Expression of the liver-type glucose transporter (GLUT2) in 3T3-L1 adipocytes: analysis of the effects of insulin on subcellular distribution

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We have expressed the liver-type facilitative glucose transporter, GLUT2, in the insulin-sensitive 3T3-L1 adipocyte clonal cell line in an effort to address the importance of transporter isoform and cellular environment on the ability of insulin to mediate glucose-transporter translocation. Analysis of non-differentiated fibroblastic cell clones transfected with the GLUT2 cDNA identified the presence of this isoform in several independent clones. These clones exhibited increased deoxyglucose and fructose transport rates compared with control cells. Upon differentiation, the fibroblastic clones selected for study achieved > 95% phenotypic

conversion into adipocytes. Expression of the GLUT2 protein was maintained throughout the differentiation protocol. Subcellular fractionation revealed that in response to insulin, unlike the native GLUT4, GLUT2 protein did not undergo significant translocation to the plasma membrane; furthermore, the subcellular distribution of the expressed GLUT2 was quite distinct from that of the endogenous GLUT4. 3T3-L1 adipocytes expressing GLUT2 only exhibited a 2-fold increase in insulin-stimulated fructose uptake, further suggesting that GLUT2 does not undergo insulin-stimulated translocation.

INTRODUCTION

Glucose transport across the plasma membrane of animal cells is mediated by a family of facilitative glucose transporters, each of which is the product of a distinct gene and is expressed in a highly co-ordinated tissue-specific fashion (for review see Bell et al., 1993; Gould and Holman, 1993). The most extensively studied of these isoforms is the so-called insulin-responsive glucose transporter, GLUT4. This transporter is expressed in adipocytes, muscle and heart, tissues which exhibit acute insulin-stimulated glucose transport (see Bell et al., 1993; Gould and Holman, 1993; and references cited therein). Studies in the early 1980s identified the mechanism of this effect as being the redistribution of cellular glucose transporters from an intracellular pool to the plasma membrane, a process termed translocation (Cushman and Wardzala, 1980; Suzuki and Kono, 1980; Blok et al. 1988; Slot et al., 1991a,b). Subsequently, studies in both isolated rat adipocytes and the 3T3-L1 adipocyte clonal cell line have demonstrated that, in resting adipocytes, ~ 90% of the GLUT4 molecules are present in an intracellular pool; in response to insulin, these transporters move to the cell surface and account for most, if not all, of the increase in transport observed in response to insulin (Zorzano et al., 1989; Holman et al., 1990; Calderhead et al., 1990; Piper et al., 1991, 1992; Slot et al., 1991a,b). Thus, in adipocytes, insulin increases the V_{max} for glucose transport by increasing the numbers of functional GLUT4 molecules at the cell surface. In contrast, insulin stimulation of resting hepatocytes does not stimulate glucose transport (Williams et al., 1968; Baur and Heldt, 1977); hepatocytes express a distinct glucose transporter isoform referred to as the liver-type glucose transporter GLUT2, which by inference does not undergo insulin-induced translocation in its native cell type (Thorens et al., 1988; Fukumoto et al., 1988).

Here, we address the question of the effect of cellular environment on the ability of glucose transporters to undergo

insulin-induced translocation. Studies have shown that GLUT4 is targeted to an intracellular location in a variety of cell lines, such as fibroblasts, PC12 cells, CHO cells, COS cells and oocytes (Haney et al., 1991; Hudson et al., 1993; Thomas et al., 1993; Czech et al., 1993; Verhey and Birnbaum, 1994). However, none of these cell systems exhibits insulin-stimulated glucose transport to the extent observed in adipocytes, and thus it has not been possible, using these cell lines, to address the influence of the cellular environment on the ability of glucose transporters to undergo insulin-stimulated translocation. We have expressed the liver-type glucose transporter, GLUT2, in adipocytes in an effort to determine whether, when expressed in a cell line which does exhibit insulin-induced translocation, this isoform undergoes insulin-induced redistribution.

The results show that when expressed in 3T3-L1 adipocytes GLUT2 does not co-localize with GLUT4, the major insulin-responsive glucose transporter, and that the majority of the expressed protein is localized to the plasma-membrane fraction (~ 55%). In response to insulin, GLUT2 undergoes only a modest increase at the cell surface, compared with the large increases seen with GLUT4. These data suggest that the property of insulin-induced transporter translocation is defined by the transporter isoform, since expression of other glucose transporter family members in an insulin-responsive cell line does not render the expressed transporters insulin-responsive.

