0163-769X/04/$20.00/0
*Printed in U.S.A.*

Endocrine Reviews 25(2):177–204

doi: 10.1210/er.2003-0011

# Regulated Membrane Trafficking of the Insulin-Responsive Glucose Transporter 4 in Adipocytes

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**Since the discovery of insulin roughly 80 yr ago, much has been learned about how target cells receive, interpret, and respond to this peptide hormone. For example, we now know that insulin activates the tyrosine kinase activity of its cell surface receptor, thereby triggering intracellular signaling cascades that regulate many cellular processes. With respect to glucose homeostasis, these include the function of insulin to suppress hepatic glucose production and to increase glucose uptake in muscle and adipose tissues, the latter resulting from the translocation of the glucose transporter 4 (GLUT4) to the cell surface membrane. Although simple in broad outline, elucidating the molecular intricacies of these receptor-signaling pathways and membrane-trafficking processes continues to challenge the creative ingenuity of scientists, and many questions remain unresolved, or even perhaps unasked. The identification and functional characterization of specific molecules required for both insulin signaling and GLUT4 vesicle trafficking remain key issues in our pursuit of developing specific therapeutic agents to treat and/or prevent this debilitating disease process. To this end, the combined efforts of numerous research groups employing a range of experimental approaches has led to a clearer molecular picture of how insulin regulates the membrane trafficking of GLUT4. (*Endocrine Reviews* 25: 177–204, 2004)**



## I. Introduction

GENERATED BY THE carbon fixation or dark reactions of photosynthesis, glucose is extremely common in nature. Indeed, one of the polymeric forms of glucose, cellulose, is likely the most abundant organic molecule in the biosphere. Therefore, it is not surprising that glucose is the primary energy source used in the animal kingdom, and multiple mechanisms have evolved for its metabolism, synthesis, and storage. Because hydrophilic carbohydrate molecules are generally excluded from the hydrophobic core of cellular membranes, numerous transport mechanisms have evolved to move glucose and other sugars across lipid bilayers (1, 2). For example, bacteria employ several symport, antiport, and uniport systems for the uptake of glucose and other carbohydrates. In contrast, mammals use $Na^+$-dependent cotransporters and facilitative uniporters. Moreover, many bacteria can survive with glucose as their only carbon source, and several mammalian tissues, notably the brain, are dependent on glucose as their primary energy source. In addition, to accommodate the glucose requirements of the

Abbreviations: aPKC, Atypical protein kinase C; APS, adaptor protein containing PH and SH2 domains; ARF, ADP ribosylation factor; CAP, Cbl-associated protein; F actin, filamentous actin; G actin, globular actin; GAP, GTPase activating protein; GEF, guanylnucleotide exchange factor; GLUT, glucose transporter; IKK$\beta$, I$\kappa$B kinase $\beta$; IR, insulin receptor; IRAP, insulin-regulated amino peptidase; IRS, insulin receptor substrate; Myo1c, myosin 1C; NSF, *N*-ethylmaleimide-sensitive fusion protein; PDK1, 3′-phosphoinositide-dependent kinase 1; PH, pleckstrin homology; PI3K, phosphatidylinositol 3-kinase; PI(3,4,5)P3, phosphatidylinositol-3,4,5-trisphosphate; PI(4,5)P2, phosphatidylinositol-4,5-bisphosphate; PKB, protein kinase B; PTEN, phosphate and tensin homolog; SHIP2, SH2-domain-containing inositol 5-phosphate; SNAP, soluble NSF attachment protein; SNARE, SNAP receptor; synip, syntaxin 4-interacting protein; SOCS, suppressor of cytokine signaling; TGN, trans-Golgi network; t-SNARE, target membrane SNARE; VCA region, verpolin, cofilin-like, and acidic region; v-SNARE, vesicle membrane SNARE; WASP, Wiskott-Aldrich syndrome protein.

***Endocrine Reviews* is published bimonthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

brain and other tissues, finely tuned regulatory systems have evolved in mammals to maintain remarkably stable plasma glucose concentrations despite intermittent food intake. Thus, during fasting periods the liver provides sufficient glucose output to maintain normal circulating glucose levels. In contrast, the increases in plasma glucose levels that occur immediately after a meal are minimized by enhanced glucose uptake in skeletal muscle and adipose tissue. This complex interplay between glucose absorption from the gut, output from the liver, and uptake by peripheral tissues is normally exquisitely balanced such that plasma glucose concentrations are maintained at 4–7 mM in humans. Deviations from this narrow range can have severe consequences. For example, low blood glucose levels can lead to seizures, coma, and death. In contrast, prolonged elevated glucose levels, as occurs in the diabetic state, can result in many complications, including renal failure, blindness, neuropathy, and cardiovascular disease.

There are several types of diabetes, with type 1 or insulin-dependent diabetes mellitus and type 2 or non-insulin-dependent diabetes mellitus accounting for the vast majority of pathological states of dysregulated glucose metabolism. Type 1 diabetes mellitus results from the autoimmune destruction of pancreatic β-cells of the islets of Langerhans, resulting in absolute insulin deficiency. In contrast, the hallmark of type 2 diabetes mellitus is peripheral insulin resistance, which refers to the inability of insulin to efficiently stimulate glucose uptake into muscle and adipose tissues and to suppress hepatic glucose production. The progression to overt diabetes in these individuals usually results when enhanced insulin secretion from pancreatic β-cells fails to compensate for peripheral insulin resistance. In addition to environmental risk factors, including diet, age, and exercise habits, type 2 diabetes is a complex polygenic disease. Type 2 diabetes accounts for more than 90% of all cases and is becoming increasing common worldwide, both in developed and developing countries. Basic research into the molecular mechanisms of insulin signaling and glucose uptake thus represents an important research area for the global human community. Since the discovery of insulin in the early 1920s, much has been learned about how this peptide hormone triggers the tyrosine kinase activity of its cell-surface receptor and initiates intracellular signaling cascades. We now know that insulin induces the translocation of the glucose transporter 4 (GLUT4) from intracellular membrane compartments to the plasma membrane, where it catalyzes the uptake of glucose into adipose and muscle cells, the rate-limiting step for glucose metabolism. Research on the mechanisms of insulin-induced GLUT4 translocation has thus progressed toward a convergence of receptor tyrosine kinase-signaling processes and regulated membrane-trafficking events.

## II. The Hexose Transporter Family

### *A. Family members, structure, and function*

In mammals, glucose is cleared from the bloodstream by facilitative transporters (GLUTs), which comprise a family of highly related 12 transmembrane domain-containing proteins (Fig. 1) (3). As facilitative carriers, the GLUT proteins transport glucose down its concentration gradient in an energy-independent manner. Most mammalian cell types are net consumers of glucose and maintain low intracellular glucose concentrations, thus favoring glucose entry. Hepatocytes, however, are net producers of glucose during the periods of reduced food intake (low circulating insulin but high glucagon levels). During fasting periods, hepatic gluconeogenesis and glycogenolysis increase the concentration of intracellular glucose above that in the blood. This results in a net efflux of glucose from the liver and provides the brain and other tissues with a steady supply of glucose despite sporadic food consumption.

An extended family of hexose transporters has evolved to meet the specific metabolic needs of various tissues. The GLUT family contains 13 known members and can be divided into three classes based upon conserved structural characteristics (4). Class I includes GLUTs 1–4, which are, by far, the best characterized transporters of the family. Class II is comprised of GLUT5 (a fructose-specific transporter), and GLUTs 7, 9, and 11, which remain poorly understood (3).

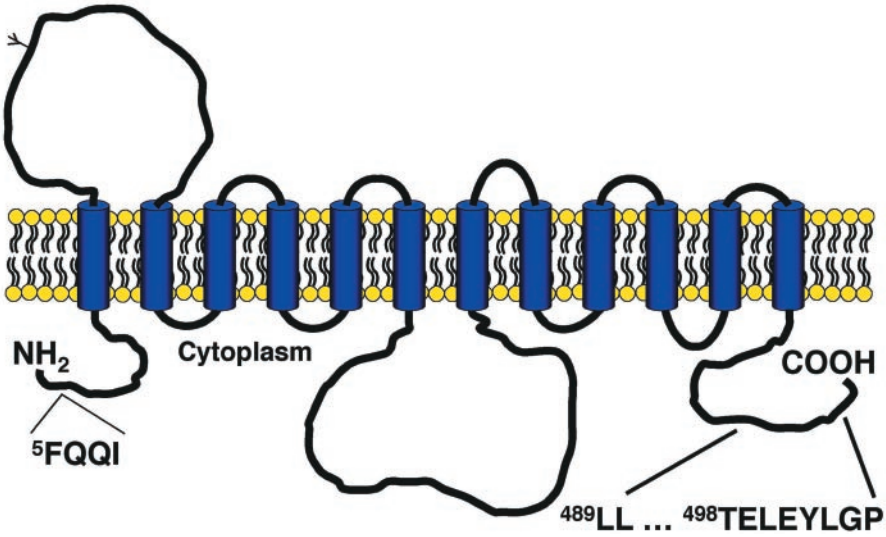


FIG. 1. Schematic diagram of the predicted membrane topology of GLUT4. Other members of the GLUT family also contain 12-transmembrane domains. The amino- and carboxyl-terminal targeting motifs of GLUT4 are indicated. See text for further details.

Class III includes GLUTs 8, 10, 12, and the proton-myoinositol symporter $H^+$-*myo*-inositol cotransporter (HMIT1), all of which are largely uncharacterized (3). Because more is known about the class I GLUTs, we will briefly summarize the properties of GLUTs 1–4.

GLUT1 is expressed to some degree in most tissues; however, it is highly expressed in endothelial cells lining the blood vessels of the brain and in human erythrocytes (5, 6). Indeed, GLUT1 represents approximately 5% of the total erythrocyte membrane protein content, which facilitated its biochemical purification by Kasahara and Hinkle (7) in 1977. GLUT1 is also expressed in adipose and muscle tissue, which are the insulin-responsive sites for glucose disposal. Here, GLUT1 appears to provide a low, constitutive level of glucose transport required for basal cellular processes, probably in combination with several other GLUT isoforms (5). GLUT2 is a low-affinity [high Michaelis-Menten constant ($K_m$)] GLUT present in liver, intestine, kidney, and pancreatic $\beta$-cells (8). This transporter functions as part of the glucose sensor system in $\beta$-cells and in the absorption of glucose by intestinal epithelial cells. GLUT3 is expressed primarily in neurons, and, together, GLUT1 and GLUT3 allow glucose to cross the blood-brain barrier and enter neurons (6). GLUT4 is expressed primarily in striated muscle and adipose tissue and, unlike most other GLUT isoforms, is sequestered in specialized intracellular membrane compartments under basal conditions (9). As the major insulin-responsive GLUT isoform, the study of the structure, function, and regulation of GLUT4 has been a major focus of workers in the diabetes field.

### *B. GLUT4*

*1. Translocation hypothesis.* In 1980, nearly a decade before the molecular identification and cloning of GLUT4, compelling evidence was presented from two independent groups that strongly supported the hypothesis that insulin causes the redistribution of what was then described as "glucose transport activity" from intracellular membrane compartments to the cell surface (10, 11). By measuring glucose binding and transport activity in membrane fractions isolated from rat adipocytes, both groups found that insulin decreased the number and function of GLUTs in the internal membrane fractions while at the same time causing a concomitant increase in transporter function in the plasma membrane fractions. Although these data were consistent with the translocation of a GLUT to the cell surface, without specific antibodies or a cloned transporter cDNA there was no unequivocal, independent means to verify these results. These early experiments, nevertheless, proved sufficient incentive, and in 1989 five independent groups reported the cloning of the cDNA encoding GLUT4 (12–16). This enabled the generation of isoform-specific antibodies and confirmed that the glucose transport activity identified in the 1980 experiments corresponded to the translocation of GLUT4 to the cell surface in response to insulin. Indeed, since its initial proposal, more than two decades of accumulated evidence has consistently supported the translocation hypothesis. However, in addition to stimulating translocation, recent work has suggested that insulin may also regulate the intrinsic transport activity of GLUT4 (reviewed in Ref. 17). Nevertheless, the redistribution of GLUT4 to the plasma membrane in response to insulin remains the dominant paradigm and can be readily visualized using a GLUT4-enhanced green fluorescent protein reporter construct transiently expressed in 3T3L1 adipocytes (Fig. 2). The molecular mechanism by which insulin causes this redistribution of GLUT4 remains unknown and is currently under intense investigation. Indeed, this research area holds promise for the identification of therapeutic agents that target specific molecules functioning proximal to the intracellular GLUT4 storage compartment, thereby bypassing the multifarious upstream insulin receptor (IR)-signaling cascades.

*2. GLUT4 knockout mouse models.* GLUT4 is the only known insulin-responsive GLUT highly and specifically expressed in muscle and adipose tissue, the major sites of postprandial glucose disposal. In addition, overexpression of the human *GLUT4* gene in muscle and fat tissue of the diabetic *db/db* mouse, which lacks the leptin receptor, protected these animals from insulin resistance and diabetes (18). However, the genetic ablation of GLUT4 did not result in severe insulin resistance or diabetes (Table 1). Indeed, although GLUT4-null mice had decreased life spans, were growth retarded, and showed cardiac and adipose tissue abnormalities, they exhibited only mild defects in glucose homeostasis and were not diabetic (19). These results suggest that GLUT4 is not absolutely required for normal glycemic control in rodent model systems. However, it was subsequently observed that male mice heterozygous for GLUT4 ($GLUT4^{+/-}$) showed progressive muscle insulin resistance accompanied by hyperinsulinemia and hyperglycemia, and by 8–12 months exhibited diabetic histopathologies of the heart and liver (20, 21). It remains unclear why the heterozygous $GLUT4^{+/-}$ mice display a more severe phenotype than the homozygous $GLUT4^{-/-}$ mice. However, the $GLUT4^{-/-}$ animals are presumably protected from diabetes by the compensatory upregulation of other GLUT isoforms (22), although this supposition has not yet been demonstrated experimentally.

Rather than globally eliminating or reducing GLUT4 in all tissues, another strategy for studying GLUT4 function in intact animals is the selective disruption of GLUT4 in specific tissues using the Cre-lox system. For example, genetic ablation of the *GLUT4* gene specifically in muscle tissue resulted in insulin resistance and glucose intolerance in mice as young as 8 wk of age (23). Moreover, disruption of GLUT4 specifically in adipose tissue resulted in impaired glucose uptake and hyperinsulinemia (24). Not surprisingly, adipose tissue showed insulin resistance and impaired glucose uptake. However, liver and muscle also developed insulin resistance, despite the fact that GLUT4 was ablated only in fat tissue. In addition, muscle tissue showed normal levels of GLUT4 expression, although insulin-induced glucose uptake was markedly reduced in this tissue. These results suggest that the GLUT4-deficient adipose tissue secretes an unknown factor(s) that travels to liver and muscle via the bloodstream. Thus, it appears that the perturbation of GLUT4 expression levels in specific tissues could be a potential contributing factor for peripheral insulin resistance.

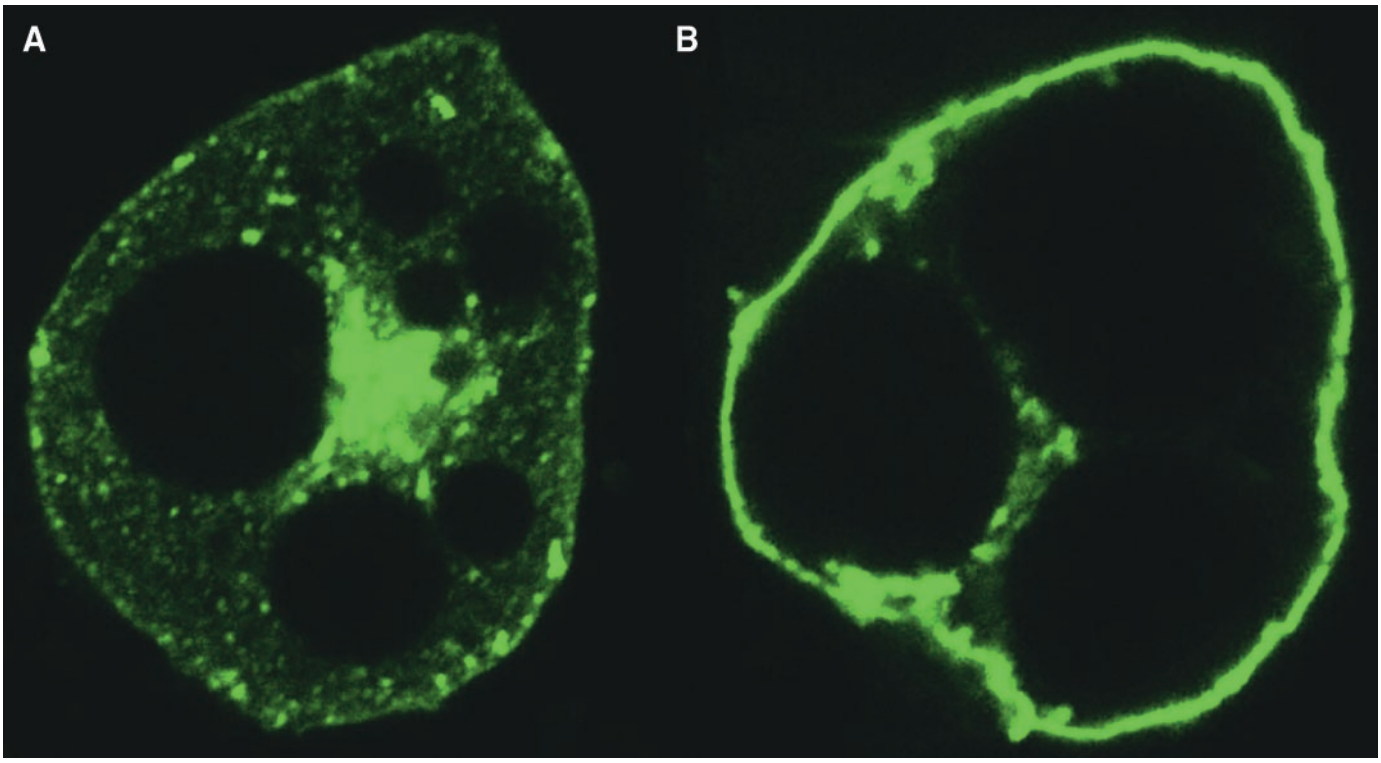


FIG. 2. Insulin stimulation results in the translocation of GLUT4 from intracellular storage sites to the plasma membrane. Differentiated 3T3L1 adipocytes were transfected with a GLUT4-enhanced green fluorescent protein fusion construct and then incubated in the absence (A) or presence (B) of insulin for 30 min. The cells were fixed and subjected to confocal fluorescent microscopy.

## III. Intracellular GLUT4 Storage Compartments

### *A. The specialized GLUT4 storage compartment*

Under basal conditions the majority of GLUT4 is excluded from the plasma membrane of adipose and muscle cells due to efficient endocytosis and intracellular sequestration within insulin-responsive storage compartments. Insulin increases the exocytosis of GLUT4 (9) and slightly inhibits endocytosis (25–28), resulting in an approximately 10- to 20-fold increase in transporter levels at the plasma membrane. Considerable attention has focused on identifying and characterizing the insulin-regulated GLUT4 storage compartment (29). In addition, several groups (30–34) have attempted to enhance our understanding of the compartmentalization and regulated trafficking of GLUT4 through comparisons with the insulin-stimulated secretion of other adipocyte proteins. For example, adipocytes secrete several small soluble proteins in response to insulin, including adipsin, leptin, lipoprotein lipase (LPL), and adipocyte complement related protein of 30 kDa (ACRP30; also known as adiponectin or adipoQ), among others. In all cases studied, GLUT4 localized to membrane compartments that were separate from those occupied by these secretory proteins. It therefore appears that adipocytes harbor several intracellular compartments that respond to insulin. Moreover, insulin stimulation results in about a 2-fold increase in the extent of exocytosis of these secreted proteins. In contrast, insulin causes a dramatic 10- to 20-fold increase in GLUT4 at the plasma membrane. Taken together, these data suggest that GLUT4 is targeted to specific membrane compartments that are exceptionally insulin responsive and that are separate from those occupied by secreted peptides.

Although the existence of a specialized insulin-responsive GLUT4 storage compartment is supported by abundant indirect evidence, it has been very difficult to biochemically or structurally characterize this compartment. In part this is because at steady state GLUT4 is distributed, at varying levels, throughout most of the endomembrane system (35). Indeed, after insulin-stimulated translocation, GLUT4 is retrieved from the plasma membrane by endocytosis and routed through a complex trafficking itinerary that appears to include endosome compartments and the *trans*-Golgi network (TGN), before returning to the insulin-responsive storage compartment. This complex itinerary has made it difficult to reliably distinguish between the insulin-responsive compartment and the general endosome recycling compartments occupied by GLUT4. In addition, there may be multiple interrelated insulin-responsive GLUT4 storage compartments with overlapping properties (36–40).

Nevertheless, several approaches including subcellular fractionation, vesicle immunoabsorption, membrane compartment ablation, and immunoelectron microscopy have begun to distinguish between the general versus specialized GLUT4 recycling compartments. For example, a horseradish peroxidase-transferrin receptor conjugate has been used in compartment ablation studies to show that the insulin-responsive pool of GLUT4 protein partitions into vesicular compartments that are largely separate from those occupied by cellubrevin and the transferrin receptor (41). Moreover, vesicle immunoabsorption experiments have demonstrated

TABLE 1. Mouse models with global or tissue-specific targeted disruptions in some genes considered potentially relevant to the maintenance of glucose homeostasis

| Genotype | Tissue | Phenotype associated with targeted gene disruption | Refs. |
|---|---|---|---|
| GLUT4$^{-/-}$ | Global | Mild insulin resistance, but not overtly diabetic | 19 |
| GLUT4$^{+/-}$ | Global | Muscle insulin resistance; hyperinsulinemia; hyperglycemia; diabetes | 20 |
| GLUT4$^{-/-}$ | Muscle | Insulin resistance; glucose intolerance | 23 |
| GLUT4$^{-/-}$ | Adipose | Adipose, liver, and muscle insulin resistance | 24 |
| IR$^{-/-}$ | Global | Death at 3–7 d after parturition | 80, 81 |
| IR$^{+/-}$ | Global | No major metabolic abnormalities, normal glucose tolerance | 80, 81 |
| IR$^{+/-}$IRS1$^{+/-}$ | Global | Diabetes at 4–6 months | 82 |
| IR$^{-/-}$ (MIRKO) | Muscle | Normal glucose tolerance, increased adipose tissue mass | 83 |
| IR$^{-/-}$ (FIRKO) | Adipose | Protected against age- and diet-induced glucose intolerance; decreased fat tissue mass; increased longevity | 86, 87 |
| IR$^{-/-}$ (LIRKO) | Liver | Reduced glucose tolerance and hyperinsulinemia | 88 |
| IR$^{-/-}$ ($\beta$IRKO) | $\beta$-cell | Impaired first-phase insulin secretion; glucose intolerance | 89 |
| IR$^{-/-}$ (NIRKO) | Neuron | Increased caloric consumption and adiposity; insulin resistance | 90 |
| IRS1$^{-/-}$ | Global | Mild insulin intolerance | 94, 95 |
| IRS2$^{-/-}$ | Global | Diabetes; liver insulin resistance; decreased $\beta$-cell mass | 92, 21 |
| IRS3$^{-/-}$ | Global | No apparent abnormalities | 96 |
| IRS4$^{-/-}$ | Global | Mild growth defects; mild glucose intolerance | 98 |
| p85$^{-/-}$ | Global | Enhanced insulin sensitivity and glucose transport; hypoglycemia | 113, 114 |
| SHIP2$^{-/-}$ | Global | Death shortly after birth; severe hypoglycemia | 117 |
| SHIP2$^{+/-}$ | Global | Improved glucose tolerance; enhanced insulin sensitivity | 117 |
| PKB$\alpha^{-/-}$ | Global | No apparent defects in glucose homeostasis | 138 |
| PKB$\beta^{-/-}$ | Global | Mild glucose intolerance; impaired glucose uptake in skeletal muscle | 135 |
| aPKC$\zeta^{+/-}$ | Global | Impaired immune system function | 148 |
| IKK$\beta^{+/-}$ ob/ob | Global | Protected against diet-induced insulin resistance | 226 |
| PTP1B$^{-/-}$ | Global | Resistance to diet-induced obesity; improved insulin sensitivity | 268, 269 |
| Syntaxin 4$^{-/-}$ | Global | Early embryonic death | 289 |
| Syntaxin 4$^{+/-}$ | Global | Impaired glucose uptake and GLUT4 translocation in skeletal muscle | 289 |
| Cellubrevin$^{-/-}$ | Global | No apparent disruption of normal glucose homeostasis | 295 |

MIRKO, Muscle IR knockout; FIRKO, fat IR knockout; LIRKO, liver IR knockout; $\beta$IRKO, $\beta$-cell IR knockout; NIRKO, neuron IR knockout.

that although some vesicle populations contain both GLUT4 and general endosomal markers, other vesicles are enriched for GLUT4 and appear to exclude endosomal proteins (41–43). Similarly, studies using quantitative immunoelectron microscopy have demonstrated that the insulin-responsive pool of GLUT4 apparently consists of a unique population of vesicles that is largely devoid of constitutively recycling proteins such as the cation-dependent mannose-6-phosphate receptor (CD-MPR) (44). Moreover, cell fractionation studies employing iodixanol (Optiprep) gradients showed that, although GLUT4 was sorted into multiple intracellular compartments, those responsive to insulin were distinct from endosomes or the TGN (45).

