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*This work was published in the Journal of *Molecular Endocrinology* 11, 442-449, 1997.

Summary

Caco-2, a human differentiated intestinal epithelial cell line, is a promising model for investigating the mechanism of polarized targeting of apical and basolateral membrane proteins. We stably transfected rat GLUT5 cDNA and rabbit GLUT1 cDNA into Caco-2 cells with an expression vector. Immunohistochemical study revealed that the GLUT5 protein expressed was localized at apical membranes and that the GLUT1 expressed was present primarily in the basolateral membranes of cells grown on permeable support. Next, to investigate the domain responsible for determining apical vs basolateral sorting in glucose transporters, we prepared several GLUT1-GLUT5 chimeric cDNAs and transfected them into Caco-2 cells. A GLUT1(N-terminus~6th transmembrane domain (TM6))-GLUT5(intracellular loop (IL)~C-terminus) chimera was observed exclusively at the apical membrane, while GLUT1(N-terminus~IL)-GLUT5(TM7~C-terminus) and GLUT1(N-terminus~TM12)-GLUT5(C-terminal domain) chimeras were observed mainly at the basolateral membrane, a localization similar to that of GLUT1. Moreover, using a recombinant adenovirus expression system, we expressed a GLUT5(N-terminus~TM6)-GLUT1(IL)-GLUT5(TM7~C-terminus) chimera, which was observed at the basolateral membrane. Based on these results, the C-terminal domain does not determine isoform-specific targeting of GLUT1 and GLUT5. Rather, it is the intracellular loop in glucose transporters that appears to play a pivotal role in apical-basolateral sorting signals in Caco-2 cells.

Introduction

Glucose is the primary source of metabolic energy for most mammalian cells, and glucose transport across the plasma membrane is the first step in glucose utilization (1). Molecular cloning studies have revealed a family of facilitated glucose transporters, GLUTs, which share a high degree of amino acid and structural homology (2-8). These transporters have been designated GLUT1/erythrocyte, GLUT2/liver, GLUT3/brain, GLUT4/muscle-fat, and GLUT5/small intestine. Among them, three different GLUTs are of special interest with regard to intestinal absorption. GLUT1 is present in colonocytes and cell lines derived from intestinal epithelial cells, and has been found in the basolateral membranes of these cells (2, 10). GLUT2 is also restricted to the basolateral side of enterocytes (11, 12). In contrast, GLUT5, which has recently been demonstrated to mediate mainly fructose transport (13-15), is localized to the brush-border membranes of human enterocytes (16). Thus, different glucose transporter isoforms are observed to be targeted to one or the other cell surface in a polarized epithelial cell, but which domains determine the apical/basolateral targeting of these glucose transporter proteins remains to be clarified.

Caco-2, a human differentiated intestinal epithelial cell line, is an excellent model for investigating the mechanisms underlying the polarized targeting of apical and basolateral membrane proteins (17, 18). This cell line is derived from a colonic adenocarcinoma, but resembles small intestine enterocytes in many respects including the expression of a Na+-dependent glucose transporter system (19). Caco-2 cells form tight monolayers when grown on permeable support, and a number of domain-specific membrane proteins such as several brush border hydrolases, Na+-K+ ATPase and basolateral glycoproteins have been identified using peptide-specific antibodies or monoclonal antibodies (18). Recent studies have revealed GLUT1 to be localized to the basolateral membranes and GLUT5 to primarily be present in the apical membranes of fully differentiated Caco-2 cells, a distribution similar to that of GLUTs in normal intestinal epithelial cells (10, 20).

In the present study, we transfected rat GLUT5 cDNA and rabbit GLUT1 cDNA into Caco-2 cells. We found that the GLUT5 protein expressed was localized at the apical membranes and that the GLUT1 expressed was present primarily in the basolateral membrane; the same cellular localizations as those of endogenous GLUT1 and GLUT5. These results strongly suggest that GLUT5 and GLUT1 follow the same sorting pathways as endogenous GLUT5 and GLUT1, two isoforms with distinct apical/basolateral sorting in Caco-2 cells. The Caco-2 cell line is thus a useful system for studying the molecular basis of the differential targeting of GLUT1 and GLUT5 glucose transporters. We therefore constructed and expressed chimeric GLUT1-5 glucose transporters, and analyzed the targeting of these chimeras.

