Subcellular trafficking of the substrate transporters GLUT4 and CD36 in cardiomyocytes

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Abstract Cardiomyocytes use glucose as well as fatty acids for ATP production. These substrates are transported into the cell by glucose transporter 4 (GLUT4) and the fatty acid transporter CD36. Besides being located at the sarcolemma, GLUT4 and CD36 are stored in intracellular compartments. Raised plasma insulin concentrations and increased cardiac work will stimulate GLUT4 as well as CD36 to translocate to the sarcolemma. As so far studied, signaling pathways that regulate GLUT4 translocation similarly affect CD36 translocation. During the development of insulin resistance and type 2 diabetes, CD36 becomes permanently localized at the sarcolemma, whereas GLUT4 internalizes. This juxtaposed positioning of GLUT4 and CD36 is important for aberrant substrate uptake in the diabetic heart: chronically increased fatty acid uptake at the expense of glucose. To explain the differences in subcellular localization of GLUT4 and CD36 in type 2 diabetes, recent research has focused on the role of proteins involved in trafficking of cargo between subcellular compartments. Several of these proteins appear to be similarly involved in both GLUT4 and CD36 translocation. Others, however, have different roles in either GLUT4 or CD36 translocation. These trafficking components, which are differently involved in GLUT4 or CD36 translocation, may be considered novel targets for the development of therapies to restore the imbalanced substrate utilization that occurs in obesity, insulin resistance and diabetic cardiomyopathy.

Keywords Cardiac metabolism · Intracellular traffic · GLUT4 · CD36 · Insulin resistance · Diabetes

Introduction

We previously reported that known signaling mechanisms, such as insulin and contraction, similarly affect myocyte GLUT4 and CD36 trafficking [1]. However, in insulin resistance and type 2 diabetes CD36 permanently relocates to the sarcolemma, while GLUT4 internalizes [2, 3]. As a corollary there must be mechanisms which selectively recruit either GLUT4 or CD36. Therefore, we began to investigate distinct intracellular processes involved in vesicular transport to uncover mechanisms that are differently involved in GLUT4 and CD36 trafficking [4, 5]. We revealed coat-proteins, actin filaments, the cellular pH gradient and vesicle-associated membrane proteins (VAMPs) to be involved in GLUT4 and CD36 trafficking, and, importantly, indeed found mechanisms that are differently involved in glucose and fatty acid uptake in cardiomyocytes.

In this review, we first describe how cardiac glucose and fatty acid uptake are regulated and which alterations occur during insulin resistance and type 2 diabetes. Then, we address signaling pathways and subcellular trafficking components that are involved in GLUT4 and CD36 translocation. Finally, we focus on components that are...
differently involved in glucose and fatty acid uptake and describe novel targets to restore metabolic disturbances in type 2 diabetes.

Regulation of cardiac glucose and fatty acid uptake

Glucose and fatty acids are of fundamental importance for energy production in all eukaryotic cells. In cardiomyocytes, the continuous supply of both substrates is especially crucial to maintain contractile activity [6]. Cardiomyocytes are metabolically flexible, i.e., they preferably use fatty acids, but can also use glucose, ketone and lactate to produce ATP [7]. These different substrates cannot sufficiently enter cardiomyocytes by diffusion and thus have to be taken up by facilitated transport. Glucose uptake into cells involves a family of glucose transport proteins—called GLUTs—which shuttle sugars across plasmalemmal membranes through their aqueous pore [8]. The GLUT family consists of several members which are expressed by various cell types [9]. In cardiomyocytes, GLUT family members 1 and 4 fulfil this function. While GLUT1 is mainly involved in basal glucose uptake, GLUT4 translocates to the plasma membrane to enhance glucose uptake in response to extracellular stimuli like insulin or increased cardiac work. Cellular fatty acid uptake is facilitated by several membrane proteins with high-affinity binding sites for fatty acids [1], but the exact mechanisms by which these proteins mediate transmembrane passage of fatty acids is not known [10]. In cardiomyocytes, the two most important fatty acid transporters are fatty acid translocase (FAT), also referred to as CD36 [10, 11], and two members of the family of 6 fatty acid transport proteins (FATP), i.e., FATP1 [12] and 6 [13].

