

MOLECULAR MECHANISMS OF INSULIN-STIMULATED GLUCOSE UPTAKE IN ADIPOCYTES

P.-H. DUCLUZEAU (1, 2), L.M. FLETCHER (1), H. VIDAL (2), M. LAVILLE (2), J.M. TAVARÉ (1)

SUMMARY - The stimulation of muscle and adipose tissue glucose metabolism, which is ultimately responsible for bringing about post-absorptive blood glucose clearance, is the primary clinically relevant action of insulin. Insulin acts on many steps of glucose metabolism, but one of the most important effects is its ability to increase the rate of cellular glucose transport. This results from the translocation of the insulin-responsive transporter isoform, GLUT4, from intra-cellular vesicular storage sites to the plasma membrane. In adipocytes, a substantial amount of cellular GLUT4 is located in a specific highly insulin-responsive storage pool, termed GLUT4 Storage Vesicles (GSVs). GLUT4 can also translocate to the plasma membrane from the recycling endosomal pool which also additionally contains the GLUT1 isoform of glucose transporter and the transferrin receptor. In this article we review the molecular mechanism by which insulin stimulates GLUT4 translocation in adipose cells, including the nature of the signaling pathways involved and the role of the cytoskeleton.

Key-words: insulin, GLUT4, signaling, adipocytes, cytoskeleton.

RÉSUMÉ - Transport du glucose induit par l'insuline dans le tissu adipeux : de la localisation à la translocation.

La principale action de l'insuline est de stimuler l'utilisation post-absorptive du glucose par les muscles et le tissu adipeux. L'insuline modifie plusieurs étapes clés du métabolisme du glucose mais l'un des effets les plus spectaculaires est sa capacité à augmenter rapidement le transport cellulaire du glucose. Ceci résulte de la translocation du transporteur insulino-dépendent du glucose, GLUT4, d'un compartiment intracellulaire à la membrane plasmique. Dans les adipocytes près de la moitié des GLUT4 sont localisés dans des vésicules intracellulaires spécifiques et très sensibles à l'insuline, appelé GLUT4 Storage Vesicles (GSV). GLUT4 peut aussi être recruté à la membrane plasmique à partir des vésicules endosomales où il est exprimé conjointement avec GLUT1 et le récepteur de la transferrine. Cette revue décrit les mécanismes moléculaires par lesquels l'insuline stimule la translocation des GLUT4 dans les adipocytes, en distinguant les différentes voies de signalisation impliquées et le rôle du cytosquelette.

Mots-clés : insuline, GLUT4, signalisation, adipocytes, cytosquelette.

 : P.-H. Ducluzeau, Unité INSERM U449, Faculté de Médecine RTH Laennec, rue Guillaume Paradin 69372 Lyon Cedex 08, France.
E-mail: ph.ducluzeau@chu-lyon.fr

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(1) Department of Biochemistry, School of Medical Sciences, University of Bristol, Bristol, BS8 1TD, U.K.

(2) Unité INSERM U449, Faculté de Médecine RTH Laennec, rue Guillaume Paradin, 69372 Lyon Cedex 08, France.

Inulin resistance is defined as an inability of insulin to promote normal cellular glucose uptake at a given insulin concentration. In muscle cells and adipocytes insulin-stimulated cellular glucose transport is driven by the appearance of the insulin responsive glucose transporter, GLUT4, at the plasma membrane [1, 2]. Importantly, in various human insulin resistant states, the expression level of the GLUT4 gene is altered, although the effects on muscle and adipose tissue GLUT4 differ. For example, adipocyte GLUT4 gene expression has been reported to be reduced in obese, glucose intolerant or diabetic subjects, whilst that in muscle is not affected [3-5]. This highlights the fact that the diminished insulin-stimulated glucose uptake observed in diabetes can not be wholly explained by a decrease in cellular GLUT4 gene expression.

The precise steps in insulin action on glucose uptake that become resistant in type 2 diabetes remain remarkably elusive. Abnormalities have been observed in the proteins that mediate GLUT4 vesicle fusion and docking with the plasma membrane. For example, it has been reported that the levels of cellubrevin, VAMP-2, and syntaxin 4 are elevated in skeletal muscle from Zucker diabetic fatty rats compared with lean controls, and that this increase is partially reversed by treatment with rosiglitazone [6]. Whether such changes occur in human diabetes remains to be fully established.