This is the first attempt, to our knowledge, to investigate the apical/basolateral sorting system of GLUTs employing molecular manipulation. Our results indicate that the intracellular loop of glucose transporters plays a pivotal role in the apical/basolateral sorting system operating in Caco-2 cells.

Material and Methods

Chimeric cDNA constructs

Chimeric cDNAs were produced according to previously described methods (32), which allowed us to prepare chimeric cDNAs at any swapping site. A rabbit GLUT1 clone (4) and a rat GLUT5 clone (15) were used as templates for PCR. Three chimeras in which the N-terminal region was GLUT1 and the C-terminal region was GLUT5 (G1-5a, G1-5b, G1-5c) were constructed. Fragments prepared by PCR from the wild-type rabbit GLUT1 cDNA and the rat GLUT5 cDNA were fully sequenced and observed to have no unexpected mutations. As shown in Figure 1, the three chimeras had the following compositions: G1-5a was constructed from GLUT1(N-terminus~TM6) and GLUT5(intracellular loop~C-terminal), G1-5b from GLUT1(N-terminus~intracellular loop) and GLUT5(TM7~C-terminus), and G1-5c from GLUT1 (N-terminus~TM12) and GLUT5(C-terminal region).

Cell Culture and Transfection

The cell line Caco-2 (at 50 passages) was kindly provided by Dr. M. Shimizu (Faculty of Agriculture, University of Tokyo). The cells were cultured in a CO2/air (1:19) atmosphere at 37°C in Dulbecco's modified Eagle's medium supplemented with 10% FCS, 1% non-essential amino acids (Gibco, Glasgow, Scotland), 4 mM L-glutamine, penicillin (50 unit/ml) and streptomycin (50 μg/ml). Cells were grown in 10 cm-plastic dishes (Corning, Corning, NY) and passaged at 60% confluence. For immunofluorescence experiments, cells were grown on Transwell filters (Costar, MA). GLUT1, GLUT5 and the three chimeric cDNAs were ligated into the expression vector pCXN (26) at the EcoRI site and subsequently transfected into Caco-2 cells with calcium phosphate precipitation as previously described (33). Caco-2 cells were observed to be most susceptible to transfection at the near confluence. Transfections were performed in 6 cm-dishes using 5 μg of DNA. Cells were selected on the basis of their resistance to 600 μg/ml of the neomycin derivative G418, (Gibco). Cell lines expressing GLUT1, GLUT5, or any of the chimeras, were isolated and used in this study.

Northern blotting

For RNA preparation, differentiated cells (15 days postconfluence) grown on plastic dishes were used. Twenty micrograms of total RNA isolated with Isogen (Nippon Gene, Japan) were separated by electrophoresis on 1% agarose-formaldehyde gels, blotted onto Biodyne nylon membranes (Pall, East Hills, NY), and hybridized with probes labeled by the Megaprime labeling system (Amersham, Buckinghamshire, UK). Hybridization was performed in a solution containing 50% deionized formamide, 5x Denhardt's solution (1x Denhardt's solution = 0.2 g/l polyvinylpyrrolidone, 0.2 g/l bovine serum albumin, 0.2 g/l Ficoll 400), 6x SSPE (1 M NaCl, 60 mM NaH_2PO_4, 6 mM EDTA) and 0.1 mg/ml salmon sperm DNA at 42°C. Blots were washed twice each for 10 min at room temperature in 2x SSC, 0.1% SDS(sodium dodecyl sulfate), and twice each for 10 min at 55°C in 0.1x SSC, 0.1% SDS.

Membrane preparation and Western blotting

For membrane preparation, differentiated cells (15 days postconfluence) grown on plastic dishes were used. Cells were harvested in cold homogenization buffer consisting of 2 mM Tris-HCl, 50 mM mannitol, and 1 mM phenylmethyl sulfonyl fluoride, pH 7.0, and homogenized in a Potter-Elvehjem glass-Teflon type homogenizer at 4°C. Brush-border-enriched fractions were prepared by the standard Ca^{2+} precipitation method(34). In brief, the homogenate was incubated for 15 min on ice with the addition of $CaCl_2$ to a final concentration of 10 mM. Subsequently, the homogenate was centrifuged at 1,000 g for 15 min at 4°C, and the resulting supernatant was centrifuged at 30,000 g for 30 min at 4°C. The pellet was then resuspended, using a syringe with a 26 gauge needle, in the suspension buffer (0.25 M sucrose, 50 mM Tris-HCl, 100 mM KCl, 5 mM $MgCl_2$, pH 7.4). This suspension was homogenized with 10 strokes of a motor-driven Teflon pestle in a volume of the suspension buffer which was 10 times the weight of the original tissue. The homogenate was centrifuged at 1,000 g for 15 min at 4°C, and the resulting supernatant was centrifuged at 30,000 g for another 30 min at 4°C. The final pellet containing purified brush border membranes was resuspended in the same buffer. Protein determination was performed with a BCA protein assay (Pierce, Rockford, IL).