Both GLUT4 and CD36 are integral membrane proteins. GLUT4 consists of 12 transmembrane domains with both termini in the cytoplasm and one large intracellular and one large extracellular loop [14]. CD36 has a hairpin-like structure with two transmembrane regions and both the C-terminal and N-terminal tails in the cytoplasm [1]. The translocation of both proteins from intracellular storage compartments to the plasma membrane, and vice versa, relies on a complex trafficking system, schematically represented in Fig. 1 [15]. Immunoadsorption studies in rat cardiomyocytes showed that GLUT4 resides in two distinct pools, one of which is called GLUT4 storage vesicles (GSV) that does not contain CD36 and is sensitive to insulin [16]. On the other hand, CD36 appears to reside in one subcellular pool which does not contain GLUT4 [16], suggesting that GLUT4 and CD36 are stored separately and travel independently.

Physiological stimuli, with circulating plasma insulin concentrations and increased cardiac work being the most important, stimulate the heart to quickly alter cardiac substrate utilization via reversible translocation of GLUT4 and CD36 from intracellular membrane compartments to the sarcolemma (Fig. 2a) [1, 10]. In contrast to CD36, the other fatty acid transporters FATP1 and FATP6 do not traffic between intracellular storage compartments and the sarcolemma in cardiomyocytes [17–19]. Therefore, FATP1 and FATP6 do not contribute to inducible fatty acid uptake.

In addition, other studies have disclosed that these transporters also have a minor contribution to basal fatty acid uptake. From experiments with cardiomyocytes from wild-type and CD36 null mice, it is known that the contribution of CD36 to fatty acid uptake is about 70% [17]. In addition, in cardiomyocytes treated with the specific CD36 inhibitor sulfo-N-succinimidyl-oleate (SSO), the insulin/
contraction-mediated increase in fatty acid uptake was totally blocked, meaning that CD36 is irreplaceable in stimulus-induced fatty acid uptake [20]. The system of regulated substrate uptake is crucial during exercise to supply cardiomyocytes with a sufficient amount of substrates, or to quickly replenish intracellular substrate storage pools after a meal. A dysfunction in this system of regulated glucose and fatty acid uptake into muscle, adipose tissue, beta cells and heart contributes to the development and progression of type 2 diabetes [10, 21]. The amount of glucose and fatty acid transporters present at the sarcolemma is a major regulatory factor of cardiac glucose and fatty acid utilization [22]. This implies that the metabolic machinery is more than adequately suited to process all incoming substrates. Accordingly, intracellular concentrations of fatty acids and glucose will remain low. This is also true for the diabetic heart in which intracellular concentrations of these substrates are not markedly increased [1, 23]. Hence, in both the healthy and the diabetic heart, the sarcomemmal presence of GLUT4 and CD36 determines cardiac substrate flux [24].

**Alterations in transporter location in insulin resistance**

Obesity, insulin resistance and type 2 diabetes show a strong association with changes in lipid metabolism [2, 25]. Permanent relocation of CD36 to the sarcolemma and increased fatty acid uptake were strongly linked in rodent models for insulin resistance [2, 26, 27] and obese humans [26, 28, 29]. Furthermore, the sarcolemmal content of CD36 correlated well with increased uptake of fatty acids, increased intramuscular triacylglycerol [28, 30, 31] and reduced insulin-stimulated GLUT4 translocation and glucose uptake [32]. Cardiac in vivo positron-emission tomography (PET) in humans with type 2 diabetes showed increased fatty acid uptake and oxidation, and reduced insulin-stimulated glucose uptake, paralleled by decreased diastolic function as compared to age-matched healthy controls [33].

The increase in intramyocardial lipid concentrations cannot just be attributed to a reduction in mitochondrial fatty acid oxidation, since studies have shown that cardiac fatty acid oxidation remained unchanged, slightly reduced, or even increased in several rodent models of obesity and insulin resistance [10]. Inhibition of CD36-mediated fatty acid uptake with SSO normalized fatty acid utilization in different rodent models of insulin resistance [26]. This indicates that the increase in intramyocardial lipid concentrations in insulin-resistant cardiomyocytes is caused by increased CD36-mediated fatty acid uptake. We and others showed that the surface presence of CD36 was increased in rodent models for insulin resistance and in skeletal muscle of obese humans [2, 3, 32]. The increase in surface presence of CD36 is not due to increased tissue expression, but instead to a permanent relocation from its intracellular storage compartment. This permanent CD36 relocation appears to be an early event in the sequence of maladaptive changes in the hearts of rodents with type 2 diabetes. We also obtained evidence that there is no decrease in cardiac mitochondrial function in this early pre-diabetic stage. However, it is very well possible that mitochondrial dysfunction develops at later stages which can lead to even

