Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 7556–7560, August 1992
Cell Biology

Polarized distribution of glucose transporter isoforms in Caco-2 cells

(sorting/targeting/epithelial cells)

DIANA S. HARRIS*, JAN W. SLOT†, HANS J. GEUZE†, AND DAVID E. JAMES*‡

*Department of Cell Biology and Physiology, Washington University School of Medicine, Box 8228, 660 South Euclid Avenue, St. Louis, MO 63110; and †Department of Cell Biology, Medical School, University of Utrecht, 3584CX Utrecht, The Netherlands

Communicated by Stuart Kornfeld, May 18, 1992

ABSTRACT **We have examined the expression and cellular location of facilitated glucose transporter proteins (GLUT1, -3, and -5) in a human colonic epithelial cell line (Caco-2) by using peptide-specific antibodies. A differential cellular distribution of these transporters was observed in differentiated (>14 days postconfluence) Caco-2 cells by immunofluorescence and immunoelectron microscopy. GLUT1 was localized primarily to the basolateral membrane, whereas GLUT3 was predominantly localized to the apical membrane. GLUT5, which was detected in only ≈40% of fully differentiated Caco-2 cells, was found primarily in the apical membrane but was also present in both basolateral and intracellular membranes. A Na^+-independent glucose transport system in the apical membrane of Caco-2 cells has been described previously [Blais, A., Bissonnette, A. & Berteloot, A. (1987) *J. Membr. Biol.* 99, 113–125], and we propose that GLUT3 mediates this process. The amino acid sequence identity (57%) and structural conservation between GLUT1 and GLUT3 may make these transporters an ideal model system for examining the molecular basis for polarized sorting of membrane proteins.**

The mechanisms involved in the targeting of cellular proteins is of key interest in cell biology. Considerable evidence supports the hypothesis that the eventual destination of a particular protein in the cell is a function of specific signals in the primary amino acid sequence of that protein (1, 2). Polarized epithelial cells have been a particularly useful system for these types of studies because the protein and lipid compositions of the apical and basolateral membranes are quite distinct (2). Many studies have focused on identifying putative sorting signals that may direct a protein to either the basolateral or the apical membrane. The first signal to be identified in epithelial cells was the glycosyl-phosphatidylinositol (GPI) anchor, which appears to confer apical sorting in epithelial cells (3). Recent studies suggest that this signal mediates clustering in the trans-Golgi reticulum, which somehow facilitates the targeting of GPI-anchored proteins to the apical domain (4). Many studies have attempted to identify polarized sorting signals in the cytoplasmic domains of non-GPI-anchored proteins (reviewed in ref. 2) by expressing recombinant proteins that have been mutated in some way. However, with the exception of the polyimmunoglobulin receptor (5), most of these studies have involved a "loss of function" analysis that may or may not have reflected the specific targeting properties of a particular domain. Analysis of chimeras between two proteins that are differentially targeted but that are structurally related may overcome many of these limitations.

A number of protein families that have been identified by molecular cloning may provide the basis for this type of analysis. One such family is that of the facilitated glucose transporter. The individual members of this family (GLUT1 through GLUT5) have identical predicted secondary structures (reviewed in ref. 6). These proteins are predicted to span the lipid bilayer 12 times, with the amino and carboxyl termini exposed on the cytoplasmic face. The length of the primary translation products is very similar (492–524 amino acids) and there is considerable amino acid homology among the GLUT isoforms. The highest degree of sequence heterogeneity occurs within the four hydrophilic domains. One function of these unique domains may be to determine isoform-specific targeting. Such specific targeting includes the preferential localization of GLUT1 to the cell surface (7), the insulin-regulated movement of GLUT4 from intracellular tubulo-vesicles to the cell surface (8), and the targeting of GLUT2 to the basolateral membrane in kidney and intestinal epithelial cells (9). By using GLUT1/GLUT4 chimeric proteins, it has recently been shown that the amino-terminal 19 amino acids of GLUT4 are both necessary and sufficient for intracellular sequestration (10).