Brush border membranes (100 μg protein) were subjected to SDS polyacrylamide (10%) gel electrophoresis and transferred onto nitrocellulose filters. Immunoblotting was performed using antisera raised in rabbits against the synthesized peptide corresponding to the COOH-terminal domain of GLUT5 (residues 491-503) as described previously in detail (15). Finally, the filters were incubated with 125I-protein A (Amersham, Buckinghamshire, UK) and subjected to autoradiography.

Transient expression of Glucose transporters with a recombinant adenovirus expression system.

The cassette cosmid for constructing recombinant adenovirus, pAdex1wt, was the generous gift of Dr Izumi Saito (Institute of Medical Science, University of Tokyo). The cDNAs encoding GLUT5 and GLUT5(N-terminus~TM6)-GLUT1(Intracellular loop domain)-GLUT5(TM7~C-terminus) were ligated into the Swa I sites of pAdex1wt. Recombinant adenoviruses were obtained as previously described (35). Caco-2 cells were infected with these viruses for 1 hr, then grown for 72 hr. As a negative control, we prepared the adenovirus expressing lacZ.

Immunofluorescence study

For immunofluorescence microscopy, Caco-2 cells (15 days postconfluence) grown on Transwell filters were fixed in 3% formaldehyde/PBS. Semithin frozen sections of 1 μm thick were made and incubated with either an antipeptide antibody against the COOH-terminal domain of GLUT1, for the detection of GLUT1, or an antipeptide antibody against the COOH-terminal domain of GLUT5, for the detection of GLUT5, and three chimeric transporters (15, 35). The sections were then incubated with lissamine rhodamine labeled affinity-purified donkey anti-rabbit IgG (Jackson Immunoresearch, West Grove, PA)(37, 38). For F-actin staining, fluorescein-phalloidin (1: 50 dilution, Molecular Probes, Eugene, OR) in PBS was added to the secondary antibody. After being washed with PBS, the sections were mounted in Perma Fluor Aqueous mountant (Lipshaw, Pittsbugh, PA). Specimens were observed with an Olympus BX-50 microscope equipped with epifluorescence and Nomarski differential-interference-contrast optics.

Results

Chimeric cDNA constructs

The cDNA constructs were prepared for the expression of GLUT1, GLUT5 and three GLUT1-5 chimeras and introduced into Caco-2 cells. The three chimeras had the following compositions: G1-5a was constructed from GLUT1(N-terminus~TM6) and GLUT5(intracellular loop~C-terminal), G1-5b from GLUT1(N-terminus~intracellular loop) and GLUT5(TM7~C-terminus), and G1-5c from GLUT1 (N-terminus~TM12) and GLUT5(C-terminal region). These swapping sites are shown in Figure 1.

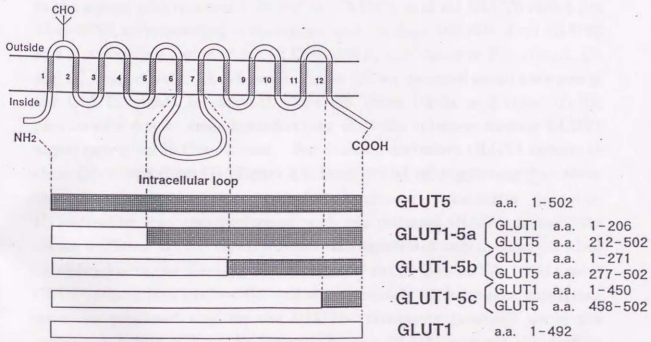

Figure 1. A model for the orientation of the chimeric glucose transporter. The putative membrane spanning domains are numbered 1-12 from the NH2 terminus to the COOH terminus. Three chimeric glucose transporters were prepared from combinations of GLUT1 and GLUT5, and termed G1-5a, G1-5b, and G1-5c. The amino acid sequences of these chimeras are indicated beside each chimeric transporter. We connected these two proteins at amino acid sequences with high homology. So, the numbers of amino acids of the swapping sites were different between GLUT1 and GLUT5.

