and disease as we rapidly approach the centennial anniversary of insulin's discovery.

Key words Diabetes - glucose transporter 4 (GLUT4) - insulin - insulin resistance - signal transduction

Following nutrient ingestion, plasma glucose triggers pancreatic β-cells to release insulin into the blood. This potent anabolic hormone regulates numerous post-prandial events, among which, controlling the cellular localization of the glucose transporter GLUT4 in muscle and fat is vital in the management of blood glucose homeostasis. Normally, in the basal state this transporter resides in an intracellular membrane compartment, and upon insulin stimulation, it rapidly populates the plasma membrane. Arrival of GLUT4 at the plasma membrane permits cellular glucose influx. In individuals with insulin resistance, this metabolic process is impaired as peripheral adipose and muscle tissues fail to respond to physiological levels of insulin. Initially, pancreatic β-cells compensate for the resistance by releasing elevated levels of insulin into the blood (hyperinsulinaemia). Over time, β-cell

function diminishes and blood glucose levels become unchecked.

Insulin resistance is the central metabolic defect in type 2 diabetes, a disease reaching epidemic proportions worldwide. This multi-organ and multi-system disease devastates its patients with increased risks of cardiovascular complications, blindness, neuropathy, renal failure, and countless other morbidities. On a molecular level, insulin resistance is associated with insufficient recruitment of GLUT4 to the plasma membrane despite normal GLUT4 protein expression[1]. This finding emphasizes the importance of understanding the molecular mechanisms of insulin-regulated GLUT4 translocation and glucose uptake so that cellular perturbations associated with insulin resistance can be elucidated and effective treatment and prevention strategies can be designed to bypass or ameliorate insulin resistance. Although details linking insulin to GLUT4 translocation have yet to be fully resolved, substantial progress has been made within the last two decades to delineate the molecular events.

In this review, we discuss current knowledge and recent advancements underpinning the insulin-regulated glucose transport system. We begin by highlighting the characteristics of the GLUT4 protein and its carrier vesicles followed by the progress in research on insulin signaling systems regulating GLUT4-containing vesicle trafficking and fusion with the plasma membrane. The review will conclude with current perspectives on the molecular events that may contribute to the insulin resistant state of obesity and type 2 diabetes.

GLUT4 protein and its carrier vesicles

GLUT4 is a facilitative glucose transporter, expressed solely in adipose and muscle tissues, that has 12 membrane-spanning regions with amino- and carboxyl-termini located intracellularly[2]. In the basal state, GLUT4 cycles continuously between the plasma membrane and one or more intracellular compartments, with 90-95 per cent of the transporter residing within the cell interior, tightly packaged into vesicles[3,4]. Activation of the insulin receptor triggers a large increase in the rate of GLUT4 vesicle exocytosis and a smaller but important decrease in the rate of internalization by endocytosis[5,6]. The overall insulin-dependent shift in the cellular dynamics of GLUT4 vesicle trafficking results in a >10-fold increase of GLUT4 protein at the cell surface[7]. Details regarding GLUT4 vesicle formation and cycling have recently been reviewed[8]. Here, we highlight important features.

GLUT4 is found within large tubulo-vesicular structures in the perinuclear region of the cell and within small vesicles dispersed throughout the cytoplasm, also known as GLUT4 storage vesicles (GSVs). Perinuclear GLUT4 is likely localized in endosomes and trans-Golgi network (TGN) structures, as evidence has shown that it co-localizes with endosomal and TGN markers, including transferrin receptor (TfR) and syntaxin 16, respectively[9]. GSVs on the other hand co-localize with insulin-responsive aminopeptidase (IRAP) and vesicle-associated membrane protein 2 (VAMP2). Upon insulin stimulation, it appears that GSVs are mobilized to the cell surface, as there is an increase of GLUT4 at the plasma membrane that is proportional to the reduction in GLUT4-containing vesicles from the cytosolic compartment[10,11], while the level of perinuclear GLUT4-containing vesicles remains relatively unaffected[10,12]. Furthermore, total internal reflection fluorescence microscopy has revealed that in basal adipocytes, GLUT4-containing vesicles are located near the plasma membrane and are recruited to the cell surface with insulin stimulation[13].

