A Role for Kinesin in Insulin-stimulated GLUT4 Glucose Transporter Translocation in 3T3-L1 Adipocytes*

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Insulin regulates glucose uptake in adipocytes and muscle by stimulating the movement of sequestered glucose transporter 4 (GLUT4) proteins from intracellular membranes to the cell surface. Here we report that optimal insulin-mediated GLUT4 translocation is dependent upon both microtubule and actin-based cytoskeletal structures in cultured adipocytes. Depolymerization of microtubules and F-actin in 3T3-L1 adipocytes causes the dispersion of perinuclear GLUT4-containing membranes and abolishes insulin action on GLUT4 movements to the plasma membrane. Furthermore, heterologous expression in 3T3-L1 adipocytes of the microtubule-binding protein hTau40, which impairs kinesin motors that move toward the plus ends of microtubules, markedly delayed the appearance of GLUT4 at the plasma membrane in response to insulin. The hTau40 protein had no detectable effect on microtubule structure or perinuclear GLUT4 localization under these conditions. These results are consistent with the hypothesis that both the actin and microtubule-based cytoskeleton, as well as a kinesin motor, direct the translocation of GLUT4 to the plasma membrane in response to insulin.

Physiological glucose homeostasis in humans is largely dependent on the actions of the hormone insulin, particularly its ability to inhibit glucose output from the liver and enhance glucose transport into fat and muscle cells. Insulin exerts this latter effect primarily through a process whereby sequestered intracellular GLUT4 glucose transporter proteins are redistributed to cell surface membranes in which they can catalyze glucose uptake into cells (1–4). In both the basal and insulin-stimulated states, GLUT4 proteins appear to cycle between intracellular membranes and plasma membrane locations (5). However, in the basal state most of the GLUT4 is diverted to intracellular perinuclear membranes, and the exocytosis rate is slow. Insulin stimulates exocytosis of GLUT4 by a mechanism that requires the p85/p110 type phosphatidylinositol 3-kinase, which is recruited to protein phosphotyrosines in response to activation of the insulin receptor tyrosine kinase (6–8). Insulin also appears to significantly inhibit GLUT4 endocytosis (5, 9, 10). However, the precise mechanism whereby insulin signals to the GLUT4 membrane trafficking machinery remains obscure.

It is known that other membrane systems, such as lysosomes (11), mitochondria (12), Golgi membranes (13, 14), and pigment granules (15), are localized within cells by molecular motors. For example, membrane vesicles containing melanin appear to be driven along microtubules over relatively long distances from their perinuclear location in unstimulated melanocytes to the cell periphery upon elevation of cAMP levels (16). The complex motor dynein drives movements along microtubules in the minus direction toward the perinuclear microtubule organizing center, whereas kinesins motors drive movements toward the plus growing ends of microtubules (17). Movements of these membranes over shorter distances seem to require actin filaments (18, 19). Based on these observations, it has been suggested that the microtubule and actin filament networks are highly integrated in discharging their organelle localization functions (20). Recent findings in our laboratory have implicated a role of the cytoskeleton in the mechanism of insulin-stimulated GLUT4 translocation in cultured 3T3-L1 adipocytes (21). These studies revealed that the microtubule protein α-tubulin and the intermediate filament protein vimentin are present in the preparations of GLUT4-containing membranes and confirmed their association with vesicles containing GLUT4 by electron microscopy. Disruption of the intermediate filaments and microtubules in 3T3-L1 adipocytes by microinjection of a vimentin-derived peptide caused the dispersion of perinuclear GLUT4. These and other findings (21) are consistent with the hypothesis that the molecular motor dynein directs the movements of GLUT4-containing membranes to the minus ends of microtubules in the juxtanuclear region of cultured adipocytes.

The major aim of the present studies was to test the hypothesis that optimal insulin-mediated GLUT4 translocation to the cell periphery requires the microtubule motor kinesin in the context of an intact microtubule and F-actin cytoskeleton. Consistent with this concept, both nocodazole and colchicine were found to inhibit GLUT4 translocation markedly as well as disrupt microtubules in 3T3-L1 adipocytes. Insulin action on GLUT4 was also dependent on intact F-actin. We took further advantage of the ability of the neuronal microtubule-associated protein hTau40 to partially inhibit the function of kinesins when it is expressed heterologously in cultured cells as described by Mandelkow and co-workers (22, 23). The hTau40 protein localized to microtubules in 3T3-L1 adipocytes and delayed the initial appearance of GLUT4 at the cell surface membrane in response to insulin. The data are consistent with the hypothesis that insulin-stimulated GLUT4 movements to the cell periphery involve one or more kinesins.