Northern blotting

We screened transfectants by northern blotting and GLUT transcripts were detected in several clones. These clones were selected and one clone from each cDNA construct was designated clone G5(wild type GLUT5), clone G1-5a(G1-5a chimera), clone G1-5b(G1-5b chimera), clone G1-5c(G1-5c chimera) and clone G1(wild type GLUT1). One of the clones transfected with pCXN alone was designated clone D. Northern blots of these clones, probed with rabbit GLUT1 cDNA (bp 955-2477, corresponding to the amino acid residues 278-492 of rabbit GLUT1 and 3' non-coding region of rabbit GLUT1 cDNA), rat GLUT5 cDNA (bp 21-656, corresponding to the amino acid residues 1-204 of rat GLUT5), and rat GLUT5 cDNA (bp 1540-2170, corresponding to the amino acid residues 500-502 of rat GLUT5 and 3' non-coding region of rat GLUT5 cDNA) are shown in Figures 2A, 2B and 2C, respectively. As shown in Figure 2A, we detected small amounts of the GLUT1 signal in clone D, clone G5, clone G1-5a and clone G1-5b, presumably due to cross-hybridization with the intrinsic human GLUT1 signal expressed in Caco-2 cells. We detected abundant GLUT1 signals in clone G1-5c and clone G1 (Figure 2A, lanes 5 and 6), suggesting that these two clones express their transfected glucose transporter proteins. Hybridization was also performed with two different GLUT5 probes. As shown in Figure 2B, the signal amount was significant only in clone G5 when hybridized with the N-terminal half region of rat GLUT5 cDNA. The size of GLUT5 transcripts in clone G5 was approximately 2.8 kilobases, consistent with the predicted size for the GLUT5 transcripts produced using the expression vector pCXN. Endogenous human GLUT5 was not observed in clone D, or in other clones transfected with chimeric cDNA, which means that we identified only transfected GLUT5 transcripts, though it is possible that a small amount of endogenous GLUT5 exists in Caco-2 cells. When hybridized with a GLUT5 cDNA fragment corresponding to the C-terminal region of GLUT5 (Figure 2C), significant signal amounts were detectable in four clones which had been transfected with cDNA containing the sequence corresponding to the C-terminal region of GLUT5 and 3' non-coding region of GLUT5 cDNA. These results demonstrate that GLUT5 and chimeric cDNAs were successfully transcribed in Caco-2 cells.

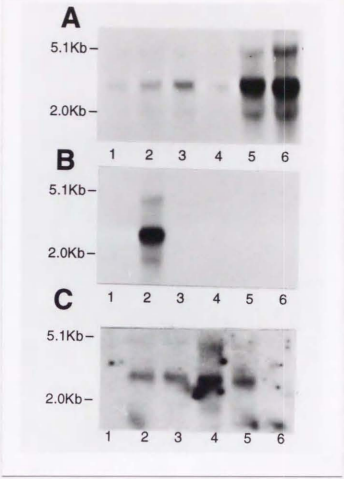

Figure 2. Expression of the transfected glucose transporter mRNA in Caco-2 cells. Twenty micrograms of total RNA were separated by electrophoresis on 1% agarose-formaldehyde gels, blotted onto a nylon membrane, and probed with (A) rabbit GLUT1 cDNA (bp 955-2477, corresponding to the amino acid residues 278-492 of rabbit GLUT1 and 3' non-coding region of rabbit GLUT1 cDNA), (B) rat GLUT5 cDNA (bp 21-656, corresponding to the amino acid residues 1-204 of rat GLUT5), (C) rat GLUT5 cDNA (bp 1540-2170, corresponding to the amino acid residues 500-502 of rat GLUT5 and 3' non-coding region of rat GLUT5 cDNA) Hybridization was performed in a solution containing 50% deionized formamide, 5x Denhardt's solution and 0.1 mg/ml salmon sperm DNA at 42°C. Blots were each washed twice for 10 min at room temperature in 2x SSC, 0.1% SDS(sodium dodecyl sulfate), and twice each for 10 min at 55°C in 0.1x SSC, 0.1% SDS. The following RNA sources were used: Lane 1, clone D; Lane 2, clone G5; Lane 3, clone G1-5a; Lane 4, clone G1-5b; Lane 5, clone G1-5c; Lane 6, clone G1.