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**Fig. 2** Alterations in transporter localisation in diabetic cardiomyocytes compared to healthy cardiomyocytes. **a** Substrate uptake in healthy cardiomyocytes. The entry of glucose and fatty acids is facilitated by glucose transporter 4 (GLUT4) and fatty acid transporter CD36. The heart can acutely react to a change in energy demand by reversible translocation of these transporters from intracellular storage compartments. Once these substrates have entered the cell they can be used for ATP production by mitochondrial oxidation or they can be stored as glycogen (not shown) or in lipid droplets. **b** Substrate uptake in diabetic cardiomyocytes: during the development of insulin resistance and type 2 diabetes, the localisation of GLUT4 and CD36 permanently alters. CD36 presence at the sarcolemma increases which associates with increased fatty acid uptake. In addition, fatty acid storage increases which is thought to interfere with insulin-stimulated glucose uptake.
more lipid storage. Moreover, it has been shown that relocation of CD36 was specific for this fatty acid transporter because such changes were not observed for plasmalemmal FABPpm, FATP1 or FATP4 [10]. Thus, permanent relocation of CD36 to the sarcolemma is important in the development toward diabetic cardiomyopathy.

It is becoming evident that the increased intramyocardial triacylglycerol content is not the main contributor to the development of insulin resistance and cardiac dysfunction. Other lipid metabolites, such as ceramides and diacylglycerols, appear to be the main contributors to reduced insulin sensitivity (see [34, 35] for excellent reviews on this topic).

Collectively, obesity, insulin resistance and type 2 diabetes impose metabolic stress on the heart [36–39], which may ultimately lead to cardiac metabolic inflexibility, lipotoxicity, and subsequent development of diabetic cardiomyopathy [3, 32] (Fig. 2b).

**Signaling pathways involved in GLUT4 and CD36 translocation**

As mentioned above, postprandial increases in circulating plasma insulin levels and an elevated cardiac work are the most important physiological stimuli to enhance cardiac glucose and fatty acid uptake via induction of GLUT4 and CD36 translocation, respectively [1]. However, the metabolic fates of glucose and fatty acids are quite different during insulin-stimulation versus contraction-stimulation. Both substrates are preferentially stored under insulin-stimulated conditions, and preferentially oxidized upon increased contraction [1]. Since translocation of GLUT4 and CD36 are similarly induced by insulin and contraction, these translocation processes do not contribute to the different metabolic fates of both substrates during insulin-versus contraction-stimulation. Rather, upon intracellular trapping of both substrates [glucose via hexokinase and fatty acids via acetyl-CoA synthase (ACS)], the metabolic fates of glucose and fatty acids are determined by the activation state of key metabolic enzymes. Specifically, glucose and fatty acids are directed towards storage via insulin-induced activation of glycogen synthase (GS) and glycerol-3-phosphate acyltransferase (GPAT), respectively [1]. In addition, the contraction-induced drop in the intracellular ATP concentrations triggers phosphofructokinase, pyruvate dehydrogenase and TCA cycle progression for acceleration of glucose oxidation, and also carnitine-palmitoyl transferase-1 for acceleration of fatty acid oxidation [1]. Despite differential effects of insulin and contraction on the metabolic machinery, both stimuli similarly induce translocation of GLUT4 and CD36 to the sarcolemma [1].

This similarity in responsiveness of both transporters to both physiological stimuli suggests that similar signaling mechanisms are involved in GLUT4 and CD36 translocation. In addition, it suggests that GLUT4 and CD36 migrate together to the sarcolemma in response to each of the stimuli. However, increasing numbers of studies report differences in the regulation of both substrate transporters, leading to a more differentiated understanding of GLUT4 and CD36 traffic.

Exactly how insulin and increased workload achieve increased abundance of GLUT4 and CD36 at the sarcolemma is unclear, but there are two primary candidates: increasing exocytosis or inhibiting endocytosis [40–43]. Insulin is proposed to stimulate fusion of intracellular GLUT4 containing-vesicles with the plasma membrane in cardiac and skeletal myocytes while it does not affect the rate of endocytosis [42, 44, 45]. In myocytes, fusion of intracellular membranes with the plasmalemmal membrane is considered to be inducible, while endocytosis of GLUT4 and CD36 is regarded a housekeeping process which cannot be regulated. However, it has been reported that decreased endocytosis in response to insulin regulates GLUT4 translocation in adipocytes [46]. Some studies found that, in line with the effect of insulin on GLUT4 translocation, contraction increases GLUT4 translocation [44, 45]. In contrast, another study found that GLUT4 endocytosis is inhibited [42]. In summary, the exact mechanism by which insulin and contraction increase presence of CD36 and GLUT4 at the plasma membrane requires further studies aiming at the rate of endocytosis and/or the rate of translocation.