The purpose of the present studies was to examine the targeting of various glucose transporter isoforms in epithelial cells in order to establish a system that would enable a molecular dissection of polarized sorting of membrane proteins. Previous studies of glucose transport kinetics in a human colonic adenocarcinoma cell line (Caco-2) have indicated the presence of a facilitated transport system at the apical and basolateral surfaces (11). Using isoform-specific antibodies, we show in these studies that Caco-2 cells express high levels of GLUT1, GLUT3, and GLUT5. Importantly, each of these isoforms displays a unique subcellular distribution in Caco-2 cells. The GLUT1 isoform localizes primarily to the basolateral membrane and GLUT3 to the apical membrane. GLUT5, on the other hand, has a pattern of distribution that is intermediate between that of GLUT1 and that of GLUT3, being found at both the apical and the basolateral membrane. These data suggest that specific information contained within the primary amino acid sequence of these related proteins confers polarized targeting in epithelial cells.

MATERIALS AND METHODS

Cell Culture. Caco-2 cells (a gift from D. Alpers, Washington University School of Medicine) were used between passages 42 and 65. They were cultured in Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol) fetal bovine serum (JRH Biosciences, Lexena, KS), nonessential amino acids [1% (vol/vol) of a stock solution; Sigma], penicillin (50 units/ml), and streptomycin (50 μg/ml). Cells were passaged at 60% confluence. For membrane preparations, cells were cultured on 100-mm dishes; for immunofluorescence experiments, cells were plated either on glass coverslips (no. 1) or on Costar Transwell filters. All exper-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

‡To whom reprint requests should be addressed.

iments using differentiated cells were performed at 14–16 days postconfluence. Day 0 was assigned as the first day of confluency.

Immunoblotting. Glucose transporter antibodies were generated against peptides corresponding to the carboxyl terminus of each transporter (GLUT1, CELFHPLGADSQV; GLUT3, CSIEPAKETTTNV; GLUT5, CEELKELPPVTSEQ) as described (12). Antibodies were affinity-purified using SulfoLink coupling gel (Pierce) according to the manufacturer's instructions. SDS/PAGE and immunoblotting were performed as described (7).

Immunofluorescence and Immunoelectron Microscopy. Immunofluorescence labeling was performed (7) using affinity-purified antibodies at a concentration of 5 μg of IgG per ml. Samples were visualized by confocal immunofluorescence microscopy. For z-line scans, we used a z-drive focus motor attached to the fluorescence microscope. Single-line sections (100–200) were taken at 0.2-μm steps, and a visual image of the x–z plane was generated using a BioRad MRC-500 confocal imaging system. Photography of images was performed directly from the microscope monitor, using identical settings.

For immunoelectron microscopy, cells were fixed for 1 hr with 2% paraformaldehyde/0.2% glutaraldehyde/0.1 M sodium phosphate buffer, pH 7.4. Fixed cells were then embedded in 10% gelatin in phosphate-buffered saline (13) and blocks were immersed in 1.6 M sucrose in phosphate-buffered saline, frozen in liquid nitrogen, dehydrated by cryosubstitution at −90°C with methanol, and embedded at −45°C in Lowicryl HM20 (Polysciences) as described (13). Ultrathin sections were labeled with GLUT1 antibody followed by protein A-gold on one face of the section, and with GLUT3 antibody and protein A-gold at the other face, as described previously (14). The sections were stained with uranyl acetate followed by lead citrate (13).

RESULTS

The specificity of the antibodies used in these studies is shown in Fig. 1. The characteristics of the GLUT1 antibody have been described previously (7). This antibody labeled an ≈45-kDa species in Caco-2 cell membranes. The GLUT3 antibody immunolabeled an ≈45-kDa species in human brain and in Caco-2 cells, but not in human jejunum. The GLUT5 antibody recognized an ≈60-kDa species in human kidney, human jejunum, and Caco-2 cell membranes. Using a GLUT4-specific antibody that immunolabels an ≈45-kDa protein in fat and muscle from human and rat (12), we were unable to detect any GLUT4 in Caco-2 membranes (data not shown). The specificity of immunolabeling for each of these antibodies was demonstrated by competitive inhibition with the respective peptide antigens (Fig. 1). Irrelevant peptides had no effect on immunolabeling. In contrast to the GLUT1 and GLUT4 antibodies, the antibodies specific for GLUT3 and GLUT5 appeared to be human-specific, because there was no crossreactivity with rat tissues. The developmental expression of these transporters in Caco-2 cells throughout differentiation is shown in Fig. 2. Whereas GLUT1 levels remained quite constant for 14 days postconfluence, a developmental increase in expression was observed for both GLUT3 and GLUT5. However, the developmental increase in GLUT5 appeared to lag behind that of GLUT3, and in contrast to GLUT3, GLUT5 levels had not plateaued by 14 days postconfluence. These data suggest that the developmental program for expression of these different transporters in Caco-2 cells must differ considerably.

