Development of an Intracellular Pool of Glucose Transporters in 3T3-L1 Cells*

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Jing Yang, Avril E. Clark, Izabela J. Kozka, Samuel W. Cushman, and Geoffrey D. Holman

From the Department of Biochemistry, the University of Bath, Claverton Down, Bath BA2 7AY United Kingdom and the Experimental Diabetes, Metabolism and Nutrition Section, Diabetes Branch, National Institutes of Diabetes and Digestive and Kidney Disease, National Institutes of Health, Bethesda, Maryland 20892

The membrane-impermeant bis-mannose photolabel 2-N-4-(1-azi-2,2,2-trifluoroethyl)benzoyl-1,3-bis-(D-mannos-4-yloxy)-2-propylamine (ATB-BMPA) has been used to study the development of an intracellular pool of glucose transporters in 3T3-L1 cells. The subcellular distributions of the transporter isoforms GLUT1 and GLUT4 were determined by comparing the labeling obtained in cells in which the impermeant reagent only had access to the cell surface and the labeling obtained in digitonin-permeabilized cells. ATB-BMPA labeling showed that only GLUT1 was present in preconfluent fibroblasts and that most of the transporters were distributed to the cell surface. In preconfluent fibroblasts, the 2-deoxy-D-glucose transport activity was ≈5 times higher than in confluent fibroblasts. ATB-BMPA labeling showed that the decrease in transport as cells reached confluence was associated with a decrease in the proportion of GLUT1 distributed to the cell surface. The sequestration of these transporters was associated with the development of an insulin-responsive transport activity which increased by ≈2.5-fold compared with unstimulated confluent cells. ATB-BMPA labeling showed that insulin stimulation resulted in an ≈2-fold increase in surface GLUT1 so that about one-half of the available transporters became recruited to the cell surface. Measurements of the changes in the distribution of both GLUT1 and GLUT4 throughout the differentiation of confluent fibroblasts into adipocytes showed that both transporters were sequestered in parallel. Basal levels of transport and photolabeling remained low throughout the differentiation period when the total pool of transporters (GLUT1 plus GLUT4) was increased by ≈5-fold. These results suggest that the sequestration process was present before new transporters were synthesized. Thus, the sequestration mechanism develops in confluent growth-arrested fibroblasts although the capacity to sequester additional transporters may increase as differentiation proceeds.

3T3-L1 cells have been used for studying insulin action on glucose transport because these cells can be differentiated to produce a reserve of internal glucose transporters which can be translocated to the cell surface in response to insulin. When the cells are grown as fibroblasts, they produce only the glucose transporter isoform GLUT1 (Kaestner et al., 1989; Garcia de Herreros and Birnbaum, 1989; Tordjman et al., 1990; Harrison et al., 1990; Reed et al., 1990; Weiland et al., 1990). The fibroblasts are induced to differentiate using insulin-responsive isoform GLUT4 (Kaestner et al., 1989; Garcia de Herreros and Birnbaum, 1989; Tordjman et al., 1990; Weiland et al., 1990). It is known from these studies that both GLUT1 and GLUT4 mRNA and total cellular protein rise in concentration over 8 days, and, at 8-11 days after initiation of differentiation, the concentrations of these transporters within the cells reach a maximum.

To examine the proportion of newly synthesized transporters which become available at the cell surface, we have extended the use of our bis-mannose photolabel to measure both cell surface and the total cellular pool of transporters. We have previously used this photolabel to measure the cell surface availability of insulin-stimulated adipose cells (Holman et al., 1990; Clark et al., 1991) and differentiated 3T3-L1 cells (Calderhead et al., 1990; Kozka et al., 1991). We show here that permeabilization of 3T3-L1 cells with digitonin allows the normally impermeant photolabel access to those transporters that are sequestered to the cell. We have therefore compared the labeling at the cell surface and the labeling of the total cellular transporter pool both before differentiation, when the 3T3-L1 cells are confluent fibroblasts, and during the subsequent period of the differentiation regime. The results suggest that mechanisms for sequestration of transporters develop before new transporters are synthesized in the course of differentiation.

The method for examining the distribution of glucose transporters that we have developed here should be a useful addition to the range of techniques that are available for studying transporter processing. The technique has the advantage that it does not depend upon homogenization and separation of membrane fractions and should be useful in studying systems where homogenization procedures are technically very difficult. In addition, it may be useful in investigations of expression systems where transporter cDNA or mRNA are introduced and in which the proportion of the expressed protein that reaches the cell surface needs to be determined.