Initial formation of GSVs is thought to involve the Golgi-localized γ-ear-containing Arf-binding protein (GGA) and sortillin, a protein located predominantly in the TGN. Presumably, sortillin interacts simultaneously with endosomal GLUT4 and GGA, pulling the transporter into the GGA-dependent budding machinery thought to be necessary for GSV formation[11,14]. Dominant negative

GGA inhibits GSV formation and subsequently blocks insulin-stimulated glucose uptake in adipocytes[15]. Layered over the complexity of defining the intracellular GLUT4 compartments exists many processes and events regulating their trafficking itinerary, which is the focus of the subsequent sections.

Insulin signaling systems regulating GLUT4

Activation of the insulin receptor triggers a cascade of phosphorylation events that ultimately promote GLUT4 vesicle exocytosis (Fig.). The classical insulin signaling pathway involves docking of the insulin receptor substrate (IRS) to the insulin receptor (IR), activation of phosphatidylinositol 3-kinase (PI3K) which leads to formation of plasma membrane phosphatidylinositol 3,4,5-trisphosphate (PI 3,4,5-P_3), subsequent PI 3,4,5-P_3-mediated activation of Akt and atypical protein kinase C (aPKC), and phosphorylation of AS160 (Akt substrate of 160 kDa) by Akt (Fig. A). Additionally, a PI3K-independent pathway involving c-Cbl, c-Cbl associated protein (CAP), and the GTPase TC10 may also regulate GLUT4 translocation (Fig. B), although this pathway appears to be exclusive to adipocytes. Here, we detail steps within both insulin signaling pathways.

IR and its substrates: Insulin action is initiated when this peptide hormone binds to its receptor. The insulin receptor is composed of two disulphide-linked heterodimers, each of which has an α and β subunit. Insulin binding to the two high-affinity extracellular a subunits leads to activation of intrinsic tyrosine kinase activity of the transmembrane β subunits and autophosphorylation of specific tyrosine residues[1,16]. Phosphorylation enhances tyrosine kinase activity of the β subunits towards a host of proteins including members of the insulin receptor substrate family (IRS-1, -2, -3, -4, -5, and -6), Gab-1, Shc, p62^{dok}, Cbl, SIRP (signal regulatory protein) family members, and APS [adapter protein containing a pleckstrin homology (PH) and Src-homology 2 (SH2) domain][17-23].

The metabolic actions of insulin in adipose and skeletal muscle tissue rely on tyrosine phosphorylation of IRS proteins. Of the six IRS proteins that have been identified to date, only IRS-1 and IRS-2 are firmly associated with glucose homeostasis in these tissues[23,24]. IRS-1-knockout mice display mild peripheral insulin resistance[25,26]. Furthermore, reducing IRS-1 via siRNA in cultured L6 myotubes significantly diminishes insulin-stimulated GLUT4 translocation and glucose uptake[24]. Ablation of IRS-2 in cultured myotubes is without effect on these parameters[24]; yet, IRS-2-knockout mice develop frank diabetes concomitant with liver insulin resistance and decreased pancreatic beta cell mass[27]. It thus appears that IRS-1 is critical in peripheral adipose and skeletal muscle insulin action while IRS-2 functions predominantly in the liver and in pancreatic β-cells. IRS-3-knockout mice are conversely without metabolic abnormalities. Ablation of IRS-4 causes mild defects in growth, reproduction, and glucose homeostasis[28]. However, since IRS-4 protein is not expressed in skeletal muscle[28], the major insulin-sensitive tissue responsible for approximately 80 per cent of whole body glucose uptake[29-31], it is unlikely that this isoform has a significant role in peripheral insulin action. Recently, IRS-5 and IRS-6 were identified within the human genome by sequence alignment[23]. Preliminary studies found that the IRS-5 gene is expressed in liver and kidney tissues and the IRS-6 gene is predominantly expressed in skeletal muscle. While both substrates are rapidly phosphorylated by insulin, neither activates the downstream effector molecule PI3K. Thus, further investigations are needed to determine whether these novel IRS proteins play a role in the metabolic actions of insulin.

