Insulin and Okadaic Acid Induce Rab4 Redistribution in Adipocytes*

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Insulin stimulation of glucose transport involves the translocation of vesicles containing the glucose transporter Glut 4 to the plasma membrane. Rab proteins, which have been implicated in the regulation of vesicular traffic, were studied in adipocytes. Rab3B, Rab3C, Rab4, and Rab8 were detected, but Rab3A was not. In the absence of insulin, Rab3B and Rab3C were cytosolic, while Rab4 and Rab8 were associated with membranes. Only Rab4 distribution was modified by insulin. In unstimulated adipocytes, most of Rab4 was found in a low density microsomal fraction, which also contained the majority of Glut 4. After insulin treatment, a 50% decrease in Rab4 content was observed, concomitantly with a departure of transporters to the plasma membrane. The dose responses for the departure of Glut 4 and Rab4 from the microsomal fractions were superimposable, half-maximal effects being obtained with 0.1 nM insulin. Rab4 was redistributed to the cytosol and its movement was reversed by insulin withdrawal. When Glut 4-containing vesicles were immunopurified with antibodies to Glut 4, Rab4 was found in the immune pellets, suggesting that Rab4 was tightly associated with the vesicles. Okadaic acid, an inhibitor of phosphatases 1 and 2A that is known to stimulate Glut 4 translocation, caused the same movement of Rab4 from low density microsomal fraction to the cytosol, while the phorbol ester 12-O-tetradecanoylphorbol-13-acetate had no effect. We suggest that insulin and okadaic acid induce a cycling of Rab4 from a vesicular fraction containing the Glut 4 transporter to the cytosol and that this cycling may participate in the insulin stimulatory action on glucose transporter translocation.

Insulin stimulation of glucose uptake in adipose tissue and skeletal muscle results mainly from the translocation of glucose transporters from an intracellular compartment to the plasma membrane (1-4). Among the different members of the glucose transporter family, Glut 4, and to a much lower extent Glut 1, are expressed in adipose tissue (5). In resting adipocytes, Glut 4 molecules are located in tubulo-vesicular organelles (6, 7), which are recovered together with endosomes and Golgi membranes in low density microsomes (LDM) following subcellular fractionation (1). Under insulin stimulation, Glut 4 molecules move to the plasma membrane (1, 3, 6, 7), a process that implies a vesicular traffic. The molecular regulation of this translocation remains to be unraveled. It is known that low molecular weight GTP-binding proteins participate in secretory processes (8), and recent observations suggest that they could be involved in Glut 4 translocation in response to insulin. First, low molecular weight GTP-binding proteins are expressed in adipocytes; some of them are located in the LDM fraction (9, 10), and have been found tightly associated with Glut 4-containing vesicles (9). Second, GTPγS, a non-hydrolyzable GTP analog, promotes Glut 4 translocation (11) and glucose metabolism (12) in adipocytes and in 3T3-L1 adipocytes (13). Third, a Rab3c isotype (Rab3D, which presents 80% homology with Rab3α, -3B, and -3C) has been found to be predominantly expressed in adipocytes and to appear during 3T3-L1 adipocyte differentiation, suggesting that it could be involved in insulin-stimulated glucose transport (14). However, the significance of the latter observation is unclear since a general increase in Rab protein expression has been described during cellular differentiation (15). Fourth, polymyxin B, which inhibited insulin-stimulated glucose transporter translocation in adipocytes, markedly modified the profile of low molecular weight GTP-binding proteins associated with plasma membranes (16).

The low molecular weight GTP-binding protein family comprises at least 50 members identified so far. They appear to be involved in controlling a diverse set of essential cellular functions. Of the Ras-related small GTP-binding proteins, the rab family seems to be critical in the regulation of the exocytotic and endocytotic pathways (8). They are highly homologous to the yeast YPT1 and SEC4, which have provided some initial insight into the role of low molecular weight GTP-binding proteins in vesicular transport (17-20). Indeed, SEC4 mutations induced a blockade in the constitutive secretory pathway, leading to accumulation of secretory vesicles (21), and YPT1 is required in the transit of the proteins from the endoplasmic reticulum through Golgi compartments (22). The subcellular localization of some Rab proteins is known and appears to be linked with their cellular function. For example, Rab2 is present at the interface between the endoplasmic reticulum and the cis-Golgi, as well as in an intermediate "salvage" compartment (23). Rab3A is expressed only in nervous and neuroendocrine tissues. It is localized on synaptic vesicles, on chromaffine granules, and on insulin-ergic synapses (24).