**EXPERIMENTAL PROCEDURES**

**Materials**—ATB-BMPA and ATB-[2-3H]BMPA (specific activity ≈ 10 Ci/mmol) were prepared as described (Clark and Holman, 1990), except that the abbreviations used are: ATB-BMPA, 2-N-4-(1-azi-2,2,2-trifluoroethyl)benzoyl-1,3-bis-(D-mannos-4-yloxy)-2-propylamine; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; C12E9, nonaethyleneglycol dodecyl ether; DMEM, Dulbecco's modified Eagle's medium.

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2-deoxy-[2,6-3H]-glucose was from Amersham International. DMEM was from Flow Laboratories. Fetal bovine serum was from Gibco Laboratories. Monocomponent porcine insulin was a gift from Dr. Ronald Chance, Eli Lilly Laboratories. Dexamethasone, isobutylmethanxanthine, and protein A-Sepharose were from Sigma. Non-aethylenglycol dodecyl ether (C12E6) was from Boehringer.

**Cell Culture—**3T3-L1 fibroblasts were seeded at a density of \(\approx 0.03 \times 10^6\) cells per 35-mm dish and cultured in DMEM with 10% newborn calf serum. Fibroblasts were differentiated to adipocytes in DMEM and 10% fetal bovine serum by treatment with insulin, dexamethasone, and isobutylmethylxanthine as described by Frost and Lane, 1975. Fully differentiated cells were washed with phosphate-buffered saline (154 mM NaCl, 12.5 mM sodium phosphate, pH 7.4) and were then incubated for 2 h in serum-free medium containing 25 mM dibutyl cAMP. This was followed by three washes in Krebs-Ringer-Hepes buffer (136 mM NaCl, 4.7 mM KCl, 1.25 mM CaCl\(_2\), 1.25 mM MgSO\(_4\), 10 mM Hepes, pH 7.4) before use in experiments to determine 2-deoxy-d-glucose transport activity or cell surface transporters. Cell numbers were determined by counting, in a cytometer, the trypan blue excluding cells harvested from trypsin treatment of the cell monolayers.

**Transport Activity—**Cells in 35-mm dishes were maintained at 37 °C either in the absence or presence of 100 nM porcine monocomponent insulin for 30 min. The cells were then incubated with 50 μM 2-deoxy-d-[2,6-3H]-glucose in 1 ml of KRH buffer at 37 °C for 5 min. Cells were then rapidly washed three times in KRH buffer, 0–4 °C, and solubilized in 1 ml of 0.1 M NaOH.

**ATB-BMPA Photolabeling—**Cells in 35-mm dishes were maintained at 37 °C either in the absence or presence of 100 nM insulin for 30 min. The dishes were washed in KRH buffer and were irradiated for 1 min in a Rayonet photochemical reactor in the presence of 100 μCi of ATB-[2-3H]BMPA in 250 μl of KRH buffer at 18 °C as described by Kozka et al., 1991. To measure labeling of the total cellular transporter pool, cells were permeabilized by treatment with 0.025% (v/v) digitonin for 8 min at 18 °C in the presence of 100 μCi of ATB-[2-3H]BMPA. The digitonin used (Sigma D1407) and suitable for use in aqueous solutions) was 50% pure, and an allowance for this was made in preparing the digitonin solution. The irradiated cells were washed four times in KRH buffer and solubilized in 1.5 ml of detergent buffer containing 2% C\(_2\)E\(_6\), 5 mM sodium phosphate, 5 mM EDTA, pH 7.2 and with the proteinase inhibitors antipain, aprotinin, pepstatin, and leupeptin, each at 1 pg/ml and maintained at 37 °C either in the absence or presence of 100 nM porcine insulin. The irridated cells were then incubated in the presence of serum-free medium for 2 h and then maintained at 37 °C for 30 is an all in the absence or presence (B) of 100 nM insulin in 1 ml of KRH buffer. The transport of 50 μM 2-deoxy-d-[2,6-3H]-glucose was then measured, and cell number was determined. Results are mean and S.E. from four independent determinations.