Insulin-induced glucose transport defects may also result from problems in GLUT4 sequestration into the GLUT4 storage vesicular pool. However, morphological examination of GLUT4 distribution does not support this hypothesis, although it is questionable whether such defects would be detectable given the resolution of current technologies [7, 8]. Currently, the most likely explanation is that decreased insulin-stimulated glucose uptake results from abnormalities in the insulin signaling pathway(s) that control GLUT4 translocation to the plasma membrane.

While insulin-stimulated glucose uptake by adipose tissue accounts for less than 10% of the whole body glucose uptake, surprising results have been observed in mice carrying an adipose-specific deletion of the GLUT4 gene. These rodents rapidly develop marked muscular and hepatic insulin resistance, preceding hyperglycaemia, despite normal fat mass, muscle and hepatic triglyceride content, and normal serum leptin levels [9]. In contrast, mice carrying a muscle-specific deletion of GLUT4 develop hepatic and adipose insulin resistance secondary to the resulting hyperglycaemia; such a resistance can be treated by correcting the glycaemia with phlorizin [10]. In the same extend lipodystrophic patients develop a generalised insulin resistance [11, 12]. Taken together these data suggest that adipose tissue plays a more important role in whole body glucose homeostasis than was previously thought. In conclusion, therefore, while adipose tissue

glucose uptake accounts for a small part of whole body glucose disposal, an understanding of the mechanism of insulin actions involved in this tissue is of utmost importance.

■ THE NATURE OF THE GLUT4-VESICLE COMPARTMENT IN ADIPOCYTES

In 1955, two pioneering studies revealed that a major metabolic effect of insulin was to stimulate the uptake of glucose into skeletal muscle and adipose tissue [13, 14]. In the ensuing years, this topic became, and remains, a major focus of research and more recent studies using nuclear magnetic resonance have clearly shown that glucose transport across the plasma membrane is the main rate limiting step for glucose metabolism in normal, obese or diabetic subjects [15, 16]. A major breakthrough occurred in 1980 when it was found that the action of insulin on glucose uptake was mediated through the translocation of a 'glucose transport activity' from an intracellular site to the plasma membrane independently of *de novo* protein synthesis [17]. The first glucose transporter was cloned in 1985 and since this time it has emerged that a large family of similar transporters exist [18, 19] (Table 1). Among this family only GLUT4 has been demonstrated to be highly insulin sensitive, with expression restricted to muscle cells and adipocytes. Nevertheless insulin also causes a translocation of GLUT1 from the recycling endosomal pool along with the receptors for IGF II/mannose-6-phosphate and transferrin.

The Insulin Responsive Amino-Peptidase (IRAP or gp160) has been shown to completely co-localise with GLUT4 and displays identical trafficking kinetics and insulin responsiveness [20]. This peculiarity makes IRAP the only *bona fide* marker of GLUT4 vesicles known, other than GLUT4 itself. Other residents of intracellular GLUT4 compartments are proteins involved in the docking and fusion process of the vesicles with the plasma membrane. This includes the vesicle-associated membrane protein (VAMP) 3/cellubrevin [21], and VAMP2 [22], small GTP-binding proteins involved in regulating membrane traffic, such as Rab4 [23], as well as proteins of unknown function including sortilin [24] and pantophysin [25]. New residents of GLUT4 compartments continue to be identified; recent studies report the association of carboxyl esterase, PKB β , PI 3-kinase and PI 4-kinase with GLUT4 vesicles, supporting the necessity of a close relation between known insulin signaling molecules and GLUT4 containing vesicles [26].

Several lines of evidence suggest that the intracellular GLUT4 pool is not a homogenous population of vesicles. Rather, GLUT4 appears to reside in at least two distinct, but inter-related, vesicular pools both in terms of protein content and sensitivity to insulin

TABLE I. Mammalian family of facilitative glucose transporters (GLUTs). Note that GLUT6 was cloned by virtue of homology with other GLUT family members but found to be a pseudogene containing multiple stop codons therefore is not believed to be expressed as a functional glucose transporter.