MATERIALS AND METHODS

Materials

Geneticin (G418), Dulbecco's modified Eagle's medium (DMEM), penicillin and streptomycin solution and Myoclon plus foetal-bovine serum were from Gibco/BRL (Paisley, Scotland, U.K.). Newborn-calf serum was from Flow Labora-

tories (Irvine, Scotland, U.K.). All other reagents were as previously described (Gould et al., 1992; Brant et al., 1992, 1993).

Cell culture

3T3-L1 fibroblasts were grown in 10% newborn-calf serum in DMEM containing penicillin and streptomycin as described by Frost and Lane (1985). Differentiation into adipocytes was induced 48 h post-confluency by addition of differentiation medium (20% foetal-bovine serum in DMEM) containing insulin, dexamethasone and isobutylmethylxanthine as described by Frost and Lane (1985). Then 48 h later, this medium was removed and replaced with differentiation medium supplemented with insulin alone. Thereafter, cells were fed every two days with 20% foetal-bovine serum in DMEM and used between days 9 and 12 after induction of differentiation. Note that, for consistency, we used this differentiation protocol for wild-type cells.

Construction of expression plasmid, transfection and clonal selection

A *Bgl*II fragment containing the entire coding region of the human GLUT2 cDNA was isolated from pHTL.217 (Kayano et al., 1990) and ligated into the *Bam*HI site of pLK444 (Gunning et al., 1987). This vector contains the β -actin gene promoter sequence, together with the gene for neomycin resistance driven by an SV40 promoter. The GLUT2 fragment orientation was confirmed by restriction digestion and also by double-stranded DNA sequencing using an ABI 373A automated DyeDeoxy DNA sequencing apparatus. The pGLUT2/LK444 plasmid was amplified in bacteria and double-stranded DNA isolated by CsCl banding (Sambrook et al., 1989).

Transfection of the constructs into 3T3-L1 fibroblasts was done with the DOTAP reagent (Boehringer-Mannheim) according to the manufacturer's instructions. Briefly, 5 μ g of plasmid DNA was used to transfect $\sim 2 \times 10^6$ cells on a 10 cm tissue-culture plate. At 24 h after transfection, the medium was aspirated and replaced with 10% newborn-calf serum in DMEM containing 0.75 μ g/ml G418 (selection medium). Cells were maintained in selection medium until such time as well-isolated colonies could be observed on the plates. Individual colonies were isolated and cultured in selection medium. After clonal expansion, all G418-resistant clones were maintained in media containing 0.25 μ g/ml G418 at all stages (except during differentiation).

2-Deoxyglucose (dGlc) and D-fructose transport assays

Six-well plates of cells were incubated in serum-free media for 2 h before assay. Monolayers were washed with 3×3 ml of Krebs Ringer phosphate buffer (KRP; 136 mM NaCl, 4.7 mM KCl, 1.25 mM MgSO_4 , 1.25 mM CaCl_2 , and 5 mM sodium phosphate, pH 7.4), and covered with 0.95 ml of the same; insulin (1 μ M) was added as indicated in the Figure legends. Uptake was initiated by addition of 50 μ l of [^3H]dGlc (final concn. 100 μ M, 0.25 μ Ci/ml) or D-[^{14}C]fructose (final concn. 1 mM, 0.5 μ Ci/ml). After incubation for the times indicated in the Figure and Table legends, uptake was terminated by aspirating the medium and washing the monolayers three times with ice-cold PBS (150 mM NaCl, 10 mM NaH_2PO_4 , pH 7.4). The cells were solubilized with 1.0 ml of 1% Triton X-100, and the radioactivity in sample was measured by liquid-scintillation spectrometry. Uptake values were corrected for the non-specific association of sugars with the cells by subtracting the uptake in the presence of 10 μ M cytochalasin B, a potent inhibitor of transport (Bloch, 1973). The

concentration of sugars and time of incubations used in these experiments were such that the rate of uptake was linear under these conditions.