EXPERIMENTAL PROCEDURES

Materials—Human Tau40 cDNA construct was kindly provided by Dr. Mandelkow (22, 23). Rabbit polyclonal anti-GLUT4 antibody was raised against the C-terminal 12 amino acids. Rabbit polyclonal anti-Tau antibody and anti-tubulin were from Sigma. Goat polyclonal anti-GLUT4 was from Santa Cruz Biotechnology (Santa Cruz, CA). The
rhodamine-conjugated or fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse rabbit antibodies were from BioSource International (Camarillo, CA). Cy3- and Cy5-conjugated donkey anti-mouse and rabbit IgG were from Jackson Immuno Research (West Grove, PA). Rhodamine-conjugated phalloidin was purchased from Molecular Probes, Inc. (Eugene, OR). Nocodazole and colchicine were purchased from Sigma, and latrunculin B was obtained from Calbiochem and dissolved in Me2SO.

**Tau Adenovirus Construct and Infection—** Recombinant adenovirus encoding the hTau40 was constructed after the method described in He et al. (24). hTau40 cDNA was cloned into the BglII-XhoI site of pAdTrack-CMV shuttle vector. This vector was linearized with PmeI and was electroporated into electrocompetent BJ5183 cells having the pAdEasy-1 plasmid to generate recombinant adenovirus. After the recombinant plasmid was amplified, DNA was digested with PacI. This adenovirus was transfected into 293 cells and monitored with green fluorescent protein expression. After the virus amplification, cell lysate was stored at −80 °C. For the adenovirus infection, 3T3-L1 adipocytes were grown and differentiated on coverslips in 6-well plates and then infected at a multiplicity of infection of 50 with either a control virus expressing green fluorescent protein only or the recombinant adenovirus encoding hTau40 adenovirus. On the next day, cells were serum-starved overnight in Dulbecco’s modified Eagle’s medium (DMEM) with 0.5% bovine serum albumin, and the experiments were started after 46 h of infection. This protocol resulted in an infection efficiency of at least 80% of adipocytes.

**Cell Culture and Treatment—** 3T3-L1 fibroblasts were grown in DMEM supplemented with 25 mM glucose, 10% fetal bovine serum, 50 mg/ml streptomycin, 50 units/ml penicillin. 3T3-L1 fibroblasts (3–4 days postconfluent) were differentiated into adipocytes by incubating with the same DMEM containing 0.5 mM isobutylmethylxanthine, 0.25 mM dexamethasone, and 4 μg/ml insulin for 3 days, grown in DMEM with 10% fetal bovine serum and 4 μg/ml insulin for 2 days and then grown in DMEM with 10% fetal bovine serum for an additional 3–6 days.

For the immunofluorescence experiments, differentiated adipocytes were replated on glass coverslips using trypsin and collagenase and then incubated in DMEM containing 25 mM glucose and 10% fetal bovine serum. Before the experiments, adipocytes were incubated overnight in serum-free medium containing 0.5% bovine serum albumin, except when rhodamine-conjugated phalloidin staining was performed. Concentrations of inhibitors used and incubation times are noted in Figs. 1–7.

**Immunofluorescence Microscopy—** 3T3-L1 adipocytes were either treated or not treated with 30 μM nocodazole, 10 μM latrunculin B for 1 h, or 10 μM colchicine for 1–3 h at 37 °C. Cells were fixed in 3.7% formaldehyde in phosphate-buffered saline (PBS) (171 mM NaCl, 10.1 mM Na2HPO4, 3.35 mM KCl, 1.84 mM KH2PO4, pH 7.2) or methanol. Expressed hTau40 constructs can be visualized by extraction with 0.5% Triton X-100 in PBS for 5–10 s before methanol fixation, which allows unbound protein to diffuse out (25). Formaldehyde fixation was not applied because the cellular localization of Tau is altered (26). Cells were incubated in PBS containing buffer A (0.5% Triton X-100 in PBS and 1% fetal bovine serum) for 15 min and then incubated for 2 h with polyclonal anti-Glut4, anti-Tau, or monoclonal anti-tubulin antibodies. The coverslips were washed extensively with buffer A and incubated in a 1:200–1:1000 dilution of the appropriate secondary antibody conjugated to FITC, rhodamine, or Cy3. For F-actin staining, cells were treated with 1 unit/ml rhodamine-conjugated phalloidin for 30 min. The coverslips were washed again extensively with buffer A, rinsed once with PBS, and mounted on slides with 90% glycerol in PBS and 2.5% DABCO. The stained cells were observed with confocal microscopy using a Nikon Diaphot 200 inverted microscope and a MRC1024 processing unit (Bio-Rad). Zoom factors of 1.0–3.0 were used, and the images were analyzed by laser-sharp processing software.