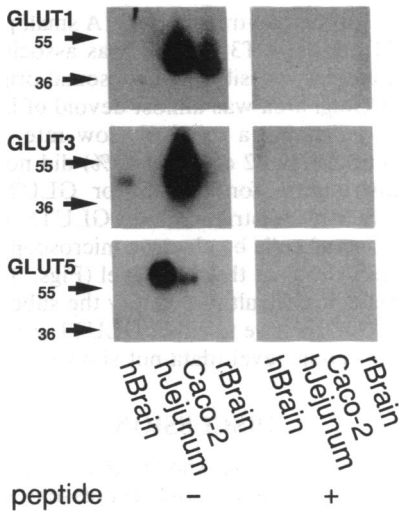

FIG. 1. Characterization of peptide-specific antibodies. Twenty micrograms of total membrane protein of human (h) and rat (r) tissues and 3 μg of total membrane protein of differentiated Caco-2 cells were immunoblotted with antibodies against human GLUT1, GLUT3, and GLUT5 in either the absence (*Left*) or the presence (*Right*) of an excess (10 μg/ml) of the peptide antigen. Incubation with irrelevant peptide had no effect on immunoreactive bands (data not shown). Migration of molecular weight standards (M_r 55,000 and 36,000) is indicated at left.

Immunofluorescence labeling of Caco-2 monolayers revealed significant differences in the cellular distribution of GLUT isoforms (Fig. 3). In fully differentiated monolayers (day 14), GLUT3 immunoreactivity was present primarily at the apical surface. In contrast, the GLUT1 isoform was predominantly lateral and basolateral in Caco-2 cells. We also observed punctate staining of GLUT1 just beneath the apical surface, which appears as a bright spot in the center of most cells (see Fig. 3*A*). This is consistent with electron microscopy immunolabeling, where labeling of endosome-like vacuoles for both GLUT1 and GLUT3 was frequently observed (data not shown). GLUT5 labeling was detected in only ≈40% of the population of differentiated Caco-2 cells (Fig. 3*E*). This labeling was often concentrated at the apical membrane but also appeared at the basolateral membrane (Fig. 3*F*), as demonstrated by the faint staining similar to that in Fig. 3*B*. Some intracellular staining was also observed in the case of GLUT5. The characteristic presence of domes in the cell monolayer creates the appearance of holes in the monolayer for any given section in the x–y plane (see Fig. 3 *A* and *B*, arrows). This lack of uniformity of cell position made it difficult to obtain a section of uniform depth in the x–y

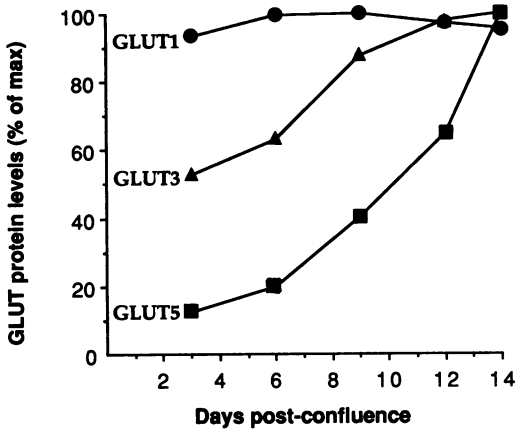

FIG. 2. Time course of expression of glucose transporters in Caco-2 cells. Day 0 corresponds to the first day of confluency. Cells were homogenized at the indicated times and equal amounts of total membrane protein (20 μg) were immunoblotted with GLUT antibodies. Immunoreactive bands were quantified by densitometry.