Subcellular fractionation and immunoblotting

The subcellular fractionation of 3T3-L1 adipocytes was performed exactly as described by Piper et al. (1991). This procedure allows the isolation of purified plasma membrane, light microsomes and heavy microsomal fractions. Antibodies to GLUT2 and GLUT4 have also been previously described and characterized (Gould et al., 1992; Brant et al., 1992, 1993). Anti-GLUT1 antibodies were purchased from East Acres Biologicals (Southbridge, MA, U.S.A.). The conditions used for immunoblotting with these antibodies were exactly as described (Gould et al., 1992; Brant et al., 1992, 1993). All immunoblots were developed by using the Enhanced Chemiluminescence (ECL) system of Amersham according to the manufacturer's instructions, or by using ^{125}I -labelled goat anti-rabbit IgG as described previously (Gould et al., 1992; Brant et al., 1992, 1993).

RESULTS AND DISCUSSION

The acute stimulation of glucose transport observed in response to insulin in adipocytes is due to the movement (translocation) of a pool of intracellular glucose transporters to the cell surface (Cushman and Wardzala, 1980; Suzuki and Kono, 1980). Recent studies from many laboratories have demonstrated that the majority of the stimulation of glucose transport is mediated by the translocation of the so-called insulin-responsive glucose transporter, GLUT4, in adipocytes, brown fat and skeletal muscle (Zorzano et al., 1989; Holman et al., 1990; Calderhead et al., 1990; Piper et al., 1991, 1992; Slot et al., 1991a,b). In contrast, the other major target tissue for insulin action, liver, expresses a distinct glucose transporter isoform (GLUT2), and

Figure 1 GLUT2 expression in 3T3-L1 fibroblasts

Immunoblot analysis of 3T3-L1 fibroblast clones transfected with GLUT2 cDNA. An immunoblot developed with anti-GLUT2 anti-peptide antibodies is shown. Two clones which were positive for GLUT2 expression (3T3/G2a and 3T3/G2b, lanes 3 and 4 respectively) and one clone which was G418-resistant but did not express the protein (3T3/ca, lane 2), together with non-transfected 3T3-L1 fibroblasts (lane 1) and rat liver membranes (as a positive control, lane 5) are shown. The positions of molecular-mass markers (kDa) is shown to the right of the blot, and the entire running gel is shown. Each lane contains ~ 30 μ g of protein from whole cell lysates of the 3T3-L1 fibroblasts clones, and 5 μ g of rat liver membrane protein (lane 5). This immunoblot was developed with the Amersham ECL system.

Table 1 Comparison of basal and insulin-stimulated dGlc and D-fructose transport rates in wild-type and GLUT2-transfected 3T3-L1 fibroblasts

dGlc or D-fructose transport was determined as described in the Materials and methods section. The data are presented as the results from a typical experiment (each point is the mean $\pm$ s.d. of triplicate determinations), and the fold increase from several independent experiments (n = number of experiments). Insulin ($1 \mu\text{M}$) was added for 60 min before the initiation of transport measurements. dGlc transport was measured for 3 min at a substrate concentration of $100 \mu\text{M}$; D-fructose uptake was measured at 1 mM for 5 min. All values have been corrected for the non-specific association of sugar with the cells by subtracting radioactivity associated with the monolayers measured in the presence of $20 \mu\text{M}$ cytochalasin B. – indicates no significant increase in response to insulin. * indicates $P \sim 0.05$, ** $P < 0.05$, *** not significant, statistical increases by paired t tests.

	dGlc transport rate (pmol/min per 10^6 cells)		
	Basal	Insulin-stimulated	Mean increase (fold)
3T3/G2a	52.2 ± 8.5	68.8 ± 8.2	
3T3/G2b	39.2 ± 3.4	53.5 ± 0.7	2.4 ± 0.3 ($n = 3$)*
3T3/ca	14.0 ± 2.3	27.5 ± 1.7	2.3 ± 0.2 ($n = 3$)**
Wild-type	17.2 ± 1.1	37.5 ± 1.3	2.6 ± 0.15 ($n = 16$)**

	D-Fructose transport rate (pmol/min per 10^6 cells)		
	Basal	Insulin-stimulated	Mean increase (fold)
3T3/G2a	46.3 ± 2.2	49.5 ± 2.0	–
3T3/G2b	11.4 ± 2.3	25.6 ± 3.5	2.5 ± 0.6 ($n = 6$)**
3T3/ca	3.9 ± 1.2	5.2 ± 2.5	–
Wild-type	4.1 ± 0.2	5.4 ± 0.2	0.35 ± 0.1 ($n = 3$)***

these cells do not exhibit acute insulin-stimulated glucose transport (Williams et al., 1968; Bauer and Heldt, 1977; Thorens et al., 1988; Fukumoto et al., 1988). Here, we set out to address the importance of transporter isoform and cellular environment on the ability of insulin to mediate glucose-transporter translocation by expressing the liver-type glucose transporter, GLUT2, in the clonal 3T3-L1 adipocyte cell line.