### *B. Insulin-regulated amino peptidase (IRAP)*

IRAP was identified as a major protein that colocalizes with GLUT4 in insulin-responsive storage vesicles (Refs. 46–49 and reviewed in Ref. 50). IRAP is a type II integral membrane protein with an amino-terminal cytoplasmic tail of 109 amino acids, a transmembrane domain, and an extracellular domain of 894 amino acids. Interestingly, the cytoplasmic domain of IRAP harbors dileucine and acidic cluster motifs that resemble those in the carboxyl terminus of GLUT4 (51). Membrane aminopeptidases are known to process regulatory peptides, and IRAP has been shown to cleave vasopressin, oxytocin, lys-bradykinin, met-enkaphalin, dynorphin, and angiotensins III and IV (50). In addition, IRAP has recently been identified as the angiotensin IV receptor (52). A possible connection between IRAP and GLUT4 was recently uncovered through the targeted ablation of IRAP (53). Interestingly, the IRAP$^{-/-}$ mice showed decreased levels of GLUT4 protein in skeletal muscle, heart, and adipose tissues, despite being normal for glucose homeostasis (53). Although the mechanism for the observed decrease in GLUT4 protein levels remains to be elucidated, these results nevertheless hint at a potential role for IRAP in regulating GLUT4 expression.

### *C. GLUT4 targeting motifs*

The data described above support a model in which GLUT4 is partitioned into specialized insulin-responsive storage compartments that represent the primary site of insulin action. In addition, this model predicts that GLUT4 contains intrinsic targeting domains that direct its localization to the insulin-responsive compartment. Consistent with this prediction, the membrane compartment distribution of GLUT4 is dramatically different from the very similar GLUT1 transporter (65% amino acid identity), which localizes predominantly to the plasma membrane, even under basal conditions (54). This difference in intracellular localization suggests that the two transporters undergo differential targeting/sorting processes. In addition, when GLUT4 is heterologously expressed in fibroblasts, it is efficiently retained in intracellular compartments. This suggests that GLUT4 may harbor intrinsic targeting signals for efficient sequestration within the cell. Understanding the mechanism

for targeting and retaining GLUT4 within intracellular compartments may provide important insights regarding the molecular mechanism by which insulin mobilizes GLUT4 vesicles. Several groups have therefore examined potential targeting motifs within the GLUT4 protein, both by mutating candidate motifs and by transplanting them onto heterologous reporter constructs (55). Attention has largely focused on the N and C termini of GLUT4, which contain sequence elements that are absent from the GLUT1 protein. However, results from several laboratories have been controversial and at times conflicting. For example, an early study found that neither the N nor the C termini contributed to the overall trafficking dynamics of GLUT4 in 3T3L1 adipocytes (56). Instead, two domains, one between residues 80 and 101 and the other between residues 316 and 343, were found to contribute to the intracellular sequestration of GLUT4. In contrast, a significant body of research has generally supported a role for either the N or the C termini in the intracellular trafficking of GLUT4 (Fig. 1). In general, the interpretation of these data is complicated by the fact that various researchers have used different cell lines and different heterologous GLUT4 chimeric constructs, making direct comparisons among investigators challenging.

*1. The amino-terminal targeting motif.* Studies carried out in the early 1990s suggested that a cytosolic N-terminal FQQI motif was important for the intracellular sequestration of GLUT4. Mutations in this sequence, particularly the aromatic phenylalanine residue, resulted in the accumulation of GLUT4 at the plasma membrane (57). Furthermore, when the FQQI motif was transplanted onto GLUT1 or the H1 subunit of the asialoglycoprotein receptor, the resulting heterologous reporter constructs were excluded from the plasma membrane and were instead retained in intracellular compartments (57). Initially, these results were interpreted as evidence that the FQQI motif played a necessary and sufficient role in targeting GLUT4 to intracellular compartments. However, by closely examining the endocytotic behavior of GLUT4-chimeric constructs, it was subsequently discovered that the FQQI motif functions during the endocytosis of GLUT4 from the plasma membrane, rather that in its intracellular retention *per se* (58, 59). Because wild-type GLUT4 undergoes efficient endocytosis, in the absence of insulin the transporter is primarily localized to intracellular compartments. Because mutations in the FQQI motif inhibited internalization, over time GLUT4 accumulated at the plasma membrane under basal conditions. Conversely, transplanting the FQQI motif led to the efficient internalization of heterologous reporter constructs from the plasma membrane, resulting in an intracellular localization under steady-state conditions.

Although the FQQI motif is required for efficient endocytosis, some mutations in this motif do not completely block the uptake of reporter constructs. Thus, it has been possible to investigate whether the FQQI motif plays additional roles in intracellular GLUT4 vesicle trafficking. For example, it was recently shown that the FQQI motif functions in the sorting of internalized cell surface GLUT4 protein into specific intracellular compartments (60). Thus, mutation of FQQI to SQQI caused the mislocalization of the mutant transporters to late endosome and lysosome compartments. Moreover, it was independently shown that mutation of the FQQI to AQQI also caused GLUT4 to localize to endosomes (in this case recycling endosomes) (61). These results suggest that, in addition to playing an important role in endocytosis, the FQQI motif may also function during the intracellular trafficking of GLUT4 from endosome compartments into the insulin-responsive perinuclear storage compartment. However, these results must be interpreted with caution because the mutant transporters may be incorrectly folded and thus targeted for degradation in lysosomes. In addition, these data are based on the steady-state distribution of reporter constructs and may therefore reflect net changes in the trafficking rate constants between membrane compartments of the normal trafficking pathway, rather than mislocalization to compartments not normally occupied by the transporter.

*2. Carboxyl-terminal targeting motifs.* In addition to the N-terminal FQQI motif, the cytoplasmic C-terminal 30-amino-acid region of GLUT4 was also initially found to be essential for the intracellular localization of the transporter (61–63). However, similar to the FQQI sequence described above, subsequent work demonstrated that a dileucine (LL) motif located within this region functioned during the endocytosis of GLUT4 from the plasma membrane (64, 65). Thus, mutating the LL motif to AA significantly inhibited internalization, resulting in the cell-surface accumulation of the mutant transporter. Moreover, studies employing chimeric proteins consisting of the extracellular domain of the transferrin receptor fused to the intracellular dileucine motifs of either GLUT4 or IRAP also support a role for this sequence during endocytosis (66). In addition to its role in endocytosis at the plasma membrane, the LL motif has also been reported to function during exit of GLUT4 from the TGN (60, 67). However, a recent study using primary adipocytes isolated from rat epididymal fat pads found that mutation of the LL motif resulted in no detectable alteration in the trafficking of a GLUT4 reporter construct (58). The apparent conflicting results regarding the LL motif may reflect important differences in the trafficking properties of GLUT4 reporter constructs expressed in different cell types.

Other studies utilizing GLUT1/GLUT4 chimeras stably expressed in 3T3L1 adipocytes found that the carboxyl-terminal 30 amino acids of GLUT4 were sufficient to correctly target chimeras to the insulin-responsive compartment (68). However, although the LL motif within this 30-amino-acid region was important for endocytosis from the plasma membrane, this motif was not critical for targeting chimeric constructs to the insulin-responsive compartment. These data are consistent with recent results implicating an acidic cluster motif (TELEYLGP) in GLUT4 trafficking. The TELEYLGP sequence is located downstream of the LL motif and appears to function in the targeting of GLUT4 from endosomes to a subdomain of the TGN enriched in the t-SNARE proteins syntaxins 6 and 16 (51, 70). Once recycled from the cell surface back to the TGN, GLUT4 may undergo additional sorting processes, perhaps involving syntaxins 6 and 16, before becoming insulin responsive (70). However, further work is needed to clarify the functional roles of syntaxin 6 and 16 in GLUT4 sorting processes.

## IV. Regulated GLUT4 Exocytosis

### *A. The IR: structure and function*

The IR is a member of the family of transmembrane receptors with intrinsic tyrosine kinase activity (71). Encompassing more than 120 kb, the IR gene harbors 22 exons, 11 of which encode the α-subunit, with the remaining 11 exons encoding the β-subunit. Synthesized as a single polypeptide, the proreceptor is cleaved in the TGN by the serine protease furin. The mature cell surface IR is composed of two extracellular α- and two transmembrane β-subunits disulfide linked into an $\alpha_2\beta_2$-heterotetrameric structure (Fig. 3) (72, 73). Moreover, owing to alternative splicing of exon 11 of the IR transcript, the human IR exists in two isoforms, IR-A and IR-B, that differ by the inclusion (IR-B) or exclusion (IR-A) of 12 amino acids at the carboxyl terminus of the α-subunit (74). IR-A is expressed predominantly in the developing fetus as well as a few adult tissues, including pancreatic β-cells (Refs. 75 and 76; reviewed in Refs. 77 and 78). In contrast, IR-B is expressed predominantly in adult tissues and mediates the metabolic effects of insulin in muscle, adipose, and liver. During development, IR-A is controlled mainly by IGF-II. In adult tissues, insulin is the major ligand for IR-B; however, in β-cells insulin appears to activate both IR-A and IR-B and may regulate different cellular processes through these receptor isoforms. Regardless of the receptor isoform, insulin binds to the extracellular α-subunits and generates a conformational change that allosterically regulates the intracellular β-subunit tyrosine kinase domain. Subsequently, the β-subunits undergo a series of intermolecular *trans*-autophosphorylation reactions that generate multiple phosphotyrosine sites, some of which serve distinct functional roles (73). For example, tyrosine phosphorylation at the juxtamembrane Y960 residue is necessary for appropriate substrate recognition (*e.g.*, insulin receptor substrates, IRS1–4), whereas tyrosine phosphorylation at residues Y1146, Y1150, and Y1151 in the kinase activation domain relieves pseudosubstrate inhibition, thereby further enhancing the receptor's tyrosine kinase activity (73).

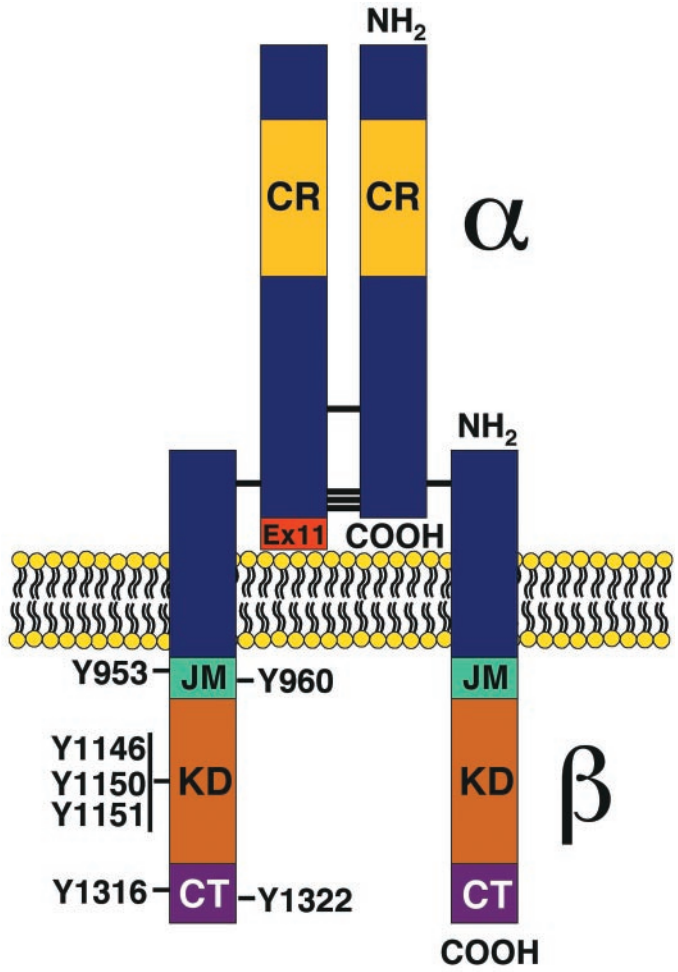


FIG. 3. Structure of the IR. The *left side* depicts IR-B, which includes the 12-amino acid alternatively spliced exon 11 (Ex11) at the carboxyl terminus of the α-subunit. The *right side* depicts IR-A. The extracellular α- and intracellular β-subunits are indicated. The *horizontal black bars* represent disulfide linkages. CR, Cysteine-rich domain; JM, juxtamembrane domain; KD, kinase domain; CT, carboxyl-terminal domain. The positions of the tyrosine autophosphorylation sites are indicated. [Adapted from L. Jongsoon and P. F. Pilch: *Am J Physiol* 266:C319–C334, 1994 (361) and P. De Meyts and J. Whittaker: *Nat Rev Drug Discov* 1:769–783, 2002 (362).]

### *B. The IR: knockout mouse models*

Mutations in the human IR are extremely rare and account for less than 5% of all cases of type 2 diabetes. Nevertheless, through genetic manipulation of the IR in mice, much has been learned about the roles specific tissues play in the pathophysiology of insulin resistance (79) (Table 1). Whereas homozygous knockout of the IR caused death at 3–7 d post parturition, heterozygous mice showed no major metabolic abnormalities and were normal for glucose tolerance (80, 81). However, mice doubly heterozygous for null alleles of the IR and IRS1 (IRS1 null animals are mildly insulin intolerant) developed frank diabetes at 4–6 months of age (82). In addition, using the Cre-lox system, a range of tissue-specific IR knockouts has been generated, sometimes with surprising results. For example, although skeletal muscle accounts for more than 80% of postprandial glucose disposal, the ablation of the IR specifically in muscle resulted in normal glucose tolerance (83). How is glucose cleared from the bloodstream in this situation? These animals have increased adipose tissue mass and appear to compensate, at least in part, with increased glucose uptake in their excess fat reserves. In addition, an increase in both IGF receptor signaling and exercise-mediated signaling has also been reported in muscle lacking the IR (84, 85). In contrast, ablation of the IR specifically in adipose tissue resulted in a protective effect against age- and diet-induced obesity and a marked resistance to the development of glucose intolerance (86). These animals also had decreased overall fat tissue mass, with a polarized distribution of large and small adipose cells. Intriguingly, the fat-specific IR knockout mice also showed increased life span in the absence of caloric restriction (87). Knockout of the IR specifically in liver resulted in reduced glucose tolerance and hyperinsulinemia (88). The β-cell IR is part of the glucose-sensing mechanism, and knockout of the receptor in this cell type resulted in a dramatic decrease in glucose-stimulated first-phase insulin secretion accompanied by progressive,

age-dependent glucose intolerance (89). Finally, ablation of the IR specifically in neurons resulted in increased caloric consumption, increased adiposity, and insulin resistance (90). Together, these studies shed light on how insulin-responsive tissues functionally interact to maintain glucose homeostasis in intact model organisms, and illustrate how insulin resistance can potentially result from the combinatorial effects of impaired insulin action in specific tissues.

### *C. The IR: complex signaling networks*

Many receptor tyrosine kinases directly recruit downstream effector molecules to their phosphorylated cytoplasmic domains. In contrast, the insulin receptor phosphorylates several scaffolding proteins that in turn recruit various downstream effector proteins (91). A number of scaffolding proteins downstream of the activated IR have been identified, including the four members of the insulin receptor substrate family (IRS1, -2, -3, and -4), Gab1, Shc, SIRPS, Cbl, and adaptor protein containing PH and SH2 domains (APS). The best studied of these scaffolding proteins is the IRS family (92), and the genetic ablation of IRS2 resulted in a diabetic phenotype (93). This resulted from a combination of peripheral insulin resistance and impaired $\beta$-cell function, similar to what occurs in typical type 2 diabetes. IRS1 knockouts showed mild insulin intolerance (94, 95), whereas ablation of IRS3 resulted in no apparent abnormalities (96). However, IRS3 does not appear to be functional in humans (97). IRS4 knockout mice showed mild growth defects and were slightly glucose intolerant (98). The insulin-dependent tyrosine phosphorylation of IRS proteins generates docking sites for several downstream effectors, including the p85-regulatory subunit of the type 1A phosphatidylinositol 3-kinase (PI3K), the SH2-containing protein tyrosine phosphatase (SHP2), the Src family member kinase Fyn, and the small adapter proteins Grb2 and Nck (91). Although the precise signaling function of these various adapters/effectors remains poorly understood, they appear to serve distinct yet overlapping biological roles in insulin signaling. For example, Grb2 primarily functions in Ras activation by insulin through engagement of the Ras guanylnucleotide exchange factor Son-of-Sevenless (SOS). This pathway leads to ERK activation (via Raf and MAPK kinase) and is an important cascade regulating several transcription events and eventually mitogenesis. In addition, Ras can also regulate the activity of the PI3K through interactions with its p110 catalytic subunit (99). The PI3K functions in many cellular responses, including the regulation of transcription, mitogenesis, antiapoptosis, protein synthesis, glycogen synthesis, and glucose transport (100–102). Moreover, PI3K functions in the regulation of the actin cytoskeleton, which is under the control of Rac and Rho family members (103). Defining the interrelationship between all these possible signaling events and the *in vivo* specificity required for a defined biological response represents a challenging problem for future investigators. Here our discussion will be limited to two independent signaling pathways that function downstream of the IR to regulate GLUT4 translocation (Fig. 4). A key player of the first, and more established, pathway is PI3K. The second pathway is characterized by the APS/CAP (Cbl-associated protein)/Cbl complex, which is compartmentalized at specialized microdomains at the plasma membrane.

### *D. The PI3K and GLUT4 translocation*

Among the many PI3K isoforms, the class 1A enzymes have been directly implicated in insulin signaling leading to the translocation of GLUT4 in adipocytes. Class 1A PI3Ks function as heterodimers and are composed of a regulatory p85 subunit and a catalytic p110 subunit (101, 102). The p85 subunit binds phosphotyrosine residues through SH2 (Src homology 2) domain interactions and allosterically regulates the catalytic activity of the p110 subunit. PI3Ks catalyze phosphorylation of the 3′-OH moiety of membrane myoinositol lipids (100). Although PI3K can phosphorylate several substrates, the type 1A isoforms prefer the substrate phosphatidylinositol-4,5-bisphosphate [PI(4,5)P2] to generate phosphatidylinositol-3,4,5-trisphosphate [PI(3,4,5)P3] (100).

Insulin stimulation results in the tyrosine phosphorylation

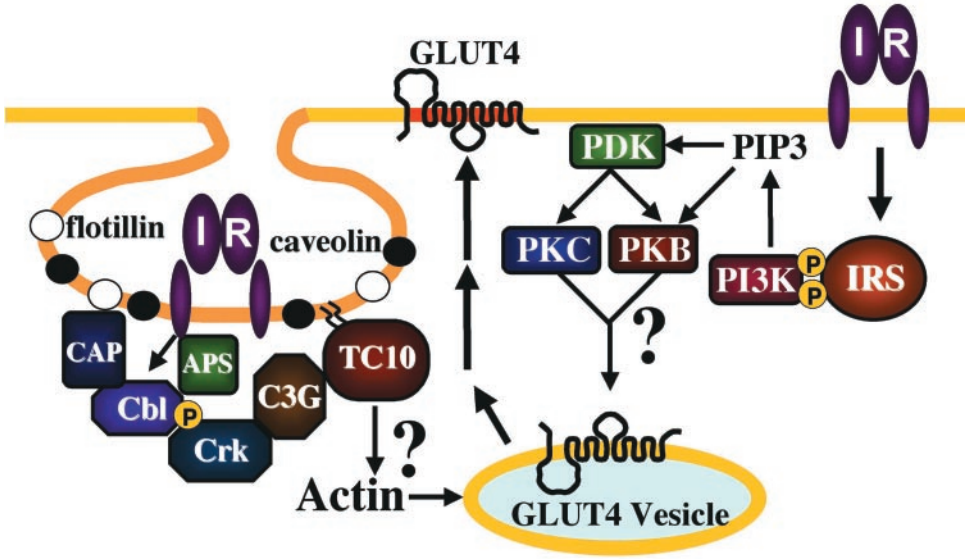


FIG. 4. Schematic model for the interrelationship between the two IR-mediated signaling pathways. Insulin stimulation results in the tyrosine phosphorylation of IRS proteins, which induces the association, activation, and/or targeting of the PI3K. Production of PI(3,4,5)P3 results in the recruitment and activation of PDK1, which phosphorylates and activates both aPKCs and PKB. In parallel, insulin appears to phosphorylate Cbl through the adaptor protein APS. CAP also associates with Cbl and targets it to lipid raft microdomains through its interaction with the caveolar protein flotillin. Cbl then recruits the CrkII-C3G complex, resulting in the regulation of the lipid raft-associated Rho GTP-binding protein, TC10.

of the IRS proteins generating docking sites for the SH2 domains of the p85-regulatory subunit (78). This interaction apparently provides a dual function by both activating the PI3K and targeting it to the plasma membrane localized substrate PI(4,5)P2. In turn, the local increase in PI(3,4,5)P3 in the membrane provides a lipid-based platform for the recruitment and anchorage of downstream signaling molecules that contain pleckstrin homology (PH) domains (100). PH domains are 100- to 120-amino acid modules with a characteristic $\beta$-sandwich fold that binds, with varying degrees of specificity and affinity, to membrane phosphoinositides (104). Although most PH domains studied to date preferentially bind various phosphoinositides, the PH domain of IRS1 has been found to mediate interaction with a recently identified protein termed PHIP for PH domain-interacting protein (105). Interestingly, this interaction appears to be required for several insulin-dependent processes, including GLUT4 translocation.

In any case, it has been well established that the insulin-stimulated production of PI(3,4,5)P3 is essential for GLUT4 translocation. For example, inhibition of PI3K activity with relatively selective pharmacological inhibitors (wortmannin and LY29004) prevents insulin-dependent GLUT4 translocation (106–108). Similarly, introduction of PI3K-blocking antibodies or expression of dominant-interfering PI3K mutants also inhibits insulin-stimulated GLUT4 translocation (109, 110). On the other hand, expression of a constitutively active p110 subunit or addition of a PI(3,4,5)P3 analog to cells induces GLUT4 translocation, albeit not to the same extent as insulin (111, 112). Surprisingly however, p85-regulatory subunit knockout mice paradoxically display enhanced insulin sensitivity and increased glucose transport in adipose and skeletal muscle, leading to a hypoglycemic phenotype (113, 114). This appears to result, at least in part, from the preferential use of the p50$\alpha$-regulatory subunit splice variant in mice lacking full-length p85. It has also been suggested that the p85 subunit may function as a competitive inhibitor of PI3K signaling in the monomeric state (115). Reduced levels of p85 may thus improve the p85/p110/IRS stoichiometry and enhance insulin signaling through this pathway.

Additional evidence favoring PI3K function in insulin action comes from investigations on the mechanisms for inactivating the PI(3,4,5)P3 second messenger. Attenuation of PI3K signaling occurs through the activity of type-II SH2-domain-containing inositol 5-phosphatase (SHIP2), a phosphatase that removes the 5′-phosphate from PI(3,4,5)P3, generating phosphatidylinositol-3,4-bisphosphate [PI(3,4)P2] (116). SHIP2 negatively regulates insulin signaling, and its genetic ablation resulted in enhanced insulin sensitivity (117). Although SHIP2-null animals died shortly after birth and were severely hypoglycemic, heterozygotes showed improved glucose tolerance and enhanced insulin sensitivity. This appeared to be due, at least in part, to enhanced insulin-dependent recruitment of GLUT4 to the plasma membrane (117). In contrast to SHIP, phosphatase and tensin homolog (PTEN) is a 3′-specific PI(3,4,5)P3 phosphatase that generates PI(4,5)P2, and overexpression of PTEN prevents the accumulation of PI(3,4,5)P3 and also inhibited insulin-stimulated GLUT4 translocation in 3T3L1 adipocytes (118). Together, these data provide compelling evidence that the PI3K lipid product, PI(3,4,5)P3 is an essential intermediate in mediating insulin-stimulated GLUT4 translocation.