PI3K-dependent signaling: Insulin-dependent tyrosine phosphorylation of IRS-1/2 creates docking sites for downstream effector molecules including Class IA PI3K[32]. This heterodimeric enzyme is composed of a p85 regulatory subunit and a p110 catalytic subunit. The p85 subunit binds phosphotryosine residues on IRS through its Src homology 2 (SH2) domain, and this interaction

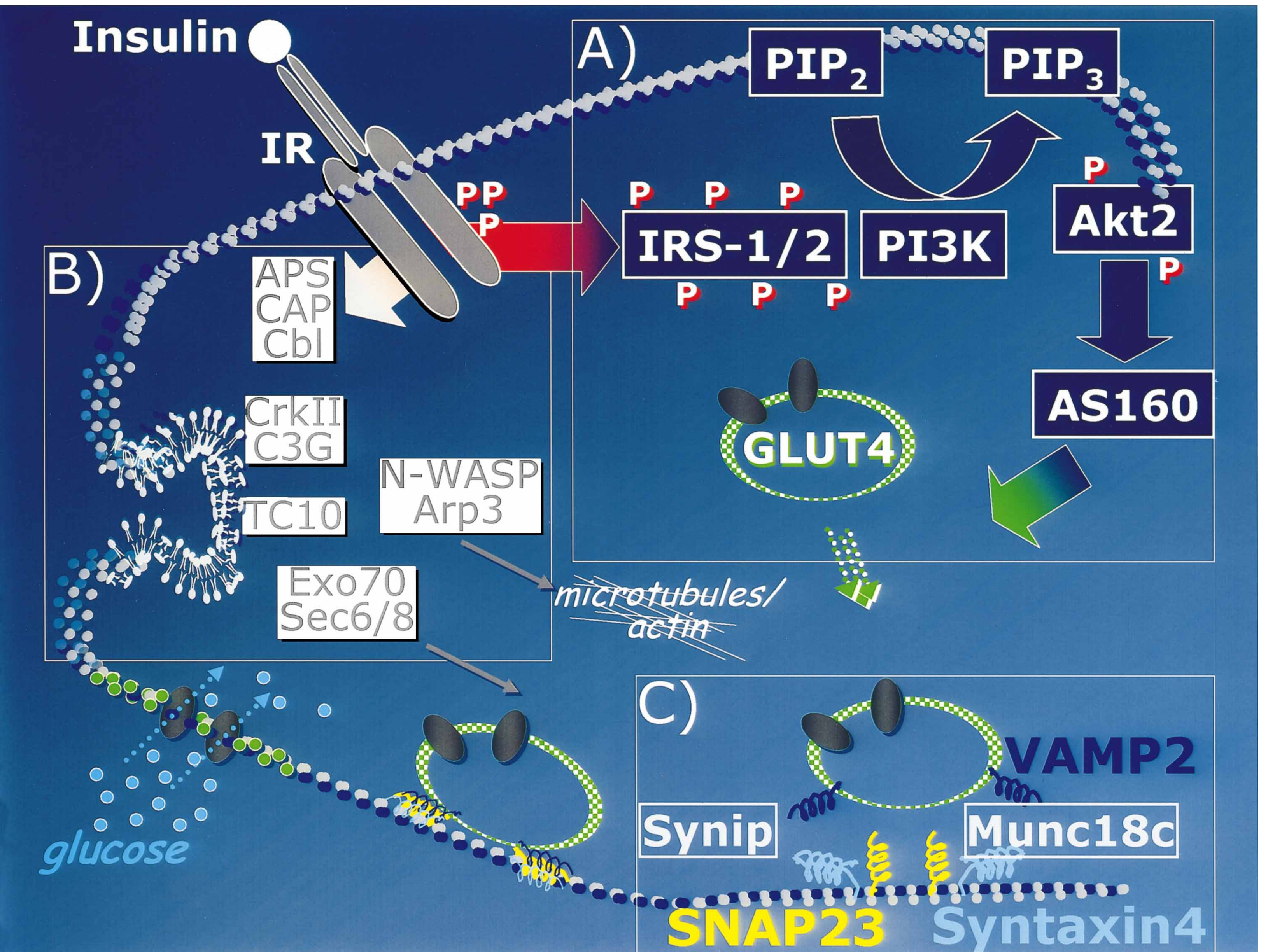

Fig. Cellular processes and insulin signals proposed to control the cellular distribution of GLUT4. **(A)** PI3K/Akt2 signal, **(B)** Cbl/TC10 pathway, and **(C)** SNARE machinery of GLUT4 regulation. See text for details.