RESULTS

We have examined the rate of 2-deoxy-d-glucose transport in fibroblasts at stages from initial seeding of dishes to the development of a confluent cell monolayer which occurs at about Day 5 after seeding (Fig. 1). One day after initial seeding of the dishes, the transport rate was 0.13 nmol/10^6 cells/min (with 0.026 × 10^6 cells/35-mm dish). This gradually fell over 7 days to 0.025 nmol/10^6 cells/min (with 0.3 × 10^6 cells/35-mm dish) and stayed at this low level for at least another 6 days. We have also examined the stage at which the transport rate in fibroblasts becomes insulin-responsive. From about 7 days after seeding, the 2-deoxy-d-glucose transport rate was increased by acute insulin stimulation to \(\approx 2.5\) times the basal level. However, even in the presence of insulin, the transport rate (0.06 nmol/10^6 cells/min) was approximately one-half the rate which was observed in preconfluent fibroblasts.

In order to determine the subcellular distribution of transporters, we have measured the cell surface availability of transporters with our impermanent photolabel ATB-BMPA and compared this with labeling that was obtained in digitonin-permeabilized cells. Fig. 2 shows the subcellular distribution of GLUT1 in confluent cells. No GLUT4 was detected in the fibroblasts. There was an \(\approx 4\)-fold increase in labeling in permeabilized cells compared with untreated cells. Insulin only increased the cell surface labeling by \(\approx 2\)-fold and did not increase the total pool of cellular GLUT1. The data from three experiments are shown in Fig. 3. This figure also shows that in preconfluent cells there is no significant increase in labeling in the presence of digitonin indicating that most of the cell transporters were at the cell surface.

Together with the transport data in Fig. 1, the labeling data show that in preconfluent 3T3-L1 fibroblasts the high transport rate is associated with the presence of most of the transporters at the cell surface. As the cells reach confluence, transporters are sequestered from the cell surface, and an intracellular reserve pool of transporters develops. Associated with this sequestration is a fall in the transport activity which can then be partially restored by acute insulin treatment which redistributes approximately one-half of the available transporters to the cell surface.

The technique for comparing cell surface and total cellular transporters has also been used to examine the distribution of the acutely insulin-sensitive isoform GLUT4 in differentiated 3T3-L1 adipocytes. Fig. 4 shows an SDS-polyacrylamide gel of ATB-BMPA-labeled and immunoprecipitated GLUT4. As shown in previous studies by Calderhead et al. (1990), Kozka et al. (1991), and Yang et al. (1992), there was very little GLUT4 at the cell surface in the basal state. Upon insulin stimulation, the GLUT4 labeling increased by \(\approx 12\)-fold in this experiment. However, as is the case with GLUT1.
in confluent fibroblasts, only about one-half of the total available transporters were returned to the cell surface upon insulin treatment. In the digitonin-permeabilized cells, there was only a slight difference between the amount of GLUT4 labeled in the basal compared with the insulin-treated condition. The small difference observed in this experiment and others (Fig. 5) was possibly due to a difference in efficiency of labeling of the intracellular pool compared with the cell surface GLUT4. This small difference was not observed in all experiments, and, over seven experiments, the mean was only slightly lower than that observed with insulin-treated cells. However, Fig. 5 also shows that the S.E. was larger for the labeling of the total cellular pool of both GLUT1 and GLUT4 in the basal cells. The greater variability of labeling of the intracellular pool (which constitutes a greater proportion of the total in the basal state) was probably a consequence of variations between cell batches in the susceptibility to digitonin permeabilization or the efficiency of which the UV light could penetrate to intracellular sites of transporter localization. Fig. 5 also shows that in the insulin-stimulated state approximately half of the total of both GLUT1 and GLUT4 was recruited to the plasma membrane. However, as previously shown by Calderhead et al. (1990) and Yang et al. (1992), the GLUT4 has a greater tendency to be internalized in the absence of insulin, and the cell surface labeling of GLUT4 is about one-third of the GLUT1 level in this basal state.

We next examined the time course for changes in the glucose transport activity and subcellular transporter distribution during the transition from confluent fibroblasts to fully differentiated adipocytes (Fig. 6). This figure shows that the basal level of 2-deoxy-D-glucose transport remained low throughout the differentiation period. The transport rate in basal cells was approximately 25 pmol/dish/min in fully differentiated cells where the cell number had also increased.