### *E. Protein kinase B (PKB) and atypical protein kinase C (aPKC)*

Insulin rapidly activates the serine/threonine kinase PKB (also called Akt). This occurs through PI3K activity and the generation of PI(3,4,5)P3, which recruits PKB to the plasma membrane through specific interactions with its amino-terminal PH domain (119). At the plasma membrane, PKB is activated when two key residues are phosphorylated, threonine 308 (T308) and serine 473 (S473) (119, 120). T308 lies within the kinase activation loop and is phosphorylated by another PH domain-containing kinase, the 3′-phosphoinositide-dependent kinase 1 (PDK1) (121). The carboxyl-terminal PH domain of PDK1 binds PI(3,4,5)P3 with high affinity, which also recruits PDK1 to the plasma membrane (121). Although PDK1 appears to be maintained in an active, phosphorylated state even under basal conditions, binding of PI(3,4,5)P3 may also potentiate the catalytic activity of PDK1 (122, 123). S473 is located near the carboxyl terminus of PKB and may undergo autophosphorylation (124). Alternatively, there has been an extensive search for a putative PDK2, and several kinases including the integrin-linked kinase, MAPK-activated protein kinase 2, protein kinase C-related kinase 2, and others have been implicated in the phosphorylation of S473 (reviewed in Ref. 119).

Although the precise activation mechanism of PKB remains unclear, results from cell culture systems have generally supported a role for PKB function in insulin-stimulated GLUT4 translocation (125–130), although others have reported evidence against such a role (131, 132). Nevertheless, stable expression of a constitutively active membrane-bound form of PKB in 3T3L1 adipocytes resulted in increased glucose transport and persistent localization of GLUT4 to the plasma membrane (72, 128, 133). In addition, coexpression of an epitope-tagged GLUT4 with dominant-interfering PKB mutants was also reported to inhibit insulin-stimulated GLUT4 translocation (134). Moreover, there are three PKB isoforms, $\alpha$, $\beta$, and $\gamma$, and knockout of PKB$\beta$ resulted in mild glucose intolerance, impaired glucose uptake in skeletal muscle, and the failure of insulin to suppress hepatic glucose production (135–137). In contrast, the ablation of PKB$\alpha$ resulted in no observable defects in glucose homeostasis (138). These results are consistent with an important role for PKB$\beta$ in insulin signaling and glucose uptake. However, although numerous substrates are known for PKB (119, 120, 139), how PKB activity influences GLUT4 translocation remains undetermined (139).

In addition to PKB, the aPKC isoforms, $\zeta$ and $\lambda/\iota$, have also been implicated in insulin-dependent GLUT4 translocation (see Ref. 140 for discussion of isoforms). Unlike the conventional and novel PKCs, aPKCs are not activated by diacylglycerol. However, although aPKCs lack PH domains, PI(3,4,5)P3 and other acidic phospholipids bind to the regulatory domains of these kinases and apparently induce conformational changes that lead to aPKC activation (141, 142). Activation of aPKC activity has also been reported to occur through autophosphorylation, phosphorylation by PDK1,

and relief from pseudosubstrate inhibition (143, 144). As with PKB, results from cell culture experiments also support a role for aPKC-ζ and -λ/ι in insulin-regulated GLUT4 translocation (140). These include inhibition of aPKC activity with a pseudosubstrate peptide (145) and with general PKC inhibitors (145, 146). In addition, expression of kinase-dead or activation-resistant aPKCs inhibited GLUT4 translocation and glucose transport, whereas expression of constitutively active aPKCs promoted glucose transport (131, 147). However, knockout of the aPKCζ isoform resulted in mice without gross abnormalities but with some impairment of immune system function (148).

### *F. A second insulin-signaling pathway regulating GLUT4 translocation?*

Although PI3K appears to be necessary for insulin-stimulated GLUT4 translocation, substantial evidence indicates that the generation of PI(3,4,5)P3 is not sufficient to recruit GLUT4 to the plasma membrane (Refs. 112, 149–152; reviewed in Ref. 153). Furthermore, expression of an active PDK1 mutant was found to phosphorylate and activate both PKB and aPKCζ/λ without any significant effect on glucose uptake (154). Together, these data imply that additional signaling pathways may function in parallel with the PI3K pathway. Recently, a candidate second signaling cascade that is functionally organized within caveolin-enriched lipid raft microdomains has been partially characterized (Fig. 4) (155–159). Caveolae are 50- to 100-nm invaginations of the plasma membrane and represent a subset of lipid raft domains (160). In addition to the caveolin and flotillin structural proteins, these liquid-ordered domains are enriched in palmitoylated signaling proteins, glycosylphosphatidylinositol (GPI)-anchored proteins, glycolipids, sphingolipids, and cholesterol (161). Lipid rafts are thought to spatially organize signaling cascades, and the tyrosine kinase activity of the IR is reportedly enhanced by virtue of its association with caveolae (162). In addition, it has recently been shown that the tyrosine phosphorylation of another IRS, the protooncogene c-Cbl, resulted in the insulin-dependent recruitment of Cbl into caveolin-enriched lipid raft compartments (155). This appears to involve two adapter proteins, APS and CAP (163, 164). CAP contains three SH3 domains that stably interact with proline-rich regions of Cbl. The CAP/Cbl heterodimer is recruited to activated IRs through interactions with APS (164). APS is also an IRS, and tyrosine-phosphorylated APS facilitates the phosphorylation of Cbl by the IR (164–166). Once Cbl is phosphorylated, the CAP/Cbl heterodimer appears to dissociate from the IR and accumulate in lipid raft domains through interactions between the Sorbin homology (SoHo) domain of CAP and the caveolae-resident protein flotillin. Dominant interfering mutants of CAP that prevent the localization of Cbl to lipid rafts specifically block insulin-stimulated GLUT4 translocation and glucose uptake, without affecting the PI3K signaling pathway (155).

Recent work has shown that Cbl recruitment to several activated tyrosine kinase receptors leads to receptor ubiquitination and down-regulation (167–169). In contrast, in the insulin signaling pathway, the phosphorylated and raft-associated Cbl recruits the SH2-containing adaptor protein CrkII to lipid microdomains. CrkII exists in a heterodimeric complex with C3G, a guanylnucleotide exchange factor (GEF) capable of activating the Rho-family GTPase TC10 (156). Recent results from cell culture experiments have implicated TC10 in both insulin-dependent and osmotic shock-induced GLUT4 translocation and glucose uptake (156, 157, 170, 171). Most Rho family members undergo geranylgeranylation and interact with GDP dissociation inhibitors. However, TC10 has a CAAX targeting domain very similar to H-Ras and is likely to be lipid modified with farnesyl and palmitoyl moieties. These posttranslational modifications target TC10 specifically to lipid raft domains (157, 170). Thus, the insulin-dependent recruitment of Cbl to lipid rafts results in the stepwise assemblage of several adapter proteins that function to target C3G to lipid microdomains. Because TC10 is a resident component of lipid rafts, this results in the insulin-dependent activation of TC10 by C3G. Indeed, the experimental mistargeting of TC10 into nonlipid-raft regions of the plasma membrane prevents its activation by insulin and abrogates the ability of TC10 to modulate insulin-stimulated GLUT4 translocation (157). Furthermore, disruption of lipid rafts with cholesterol-extracting drugs or by expression of inhibitory forms of caveolin effectively blocks insulin-dependent TC10 activation and GLUT4 translocation (157). Together, these results suggest that TC10 may be a key molecule in a signaling cascade that functions independently of the PI3K pathway. Nevertheless, a key question remains: How is insulin-dependent TC10 activation linked to GLUT4 translocation? Rho-family proteins are well known for their roles in controlling actin dynamics, and TC10 directly interacts with several effector molecules that regulate actin cytoskeletal function. The potential for TC10 to regulate the actin cytoskeleton during insulin-dependent GLUT4 translocation is discussed below in *Section VI*.

## V. Role of the Cytoskeleton in Insulin-Stimulated GLUT4 Translocation

It is generally recognized that the cell cytoskeleton plays important roles in various membrane trafficking events and in the retention of organelles at specific locations within cells (172–175). In the case of GLUT4, the integrity of the GLUT4 storage compartment is disrupted by cytoskeleton-perturbing reagents such as nocodazole (for microtubules) and latrunculin (for actin filaments) (176, 177), and subsequently insulin-induced glucose uptake and GLUT4 translocation are severely impaired by these treatments (158, 177–184). Nevertheless, recent studies have revealed the importance of the actin cytoskeleton on the acute metabolic actions of insulin (185, 186). Furthermore, the PI3K and TC10 insulin signaling pathways have profound influence on the actin cytoskeletal organization in various cell types (111, 187). Although a substantial amount of evidence strongly supports a critical role of the cytoskeleton in insulin-induced GLUT4 translocation, the precise functional roles and cytoskeleton-regulatory mechanisms remain enigmatic.

### *A. Actin*

Monomeric globular actin (G actin) is highly conserved and is the most abundant cytoskeletal protein in mammalian

cells. G actin polymerizes in a head to tail fashion to form filamentous actin (F actin) (188). Currently, more that 150 actin-binding proteins have been identified, which not only modulate the behavior of actin but also directly impinge upon the function of intracellular signaling cascades (189, 190). Stimuli such as attachment to the extracellular matrix and growth factors including insulin integrate these various actin-binding proteins to regulate the actin-based cytoskeletal system. Spatial and temporal regulation of dynamic actin rearrangements play important roles in a wide array of cell functions such as motility, chemotaxis, cytokinesis, phagocytosis, and intracellular vesicle trafficking, as well as for cell polarity and differentiation processes (191–194).

In fibroblasts, F actin is well known to form stress fibers, lamellipodia, and filipodia. However, the actin cytoskeleton is dramatically changed during the differentiation of preadipocytes (fibroblast-like) into adipocytes (Fig. 5). Although preadipocytes contain well-defined stress fibers, after differentiation into adipocytes this F actin converts to a cortical actin lining the inner face of the cell surface membrane (158, 195). Concurrent with the changes in F actin during this differentiation process, the levels of caveolin mRNA and protein expression increase 20-fold, and the number of caveolae increases 10-fold (196, 197). This marked induction of caveolin, and hence caveolae, results in the clustering of the individual caveolae (50–80 nm) into large ring-like arrays (caveolae rosettes) that can be visualized by fluorescent microscopy (157, 195, 197, 198). A recent report demonstrates that these caveolae-rosette structures are a complex of both caveolae and other membrane components that form large caves (199). Intriguingly, fully differentiated 3T3L1 adipocytes display patches of punctate F actin that emanate from the organized caveolae-rosette/cave structure composing the relatively thick cortical actin (Fig. 5) (158, 195). In addition, the F actin localized along the inner circumference of the caveolae-rosettes/caves disappears after the disruption of caveolae by depleting cholesterol from the cells. This unique F actin structure has therefore been designated as caveolin-associated F actin (Cav-actin) (195).

Currently, the molecular basis underlying the conversion from stress fiber type F actin to the Cav-actin structure in adipocytes has not been identified. However, this dramatic structural change suggests that actin modulators and their regulatory mechanisms are essential for normal adipocyte function. Indeed, the insulin-induced rapid stress fiber break down and appearance of lamellipodia/membrane ruffling are observed only in predifferentiated and relatively immature 3T3L1 cells, whereas fully differentiated 3T3L1 adipo-

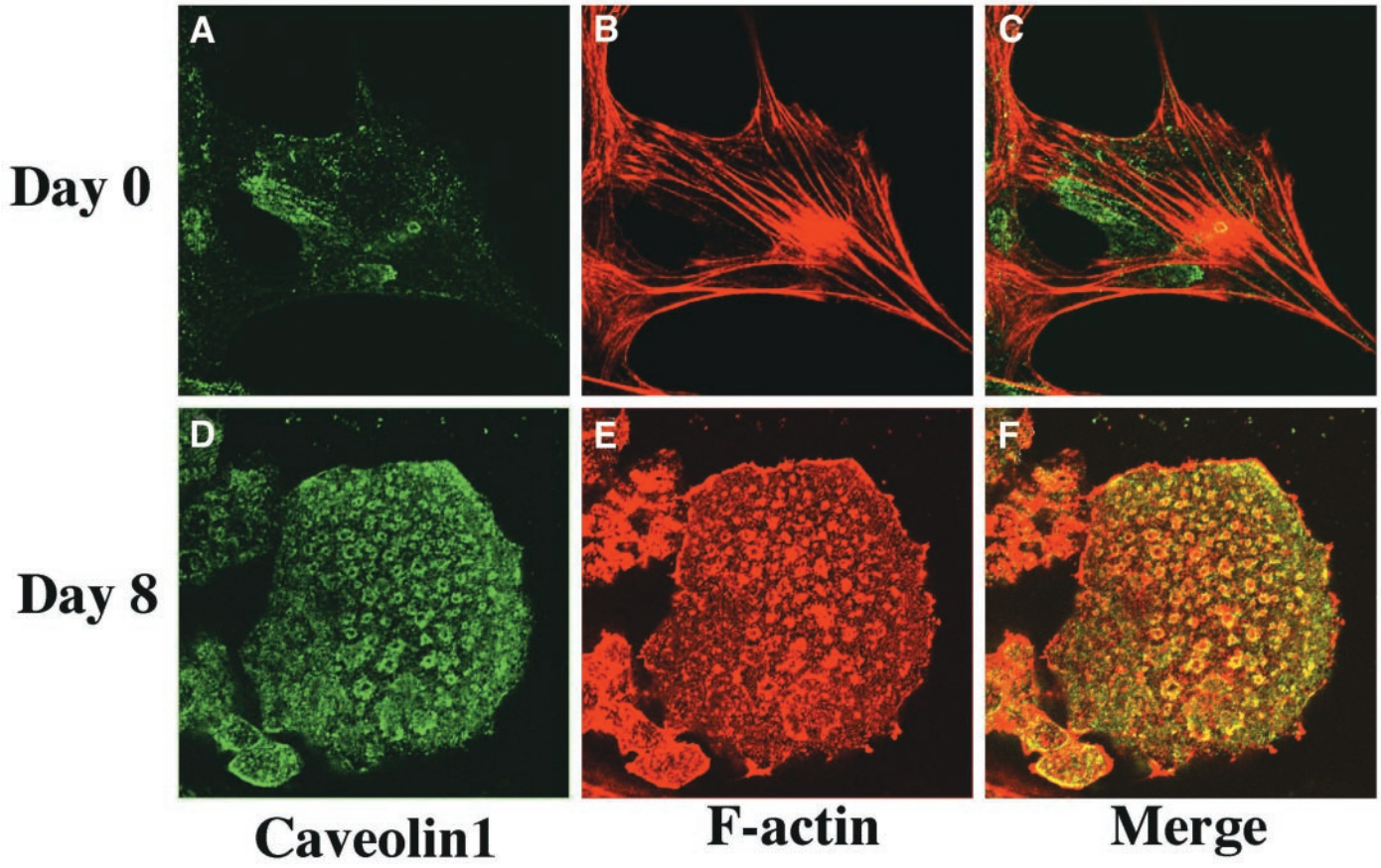


FIG. 5. Adipocyte-differentiation is accompanied by reorganization of F actin. 3T3L1 Fibroblasts express relatively low levels of caveolin 1, which is dispersed along the plasma membrane (panel A). Phalloidin labeling of F actin demonstrates that these cells display typical stress fibers (panel B) that do not colocalize with caveolin 1 (panel C). In contrast, after adipocyte differentiation, caveolin 1 expression is induced and is concentrated in large ring-like rosette structures at the plasma membrane (panel D). F actin is also remodeled and concentrates at the sites of the caveolin 1 rosettes (panels E and F). [Adapted with permission from M. Kanzaki and J. E. Pessin: *J. Biol. Chem.* 277:25867–25869, 2002 (195).]

cytes do not display pronounced lamellipodia/membrane ruffling (158). Similarly, isolated primary rat adipocytes do not have significant amounts of stress fibers, lamellapodia, or filopodia but instead they have a layer of cortical F actin and diffuse reticular F actin near the cell pole (181).

The role of F actin in relation to insulin action has been examined using actin depolymerizing agents such as cytochalasin D, latrunculin A and B, and all these compounds inhibited insulin-induced glucose uptake and GLUT4 translocation (178–182). More recently, using enhanced yellow fluorescent protein-tagged G actin and real-time imaging techniques, insulin was shown to induce dynamic actin rearrangements at both the cortical and perinuclear regions in differentiated 3T3L1 adipocytes (158). Consistent with these findings, latrunculin, which directly binds to the monomeric G actin to prevent its polymerization (200), has a more potent inhibitory effect on GLUT4 translocation than cytochalasin D, which inhibits further polymerization of the existing F actin by capping the elongating ends (181). These results suggest that *de novo* actin polymerization may play an important role in insulin-dependent GLUT4 translocation. Furthermore, jasplakinolide, a cell-permeable F actin stabilizer, results in massive actin polymerization at both cortical and the perinuclear regions and inhibits insulin-induced dynamic actin rearrangements and GLUT4 translocation (158). The necessity of actin dynamics has been further supported by the recent observation that myosin 1C (Myo1c) function is required for insulin-stimulated GLUT4 translocation (201). In a series of experiments, it was shown that expression of wild-type Myo1c enhanced insulin-dependent GLUT4 translocation, whereas a dominant-interfering mutant form of Myo1c inhibited GLUT4 translocation. Moreover, small interfering RNAs were used to reduce the expression of endogenous Myo1c, and this maneuver inhibited insulin-stimulated glucose uptake.

In addition to the importance of nonconventional myosin families, multiple mechanisms have been proposed for the function of actin in the control of vesicle-trafficking events (174). For example, several studies have shown that a proper actin regulation is required for the TGN exit (202, 203), spatial targeting of secretory proteins, and the maintenance of the Golgi complex structure (204–206) including the GLUT4 storage compartment (177). In different cell systems, regulated secretion events apparently require distinct properties of actin with some fusion events promoted by F actin stabilization (207) and others by F actin disassembly (208) or dynamic actin rearrangements such as depolymerization/repolymerization (209, 210). Recently, one exciting mechanism has emerged from the study of the N-WASP isoform of the Wiskott-Aldrich syndrome protein (WASP) (211). N-WASP, a ubiquitously expressed member of the WASP family, contains several multifunctional domains including the WASP homology/Ena vasodilator-stimulated phosphoprotein homology domain, IQ motif, basic region, Cdc42/Rac interactive binding/GTPase binding domain, proline-rich region, and VCA (verpolin, cofilin-like, and acidic) region (212, 213). In concert with PI(4,5)P2, N-WASP is regulated by direct binding of the Rho family small GTP binding proteins such as Cdc42 (211, 212, 214), TC10 (187, 215), and recently identified RhoT (216). The interaction of activated Rho proteins with N-WASP induces a conformational change in N-WASP that exposes its VCA region. In turn, this activates the Arp2/3 complex, resulting in a burst of *de novo* actin polymerization in response to extracellular stimuli (188, 213). This N-WASP-dependent actin polymerization, referred to as actin-comet tails, has been shown to provide a driving motor force to propel vesicles (159, 217–219), viruses, and infectious bacteria (220–222).

*In vitro* reconstitution with endogenous vesicles and exogenous artificial vesicles containing PI(4,5)P2 or PI(3,4,5)P3 resulted in the nucleation of actin comet-tails and actin-based motility in a manner dependent upon Cdc42, N-WASP, Arp2/3, and tyrosine phosphorylation (214, 217, 219). Similarly, TC10 appeared to be capable of inducing the nucleation of actin-comet tails on the vesicles in an N-WASP-dependent manner (187). Intriguingly, using the same *in vitro* reconstituted system, GLUT4-containing vesicles were also found to induce actin-comet tails and actin-based motility stimulated by insulin stimulation (159). Furthermore, GTPγS plus sodium vanadate treatment is known to induce insulin-like actions including glucose uptake and GLUT4 translocation in adipocytes and muscle cells (223–225). These agonists also induced actin-comet tails on the GLUT4 vesicle and actin-based motility in the *in vitro* reconstituted system. The GLUT4 vesicle mobility was inhibited by latrunculin B, toxin B, and a dominant-interfering N-WASP/ΔVCA-lacking VCA region that is necessary for Arp2/3-dependent actin polymerization (159). Furthermore, expression of N-WASP/ΔVCA in 3T3L1 adipocytes partially, but significantly, inhibited insulin-stimulated GLUT4 translocation (159, 226). Taken together, these data suggest that the N-WASP-dependent actin polymerization on the GLUT4-containing compartments somehow plays a role in the process of the translocation of GLUT4 protein from the intracellular storage compartments to the plasma membrane.

Although these findings support a required role for actin function in the regulation of insulin-stimulated GLUT4 vesicle trafficking, they also raise several questions, especially with regard to the insulin-signaling pathways involved. Actin is an exceedingly complex molecule in terms of its dynamics and interactions with other proteins; however, two key regulators are well established: as mentioned above, one is phosphoinositides and another is the Rho family small GTP-binding proteins (191, 227). The insulin-induced dynamic actin rearrangements necessary for GLUT4 translocation apparently result from a combination of the PI3K signal and spatially compartmentalized TC10 signal that both are activated by insulin stimulation.

It is well established that phosphoinositides play critical roles in modulating actin cytoskeleton and membrane trafficking (227, 228). The concentration and composition of cellular phosphoinositides are tightly regulated by both inositol lipid kinases including the type IA p85/P110 PI3K and phosphatases such as PTEN and SHIPs. In particular, the PI(4,5)P2 and PI(3,4,5)P3 appeared to be important regulators of the actin cytoskeleton in response to multiple extracellular stimuli (229). In many cell types, activation of PI3K and subsequent production of PI(3,4,5)P3 from PI(4,5)P2 stimulated by growth factors/hormones including insulin induce dynamic actin rearrangements (111, 230). The most recent link of PI3K

to the actin cytoskeleton is a role for these enzymes in the regulation of chemotaxis, the migration of cells along a chemical gradient (231–233). There are several potential pathways for these lipid products to regulate the actin cytoskeleton. For example, Rac-GEFs such as Vav-1 and Tiam-1 interact with PI(3,4,5)P3 through their PH domains and are thereby activated (234, 235). Consistent with these results, treatment of cells with PI3K inhibitors blocks Rac activation by Vav-1 and Tiam-1 and PI3K-dependent actin rearrangements in fibroblasts (235, 236).

Another important molecular link between PI3K and actin includes members of the ARF (ADP ribosylation factor) family of small GTP-binding proteins. ARFs are well established as important regulators of intracellular membrane trafficking through controlling the assembly and disassembly of vesicle coat proteins (237, 238). Recent evidence has emerged that ARF1 and ARF6 are involved in reorganizing the actin cytoskeleton (239–242). ARFs are regulated by ARF-GEFs and ARF-GAPs (GTPase-activating proteins) (237, 238). Two classes of ARF-GEFs can be distinguished on the basis of their sensitivity to brefeldin A. Although members of the brefeldin-sensitive type do not contain a PH domain and control Golgi apparatus traffic and integrity, the cytohesin family of ARF-GEFs that is insensitive to brefeldin contains a PH domain, a coiled-coil domain, and a Sec7 catalytic exchange factor domain (243). The PH domains of ARF nucleotide-binding site opener, cytohesin-1, and general receptor for phosphoinositides (also known as cytohesin-3) preferentially bind PI(3,4,5)P3 over PI(3,4)P2 and other phosphoinositides (244, 245). On the other hand, the PH domain-containing ARF-GAPs have recently been grouped in the centaurin family (246). These proteins display a PI3K-dependent recruitment to the plasma membrane through their PH domains upon activation of PI3K (247). Although the precise roles played by ARF1 and ARF6 during actin reorganization remain unclear, some evidence indicates that ARF1 may potentiate Rho-stimulated stress fiber formation (242). In contrast, ARF6 may function in Rac-stimulated membrane ruffling (248).

In addition to these possible roles for PI(3,4,5)P3 function, PI(4,5)P2 also plays an important role in regulating actin organization through modulating the activities of multiple actin-binding proteins. For example, PI(4,5)P2 inhibits the activities of actin-severing proteins (*e.g.*, ADF/cofilin), capping proteins (*e.g.*, gelsolin), and profilin. In contrast, PI(4,5)P2 activates actin cross-linking proteins (*e.g.*, α-actinin) and links actin to the plasma membrane through vinculin, talin, and ezrin/radixin/moesin proteins (228). Recent studies have revealed that WASP family proteins are activated by PI(4,5)P2 in conjunction with Cdc42 and TC10 leading to Arp2/3 complex-induced actin nucleation (187, 249, 250). As mentioned above, phosphatidylinositol 4-phosphate 5-kinase α expression induces actin-comet tails that are predominately nucleated from sphingolipid-cholesterol-rich rafts (217). Moreover, local increases in PI(4,5)P2 production might facilitate vesicle budding by enhancing membrane curvature (251).