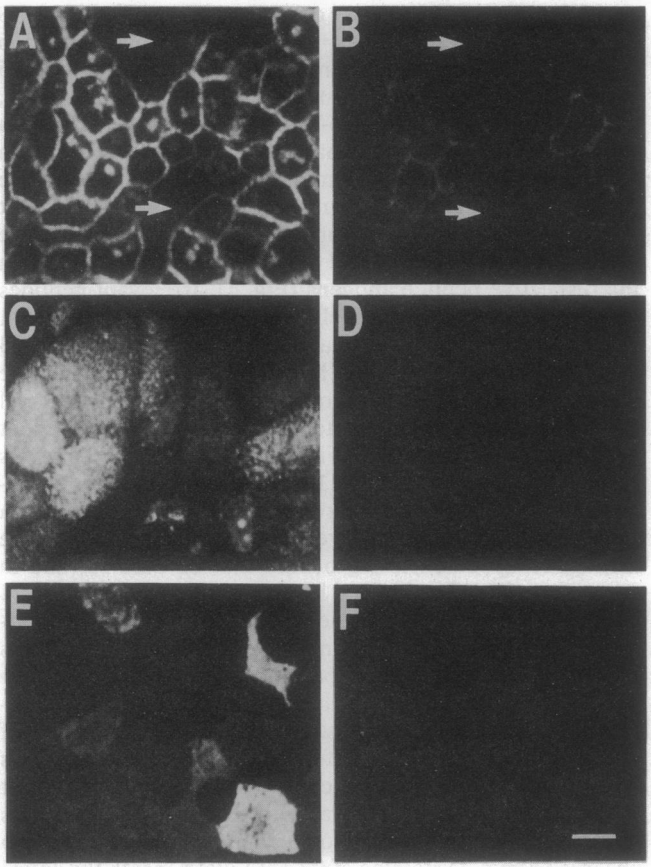

FIG. 3. Confocal immunofluorescence microscopy of Caco-2 monolayers. Fully differentiated cell monolayers were labeled with antibodies to GLUT1 (*A* and *B*), GLUT3 (*C* and *D*), or GLUT5 (*E* and *F*), and images were generated with a laser scanning immunofluorescence microscope system. Thin-section images were taken through the *x–y* plane of the monolayer at the cell surface (*A*, *C*, and *E*) or 4 μm below the cell surface (*B*, *D*, and *F*). Arrows point to regions occupied by domes, where the cells have detached from the matrix. These appear as "holes" in the monolayer due to the depth of the section. Bright punctate areas in the center of each cell (*A*) are most probably populations of endosomes just below the apical surface. Although labeling of GLUT5 in lateral membranes is low (*F*), it mimics labeling of GLUT1 (*B*). (Bar = 10 μm.)

plane of the immunofluorescence microscope. To circumvent this problem we obtained *z*-line images by using confocal laser microscopy (Fig. 4). With this approach the distinct distribution of the transporters was clearly observed. GLUT3 was localized to the apical membrane with very little labeling of the lateral membrane (Fig. 4*B*). Conversely, GLUT1 was found primarily at the lateral membrane with no detectable staining of the apical membrane (Fig. 4*A*). This polarized distribution of GLUT1 and GLUT3 was evident at early times (day 3 postconfluence) as well as at late times (day 14) (data not shown). GLUT5 was not detected at day 3 (Fig. 2) and exhibited a heterogeneous distribution both between individual cells and within a given cell at day 14 (Fig. 4*C*).

To obtain a more quantitative impression of the distribution of GLUT1 and GLUT3 in Caco-2 cells, we performed double immunogold labeling and electron microscopy (Fig. 5). Both transporters were detected mainly at the cell surface. GLUT3 dominated at the apical membrane and GLUT1 at the basolateral membrane. Immunogold labeling of the apical and the adjacent lateral membranes were quantitated in 10 different cells. The GLUT3/GLUT1 labeling ratio at the apical membrane was 41.3 ± 6.2 (mean ± SD), and the GLUT3/GLUT1 ratio in the adjacent lateral membranes was 0.19 ± 0.04. Therefore, the total apical enrichment of GLUT3 vs.