3T3-L1 fibroblasts were transfected with a plasmid containing the GLUT2 cDNA downstream of the β -actin promoter and containing the neomycin-resistance gene. Stably transfected clones were isolated and analysed for expression of the GLUT2 protein by using antibodies against the C-terminus of this isoform (Brant et al., 1992). Three independent clones were chosen for study: two (3T3/G2a and 3T3/G2b) were found to express GLUT2 protein, and another G418-resistant clone (3T3/ca) was chosen as a control, since no detectable GLUT2 protein was observed in this clone. Immunological analysis of these clones indicated that 3T3/G2a expressed significantly higher levels of GLUT2 protein than did 3T3/G2b, and thus comparison of these two clones acts as a control for potential artefacts of over-expression (Figure 1).

Studies of GLUT2 function in oocytes have shown that GLUT2 is unique among GLUT1–4 in its ability to transport D-fructose in a cytochalasin B-inhibitable fashion in addition to dGlc (Gould et al., 1991; Colville et al., 1993). Thus, in order to determine whether GLUT2 is functionally expressed in the clones isolated above, we have assayed fructose and dGlc transport in these cells. The results (Table 1) show that clone 3T3/G2a exhibited a large (~ 3 – 5 -fold) increase in dGlc transport com-

Table 2 Comparison of basal and insulin-stimulated dGlc and D-fructose transport rates in wild-type and GLUT2-transfected 3T3-L1 adipocytes

dGlc or D-fructose transport was determined as described in the Materials and methods section. The data are presented as the results from a typical experiment (each point is the mean $\pm$ s.d. of triplicate determinations), and the fold increase from several independent experiments (n = number of experiments). dGlc transport was measured for 3 min at a substrate concentration of $100 \mu\text{M}$; D-fructose uptake was measured at 1 mM for 3 min. All values have been corrected for the non-specific association of sugar with the cells by subtracting radioactivity associated with the monolayers measured in the presence of $20 \mu\text{M}$ cytochalasin B. Note that cytochalasin B-inhibitable D-fructose transport was not detected in any clone or wild-type cells, except 3T3/G2a (see the Results and discussion section). The marginally decreased insulin-stimulated glucose transport rates observed in 3T3/G2a and 3T3/G2b were not accompanied by a decrease in native GLUT1 or GLUT4 levels (results not shown). Statistically significant fold increases are as follows: * $P < 0.05$, ** $P = 0.01$, $P \sim 0.05$, as determined by paired t tests.

	dGlc transport rate (pmol/min per 10^6 cells)		
	Basal	Insulin-stimulated	Mean increase (fold)
3T3/G2a	36 ± 4.3	132 ± 18	3.0 ± 0.6 * ($n = 6$)
3T3/G2b	23 ± 2.4	140 ± 32	9.6 ± 1.9 * ($n = 5$)
3T3/ca	12.9 ± 2.1	192 ± 31	17.2 ± 3.1 ** ($n = 3$)
Wild-type	9.3 ± 1.5	193 ± 16	19.3 ± 2.3 ** ($n = 4$)

	D-Fructose transport rate (pmol/min per 10^6 cells)		
	Basal	Insulin-stimulated	Mean increase (fold)
3T3/G2a	44 ± 6.4	75 ± 14	1.9 ± 0.4 *** ($n = 6$)

pared with cells not expressing GLUT2 protein; a smaller increase in dGlc transport was observed in 3T3/G2b (~ 3 -fold), presumably because the level of expression of the protein is significantly lower (see immunoblot analysis in Figure 1).

We (Merrall et al., 1993) and others (e.g. Yang et al., 1992) have shown that insulin treatment of 3T3-L1 fibroblasts increases dGlc transport about 2–3-fold within 60 min of exposure. This effect is mediated predominantly by translocation of GLUT1 to the cell surface (Yang et al., 1992). In the control cell line 3T3/ca and wild-type 3T3-L1 fibroblasts, 2–3-fold stimulations of dGlc transport were observed. In 3T3/G2a, no significant increase in response to insulin was measured, and the increase in 3T3/G2b was not significantly greater than that observed in either 3T3/ca or wild-type cells (Table 1). Therefore, we conclude from these activity studies that GLUT2 is expressed at the cell surface, and is relatively insensitive to insulin.