**Insulin-signaling**

From all stimulus-induced transporter translocation processes, insulin-induced GLUT4 translocation is the most intensively studied [47]. Several of the kinases involved in insulin-induced GLUT4 translocation have been tested on their additional involvement in insulin-induced CD36 translocation. So far, these kinases seem to play a similar role in both translocation events, as detailed below. The insulin-signaling axis is initiated by the binding of insulin to its receptor and subsequent activation of insulin receptor substrate (IRS) 1 and IRS2 ([48] and [49]) (Fig. 3a). These activate the regulatory subunit of phosphatidylinositol-3-kinase (PI3K), which consists of a catalytic p110 subunit and a regulatory p85 subunit ([50]. Pharmacological inhibitors (most notably wortmannin) have greatly facilitated the investigation of the role of PI3K in GLUT4 and CD36 translocation, and have pinpointed this lipid kinase as a key component ([51–53]. The main phosphatidylinositol (PI)-phosphate generated by PI3K during insulin action is PI-3,4,5-trisphosphate (PIP3) ([54]. Generation of
PIP3 at the plasma membrane directly drives the activation of a number of different protein kinases with lipid binding pleckstrin homology domains [55]. Three of these kinases play an essential role in insulin-induced glucose uptake: (1) Akt/protein kinase B (PKB)-isoform 2, (2) protein kinase C (PKC)-βI/βII and (3) 3-phosphoinositide-dependent protein kinase (PDK) [56–59]. Akt/PKB-2 and PKC-βI have additionally been implicated in insulin-induced CD36 translocation [24, 59].

Activation of Akt requires dual phosphorylation at Ser473 and Thr308 in addition to PI3K-mediated recruitment to the plasma membrane [1]. The Thr308 phosphorylation within the activation loop of Akt is mediated by PDK1 [60] and the Ser473 phosphorylation by a putative PDK2 [61]. Upon its activation, Akt phosphorylates TBC1D1 and TBC1D4, also referred to as AS160, as a final step to induce GLUT4 translocation [62, 63]. Next to Akt, PDK1 also activates PKC-ζ upon unfolding of its pseudosubstrate domain and exposure of the activation loop [64]. The simultaneous and combined activation of Akt and PKC-ζ is necessary for insulin-induced GLUT4 translocation in both heart [65] and skeletal muscle [66], and likely also essential for insulin-induced CD36 translocation.

To date, GLUT4 and CD36 translocation seem identically regulated by insulin. However, it is possible to translocate GLUT4 from insulin-responsive intracellular compartments in cardiomyocytes without changes in subcellular CD36 localization using the thiol-modifying agent arsenite [67]. Hence, there are arsenite-sensitive proteins that contribute to insulin-induced GLUT4 translocation without affecting CD36 dynamics.

Contraction-signaling

Increased cardiac work results in a rapid rise of both glucose and fatty acid uptake in heart and muscle [1, 68]. Upon increased contraction, the intracellular concentration of adenosine-monophosphate (AMP), cyclic AMP, reactive oxygen species (ROS) and calcium increase. Together, these second messengers activate a complex signaling system that involves AMP-activated protein kinase (AMPK), protein kinase A (PKA), atypical PKCs, protein kinase D (PKD), calcium–calmodulin-dependent protein kinases (CaMK), and the extracellularly regulated protein kinases (ERK) 1 and 2 [68] (Fig. 3b). Of all of these, AMPK, PKD and CaMK have been studied for their effects on metabolic processes.

AMPK is a heterotrimer consisting of a catalytic α-domain, a glycogen-binding regulatory β-domain and an AMP-binding regulatory γ-domain. Binding of AMP to the regulatory γ-subunit of AMPK leads to a conformational change of the kinase, which makes it accessible for upstream AMPK kinases (AMPKK). These phosphorylate AMPK at threonine-172 and consequently activate this kinase. In the heart, activation of the α2 isoform of AMPK is essential for contraction-induced GLUT4 and CD36 translocation [69]. Kinases with suggested AMPKK activity include CaMK-kinase (CaMKK) and the tumor suppressor protein LKB1 [70]. In LKB1-null mice contraction-induced GLUT4 and CD36 translocation are completely abrogated, indicating that LKB1 is essential for both translocation processes [69]. Additionally, LKB1 is involved in hypoxia-induced AMPK-mediated cardiac GLUT4 translocation [71].