activates catalytic activity of p110 and simultaneously localizes the kinase to the plasma membrane, in close proximity to its substrate phosphatidylinositol 4,5-bisphosphate (PI 4,5-P_2). Activated PI3K catalyzes the phosphorylation of PI 4,5-P_2 on the 3 position of the inositol ring, forming PI 3,4,5-P_3. Increased membrane PI 3,4,5-P_3 is essential for insulin-stimulated GLUT4 translocation, as this phospholipid provides docking sites for downstream molecules via their pleckstrin homology (PH) domains. Inhibiting the formation of PI 3,4,5-P_3 with wortmannin or LY29004 effectively blocks insulin-stimulated GLUT4 translocation and glucose transport[33,34]. Phosphatase regulators of PI3K-generated lipids include PTEN and SHIP2. The former catalyzes the dephosphorylation of phosphatidylinositols at the D3 position, while the latter catalyzes dephosphorylation of PI 3,4,5-P_3 to yield PI 3,4-P_2[35].

PI 3,4,5-P_3 formation mediates the plasma membrane translocation of two PH domain-containing proteins crucial for insulin-regulated glucose uptake: Akt (protein kinase B, PKB) and phosphoinositide-dependent-kinase-1 (PDK1)[35]. Following membrane recruitment, the 60 kDa serine/threonine kinase Akt is phosphorylated at two key sites, threonine 308 (Thr308) and serine 473 (Ser473), resulting in enzyme activation. Ser473 phosphorylation occurs first and is accomplished by the once elusive PDK2, which was recently identified as the protein kinase mTOR (mammalian target of rapamycin) complexed to the regulatory protein rictor[36-38]. This phosphorylation event appears to promote interaction between the C-terminal hydrophobic motif (HM) of Akt and the serine/threonine kinase PDK1, thus stabilizing and activating PDK1 to phosphorylate Akt on Thr308[39,40]. This second and final phosphorylation step increases the affinity of the Akt HM for itself, causing dissociation of the active Akt from PDK1[35].

There are three existing Akt isoforms (Akt1-3)[41], and studies have identified Akt2 as the relevant isoform in insulin-stimulated GLUT4 translocation. Akt2-knockout mice exhibit a diabetes-like phenotype[42], while mice devoid of Akt1 are small in size with normal glucose handling capabilities[43,44]. Furthermore, primary adipocytes lacking Akt2 display significantly impaired glucose uptake[41], whereas loss of Akt1 does not produce this defect. Intracellular localization of Akt1 and Akt2 lend further support towards Akt2 as the relevant metabolic isoform. Adipocyte model systems have demonstrated that Akt1 exists primarily in the cytosol while Akt2 is localized to both cytosolic and membrane components[45]. This finding led to the discovery that Akt2 is tightly associated with GLUT4-containing vesicles in the basal state and that insulin increases Akt2-GLUT4 vesicle association while simultaneously activating Akt2 to phosphorylate numerous GSV component proteins[45,46]. It appears that GLUT4-containing vesicle exocytosis is dependent on Akt2[47], thus this kinase may serve as a link between the insulin signaling cascade and GLUT4 fusion events.