Western blotting

The immunoblotting of cellular homogenates of these transfectants with an antibody raised against the COOH-terminal peptide of rat GLUT5 showed the presence of large amounts of the expressed proteins in clone G5, clone G1-5a, clone G1-5b and clone G1-5c (Figure 3), while the signal was barely observable in clones D and G1. The apparent molecular mass of the proteins detected was 45~80 kDa. These results suggest that GLUT5 and chimeric proteins were expressed in Caco-2 cells and that our antibody detected only transfected proteins in these clones.

Localization of the wild-type glucose transporters

Immunofluorescence labeling of Caco-2 monolayers revealed marked differences in the cellular distributions of GLUT1 and GLUT5 (Figure 4). GLUT5 was observed on the apical side in clone G5 (Figure 4-a). In double-labeling experiments, the GLUT5 labeling was co-localized with the intense labeling of F-actin, a marker of the brush border (Figure 4-b), indicating that GLUT5 is expressed in the brush border of the apical membrane. We also investigated two other clones expressing rat wild-type GLUT5 and obtained results similar to those of clone G5 (data not shown). Almost no GLUT5 signal was observed in clone D (Figure 4-g). In contrast to the distribution of GLUT5, GLUT1 was observed predominantly on the lateral and basolateral sides in clone G1 which overexpressed rabbit GLUT1, while weak signals were observed on the apical side (Figure 4-d). We also observed a similar distribution of GLUT1 in clone D which expressed endogenous GLUT1 (data not shown). The cellular distributions of the GLUT1 and GLUT5 expressed were compatible with those seen in previous studies on the distribution of endogenous GLUT1 and GLUT5 (10, 20).

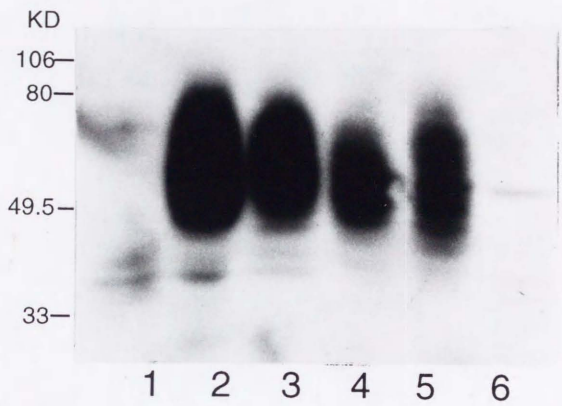

Figure 3 Immunoblot analysis of glucose transporters in Caco-2 cells. For membrane preparation, differentiated cells (15 days postconfluence) grown on plastic dishes were used. One hundred micrograms of brush border membranes (lanes 2 and 3) and crude homogenates (lanes 1, 4, 5, and 6) were subjected to SDS polyacrylamide (10%) gel electrophoresis and transferred onto nitrocellulose filters. Immunoblotting was performed using antisera raised in rabbits against the synthesized peptide corresponding to the COOH-terminal domain of GLUT5 (residues 491-503), as has been described in detail previously (15). The filters were incubated with 125I-protein A (Amersham) and subjected to autoradiography. The following membrane sources were employed: Lane 1, clone D; Lane 2, clone G5; Lane 3, clone G1-5a; Lane 4, clone G1-5b; Lane 5, clone G1-5c; Lane 6, clone G1.