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1The abbreviations used are: LDM, low density microsomes; HDM, high density microsomes; PM, plasma membrane; GTPγS, guanosine 5′-O-(thiotriphosphate); TPA, 12-O-tetradecanoylphorbol-13-acetate; CHO, Chinese hamster ovary; BSA, bovine serum albumin; PAGE, polyacrylamide gel electrophoresis.
containing granules (24-27). Studies with isolated synaptosomes suggest that it could be involved in the secretion of neurotransmitters. Rab4 has been found to be preferentially associated with early endosomes and endocytic vesicles containing transferrin receptors in 3T3-L1 cells (28) and may play a role in the function and/or formation of the multivesicular bodies involved in recycling (29). Rab5 is tightly linked with plasma membranes, primary endosomes, and clathrin-coated vesicles and is implicated mostly in early endocytic pathway (23, 30, 31), while Rab6 is located in the trans-Golgi network (32).

Since members of the Rab family appear as plausible actors in insulin-stimulated glucose transport translocation, we decided to test this hypothesis using isolated adipocytes. We first studied their expression and their subcellular localization with antibodies to members of the Rab family. Second, taking advantage of this highly insulin-responsive system, we searched for an effect of insulin on their subcellular distribution and for a possible colocalization of those proteins with the Glut4-containing vesicles. Finally, we compared insulin effect with the action of two insulinomimetic agents on glucose transport in adipocytes, the phorbol ester TPA and the protein phosphatase inhibitor okadaic acid.

**EXPERIMENTAL PROCEDURES**

**Preparation of Adipocytes**—Adipocytes were isolated from the epididymal fat pads of 150-200-g male Wistar rats. Finely cut tissue pieces were incubated for 45 min at 37 °C with collagenase (1 mg/ml, Boehringer, Mannheim, Germany) in Krebs-Ringer bicarbonate buffer, 30 mM Hepes, pH 7.4, containing 0.3 mM glucose and 1% (w/v) bovine serum albumin (BSA, fraction V, Interco, Perugia, NY). After 3 washes, tissue pieces were filtered through a 250-μm nylon screen, and the cells were washed three times by flotation. The above mentioned buffer without glucose and BSA. Cells were resuspended in the Krebs-Ringer bicarbonate Hepes buffer with 1% BSA and were preincubated for 30 min at 37 °C before the beginning of the experiments. (Glucose 0.3 mM) was added to the medium when deoxyglucose uptake was not measured.

Measurement of [2-3H]Deoxyglucose Uptake—Cells (170 μl) were incubated for 20 min without or with insulin, or for 30 min with TPA (1 μM) or okadaic acid (2 μM) (LC Services Corp., Woburn, MA). [2-3H]Deoxyglucose (0.2 μCi, 0.1 mM) was then added, and the uptake was allowed to occur for 90 s before the addition of cytochalasin B (50 μg/ml, final concentration). Cells were separated from the medium by centrifugation through dinonyl phthalate and the radioactivity associated with the cells was determined. For each condition, [2-3H]deoxyglucose transport via the glucose transporter was obtained by subtracting the nonspecific value measured when cytochalasin B was associated with the cells.

**Preparation of Total Cellular Membranes from Isolated Adipocytes**—Adipocytes from two rats were incubated in 8 ml of Krebs-Ringer bicarbonate buffer containing 1% BSA, in absence or presence of different agents (as described in legends to figures) for various lengths of time at 37 °C. After three rapid washes with the same buffer without BSA, adipocytes were resuspended in 8 ml of buffer (50 mM Tris, 1 mM EDTA, 250 mM sucrose, pH 7.4, 100 units/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride) and homogenized 10 times in a Thomas Potter (pestle C). Homogenates were first centrifuged at 500 rpm at 4 °C to eliminate the fat cake and then ultracentrifuged at 210,000 × g for 75 min. Supernatant (cytosol) and pellet (referred to as total cellular membranes) were resuspended in Tris-EDTA-sucrose buffer containing protease inhibitors. Crude membranes from brain and CHO cells have been described as previously (33). Protein content of each fraction was determined by the Bio-Rad assay using bovine serum albumin as standard.