CaMK-kinase (CaMKK) is described as an alternative upstream kinase of AMPK [72]. However, evidence for the physiological importance of CaMKK in cardiomyocyte metabolism is scarce. One study reported basal activity and phosphorylation of AMPK in LKB1-deficient cells that could be further stimulated by calcium ionophores [72]. Another study, in which CaMKKβ was overexpressed or pharmacologically inhibited, showed that AMPK can also be activated by CaMKK [73]. In addition, it was shown that CaMKK is important for contraction-mediated GLUT4 and CD36 translocation in skeletal muscle [74]. However,
the importance of CaMKK in cardiac GLUT4 and CD36 translocation remains to be established.

Downstream events of AMPK involve atypical PKCs, ERK1/2, TBC1D1 and TBC1D4. It was shown that exercise increases glucose transport in skeletal muscle via AMPK through atypical PKCs. Furthermore, these effects of atypical PKCs were dependent on the ERK1/2 pathway, activation of proline-rich tyrosine kinase-2 (PYK2), and phospholipase D (PLD) [75]. In a muscle-specific knockout mouse model for the atypical PKC PKCζ, glucose transport was impaired and mice developed the metabolic syndrome [76]. In rat skeletal muscle, ERK1/2 inhibitors decreased contraction-induced fatty acid uptake and CD36 translocation [77]. Hence, atypical PKCs and ERKs are involved in contraction-stimulated substrate uptake in skeletal muscle. However, their function in substrate uptake of the heart still needs to be disclosed.

We recently identified involvement of the contraction-activated protein PKD to be involved in contraction-induced GLUT4 translocation and glucose uptake [78]. Pharmacological inhibition of PKD1 in cardiomyocytes inhibited contraction-stimulated GLUT4 translocation and glucose uptake [78]. Furthermore, contraction-stimulated PKD1 activation was still able to induce GLUT4 translocation and glucose uptake in AMPKζ2 knockout mice suggesting that PKD1 acts independently of AMPK [78]. These findings suggest that contraction-mediated translocation of GLUT4 is a dual input mechanism which needs both the input of AMPK and PKD. Future experiments should reveal the role of PKD in contraction-induced CD36 translocation and fatty acid uptake. Additionally, upstream and downstream proteins of PKD1 have not yet been elucidated.

Surprisingly, the two Akt substrates TBC1D1 and TBC1D4 are also substrates of activated AMPK and therefore also part of the contraction signaling cascade [66, 79]. Although these data suggest a complementary role of both TBC1D isoforms, the high expression of TBC1D1 in muscle compared to fat tissue indicates a pivotal role of this protein in contractile tissue [79]. Still, further research is needed to unravel (1) whether the two isoforms are physiologically relevant, and (2) whether they fulfil similar functions in CD36 translocation.

In summary, GLUT4 and CD36 translocation seem identically regulated by increased workload, but the roles of CaMKK and PKD in contraction-induced CD36 translocation still await exploration.

** Trafficking machinery involved in GLUT4 and CD36 translocation**

The trafficking machinery involved in GLUT4 translocation [45, 80–85], especially in adipocytes and muscle cells, has gained much more attention than the trafficking machinery involved in CD36 translocation [4, 5]. The isolation of GLUT4 vesicles revealed approximately 50 proteins that regulate vesicle fission, transport and fusion, as well as the specificity of GLUT4 transport [86]. CD36-containing vesicles have been isolated [16], but not so far extensively studied with a proteomics approach.

Cellular protein traffic generally involves three major steps. Firstly, vesicle fission: at the donor compartment, the membranes will be curved into a bud that will subsequently excise. This process is dependent on bilayer destabilizing proteins, coat proteins, Rab GTPases and a number of adapter proteins forming a fission complex. Secondly, subcellular vesicle transport: these newly formed transport vesicles move along one of the cytoskeletal networks with the aid of motor proteins which are regulated by Rab GTPases. And thirdly, vesicle fusion: at the acceptor compartment, the vesicle membranes fuse with the acceptor lipid bilayer requiring the formation of a SNARE complex and is modulated by Rab GTPases. Both translocation and endocytotic routes between endosomes and the sarcolemma proceed accordingly (Figs. 1, 4).

Although it is generally believed that GLUT4 and CD36 translocation are vesicle-mediated endosomal processes, GLUT4 localization overlaps only for 30–40% with markers of this endocytosis system, e.g., the transferrin receptor and Rab5. In addition, chemical ablation of endosomes does not fully block insulin-stimulated GLUT4 translocation in adipocytes [87].

Recently, posttranslational modification of GLUT4 by ubiquitination was found to be important for its intracellular sorting [88]. CD36 can also be ubiquitinated, but in this case ubiquitination affects protein expression rather than localization [89].