As indicated above, phosphoinositide-based actin polymerization appears to function in cooperation with small GTP-binding proteins of the Rho family, particularly, RhoA, Rac, and Cdc42. As previously discussed, TC10 is an unusual member of this family and has been implicated in the insulin regulation of GLUT4 translocation (156, 157). *In vitro* binding assays have indicated that active GTP-bound TC10 can bind several potential effectors that were originally identified as binding partners for Cdc42 and/or Rac. These include mixed-lineage kinase 2 (MLK2), myotonic dystrophy-related Cdc42 kinase (MRCK), p21-activated protein kinases (PAK), the Borg family of interacting proteins, the mammalian partition-defective homolog Par6, and N-WASP (215, 252–255). To date, more than 20 Cdc42 target proteins have been identified in mammalian cells, and most of these are also potentially able to interact with TC10. However, despite their high degree of sequence similarity, TC10 and Cdc42 may play distinct functional roles in adipocytes and other cell types (156, 157, 187, 256).

For example, overexpression of Cdc42 wild type (Cdc42/WT) in fibroblasts caused a decrease in actin stress fibers along with the formation of plasma membrane microspikes, whereas overexpression of wild-type TC10 (TC10/WT) was without effect (255). However, expression of constitutively active TC10 (TC10/Q75L) is fully capable of inducing plasma membrane microspikes in fibroblasts. However, expression of constitutively active TC10 (TC10/Q75L) is fully capable of inducing plasma membrane microspikes. These data suggest that the signaling pathways required for TC10 activation are not present and/or functional in fibroblasts. This is in marked contrast to adipocytes where expression of Cdc42 has no significant effect on the actin cytoskeleton or insulin-induced GLUT4 translocation (156, 187). In contrast, a more recent report has indicated that expression of a constitutively active mutant version of Cdc42 (V12) can induce GLUT4 translocation (257). In any case, overexpression of TC10/WT, TC10/Q75L, or even the GDP-bound inactive mutant (TC10T31N) results in a total disruption of cortical and Cav-actin, leading to a marked inhibition of insulin-induced GLUT4 translocation. One interpretation of these data is that the ability of TC10 to regulate actin function is dependent upon specific cell types that express unique subsets of actin regulators. In addition, the existence and amount of lipid raft/caveolae are also crucial for actin regulation by TC10, because targeting to these specialized microdomains through C-terminal lipid modification is necessary for TC10 activation and its subsequent biological effects (157). Consistent with a role of TC10 in the control of Cav-actin, the Rho family-specific inhibitor, *Clostridium difficile* toxin B, blocks TC10 activation, disrupts Cav-actin, and prevents insulin-stimulated GLUT4 translocation without affecting PI3K signaling (158).

### *B. Microtubules*

Microtubules are composed of repeating subunits of α and β-tubulin polymerized into long, hollow cylinders. Typically, microtubules have one end attached to a single microtubule-organizing center (MTOC) called a centrosome. Microtubules play critical roles in the long-range transport of intracellular organelles and vesicles in neurons (258). The balance of molecular motor activity directed toward the plus ends (cell periphery) *vs.* the minus ends (perinuclear region) of micro-

tubules is an important mechanism implicated in the regulation of membrane trafficking (172).

Several studies have implicated the microtubule cytoskeletal network as important structural and regulatory elements in insulin-induced GLUT4 translocation (176, 177, 183, 184, 259). For example, α-tubulin and the intermediate filament protein vimentin have been copurified with intracellular GLUT4-containing vesicles (176), and expression of a dominant-interfering vimentin peptide dispersed the perinuclear localized GLUT4 protein. Furthermore, inhibition of the microtubule motor proteins dynein and kinesin reduced insulin-stimulated GLUT4 translocation (176, 184). Most recent work has demonstrated that conventional kinesin KIF5B is highly expressed in adipocytes, and dominant negative mutants of conventional kinesin light chain blocked GLUT4 translocation in response to insulin (260). In addition, microtubule-depolymerizing agents (nocodazole, colchicine, and vinblastine) dispersed the perinuclear localized GLUT4 protein and partially inhibited insulin-stimulated glucose uptake and GLUT4 translocation (177, 183, 184, 259). Furthermore, the microtubule minus end motor dynein has been found to function with Rab5 in regulating the endocytosis of GLUT4 (28). These data support a model wherein the microtubule cytoskeleton participates in insulin-induced GLUT4 translocation.

In contrast, other studies have demonstrated that disruption or stabilization of microtubule structure has no significant effect on insulin-stimulated GLUT4 translocation (185, 186). In addition, although the initial rate of GLUT4 endocytosis was not affected by microtubule disruption, the internalized transporter was not correctly recycled back to the perinuclear region. Furthermore, nocodazole appears to inhibit glucose uptake through a direct interaction with the transporter itself, without affecting the ability of GLUT4 to translocate to the plasma membrane in response to insulin (185, 186). Together, these data demonstrate that microtubular organization may play a role in the recycling of endocytosed GLUT4 from early endosome compartments back to the perinuclear region and in the retention of GLUT4 storage compartments under basal conditions. However, the potential role of microtubules in insulin-stimulated translocation of GLUT4 to the cell surface remains unclear. Because microtubule depolymerization generates short, disorganized microtubule tracks, additional studies are still needed to clarify the potential role of microtubule motors in the process of GLUT4 translocation.

## VI. The IR: Attenuation of Receptor Signaling

Two basic mechanisms are known for attenuating IR signaling: 1) serine phosphorylation of the IR and IRS proteins, and 2) tyrosine dephosphorylation of the receptor and its substrates (261). Although a great deal of attention has recently focused on the ubiquitination of receptor tyrosine kinases, particularly the epidermal growth factor receptor (262), so far this mechanism has not been demonstrably associated with the IR (see, however, Ref. 165). IR serine phosphorylation appears to decrease insulin-stimulated tyrosine kinase substrate activity, leading to a reduction in IRS tyrosine phosphorylation (78). Similarly, serine/threonine phosphorylation of IRS has been reported to decrease its effectiveness as an IRS and may also promote interactions between 14-3-3 proteins (263). 14-3-3 Proteins bind phosphoserine/threonine residues and have been proposed to desensitize insulin signaling, although the mechanism remains unclear (264).

Several serine/threonine kinases have been implicated in the desensitization process, including PKB, PI3K, glycogen synthase kinase-3, mammalian target of rapamycin, MAPKs (ERK and c-Jun N-terminal kinase), and the inflammatory kinase pathway mediated through IκB kinase β (IKKβ). Evidence that the IKKβ pathway plays a critical role in the development of insulin resistance has been provided by historical clinical studies coupled with the use of reverse mouse genetics. Several historical clinical studies had observed that treatment of patients with high-dose salicylates improved glucose tolerance (reviewed in Ref. 265). More recently, it was observed that salicylates at these doses are inhibitors of IKKβ and that salicylate treatment of rodents prevents lipid infusion-induced insulin resistance (266). Moreover, overexpression of IKKβ in cultured cells inhibited insulin signaling, and inhibition of IKKβ activity reversed insulin resistance (266). The role for IKKβ in insulin resistance was further substantiated in IKKβ(+/−) heterozygotic mice that were protected against high-fat diet-induced insulin resistance when crossed into the obese (ob/ob) background (266). Although substantial work is still needed to establish the IKKβ signaling cascade in mediating insulin resistance, this pathway is quite appealing, as many other manipulations known to cause insulin resistance, such as increased fatty acid levels, activation of PKC-θ, and proinflammatory cytokines, all result in activation of IKKβ.

In addition to serine/threonine phosphorylation as a negative regulator, several protein tyrosine phosphatases have also been associated with the attenuation of IR signaling. Recently, considerable attention has focused on PTP1B. The activated IR tyrosine phosphorylates PTP1B, thereby increasing its enzymatic activity (267). Furthermore, PTP1B associates only with activated receptors and can dephosphorylate both the IR and IRS proteins. Genetic ablation of PTP1B resulted in resistance to diet-induced obesity, enhanced tyrosine phosphorylation of the IR and IRS proteins, and improved insulin sensitivity (268, 269). More recently, it has been suggested that increased insulin sensitivity in PTP1B deficiency *in vivo* is an indirect effect due to enhanced leptin signaling (270). In either case, the pharmacological inhibition of PTP1B holds promise for enhancing insulin sensitivity. Indeed, this phosphatase has become an attractive target for the development of small-molecule inhibitors that could potentially be used for the treatment of type 2 diabetes.

Several receptor tyrosine kinases (*e.g.*, platelet-derived growth factor, c-Met, and epidermal growth factor) are thought to undergo rapid homologous down-regulation through ubiquitination and lysosomal degradation (167–169). However, the IR undergoes a very slow desensitization and down-regulation process. Although one study has reported an insulin-induced ubiquitination of the IR, this is unlikely to account for impaired insulin signaling (165). On the other hand, IRS protein levels are reduced in several

rodent models of insulin resistance and are down-regulated in cultured cell systems with impaired insulin signaling (92, 271). More recently, the IRS proteins were observed to undergo ubiquitination followed by 26S proteasome degradation during insulin stimulation or during cellular stress (272). Interestingly, proinflammatory cytokines not only activate the IKK$\beta$ pathway, they are also potent inducers of the SOCS (suppressor of cytokine signaling) family of proteins that were originally identified as negative regulators of cytokine receptor signaling (273). More recently, insulin has been observed to induce the up-regulation of SOCS3, and several SOCS proteins have been found to promote ubiquitination of IRS1 and IRS2 (272) and to negatively regulate insulin signaling (274). This may provide an additional mechanism to inhibit insulin action and promote glucose intolerance during chronic states of metabolic stress, infection, or inflammation.

## VII. GLUT4 Vesicle Docking and Fusion with the Plasma Membrane

### *A. SNARE proteins*

Results from classic membrane trafficking studies suggested that specific interactions between cognate receptor proteins expressed on the cytoplasmic surfaces of vesicle and target membranes control lipid bilayer fusion events. Several key components of the fusion machinery were initially identified in experiments that reconstituted intercisternal Golgi transport *in vitro*, including two cytosolic proteins, the N-ethylmaleimide (NEM)-sensitive fusion protein (NSF) and the soluble NSF attachment proteins (SNAPs) (275). With the soluble components identified, their membrane-bound partners, termed SNARES for SNAP receptors, were soon discovered. These studies, together with results from purified synaptic vesicles, led to the discovery of the target membrane SNAP receptors (t-SNAREs), which are members of the syntaxin family, and the vesicle membrane SNAP receptors (v-SNAREs), which are members of the VAMP family. Subsequently, another target membrane SNARE, called SNAP25 for synaptosome-associated protein of 25 kDa, was found to stably interact with syntaxin. The key functional significance of v- and t-SNAREs for synaptic vesicle exocytosis was shown in neurons by utilizing the tetanus and botulinum clostridial neurotoxins, which block neurotransmitter release in an irreversible manner by selectively cleaving VAMP2, syntaxin 1, or SNAP25 (276). Moreover, *in vitro* studies using v- and t-SNARE partners reconstituted into separate liposome populations demonstrated that interactions between cognate SNARE pairs form a molecular bridge that brings the respective vesicles into sufficiently close proximity to induce lipid bilayer mixing (277). In addition, structural studies have shown that cognate v- and t-SNAREs interact through coiled-coil domains to form a very stable four-helix bundle that may be sufficient to overcome the energetic barrier to membrane fusion (278, 279). Thus, v- and t-SNAREs may represent the minimal physiologic machinery required for membrane fusion. In this model, the v-/t-SNARE complex forms first during the fusion event, allowing the subsequent binding of SNAPs followed by NSF. The SNARE complex is subsequently disassembled through ATP hydrolysis by NSF, thereby freeing the SNAREs for another round of membrane fusion.

Although v- and t-SNAREs may have the potential to interact promiscuously *in vitro*, under *in vivo* conditions specific pairing of SNARE partners appears to contribute to the fidelity of the membrane fusion process (280, 281). Both the v- and t-SNAREs represent large families of proteins that localize to specific intracellular membrane compartments. SNARE proteins may therefore help to demarcate the membrane compartments that have the potential to fuse with each other. Because the distribution of SNAREs may be critical for maintaining membrane compartment identity, several investigators have examined the targeting mechanism by which SNAREs localize to specific membrane compartments. For VAMP2, a specific signal within an $\alpha$-helical domain was shown to specify localization to synaptic vesicles (282). More recently, two independent signals that cooperate to maintain syntaxin 6 in the TGN were identified, including an $\alpha$-helical motif that traps syntaxin 6 in the TGN and a retrieval signal that returns wayward syntaxin 6 molecules from the plasma membrane back to the TGN (283). These results suggest that cells actively maintain the specific membrane compartment localization of various SNARE proteins. Thus, the specificity of membrane fusion events may be maintained at least in part by localizing the fusogenic SNARE proteins in spatially segregated compartments. Maintaining membrane compartment identity and fusion specificity is particularly important for GLUT4 and other proteins that traffic through multiple compartments during their biogenesis, intracellular storage, exocytosis, and retrieval from the cell surface. Although SNAREs play central roles in membrane fusion, several additional proteins also regulate the fusion process and may help ensure membrane fusion specificity (284, 285).

Syntaxin 4 is the only syntaxin isoform that has been implicated in insulin-stimulated GLUT4 vesicle trafficking in adipocytes (Fig. 6) (reviewed in Refs. 286–288). Endogenous syntaxin 4 function has been disrupted by several experimental approaches in cell culture systems, including overexpression of the cytosolic domain of syntaxin 4 or introduction of inhibitory syntaxin 4 antibodies. These experimental maneuvers inhibited insulin-stimulated GLUT4 translocation, apparently by blocking the docking or fusion of GLUT4 vesicles with the plasma membrane. In addition, although the homozygous knockout of syntaxin 4 caused early embryonic lethality, heterozygous mice showed impaired insulin-stimulated glucose uptake and GLUT4 translocation in skeletal muscle (289). Moreover, the syntaxin 4 binding partner SNAP23 (a SNAP25 homolog in adipocytes) is a functional component of the t-SNARE complex and also has been implicated in GLUT4 vesicle fusion with the plasma membrane (290).

Synaptobrevin 2 (also known as VAMP2) and cellubrevin (also known as VAMP3) are two potential GLUT4 vesicle v-SNAREs expressed in adipocytes. Both of these isoforms partially colocalize in intracellular membrane compartments with GLUT4 and both translocate to the plasma membrane after insulin stimulation. In addition, these two v-SNAREs can form stable complexes with syntaxin 4, and expression of the cytosolic domains of either synaptobrevin 2 or cellubrevin inhibited insulin-stimulated GLUT4 translocation.

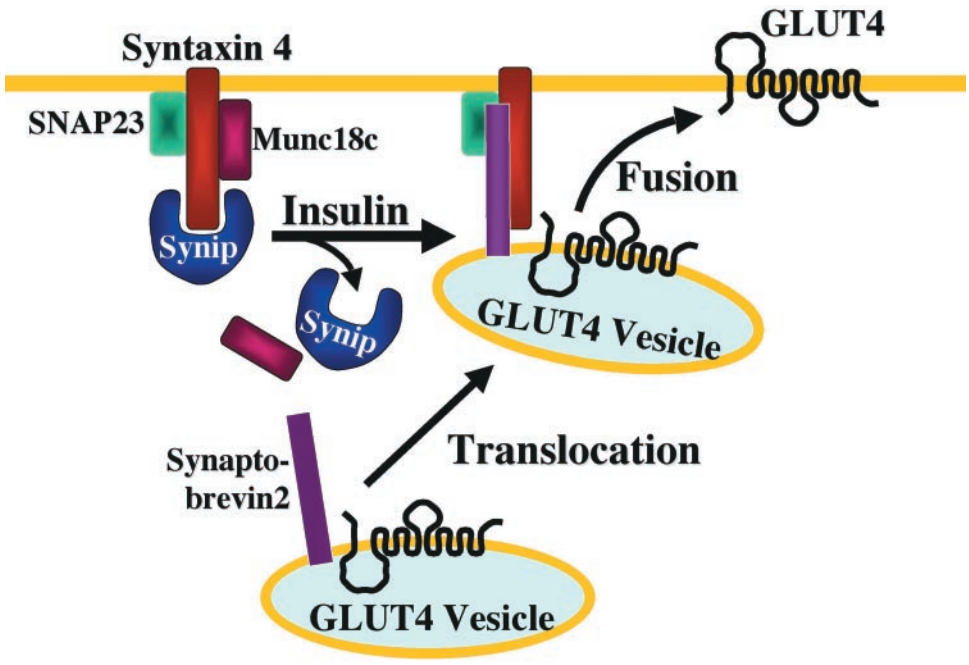


FIG. 6. SNARE and associated proteins implicated in GLUT4 trafficking. The v-SNARE synaptobrevin 2 is present in GLUT4-containing compartments. After insulin stimulation, synaptobrevin 2 interacts with the t-SNAREs syntaxin 4 and SNAP23 at the plasma membrane. This interaction brings the GLUT4 vesicle into close contact with the plasma membrane and may be sufficient to drive bilayer fusion. Although the exact sequence of events remains to be elucidated, synip and Munc18c may dissociate from syntaxin 4 after insulin stimulation, thus allowing the productive formation of the synaptobrevin 2/SNAP23/syntaxin 4 heterotrimeric fusion-competent complex.

Although these two proteins are divergent at their N termini, they differ by just one amino acid in their central t-SNARE binding coiled-coil domains. Based upon these close structural properties, a series of elegant experimental approaches has been employed to distinguish the functional differences between synaptobrevin 2 and cellubrevin. Initial studies using relatively selective proteases found that cleavage of synaptobrevin 2, but not cellubrevin, inhibited insulin-stimulated GLUT4 translocation (291). Consistent with a specific role for synaptobrevin 2, introduction of short peptides corresponding to the unique amino-terminal extension of this v-SNARE had a small inhibitory effect on the translocation of GLUT4 to the plasma membrane (290). In addition, membrane compartment ablation studies showed that cellubrevin localized predominantly to endosomal compartments defined by the transferrin receptor (292–294). These compartments contained about 50% of the GLUT4 protein but only a minor fraction of the total population of synaptobrevin 2 protein. Furthermore, ablation of the endosomal population enriched for cellubrevin and the transferrin receptor resulted in a minor effect on insulin-stimulated GLUT4 translocation but significantly inhibited GTPγS-stimulated GLUT4 translocation (294). Together, these results strongly indicate that synaptobrevin 2 is the primary v-SNARE for insulin-stimulated GLUT4 translocation. In contrast, other stimuli such as GTPγS appear to function through the recruitment of alternative GLUT4 transport vesicles and/or other SNARE isoforms. Consistent with this hypothesis, the genetic ablation of cellubrevin caused no observable disruption of glucose homeostasis (295).

### *B. SNARE-associated proteins in GLUT4 translocation*

Several proteins in addition to NSF, SNAPs, and SNAREs participate in the membrane fusion process. One important syntaxin interacting partner was initially identified in yeast as *sec1* and subsequently as *unc18* in *Caenorhabditis elegans*, ROP in *Drosophila melanogaster*, and nSec1 or Munc18a in mammals (296). In *in vitro* assays, nSec1 interacts with high affinity with syntaxin 1 and competes with the ability of syntaxin1 to bind synaptobrevin 2 and SNAP25. Thus, nSec1 effectively prevents the formation of the syntaxin 1-synaptobrevin 2-SNAP25 ternary complex (297, 298). Moreover, in *D. melanogaster* increased ROP expression inhibited neurotransmitter release *in vivo*, consistent with a negative regulatory role for nSec1 (299). However, in contrast to the above results, null or temperature-sensitive mutants of Sec1 homologs in *Saccharomyces cerevisiae*, *C. elegans*, and *D. melanogaster* blocked secretion at specific steps. These results suggest that Sec1 proteins play a positive role during membrane fusion (300–302). Recent structural analyses of the nSec1-syntaxin 1 complex have suggested that nSec1 maintains syntaxin 1 in a closed, inactive conformational state that prevents promiscuous membrane fusion (303). In this model, nSec1 is postulated to undergo a conformation change that leads to the conversion of syntaxin 1 to the open conformational state, thereby allowing membrane fusion. nSec1 may therefore regulate the transition state between the open and closed conformations of syntaxin 1.

Although nSec1/Munc18a was originally characterized as a neuronal-specific isoform, two additional ubiquitously expressed isoforms, termed Munc18b and Munc18c, were subsequently identified. Because only Munc18c binds to syntaxin 4 with high affinity, substantial attention has therefore focused on this isoform for its potential role in GLUT4 vesicle trafficking. Several groups have shown that overexpression of Munc18c inhibits GLUT4 vesicle translocation, apparently by binding to syntaxin 4 and blocking its ability to interact with synaptobrevin 2 (304–306). Consistent with this hypothesis, Munc18c blocked the interaction of synaptobrevin 2 with syntaxin 4 in both the yeast two-hybrid assay and in

*in vitro* binding experiments. However, although overexpression studies have generated important information regarding the potential function of endogenous Munc18c, it is also important to study Munc18c under more physiologically relevant conditions. This is especially true given the conflicting data and possible dual functional roles played by other Sec1 family members during membrane fusion. To address this issue, short peptides corresponding to specific regions of Munc18 isoforms have been employed to study the functional significance of the syntaxin 4-Munc18c complex in 3T3L1 adipocytes (306). These studies found that a short peptide corresponding to amino acids 459–483 of Munc18c inhibited the ability of GLUT4 vesicles to integrate into the plasma membrane but did not block the trafficking of GLUT 4 vesicles to the cell surface. Instead, GLUT4 vesicles accumulated beneath the plasma membrane, suggesting that an early stage of the membrane fusion process was inhibited. In addition, the endogenous Munc18c-syntaxin 4 complex was disrupted by the Munc18c peptide. Together, the above results suggest that Munc18c plays a positive or facilitative role during the fusion of GLUT4 vesicles with the plasma membrane, possibly by keeping syntaxin 4 in a conformation that favors interactions with synaptobrevin 2. According to this model, in the basal state Munc18c interacts with syntaxin 4 and inhibits GLUT4 vesicle fusion with the plasma membrane, perhaps by maintaining syntaxin 4 in the inactive, closed conformation. After insulin stimulation, Munc18c may undergo a conformation change that in turn facilitates interactions between syntaxin 4 and synaptobrevin 2. Thus, the superphysiological doses of Munc18c that occur upon overexpression may shift the equilibrium such that syntaxin 4 is maintained in a closed conformation, leading to the observed inhibition of insulin-stimulated GLUT4 translocation. Although this is an appealing model, there is currently no structural data regarding Munc18c/syntaxin 4 interactions, nor is there evidence that insulin can modulate these interactions on a time scale consistent with GLUT4 translocation.

In continuing efforts to identify additional regulatory components necessary for GLUT4 translocation, several syntaxin 4-interacting partners have been identified. One binding partner, termed synip (syntaxin 4-interacting protein), was isolated using the yeast two-hybrid assay system (307). Synip is predicted to contain PDZ and EF hand domains at its amino terminus, two tandem coiled-coil domains in its central region, and a WW motif at its carboxyl terminus. Among syntaxin isoforms, synip interacts preferentially with syntaxin 4 (presumably through the coiled-coil domains). When overexpressed, wild-type synip had no significant effect on insulin-stimulated GLUT4 vesicle translocation. However, overexpression of the carboxyl-terminal half of synip (which contains the coiled-coil and WW domains) significantly inhibited GLUT4 translocation. Furthermore, insulin caused the dissociation of full-length synip from syntaxin 4, suggesting that the carboxyl-terminal region interacts with syntaxin 4 and that the amino-terminal region provides the insulin regulatory sites required for the dissociation of the synip-syntaxin 4 complex. Synip may thus function as a fusion control switch at the plasma membrane. In the absence of insulin, synip binds to and masks the syntaxin 4 molecule, thereby preventing nonspecific vesicle fusion. In this model, insulin stimulation releases synip and exposes the syntaxin 4 coiled-coil domains, making them available for interactions with synaptobrevin 2 (Fig. 6). Clearly, additional studies are needed to test this model and we are currently investigating the upstream signaling mechanisms by which insulin causesthe dissociation of the synip-syntaxin 4 complex.