With the discovery of AS160 (Akt substrate of 160 kDa) in 2002 by Kane *et al*[48], knowledge of the PI3K-dependent insulin signaling pathway became more complete. This group utilized an antibody that recognizes the Akt phosphomotif RXRXXpS/T to identify and help characterize AS160. From that time, AS160 protein expression has been confirmed in cultured adipocytes, rat and human skeletal muscle[48-51], and L6 myotubes[52]. Also, a number of key properties of the protein have since been elucidated. First, AS160 contains a GTPase-activating domain (GAP) for Rabs, small G proteins involved in vesicle trafficking[49,53]. 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[9,54]. In all cargo vesicle trafficking systems examined to date, small GTP-binding proteins are necessary effectors in trafficking, docking and fusion steps[55]. Thus, through Rab function, AS160 may represent a convergence between insulin signaling and vesicle trafficking. Second, two separate sets of studies have

identified AS160 as a negative regulator of basal GLUT4 exocytosis[9,56]. AS160 knockdown in adipocytes causes increased basal plasma membrane GLUT4 via increased exocytosis, a phenotype rescued by transiently transfecting human AS160. This phenotype, however, is not rescued when AS160 mutated in the GAP domain is transfected[55]. 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[9,56-57].

Along with recruiting and activating Akt, PI 3,4,5-P_3 formation in concert with PDK1 also leads to the activation of atypical protein kinase C (aPKC), and both aPKC isoforms ζ and λ have been implicated in GLUT4 translocation. As recently reviewed[58], aPKC knockout models and expression of kinase-inactive PKC-ζ/λ inhibits GLUT4 translocation and glucose uptake in a variety of cell types, and this phenotype can be reversed in PKC-$\lambda^{-/-}$ cells by expressing wild-type aPKC. Furthermore, expression of constitutively active aPKC recapitulates the effects of insulin on GLUT4 translocation and glucose transport. As our understanding of Akt2/AS160 expands, investigations into the aPKC branch of signaling have remained sparse. Further studies are needed to elucidate possible downstream effector molecules of aPKC necessary for insulin action.

PI3K-independent signaling: It is well established that activation of GLUT4 translocation by insulin requires a PI3K signal involving the upstream IR and IRS activators and the downstream Akt and PKC target enzymes and AS160 protein as presented above (Fig.A). Some studies over the past decade have also suggested that a second pathway (Fig.B) occurs as a consequence of Cbl tyrosine phosphorylation[59,60]. Cbl and the adaptor protein CAP are recruited to the insulin receptor by APS[61]. Once tyrosine phosphorylated by the receptor, Cbl can recruit the adaptor protein CrkII to lipid rafts, along with the guanyl nucleotide exchange factor C3G[62]. C3G can then activate the GTP-binding protein TC10, which resides in lipid rafts[63]. 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[64]. However, the involvement of this pathway has been debated by recent RNA interference-based analysis of Cbl, CAP, and CrkII function in regulation of GLUT4 by insulin[65]. Gene silencing of Cbl or CAP or CrkII in 3T3-L1 adipocytes fails to attenuate insulin-stimulated glucose transport or myc-tagged GLUT4-GFP translocation at either sub-maximal or maximal concentrations of insulin. The dose-response relationship for insulin stimulation of glucose transport in primary adipocytes derived from Cbl knockout mice is also identical to insulin action on adipocytes from wild type mice[65]. Furthermore, the likelihood that the Cbl cascade plays an important role in GLUT4 regulation is also dampened by studies in skeletal muscle examining this pathway[66-68].

Nevertheless, investigation suggests a role of TC10 in the regulation of actin dynamics[69-74] and phosphoinositides[75]. In particular, the actin-regulatory neural Wiskott-Aldrich syndrome protein (N-WASP)[71], the actin related protein-3 (Arp3)[71], and the exocyst protein complex[70] appear to be downstream targets of TC10. Whereas N-WASP/Arp3 apparently regulates actin polymerization[71], the exocyst protein complex is postulated to mediate the tethering/docking of GLUT4 vesicles at the plasma membrane[70]. However, the importance of TC10-regulated actin remains unclear as dominant-negative TC10 mutants do not prevent insulin-induced actin remodeling in either myoblasts or myotubes and do not interfere with insulin-mediated recruitment of c-myc epitope-tagged GLUT4 to the cell surface[67]. Certainly, since both cytoskeletal and SNARE

networks participate in GLUT4 regulation, the exact mechanism by which insulin signaling is coupled to cytoskeletal and membrane fusion events is particularly interesting. We address the importance of these networks below.