Recent data, which will be discussed below, have revealed the role of these trafficking components in GLUT4 and CD36 translocation.

**Coat proteins**

Coat proteins are essential players in vesicle fission and fusion. To initiate vesicle fission, these proteins are recruited to membrane spots where cargo is concentrated by adaptor proteins. There, coat proteins form a ‘bulb’ in the membrane and thus start budding of a vesicle. Once the vesicle is formed and detached from the organelle, the coat proteins are released. The following protein families are known to function as coat proteins: coat protein complex (COP), clathrin and caveolin, which reside in specific subcellular compartments [15, 90].

COPI and II are involved in transport from the ER to the Golgi apparatus, where sorting receptors couple specific cargo to COPI and COPII transport vesicles [15, 91]. Much
of the knowledge on COPI vesicle formation is derived from pharmacological studies using brefeldin-A as a non-competitive inhibitor of Arf1 \[92, 93\], although it has also been reported that brefeldin-A affects clathrin functioning \[94\]. Treatment of cells with brefeldin-A results in rapid fusion of the Golgi apparatus with the ER, which suggests an important role of these coat proteins in the maintenance of the distinct organelles \[92\]. However, the use of brefeldin-A in rat adipocytes could not clearly prove an involvement of COPI in insulin-stimulated GLUT4 translocation to the plasma membrane \[95–98\]. Studies performed in cardiomyocytes show that COP proteins and/or clathrin function in stimulus-induced glucose and fatty acid uptake in cardiomyocytes \[4\]. Hence, GLUT4 and CD36 translocation in cardiomyocytes are both closely related vesicle-mediated processes (Figs. 1, 4).

Caveolins—caveolin-1, -2 and -3—reside in cholesterol-enriched lipid rafts of the plasma membrane called caveolae. Caveolins could play a role in plasmalemmal docking of GLUT4- or CD36-containing vesicles, and could be involved in GLUT4 or CD36 internalization \[99, 100\]. Caveolin-1 \[101, 102\] has been proposed to play a role in initiation of GLUT4 endocytosis in adipocytes, but in skeletal muscle, the muscle-specific isoform caveolin-3 does not colocalize with GLUT4 \[103\]. Hence, the role of caveolins in GLUT4 trafficking is unclear. Caveolin-1 has
been shown to be involved in CD36 localization and function in smooth muscle cells [104] and fibroblast [105]. However, caveolin-3 does not seem to play a role in CD36 translocation in muscle cells because regulation of cardiac LCFA uptake was not altered in caveolin-3 knockout mice. In conclusion, there is evidence that caveolin-1 is involved in GLUT4 and CD36 translocation in non-muscle cells, but caveolin-3 most likely does not play a role in translocation of both transporters in (cardiac) myocytes.

Cytoskeletal filaments

It is well established that reorganization of filamentous actin beneath the plasma membrane plays a role in insulin-induced GLUT4 translocation [83, 106–108]. Insulin signaling bifurcates at the level of PI3K towards Akt and Rac. Insulin activates GTP loading of Rac within 5 min and then induces reorganization of actin. It has been shown that agents that disturb actin polymerization, e.g., latrunculin B or cytochalasin D, inhibit insulin-induced GLUT4 translocation in adipocytes, skeletal muscle cells and cardiomyocytes [4, 82, 83, 109]. Findings obtained by these pharmacological approaches were confirmed by overexpression of a dominant negative Rac mutant [110] or siRNA-mediated knockdown of Rac [111]. Recently, it was shown that Arp2/3 is a downstream effector of Rac and that coflin regulates actin depolymerization which again proposes that active actin cycling is essential for insulin-stimulated GLUT4 translocation [83]. In contrast, latrunculin B did not inhibit stimulus-induced CD36 translocation in cardiomyocytes [4]. Hence, actin filaments are involved in GLUT4 translocation, but not in CD36 translocation in the heart.

Microtubule involvement in GLUT4 translocation has mainly been studied in adipocytes. Controversy exists about their role in stimulus-induced GLUT4 translocation. Some groups reported that microtubule-disrupting agents inhibited insulin-induced GLUT4 translocation in 3T3-adipocytes [81, 112], while others did not find any effect on this process [113]. However, microtubules do not seem to play a role in stimulus-induced GLUT4 or CD36 translocation in skeletal muscle and cardiomyocytes [4, 114].