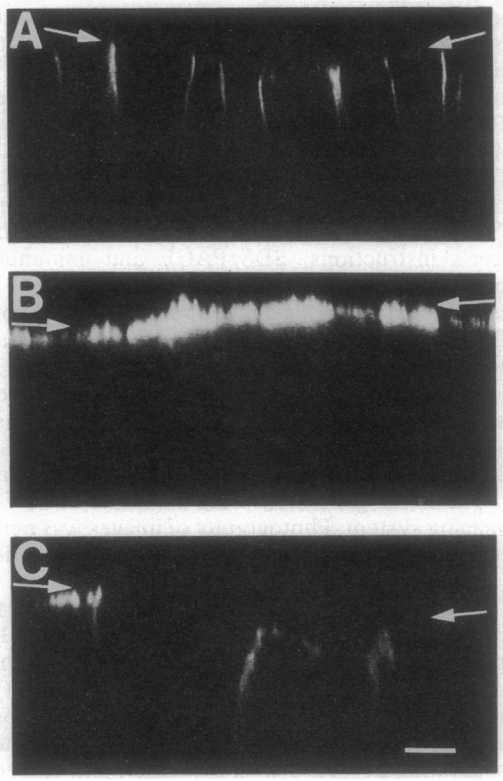

FIG. 4. Vertical slice (*x–z* plane) image of immunofluorescence labeling in differentiated Caco-2 monolayers. Caco-2 cells were labeled with antibodies specific for GLUT1 (*A*), GLUT3 (*B*), or GLUT5 (*C*), and images of the *x–z* plane were obtained with the confocal laser microscope. Arrows indicate apical surface of the monolayer. Note the homogeneity of the pattern of distribution in *A* and *B*, and the heterogeneity of labeling (both within and between cells) in *C*. (Bar = 10 μm.)

GLUT1 was 276.6 ± 44.8. However, these ratios reflect only the relative levels of each transporter in either membrane because the GLUT1 immunoreactivity appeared more sensitive to the tissue processing, so that the labeling efficiency was different for the two transporters. A small percentage of both GLUT1 and GLUT3 labeling was associated with intracellular vacuoles, possibly of endosomal origin (data not shown). The Golgi area was almost devoid of label for both transporters, indicating a relatively low rate of synthesis. Some cells in the Caco-2 culture (<5%) did not exhibit the polarized distribution for GLUT1 or GLUT3 (data not shown). A variable distribution of GLUT5 labeling was observed in Caco-2 cells by electron microscopy, consistent with the observations at the light level (Figs. 3 and 4). This variability made it difficult to quantify the subcellular distribution of GLUT5 relative to either GLUT1 or GLUT3 at the electron microscopic level (data not shown).

DISCUSSION

In these studies we have shown that a human colonic adenocarcinoma cell line (Caco-2) expresses significant levels of GLUT1, GLUT3, and GLUT5. Most notably, GLUT1 and GLUT3 undertake a marked polarity in their distribution. In well-differentiated cells GLUT1 was localized to the basolateral membrane whereas GLUT3 was localized to the apical membrane (Figs. 3–5). In view of the high degree of amino acid and structural homology between these transporter isoforms (6), this should be an ideal system to elucidate putative targeting signals that confer apical/basolateral targeting.