**Subcellular Fractionation of Isolated Adipocytes**—Cells from eight rats (in each experimental conditions) were incubated at 37 °C in absence or presence of insulin (at the concentration indicated in the legends to figures) for 20 min. After washes, the adipocytes were hemogenized as above, and subcellular fractions were prepared as previously described (1, 33). Briefly, homogenates were centrifuged at 16,000 × g for 15 min. Following centrifugation of the supernatant for 30 min at 44,000 × g, the pellet was kept (high density microsomes, HDL) and the supernatant recentrifuged at 210,000 × g for 75 min.

Following this second centrifugation both the pellet (referred to as low density microsomes, LDM) and the microsomal supernatant were analyzed. In parallel, the pellet of the 16,000 × g centrifugation was rehomogenized in the same buffer, washed once, and centrifuged for 60 min at 100,000 × g on a sucrose cushion (38% in Tris-EDTA-protease inhibitor buffer). The membranes between the two layers were washed, deoxyglucose, diluted, homogenized, and centrifuged for 45 min at 210,000 × g; the pellet will be referred as plasma membrane (PM). In insulin dose-response curves, HDM and LDM were combined. Protein content of the fractions was determined by the Bio-Rad assay with BSA as standard.

**Immunodetection of Glut4 Transporters and Low Molecular Weight GTP-binding Proteins**—Crude membranes or the different subcellular fractions (100-300 μg) were digested in Laemmli buffer (3% SDS, 70 mM Tris, 11% glycerol) with bromphenol blue (0.05%) and β-mercaptoethanol (0.7 M) and run on a 10% SDS-PAGE with a 5% polyacrylamide stacking gel (64). After transfer to nitrocellulose (Hybond C super, Amersham, Bucks, United Kingdom), the blots were saturated with blocking buffer (5% nonfat milk in phosphate-buffered saline) for 2 h at room temperature. The nitrocellulose sheet was then cut between the standards of 45 and 30 kDa, the upper and the lower parts being used for Glut4 and Rab proteins immunodetection, respectively. Nitrocellulose sheets were successively incubated overnight at 4 °C with the different antibodies, washed three times for 10 min in phosphate-buffered saline containing 1% Triton X-100, incubated with 125I-protein A (500,000 cpm/ml blocking buffer), and washed as above.

The antibodies to the glucose transporter Glut4 were obtained from a rabbit injected with a peptide corresponding to the 12 amino acids of the C terminus of Glut4 and coupled to keyhole limpet hemocyanin (35). The antipeptide antibodies were affinity-purified on a peptide column as described. The antibodies to Rab5A and Rab4 were obtained by immunizing rabbits with the purified proteins produced in E. coli. The antibodies to Rab3B, Rab3C, and Rab8 are antibodies against the highly divergent C terminus of the corresponding proteins coupled to ovalbumin. Quantiification of Glut4 was performed by counting the radioactivity of the 45-50-kDa band, and Rab4 amounts were quantified by scanning (Hoeffer Scientific Instruments). In total cellular membrane fractions, in order to overcome small differences in the amount of proteins analyzed by SDS-PAGE, Rab4 amount was normalized to the amount of Glut4 present in the same fractions.

**Immunopurification of Glut 4-containing Vesicles**—Immunopurification of Glut 4-containing vesicles was performed as previously described (9) with some modifications. LDM fractions were prepared as above from adipocytes obtained from four rats in each condition and immediately used for immunopurification. Fractions were resuspended in 4 ml of phosphate-buffered saline containing protease inhibitors, and incubated with 200 μg of affinity-purified immunoglobulins against Glut4 preadsorbed on 80 μl of protein A-Sepharose beads, or with the same amount of non-immune immunoglobulins. Following immunoadsorption for 4 h at 4 °C, pellets were centrifuged at 1000 rpm for 30 s, washed twice with phosphate-buffered saline, 25 mM, and three times with phosphate-buffered saline. Pellets were treated with 3% SDS, 70 mM Tris, 11% glycerol, 10 μl diethyrlitol for 30 min at 22 °C and finally alkylated in the presence of 25 mM iodoacetic acid for another 30 min at 23 °C (36). Bromphenol blue (0.05%) was added before SDS-PAGE. Transfer to nitrocellulose and immunodetection of Glut4 and Rab4 were performed as above.