Name	Tissue distribution	Functions
GLUT1	Wide distribution, abundant in red blood cells, endothelial cells and tissue culture cell lines	Basal glucose uptake in many cells (incl. insulin sensitive cells), transport in growing cells and across blood-brain barrier
GLUT2	Limited to pancreatic β cells, hepatocytes, intestine, kidney	Glucose-sensing in β cells, high capacity transport, trans-epithelial transport, major transporter in liver
GLUT3	Wide distribution in humans, limited to brain in some species	Basal transport, uptake from cerebral fluid
GLUT4	Largely expressed in insulin-responsive tissues of skeletal and cardiac muscle, and adipose	Insulin-sensitive glucose uptake, vital in postprandial glucose disposal
GLUT5	Primarily intestine, small amounts in adipose, muscle, brain, kidney	Absorption of fructose in intestine
GLUT7	Gluconeogenic tissues: hepatocytes	Release of glucose from gluconeogenesis from ER lumen
GLUT8	Blastocyst, possibly other tissues	Insulin-stimulated glucose uptake into blastocyst and possible other tissues lacking GLUT4
GLUTX1	High in testis, moderate in central nervous system, low in insulin responsive tissues	? Sequestered intracellularly therefore may play a role in regulatable glucose uptake

stimulation. Electron microscopy of rat adipocytes or 3T3-L1 adipocytes has revealed multiple membrane sub-compartments including clathrin-coated pits, small vesicles close to the plasma membrane, and tubulo-vesicular structures in the perinuclear region. Morphological analysis by immunofluorescence, and subcellular fractionation, suggests that a significant proportion of the GLUT4 vesicles in adipocytes reside in an endosomal pool that is continuously recycling between the plasma membrane and the cell interior [27]. Consistent with this, insulin also causes translocation of other proteins resident in the recycling endosomal system such as the transferrin receptor and GLUT1 [28, 29].

GLUT4 also resides in a compartment that appears to lack endosomal markers. This pool has been called the "GLUT4 storage vesicle" (GSV) compartment by James and his colleagues and may contain up to 60% of the insulin-responsive GLUT4 present in adipocytes [30]. The precise machinery required for the formation of the highly insulin-responsive compartment (the GSV) is still a matter of debate; nonetheless the GSV pool may act as a reservoir of GLUT4 that is much more efficiently mobilised by insulin compared to the GLUT4 residing in recycling endosomes (Fig. 1).

This specific compartmentalisation of GLUT4 provides a mechanism by which insulin can stimulate a pronounced translocation of GLUT4 while only mildly stimulating the translocation of recycling pro-

teins. In the basal state, GLUT4 cycles slowly between the plasma membrane and, at least, the endosomal compartment, thus the majority of the GLUT4 is located within the cell interior [31]. Insulin stimulation triggers a large increase in the rate of GLUT4 vesicle exocytosis (mainly from the GSV pool), and a small decrease in the rate of internalisation by endocytosis which together result in a net increase in GLUT4 protein content in the plasma membrane, and thus glucose uptake.

■ ROLE OF THE CYTOSKELETON

It has become increasingly clear that the cytoskeleton plays a crucial role in vesicle trafficking and recent studies using cytoskeletal disrupting agents have implicated these structures in GLUT4 vesicle trafficking itself [32, 33]. For example, colchicine, vinblastine, or nocodazole have been reported to block GLUT4 vesicle movements in living cells and to inhibit insulin-stimulated glucose uptake and GLUT4 translocation [34, 35]. This appears to exclusively affect the trafficking of GLUT4 present in the GSV pool, but not the endosomal pool, because microtubule disruption did not inhibit insulin-stimulated transferrin receptor translocation [32]. These data were interpreted in terms of a role for microtubules in maintaining, if not generating, the organisation of the GSV compartment in the basal state. It is currently unclear whether microtubules play any role in directly trans-



FIG. 1. Possible model by which insulin may stimulate GLUT4 translocation and cellular glucose uptake. GLUT4 is found in both a unique pool (GLUT4 storage vesicles; GSVs) as well as in the recycling endosomes where it co-localises with GLUT1 and transferrin receptors. The GSVs are stimulated to move directly to the surface in response to insulin. Insulin also increases the rate of recycling of the endosomal system.

locating GLUT4 vesicles to the plasma membrane as, unlike in fibroblasts, microtubules are highly fragmented in 3T3-L1 adipocytes and thus unlikely to be capable of playing such a role (Fig. 2).