Although the cytoskeleton is clearly involved in GLUT4 and CD36 translocation in cardiomyocytes, its role in the altered transporter localization in diabetes has not yet been studied. However, investigators in other fields of research have studied the cytoskeleton in the diabetic heart. For example, in ventricular cells from streptozotocin-induced diabetic rats, impairment of cytoskeletal function and structure—actin and microtubules—was found. In their study, the insulin-deficient conditions affected the cardiac potassium-current in a cytoskeleton-dependent manner [115], suggesting that this may also affect insulin-dependent GLUT4 translocation.

Endosomal pH

The acidity of intracellular compartments, such as endosomes, is essential for various cellular processes, including endosomal function and vesicular trafficking [116, 117]. Endosomal acidification is regulated by vacular-ATPase (v-ATPase or V1V0-ATPase), a large multisubunit complex that functions as an ATP-driven proton pump. It has a similar build-up as the F1F0-ATPase located at the mitochondrial membrane; however, it needs ATP to pump protons whereas the F1F0 needs protons to produce ATP [118].

Surprisingly, not much is known about the role of endosomal acidity in trafficking of GLUT4 or CD36. The role of v-ATPases in GLUT4 translocation has been studied in 3T3-L1 adipocytes [119], and in cardiomyocytes [120]. Upon inhibition of v-ATPase with bafilomycin A1 in 3T3-L1 adipocytes, insulin-stimulated glucose uptake was disrupted and GLUT4 accumulated in intracellular membranes, while Akt and IRS1 signaling were still intact. From these data, it was concluded that proper regulation of endosomal pH is important for the formation of small insulin-responsive vesicles [119]. This has also been studied in cardiomyocytes, and in these cells, v-ATPases in the GLUT4-containing vesicles may play a role in insulin-stimulated increase of GLUT4 translocation and glucose uptake [120, 121]. When examining GLUT1 in mouse mammary epithelial cells, it was found that endosomal acidification was important for directed trafficking of GLUT1 [122].

We recently used the specific v-ATPase inhibitor bafilomycin-A and the proton ionophore monensin to study the role of endosomal acidification in CD36 translocation. While reducing glucose uptake only in the acutely stimulated state, both compounds already increased basal CD36 translocation and subsequent fatty acid uptake [4]. Thus, v-ATPase seems to be involved in stimulus-induced, but not in basal, GLUT4 translocation. This seems to be opposite for CD36 translocation, where v-ATPase is involved in basal, but not in stimulus-induced, processes (Figs. 4 and 5). A novel function of v-ATPase is the modulation of protein traffic between early and late endosomes. Here, vATPase acts as a pH sensor interacting with proteins such as small GTPases of the Arf-family and their regulatory proteins [123]. Whether this function of the v-ATPases is involved in GLUT4 and CD36 translocation is not yet known. However, the proton pump function of the v-ATPases is clearly important since bafilomycin A and monensin similarly affect GLUT4 and CD36 localization.
Taken together, regulation of the endosomal pH seems an interesting target to restore the metabolic substrate balance in type 2 diabetes.

Rab proteins

Rab-GTPases are regulators of multiple steps of vesicular transport (e.g., vesicle formation, transport along the actin- and microtubule-based cytoskeleton and fusion with target membranes) by facilitating the formation of SNARE complexes [124, 125]. Therefore, Rab-GTPases are considered to play a key role in the control of GLUT4 and CD36 vesicle trafficking [126]. Adding to the complexity, 19 isoforms of the more than 60 currently known Rabs are present on GLUT4 vesicles [62]. Examples are Rab4 and Rab11a, which are involved in the intracellular GLUT4 sequestration and endocytosis of both GLUT4 and CD36, respectively [127–129]. Interestingly, Rab11a was activated and recruited to GLUT4 vesicles upon insulin stimulation [130, 131]. In this context, it was also observed that Rab11a shifted from microsomal fractions to the plasma membrane after insulin stimulation. In addition, Rab4 GTP-loading is also stimulated by insulin, and this complex is known to bind syntaxin4, which functions in the docking and fusion of vesicles with the plasma membrane [132, 133]. Two proteins interacting with Rab11a, i.e., FIP2 and Rip11, have been investigated for their role in GLUT4 and CD36 recycling. FIP2, which functions as an adaptor for interaction of Rab11 with the motor protein myosin-Vb [134], mediates endocytosis of both GLUT4 and CD36 in cardiac myoblast cultures [128]. In the same cell system, Rip11, which colocalizes with Rab11 in endosomal membranes [135], is involved in CD36 endocytosis, but does not influence GLUT4 dynamics [128]. However, in cultured adipocytes, Rip11 does influence GLUT4 traffic, indicating a cell type-specific function of this Rab11a regulator [136].