Fructose transport is elevated by ~ 8 -fold in 3T3/G2a and ~ 3 -fold in 3T3/G2b. Interestingly, insulin was without effect on fructose transport in 3T3/G2a, but increased fructose transport in 3T3/G2b about 2.5-fold. This increase is likely to be mediated by GLUT2, since the fructose transport rate in 3T3/ca and wild-type cells is not significantly increased by insulin (Table 1). This relatively small increase in fructose transport in response to insulin (~ 2.5 -fold) is similar in magnitude to the increase in glucose transport observed in these cells, and thus we speculate that this may be the result of a small degree of translocation of GLUT2 to the cell surface in response to insulin in a fashion similar to the native GLUT1 (Yang et al., 1992). The larger increases in GLUT2 activity measured by fructose transport are

Figure 2 Subcellular fractionation of 3T3/G2a: analysis of the effect of insulin on GLUT2 and GLUT4 distribution

Fractions corresponding to the plasma membrane (PM) and low-density microsomal membranes (LDM) were isolated from basal or insulin-stimulated ($1 \mu\text{M}$, 30 min) clone 3T3/G2a as described in the text. $10 \mu\text{g}$ of protein was loaded in each lane, and the proteins were separated by SDS/PAGE. Following transfer to nitrocellulose, the immunoblots were developed with antibodies specific for GLUT4 (a) or GLUT2 (b). Quantification of the relative changes in response to insulin in each fraction was performed as described, and the results are presented in Table 3. Shown is a representative blot using clone 3T3/G2a; similar results (not shown) were obtained for clone 3T3/G2b.

not manifest when using dGlc to assess activity, due to the high background of native GLUT1 expression in these cells.

The 3T3-L1 cell line was chosen for this study as this cell is readily differentiated into adipocytes by appropriate hormonal treatment (Frost and Lane, 1985). Consequently, we have examined the behaviour of GLUT2 in the differentiated adipocyte counterparts of these fibroblastic clones. 3T3-L1 fibroblasts were differentiated into adipocytes as described by Frost and Lane (1985). Some modifications to the published protocols were used. Firstly, cells were plated on to collagen-coated plastic-ware. This was found to be necessary, as the cells undergoing phenotypic conversion were very loosely attached to the plate. In a previous study, similar treatment was also determined to be necessary with transfected 3T3-L1 cells (Gould et al., 1989). Secondly, we used higher concentrations of insulin ($2.5 \mu\text{g/ml}$ rather than $1 \mu\text{g/ml}$) to induce differentiation; a similar increase had also proved necessary in a previous study (Gould et al., 1989). In addition, we routinely found that the use of higher concentrations of fetal-bovine serum supported more complete differentiation. As assessed by eye, the fibroblasts from 3T3/G2a, 3T3/G2b and 3T3/ca underwent essentially complete ($> 95\%$) phenotypic conversion, as determined by the presence of triacylglycerol droplets in the cytoplasm (results not shown). For consistency, we have used wild-type 3T3-L1 adipocytes differentiated under the same condition as control (non-transfected) cells.

We assayed dGlc and fructose transport in the adipocyte counterparts of clones 3T3/G2a and 3T3/G2b and compared the results with data obtained from non-transfected 3T3-L1 adipocytes and clone 3T3/ca. The results are shown in Table 2. As expected, an elevated level of basal dGlc transport was measured in both GLUT2-positive clones relative to wild-type 3T3-L1 adipocytes or clone 3T3/ca. Similar maximal insulin-stimulated rates were observed in all cell lines assayed.

Analysis of the fructose transport rates in 3T3-L1 adipocytes was hampered by our observation that, upon differentiation into adipocytes, these cells exhibited profoundly elevated rates of

Table 3 Quantification of GLUT2 and GLUT4 levels in subcellular fractions of 3T3-L1 adipocyte clones 3T3/G2a and 3T3/G2b

Subcellular fractions corresponding to plasma membranes or low-density microsomal membranes were isolated from basal or insulin-stimulated ($1 \mu\text{M}$, 15 min) adipocytes as described in the text. Samples were immunoblotted with antisera against GLUT4 and quantified by using radioiodinated goat anti-rabbit antibody as described. For examples analysed with anti-GLUT2 antibodies, the immunoblots were developed with the ECL system, and scanned by densitometry. In all cases, care was taken to ensure that the signal obtained from the immunoblot was on the linear range of the development curve. The data are expressed as a percentage of the signal obtained from both fractions. An immunoblot of the high-density microsomal fraction indicated that this fraction contained less than 5% of the total cellular GLUT4 and GLUT2 in all cell lines (results not shown). * indicates a statistically significant change with respect to the basal condition ($P \sim 0.05$).