![Figure 2](Image)

**FIG. 2.** ATB-BMPA labeling of cell surface and total cellular pools of GLUT1 in confluent 3T3-L1 fibroblasts. Cells were maintained in culture for 11 days in DMEM with 10% newborn calf serum and then incubated in serum-free medium for 2 h. Cells were then maintained at 37 °C for 30 min either in the absence (circles) or in the presence (triangles) of 100 nM insulin in 1 ml of KRH buffer. Cells were then labeled with 100 μCi of ATB-[2-3H]BMPA in 250 μl of KRH buffer at 18 °C either without (open symbols) or with (closed symbols) 0.025% digitonin. Labeled cells were immediately solubilized in C₆Es detergent buffer, and the labeled proteins were subjected to immunoprecipitation with GLUT1 C-terminal peptide antibody and then analyzed by electrophoresis.

![Figure 3](Image)

**FIG. 3.** Comparison of GLUT1 cellular distribution in preconfluent and confluent 3T3-L1 fibroblasts. In a, 3T3-L1 fibroblasts were harvested and plated into two 35-mm dishes to give a total of 0.28 × 10⁶ subconfluent cells after 1 day in culture. Cells in each dish were incubated in serum-free medium for 2 h and then labeled with 100 μCi of ATB-[2-3H]BMPA in 250 μl of KRH buffer at 18 °C either in the absence (B(sur)) or presence (B(tot)) of 0.025% digitonin. Labeled cells from the two dishes were then immediately solubilized in C₆Es detergent buffer, and the labeled proteins were subjected to immunoprecipitation with GLUT1 C-terminal peptide antiserum followed by electrophoresis. Results are the mean and S.E. from four experiments. In b, 3T3-L1 fibroblasts were maintained in culture for 11 days and at confluence reached a density of 0.28 × 10⁶ cells/35-mm dish. The cells were then labeled with 100 μCi of ATB-[2-3H]BMPA in 250 μl of KRH buffer at 18 °C. Cells were then labeled with 100 μCi of ATB-[2-3H]BMPA in 250 μl of KRH buffer at 18 °C either in the absence (sur) and (tot) of digitonin, and GLUT1 immunoprecipitated as described in the Fig. 2 legend. Results are the mean and S.E. of three experiments.
increased to 0.6 × 10^6 cells/dish. During the differentiation, the amount of protein per 35-mm dish increased enormously from 88 μg/dish to 1.1 mg/dish. The insulin-stimulated rate of transport was approximately double the basal rate for about 4 days after the initiation of differentiation. By 6 days, there was a very marked increase in the insulin-stimulated rate of transport to reach by 11 days, a rate which was ≈20 times the basal rate.

Fig. 7a shows the distribution of GLUT1 throughout this differentiation period. During the first 4 days, the cells behaved in a manner similar to that observed in confluent fibroblasts. The basal level of labeling was approximately 2.5-fold lower than the insulin-stimulated level. The total cellular levels were approximately double the level found at the cell surface in the insulin-stimulated state. Between 6 and 11 days, there was a marked rise in the total cellular GLUT1. The cell surface GLUT1 in the basal cells rose slightly throughout this period, and this may have been a consequence of the increased cellular level of this transporter. The GLUT4 was produced in parallel with the rise in GLUT1 (Fig. 7b). Thus, there was little detectable GLUT4 over the first 2 days of differentiation and then this rose steeply between days 6 and 11.

In contrast with the rise in the basal level of labeled GLUT1, labeling showed that the GLUT4 that was newly synthesized was very effectively sequestered inside the cell. The basal GLUT4 labeling remained consistently low from days 4–11 after the initiation of differentiation while the total cellular GLUT4 rose by >10-fold over this period.

**DISCUSSION**

The sequestration of glucose transporters in an intracellular pool is likely to be a general mechanism by which cells regulate the supply of glucose to metabolic enzymes in line with the growth demands and cellular metabolism requirements of the cell. The phenomenon of intracellular transporter sequestration was first demonstrated in insulin-sensitive rat adipose cells by Cushman and Wardzala (1980) and Suzuki and Kono (1980) and has subsequently been found in other insulin-responsive tissues such as brown adipose tissue (Slot et al., 1991a), heart muscle (Watanabe et al., 1984; Slot et al., 1991b), diaphragm muscle (Wardzala and Jeannerauld, 1983), and skeletal muscle (Klip et al., 1987; Hirshman et al., 1990; Klip et al., 1990). In addition, it has been shown that virus infection of BHK cells (Widnell et al., 1990) leads to a redistribution of internalized GLUT1 transporters to the cell surface. Widnell et al. (1990) have also shown that in BHK cells the sequestered GLUT1 transporters redistribute to the cell surface in response to stress stimuli such as arsenite and heat shock. Haspel et al., 1986 have shown that in fibroblasts GLUT1 redistribution can occur in response to glucose starvation. Although the cellular redistributions of glucose transporters can be followed by subcellular fractionation and separation of plasma membrane from the light microscope membranes, this is not a technique that can be applied readily to cells and tissues that are difficult to successfully homogenize and fractionate such as skeletal muscle (Klip et al., 1987) and cultured cells (Calderhead et al., 1990). Immunochemical tech-
unpublished results.