Rab-GTPase activating proteins (Rab-GAPs) silence Rab activity by keeping them in an inactive state [137]. TBC1D1 and TBC1D4, two proteins mentioned as Akt- and AMPK substrates in “Signalling pathways involved in GLUT4 and CD36 translocation”, are Rab-GAPs that silence Rab-function on GLUT4 translocation [62, 138]. By doing so, they restrain GLUT4 in its intracellular stores under basal conditions. Insulin releases this brake on GLUT4 translocation by stimulating Akt-mediated phosphorylation and inactivation of TBC1D1 and 4. This will promote the formation of GLUT4 transport vesicles and eventually their translocation to the plasma membrane [63, 139]. As detailed in “Contraction-signaling”, under conditions of increased contractions, activated AMPK acts analogous to Akt, indicating a common mechanism of GLUT4 mobilization that is shared by different signaling pathways [140]. Which Rab isoforms are regulated by TCB1D1 and TCB1D4, respectively? In vitro Rab-GAP assays revealed a strong activity of both GAPs against Rab2a, -8, -10 and -14, and no activity against the above-mentioned Rab4 and Rab11a [62, 63]. Still, identifying one Rab protein to be the crucial isoform for GLUT4 translocation remains challenging, as Rab8a, -10 and -13 all appear to be essential for complete insulin-dependent GLUT4 translocation to plasma membrane [141–143]. In addition, if and how TBC1D1, TBC1D4 and Rabs are involved in CD36 translocation needs further study.

SNARE proteins

Integration of vesicular GLUT4 and CD36 into the plasma membrane is regulated by soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), the mechanistic core complexes of membrane fusion [144]. Subtypes of the SNARE proteins have been classified depending on the amino acid residue within the SNARE motifs which is either a glutamine (Q-SNAREs) or an arginine (R-SNAREs) [145]. When the vesicle-associated R-SNAREs interact with a specific subset of Q-SNAREs at the target membrane, a hetero-oligomeric SNARE complex is formed which catalyzes the fusion of the vesicle with the target membrane [145].
It is well accepted that insulin-stimulated translocation of GLUT4 in adipocytes, skeletal- and cardiac myocytes involves the R-SNARE VAMP2 [146], which interacts with the Q-SNAREs syntaxin4 and SNAP23 at the plasma membrane (Fig. 4) [147, 148]. We have recently shown that insulin-stimulated CD36 translocation is also dependent on VAMP2 [5]. Another VAMP isoform, VAMP3, links contraction signaling to GLUT4 and CD36 translocation, disclosing a strong resemblance of the mechanisms of GLUT4 and CD36 trafficking [5]. Although present on GLUT4 vesicles, VAMP3 does not translocate to the plasma membrane upon increased contraction, pointing to a rather unclear function of this VAMP isoform in GLUT4 translocation [149]. However, other VAMP isoforms, like VAMP4 and VAMP7, are differentially involved in the regulation of GLUT4 and CD36 traffic and could be the basis for selective regulation of transporter distribution [5]. Still, their physiological function needs to be disclosed.

Concluding remarks

A complex interplay between signaling pathways and trafficking components is involved in the regulation of GLUT4 and CD36 translocation (Fig. 4). The signaling pathways appear to similarly affect GLUT4 and CD36 translocation and thus are unsuitable targets for restoring the improper subcellular localization of both substrate transporters in the diabetic heart, for example by bringing GLUT4 to the cell surface and internalizing CD36. However, the subcellular trafficking machinery is able to discriminate between regulation of GLUT4 and CD36 translocation, and therefore could form the basis of a novel approach to restore cardiac substrate preference in metabolic diseases with altered substrate utilization. Coat proteins are similarly involved in GLUT4 and CD36 translocation. Other trafficking components are differentially involved in both processes. In detail, actin organisation and v-ATPase are specifically involved in GLUT4 translocation, while v-ATPase is specifically involved in CD36 endocytosis. Future experiments should explore the possible disturbance of these subcellular trafficking components in animal models and in biopsies from patients with type 2 diabetes.

Importantly, all these trafficking components that differentiate between GLUT4 and CD36 translocation are novel targets for the development of therapies to restore the metabolic balance in cardiomyocytes during disease characterized by unbalanced substrate usage (e.g., insulin resistance, diabetic cardiomyopathy and heart failure). Another advantage in therapeutically targeting the GLUT4 and CD36 trafficking machineries is that this can be achieved in a tissue-specific manner because many members of the major trafficking protein families display a tissue-specific distribution pattern [144, 150]. Future research will undoubtedly unmask more GLUT4 and CD36-dedicated trafficking proteins that could be added to the list of novel anti-diabetic targets.

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