Regulatory input of membrane and cytoskeletal systems

Strong evidence supports a vital role for the cellular cytoskeleton in insulin-regulated glucose transport. Both microtubules and cortical actin appear essential in the GLUT4 translocation process, and disruption of either structure results in diminished GLUT4 at the plasma membrane. The following two subsections will highlight the basic properties of each protein and discuss investigations concerning the GLUT4-cytoskeletal-membrane relation.

Cortical actin: Cellular cortical actin exists in two forms: monomeric globular actin (G-actin) and filamentous actin (F-actin). Actin monomers are each bound to ATP, and polymerization into filaments requires ATP hydrolysis. Resulting filaments are polar, containing a fast-growing end (the barbed or plus (+) end) where further polymerization typically occurs, and a slower-growing end (the pointed or minus (–) end)[76]. In 3T3-L1 adipocytes and L6 myotubes, insulin has been shown to elicit F-actin formation[77-79]. Actin filaments have been demonstrated to play a critical role in insulin-induced exocytotic recruitment but not in endocytosis of GLUT4 in isolated rat adipocytes[80]. Disrupting the actin network in cultured cells or in intact rat skeletal muscle with the actin-depolymerizing agent cytochalasin D, or the actin monomer binding Red Sea Sponge toxins Latrunculin A or B, inhibits insulin-stimulated GLUT4 translocation and glucose uptake[77,80,81]. In these model systems, pharmacological disruption of actin does not appear to affect proximal insulin signaling, such as the insulin-stimulated activities of PI3K and Akt[72,77,81]. Interestingly, use of the F-actin stabilizing compound jasplakinolide also impairs GLUT4 translocation, suggesting that dynamic reorganization of actin is necessary in the metabolic actions of insulin[72]. Overall, these studies have given rise to two potentially overlapping models hypothesizing the role of actin in glucose uptake. The first proposes that insulin causes cortical actin remodeling, such that incoming vesicles can travel through the peripheral actin mesh to fuse with the plasma membrane. The second suggests that actin filaments function as "highways," upon which vesicles travel to reach the plasma membrane[71]. Regardless of the exact actin function, it is apparent that insulin signaling to rearrange cortical actin represents a required pathway for optimal movement or fusion of GLUT4-containing vesicles and plasma membranes.

Regulation of F-actin is accomplished by actin-binding proteins and membrane phospholipids. As mentioned in the previous subsection (PI3K-independent signaling), insulin activation of TC10 appears to be an important component of F-actin formation in adipocytes[67,71,74,82,83], but may not operate in myocytes[67]. Studies have proposed that the ubiquitously expressed N-WASP is a downstream effector of TC10, and N-WASP subsequently activates Arp2/3 to polymerize actin[71]. Additionally, membrane phosphoinositides, especially PI 4,5-P_2, are also involved in modulating the actin cytoskeleton. PI 4,5-P_2 is synthesized predominantly by PI4P 5-kinase and is present at relatively high concentrations in the plasma membrane, representing approximately 1 per cent of total plasma membrane phospholipids[84]. The exact plasma membrane distribution of PI 4,5-P_2 remains controversial. Studies analyzing PI 4,5-P_2 synthesis or visualizing the phospholipid with a specific antibody suggest that it is localized to membrane regions coinciding with lipid rafts[85-87]. Consistent with this observation, Kanzaki and Pessin[73] clearly visualize actin membrane connections in this membrane microdomain. Other investigations visualizing PI 4,5-P_2 via the PH domain of PLCδ, which specifically interacts with PI 4,5-P2, or via electron microscopy have demonstrated homogenous plasma membrane distribution[88,89]. Irrespective of the exact PI 4,5-P_2

address, clear correlations exist between PI 4,5-P_2 and actin organization. Multiple studies have shown that sequestering[90-92] or hydrolyzing[93,94] PI 4,5-P_2 reduces plasma membrane-cytoskeletal interactions, which in turn depletes cortical F-actin. Furthermore, reconstitutions with endogenous vesicles and exogenous artificial vesicles containing PI 4,5-P_2 result in nucleation of actin comet-tails and vesicle movement[95,96].