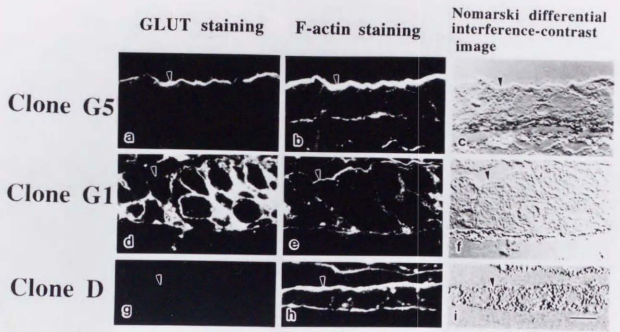

Figure 4. Immunofluorescence localization of glucose transporters in clone G5 (upper), clone G1 (middle), and clone D (lower). Wild-type rat GLUT5 cDNA, wild-type rabbit GLUT1 cDNA, and pCXN vector alone were respectively transfected into these clones. For immunofluorescence microscopy, Caco-2 cells (15 days postconfluence) grown on Transwell filters were fixed in 3% formaldehyde/PBS. Semithin frozen sections of 1 μm thick were made and incubated with either antipeptide antibody against the COOH-terminal domain of GLUT1 (d) and antipeptide antibody against the COOH-terminal domain of GLUT5 (a, g). The sections were then incubated with lissamine rhodamine labeled affinity-purified donkey anti-rabbit IgG. For staining of F-actin, a brush border marker, fluorescein-phalloidin in PBS was added to the secondary antibody (b, e, h). The arrowheads point to the apical sides of Caco-2 cells, which are rich in F-actin and thus intensely stained. Nomarski differential interference-contrast images are also shown (c, f, i). Bar=10 μm

Localization of the GLUT1-GLUT5 glucose transporters

For comparison with the distribution of wild-type GLUT1 or GLUT5, stable transfectants expressing GLUT1-5 chimeric protein were likewise studied. Figure 5 shows the results of immunofluorescence studies of clone G1-5a, clone G1-5b and clone G1-5c, obtained using an anti-GLUT5 C-terminal antibody. Interestingly, in clone G1-5a, the chimeric glucose transporter composed of GLUT1(N-terminus~TM6) and GLUT5(intracellular loop~C-terminal) was localized to the apical membrane, a distribution similar to that of the wild-type GLUT5. In sharp contrast, the G1-5b chimeric transporter, composed of GLUT1(N-terminus~intracellular loop) and GLUT5(TM7~C-terminus), and the G1-5c chimeric glucose transporter, composed of GLUT1 (N-terminus~TM12) and GLUT5(C-terminal region), were observed primarily on the basolateral sides of Caco-2 cells. The distribution of these two constructs was essentially the same as that of expressed, i.e. intrinsic, GLUT1.

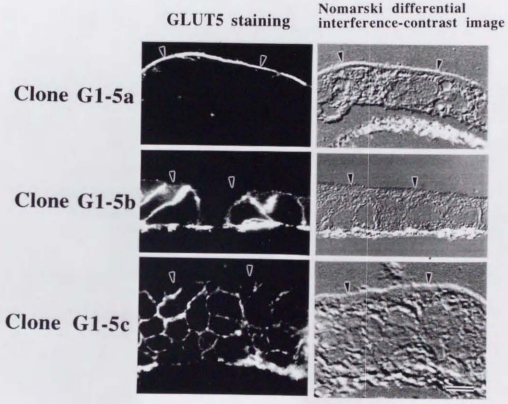

Figure 5. Immunofluorescence localization of glucose transporters in clone G1-5a (upper), clone G1-5b (middle), and clone G1-5c (lower). Ultrathin frozen sections were incubated with antipeptide antibody against the COOH-terminal domain of GLUT5 (a, c, e). The sections were then incubated with rhodamine-labeled affinity-purified donkey anti-rabbit IgG. The arrowheads point to the apical sides of Caco-2 cells. Nomarski differential interference-contrast images are also shown (b, d, f).

Localization of the GLUT5-1-5 glucose transporters

In order to clarify the role of the GLUT intracellular loop domain, we investigated the targeting of another chimeric glucose transporter, which has two reciprocal swapping sites; GLUT5(N-terminus~TM6, amino acid residues, 1-211)-GLUT1(Intracellular loop domain, amino acid residues, 207-271)-GLUT5(TM7~C-terminus, amino acid residues, 277-502), designated G5-1-5 (Figure 6). We prepared the cDNA constructs encoding G5 and G5-1-5 cDNA and transfected them transiently into Caco-2 cells with a recombinant adenovirus expression system. Expression of these proteins was confirmed by western blotting with the antibody against GLUT5(data not shown). On immunohistochemical study, G5-1-5 protein was mainly targeted to the basolateral membrane, while the wild type GLUT5 was mainly targeted to the apical membrane (Figure 7).