Actin stress fibres in 3T3-L1 adipocytes are also largely depolymerized during the differentiation process of 3T3-L1 adipocytes (Fig. 2). In adipocytes the predominant actin structures that remain are those that make up the cortical actin network underlying the plasma membrane. Interestingly, the actin cytoskeleton has also been proposed to play an important role in insulin-stimulated GLUT4 translocation, and insulin has been shown to promote a significant accumulation of cortical actin in 3T3-L1 adipocytes [36]. Actin disrupting agents (e.g. cytochalasin D or latrunculin A) partially inhibit insulin-stimulated GLUT4 translocation and glucose uptake, suggesting that this cortical actin plays an important role. While the precise molecular details of this role have not been established, several possibilities can be proposed. This includes a role as a scaffold for the assembly of signaling molecules such as IRS-1 immediately beneath the plasma membrane [37]. Consistent with this hypothesis, disruption of the actin cytoskeleton in 3T3-L1 adipocytes results in a significant loss in insulin-induced activation of PKB [38], a process which is IRS-1 dependent. Alternatively, the cortical actin network could act to trap GLUT4 vesicles close to the plasma membrane thus indirectly increasing the frequency of fusion events with the plasma membrane. Indeed, by time-lapse confocal microscopy rapid movements of GFP-GLUT4 containing vesicles can be readily observed in the gap region between the plasma membrane and the cortical actin network (J.M. Tavaré, unpublished data).

■ SIGNALING TO GLUT4 THROUGH A PI 3-KINASE-DEPENDENT PATHWAYS

Insulin fixation on its plasma membrane receptor activates the β -subunit kinase activity and induce tyrosine phosphorylation of IRS family members (IRS1, 2, 3 and/or 4). The phosphorylated IRS proteins recruit the phosphatidylinositol 3-kinase (PI3K) by its associations with the regulatory sub-unit p85 α . Central to the actions of insulin is the lipid kinase, PI 3-kinase. This enzyme, activated by insulin, rapidly generates from cellular membrane the lipid mediator of insulin action on glucose transport, phosphatidylinositol-3,4,5-triphosphate (PIP₃). Blocking insulin-induced PIP₃ generation with wortmannin, an irreversible PI 3-kinase inhibitor, or a dominant-negative PI 3-kinase mutant, almost completely blocks the ability of insulin to induce GLUT4 translocation in adipocytes [39]. This demonstrates the absolute requirement for the activation of PI 3-kinase in insulin-induced translocation of GLUT4 from both the endosomal and GSV pools.

Insulin-stimulated PIP₃ generation leads to the recruitment of the Akt/protein kinase B (PKB) to the plasma membrane where PIP₃ dependent kinase 1 (PDK1) fully activate the serine/threonine kinase PKB by phosphorylating critical threonine residues in its activation loops [40]. PIP₃ alone and PDK1 are also involved in the insulin activation of atypical protein kinase Cs (PKC λ/ζ) [41]. Recent findings suggest that atypical PKCs and PKB serve as important positive regulators of insulin-stimulated glucose metabolism, and active forms of these two enzymes appears to be required for insulin-induced GLUT4 translocation to the plasma membrane [42] (Fig. 3). We have previ-