### *C. Exocyst in GLUT4 translocation*

As discussed above, SNARE proteins are thought to play an essential role during membrane fusion. However, before SNARE-mediated fusion, vesicles appear to undergo a tethering (pre-SNARE binding) step with target membranes. A recent study has suggested that the exocyst protein complex may function in such a manner for GLUT4 vesicles (308). The exocyst is an evolutionarily conserved octameric protein complex composed of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84 (309). The exocyst component Exo70 has been identified as a direct target for activated TC10, and insulin activation of TC10 through CAP-Cbl signaling cascade recruits Exo70 together with Sec6 and Sec8 (presumably with other exocyst proteins) to the plasma membrane. Although Exo70 does not possess an identifiable CRIB domain, the amino-terminal region of Exo70 contains a coiled-coil domain that is required for the interaction with TC10. In addition, the carboxy-terminal region of Exo70 appears necessary for its recruitment to the plasma membrane, probably due to an interaction with other proteins. Intriguingly, the amino-terminal fragment of Exo70 specifically prevented the tethering and/or docking of GLUT4-containing vesicles, a necessary prerequisite for plasma membrane fusion. However, the amino-terminal fragment did not disrupt the intracellular membrane trafficking of GLUT4 *en route* to the cell surface, because GLUT4 vesicles accumulated underneath the plasma membrane. These data suggest that TC10 and Exo70 recruit the exocyst complex during the final steps of GLUT4 exocytosis.

In addition to TC10, recent studies have demonstrated that the exocyst complex is regulated by several small GTP-binding proteins, including Rab, and Rho (309). Sec4, a member of yeast Rab family, was originally reported to interact with Sec15 (310). In the case of the yeast Rho family, Rho1 and Cdc42 interact with Sec3, whereas Rho3 has been shown to associate with Exo70 (311, 312**)**. Mammalian exocyst Sec5 has been reported to interact with Ral A small GTP-binding protein (313, 314), and this Ral A-Sec5 interaction appeared to be involved in basolateral, but not apical, membrane traffic in polarized epithelial cells (314). Thus, the exocyst appears to play an important role in multiple aspects of exocytosis, apparently being regulated by interactions between specific exocyst components. Further work is clearly needed to identify a functional role of the exocyst complex in the insulin-induced GLUT4 translocation process.

### *D. Rab and ARF proteins in GLUT4 translocation*

With more than 60 known mammalian isoforms, Rab proteins comprise the largest branch of the Ras superfamily of small GTP-binding proteins. Rabs localize to the cytoplasmic

surfaces of intracellular compartments and regulate membrane transport between organelles. In addition, distinct Rab isoforms localize to specific intracellular compartments and may contribute to the specificity of bilayer fusion events (reviewed in Refs. 315 and 316). Like other small GTPases, Rabs are considered molecular switches that oscillate between a GDP-bound (off) and a GTP-bound (on) state. In the GTP-bound state, Rabs interact with effector proteins that regulate several steps of membrane transport, including vesicle budding, motility, tethering, and fusion (311, 312). After a round of vesicle fusion, the Rab protein returns to its GDP-bound state. In addition, GDP-Rabs are extracted from their target membrane by soluble guanylnucleotide dissociation inhibitor proteins and recycled back to the donor membrane for another round of vesicle transport (311, 312).

Although insulin-responsive cells express many Rab isoforms, to date Rab4, Rab5, and Rab11 have been implicated in GLUT4 trafficking processes (reviewed in Refs. 286, 288, and 317). In addition, a Rab GAP has also recently been implicated in insulin-stimulated GLUT4 trafficking (139). In fibroblasts, Rab4 localizes to early endosomes and has been implicated in protein sorting and recycling processes (315). In addition, in cell fractionation studies Rab4 colocalized with GLUT4 in the low-density microsome fraction of adipocytes (318) and muscle (319). Moreover, insulin stimulated nucleotide exchange on Rab4 and caused the redistribution of Rab4 from the microsome fraction to the cytosolic fraction, and this occurred in parallel with the translocation of GLUT4 to the cell surface (318, 320). Insulin-simulated GLUT4 translocation was also inhibited both by the introduction of a peptide corresponding to the carboxyl terminus of Rab4 and by expression of a carboxyl-terminal Rab4 truncation mutant (321–323). In addition, overexpression of wild-type Rab4 or a hydrolysis-deficient (GTP-bound) mutant also inhibited GLUT4 translocation, presumably because the cytosolic forms of these proteins function as dominant interfering inhibitors when present at high levels (323–325). Interestingly, Rab4 has been reported to interact directly with syntaxin 4 and to regulate SNARE protein interactions in a GTP/GDP-dependent manner (326).

Rab5 was originally identified as a regulator of the fusion between internalized vesicles and early endosomes. Recent work has shown that insulin may inhibit Rab5 activity, thereby decreasing the rate of GLUT4 endocytosis and favoring plasma membrane localization of the transporter (28). Rab11 localizes to perinuclear recycling endosomes of fibroblasts. In rat cardiomyocytes, Rab11 has been shown to colocalize with GLUT4 vesicles and to redistribute to the plasma membrane in response to insulin (327). However, recent work has indicated that Rab11 may function in the endosomal sorting steps that return GLUT4 to the insulin-responsive compartment (328).

The ADP-ribosylation factors (ARFs) are another important Ras subfamily of small GTP binding proteins involved in vesicle trafficking (329). ARFs are thought to function in the early stages of vesicle budding, during the recruitment of coat proteins to the donor membrane compartment. In the GDP-bound state, ARFs remain in the cytosol; however, upon GTP loading they associate with donor membranes and recruit cytosolic coat components to the site of vesicle bud formation. The coat proteins then deform the donor membrane into a nascent vesicle and interact with cargo molecules. Given their widespread roles in membrane trafficking processes, it seems likely that one or more ARF isoforms participate in the complex intracellular trafficking itinerary of GLUT4. Indeed, ARF6 has been implicated in GLUT4 vesicle trafficking; however, results have not always been consistent. For example, the introduction of myristoylated peptides corresponding to the amino terminus of ARF6 inhibited GLUT4 vesicle translocation by approximately 50% in permeabilized adipocytes (330). In contrast, other studies using a dominant-interfering mutant of ARF6 observed no significant effect on basal or insulin-stimulated glucose transport (331). Thus, although members of the ARF family are likely to participate in GLUT4 vesicle trafficking, additional work is needed to clarify our understanding of the potential roles played by ARF6 and perhaps other ARF isoforms.

## VIII. GLUT4 Endocytosis

Although insulin is well known to enhance the rate of GLUT4 exocytosis, insulin also decreases the rate of GLUT4 endocytosis approximately 2- to 3-fold (332–334), and this may occur at least in part through inhibition of Rab5 activity (28). A complete understanding of the molecular mechanism underlying GLUT4 endocytosis could create opportunities for developing novel drug therapies aimed at slowing the rate of GLUT4 internalization and thus enhancing glucose uptake under diabetic conditions. Currently, we have only a rudimentary understanding of the molecular mechanisms responsible for GLUT4 endocytosis. Nevertheless, several lines of evidence suggest that GLUT4 is internalized through clathrin-coated pits. For example, GLUT4 localizes to clathrin-coated pits as determined by immunofluorescence and electron microscopy analysis (42, 335, 336). In addition, inhibition of clathrin-mediated endocytosis by a variety of methods also prevents GLUT4 internalization (65, 337).

The mechanism by which clathrin-coated pits invaginate and form free vesicles remains unclear. However, the GTPase dynamin plays a key role in this process. Dynamin is a 100-kDa cytosolic protein with an amino-terminal GTPase domain, a central pleckstrin homology domain, and a carboxyl-terminal proline-rich region (338). Dynamin encircles the necks of invaginating vesicles in a spiral conformation, a process that was initially observed in studies examining the *D. melanogaster* dynamin homolog *shibire*. Temperature-sensitive mutations in *shibire* result in a paralytic phenotype at the nonpermissive temperature (339). Analysis of the presynaptic termini of these flies revealed an absence of synaptic vesicles and an abundance of clathrin-coated pits with electron-dense collars around their necks, suggesting that a late stage of endocytosis was inhibited (340). The *shibire* temperature-sensitive mutation occurs near the GTPase domain, and together with experiments employing GTP$\gamma$S it was proposed that dynamin's GTPase activity was necessary for the vesicle scission process. In this model, GTP hydrolysis causes a conformational change in dynamin such that the necks of invaginating vesicles are constricted to the point

where membrane scission occurs and free vesicles released (341). However, in another model for dynamin function, the energy derived from GTP hydrolysis has been proposed to cause the elongation of dynamin spirals, resulting in the stretching and eventual scission of the vesicle neck (342). Alternatively, dynamin may instead function as a classical GTPase molecular switch that recruits downstream effector molecules, such as endophilin, which then participate in the formation of released vesicles (343).

Although the details of its molecular mechanism remain controversial, dynamin clearly plays an important role in GLUT4 endocytosis. Several studies have used dominant-interfering dynamin mutants or specific peptides to disrupt dynamin function, resulting in a significant inhibition of GLUT4 endocytosis (344–346). However, it remains unclear how insulin might regulate dynamin function. Nevertheless, the proline-rich domain of dynamin is known to associate with various SH3 domain-containing proteins, which in turn enhance dynamin's GTPase activity (347). For example, Grb2 associates with dynamin and induces the binding of the dynamin-Grb2 complex to tyrosine-phosphorylated Shc and IRS1 proteins (348). These results suggest that this higher-order complex either directly inhibits dynamin activity and/or results in the sequestration of dynamin away from the GLUT4-containing clathrin-coated pits. Alternatively, insulin reportedly induces the tyrosine phosphorylation of dynamin (349). In either case, the functional significance of these events remains to be determined, as there is currently no *in vivo* evidence that insulin regulates the localization, GTPase activity, or pinchase function of dynamin.

## IX. GLUT4 Activation

Although it is clear that insulin induces the translocation of GLUT4 from intracellular compartments to the plasma membrane, it remains possible that the intrinsic glucose transport activity of GLUT4 may also be regulated (reviewed in Ref. 17). For example, early studies showed that isoproterenol and other β-adrenergic agonists inhibit insulin-stimulated glucose transport in muscle and adipose tissue, without changing the plasma membrane content of the transporter. In addition, agents that stimulate cAMP production (*e.g.*, forskolin), inhibit cAMP phosphodiesterase (*e.g.*, 3-isobutyl-1-methylxanthine), or mimic cAMP (dibutyryl-cAMP) all inhibit glucose transport (350–354). One interpretation of these data is that isoproterenol and other cAMP stimulators modulate the intrinsic transport activity of GLUT4. Indeed, it was subsequently demonstrated that isoproterenol can stimulate phosphorylation of the carboxyl terminus of GLUT4 at serine 488 *in vivo*, and that the cAMP-dependent protein kinase A can phosphorylate the same site *in vitro* (355). These data suggest that the observed attenuation of glucose transport activity upon β-adrenergic stimulation may result from phosphorylation of GLUT4. However, other models have been proposed to explain the effects of isoproterenol on glucose transport, including the occlusion or incomplete fusion of GLUT4-containing vesicles at the plasma membrane (356, 357).

In addition to the possible modulator effects of β-adrenergic agonists on intrinsic GLUT4 transport activity, a number of investigators have reported a discrepancy between the fold increase in glucose uptake with the increase in GLUT4 transporter translocation (reviewed in Ref. 17). In general, the reported fold increase in glucose uptake exceeded the fold increase in translocation, although results varied considerably. In addition, time course experiments employing a GLUT4 reporter construct with an engineered exofacial myc epitope have shown that GLUT4 translocation occurs before glucose transport (358). One interpretation of these data is that insulin enhances the glucose transport activity of GLUT4 transporters at the plasma membrane above basal activity levels. Moreover, recent evidence has implicated p38 MAPKs in GLUT4 activation. For example, it was shown that the SB202190 and SB203580 inhibitors of p38 MAPK attenuate glucose transport without affecting the ability of GLUT4 to translocate to the cell surface in response to insulin (359). However, although insulin increases p38 MAPK activity (360), insulin is also known to reduce the amount of phosphorylated GLUT4 at the plasma membrane (353). Thus, future work is necessary to resolve the potential mechanism by which p38 MAPK may regulate the intrinsic transport activity of GLUT4.

## X. Summary and Future Directions

Since the discovery of insulin in the early 1920s it took an additional 60 yr to make the seminal observation that insulin stimulates glucose uptake by inducing the translocation of GLUT proteins from intracellular storage sites to the plasma membrane. This discovery provided the conceptual framework that continues to guide the research efforts of workers in the insulin-signaling and GLUT4-trafficking fields. Indeed, in the intervening decades since the translocation hypothesis was proposed, we have identified multiple members of the facilitative GLUT family and demonstrated that the GLUT4 isoform is the predominant insulin-responsive transporter in striated muscle and adipose tissue. Moreover, inroads have been made with regard to the signaling processes downstream of the IR, and it now appears that two distinct cascades are required to stimulate GLUT4 translocation. One pathway, characterized by the PI3K, has been intensely studied, and several downstream effectors including PKB, PDK, and aPKC isoforms, have been implicated in GLUT4 translocation. The second pathway, characterized by the Rho-family GTPase TC10, has only recently been implicated in GLUT4 translocation, and much remains to be learned regarding downstream signaling molecules.

In addition, over the past several years gene knockout and transgenic technologies have provided important new insights into how specific molecules function with respect to insulin signaling in the context of intact model organisms. Furthermore, the genetic disruption of individual genes has yielded valuable and, at times, surprising new information on how specific tissue types interact to regulate glucose disposal. In particular, it has become increasingly clear that cross-talk exists between adipose and other tissues, and that adipocytes may secrete factors that regulate glucose uptake in muscle tissue. Newer technologies, including microchip arrays and RNA interference, are also likely to yield impor-

tant and surprising insights into insulin signaling and GLUT4 translocation.

Despite these advances, it remains completely unknown how insulin mobilizes GLUT4-containing vesicles. This is an important area for future investigations as is the elucidation of insulin signaling events occurring proximal to the GLUT4 storage compartment. Indeed, a key goal is to characterize the GLUT4 storage compartment at the molecular level, with the aim of identifying the molecular machinery that allows the insulin-dependent mobilization of GLUT4 vesicles. Unlike synaptic vesicles, the small, uniform size of which facilitated their isolation and characterization in almost unprecedented molecular detail, the apparently heterogeneous insulin-responsive GLUT4 storage compartment has so far remained recalcitrant to biochemical isolation and characterization. This represents a major technical hurdle for future work, because the ability to isolate GLUT4 vesicles and reconstitute insulin-dependent GLUT4 trafficking steps *in vitro* holds tremendous promise for identifying the specific molecules that link insulin signaling processes to GLUT4 trafficking events. The identification of molecules that act directly on the GLUT4 storage compartment and regulate transporter mobilization in response to insulin could potentially serve as targets for the rational design of therapeutic interventions for treating or preventing the development and progression of type 2 diabetes.

## Acknowledgments

We thank the numerous researchers in the diverse fields of insulin signaling and GLUT4 trafficking for their important scientific contributions. In attempting to summarize a very broad body of data with restricted space limitations, we were regrettably unable to include the important work of many laboratories and colleagues.

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## References

1. **Walmsley AR, Barrett MP, Bringaud F, Gould GW** 1998 Sugar transporters from bacteria, parasites, and mammals: structure-activity relationships. Trends Biochem Sci 23:476–481
2. **Tetaud E, Barrett MP, Baltz T** 1997 Kinetoplastid glucose transporters. Biochem J 325:569–580
3. **Joost HG, Thorens B** 2001 The extended GLUT-family of sugar/polyol transport facilitators: nomenclature, sequence characteristics, and potential function of its novel members. Mol Membr Biol 18:247–256 (Review)
4. **Joost H-G, Bell GI, Best JD, Birnbaum MJ, Charron MJ, Chen YT, Doege H, James DE, Lodish HF, Moley KH, Moley JF, Mueckler M, Rogers S, Schurmann A, Seino S, Thorens B** 2002 Nomenclature of the GLUT/SLC2A family of sugar/polyol transport facilitators. Am J Physiol 282:E974–E976
5. **Mueckler M, Hresko RC, Sato M** 1997 Structure, function and biosynthesis of GLUT1. Biochem Soc Trans 25:951–954
6. **Vannucci SJ, Maher F, Simpson IA** 1997 Glucose transporter proteins in brain: delivery of glucose to neurons and glia. Glia 21:2–21
7. **Kasahara M, Hinkle PC** 1977 Reconstitution and purification of the D-glucose transporter from human erythrocytes. J Biol Chem 252: 7384–7390
8. **Olson AL, Pessin JE** 1996 Structure, function and regulation of the mammalian facilitative glucose transporter gene family. Ann Rev Nutr 16:235–256
9. **Bryant NJ, Govers R, James DE** 2002 Regulated transport of the glucose transporter GLUT4. Nat Rev Mol Cell Biol 3:267–277
10. **Cushman SW, Wardzala LJ** 1980 Potential mechanism of insulin action on glucose transport in the isolated rat adipose cell. Apparent translocation of intracellular transport systems to the plasma membrane. J Biol Chem 255:4758–4762
11. **Suzuki K, Kono T** 1980 Evidence that insulin causes translocation of glucose transport activity to the plasma membrane from an intracellular storage site. Proc Natl Acad Sci USA 77:2542–2545
12. **Birnbaum MJ** 1989 Identification of a novel gene encoding an insulin-responsive glucose transporter protein. Cell 57:305–315
13. **Fukumoto H, Kayanol T, Busel JB, Edwards Y, Pilch PF, Bell GI, Seino S** 1989 Cloning and characterization of the major insulin-responsive glucose transporter expressed in human skeletal muscle and other insulin-responsive tissues. J Biol Chem 264:7776–7779
14. **James DE, Strube M, Mueckler M** 1989 Molecular cloning and characterization of an insulin-regulatable glucose transporter. Nature 338:83–87
15. **Kaestner KH, Christy RJ, McLenithan JC, Braiterman LT, Cornelius P, Pekala PJ, Lane MD** 1989 Sequences, tissue distribution, and differential expression of mRNA for a putative insulin-responsive glucose transporter in mouse 3T3–L1 adipocytes. Proc Natl Acad Sci USA 86:3150–3154
16. **Charron MJ, Brosius III FC, Alper SL, Lodish HF** 1989 A glucose transport protein expressed predominately in insulin-responsive tissues. Proc Natl Acad Sci USA 86:2535–2539
17. **Furtado LM, Somwar R, Sweeney G, Niu W, Klip A** 2002 Activation of the glucose transporter GLUT4 by insulin. Biochem Cell Biol 80:569–578
18. **Brozinick Jr JT, McCoid SC, Reynolds TH, Nardone NA, Hargrove DM, Stevenson RW, Cushman SW, Gibbs EM** 2001 GLUT4 overexpression in db/db mice dose-dependently ameliorates diabetes but is not a lifelong cure. Diabetes 50:593–600
19. **Katz EB, Stenbit AE, Hatton K, DePhino R, Charron MJ** 1995 Cardiac and adipose tissue abnormalities but not diabetes in mice deficient in GLUT4. Nature 377:151–155
20. **Stenbit AE, Tsao TS, Li J, Burcelin R, Geenen DL, Factor SM, Houseknecht K, Katz EB, Charron MJ** 1997 GLUT4 heterozygous knockout mice develop muscle insulin resistance and diabetes. Nat Med 3:1096–1101
21. **Kubota N, Tobe K, Terauchi Y, Eto K, Yamauchi T, Suzuki R, Tsubamoto Y, Komeda K, Nakano R, Miki H, Satoh S, Sekihara H, Sciacchitano S, Lesniak M, Aizawa S, Nagai R, Kimura S, Akanuma Y, Taylor SI, Kadowaki T** 2000 Disruption of insulin receptor substrate 2 causes type 2 diabetes because of liver insulin resistance and lack of compensatory $\beta$-cell hyperplasia. Diabetes 49:1880–1889
22. **Wallberg-Henriksson H, Zierath JR** 2001 GLUT4: a key player regulating glucose homeostasis? Insights from transgenic and knockout mice. Mol Membr Biol 18:205–211 (Review)
23. **Zisman A, Peroni OD, Abel ED, Michael MD, Mauvais-Jarvis F, Lowell BB, Wojtaszewski JFP, Hirshman MF, Virkamaki A, Goodyear LJ, Kahn CR, Kahn BB** 2000 Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. Nat Med 6:924–928
24. **Abel ED, Peroni O, Kim JK, Kim YB, Boss O, Hadro E, Mennemann T, Shulman GI, Kahn BB** 2001 Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. Nature 409:729–733
25. **Jhun BH, Rampal AL, Liu H, Lachaal M, Jung CY** 1992 Effects of insulin on steady state kinetics of GLUT4 subcellular distribution in rat adipocytes. Evidence of constitutive GLUT4 recycling. J Biol Chem 267:17710–17715
26. **Yang J, Holman GD** 1993 Comparison of GLUT4 and GLUT1 subcellular trafficking in basal and insulin-stimulated 3T3–L1 cells. J Biol Chem 268:4600–4603
27. **Czech MP, Buxton JM** 1993 Insulin action on the internalization of the GLUT4 glucose transporter in isolated rat adipocytes. J Biol Chem 268:9187–9190
28. **Huang J, Imamura T, Olefsky JM** 2001 Insulin can regulate GLUT4 internalization by signaling to Rab5 and the motor protein dynein. Proc Natl Acad Sci USA 98:13084–13089