these stages, insulin produced a redistribution of about 50% of both the transsected and endogenous murine GLUT1 were sequestered in intracellular membranes, and that both human GLUT1 and endogenous GLUT1 responded equally to insulin stimulation of translocation.

Other investigators have shown that there is either no change in the basal transport activity throughout differentiation (Kaestner et al., 1989) or that basal activity falls in the first few days after the initiation of differentiation (Weiland et al., 1990; Harrison et al., 1990). Our results show that basal activity can fall markedly if cells are maintained at full confluence for several days without the initiation of differentiation. Thus, the studies cited above can be reconciled if there is a variable time after confluence was reached and before the differentiation regime was initiated. Thus, if the initiation program was started earlier than in our experiments, the basal rate would have fallen during the first few days of differentiation. However, our results suggest that the increase in transporter sequestration and the fall in the transport activity is not a direct consequence of the differentiation regime but develops because cells are in a growth-arrested phase of the cell cycle.

Harrison et al. (1990, 1991a, 1991b) and Clancy et al. (1991) have recently described evidence that the intrinsic activity of glucose transporters in 3T3-L1 cells and particularly that of GLUT1 is suppressed during differentiation. Their evidence is based on an apparent higher insulin-stimulated glucose transport activity in fibroblasts where GLUT1 is present, compared with differentiated 3T3-L1 cells where both GLUT1 and GLUT4 are present. Our results, and those of other investigators (Kaestner et al., 1989; García de Herreros and Birnbaum, 1989; Weiland et al., 1990), show, however, that insulin-stimulated transport activity in adipocytes is much higher than in fibroblasts. In addition, however, Harrison et al. (1990, 1991a, 1991b) argue that as the binding of their exofacial GLUT1 antibody, the β-antibody, is increased 2.6-fold in adipocytes in the basal state compared with fibroblasts while the transport activity is reduced by ~2.5-fold, the GLUT1 intrinsic activity is suppressed by 90%. They suggest that inactivation is due to interaction with an inhibitory protein (Harrison et al., 1981b; Clancy et al., 1991). Our results also show a discrepancy between transport activity and labeled transporter level in comparing fibroblasts with differentiated adipocytes in the basal state but suggest that the discrepancy is smaller than the 90% (10-fold) discrepancy calculated by Harrison et al., 1990. The ratio of transport activity to GLUT1 labeling in fibroblasts can be calculated as 130 pmol/10^6 cells/min divided by GLUT1 labeling of 970 dpm/10^6 cells or 0.044 pmol/10^6 cells (the specific activity is 10 Ci/mmol). This intrinsic activity ratio is 41 pmol/10^6 cells/min divided by GLUT1 labeling of 590 dpm/10^6 cells or 0.026 pmol/10^6 cells in differentiated cells. Thus, during the transition from preconfluent fibroblasts to differentiated adipocytes, the intrinsic activity ratio falls from 2594 min^-1 in fibroblasts to 1576 min^-1 in adipocytes in the basal state. Any GLUT4 present at the surface of basal adipocytes would lower this ratio. Thus these results suggest that the activity of GLUT1 and probably GLUT4 is suppressed by 40–50% or by 1.5–2-fold and are consistent with estimates of the intrinsic activity of GLUT1 and GLUT4 previously reported for the basal state (Holman et al., 1990; Kozka et al., 1991; Clark et al., 1991; Palfreyman et al., 1992).