Microtubules: New perspective on the role of microtubules and motor proteins in GLUT4-containing vesicle motility has been provided by several recent studies[14,97-100]. Although some controversy existed as to whether the microtubule network is involved in GLUT4 regulation[97,101-104], recent live cell imaging analyses seem to convincingly show insulin-regulated GLUT4-containing vesicle long-range movement on microtubules[100,105]. Consistent with this, microtubule minus-end motor dynein or plus-end motor kinesins (KIF3 and KIF5b), have been found to regulate GLUT4 vesicle endocytosis and exocytosis, respectively[98-100]. Also highlighted in these motor protein studies is that both GLUT4 exocytosis and GLUT4 endocytosis are regulated by the microtubule network[100].

SNAREing GLUT4

Following the insulin-mediated arrival of GLUT4-containing vesicles from intracellular storage sites to the plasma membrane, regulated fusion of these vesicles ensues. Exocytosis of GLUT4-containing vesicles is mediated by interactions between specific vesicular and plasma membrane protein complexes known as SNAREs (Fig. C). Vesicle SNAREs (v-SNARES, vesicle soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors) bind target membrane SNAREs (t-SNAREs) in company with numerous accessory proteins. Syntaxin4 and SNAP23 (23 kDa synaptosomal-associated protein) are the t-SNARES and VAMP2 is the v-SNARE involved in GLUT4 vesicle fusion[106]. While SNAREs are essential in GLUT4 exocytosis, they themselves do not appear to be direct targets of insulin action. Rather, studies suggest that the accessory proteins Munc18 and Synip may be regulated by insulin to accomplish fusion events. Three Munc18 isoforms (Munc18a-c) have been identified in mammalian cells: Munc18a is a neuronal isoform and Munc18b and Munc18c are expressed in muscle and fat. Munc18c interacts directly with syntaxin4 suggesting that this is the relevant isoform in insulin-regulated GLUT4 translocation[107,108]. Further studies have unveiled the functional role of this accessory protein. Munc18c overexpression inhibits insulin-stimulated GLUT4 translocation[109,110], and Munc18c-null mouse adipocytes exhibit enhanced insulin-stimulated GLUT4 externalization[111]. These findings support a model whereby insulin causes Munc18c dissociation from syntaxin4, allowing syntaxin4 to interact with SNAP23 and VAMP2 and thus permitting GLUT4 vesicles to fuse with the plasma membrane. In addition to Munc18c, the accessory protein Synip may also play a role in insulin-stimulated GLUT4 vesicle fusion, although data are conflicting. Synip was first identified by Min and colleagues[112], who determined that this protein dissociates from syntaxin4 in an insulin-dependent manner and is directly involved in GLUT4 translocation. Recently, it was reported that Akt2 phosphorylates Synip on serine 99 and this phosphorylation mediates the Synip-syntaxin4 dissociation necessary for GLUT4 vesicle exocytosis[113]. However, recent studies argue against this possibility and show that a serine-to-alanine Synip mutant (S99A) does not impair GLUT4 translocation[114]. Further studies are needed to clarify this discrepancy.

A variety of other molecules have also been implicated in the fusion process. For example, the enzyme phospholipase D1 (PLD1) has been found on GLUT4 vesicles, and together with its lipid product phosphatidic acid (PA), PLD1 appears to be necessary for GLUT4 vesicle fusion[115]. Enzyme activity is proportional to glucose uptake, and reduced PA production hinders appearance of

GLUT4 at the cell surface. Other studies have utilized a novel cell-free approach to determine that, in addition to SNARE proteins, fusion also depends on Akt and yet unidentified cell cytosolic components[115]. Akt translocation to the plasma membrane and phosphorylation appear to be crucial insulin-sensitive steps necessary for activation of fusion[35,116]. Interestingly, this approach also revealed that fusion is actin-independent, suggesting that the cytoskeleton functions at a step(s) before membrane fusion of GLUT4. Such recently developed molecular techniques will no doubt continue to aid investigations into the GLUT4 vesicle fusion process.