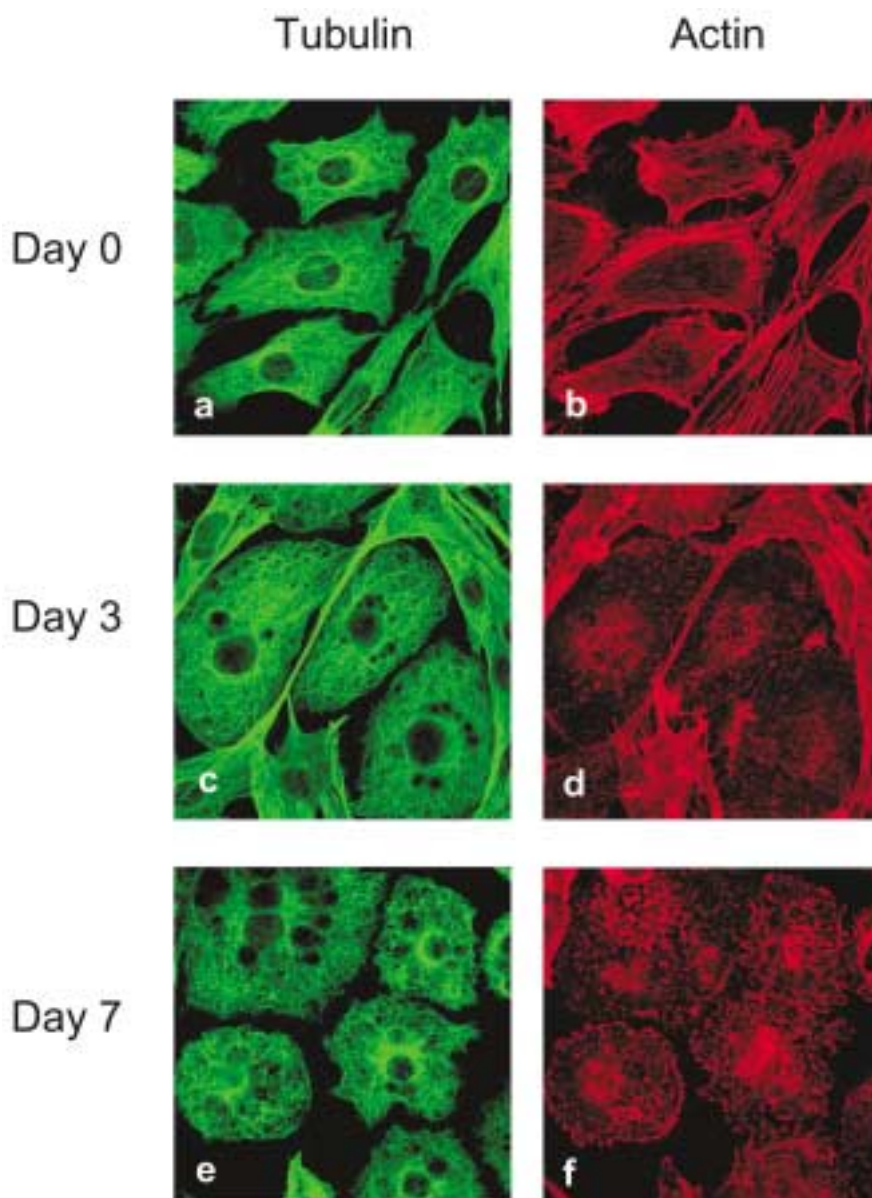


FIG. 2. Sub-cellular distribution of microtubules and actin filaments during the differentiation of 3T3-L1 fibroblasts into adipocytes. 3T3-L1 fibroblasts were induced to differentiate in adipocytes. The cells were fixed at day 0 (a, b), day 3 (c, d) or day 7 (e, f) and co-stained for the presence of microtubules, using anti-tubulin antibodies (a, c, e), or actin filaments, using rhodamine-phalloidin (b, d, f). Images are presented as projections, captured using a Leica laser scanning Confocal Imaging Spectrophotometer. During the course of differentiation, significant changes in the structure of the cytoskeleton occurs such that microtubules become fragmented and actin filaments are largely depolymerised.

ously shown that constitutively-active forms of protein kinase B induce the translocation of GLUT4 present only in the GSV compartment, but not of the GLUT4 that resides in recycling endosomes [43]. This argues for the existence of distinct mechanisms operating downstream of PI 3-kinase mediating insulin effect on GLUT4 vesicles issued from the GSV or from the endosomal pool.