Harrison et al. (1991b) have suggested that the exofacial probe that we have used may react only with transporters that are catalytically active. They suggest that in basal cells...
Glucose Transporter Pool in 3T3-L1 Cells

![Graphs of GLUT1 and GLUT4](https://example.com/graphs)

**Day of differentiation**

**a)** GLUT1  
**b)** GLUT4

**Fig. 7.** Time course for the increase in cell surface and total cellular pools of GLUT1 and GLUT4 in 3T3-L1 cells. Cells in 35-mm dishes were treated with dexamethasone, isobutylmethylxanthine, and insulin in DMEM with 10% fetal bovine serum to initiate differentiation. On the indicated days, cells were incubated in serum-free medium for 2 h and were then maintained at 37 °C for 30 min either in the absence (squares) or presence (triangles) of 100 nM insulin in 1 ml of KRH buffer. In GLUT1 (a) and GLUT4 (b), distribution was determined by labeling with 100 μCi (closed symbols) or total cellular levels in cells permeabilized with 0.025% digitonin (open symbols). Cells were washed twice in KRH buffer and then solubilized in C₄E₆ detergent buffer except Day 0 cells which were immediately solubilized in detergent buffer. The isoforms were then immunoprecipitated with anti-C-terminal peptide antibodies and analyzed by electrophoresis to obtain the total disintegrations/min under the gel peaks. Results are the mean from two experiments.

A pool of transporters exists which has suppressed activity due to an inactivating modifier protein. However, several studies using the photolabel (as cited above) have shown an ≈2-fold discrepancy between photolabeling and transport and have shown a lag between the appearance of transporters that can be photolabeled and the increase in transport activity following insulin stimulation of rat adipose cells (Clark et al., 1991; Satoh et al., 1991) and 3T3-L1 cells (Yang et al., 1992). These studies show that ATB-BMPA binds to transporters that are inactive in transport catalysis. We have suggested that it is this form of the transporter that may be associated with modifier proteins such as those involved in trafficking events in the normal translocation pathway (Yang et al., 1992). In addition we have suggested that where there is a further discrepancy between transporters that are detected by labeling compared with Western blotting, as in isoproterenol-treated insulin-stimulated rat adipocytes and phenylarsine oxide-treated insulin-stimulated 3T3-L1 cells (Yang et al., 1992), that this is due to the formation of plasma membrane-associated but occluded vesicles. Thus, we, to some extent concur, with the hypothesis described by Harrison et al. (1990, 1991a, 1991b), but suggest that the transport-inactive form at the plasma membrane is a relatively small fraction of the total plasma membrane pool, that these forms represent intermediates in normal transporter trafficking and that translocation is the major mechanism by which glucose transport is stimulated in insulin-sensitive cells.

Our experiments in which we have used digitonin suggest that the photolabel interacts with equal amounts of transporter in the basal and insulin-stimulated states and that this provides additional evidence, to that previously described (Calderhead et al., 1990; Holman et al., 1989), which suggests that basal and insulin-stimulated transporters do not have differing affinities for the photolabel. If an inhibitory protein were present in basal cells which produced a large increase in the proportion of transporters in a catalytically inactive state, then this association would be expected to reduce the apparent labeling of the total cellular transporter pool of basal cells. Thus, these experiments in which cells are digitonin-permeabilized also suggest that the proportion of transporters with suppressed activity is small and that the low surface levels of transporters detected by labeling of cells in the basal state is due to transporter sequestration within the intracellular pool.

During the process of 3T3-L1 cell differentiation, over 95% of the total cellular GLUT4 in the intracellular pool was sequestered. This is similar to the level of sequestration of GLUT4 seen in other insulin-responsive tissues (Slot et al., 1991a, 1991b). The greater sequestration of GLUT4 may be related to the targeting of this isoform to separate intracellular vesicles (Zorzano et al., 1989), although Calderhead et al., 1990, have reported that GLUT1 and GLUT4 were detected in the same vesicles. It seems likely that the two isoforms leave the cell surface by the same endocytosis route as we have shown that both isoforms are endocytosed with the same half-time when insulin is removed. This has been observed in rat adipocytes (Clark et al., 1991) and in 3T3-L1 cells (Yang et al., 1992).
et al., 1992). However, we have observed that, in insulin-stimulated 3T3-L1 cells which are treated with the vicinal dithiol inactivating reagent phenylarsine oxide, there may be some separation of the transporter vesicle processing as GLUT4 re-exocytosis appeared to be blocked by this reagent, but GLUT1 re-exocytosis was not (Yang et al., 1992). Cellular mechanisms for the separate localization and processing of GLUT4 must be very dependent on the GLUT4 protein structure as GLUT4 transfected into expression systems probably enter a preformed sequestration process. Further studies will be required to determine whether growth arrest alone is sufficient to induce transporter sequestration or whether cell confluence and cell-cell contact phenomena result in morphological changes in the cell membrane system responsible for down-regulation of transporters and transport activity.

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