**GLUT4 Translocation Assay—** GLUT4 translocation was assayed using plasma membrane lawns as described previously (27). 3T3-L1 adipocytes cultured on glass coverslips were treated as described in the figure legends. At the end of each experiment, cells were rapidly washed in PBS followed by a 40-s treatment in PBS containing 0.5 mg/ml poly-L-lysine (Sigma). The cells were swollen by three rapid washes in hypotonic buffer (one-third of buffer B) transferred to buffer B (70 mM KCl, 30 mM HEPES, 5 mM MgCl2, 3 mM EGTA, pH 7.4), and sonicated using a probe sonicator to generate a lawn of plasma membrane fragments attached to the glass. The membranes were then fixed for 15 min with 3.7% formaldehyde, washed three times with PBS, and blocked with PBS containing 2% bovine serum albumin for 40 min. To quantify GLUT4 on lawns, coverslips were incubated with anti-Glut4 antibody diluted 1:1000 in PBS containing 0.05% Tween 20 for 2 h at room temperature. Coverslips were washed five times for 3 min each, and they were incubated with FITC-conjugated goat anti-rabbit IgG (1:1000) mixed with 10 μg/ml rhodamine-conjugated wheat germ agglutinin (to identify plasma membranes). Coverslips were then washed as described earlier and postfixed for 10 min with 3.7% formaldehyde followed by a final wash with PBS before mounting on slides with DABCO. Stained cells were observed using the confocal microscopy system as described above. For each experiment, samples were run in duplicate, and 10 random representative images were collected from each coverslip. Fluorescence intensity was quantified using Adobe Photoshop analysis software.

**RESULTS AND DISCUSSION**

**Intracellular Localization of GLUT4 Is Disrupted by Nocodazole or Latrunculin B—** Initial experiments were conducted using confocal microscopy to test the effects of the F-actin-disrupting agent latrunculin B and the microtubule depolymerizing agent nocodazole on GLUT4 disposition in 3T3-L1 adipocytes. Minimum concentrations of these agents required for disrupting the cytoskeleton were determined (data not shown). As depicted in Fig. 1A, cultured adipocytes do not

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1. The abbreviations used are: GLUT4, glucose transporter 4; FITC, fluorescein isothiocyanate; DMEM, Dulbecco’s modified Eagle’s medium; PBS, phosphate-buffered saline; Dabco, 1,4-diazabicyclo[2.2.2]octane; CLIP, cytoplasmic linker protein.
displays actin stress fibers, but cortical actin and thin actin filaments, especially at the lower focal planes of the cells, can be visualized by phalloidin staining. Incubation of the cells with 10 μM latrunculin B for 60 min causes dispersion of the actin filaments and much of the cortical actin. Interestingly, the normally concentrated perinuclear GLUT4 in these treated cells appears to be partially dispersed and more granular. In addition, many large vesicular structures containing GLUT4 are visualized throughout the latrunculin B-treated cells (Fig. 1A). Treatment with 30 μM nocodazole causes a virtually complete disassembly of microtubules in 3T3-L1 adipocytes as well as a marked dispersion of perinuclear GLUT4 into a fine punctate appearance throughout the cytoplasm. The addition of both agents to the cultured adipocytes yields a disposition of GLUT4 that is also dispersed throughout the cells. These alterations in GLUT4-containing membrane localizations do not appear to originate from nonspecific cell damage because a normal perinuclear GLUT4 morphology is restored within 3 h after the removal of the cytoskeleton-disrupting agents. These data indicate that the integrity of both F-actin and microtubules is required to concentrate GLUT4 in the perinuclear region of 3T3-L1 adipocytes.