GLUT4 dysregulation in obesity and type 2 diabetes

Abundant studies of insulin resistance demonstrate defects at numerous levels in the insulin-regulated glucose transport pathway. Insulin sensitivity is profoundly affected by both genetic and environmental components. Mutations in the insulin receptor are rare but result in extremely severe insulin resistance. These include Leprechaunism, Rabson-Mendenhall Syndrome, and the type A syndrome of insulin resistance[117]. Type 2 diabetes is polygenic, probably involving defects at numerous points in the glucose regulation system. For example, skeletal muscle analyzed from type 2 diabetic subjects versus lean controls displays diminished insulin-stimulated IRS-1 tyrosine phosphorylation and decreased PI3K activity coupled to impaired glucose transport[118]. These defects could not be explained by alterations in protein expression. Likewise, skeletal muscle and adipocytes from obese, insulin-resistant individuals demonstrate impaired insulin-triggered IRS-1 associated PI3K activity compared to matching tissue from lean individuals[119,120]. Impaired insulin signaling through downstream Akt2 and AS160 proteins has also been reported in skeletal muscle[51,120]. Furthermore, the fatty acid metabolite ceramide causes insulin resistance that is coupled to impaired membrane recruitment and phosphorylation of Akt[121]. Knowledge on mechanisms of such defects has remained underdeveloped. One possible culprit could be the protein tyrosine phosphatases (PTP), enzymes that dephosphorylate IR and IRS-1 to halt signal propagation. Increased PTP expression and/or activity could account for diminished tyrosine phosphorylation seen in insulin-resistant states[122,123]. Increased IRS-1 serine phosphorylation may also help explain insulin resistance, as phosphorylated serine residues are thought to sterically hinder interactions with downstream PI3K. Dysregulated PKC activity in insulin resistance could increase serine phosphorylation[124], and PKC knockout mice are protected from insulin resistance[125].

Membrane and cytoskeletal defects are also a possible basis of insulin resistance. We now know that moderate increases in plasma membrane fluidity increase glucose transport[126-128]. Furthermore, it has been shown that basal glucose transport is not fully active in fat cells and that it can be increased further by augmenting membrane fluidity[126]. Consistent with membrane fluidity influencing insulin responsiveness, insulin-stimulated glucose transport is decreased when fluidity diminishes[127]. Recent data suggest that the anti-diabetic drug metformin enhances insulin action by increasing membrane fluidity[129,130]. Interestingly, the beneficial effects of chromium supplementation on insulin responsiveness may also be linked to membrane fluidity[131-133]. With regards to cytoskeletal defects, recent study of various cell culture models of insulin resistance suggests that an underlying basis of reduced cellular insulin sensitivity may be perturbations in phosphoinositide-regulated cortical F-actin structure. In particular, PI 4,5-P_2 control of cortical F-actin is disturbed by hyperinsulinaemic[134] and hyperendothelinaemic[135,136] insulin-resistant conditions and reversal of these changes by experimental manipulation of PI 4,5-P_2 corresponds with a restoration in insulin sensitivity. Furthermore, isolated adipocytes from ethanol-induced insulin-resistant Wistar rats[82] and skeletal muscle from obese insulin resistant Zucker rats[137] display altered actin polymerization. These findings agree with the necessity of an intact cytoskeleton for proper glucose regulation and suggest a membrane/cytoskeletal

component of insulin resistance. Finally, some study has also revealed that insulin-resistant conditions are associated with defects in the SNARE machinery[138]. As future research continues to expand our understanding of the signaling pathways of insulin-regulated GLUT4 translocation and glucose transport, our ability to develop interventions to prevent, reverse, and ameliorate insulin resistance in obesity and type 2 diabetes will be favourably impacted.

Perspectives

In this time of an exploding prevalence of obesity and type 2 diabetes, understanding and familiarity with the underlying molecular mechanisms of insulin signaling and glucose transport in the healthy and diseased states are crucial. There has been remarkable progress over the past two decades in elucidating the metabolic actions of insulin and the dysfunctions that give rise to insulin resistance. Nonetheless, gaps in our knowledge remain. Current expertise must guide ongoing investigations aimed at the eventual prevention and resolution of diabetes and insulin resistance.

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