Despite this, it has been known for some time that the addition of platelet-derived growth factor to 3T3-L1 adipocytes promotes PI 3-kinase activation but does not stimulate glucose uptake or cause

GLUT4 translocation [44]. While PDGF can activate PI3-kinase it does not appear to induce the generation of significant amounts of PIP_3 in the plasma membrane, for reasons which are not yet apparent [45]. Furthermore, a cell-permeant analogue of PIP_3 cannot induce GLUT4 translocation in adipocytes unless added simultaneously with insulin [46]. Taken together, the data suggest that while there is an absolute requirement for PI 3-kinase-mediated PIP_3 generation in insulin-stimulated glucose uptake, the activation of PI 3-kinase alone is not sufficient. Consistent with this, constitutively-active mutants of PI 3-kinase are

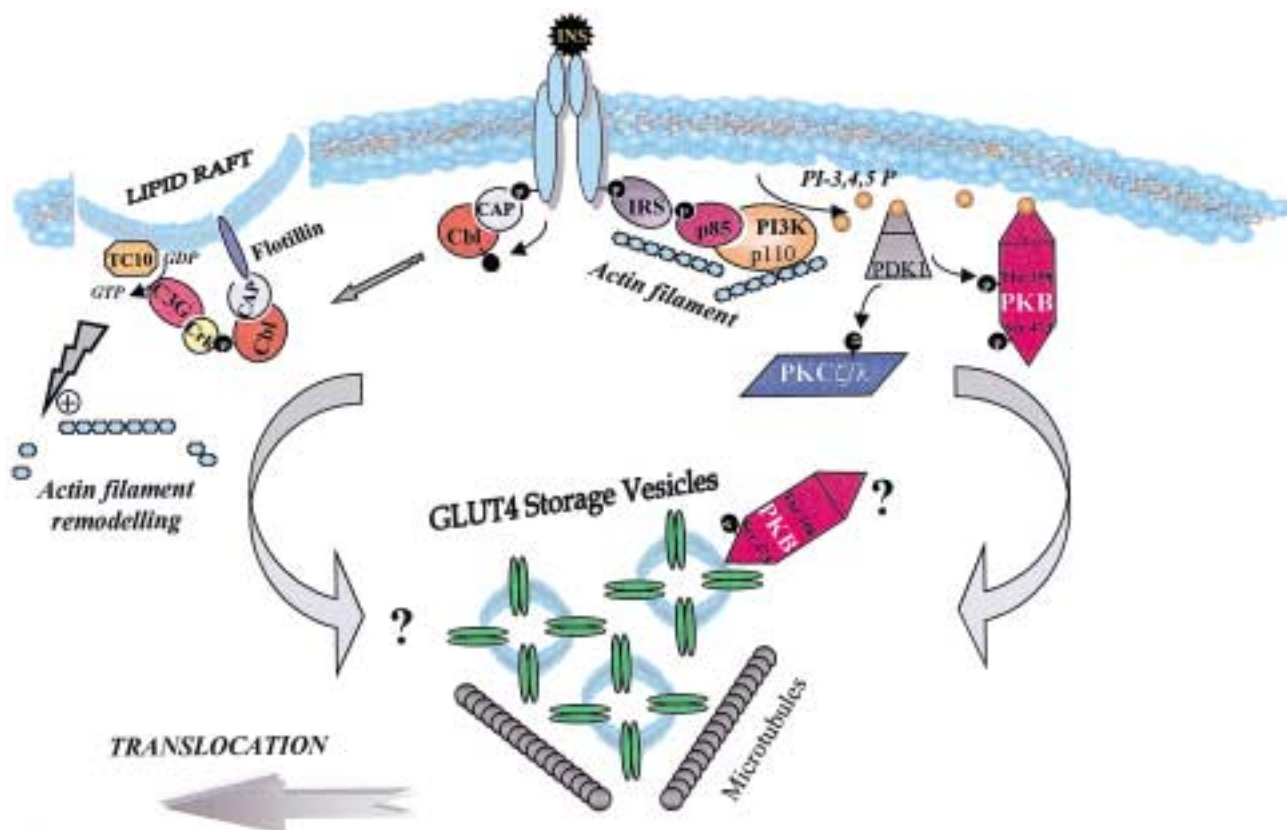


FIG. 3. Insulin signaling pathways involved in the GLUT4 translocation process. Protein kinase B as well as atypical PKC seems to be involved in the first insulin effects on the GLUT4 translocation process, may be at the level of GSV themselves. This involved an insulin-dependent activation of the PI 3-kinase. In addition, insulin-induced TC10 activation at the level of lipid raft, independent of PI 3-Kinase activity, is also necessary for a complete and efficient GLUT4 translocation. The second pathway probably involved insulin-stimulated actin filament remodeling that could imply in the final steps of the translocation process.

often not very effective at inducing glucose uptake. This suggests that a second PI 3-kinase-independent insulin signaling cascade is additionally required in addition to a PI 3-kinase-dependent pathway to fully induce glucose uptake.