**Disruption of Both Microtubules and F-actin Abolishes GLUT4 Translocation in Response to Insulin—**To determine whether tubulin- and actin-based cytoskeletal elements are necessary for optimal GLUT4 movements to the cell surface in response to insulin, the effects of latrunculin B and nocodazole on the appearance of GLUT4 on plasma membrane are visualized throughout the latrunculin B-treated cells (Fig. 4). Treatment of the cultured adipocytes with either nocodazole or latrunculin B leads to an approximately 50% inhibition of insulin-stimulated GLUT4 translocation. Remarkably, incubation of 3T3-L1 adipocytes with the combination of these agents for 60 min completely blocked insulin action on GLUT4 translocation. Taken together, these results demonstrate the requirement of either intact microtubules or intact F-actin in cultured adipocytes for partial responsiveness of GLUT4 to insulin. In the absence of both cytoskeletal systems, GLUT4 movements to the cell surface are abolished.

The data depicted in Figs. 1 and 2 are consistent with the recent work on several other cell types revealing that the

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**FIG. 2.** Treatment of 3T3-L1 adipocytes with nocodazole and latrunculin B abolishes GLUT4 translocation in response to insulin. A, after the serum starvation, 3T3-L1 adipocytes were treated without or with 30 μM nocodazole (Noc) or 10 μM latrunculin B (LB) for 60 min at 37 °C and then they were treated with or without 100 nM insulin for 15 min. Lawns of adipocyte plasma membranes were generated as described under “Experimental Procedures.” The lawns were incubated with rabbit anti-GLUT4 antibody followed by FITC-conjugated secondary antibody. These images are representative fields from three independent experiments. B, translocated GLUT4 protein in the lawns as shown in A were quantified by measuring the fluorescence intensity using Adobe Photoshop software. These values were obtained by random counting of >300 cell sheets from three independent experiments and were normalized to total cell sheet numbers in each experiment. Results are the means ± S.D.

**FIG. 3.** Effects of nocodazole treatment and overexpression of hTau40 protein on microtubules and GLUT4 localization in 3T3-L1 adipocytes. A, bar diagram of the long human Tau isoform (hTau40) used for expression in 3T3-L1 adipocytes in this study. The hTau40 protein can be subdivided into an N-terminal projection domain and a C-terminal microtubule binding domain. hTau40 contains four repeats (R1–R4) that are flanked by a proline-rich region (P1 and P2) that extends into the projection domain and harbors most of the Ser-Thr-Pro motifs. B, differentiated 3T3-L1 adipocytes were infected with hTau40 recombinant adenovirus (c, d, g, h, k, l, o, and p) or control virus with no insert (a, b, e, f, i, j, m, and n). The cells were then treated with 30 μM nocodazole (-/- Noc) (e–k) and m–p) for 60 min at 37 °C. The cells were fixed with methanol (see “Experimental Procedures”) and subjected to confocal fluorescence microscopy. Shown are representative fields from three to four independent experiments. Note that nocodazole disrupted the microtubules and dispersed GLUT4 vesicles, but hTau40 overexpression had little effect on GLUT4 morphology.
functions of microtubules and F-actin in localizing intracellular membranes are highly coordinated (for review see Ref. 20). Axoplasmic vesicles in the giant squid axon were shown to translocate on both microtubules and actin filaments, and these vesicles apparently can switch from one system to the other during movements (28). Early endosomes containing Rab5 appear to be regulated initially by cortical actin and then by microtubule-based motors as they move within the cytoplasm (29). Furthermore, regulated exocytosis in embryonic sea urchin cells in response to wounding was found to require both microtubule- and actin-dependent stages based on sensitivity to anti-kinesin antibodies and a myosin ATPase inhibitor (30). Pigment granules within frog melanocytes display short range movements that are dependent on F-actin integrity (19), and depolymerization of actin causes exaggerated microtubule-dependent aggregation of pigment granules in unstimulated cells (18). When cells are stimulated under these conditions, granule dispersion is restricted to the plus ends of microtubules rather than being spread out. These and other observations have led to the hypothesis that specific molecular mechanisms, possibly involving unconventional myosin, CLIP-170, and dynein, operate to switch vesicles between microtubule and F-

actin tracks (20). Further experiments will be required to test this concept vigorously with respect to GLUT4 translocation.