29. **Rea S, James DE** 1997 Moving GLUT4: the biogenesis and trafficking of GLUT4. storage vesicles. Diabetes 46:1667–1677
30. **Barr VA, Malide D, Zarnowski MJ, Taylor SI, Cushman SW** 1997 Insulin stimulates both leptin secretion and production by rat white adipose tissue. Endocrinology 138:4463–4472
31. **Bogan JS, Lodish HF** 1999 Two compartments for insulin-stimulated exocytosis in 3T3–L1 adipocytes defined by endogenous ACRP30 and GLUT4. J Cell Biol 146:609–620
32. **Roh C, Roduit R, Thorens B, Fried S, Kandror KV** 2001 Lipoprotein lipase and leptin are accumulated in different secretory compartments in rat adipocytes. J Biol Chem 276:35990–35994
33. **Roh C, Thoidis G, Farmer SR, Kandror KV** 2000 Identification and characterization of leptin-containing intracellular compartment in rat adipose cells. Am J Physiol 279:E893–E899
34. **Millar CA, Meerloo T, Martin S, Hickson GR, Shimwell NJ, Wakelam MJ, James DE, Gould GW** 2000 Adipsin and the glucose transporter GLUT4 traffic to the cell surface via independent pathways in adipocytes. Traffic 1:141–151
35. **Kandror KV, Pilch PF** 1998 Multiple endosomal recycling pathways in rat adipose cells. Biochem J 331:829–835
36. **Holman GD, Leggio LL, Cushman SW** 1994 Insulin-stimulated GLUT4 glucose transporter recycling—a problem in membrane protein subcellular trafficking through multiple pools. J Biol Chem 269:17516–17524
37. **Aledo JC, Lavoie L, Volchuk A, Keller SR, Klip A, Hundal HS** 1997 Identification and characterization of two distinct intracellular GLUT4 pools in rat skeletal muscle: evidence for an endosomal and an insulin-sensitive GLUT4 compartment. Biochem J 325:727–732
38. **Ploug T, van Deurs B, Ai H, Cushman SW, Ralston E** 1998 Analysis of GLUT4 distribution in whole skeletal muscle fibers: identification of distinct storage compartments that are recruited by insulin and muscle contractions. J Cell Biol 142:1429–1446
39. **Lee W, Ryu J, Spangler RA, Jung CY** 2000 Modulation of GLUT4 and GLUT1 recycling by insulin in rat adipocytes: kinetic analysis based on the involvement of multiple intracellular compartments. Biochemistry 39:9358–9366
40. **Kupriyanova TA, Kandror V, Kandror KV** 2002 Isolation and characterization of the two major intracellular Glut4 storage compartments. J Biol Chem 277:9133–9138
41. **Livingstone C, James DE, Rice JE, Gould GW** 1996 Compartment ablation analysis of the insulin-responsive glucose transporter (GLUT4) in 3T3L1 adipocytes. Biochem J 315:487–495
42. **Robinson LJ, Pang S, Harris DS, Heuser J, James DE** 1992 Translocation of the glucose transporter (GLUT4) to the cell surface in permeabilized 3T3–L1 adipocytes: effects of ATP and GTPγS and localization of GLUT4 to clathrin lattices. J Cell Biol 117:1181–1196
43. **Zorzano A, Wilkinson W, Kotliar N, Thorodias G, Wadzinkski BE, Ruoho AE, Pilch PF** 1989 Insulin-regulated glucose uptake in rat adipocytes is mediated by two transporter isoforms present in at least two vesicle populations. J Biol Chem 264:12358–12363
44. **Malide D, Ramm G, Cushman SW, Slot JW** 2000 Immunoelectron microscopic evidence that GLUT4 translocation explains the stimulation of glucose transport in isolated rat white adipose cells. J Cell Sci 113:4203–4210
45. **Hashiramoto M, James DE** 2000 Characterization of insulin-responsive GLUT4 storage vesicles isolated from 3T3–L1 adipocytes. Mol Cell Biol 20:416–427
46. **Mastick CC, Aebersold R, Lienhard GE** 1994 Characterization of a major protein in Glut4 vesicles—concentration in the vesicles and insulin-stimulated translocation to the plasma membrane. J Biol Chem 269:6089–6092
47. **Kandror KV, Pilch PF** 1994 gp160, A tissue-specific marker for insulin-activated glucose transport. Proc Natl Acad Sci USA 91: 8017–8021
48. **Kandror K, Pilch PF** 1994 Identification and isolation of glycoproteins that translocate to the cell surface from GLUT4-enriched vesicles in an insulin-dependent fashion. J Biol Chem 269:138–142
49. **Kandror KV, Yu L, Pilch PF** 1994 The major protein of GLUT4-containing vesicles, gp160, has aminopeptidase activity. J Biol Chem 269:30777–30780
50. **Keller SR** 2003 The insulin-regulated aminopeptidase: a companion and regulator of GLUT4. Front Biosci 8:S410–S420
51. **Shewan AM, Marsh BJ, Melvin DR, Martin S, Gould GW, James DE** 2000 The cytosolic C-terminus of the glucose transporter GLUT4 contains an acidic cluster endosomal targeting motif distal to the dileucine signal. Biochem J 350:99–107
52. **Albiston AL, McDowall SG, Matsacos D, Sim P, Clune E, Mustafa T, Lee J, Mendelsohn FA, Simpson RJ, Connolly LM, Chai SY** 2001 Evidence that the angiotensin IV (AT(4)) receptor is the enzyme insulin-regulated aminopeptidase. J Biol Chem 276:48623–48626
53. **Keller SR, Davis AC, Clairmont KB** 2002 Mice deficient in the insulin-regulated membrane aminopeptidase show substantial decreases in glucose transporter GLUT4 levels but maintain normal glucose homeostasis. J Biol Chem 277:17677–17686
54. **Hudson AW, Ruiz M, Birnbaum MJ** 1992 Isoform-specific subcellular targeting of glucose transporters in mouse fibroblasts. J Cell Biol 116:785–797
55. **Holman GD, Sandoval IV** 2001 Moving the insulin-regulated glucose transporter GLUT4 into and out of storage. Trends Cell Biol 11:173–179
56. **Asano T, Takata K, Katagiri H, Tsukuda K, Lin J-L, Ishihara H, Inukai K, Hirano H, Yazaki Y, Oka Y** 1992 Domains responsible for the differential targeting of glucose transporter isoforms. J Biol Chem 267:19636–19641
57. **Piper RC, Tai C, Kulesza P, Pang SH, Warnock D, Baenziger J, Slot JW, Geuze HJ, Puri C, James DE** 1993 GLUT-4 NH2 terminus contains a phenylalanine-based targeting motif that regulates intracellular sequestration. J Cell Biol 121:1221–1232
58. **Garippa RJ, Judge TW, James DE, McGraw TE** 1994 The amino terminus of GLUT4 functions as an internalization motif but not an intracellular retention signal when substituted for the transferrin receptor cytoplasmic domain. J Cell Biol 124:705–715
59. **Al-Hasani H, Kunamneni RK, Dawson K, Hinck CS, Muller-Wieland D, Cushman SW** 2002 Roles of the N- and C-termini of GLUT4 in endocytosis. J Cell Sci 115:131–140
60. **Palacios S, Lalioti V, Martinez-Arca S, Chattopadhyay S, Sandoval IV** 2001 Recycling of the insulin-sensitive glucose transporter GLUT4. Access of surface internalized GLUT4 molecules to the perinuclear storage compartment is mediated by the Phe5-Gln6-Gln7-Ile8 motif. J Biol Chem 276:3371–3383
61. **Melvin DR, Marsh BJ, Walmsley AR, James DE, Gould GW** 1999 Analysis of amino and carboxy terminal GLUT-4 targeting motifs in 3T3–L1 adipocytes using an endosomal ablation technique. Biochemistry 38:1456–1462
62. **Czech MP, Chawla A, Wook CW, Buxton J, Armoni M, Tang W, Joly M, Corvera S** 1993 Exofacial epitope-tagged glucose transporter chimeras reveal COOH-terminal sequences governing cellular localization. J Cell Biol 123:127–135
63. **Marshall BA, Murata H, Hresko RC, Mueckler M** 1993 Domains that confer intracellular sequestration of the Glut4 glucose transporter in *Xenopus* oocytes. J Biol Chem 268:26193–26199
64. **Hudson AW, Fingar DC, Seidner GA, Griffiths G, Burke B, Birnbaum MJ** 1993 Targeting of the insulin-responsive glucose transporter (GLUT4) to the regulated secretory pathway in PC12 cells. J Cell Biol 122:579–588
65. **Garippa RJ, Johnson A, Park J, Petrush RL, McGraw TE** 1996 The carboxyl terminus of GLUT4 contains a serine-leucine-leucine sequence that functions as a potent internalization motif in Chinese hamster ovary cells. J Biol Chem 271:20660–20668
66. **Corvera S, Chawla A, Chakrabarti R, Joly M, Buxton J, Czech MP** 1994 A double leucine within the GLUT4 glucose transporter COOH-terminal domain functions as an endocytosis signal. J Cell Biol 126:979–989
67. **Subtil A, Lampson MA, Keller SR, McGraw TE** 2000 Characterization of the insulin-regulated endocytic recycling mechanism in 3T3–L1 adipocytes using a novel reporter molecule. J Biol Chem 275:4787
68. **Martinez-Arca S, Lalioti VS, Sandoval IV** 2000 Intracellular targeting and retention of the glucose transporter GLUT4 by the perinuclear storage compartment involves distinct carboxyl-tail motifs. J Cell Sci 113:1705–1715
69. **Verhey KJ, Hausdorff SF, Birnbaum MJ** 1993 Identification of the carboxyl terminus as important for the isoform-specific subcellular targeting of glucose transporter proteins. J Cell Biol 123:137–147
70. **Shewan AM, Van Dam EM, Martin S, Luen TB, Hong W, Bryant NJ, James DE** 2003 GLUT4 recycles via a trans-Golgi network

(TGN) subdomain enriched in syntaxins 6 and 16 but not TGN38: involvement of an acidic targeting motif. Mol Biol Cell 14:973–986
71. **Ullrich A, Schlessinger J** 1990 Signal transduction by receptors with tyrosine kinase activity. Cell 61:203–212
72. **Czech MP, Corvera S** 1999 Signaling mechanisms that regulate glucose transport. J Biol Chem 274:1865–1868
73. **Lee JS, Pilch PF** 1994 The insulin receptor—structure, function, and signaling. Am J Physiol 266:C319–C334
74. **Moller DE, Yokota A, Caro JF, Flier JS** 1989 Tissue-specific expression of two alternatively spliced insulin receptor mRNAs in man. Mol Endocrinol 3:1263–1269
75. **Frasca F, Pandini G, Scalia P, Sciacca L, Mineo R, Costantino A, Goldfine ID, Belfiore A, Vigneri R** 1999 Insulin receptor isoform A, a newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells. Mol Cell Biol 19:3278–3288
76. **Pandini G, Frasca F, Mineo R, Sciacca L, Vigneri R, Belfiore A** 2002 Insulin/insulin-like growth factor I hybrid receptors have different biological characteristics depending on the insulin receptor isoform involved. J Biol Chem 277:39684–39695
77. **Joost HG** 1995 Structural and functional heterogeneity of insulin receptors. Cell Signal 7:85–91
78. **White MF** 2002 IRS proteins and the common path to diabetes. Am J Physiol 283:E413–E422
79. **Kitamura T, Kahn CR, Accili D** 2003 Insulin receptor knockout mice. Annu Rev Physiol 65:313–332
80. **Joshi RL, Lamothe B, Cordonnier N, Mesbah K, Monthioux E, Jami J, Bucchini D** 1996 Targeted disruption of the insulin receptor gene in the mouse results in neonatal lethality. EMBO J 15:1542–1547
81. **Accili D, Drago J, Lee EJ, Johnson MD, Cool MH, Salvatore P, Asico LD, Jose PA, Taylor SI, Westphal H** 1996 Early neonatal death in mice homozygous for a null allele of the insulin receptor gene. Nat Genet 12:106–109
82. **Bruning JC, Winnay J, Bonner-Weir S, Taylor SI, Accili D, Kahn CR** 1997 Development of a novel polygenic model of NIDDM in mice heterozygous for IR and IRS-1 null alleles. Cell 88:561–572
83. **Bruning JC, Michael MD, Winnay JN, Hayashi T, Horsch D, Accili D, Goodyear LJ, Kahn CR** 1998 A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. Mol Cell 2:559–569
84. **Wojtaszewski JF, Higaki Y, Hirshman MF, Michael MD, Dufresne SD, Kahn CR, Goodyear LJ** 1999 Exercise modulates postreceptor insulin signaling and glucose transport in muscle-specific insulin receptor knockout mice. J Clin Invest 104:1257–1264
85. **Shefi-Friedman L, Wertheimer E, Shen S, Bak A, Accili D, Sampson SR** 2001 Increased IGFR activity and glucose transport in cultured skeletal muscle from insulin receptor null mice. Am J Physiol 281:E16–E24
86. **Bluher M, Michael MD, Peroni OD, Ueki K, Carter N, Kahn BB, Kahn CR** 2002 Adipose tissue selective insulin receptor knockout protects against obesity and obesity-related glucose intolerance. Dev Cell 3:25–38
87. **Bluher M, Kahn BB, Kahn CR** 2003 Extended longevity in mice lacking the insulin receptor in adipose tissue. Science 299:572–574
88. **Michael MD, Kulkarni RN, Postic C, Previs SF, Shulman GI, Magnuson MA, Kahn CR** 2000 Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. Mol Cell 6:87–97
89. **Kulkarni RN, Bruning JC, Winnay JN, Postic C, Magnuson MA, Kahn CR** 1999 Tissue-specific knockout of the insulin receptor in pancreatic $\beta$ cells creates an insulin secretory defect similar to that in type 2 diabetes. Cell 96:329–339
90. **Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Krone W, Muller-Wieland D, Kahn CR** 2000 Role of brain insulin receptor in control of body weight and reproduction. Science 289:2122–2125
91. **Saltiel AR, Kahn CR** 2001 Insulin signalling and the regulation of glucose and lipid metabolism. Nature 414:799–806
92. **White MF** 2002 IRS proteins and the common path to diabetes. Am J Physiol 283:E413–E422
93. **Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI, Bonner-Weir S, White MF** 1998 Disruption of IRS-2 causes type 2 diabetes in mice. Nature 391:900–904
94. **Araki E, Lipes MA, Patti ME, Bruning JC, Haag B, Johnson RS, Kahn CR** 1994 Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. Nature 372:186–190
95. **Tamemoto H, Kadowaki T, Tobe K, Yagi T, Sakura H, Hayakawa T, Terauchi Y, Ueki K, Kaburagi Y, Satoh S, Sekihara H, Yoshioka S, Horikoshi H, Furuta Y, Ikawa Y, Kasuga M, Yazaki Y, Aizawa S** 1994 Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1. Nature 372:182–186
96. **Liu SC, Wang Q, Lienhard GE, Keller SR** 1999 Insulin receptor substrate 3 is not essential for growth or glucose homeostasis. J Biol Chem 274:18093–18099
97. **Bjornholm M, He AR, Attersand A, Lake S, Liu SC, Lienhard GE, Taylor S, Arner P, Zierath JR** 2002 Absence of functional insulin receptor substrate-3 (IRS-3) gene in humans. Diabetologia 45:1697–1702
98. **Fantin VR, Wang Q, Lienhard GE, Keller SR** 2000 Mice lacking insulin receptor substrate 4 exhibit mild defects in growth, reproduction, and glucose homeostasis. Am J Physiol 278:E127–E133
99. **Avruch J** 1998 Insulin signal transduction through protein kinase cascades. Mol Cell Biochem 182:31–48
100. **Cantley LC** 2002 The phosphoinositide 3-kinase pathway. Science 296:1655–1657
101. **Alessi DR, Downes CP** 1998 The role of PI 3-kinase in insulin action. Biochim Biophys Acta 1436:151–164
102. **Corvera S, Czech MP** 1998 Direct targets of phosphoinositide 3-kinase products in membrane traffic and signal transduction. Trends Cell Biol 8:442–446
103. **Ridley AJ** 2001 Rho proteins: linking signaling with membrane trafficking. Traffic 2:303–310
104. **Rebecchi MJ, Scarlata S** 1998 Pleckstrin homology domains: a common fold with diverse functions. Annu Rev Biophys Biomol Struct 27:503–528
105. **Farhang-Fallah J, Randhawa VK, Nimnual A, Klip A, Bar-Sagi D, Rozakis-Adcock M** 2002 The pleckstrin homology (PH) domain-interacting protein couples the insulin receptor substrate 1 PH domain to insulin signaling pathways leading to mitogenesis and GLUT4 translocation. Mol Cell Biol 22:7325–7336
106. **Okada T, Kawano Y, Sakakibara T, Hazeki O, Ui M** 1994 Essential role of phosphatidylinositol 3-kinase in insulin-induced glucose transport and antilipolysis in rat adipocytes. Studies with a selective inhibitor wortmannin. J Biol Chem 269:3568–3573
107. **Cheatham B, Vlahos CJ, Cheatham L, Wang L, Blenis J, Kahn CR** 1994 Phosphatidylinositol 3-kinase activation is required for insulin stimulation of pp70 S6 kinase, DNA synthesis, and glucose transporter translocation. Mol Cell Biol 14:4902–4911
108. **Clarke JF, Young PW, Yonezawa K, Kasuga M, Holman GD** 1994 Inhibition of the translocation of GLUT1 and GLUT4 in 3T3–L1 cells by the phosphatidylinositol 3-kinase inhibitor, wortmannin. Biochem J 300:631–635
109. **Kotani K, Carozzi AJ, Sakaue H, Hara K, Robinson LJ, Clark SF, Yonezawa K, James DE, Kasuga M** 1995 Requirement for phosphoinositide 3-kinase in insulin-stimulated GLUT4 translocation in 3T3–L1 adipocytes. Biochem Biophys Res Commun 209:343–348
110. **Sharma PM, Egawa K, Huang Y, Martin JL, Huvar I, Boss GR, Olefsky JM** 1998 Inhibition of phosphatidylinositol 3-kinase activity by adenovirus-mediated gene transfer and its effect on insulin action. J Biol Chem 273:18528–18537
111. **Martin SS, Haruta T, Morris AJ, Klippel A, Williams LT, Olefsky JM** 1996 Activated phosphatidylinositol 3-kinase is sufficient to mediate actin rearrangement and GLUT4 translocation in 3T3–L1 adipocytes. J Biol Chem 271:17605–17608
112. **Jiang T, Sweeney G, Rudolf MT, Klip A, Traynor-Kaplan A, Tsien RY** 1998 Membrane-permeant esters of phosphatidylinositol 3,4,5-trisphosphate. J Biol Chem 273:11017–11024
113. **Terauchi Y, Tsuji Y, Satoh S, Minoura H, Murakami K, Okuno A, Inukai K, Asano T, Kaburagi Y, Ueki K, Nakajima H, Hanafusa T, Matsuzawa Y, Sekihara H, Yin Y, Barrett JC, Oda H, Ishikawa T, Akanuma Y, Komuro I, Suzuki M, Yamamura K, Kodama T, Suzuki H, Kadowaki T** 1999 Increased insulin sensitivity and hypoglycaemia in mice lacking the p85 $\alpha$ subunit of phosphoinositide 3-kinase. Nat Genet 21:230–235

114. **Fruman DA, Mauvais-Jarvis F, Pollard DA, Yballe CM, Brazil D, Bronson RT, Kahn CR, Cantley LC** 2000 Hypoglycaemia, liver necrosis and perinatal death in mice lacking all isoforms of phosphoinositide 3-kinase p85 α. Nat Genet 26:379–382
115. **Mauvais-Jarvis F, Ueki K, Fruman DA, Hirshman MF, Sakamoto K, Goodyear LJ, Iannacone M, Accili D, Cantley LC, Kahn CR** 2002 Reduced expression of the murine p85α subunit of phosphoinositide 3-kinase improves insulin signaling and ameliorates diabetes. J Clin Invest 109:141–149
116. **Jiang G, Zhang BB** 2002 Pi 3-kinase and its up- and down-stream modulators as potential targets for the treatment of type II diabetes. Front Biosci 7:d903–d907
117. **Clement S, Krause U, Desmedt F, Tanti JF, Behrends J, Pesesse X, Sasaki T, Penninger J, Doherty M, Malaisse W, Dumont JE, Le Marchand-Brustel Y, Erneux C, Hue L, Schurmans S** 2001 The lipid phosphatase SHIP2 controls insulin sensitivity. Nature 409: 92–97
118. **Nakashima N, Sharma PM, Imamura T, Bookstein R, Olefsky JM** 2000 The tumor suppressor PTEN negatively regulates insulin signaling in 3T3–L1 adipocytes. J Biol Chem 275:12889–12895
119. **Brazil DP, Hemmings BA** 2001 Ten years of protein kinase B signalling: a hard Akt to follow. Trends Biochem Sci 26:657–664
120. **Brazil DP, Park J, Hemmings BA** 2002 PKB binding proteins. Getting in on the Akt. Cell 111:293–303
121. **Storz P, Toker A** 2002 3′-Phosphoinositide-dependent kinase-1 (PDK-1) in PI 3-kinase signaling. Front Biosci 7:d886–d902
122. **Stokoe D, Stephens LR, Copeland T, Gaffney PR, Reese CB, Painter GF, Holmes AB, McCormick F, Hawkins PT** 1997 Dual role of phosphatidylinositol-3,4,5-trisphosphate in the activation of protein kinase B. Science 277:567–570
123. **Le Good JA, Ziegler WH, Parekh DB, Alessi DR, Cohen P, Parker PJ** 1998 Protein kinase C isotypes controlled by phosphoinositide 3-kinase through the protein kinase PDK1. Science 281:2042–2045
124. **Toker A, Newton AC** 2000 Akt/protein kinase B is regulated by autophosphorylation at the hypothetical PDK-2 site. J Biol Chem 275:8271–8274
125. **Ueki K, Yamamoto-Honda R, Kaburagi Y, Yamauchi T, Tobe K, Burgering BM, Coffer PJ, Komuro I, Akanuma Y, Yazaki Y, Kadowaki T** 1998 Potential role of protein kinase B in insulin-induced glucose transport, glycogen synthesis, and protein synthesis. J Biol Chem 273:5315–5322
126. **Tanti JF, Grillo S, Gremeaux T, Coffer PJ, Van Obberghen E, Le Marchand-Brustel Y** 1997 Potential role of protein kinase B in glucose transporter 4 translocation in adipocytes. Endocrinology 138:2005–2010
127. **Wang Q, Somwar R, Bilan PJ, Liu Z, Jin J, Woodgett JR, Klip A** 1999 Protein kinase B/Akt participates in GLUT4 translocation by insulin in L6 myoblasts. Mol Cell Biol 19:4008–4018
128. **Kohn AD, Summers SA, Birnbaum MJ, Roth RA** 1996 Expression of a constitutively active Akt ser/thr kinase in 3T3–L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation. J Biol Chem 271:31372–31378
129. **Hill MM, Clark SF, Tucker DF, Birnbaum MJ, James DE, Macaulay SL** 1999 A role for protein kinase Bβ/Akt2 in insulin-stimulated GLUT4 translocation in adipocytes. Mol Cell Biol 19:7771–7781
130. **Ducluzeau PH, Fletcher LM, Welsh GI, Tavare JM** 2002 Functional consequence of targeting protein kinase B/Akt to GLUT4 vesicles. J Cell Sci 115:2857–2866
131. **Kotani K, Ogawa W, Matsumoto M, Kitamura T, Sakaue H, Hino Y, Miyake K, Sano W, Akimoto K, Ohno S, Kasuga M** 1998 Requirement of atypical protein kinase Cλ for insulin stimulation of glucose uptake but not for Akt activation in 3T3–L1 adipocytes. Mol Cell Biol 18:6971–6982
132. **Kitamura T, Ogawa W, Sakaue H, Hino Y, Kuroda S, Takata M, Matsumoto M, Maeda T, Konishi H, Kikkawa U, Kasuga M** 1998 Requirement for activation of the serine-threonine kinase Akt (protein kinase B) in insulin stimulation of protein synthesis but not of glucose transport. Mol Cell Biol 18:3708–3717
133. **Kohn AD, Barthel A, Kovacina KS, Boge A, Wallach B, Summers SA, Birnbaum MJ, Scott PH, Lawrence JCJ, Roth RA** 1998 Construction and characterization of a conditionally active version of the serine/threonine kinase Akt. J Biol Chem 273:11937–11943
134. **Cong LN, Chen H, Li Y, Zhou L, McGibbon MA, Taylor SI, Quon MJ** 1997 Physiological role of Akt in insulin-stimulated translocation of GLUT4 in transfected rat adipose cells. Mol Endocrinol 11:1881–1890
135. **Cho H, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Crenshaw 3rd EB, Kaestner KH, Bartolomei MS, Shulman GI, Birnbaum MJ** 2001 Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB β). Science 292:1728–1731
136. **Bae SS, Cho H, Mu J, Birnbaum MJ** 2003 Isoform-specific regulation of insulin-dependent glucose uptake by Akt/protein kinase B. J Biol Chem 278:49530–49536
137. **Jiang ZY, Zhou QL, Coleman KA, Chouinard M, Boese Q, Czech MP** 2003 Insulin signaling through Akt/protein kinase B analyzed by small interfering RNA-mediated gene silencing. Proc Natl Acad Sci USA 100:7569–7574
138. **Cho H, Thorvaldsen JL, Chu Q, Feng F, Birnbaum MJ** 2001 Akt1/PKBα is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. J Biol Chem 276:38349–38352
139. **Sano H, Kane S, Sano E, Miinea CP, Asara JM, Lane WS, Garner CW, Lienhard GE** 2003 Insulin-stimulated phosphorylation of a Rab GTPase-activating protein regulates GLUT4 translocation. J Biol Chem 278:14599–14602
140. **Farese RV** 2002 Function and dysfunction of aPKC isoforms for glucose transport in insulin-sensitive and insulin-resistant states. Am J Physiol Endocrinol Metab 283:E1–E11
141. **Standaert ML, Bandyopadhyay G, Kanoh Y, Sajan MP, Farese RV** 2001 Insulin and PIP3 activate PKC-ζ by mechanisms that are both dependent and independent of phosphorylation of activation loop (T410) and autophosphorylation (T560) sites. Biochemistry 40:249–255
142. **Limatola C, Schaap D, Moolenaar WH, van Blitterswijk WJ** 1994 Phosphatidic acid activation of protein kinase C-ζ overexpressed in COS cells: comparison with other protein kinase C isotypes and other acidic lipids. Biochem J 304:1001–1008
143. **Standaert ML, Bandyopadhyay G, Perez L, Price D, Galloway L, Poklepovic A, Sajan MP, Cenni V, Sirri A, Moscat J, Toker A, Farese RV** 1999 Insulin activates protein kinases C-ζ and C-λ by an autophosphorylation-dependent mechanism and stimulates their translocation to GLUT4 vesicles and other membrane fractions in rat adipocytes. J Biol Chem 274:25308–25316
144. **Chou MM, Hou W, Johnson J, Graham LK, Lee MH, Chen CS, Newton AC, Schaffhausen BS, Toker A** 1998 Regulation of protein kinase C ζ by PI 3-kinase and PDK-1. Curr Biol 8:1069–1077
145. **Bandyopadhyay G, Standaert ML, Galloway L, Moscat J, Farese RV** 1997 Evidence for involvement of protein kinase C (PKC)-ζ and noninvolvement of diacylglycerol-sensitive PKCs in insulin-stimulated glucose transport in L6 myotubes. Endocrinology 138:4721–4731
146. **Bandyopadhyay G, Standaert ML, Zhao L, Yu B, Avignon A, Galloway L, Karnam P, Moscat J, Farese RV** 1997 Activation of protein kinase C (α, β, and ζ) by insulin in 3T3/L1 cells. Transfection studies suggest a role for PKC-ζ in glucose transport. J Biol Chem 272:2551–2558
147. **Bandyopadhyay G, Kanoh Y, Sajan MP, Standaert ML, Farese RV** 2000 Effects of adenoviral gene transfer of wild-type, constitutively active, and kinase-defective protein kinase C-λ on insulin-stimulated glucose transport in L6 myotubes. Endocrinology 141:4120–4127
148. **Leitges M, Sanz L, Martin P, Duran A, Braun U, Garcia JF, Camacho F, Diaz-Meco MT, Rennert PD, Moscat J** 2001 Targeted disruption of the ζPKC gene results in the impairment of the NF-κB pathway. Mol Cell 8:771–780
149. **Guilherme A, Czech MP** 1998 Stimulation of IRS-1-associated phosphatidylinositol 3-kinase and Akt/protein kinase B but not glucose transport by β1-integrin signaling in rat adipocytes. J Biol Chem 273:33119–33122
150. **Isakoff SJ, Taha C, Rose E, Marcusohn J, Klip A, Skolnik EY** 1995 The inability of phosphatidylinositol 3-kinase activation to stimulate GLUT4 translocation indicates additional signaling pathways are required for insulin-stimulated glucose uptake. Proc Natl Acad Sci USA 92:10247–10251
151. **Staubs PA, Nelson JG, Reichart DR, Olefsky JM** 1998 Platelet-derived growth factor inhibits insulin stimulation of insulin re-