**RESULTS**

**Identification and Subcellular Localization of the Rab Family Proteins in Adipocytes**—We first looked for the expression of the different members of the Rab family in isolated adipocytes. Proteins from total homogenates were separated by SDS-PAGE, transferred to nitrocellulose, and incubated with specific polyclonal antisera to Rab3A, Rab3B, Rab3C, Rab4, and Rab8. Brain membranes were used as control for Rab3A, Rab3B, Rab3C, and Rab8 (17), and CHO cell membranes served as a control for the detection of Rab4 (28). A typical autoradiogram is shown in Fig. 1. Antibodies to Rab3A, which recognized a 24-kDa protein in brain membranes, did not detect proteins in adipocytes. Antibodies to Rab3B and Rab3C visualized proteins with an apparent molecular weight of 28,000 in adipocytes and in brain membranes. Antibodies to
**Effect of Insulin on Rab4 Localization in Adipocytes**

Adipocytes were incubated without or with 20 nM insulin for 20 min. Then, they were fractionated as described above. 200 μg of protein from homogenates and 100 μg of protein from the different fractions from control or insulin-treated adipocytes were analyzed by SDS-PAGE on a 10% polyacrylamide gel, transferred to nitrocellulose, and incubated with specific antisera (dilution 1/1000). Binding of antibodies to protein was revealed by 125I-protein A. Autoradiograms obtained with anti-Rab3A, anti-Rab3B, and anti-Rab3C antisera were exposed for 18 h at -80 °C, while those obtained with anti-Rab4 and anti-Rab8 antisera were exposed for at least 2 days at -80 °C.

![Fig. 1. Characterization of members of the Rab protein family in adipocytes.](image)

**Fig. 1.** Characterization of members of the Rab protein family in adipocytes. 200 μg of proteins from total homogenates of adipocytes (Ad) and crude membranes of brain (Br) or CHO cells were analyzed by SDS-PAGE on a 10% polyacrylamide gel, transferred to nitrocellulose, and incubated with specific antisera (dilution 1/1000). Binding of antibodies to protein was revealed by 125I-protein A. Autoradiograms obtained with anti-Rab3A, anti-Rab3B, and anti-Rab3C antisera were exposed for 18 h at -80 °C, while those obtained with anti-Rab4 and anti-Rab8 antisera were exposed for at least 2 days at -80 °C.

Rab4 detected a 24-kDa protein in adipocytes, which comigrated with the Rab4 protein expressed in CHO cells. Anti-Rab8 antibodies detected a 26-kDa protein in adipocyte homogenates and in brain membranes.

To localize more precisely the Rab proteins expressed in adipocytes, subcellular fractionation was performed. Three membrane fractions (PM, LDM, and HDM), as well as the microsomal supernatant, were separated. Proteins from each fraction were analyzed by SDS-PAGE, transferred to nitrocellulose, and immunoblotted with the different anti-Rab antibodies as above (Fig. 2). Rab3B and Rab3C were nearly exclusively present in the supernatant. Rab4 was essentially detected in the LDM and HDM fractions; very faint signals were observed in the PM and the supernatant. Rab8 was found to be located primarily in the HDM but was also detected in PM and in LDM.