The hTau40 Protein Associates with Microtubules and Delays GLUT4 Translocation to the Cell Surface Membrane—If GLUT4-containing vesicles translocate on microtubule tracks in response to insulin before connecting to an actin-based system of movement to the cell surface, one might expect a more complete inhibition of insulin action upon the destruction of microtubules by nocodazole (see Fig. 2). However, nocodazole itself releases GLUT4-containing vesicles from the juxtanuclear region of adipocytes, presumably due to disruption of dynein motors that concentrate GLUT4-containing vesicles, and thus may partially mimic the ability of insulin to engage GLUT4-containing vesicles with peripheral actin filaments. According to this model, insulin promotes the movement of GLUT4 toward the plus ends of microtubules through the actions of kinesin motors on microtubules, whereas nocodazole promotes outward mobility of GLUT4 through removal of the normally predominant minus end-directed movement.

To test this hypothesis, we performed heterologous expression of the microtubule-binding human Tau40 protein (Fig. 3A) in cultured adipocytes through recombinant adenovirus infection. Tau40 is normally a neuronal protein, but its expression in cultured Chinese hamster ovary cells has been shown to disrupt kinesin-mediated movements of intracellular membranes to the cell periphery (22, 23). For example, mitochondria and intermediate filaments are inappropriately localized to the juxtanuclear region of these cells stably expressing Tau, and the expansion of the endoplasmic reticulum throughout the cytoplasm in neuroblastoma cells expressing high levels of Tau is inhibited. Importantly, the expression of Tau has also been shown to inhibit the rate of exocytosis of vesicles containing the transferrin receptor (22). These effects of Tau are reported to result from the ability of the protein to affect the frequencies of attachment and detachment of vesicles to microtubules rather

<Fig. 4. Expression of microtubule-binding protein hTau40 delays GLUT4 translocation in response to insulin in 3T3-L1 adipocytes. A, 3T3-L1 adipocytes were infected with hTau40 adenovirus or control virus as described under “Experimental Procedures”. After the overnight serum starvation, cells were treated without or with 100 nM insulin for 3–15 min. Lawns of plasma membrane were then generated as described under “Experimental Procedures”. The lawns were incubated with rabbit anti-GLUT4 antibody followed by FITC-conjugated secondary antibody. Shown are representative fields from five different experiments. B, translocated GLUT4 protein in the lawns as shown in A were quantified by measuring the fluorescence intensity using Adobe Photoshop software. Fluorescence intensity was normalized to total cell numbers in each experiment. These data represent averages of five independent experiments (500–1000 cell sheets/condition). A significant decrease of translocation can be seen at 3 min after insulin stimulation in Tau-expressing cells (open circles) and control virus-infected cells (closed circles). Results are the means ± S.D. Asterisk, p < 0.01 versus control, n = 5.

<Fig. 5. Effect of colchicine treatment on the cytoskeleton and GLUT4 localization in 3T3-L1 adipocytes. After the serum starvation, differentiated 3T3-L1 adipocytes were treated with or without 10 μM colchicine for 1 or 2 h at 37 °C, and microtubules (αTubulin) and GLUT4 were visualized. A 1- or 2-h treatment with colchicine disrupted microtubules almost completely, but in a 1-h treated cell, perinuclear GLUT4 vesicles still existed.
than the speed of microtubule-based motors. In particular, reversals of vesicle movements from minus end to plus end are greatly reduced by the expression of Tau, effectively favoring movements in the minus end direction. By reducing attachment of kinesins to microtubules, we reasoned that Tau expression may partially inhibit insulin-mediated GLUT4 translocation.

Fig. 3B shows the localization of expressed hTau40 protein in cultured adipocytes. In the absence of nocodazole, hTau40 was observed to localize with microtubules, whereas in the presence of the drug, the structural features of both microtubules and hTau40 were dispersed. Thus, the expression of hTau40 decorates rather than disrupts microtubule structures, which is consistent with previous results (22, 23). Similarly, the expression of hTau40 had no detectable effect on the perinuclear disposition of GLUT4 in 3T3-L1 adipocytes, whereas under similar conditions nocodazole treatment dispersed GLUT4-containing vesicles throughout the cytoplasm (Fig. 3B). These results show that heterologously expressed hTau40 protein associates with microtubules in cultured adipocytes, consistent with its reported role as a modulator of motility on these cytoskeletal tracks. Importantly, as expected hTau40 protein does not detectably disrupt microtubules in 3T3-L1 adipocytes (Fig. 3B).