	GLUT4		
	Wild-type	3T3-G2a	3T3-G2b
Plasma membrane, basal	12 ± 2	10 ± 4	16 ± 7
Plasma membrane, insulin	$49 \pm 4^*$	$56 \pm 10^*$	$52 \pm 9^*$
Low-density microsomes, basal	83 ± 6	88 ± 9	73 ± 12
Low-density microsomes, insulin	$41 \pm 8^*$	$43 \pm 7^*$	$47 \pm 5^*$

	GLUT2		
	Wild-type	3T3-G2a	3T3-G2b
Plasma membrane, basal	—	55 ± 15	55 ± 8
Plasma membrane, insulin	—	47 ± 12	75 ± 16
Low-density microsomes, basal	—	36 ± 5	42 ± 9
Low-density microsomes, insulin	—	40 ± 10	23 ± 8

fructose transport (A. M. Brant and G. W. Gould, unpublished work). This fructose transport was insensitive to cytochalasin B. Only 3T3/G2a exhibited a sufficiently high level of GLUT2 expression to allow measurement of cytochalasin B-inhibitable fructose transport (i.e. GLUT2-mediated fructose transport) (Table 2). The results from this clone demonstrated that GLUT2-mediated fructose transport was stimulated only about 2-fold by insulin.

Immunoblot analysis was undertaken by using anti-peptide antibodies specific for GLUT4 or GLUT2 of fractions corresponding to the plasma membranes and low-density microsomal membranes isolated from basal and insulin-treated adipocytes; the results from a representative experiment with clone 3T3/G2a are presented in Figure 2. The analysis showed that, as expected, GLUT4 undergoes a pronounced increase in the plasma-membrane fraction in response to insulin, with a concomitant decrease in the low-density microsomal fraction (Figure 2a). In contrast, GLUT2 was expressed predominantly in the plasma membrane, and its distribution did not change in response to insulin (Figure 2b). Similar results (not shown) were obtained with clone 3T3/G2b.

Quantification of a series of immunoblots for GLUT2 and GLUT4 in wild-type, 3T3/G2a and 3T3/G2b adipocytes is presented in Table 3. The inability to immunoprecipitate GLUT2 with the antibodies described herein precludes an analysis of translocation by any of the presently available cell-surface labelling procedures (see also Jordan and Holman, 1992). Analysis of these data demonstrated that GLUT2 expression in the subcellular fractions was quite distinct from the native GLUT4 expression. In resting (basal) adipocytes, GLUT4 was predominantly localized to the low-density microsomal fraction ($> 80\%$); upon insulin stimulation, the amount of GLUT4 in

this fraction was much decreased, consistent with translocation of GLUT4 to the cell surface (Table 3). In contrast, GLUT2 was present at high levels in the plasma-membrane fraction, and did not appear to undergo translocation in response to insulin (Table 3). These results demonstrate that (i) GLUT2 does not undergo insulin-stimulated translocation to the plasma membrane in fashion analogous to the native GLUT4, and (ii) the subcellular distribution of GLUT2 is quite distinct from the endogenous GLUT4.

Several studies have shown that GLUT4 is targeted to an intracellular location in a variety of cell lines, such as fibroblasts, PC12 cells, CHO cells, COS cells and oocytes (Haney et al., 1991; Hudson et al., 1993; Thomas et al., 1993; Czech et al., 1993; Verhey and Birnbaum, 1994). However, none of these cell systems exhibits insulin-stimulated glucose transport to the extent observed in adipocytes, and thus it has not been possible, using these cell lines, to address the influence of the cellular environment on the ability of glucose transporters to undergo insulin-stimulated translocation. We have used an alternative approach to address this issue. We have expressed a non-insulin-responsive glucose transporter, GLUT2, in a cellular environment within which translocation of GLUT4 has been unequivocally demonstrated. We have shown that the expressed GLUT2, unlike the endogenous GLUT4, is targeted to the plasma membrane, and does not undergo insulin-stimulated glucose transport. Thus, from such analyses, we conclude that the property of insulin-stimulated glucose transport is defined by the GLUT4 isoform, since the expression of GLUT2 in an adipocyte environment does not render this transporter insulin-sensitive.

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