**Effect of Insulin on the Subcellular Distribution of Rab4—**

To look for a possible effect of insulin on the subcellular localization of the different Rab proteins in adipocytes, cells were incubated without or with insulin for 20 min before subcellular fractionation. Insulin did not modify the cytosolic distribution of Rab3B or Rab3C (data not shown). Similarly, there was no significant hormonal effect on the distribution of Rab8 in the different membrane compartments (Fig. 3, **middle panel**). By contrast, insulin induced a modification in Rab4 distribution, in parallel to the translocation of the transporter Glut 4 (Fig. 3). Indeed, as already described, insulin promoted the translocation of Glut 4 molecules from the LDM compartment to the PM (Fig. 3, **upper panel**). Similarly, Rab4 left the LDM compartment upon insulin stimulation, but no significant changes were observed in the plasma membrane fraction (Fig. 3, **lower panel**). A quantification of this insulin effect, performed in seven different fractionations, indicated that Rab4 content in LDM from insulin-treated adipocytes was 46 ± 8% of the amount present in LDM from control adipocytes. Parallel decreases in Glut 4 and Rab4 contents were observed in the HDM fraction, but those proteins were less abundant in HDM than in LDM. As expected, Rab4 and Glut 4 contents were similar in homogenates from control and insulin-treated adipocytes. The insulin dose-response curves for Glut 4 and Rab4 departures from the microsomal (HDM + LDM) compartment were compared. As shown in Fig. 4, the two dose responses were superimposable, maximal and half-maximal insulin effects being obtained at 1 and 0.1 nM, respectively. These dose responses are similar to that observed for the stimulation of glucose transport (data not shown).

These first series of experiments suggested that upon insulin stimulation, Rab4 was leaving the microsomal compartment but, in contrast to Glut 4, did not appear in the PM. With this subcellular fractionation procedure, the microsomal supernatant fraction was too diluted to allow for a quantitative detection of Rab4 (see Fig. 2). To overcome this problem, following homogenization, adipocytes were separated only in the two following fractions: total membranes (including HDM, LDM, and PM) and cytosol. Such an experiment is shown in Fig. 5 (**upper panel**). Insulin treatment of adipocytes induced a 50% decrease of the Rab4 content of total membranes compared to control cells. Concomitantly with this decrease in the total membrane fraction, Rab4 amount was higher in the cytosolic fraction of insulin-treated adipocytes compared to control. It should be noted that similar results were obtained for Rab4 immunodetection using affinity-purified immunoglobulins and this immunodetection was prevented by the addition of the purified Rab4 protein (data not shown). To test the reversibility of this movement of Rab4 from microsomes to cytosol, adipocytes were incubated without or with 1 nM insulin for 20 min. After three washes, adipocytes were further incubated without or with 1 nM

![Fig. 2. Subcellular distribution of Rab proteins in adipocytes.](image)

**Fig. 2.** Subcellular distribution of Rab proteins in adipocytes. Adipocytes were fractionated, as described under "Experimental Procedures," in PM, LDM, HDM, and microsomal supernatant (SN). 100 μg of protein from each fraction were analyzed by SDS-PAGE. After transfer to nitrocellulose, incubations with antisera against Rab3B, Rab3C, Rab4, Rab8, and 125I-protein A, the nitrocellulose sheets were submitted to autoradiography for 20 h to 3 days at -80 °C.

![Fig. 3. Effect of insulin on Rab4 distribution in adipocytes.](image)

**Fig. 3.** Effect of insulin on Rab4 distribution in adipocytes. Adipocytes were incubated without or with 20 nM insulin for 20 min. Then, they were fractionated as before. 200 μg of protein from homogenates and 100 μg of protein from the different fractions from control or insulin-treated adipocytes were analyzed by SDS-PAGE on a 10% polyacrylamide gel, transferred to nitrocellulose, and immunoblotted for Glut 4 (**upper panel**), Rab8 (**middle panel**), and Rab4 (**lower panel**). The nitrocellulose sheets were incubated with 125I-protein A and autoradiographed for 24 h at -80 °C.
Effect of Insulin on Rab4 Localization in Adipocytes

A specific subset of vesicles markedly enriched in Glut 4 molecules, which can be purified by immunoadsorption. To study whether Rab4 was colocalized with the transporters, LDM fractions were immunoprecipitated with immunoglobulins to the C-terminal portion of Glut 4 or exposed to non-immune immunoglobulins, and thereafter the presence of Rab4 was studied in the immune pellet. As shown in Fig. 6, the immunoprecipitate obtained with antibodies to Glut 4 also contained a significant amount of Rab4, while no proteins were present in non-immune pellets. This appearance of Glut 4 and Rab4 in the immune pellets was accompanied by a parallel decrease in the concentration of Glut 4 and Rab4 proteins in the supernatants (data not shown). It should be noted that Rab8 was not found in the immune pellets and that its concentration did not change in the supernatant (data not shown).