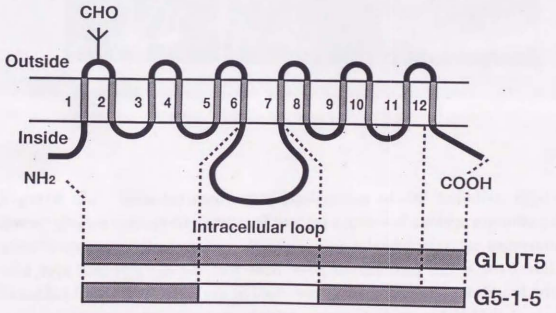

Figure 6. Schematic representation of G5-1-5 chimeric protein.

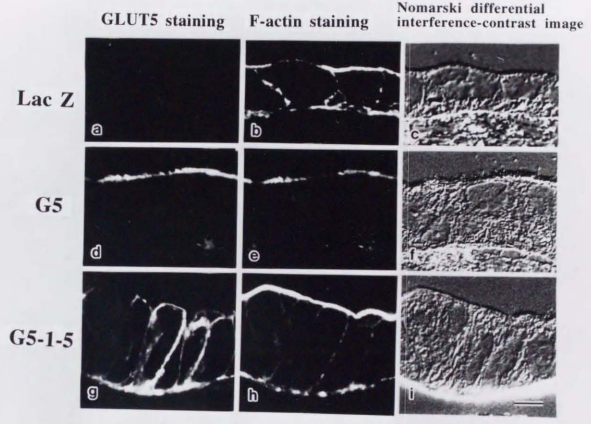

Figure 7. Immunofluorescence localization of G5 (middle), G5-1-5 (lower) glucose transporter and lacZ (upper) expressed using a recombinant adenovirus expression system. Recombinant adenoviruses for expressing wild type GLUT5, G5-1-5 and lacZ were transfected into Caco-2 cells. Semithin frozen sections, 1μm in thickness, were made and incubated with antipeptide antibody against the COOH-terminal domain of GLUT5 (a, d, g). The sections were then incubated with lissamine rhodamine labeled affinity-purified donkey anti-rabbit IgG. The apical sides of Caco-2 cells are rich in F-actin and thus intensely stained (b, e, h). Nomarski differential interference-contrast images are also shown (c, f, i). Bar=10 μm

Discussion

Most studies on GLUT sorting have focused on the unique sequestration of GLUT4. A variety of molecular manipulations such as site-directed mutagenesis and chimeric formation have been employed to examine the GLUT4 molecule and the mechanism of its intracellular sequestration has been clarified to some extent (21-23). In polarized epithelial cells, recent studies have shown that glucose transporter isoforms are sorted to either the apical or the basolateral surface (10-12, 16, 20). However, the domains responsible for this apical/basolateral sorting have not been determined. In the present study, we first confirmed that GLUT1 and GLUT5, when expressed with an expression vector, show the same cellular localization as endogenous GLUT1 and GLUT5. We then prepared chimeric proteins combining these two GLUT isoforms and investigated their targeting in polarized cells.

The human colon carcinoma cell line Caco-2 exhibits, at late confluence, the same morphological characteristics as differentiated small-intestinal enterocytes (24). The facilitative glucose transporters GLUT1 and GLUT3, and the fructose transporter GLUT5 are expressed in these cells (10). However, the expression of GLUT5 in Caco-2 cells is considered to be controversial. A clonal difference was previously suggested as to the level of expression of intrinsic GLUT5, based on the observation that GLUT5 was expressed only in low-glucose-consuming clones (25). Moreover, considerable developmental variations were observed in GLUT5 expression. In particular, an increase in GLUT5 expression was observed after confluence had been reached and the expression level also depends on the passage number of the Caco-2 cells (20). For instance, GLUT5 was detected in only 40% of Caco-2 cells (passage 60) at 15 days after confluence. These earlier observations suggest that Caco-2 cells show heterogeneity and that the regulation of GLUT5 expression depends on the degree of differentiation in Caco-2 cells.

In the present study, we ligated rat GLUT5 cDNA into the expression vector pCXN and transfected rat GLUT5 into Caco-2 cells. This vector has the actin promoter and CMV enhancer sequences (26). Thus, the regulation system for expression of transfected GLUT5 is assumed to be considerably different from that of endogenous GLUT5. We found that the transfected GLUT5 was expressed even in the the early postconfluence stage (data not shown), and was observed most abundantly on the apical side in essentially all cells at 15 days after reaching confluence. These results were obtained with expressed GLUT5, and are somewhat inconsistent with those of a previous report on endogenous GLUT5, indicating that clone G5 has the property of homogeneity and that its GLUT5 expression is regulated by a vector program.