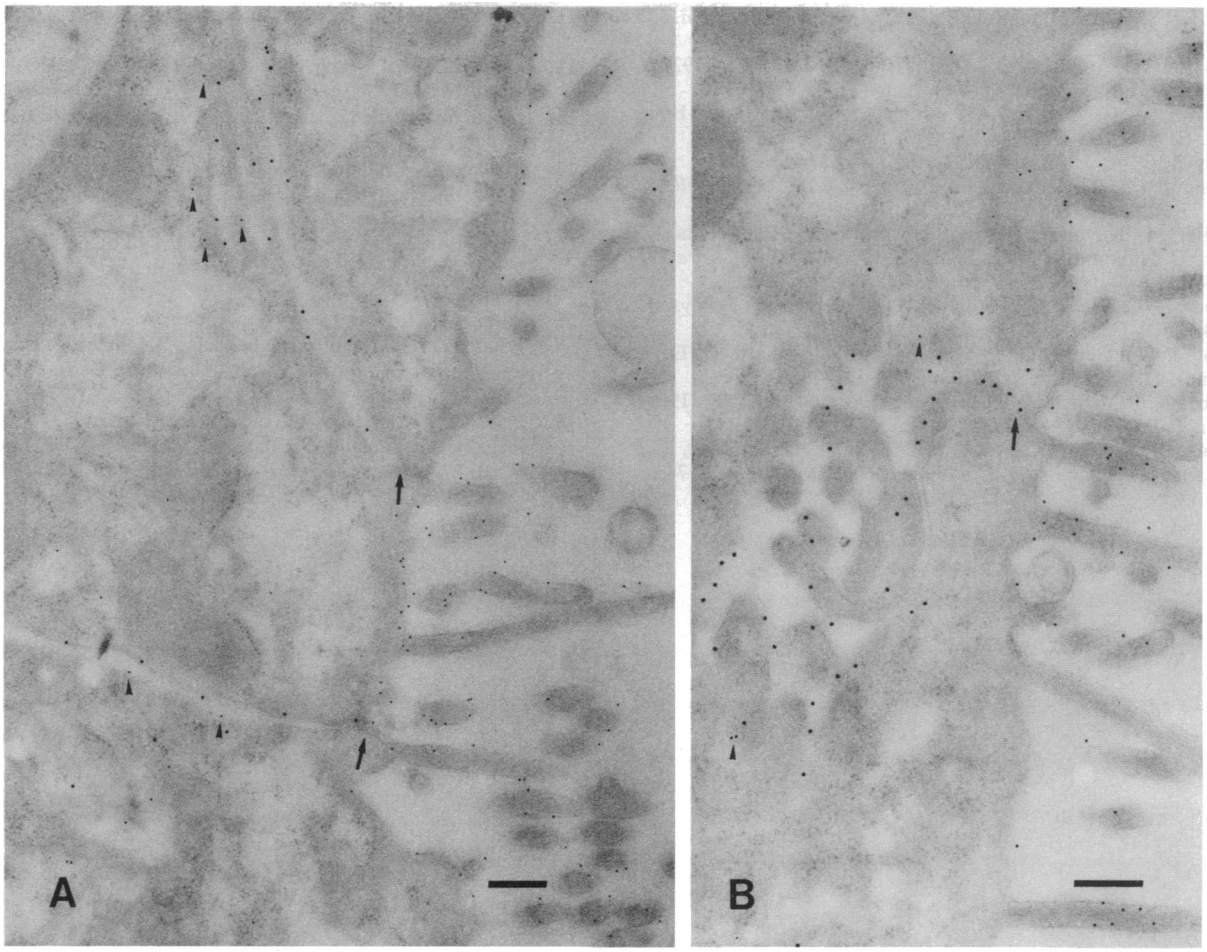

FIG. 5. Lowicryl section of Caco-2 cells labeled at one side for GLUT3 with 10-nm gold and for GLUT1 with 15-nm gold at the other side. (*A*) GLUT1 labeling is present at the lateral surface and in an endosome-like vacuole. The apical cell surface with microvilli is strongly labeled for GLUT3. (*B*) A striking example of polarized localization of GLUT1 (15 nm) laterally and GLUT3 (10 nm) apically. Arrowheads, a small amount of GLUT3 is present at the lateral membrane; arrows, junctional complexes. (Bar = 250 nm.)

The presence of GLUT3 in polarized epithelial cells was surprising in view of the limited tissue distribution of this transporter *in vivo* (15). GLUT3 is expressed at high levels in brain parenchymal cells (15). This is of interest from a targeting standpoint because it has been suggested that the mechanism of targeting may be conserved between neurons and epithelial cells. Membrane proteins that are sorted to the apical membrane in epithelial cells appear to be preferentially localized to the axon in neurons, whereas basolateral proteins distribute to the cell body and dendrites (16). Our studies provide further support for this hypothesis, because previous work has shown that GLUT3 labeling of human cerebellum sections colocalizes with neurofilament labeling (15).

Whereas the distribution of GLUT1 and GLUT3 in Caco-2 cells was distinctly polarized, this was not the case for GLUT5. Both at the light level (Figs. 3 and 4) and at the electron microscope level (data not shown) we observed significant GLUT5 immunoreactivity in the apical membrane, as well as in the basolateral membrane and within intracellular structures. A similar distribution has been observed for GLUT5 in human duodenum (J.W.S., unpublished data). Furthermore, Davidson *et al.* (16) have recently reported that GLUT5 is localized primarily to the brush border in human intestine. The temporal pattern of GLUT5 expression during differentiation of Caco-2 cells also differed significantly from that of GLUT1 and GLUT3 (Fig. 2). In contrast to GLUT1 and GLUT3, only a subset of differentiated Caco-2 cells exhibited positive GLUT5 labeling, suggesting that at least under our experimental conditions these cells are not uniformly differentiated. It is not known whether this reflects nonuniform differentiation or the presence of phenotypically distinct cell types. Other studies have confirmed that Caco-2 cells do not display the biochemical characteristics of any one type of intestinal tissue. Rather, they retain aspects of normal adult small intestine, fetal colon, and cancerous adult colon (17).