ceptor substrate-1-associated phosphatidylinositol 3-kinase in 3T3–L1 adipocytes without affecting glucose transport. J Biol Chem 273:25139–25147
152. **Krook A, Whitehead JP, Dobson SP, Griffiths MR, Ouwens M, Baker C, Hayward AC, Sen SK, Maassen JA, Siddle K, Tavare JM, O'Rahilly S** 1997 Two naturally occurring insulin receptor tyrosine kinase domain mutants provide evidence that phosphoinositide 3-kinase activation alone is not sufficient for the mediation of insulin's metabolic and mitogenic effects. J Biol Chem 272:30208–30214
153. **Watson RT, Pessin JE** 2001 Intracellular organization of insulin signaling and GLUT4 translocation. Recent Prog Horm Res 56:175–193
154. **Egawa K, Maegawa H, Shi K, Nakamura T, Obata T, Yoshizaki T, Morino K, Shimizu S, Nishio Y, Suzuki E, Kashiwagi A** 2002 Membrane localization of 3-phosphoinositide-dependent protein kinase-1 stimulates activities of Akt and atypical protein kinase C but does not stimulate glucose transport and glycogen synthesis in 3T3–L1 adipocytes. J Biol Chem 277:38863–38869
155. **Baumann CA, Ribon V, Kanzaki M, Thurmond DC, Mora S, Shigematsu S, Bickel PE, Pessin JE, Saltiel AR** 2000 CAP defines a second signalling pathway required for insulin-stimulated glucose transport. Nature 407:202–207
156. **Chiang SH, Baumann CA, Kanzaki M, Thurmond DC, Watson RT, Neudauer CL, Macara IG, Pessin JE, Saltiel AR** 2001 Insulin-stimulated GLUT4 translocation requires the CAP-dependent activation of TC10. Nature 410:944–948
157. **Watson RT, Shigematsu S, Chiang SH, Mora S, Kanzaki M, Macara IG, Saltiel AR, Pessin JE** 2001 Lipid raft microdomain compartmentalization of TC10 is required for insulin signaling and GLUT4 translocation. J Cell Biol 154:829–840
158. **Kanzaki M, Pessin JE** 2001 Insulin-stimulated GLUT4 translocation in adipocytes is dependent upon cortical actin remodeling. J Biol Chem 276:42436–42444
159. **Kanzaki M, Watson RT, Khan AH, Pessin JE** 2001 Insulin stimulates actin comet tails on intracellular GLUT4-containing compartments in differentiated 3T3L1 adipocytes. J Biol Chem 276: 49331–49336
160. **Anderson RG** 1998 The caveolae membrane system. Annu Rev Biochem 67:199–225
161. **Galbiati F, Razani B, Lisanti MP** 2001 Emerging themes in lipid rafts and caveolae. Cell 106:403–411
162. **Yamamoto M, Toya Y, Schwencke C, Lisanti MP, Myers Jr MG, Ishikawa Y** 1998 Caveolin is an activator of insulin receptor signaling. J Biol Chem 273:26962–26968
163. **Kimura A, Baumann CA, Chiang SH, Saltiel AR** 2001 The sorbin homology domain: a motif for the targeting of proteins to lipid rafts. Proc Natl Acad Sci USA 98:9098–9103
164. **Liu J, Kimura A, Baumann CA, Saltiel AR** 2002 APS facilitates c-Cbl tyrosine phosphorylation and GLUT4 translocation in response to insulin in 3T3–L1 adipocytes. Mol Cell Biol 22:3599–3609
165. **Ahmed Z, Smith BJ, Pillay TS** 2000 The APS adapter protein couples the insulin receptor to the phosphorylation of c-Cbl and facilitates ligand-stimulated ubiquitination of the insulin receptor. FEBS Lett 475:31–34
166. **Ahmed Z, Pillay TS** 2003 Adapter protein with a PH and SH2 domain (APS) and SH2-B enhance insulin receptor autophosphorylation, extracellular signal-regulated kinase and phosphoinositide 3-kinase-dependent signalling. Biochem J 371:405–412
167. **Soubeyran P, Kowanetz K, Szymkiewicz I, Langdon WY, Dikic I** 2002 Cbl-CIN85-endophilin complex mediates ligand-induced downregulation of EGF receptors. Nature 416:183–187
168. **Petrelli A, Gilestro GF, Lanzardo S, Comoglio PM, Migone N, Giordano S** 2002 The endophilin-CIN85-Cbl complex mediates ligand-dependent downregulation of c-Met. Nature 416:187–190
169. **Szymkiewicz I, Kowanetz K, Soubeyran P, Dinarina A, Lipkowitz S, Dikic I** 2002 CIN85 participates in Cbl-b-mediated down-regulation of receptor tyrosine kinases. J Biol Chem 277:39666–39672
170. **Watson RT, Furukawa M, Chiang SH, Boeglin D, Kanzaki M, Saltiel AR, Pessin JE** 2003 The exocytotic trafficking of TC10 occurs through both classical and nonclassical secretory transport pathways in 3T3L1 adipocytes. Mol Cell Biol 23:961–974
171. **Gual P, Shigematsu S, Kanzaki M, Gremeaux T, Gonzalez T, Pessin JE, Le Marchand-Brustel Y, Tanti JF** 2002 A Crk-II/TC10 signaling pathway is required for osmotic shock-stimulated glucose transport. J Biol Chem 277:43980–43986
172. **Terada S, Hirokawa N** 2000 Moving on to the cargo problem of microtubule-dependent motors in neurons. Curr Opin Neurobiol 10:566–573
173. **Apodaca G** 2001 Endocytic traffic in polarized epithelial cells: role of the actin and microtubule cytoskeleton. Traffic 2:149–159
174. **Stamnes M** 2002 Regulating the actin cytoskeleton during vesicular transport. Curr Opin Cell Biol 14:428–433
175. **Schafer DA** 2002 Coupling actin dynamics and membrane dynamics during endocytosis. Curr Opin Cell Biol 14:76–81
176. **Guilherme A, Emoto M, Buxton JM, Bose S, Sabini R, Theurkauf WE, Leszyk J, Czech MP** 2000 Perinuclear localization and insulin responsiveness of GLUT4 requires cytoskeletal integrity in 3T3–L1 adipocytes. J Biol Chem 275:38151–38159
177. **Patki V, Buxton J, Chawla A, Lifshitz L, Fogarty K, Carrington W, Tuft R, Corvera S** 2001 Insulin action on GLUT4 traffic visualized in single 3T3–l1 adipocytes by using ultra-fast microscopy. Mol Biol Cell 12:129–141
178. **Tsakiridis T, Vranic M, Klip A** 1994 Disassembly of the actin network inhibits insulin-dependent stimulation of glucose transport and prevents recruitment of glucose transporters to the plasma membrane. J Biol Chem 269:29934–29942
179. **Tsakiridis T, Tong P, Matthews B, Tsiani E, Bilan PJ, Klip A, Downey GP** 1999 Role of the actin cytoskeleton in insulin action. Microsc Res Tech 47:79–92
180. **Wang Q, Bilan PJ, Tsakiridis T, Hinek A, Klip A** 1998 Actin filaments participate in the relocalization of phosphatidylinositol 3-kinase to glucose transporter-containing compartments and in the stimulation of glucose uptake in 3T3–L1 adipocytes. Biochem J 331:917–928
181. **Omata W, Shibata H, Li L, Takata K, Kojima I** 2000 Actin filaments play a critical role in insulin-induced exocytotic recruitment but not in endocytosis of GLUT4 in isolated rat adipocytes. Biochem J 346:321–328
182. **Tong P, Khayat ZA, Huang C, Patel N, Ueyama A, Klip A** 2001 Insulin-induced cortical actin remodeling promotes GLUT4 insertion at muscle cell membrane ruffles. J Clin Invest 108:371–381
183. **Olson AL, Trumbly AR, Gibson GV** 2001 Insulin-mediated GLUT4 translocation is dependent on the microtubule network. J Biol Chem 276:10706–10714
184. **Emoto M, Langille SE, Czech MP** 2001 A role for kinesin in insulin-stimulated GLUT4 glucose transporter translocation in 3t3–L1 adipocytes. J Biol Chem 276:10677–10682
185. **Molero JC, Whitehead JP, Meerloo T, James DE** 2001 Nocodazole inhibits insulin-stimulated glucose transport in 3T3–L1 adipocytes via a microtubule-independent mechanism. J Biol Chem 276:43829–43835
186. **Shigematsu S, Khan AH, Kanzaki M, Pessin JE** 2002 Intracellular insulin-responsive glucose transporter (GLUT4) distribution but not insulin-stimulated GLUT4 exocytosis and recycling are microtubule dependent. Mol Endocrinol 16:1060–1068
187. **Kanzaki M, Watson RT, Hou JC, Stamnes M, Saltiel AR, Pessin JE** 2002 Small GTP-binding protein TC10 differentially regulates two distinct populations of filamentous actin in 3T3L1 adipocytes. Mol Biol Cell 13:2334–2346
188. **Welch MD, Mullins RD** 2002 Cellular control of actin nucleation. Annu Rev Cell Dev Biol 18:247–288
189. **Pollard TD, Almo S, Quirk S, Vinson V, Lattman EE** 1994 Structure of actin binding proteins: insights about function at atomic resolution. Annu Rev Cell Biol 10:207–249
190. **Pollard TD, Blanchoin L, Mullins RD** 2000 Molecular mechanisms controlling actin filament dynamics in nonmuscle cells. Annu Rev Biophys Biomol Struct 29:545–576
191. **Etienne-Manneville S, Hall A** 2002 Rho GTPases in cell biology. Nature 420:629–635
192. **Insall RH, Weiner OD** 2001 PIP3, PIP2, and cell movement—similar messages, different meanings? Dev Cell 1:743–747
193. **Jeng RL, Welch MD** 2001 Cytoskeleton: actin and endocytosis—no longer the weakest link. Curr Biol 11:R691–R694
194. **Qualmann B, Kessels MM** 2002 Endocytosis and the cytoskeleton. Int Rev Cytol 220:93–144
195. **Kanzaki M, Pessin JE** 2002 Caveolin-associated filamentous actin

(Cav-actin) defines a novel F-actin structure in adipocytes. J Biol Chem 277:25867–25869
196. **Rothberg KG, Heuser JE, Donzell WC, Ying YS, Glenney JR, Anderson RG** 1992 Caveolin, a protein component of caveolae membrane coats. Cell 68:673–682
197. **Scherer PE, Lisanti MP, Baldini G, Sargiacomo M, Mastick CC, Lodish HF** 1994 Induction of caveolin during adipogenesis and association of GLUT4 with caveolin-rich vesicles. J Cell Biol 127: 1233–1243
198. **Parpal S, Karlsson M, Thorn H, Stralfors P** 2001 Cholesterol depletion disrupts caveolae and insulin receptor signaling for metabolic control via insulin receptor substrate-1, but not for mitogen-activated protein kinase control. J Biol Chem 276:9670–9678
199. **Parton RG, Molero JC, Floetenmeyer M, Green KM, James DE** 2002 Characterization of a distinct plasma membrane macrodomain in differentiated adipocytes. J Biol Chem 277:46769–46778
200. **Morton WM, Ayscough KR, McLaughlin PJ** 2000 Latrunculin alters the actin-monomer subunit interface to prevent polymerization. Nat Cell Biol 2:376–378
201. **Bose A, Guilherme A, Robida SI, Nicoloro SM, Zhou QL, Jiang ZY, Pomerleau DP, Czech MP** 2002 Glucose transporter recycling in response to insulin is facilitated by myosin Myo1c. Nature 420:821–824
202. **Musch A, Cohen D, Kreitzer G, Rodriguez-Boulan E** 2001 cdc42 Regulates the exit of apical and basolateral proteins from the trans-Golgi network. EMBO J 20:2171–2179
203. **Luna A, Matas OB, Martinez-Menarguez JA, Mato E, Duran JM, Ballesta J, Way M, Egea G** 2002 Regulation of protein transport from the Golgi complex to the endoplasmic reticulum by CDC42 and N-WASP. Mol Biol Cell 13:866–879
204. **di Campli A, Valderrama F, Babia T, De Matteis MA, Luini A, Egea G** 1999 Morphological changes in the Golgi complex correlate with actin cytoskeleton rearrangements. Cell Motil Cytoskeleton 43:334–348
205. **Valderrama F, Babia T, Ayala I, Kok JW, Renau-Piqueras J, Egea G** 1998 Actin microfilaments are essential for the cytological positioning and morphology of the Golgi complex. Eur J Cell Biol 76:9–17
206. **Valderrama F, Duran JM, Babia T, Barth H, Renau-Piqueras J, Egea G** 2001 Actin microfilaments facilitate the retrograde transport from the Golgi complex to the endoplasmic reticulum in mammalian cells. Traffic 2:717–726
207. **Jahraus A, Egeberg M, Hinner B, Habermann A, Sackman E, Pralle A, Faulstich H, Rybin V, Defacque H, Griffiths G** 2001 ATP-dependent membrane assembly of F-actin facilitates membrane fusion. Mol Biol Cell 12:155–170
208. **Muallem S, Kwiatkowska K, Xu X, Yin HL** 1995 Actin filament disassembly is a sufficient final trigger for exocytosis in nonexcitable cells. J Cell Biol 128:589–598
209. **Bernstein BW, Bamburg JR** 1989 Cycling of actin assembly in synaptosomes and neurotransmitter release. Neuron 3:257–265
210. **Lang T, Wacker I, Wunderlich I, Rohrbach A, Giese G, Soldati T, Almers W** 2000 Role of actin cortex in the subplasmalemmal transport of secretory granules in PC-12 cells. Biophys J 78:2863–2877
211. **Rohatgi R, Ma L, Miki H, Lopez M, Kirchhausen T, Takenawa T, Kirschner MW** 1999 The interaction between N-WASP and the Arp2/3 complex links Cdc42-dependent signals to actin assembly. Cell 97:221–231
212. **Fawcett J, Pawson T** 2000 Signal transduction. N-WASP regulation—the sting in the tail. Science 290:725–726
213. **Prehoda KE, Scott JA, Mullins RD, Lim WA** 2000 Integration of multiple signals through cooperative regulation of the N-WASP-Arp2/3 complex. Science 290:801–806
214. **Rohatgi R, Ho HY, Kirschner MW** 2000 Mechanism of N-WASP activation by CDC42 and phosphatidylinositol 4,5-bisphosphate. J Cell Biol 150:1299–1310
215. **Neudauer CL, Joberty G, Tatsis N, Macara IG** 1998 Distinct cellular effects and interactions of the Rho-family GTPase TC10. Curr Biol 8:1151–1160
216. **Abe T, Kato M, Miki H, Takenawa T, Endo T** 2003 Small GTPase Tc10 and its homologue RhoT induce N-WASP-mediated long process formation and neurite outgrowth. J Cell Sci 116:155–168
217. **Rozelle AL, Machesky LM, Yamamoto M, Driessens MH, Insall RH, Roth MG, Luby-Phelps K, Marriott G, Hall A, Yin HL** 2000 Phosphatidylinositol 4,5-bisphosphate induces actin-based movement of raft-enriched vesicles through WASP-Arp2/3. Curr Biol 10:311–320
218. **Taunton J, Rowning BA, Coughlin ML, Wu M, Moon RT, Mitchison TJ, Larabell CA** 2000 Actin-dependent propulsion of endosomes and lysosomes by recruitment of N-WASP. J Cell Biol 148: 519–530
219. **Benesch S, Lommel S, Steffen A, Stradal TE, Scaplehorn N, Way M, Wehland J, Rottner K** 2002 Phosphatidylinositol 4,5-biphosphate (PIP2)-induced vesicle movement depends on N-WASP and involves Nck, WIP, and Grb2. J Biol Chem 277:37771–37776
220. **Suzuki T, Miki H, Takenawa T, Sasakawa C** 1998 Neural Wiskott-Aldrich syndrome protein is implicated in the actin-based motility of *Shigella flexneri*. EMBO J 17:2767–2776
221. **Egile C, Loisel TP, Laurent V, Li R, Pantaloni D, Sansonetti PJ, Carlier MF** 1999 Activation of the CDC42 effector N-WASP by the *Shigella flexneri* IcsA protein promotes actin nucleation by Arp2/3 complex and bacterial actin-based motility. J Cell Biol 146:1319–1332
222. **Loisel TP, Boujemaa R, Pantaloni D, Carlier MF** 1999 Reconstitution of actin-based motility of *Listeria* and *Shigella* using pure proteins. Nature 401:613–616
223. **Shibata H, Suzuki Y, Omata W, Tanaka S, Kojima I** 1995 Dissection of GLUT4 recycling pathway into exocytosis and endocytosis in rat adipocytes: evidence that GTP-binding proteins are involved in both processes. J Biol Chem 270:11489–11495
224. **Elmendorf JS, Chen D, Pessin JE** 1998 Guanosine 5′-O-(3-thiotriphosphate) (GTPγS) stimulation of GLUT4 translocation is tyrosine kinase-dependent. J Biol Chem 273:13289–13296
225. **Tsiani E, Bogdanovic E, Sorisky A, Nagy L, Fantus IG** 1998 Tyrosine phosphatase inhibitors, vanadate and pervanadate, stimulate glucose transport and GLUT translocation in muscle cells by a mechanism independent of phosphatidylinositol 3-kinase and protein kinase C. Diabetes 47:1676–1686
226. **Jiang ZY, Chawla A, Bose A, Way M, Czech MP** 2002 A phosphatidylinositol 3-kinase-independent insulin signaling pathway to N-WASP/Arp2/3/F-actin required for GLUT4 glucose transporter recycling. J Biol Chem 277:509–515
227. **Yin HL, Janmey PA** 2003 Phosphoinositide regulation of the actin cytoskeleton. Annu Rev Physiol 65:761–789
228. **Takenawa T, Itoh T** 2001 Phosphoinositides, key molecules for regulation of actin cytoskeletal organization and membrane traffic from the plasma membrane. Biochim Biophys Acta 1533:190–206
229. **Vanhaesebroeck B, Leevers SJ, Ahmadi K, Timms J, Katso R, Driscoll PC, Woscholski R, Parker PJ, Waterfield MD** 2001 Synthesis and function of 3-phosphorylated inositol lipids. Annu Rev Biochem 70:535–602
230. **Vollenweider P, Clodi M, Martin SS, Imamura T, Kavanaugh WM, Olefsky JM** 1999 An SH2 domain-containing 5′ inositolphosphatase inhibits insulin-induced GLUT4 translocation and growth factor-induced actin filament rearrangement. Mol Cell Biol 19:1081–1091
231. **Funamoto S, Meili R, Lee S, Parry L, Firtel RA** 2002 Spatial and temporal regulation of 3-phosphoinositides by PI 3-kinase and PTEN mediates chemotaxis. Cell 109:611–623
232. **Iijima M, Devreotes P** 2002 Tumor suppressor PTEN mediates sensing of chemoattractant gradients. Cell 109:599–610
233. **Wang F, Herzmark P, Weiner OD, Srinivasan S, Servant G, Bourne HR** 2002 Lipid products of PI(3)Ks maintain persistent cell polarity and directed motility in neutrophils. Nat Cell Biol 4:513–518
234. **Das B, Shu X, Day GJ, Han J, Krishna UM, Falck JR, Broek D** 2000 Control of intramolecular interactions between the pleckstrin homology and Dbl homology domains of Vav and Sos1 regulates Rac binding. J Biol Chem 275:15074–15081
235. **Michiels F, Stam JC, Hordijk PL, van der Kammen RA, Ruuls-Van Stalle L, Feltkamp CA, Collard JG** 1997 Regulated membrane localization of Tiam1, mediated by the NH2-terminal pleckstrin homology domain, is required for Rac-dependent membrane ruffling and C-Jun NH2-terminal kinase activation. J Cell Biol 137: 387–398
236. **Ma AD, Metjian A, Bagrodia S, Taylor S, Abrams CS** 1998 Cytoskeletal reorganization by G protein-coupled receptors is dependent on phosphoinositide 3-kinase γ, a Rac guanosine exchange factor, and Rac. Mol Cell Biol 18:4744–4751