Effect of Insulinomimetic Agents on Rab4 Subcellular Distribution—In an attempt to find a correlation between glucose transporter translocation and the subcellular redistribution of Rab4, we studied the effect of two agents, the phorbol ester TPA, an activator of protein kinase C, and okadaic acid, an inhibitor of phosphatases 1 and 2A (37). As shown in Fig. 7 (lower panel), TPA and okadaic acid increased deoxyglucose uptake in adipocytes but less efficiently than insulin. Indeed, insulin stimulated 5–6-fold the uptake of deoxyglucose, while TPA and okadaic acid induced only 1.6- and 2.2-fold increases, respectively. Total cellular membranes were then prepared from adipocytes incubated without or with insulin, TPA and okadaic acid. A typical autoradiogram is shown in Fig. 7 (upper panel), and the quantification of 3–5 experiments in Fig. 7 (middle panel). While TPA did not change the Rab4 content of the total crude membranes, okadaic acid was as efficient as insulin in decreasing Rab4 concentration in the membranes. None of those agents modified the total amount of Rab4 quantified in their respective homogenates (data not shown). The lack of TPA effect was also confirmed when the more elaborate subcellular fractionation was performed. The Rab4 amount in LDM prepared from TPA-treated adipocytes was 88.6 ± 3.3% (n = 3) of the Rab4 present in LDM from control cells. Since deoxyglucose uptake was assayed in adipocytes incubated without glucose for approximately 30 min, and the subcellular fractionation was routinely performed with adipocytes incubated with glucose, we checked whether the presence or absence of glucose modified the distribution of Rab4 in response to insulin, TPA, and okadaic acid. No differences in the extent of the Rab4 movement from mem-

![Image](Image0x0)
branes to cytosol were observed whether adipocytes were incubated without or with glucose (data not shown).

DISCUSSION

In the present work the following four key findings were made. (i) Different members of the Rab protein family are expressed in adipocytes, a major insulin-sensitive tissue. (ii) Insulin stimulation induces in parallel the translocation of the transporter Glut 4 to the PM and the passage of Rab4 from a membrane compartment to the cytosol. (iii) Rab4 colocalizes with the vesicles containing Glut 4. (iv) Okadaic acid, an inhibitor of phosphatases 2A and 1, mimics these insulin effects on glucose transporter translocation and Rab4 cycling from membranes to cytosol.

**Different Rab Proteins Are Expressed in Freshly Isolated Adipocytes—**As reviewed in the Introduction, several lines of evidence point to a role of low molecular weight GTP-binding proteins in the insulin stimulation of glucose transport. The Glut 4 translocation process presents analogies with exocytotic events such as those induced in the secretion of neurotransmitters in neuronal cells or hormones by endocrine cells. The recent observation that members of the VAMP (vesicle-associated membrane protein) family are associated with the Glut 4-containing vesicles further underlines the similarities between those processes (38). Moreover, in a previous study, we showed the presence of low molecular weight GTP-binding proteins in LDM fractions, using labeled GTP binding after SDS-polyacrylamide gel electrophoresis and transfer to nitrocellulose. These proteins were more specifically associated with the Glut 4-containing vesicles (9). However, this technique has a number of shortcomings. Indeed, all low molecular weight GTP-binding proteins are not visualized and one labeled band could correspond to different proteins with the same apparent molecular weight. This led us to try to identify some of those proteins using antipeptide antisera to hRab3B, hRab9C, or hRab8 or to the whole proteins hRab3A or hRab4.

Our study was performed in isolated rat adipocytes, a physiological system for studying insulin action. In addition, for this tissue, conventional fractionation procedures have been described, allowing for the separation of relatively pure PM and low and high density microsomes (1, 39, 40). The low density microsomal fraction is an heterogeneous membrane population enriched in marker enzymes characteristic of the Golgi apparatus, but contaminated with endoplasmic reticulum, endosomes, and lysosomes (40, 41). The high density microsomal fraction is enriched with Golgi elements and endoplasmic reticulum (40). Rab3A was not detected in adipocytes, in accordance with the previous observations that its distribution was restricted to neuronal cells and neuroendocrine tissues (24–27). Rab3B and Rab3C were abundant in adipocytes but were present only in the cytosol. Rab4 and Rab8 were mostly found in the LDM and in the HDL, respectively. It should, however, be noted that, since the Rab proteins belong to a rapidly growing family, it cannot be totally excluded that some of the protein(s) detected by our antisera might be very closely related proteins.