■ SIGNALING TO GLUT4 THROUGH A PI 3-KINASE-INDEPENDENT PATHWAYS

A recent series of experiments have begun to unravel this PI 3-kinase-independent pathway. The adipocyte plasma membrane is characterised by the presence of multiple caveolae [47]; large vesicular invaginations of the plasma membrane. Caveolae have recently been proposed as being crucial in initiating the PI 3-kinase-independent signal transduction pathway. Caveolae are found in specialised cholesterol-rich regions of the plasma membrane termed "lipid rafts". The cholesterol content of lipid rafts is known to be important for their structure and function. Inter-

estingly, depletion of membrane cholesterol from lipid rafts using methyl- β -cyclodextrin markedly inhibits insulin-stimulated glucose uptake [48].

Caveolae appear to play an important role in the insulin-induced tyrosine phosphorylation of a protein called c-Cbl by the activated insulin receptor [49]. This protein is recruited to lipid rafts as a complex with an adaptor protein called CAP, that bind the flotillin (Fig. 3). As such, CAP also appears to aid c-Cbl recruitment to, and phosphorylation by, activated insulin receptors. This pathway is of particular interest because CAP expression is restricted to the adipose tissue and its expression is increased by the thiazolidinediones, which may provide another explanation for their facilitating action on insulin sensitivity in this tissue [50]. Moreover, over-expression of a dominant-negative CAP in adipocytes blocks insulin-stimulated glucose uptake [51].

Our understanding of the role of the CAP pathway was recently boosted by the observations that it controls the activity of another protein associated with

lipid rafts called TC10, a Rho family GTPase expressed in muscle and adipose tissue [52]. Phosphorylated c-Cbl appears to recruit C3G (a guanylnucleotide exchange factor acting on TC10), via an SH2 domain-containing adaptor, CrkII. Thus the insulin-induced localisation of the CAP/Cbl/CrkII/C3G complex promotes the conversion of GDP-bound TC10 to its active GTP-bound state. While the molecular target of TC10 is currently unknown, TC10 activation seems to be required for insulin-stimulated GLUT4 translocation from the GSV pool, with no apparent effect on GLUT4 in the endosomal pool [53]. Interestingly, TC10 has also been proposed to be responsible for the actin remodelling effects of insulin.

Importantly, growth factors other than insulin are not able to activate TC10 in adipocytes, providing a possible explanation for the ability of growth factors to stimulate PI 3-kinase but not glucose transport.

■ CONCLUSION

Cellular glucose uptake is the major rate limiting step in the regulation of glucose metabolism by insulin and, as such, defects in its regulation lead to insulin resistant states. Since the early 1920's science has striven to elucidate insulin's mechanism of action. Only now, however, is an understanding of this process becoming unravelled to a point at which we are beginning to identify some of the signaling proteins that may be defective in insulin resistance in humans and which may represent future targets for therapy.

Indeed, the biology underlying insulin action on glucose transport is immensely complex, which means that we still have a long way to go until we have a complete picture. At least in adipocytes, this insulin-stimulated glucose transport process involves at least two distinct but complementary signaling cascades. One route involves the PI 3-kinase dependent activation of specific serine/threonine kinases (PKB and atypical PKCs). The other appears to involve remodelling of the cortical actin cytoskeleton in a TC10-dependent manner. Hence, a "new" role is emerging for the cytoskeleton in cells that lost their ability to move during their differentiation process (fibroblast in adipocyte and myoblast in myotube). Any biochemical or morphological disruption of these complex interrelationships could generate an insulin resistant state. Furthermore, obesity is characterised by a dramatic increase in adipose cell size, which could turn up a such disruption, between cell cytoskeleton and insulin signaling molecules, then, exacerbating an obesity-induced insulin resistance state. The next goal must be to confirm and extend these findings and finally identify the molecular target(s) that are defective in type II diabetes — a holy grail for diabetes research.

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