The distributions of GLUT1, GLUT5 and the three chimeric proteins used herein showed two distinct patterns. The wild-type GLUT5 and the G1-5a chimeric protein were localized on the apical side, while the wild-type GLUT1, the G1-5b and the G1-5c chimeric protein were observed mainly on the lateral and basolateral sides. These results suggest that the intracellular loop region of these glucose transporter isoforms determines apical/basolateral sorting in Caco-2 cells. These chimeric transporters were properly sorted to the plasma membrane in a fashion similar to that of the wild-type in CHO cells (27), suggesting that the secondary structure of chimeric proteins was not markedly impaired.

In order to further elucidate the role of the GLUT's intracellular loop region, we studied the targeting of G5-1-5 protein, GLUT5 in which the intracellular loop domain had been replaced with that of GLUT1. First, we prepared G5-1-5 cDNA and transfected it into Caco-2 cells with an expression vector using the calcium phosphate method. However, we could not obtain a clone expressing an adequate amount of G5-1-5 protein for immunofluorescense study. The reason for this is not clear. We then employed a recombinant adenovirus expression system and obtained adequate amounts of the expressed proteins. Interestingly, G5-1-5 protein was targeted to the basolateral membrane, while the wild type GLUT5 was mainly targeted to the apical membrane (Figure 7).

It is possible that GLUT1 contains the basolateral sorting signal in its intracellular loop region. Several basolateral sorting signals such as the tyrosine motif and the di-leucine motif have been identified in another polarized model system, MDCK cells (28-30). A recent report indicated, however, that a specific sorting signal is not likely to be required for intracellular vesicle traffic to the basolateral membrane in Caco-2 cells (31). Thus, an alternative, and quite plausible, explanation is that GLUT5 contains the apical sorting signal in its intracellular loop region and that the delivery of GLUT1 to the basolateral membrane represents the default pathway.

A recent study has shown that newly synthesized proteins in Caco-2 cells are sorted to the apical membrane via two different routes, an exocytic route and an endocytic-transcytotic route (18). Regarding the GLUT5 traffic, we have not analyzed which route is taken by GLUT5. Based on the observation that GLUT5 is also present in the basolateral membranes of some undifferentiated Caco-2 cells (10), it is tempting to speculate that fully differentiated Caco-2 cells, in which the transcytotic route is well developed, can effectively carry GLUT5-containing vesicles from the basolateral endosome to the apical plasma membrane. In this context, it is reasonable to speculate that the intracellular loop domain of GLUT5 actually contains the transcytotic and apical signal. The basolateral sorting of G5-1-5 protein is consistent with the idea that the apical sorting signal exists in the intracellular loop domain of GLUT5, and not in that of GLUT1. Though further study is expected to clarify in greater detail the transporting mechanism of GLUT5, our results indicate that the intracellular loop domains of GLUT1 and GLUT5 play pivotal roles in the apical/basolateral sorting in the polarized cell line Caco-2.

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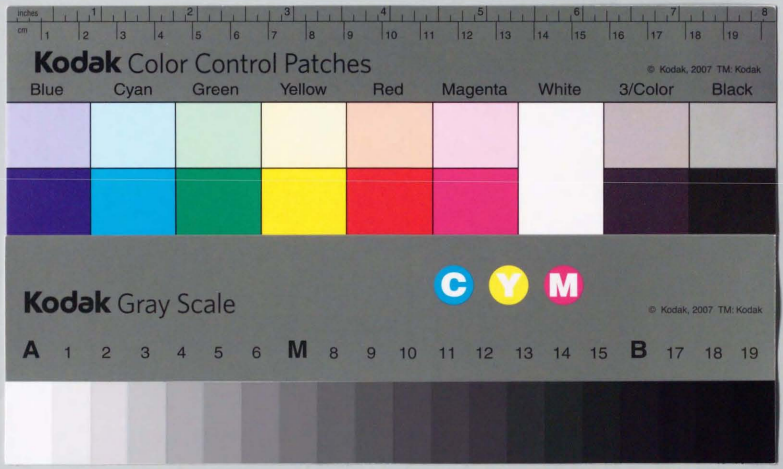
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