**Effect of Insulin on Rab4 Subcellular Distribution in Adipocytes—**When this study was initiated, it was assumed that, if a low molecular weight GTP-binding protein was implicated in the insulin-stimulated translocation of Glut 4-containing vesicles, the hormone would also induce a redistribution of this GTP-binding protein. The localization of Rab3B, Rab3C, and Rab8 was unchanged following insulin treatment of adipocytes. By contrast, insulin induced a 50% decrease in the Rab4 amount of the LDM fraction compared to control adipocytes, in parallel with the translocation of Glut 4 molecules from the LDM to the PM. Furthermore, the dose responses of Glut 4 and Rab4 departure from the microsomal compartment were similar and reflected the insulin effect on glucose transport in adipocytes. Interestingly, in contrast to Glut 4, Rab4 did not translocate to the PM of insulin-treated adipocytes. Indeed, as shown by the two compartments fractionation procedure yielding total membranes (including mitochondria, nuclei, PM, and microsomes) and cytosol, Rab4 left the membranes and was recovered in the cytosol following insulin stimulation. The major advantage of this simple separation method is its rapid execution, which allows for Rab4 detection in the cytosol. Indeed, despite the presence of protease inhibitors, Rab4 was quickly degraded in the homogenate (data not shown) and was barely detectable in the microsomal supernatant following the complete subcellular fractionation. As expected for a low molecular weight GTP-binding protein involved in insulin-stimulated glucose transporter trafficking, the movement of Rab4 from the membranes to the cytosol is reversible. Upon insulin withdrawal from the incubation medium, Glut 4 is sorted from other membrane proteins in the endocytotic pathway and again accumulates in specialized intracellular vesicles (6), and concurrently Rab4 returns to the membrane fraction. Based on these data, we suggest that Rab4 participates in the insulin stimulatory action on glucose transporter translocation.

As pointed out above, LDM are heterogeneous and include different types of vesicles containing Glut 4, Glut 1, insulin-like growth factor II and transferrin receptors, which are all able to translocate in response to insulin (42–44). Glut 4-containing vesicles represent 5–10% of the total fraction proteins (9, 43). To further document a possible role of Rab4 in Glut 4 translocation, we looked for an association of those proteins in the same vesicular fraction. Indeed, when the vesicles containing the Glut 4 transporter were isolated by immunopurification with an antibody to Glut 4, a significant
In conclusion, as schematized in Fig. 8, insulin and okadaic acid promote the translocation of various types of vesicles. Simultaneously, they induce, by an as yet unknown mechanism, a cycling of Rab4 between a membrane compartment where Rab4 and Glut 4 would be colocalized and the cytosol. Those results are reminiscent of the observations made with isolated nerve termini, in which Rab3A has been shown to dissociate from the synaptic vesicle membranes during exocytosis induced by a calcium ionophore (58). This phenomenon was also reversible in that Rab3A could reassociate to the vesicles, suggesting that an association-dissociation cycle of a low molecular weight GTP-binding protein occurred during vesicle trafficking (58). It has also been shown that during neutrophil activation, the Rap1 and Rap2 proteins are translocated to the plasma membranes during granule exocytosis and thus, in contrast to our results, do not appear as a soluble form in the cytosol (59). Our results are to our knowledge the first demonstration of a low molecular weight GTP-binding protein cycling between membrane and cytosol occurring in an intact cell. They should help clarify the picture of how insulin activates one of its key biological responses, i.e., stimulation of glucose transport.

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Fig. 8. Schematic representation of insulin and okadaic acid effect in adipocytes. See “Discussion” for comments. Glut, glucose transporters; IGF II-R, receptor for insulin-like growth factor II; PP2A and PP1, protein phosphatases 2A and 1.
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