237. **Moss J, Vaughan M** 1998 Molecules in the ARF orbit. J Biol Chem 273:21431–21434
238. **Jackson CL, Casanova JE** 2000 Turning on ARF: the Sec7 family of guanine-nucleotide-exchange factors. Trends Cell Biol 10:60–67
239. **Fucini RV, Chen JL, Sharma C, Kessels MM, Stamnes M** 2002 Golgi vesicle proteins are linked to the assembly of an actin complex defined by mAbp1. Mol Biol Cell 13:621–631
240. **Jackson TR, Brown FD, Nie Z, Miura K, Foroni L, Sun J, Hsu VW, Donaldson JG, Randazzo PA** 2000 ACAPs are arf6 GTPase-activating proteins that function in the cell periphery. J Cell Biol 151: 627–638
241. **Franco M, Peters PJ, Boretto J, van Donselaar E, Neri A, D'Souza-Schorey C, Chavrier P** 1999 EFA6, a sec7 domain-containing exchange factor for ARF6, coordinates membrane recycling and actin cytoskeleton organization. EMBO J 18:1480–1491
242. **Norman JC, Jones D, Barry ST, Holt MR, Cockcroft S, Critchley DR** 1998 ARF1 mediates paxillin recruitment to focal adhesions and potentiates Rho-stimulated stress fiber formation in intact and permeabilized Swiss 3T3 fibroblasts. J Cell Biol 143:1981–1995
243. **Mossessova E, Gulbis JM, Goldberg J** 1998 Structure of the guanine nucleotide exchange factor Sec7 domain of human arno and analysis of the interaction with ARF GTPase. Cell 92:415–423
244. **Macia E, Paris S, Chabre M** 2000 Binding of the PH and polybasic C-terminal domains of ARNO to phosphoinositides and to acidic lipids. Biochemistry 39:5893–5901
245. **Lietzke SE, Bose S, Cronin T, Klarlund J, Chawla A, Czech MP, Lambright DG** 2000 Structural basis of 3-phosphoinositide recognition by pleckstrin homology domains. Mol Cell 6:385–394
246. **Jackson TR, Kearns BG, Theibert AB** 2000 Cytohesins and centaurins: mediators of PI 3-kinase-regulated ARF signaling. Trends Biochem Sci 25:489–495
247. **Venkateswarlu K, Oatey PB, Tavare JM, Jackson TR, Cullen PJ** 1999 Identification of centaurin-$\alpha$1 as a potential in vivo phosphatidylinositol 3,4,5-trisphosphate-binding protein that is functionally homologous to the yeast ADP-ribosylation factor (ARF) GTPase-activating protein, Gcs1. Biochem J 340:359–363
248. **Radhakrishna H, Al-Awar O, Khachikian Z, Donaldson JG** 1999 ARF6 requirement for Rac ruffling suggests a role for membrane trafficking in cortical actin rearrangements. J Cell Sci 112:855–866
249. **Ma L, Cantley LC, Janmey PA, Kirschner MW** 1998 Corequirement of specific phosphoinositides and small GTP-binding protein Cdc42 in inducing actin assembly in *Xenopus* egg extracts. J Cell Biol 140:1125–1136
250. **Moreau V, Way M** 1998 Cdc42 is required for membrane dependent actin polymerization in vitro. FEBS Lett 427:353–356
251. **Ford MG, Mills IG, Peter BJ, Vallis Y, Praefcke GJ, Evans PR, McMahon HT** 2002 Curvature of clathrin-coated pits driven by epsin. Nature 419:361–366
252. **Joberty G, Perlungher RR, Macara IG** 1999 The Borgs, a new family of Cdc42 and TC10 GTPase-interacting proteins. Mol Cell Biol 19:6585–6597
253. **Joberty G, Petersen C, Gao L, Macara IG** 2000 The cell-polarity protein Par6 links Par3 and atypical protein kinase C to Cdc42. Nat Cell Biol 2:531–539
254. **Lin D, Edwards AS, Fawcett JP, Mbamalu G, Scott JD, Pawson T** 2000 A mammalian PAR-3-PAR-6 complex implicated in Cdc42/Rac1 and aPKC signalling and cell polarity. Nat Cell Biol 2:540–547
255. **Murphy GA, Solski PA, Jillian SA, Perez de la Ossa P, D'Eustachio P, Der CJ, Rush MG** 1999 Cellular functions of TC10, a Rho family GTPase: regulation of morphology, signal transduction and cell growth. Oncogene 18:3831–3845
256. **Murphy GA, Jillian SA, Michaelson D, Philips MR, D'Eustachio P, Rush MG** 2001 Signaling mediated by the closely related mammalian Rho family GTPases TC10 and Cdc42 suggests distinct functional pathways. Cell Growth Differ 12:157–167
257. **Usui I, Imamura T, Huang J, Satoh H, Olefsky JM** 2003 Cdc42 is a Rho GTPase family member that can mediate insulin signaling to glucose transport in 3T3–L1 adipocytes. J Biol Chem 278:13765–13774
258. **Rogers SL, Gelfand VI** 2000 Membrane trafficking, organelle transport, and the cytoskeleton. Curr Opin Cell Biol 12:57–62
259. **Fletcher LM, Welsh GI, Oatey PB, Tavare JM** 2000 Role for the microtubule cytoskeleton in GLUT4 vesicle trafficking and in the regulation of insulin-stimulated glucose uptake. Biochem J 352:267–276
260. **Semiz S, Park JG, Nicoloro SM, Furcinitti P, Zhang C, Chawla A, Leszyk J, Czech MP** 2003 Conventional kinesin KIF5B mediates insulin-stimulated GLUT4 movements on microtubules. EMBO J 22:2387–2399
261. **Zick Y** 2001 Insulin resistance: a phosphorylation-based uncoupling of insulin signaling. Trends Cell Biol 11:437–441
262. **Oved S, Yarden Y** 2002 Signal transduction: molecular ticket to enter cells. Nature 416:133–136
263. **Kosaki A, Yamada K, Suga J, Otaka A, Kuzuya H** 1998 14-3-3$\beta$ protein associates with insulin receptor substrate 1 and decreases insulin-stimulated phosphatidylinositol 3′-kinase activity in 3T3L1 adipocytes. J Biol Chem 273:940–944
264. **Xiang X, Yuan M, Song Y, Ruderman N, Wen R, Luo Z** 2002 14-3-3 Facilitates insulin-stimulated intracellular trafficking of insulin receptor substrate 1. Mol Endocrinol 16:552–562
265. **Baron SH** 1982 Salicylates as hypoglycemic agents. Diabetes Care 5:64–71
266. **Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, Shoelson SE** 2001 Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikk$\beta$. Science 293:1673–1677
267. **Dadke S, Kusari A, Kusari J** 2001 Phosphorylation and activation of protein tyrosine phosphatase (PTP) 1B by insulin receptor. Mol Cell Biochem 221:147–154
268. **Klaman LD, Boss O, Peroni OD, Kim JK, Martino JL, Zabolotny JM, Moghal N, Lubkin M, Kim YB, Sharpe AH, Stricker-Krongrad A, Shulman GI, Neel BG, Kahn BB** 2000 Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. Mol Cell Biol 20:5479–5489
269. **Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, Normandin D, Cheng A, Himms-Hagen J, Chan C-C, Ramachandran C, Gresser MJ, Tremblay ML, Kennedy BP** 1999 Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science 283:1544–1548
270. **Zabolotny JM, Bence-Hanulec KK, Stricker-Krongrad A, Haj F, Wang Y, Minokoshi Y, Kim YB, Elmquist JK, Tartaglia LA, Kahn BB, Neel BG** 2002 PTP1B regulates leptin signal transduction in vivo. Dev Cell 2:489–495
271. **Kaburagi Y, Yamauchi T, Yamamoto-Honda R, Ueki K, Tobe K, Akanuma Y, Yazaki Y, Kadowaki T** 1999 The mechanism of insulin-induced signal transduction mediated by the insulin receptor substrate family. Endocr J 46:S25–S34
272. **Rui L, Yuan M, Frantz D, Shoelson S, White MF** 2002 SOCS-1 and SOCS-3 block insulin signaling by ubiquitin-mediated degradation of IRS1 and IRS2. J Biol Chem 277:42394–42398
273. **Yasukawa H, Sasaki A, Yoshimura A** 2000 Negative regulation of cytokine signaling pathways. Annu Rev Immunol 18:143–164
274. **Emanuelli B, Peraldi P, Filloux C, Sawka-Verhelle D, Hilton D, Van Obberghen E** 2000 SOCS-3 is an insulin-induced negative regulator of insulin signaling. J Biol Chem 275:15985–15991
275. **Calakos N, Scheller RH** 1996 Synaptic vesicle biogenesis, docking, and fusion: a molecular description. Physiol Rev 76:1–29
276. **Nieman H, Blasi J, Jahn R** 1994 Clostridial neurotoxins: new tools for dissecting exocytosis. Trends Cell Biol 4:179–185
277. **Weber T, Zemelman BV, McNew JA, Westermann B, Gmachl M, Parlati F, Sollner TH, Rothman JE** 1998 SNAREpins: minimal machinery for membrane fusion. Cell 92:759–772
278. **Hanson PI, Roth R, Morisaki H, Jahn R, Heuser JE** 1997 Structure and conformational changes in NSF and its membrane receptor complexes visualized by quick-freeze/deep-etch electron microscopy. Cell 90:523–535
279. **Sutton R, Fasshauer D, Jahn R, Brunger A** 1998 Crystal structure of a SNARE complex involved in synaptic exocytosis at 2.4 A resolution. Nature 395:347–353
280. **Calakos N, Bennett MK, Peterson KE** 1994 Protein-protein interactions contributing to the specificity of intracellular vesicular trafficking. Science 263:1146–1149
281. **Scales SJ, Chen YA, Yoo BY, Patel SM, Doung Y-C, Scheller RH** 2000 SNAREs contribute to the specificity of membrane fusion. Neuron 26:457–464

282. **Grote E, Hao JC, Bennett MK, Kelly RB** 1995 A targeting signal in VAMP regulating transport to synaptic vesicles. Cell 81:581–589
283. **Watson RT, Pessin JE** 2000 Functional cooperation of two independent targeting domains in syntaxin 6 is required for its efficient localization in the trans-Golgi network of 3T3L1 adipocytes. J Biol Chem 275:1261–1268
284. **Mayer A** 1999 Intracellular membrane fusion: SNAREs only? Curr Opin Cell Biol 11:447–452
285. **Pfeffer SR** 1999 Transport-vesicle targeting: tethers before SNAREs. Nat Cell Biol 1:E17–E22
286. **Foster LJ, Klip A** 2000 Mechanism and regulation of GLUT-4 vesicle fusion in muscle and fat cells. Am J Physiol 279:C877–C890
287. **Thurmond DC, Pessin JE** 2001 Molecular machinery involved in the insulin-regulated fusion of GLUT4-containing vesicles with the plasma membrane. Mol Membr Biol 18:237–245 (Review)
288. **Grusovin JM, Macaulay SL** 2003 Snares for glut4-mechanisms directing vesicular trafficking of glut4. Front Biosci 8:D620–D641
289. **Yang C, Coker KJ, Kim JK, Mora S, Thurmond DC, Davis AC, Yang B, Williamson RA, Shulman GI, Pessin JE** 2001 Syntaxin 4 heterozygous knockout mice develop muscle insulin resistance. J Clin Invest 107:1311–1318
290. **Rea S, Martin LB, McIntosh S, Macaulay SL, Ramsdale T, Baldini G, James DE** 1998 Syndet, an adipocyte target SNARE involved in the insulin-induced translocation of GLUT4 to the cell surface. J Biol Chem 273:18784–18792
291. **Cheatham B, Volchuk A, Kahn CR, Wang L, Rhodes CJ, Klip A** 1996 Insulin-stimulated translocation of GLUT4 glucose transporters requires SNARE-complex proteins. Proc Natl Acad Sci USA 93:15169–15173
292. **Martin S, Tellam J, Livingstone C, Slot JW, Gould GW, James DE** 1996 The glucose transporter (GLUT-4) and vesicle-associated membrane protein-2 (VAMP-2) are segregated from recycling endosomes in insulin-sensitive cells. J Cell Biol 134:625–635
293. **Martin LB, Shewan A, Millar CA, Gould GW, James DE** 1998 Vesicle-associated membrane protein 2 plays a specific role in the insulin-dependent trafficking of the facilitative glucose transporter GLUT4 in 3T3–L1 adipocytes. J Biol Chem 273:1444–1452
294. **Millar CA, Shewan A, Hickson GR, James DE, Gould GW** 1999 Differential regulation of secretory compartments containing the insulin-responsive glucose transporter 4 in 3T3–L1 adipocytes. Mol Biol Cell 10:3675–3688
295. **Yang C, Mora S, Ryder JW, Coker KJ, Hansen P, Allen LA, Pessin JE** 2001 VAMP3 null mice display normal constitutive, insulin- and exercise-regulated vesicle trafficking. Mol Cell Biol 21:1573–1580
296. **Halachmi N, Lev Z** 1996 The Sec1 family: a novel family of proteins involved in synaptic transmission and general secretion. J Neurochem 66:889–897
297. **Fujita Y, Sasaki T, Fukui K, Kotani H, Kimura T, Hata Y, Sudhof TC, Scheller RH, Takai Y** 1996 Phosphorylation of Munc-18/n-Sec1/rbSec1 by protein kinase C. J Biol Chem 271:7265–7268
298. **Pevsner J, Hsu S-C, Scheller RH** 1994 N-Sec1: a neural-specific syntaxin-binding protein. Proc Natl Acad Sci USA 91:1445–1449
299. **Schulze KL, Littleton JT, Salzberg A, Halachmi N, Stern M, Lev Z, Bellen HJ** 1994 rop, a *Drosophila* homolog of yeast Sec1 and vertebrate n-Sec1/Munc-18 proteins, is a negative regulator of neurotransmitter release in vivo. Neuron 13:1099–1108
300. **Harrison SD, Broadie K, van de Goor J, Rubin GM** 1994 Mutations in the *Drosophila* Rop gene suggest a function in general secretion and synaptic transmission. Neuron 13:555–566
301. **Hosono R, Hekimi S, Kamuya Y, Sassa T, Murakami S, Nishiwaki K, Miwa J, Taketo A, Kodaira KI** 1992 The unc-18 gene encodes a novel protein affecting the kinetics of acetylcholine metabolism in the nematode *Caenorhabditis elegans*. J Neurochem 58:1517–1525
302. **Novick P, Schekman R** 1979 Secretion and cell-surface growth are blocked in a temperature-sensitive mutant of *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 76:1858–1862
303. **Misura KM, Scheller RH, Weis WI** 2000 Three-dimensional structure of the neuronal-Sec1-syntaxin 1a complex. Nature 404:355–362
304. **Tellam JT, Macaulay SL, McIntosh S, Hewish DR, Ward CW, James DE** 1997 Characterization of Munc-18c and syntaxin-4 in 3T3–L1 adipocytes. Putative role in insulin-dependent movement of GLUT-4. J Biol Chem 272:6179–6186
305. **Thurmond DC, Ceresa BP, Okada S, Elmendorf JS, Coker K, Pessin JE** 1998 Regulation of insulin-stimulated GLUT4 translocation by munc18c in 3T3L1 adipocytes. J Biol Chem 273:33876–33883
306. **Thurmond DC, Kanzaki M, Khan AH, Pessin JE** 2000 Munc18c function is required for insulin-stimulated plasma membrane fusion of GLUT4 and insulin-responsive amino peptidase storage vesicles. Mol Cell Biol 20:379–388
307. **Min J, Okada S, Coker K, Ceresa BP, Elmendorf JS, Syu L-J, Noda Y, Saltiel AR, Pessin JE** 1999 Synip: a novel insulin-regulated syntaxin 4 binding protein mediating GLUT4 translocation in adipocytes. Mol Cell 3:751–760
308. **Inoue M, Chang L, Hwang J, Chiang SH, Saltiel AR** 2003 The exocyst complex is required for targeting of Glut4 to the plasma membrane by insulin. Nature 422:629–633
309. **Lipschutz JH, Mostov KE** 2002 Exocytosis: the many masters of the exocyst. Curr Biol 12:R212–R214
310. **Guo W, Roth D, Walch-Solimena C, Novick P** 1999 The exocyst is an effector for Sec4p, targeting secretory vesicles to sites of exocytosis. EMBO J 18:1071–1080
311. **Adamo JE, Rossi G, Brennwald P** 1999 The Rho GTPase Rho3 has a direct role in exocytosis that is distinct from its role in actin polarity. Mol Biol Cell 10:4121–4133
312. **Robinson NG, Guo L, Imai J, Toh EA, Matsui Y, Tamanoi F** 1999 Rho3 of *Saccharomyces cerevisiae*, which regulates the actin cytoskeleton and exocytosis, is a GTPase which interacts with Myo2 and Exo70. Mol Cell Biol 19:3580–3587
313. **Sugihara K, Asano S, Tanaka K, Iwamatsu A, Okawa K, Ohta Y** 2002 The exocyst complex binds the small GTPase RalA to mediate filopodia formation. Nat Cell Biol 4:73–78
314. **Moskalenko S, Henry DO, Rosse C, Mirey G, Camonis JH, White MA** 2002 The exocyst is a Ral effector complex. Nat Cell Biol 4:66–72
315. **Zerial M, McBride H** 2001 Rab proteins as membrane organizers. Nat Rev Mol Cell Biol 2:107–117
316. **Pfeffer S** 2003 Membrane domains in the secretory and endocytic pathways. Cell 112:507–517
317. **Cormont M, Le Marchand-Brustel Y** 2001 The role of small G-proteins in the regulation of glucose transport. Mol Membr Biol 18:213–220 (Review)
318. **Cormont M, Tanti JF, Zahraoui A, Vanobberghen E, Tavitian A, Le Marchand-Brustel Y** 1993 Insulin and okadaic acid induce Rab4 redistribution in adipocytes. J Biol Chem 268:19491–19497
319. **Aledo JC, Darakhshan F, Hundal HS** 1995 Rab4, but not the transferrin receptor, is colocalized with GLUT4 in an insulin-sensitive intracellular compartment in rat skeletal muscle. Biochem Biophys Res Commun 215:321–328
320. **Shibata H, Omata W, Kojima I** 1997 Insulin stimulates guanine nucleotide exchange on Rab4 via a wortmannin-sensitive signaling pathway in rat adipocytes. J Biol Chem 272:14542–14546
321. **Knight JB, Cao KT, Gibson GV, Olson AL** 2000 Expression of a prenylation-deficient Rab4 interferes with propagation of insulin signaling through insulin receptor substrate-1. Endocrinology 141:208–218
322. **Shibata H, Omata W, Suzuki Y, Tanaka S, Kojima I** 1996 A synthetic peptide corresponding to the Rab4 hypervariable carboxyl-terminal domain inhibits insulin action on glucose transport in rat adipocytes. J Biol Chem 271:9704–9709
323. **Cormont M, Bortoluzzi MN, Gautier N, Mari M, Van Obberghen E, Le Marchand-Brustel Y** 1996 Potential role of Rab4 in the regulation of subcellular localization of Glut4 in adipocytes. Mol Cell Biol 16:6879–6886
324. **Cormont M, Gautier N, Ilc K, le Marchand-Brustel Y** 2001 Expression of a prenylation-deficient Rab4 inhibits the GLUT4 translocation induced by active phosphatidylinositol 3-kinase and protein kinase B. Biochem J 356:143–149
325. **Mora S, Monden I, Zorzano A, Keller K** 1997 Heterologous expression of rab4 reduces glucose transport and GLUT4 abundance at the cell surface in oocytes. Biochem J 324:455–459
326. **Li L, Omata W, Kojima I, Shibata H** 2001 Direct interaction of Rab4 with syntaxin 4. J Biol Chem 276:5265–5273
327. **Kessler A, Tomas E, Immler D, Meyer HE, Zorzano A, Eckel J** 2000 Rab11 is associated with GLUT4-containing vesicles and redistributes in response to insulin. Diabetologia 43:1518–1527
328. **Zeigerer A, Lampson MA, Karylowski O, Sabatini DD, Adesnik M, Ren M, McGraw TE** 2002 GLUT4 retention in adipocytes re-

quires two intracellular insulin-regulated transport steps. Mol Biol Cell 13:2421–2435
329. **Chavrier P, Goud B** 1999 The role of ARF and Rab GTPases in membrane transport. Curr Opin Cell Biol 11:466–475
330. **Millar CA, Powell KA, Hickson GR, Bader MF, Gould GW** 1999 Evidence for a role for ADP-ribosylation factor 6 in insulin-stimulated glucose transporter-4 (GLUT4) trafficking in 3T3–L1 adipocytes. J Biol Chem 274:17619–17625
331. **Yang CZ, Mueckler M** 1999 ADP-ribosylation factor 6 (ARF6) defines two insulin-regulated secretory pathways in adipocytes. J Biol Chem 274:25297–25300
332. **Czech MP** 1995 Molecular actions of insulin on glucose transport. Annu Rev Nutr 15:441–471
333. **Holman GD, Cushman SW** 1994 Subcellular localization and trafficking of the GLUT4 glucose transporter isoform in insulin-responsive cells. Bioessays 16:753–759
334. **Kandror KV, Pilch PF** 1996 Compartmentalization of protein traffic in insulin-sensitive cells. Am J Physiol 271:E1–E14
335. **Slot JW, Geuze HJ, Gigengack S, Lienhard GE, James DE** 1991 Immuno-localization of the insulin regulatable glucose transporter in brown adipose tissue of the rat. J Cell Biol 113:123–135
336. **Slot JW, Geuze HJ, Gigengack S, James DE, Lienhard GE** 1991 Translocation of the glucose transporter GLUT4 in cardiac myocytes of the rat. Proc Natl Acad Sci USA 88:7815–7819
337. **Nishimura H, Zarnowski MJ, Simpson IA** 1993 Glucose transporter recycling in rat adipose cells. Effects of potassium depletion. J Biol Chem 268:19246–19253
338. **Muhlberg AB, Warnock DE, Schmid SL** 1997 Domain structure and intramolecular regulation of dynamin GTPase. EMBO J 16:6676–6683
339. **van der Bliek AM, Meyerowitz EM** 1991 Dynamin-like protein encoded by the *Drosophila* shibire gene associated with vesicular traffic. Nature 351:411–414
340. **Kosaka T, Ikeda K** 1983 Possible temperature-dependent blockage of synaptic vesicle recycling induced by a single gene mutation in *Drosophila*. J Neurobiol 14:207–225
341. **McNiven MA** 1998 Dynamin: a molecular motor with pinchase action. Cell 94:151–154
342. **McNiven MA, Cao H, Pitts KR, Yoon Y** 2000 The dynamin family of mechanoenzymes: pinching in new places. Trends Biochem Sci 25: 115–120
343. **Ringstad N, Gad H, Low P, Di Paolo G, Brodin L, Shupliakov O, De Camilli P** 1999 Endophilin/SH3p4 is required for the transition from early to late stages in clathrin-mediated synaptic vesicle endocytosis. Neuron 24:143–154
344. **Al-Hasani H, Hinck CS, Cushman SW** 1998 Endocytosis of the glucose transporter GLUT4 is mediated by the GTPase dynamin. J Biol Chem 273:17504–17510
345. **Kao AW, Ceresa BP, Santeler SR, Pessin JE** 1998 Expression of a dominant interfering dynamin mutant in 3T3L1 adipocytes inhibits GLUT4 endocytosis without affecting insulin signaling. J Biol Chem 273:25450–25457
346. **Volchuk A, Narine S, Foster LJ, Grabs D, De Camilli P, Klip A** 1998 Perturbation of dynamin II with an amphiphysin SH3 domain increases GLUT4 glucose transporters at the plasma membrane in 3T3–L1 adipocytes. Dynamin II participates in GLUT4 endocytosis. J Biol Chem 273:8169–8176
347. **Hinshaw JE** 2000 Dynamin and its role in membrane fission. Annu Rev Cell Dev Biol 16:483–519
348. **Ando A, Yonezawa K, Gout I, Nakata T, Ueda H, Hara K, Kitamura Y, Noda Y, Takenawa T, Hirokawa N, Waterfield MD, Kasuga M** 1994 A complex of GRB2 dynamin binds to tyrosine-phosphorylated insulin receptor substrate-1 after insulin treatment. EMBO J 13:3033–3038
349. **Baron V, Alengrin F, Van Obberghen E** 1998 Dynamin associates with Src-homology collagen (Shc) and becomes tyrosine phosphorylated in response to insulin. Endocrinology 139:3034–3037
350. **Smith U, Kuroda M, Simpson IA** 1984 Counter-regulation of insulin-stimulated glucose transport by catecholamines in the isolated rat adipose cell. J Biol Chem 259:8758–8763
351. **Kuroda M, Honnor RC, Cushman SW, Londos C, Simpson IA** 1987 Regulation of insulin-stimulated glucose transport in the isolated rat adipocyte. cAMP-independent effects of lipolytic and antilipolytic agents. J Biol Chem 262:245–253
352. **Kashiwagi A, Huecksteadt TP, Foley JE** 1983 The regulation of glucose transport by cAMP stimulators via three different mechanisms in rat and human adipocytes. J Biol Chem 258:13685–13692
353. **James DE, Hiken J, Lawrence Jr JC** 1989 Isoproterenol stimulates phosphorylation of the insulin-regulatable glucose transporter in rat adipocytes. Proc Natl Acad Sci USA 86:8368–8372
354. **Piper RC, James DE, Slot JW, Puri C, Lawrence Jr JC** 1993 GLUT4 phosphorylation and inhibition of glucose transport by dibutyryl cAMP. J Biol Chem 268:16557–16563
355. **Lawrence Jr JC, Hiken JF, James DE** 1990 Phosphorylation of the glucose transporter in rat adipocytes. Identification of the intracellular domain at the carboxyl terminus as a target for phosphorylation in intact-cells and in vitro. J Biol Chem 265:2324–2332
356. **Vannucci SJ, Nishimura H, Satoh S, Cushman SW, Holman GD, Simpson IA** 1992 Cell surface accessibility of GLUT4 glucose transporters in insulin-stimulated rat adipose cells. Modulation by isoprenaline and adenosine. Biochem J 288:325–330
357. **Yang J, Hodel A, Holman GD** 2002 Insulin and isoproterenol have opposing roles in the maintenance of cytosol pH and optimal fusion of GLUT4 vesicles with the plasma membrane. J Biol Chem 277: 6559–6566
358. **Somwar R, Kim DY, Sweeney G, Huang C, Niu W, Lador C, Ramlal T, Klip A** 2001 GLUT4 translocation precedes the stimulation of glucose uptake by insulin in muscle cells: potential activation of GLUT4 via p38 mitogen-activated protein kinase. Biochem J 359:639–649
359. **Sweeney G, Somwar R, Ramlal T, Volchuk A, Ueyama A, Klip A** 1999 An inhibitor of p38 mitogen-activated protein kinase prevents insulin-stimulated glucose transport but not glucose transporter translocation in 3T3–L1 adipocytes and L6 myotubes. J Biol Chem 274:10071–10078
360. **Somwar R, Perreault M, Kapur S, Taha C, Sweeney G, Ramlal T, Kim DY, Keen J, Cote CH, Klip A, Marette A** 2000 Activation of p38 mitogen-activated protein kinase $\alpha$ and $\beta$ by insulin and contraction in rat skeletal muscle: potential role in the stimulation of glucose transport. Diabetes 49:1794–1800
361. **Jongsoon L, Pilch PF** 1994 The insulin receptor: structure, function, and signaling. Am J Physiol 266:C319–C334
362. **De Meyts P, Whittaker J** 2002 Structural biology of insulin and IGF1 receptors: implications for drug design. Nat Rev Drug Discov 1:769–783