With respect to the overexpression of GLUT1 and GLUT3 in these cells, our studies have confirmed the previous assertion that the expression of certain glucose transporter isoforms is switched on in tumor cells of epithelial origin. Yamamoto *et al.* (18) have reported a marked increase in the levels of both GLUT1 and GLUT3 mRNA in human cancers of the digestive system. We have also observed expression of GLUT3 in BeWo and HepG2 cell lines, which originate from a human choriocarcinoma and a human hepatoma, respectively (J.W.S., unpublished data), and which also exhibit a polarized phenotype in culture. The overexpression of the GLUT1 isoform in many cancerous tissues was also reported (18). These data suggest a role for the glucose transporter in the transformed phenotype.

We thank Drs. Sherin Devaskar, Jeff Gordon, and David Hanpeter for providing human tissues. This work was supported by a Lucille P. Markey Foundation grant to D.E.J. and by the Diabetes Research Training Center at Washington University. D.E.J. is a recipient of a career development award from the Juvenile Diabetes Foundation International.

1. Pfeffer, S. R. & Rothman, J. E. (1987) *Annu. Rev. Biochem.* **56,** 829–852.
2. Mostov, K., Apodaca, G., Aroeti, B. & Okamoto, C. (1992) *J. Cell Biol.* **116,** 577–583.
3. Lisanti, M. P., LeBivic, A., Saltiel, A. R. & Rodriguez-Boulan, E. (1990) *J. Membr. Biol.* **113,** 155–167.
4. Brown, D. A. & Rose, J. K. (1992) *Cell* **68,** 533–544.
5. Casanova, J. E., Apodaca, G. & Mostov, K. E. (1991) *Cell* **66,** 65–75.
6. Bell, G. I., Kayano, T., Buse, J. B., Burant, C. F., Takeda, J., Lin, D., Fukumoto, H. & Seino, S. (1990) *Diabetes Care* **13,** 198–208.
7. Piper, R. C., Hess, L. J. & James, D. E. (1991) *Am. J. Physiol.* **260,** C570–C580.
8. Slot, J. W., Geuze, H. J., Gigengack, S., Lienhard, G. E. & James, D. E. (1991) *J. Cell Biol.* **113,** 123–135.
9. Thorens, B., Cheng, Z.-Q., Brown, D. & Lodish, H. F. (1990) *Am. J. Physiol.* **259,** C279–C285.
10. Piper, R. C., Tai, C., Slot, J. W., Hahn, C. S., Rice, C., Huang, H. & James, D. E. (1992) *J. Cell Biol.* **117,** 729–743.
11. Blais, A., Bissonnette, A. & Berteloot, A. (1987) *J. Membr. Biol.* **99,** 113–125.
12. James, D. E., Strube, M. & Mueckler, M. (1989) *Nature (London)* **338,** 83–87.
13. van Genderen, I. L., van Meer, G., Slot, J. W., Geuze, H. J. & Voorhout, W. F. (1991) *J. Cell Biol.* **115,** 1009–1019.
14. Bendayan, M. (1982) *J. Histochem. Cytochem.* **30,** 81–85.
15. Mantych, G. J., James, D. E., Chung, H. D. & Devaskar, S. U. (1992) *Endocrinology*, in press.
16. Davidson, N. O., Hausman, A. M. L., Ifkovits, C. A., Buse, J. B., Gould, G. W., Burant, C. F. & Bell, G. I. (1992) *Am. J. Physiol.* **262,** C795–C800.
17. Pinto, M., Robine-Leon, S., Appay, M.-D., Kedinger, M., Triadou, N., Dussaulx, E., Lacroix, B., Simon-Assmann, P., Haffen, K., Fogh, J. & Zweibaum, A. (1983) *Biol. Cell.* **47,** 323–330.
18. Yamamoto, T., Seino, Y., Fukumoto, H., Koh, G., Yano, H., Inagaki, N., Yamada, Y., Inoue, K., Manabe, T. & Imura, H. (1990) *Biochem. Biophys. Res. Commun.* **170,** 223–230.