The effect of hTau protein expression on GLUT4 responsiveness to insulin in 3T3-L1 adipocytes was then tested using the plasma membrane sheet assay as shown in Fig. 2. After 12 or 15 min of insulin stimulation, GLUT4 at the plasma membrane was maximally elevated and was no different in adipocytes infected with control adenovirus versus hTau40 adenovirus. However, expression of hTau40 in the cultured adipocytes caused a significant inhibition of approximately 65% in the GLUT4 translocated to the cell surface 3 min after the addition of insulin. This delay in insulin-stimulated GLUT4 transloca-

![Image](http://www.jbc.org/)
tion due to hTau40 expression is similar to the delay in exocytosis of transferrin-receptor-containing vesicles in Tau-expressing Chinese hamster ovary cells (22). These data are consistent with the hypothesis that hTau40 interference with kinesin-mediated transport along microtubules retards the rate of GLUT4 exocytosis in response to insulin.

Our data show a good correlation between the reported ability of hTau40 to partially inhibit kinesin activity (22, 23) and its partial disruption of GLUT4 translocation, as reflected in the retardation of GLUT4 movements to the cell surface (Fig. 4). Furthermore, the data in Fig. 2 show a requirement of microtubule integrity for optimal GLUT4 translocation. Taken together, these data strongly support an obligatory role of both microtubules and kinesin motor activity in directing GLUT4 movements toward the plasma membrane. These considerations lead to the prediction that the full effect of insulin on the increase in cell surface GLUT4 would actually be permanently inhibited rather than be simply delayed by depolymerization of microtubules in cultured adipocytes. We tested this prediction by monitoring plasma membrane GLUT4 during prolonged insulin treatments of cultured adipocytes. For these experiments, we used either colchicine or nocodazole to disrupt microtubules in these cells. As shown in Fig. 5, a low concentration (10 μM) of colchicine disrupts microtubules in 3T3-L1 adipocytes in 1–2 h, whereas by 2 h of treatment, the perinuclear GLUT4 also is dispersed fully. In contrast to the transient effect observed for hTau40 (Fig. 4), colchicine or nocodazole inhibits the effect of insulin by 50–70% even after cells are exposed to insulin for 60 min (Fig. 6). Similar experiments were conducted using latrunculin B to disrupt F-actin in cultured adipocytes (Fig. 7). The results of such experiments were consistent with the results obtained by depolymerization of microtubules, stable inhibition of full GLUT4 translocation even after a 60-min exposure of 3T3-L1 adipocytes to insulin. These data indicate that the full effect of insulin on translocation of GLUT4 is dependent upon intact microtubules as well as intact F-actin.

It is noteworthy that a previous report appeared indicating that colchicine treatment of adipocytes caused a delay in the full effect of insulin on hexose transport activity, but that the stimulatory effect of prolonged treatment with insulin was not affected (31). Our data indicate that GLUT4 movements are stably inhibited by colchicine (Fig. 6) and that insulin-stimulated deoxyglucose uptake likewise is stably inhibited by colchicine (data not shown). The previous work did not monitor GLUT4 itself, and it is not clear that glucose transport activity simply reflects plasma membrane GLUT4 in those studies. Further work will be required to evaluate whether the effects of other glucose transporter isoforms might have contributed to the hexose uptake measured under the experimental conditions used in the previous studies.

Taken together, the data presented here provide a compelling case for the involvement of the cytoskeleton in both the perinuclear localization of GLUT4 and its movements in response to insulin. The cytoplasmic dispersion of perinuclear GLUT4 in response to nocodazole (Fig. 1) or colchicine (Fig. 5) and its partial inhibition of GLUT4 translocation (Fig. 2) indicate that both plus end-directed and minus end-directed microtubule-based motors are involved in GLUT4 localization and movements. It is unlikely that nonspecific effects of nocodazole and colchicine account for these effects, because perinuclear GLUT4 dispersion is observed when microtubules are disrupted by an alternative approach (21), and in the present studies, GLUT4 translocation is also modulated by the disruption of kinesin function (Fig. 4). A recent report also describes the use of colchicine to implicate a role of microtubules in GLUT4 movements (32). It is noted that in addition to microtubule depolymerization, the effects of nocodazole but not colchicine can also partially inhibit insulin signaling to Akt. The data we present implicating actin filament involvement also are consistent with recent findings showing an association of GLUT4 with actin bundles in cultured muscle cells (33) and with the reported dependence of GLUT4 translocation in primary adipocytes on intact F-actin (34). An important question for future investigation is how insulin signaling directly regulates the interactions between GLUT4-containing membranes and cytoskeletal elements that direct their movements.

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