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Regulation of Glucose Transporters in LLC-PK$_1$ Cells: Effects of D-Glucose and Monosaccharides

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Regulation of D-glucose transport in the porcine kidney epithelial cell line LLC-PK$_1$ was examined. To identify the sodium-coupled glucose transporter (SGLT), we cloned and sequenced several partial cDNAs homologous to SGLT1 from rabbit small intestine (M. A. Hediger, M. J. Coady, T. S. Ikeda, and E. M. Wright, Nature (London) 330:379–381, 1987). The extensive homology of the two sequences leads us to suggest that the high-affinity SGLT expressed by LLC-PK$_1$ cells is SGLT1. SGLT1 mRNA levels were highest when the D-glucose concentration in the culture medium was 5 to 10 mM. Addition of D-mannose or D-fructose, but not D-galactose, in the presence of 5 mM D-glucose suppressed SGLT1 mRNA levels. SGLT1 activity, measured by methyl α-D-glucopyranoside uptake, paralleled message levels except in cultures containing D-galactose. Therefore, SGLT1 gene expression may respond either to the cellular energy status or to the concentration of a hexose metabolite(s). By isolating several cDNAs homologous to rat GLUT-1, we identified the facilitated glucose transporter in LLC-PK$_1$ cells as the erythroid/brain type GLUT-1. High-stringency hybridization of a single mRNA transcript to the rat GLUT-1 cDNA probe and failure to observe additional transcripts hybridizing either to GLUT-1 or to GLUT-2 probes at low stringency provide evidence that GLUT-1 is the major facilitated glucose transporter in this cell line. LLC-PK$_1$ GLUT-1 mRNAs were highest at medium D-glucose concentrations of ≤2 mM. D-Fructose, D-mannose, and to a lesser extent D-galactose all suppressed GLUT-1 mRNA levels. Since the pattern of SGLT1 and GLUT-1 expression differed, particularly in low D-glucose or in the presence of D-galactose, we suggest that the two transporters are regulated independently.

Glucose transport by mammalian cells is accomplished by two classes of transporters: a sodium-coupled glucose transporter(s) (SGLT) and facilitated glucose transporters (GLUT). Since the plasma glucose level is tightly regulated, most cells need to express only facilitated glucose transport. This pattern is also observed for gluconeogenic cells such as hepatocytes. However, specialized epithelial cells of the brush border membranes of the small intestine and kidney proximal tubules express SGLT on their apical surface in addition to GLUT on the basolateral surface. The presence of both types of transporters allows these cells to perform concentrative uptake from the lumen as well as transepithelial transport of glucose. We have been interested in how cells expressing both types of transporter regulate their expression.

Several recent advances have begun to provide a molecular picture of glucose transport in various tissues. A cDNA encoding a high-affinity SGLT (SGLT1) was isolated from rabbit small intestine (18) and later from human small intestine (19). RNA blot analysis and polymerase chain reaction amplification have indicated the presence of the same or a similar transcript in kidney (M. Hediger, personal communication). Isolation of an SGLT1 partial cDNA from a rabbit renal cortex library confirms the presence of SGLT1 in kidney (7a). This transporter also transports galactose (21); inherited glucose-galactose malabsorption syndrome may be due to a defect in the SGLT1 gene. The SGLT1 gene maps to human chromosome 22 (17). Genetic evidence for the presence of a second SGLT in kidney, first suggested by Elsas et al. (9, 10), is provided by the occurrence of familial renal glycosuria, a syndrome in which D-glucose resorption is also defective. Inheritance of renal glycosuria is linked to HLA genes and therefore probably maps to chromosome 6 (8). Kinetic evidence for the additional SGLT in kidney differing in affinity and sodium:glucose stoichiometry from SGLT1 has been provided by the elegant studies of Barfuss and Schafer with perfused renal tubules (1) and of Turner and Moran with isolated brush border membranes (41, 42). Since most patients with glucose-galactose malabsorption improve with age (37) and renal glycosuria does not affect the intestine (39), a putative second intestinal SGLT may predominate after weaning, when galactose intake is generally reduced. Independent evidence for this second transporter in the gut has been obtained from studies on guinea pigs in which sodium-coupled glucose transport activities differing in affinity for D-glucose were separately regulated by nutritional status (6).

Glucose transport to and from plasma is carried out via a family of GLUT. GLUT-1 was first cloned from human hepatoblastoma HepG2 cells (30) and later from rat brain (5) and is widely distributed. Subsequently, four additional GLUT were cloned: GLUT-2 was cloned from rat liver (12) and is present also in intestine, kidney, and the islets of Langerhans; GLUT-3 was cloned from human fetal muscle (23) and is widely distributed; GLUT-4 was cloned from rat adipocytes (22) and skeletal muscle (4) and is the insulin-responsive transporter; and GLUT-5 was cloned from human small intestine and is also found in kidney (2). These transporters are homologous in both sequence and structure and have the characteristics of a gene family (2).

LLC-PK$_1$, a well-differentiated epithelial cell line derived from porcine proximal tubule cells (20), has been widely used as a model system for studying functions of polarized

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monolayer cells. When a confluent monolayer is formed, LLC-PK_1 cells form tight junctions and microvilli and express several proximal tubule marker enzymes (14). Characteristic of the distal portion of the proximal tubule, LLC-PK_1 cells also possess vasopressin responsiveness (13); unidirectional transepithelial transport of phosphate (3), amino acids (35, 36), and salt and water results in formation of characteristic domes (20). LLC-PK_1 cells also possess a sodium-coupled glucose transport activity (34) located at the apical surface (33). Kinetic studies (27) suggest that this latter activity is due to the high-affinity SGLT1, although this identification has not been established by cloning and sequence analysis. Moreover, LLC-PK_1 cells constitutively express facilitated glucose transport (40).

To understand how LLC-PK_1 cells regulate two glucose transporters, we have begun to characterize their identity and expression. Using rabbit SGLT1 cDNA as a probe, we have isolated partial cDNA clones from two LLC-PK_1 cDNA libraries and report the sequence here. Since the coding sequence of the porcine transcript is highly homologous to that of rabbit SGLT1 (84%), it is reasonable to suggest that the sodium-coupled glucose transport activity observed in LLC-PK_1 cells is SGLT1. We have also isolated cDNAs homologous to rat GLUT-1 and thus refer to the GLUT-1 hybridizing transcripts as GLUT-1. Using the porcine SGLT1 cDNA and the rat GLUT-1 cDNA as probes, we have studied changes of mRNA levels in glucose transporters in response to various concentrations of medium glucose and various other monosaccharides. We also report transport assays of SGLT1. High medium glucose (>15 mM) down-regulates expression of both transporters; optimum expression is observed between 5 and 10 mM for SGLT1 but ≤2 mM for GLUT-1. Our results with SGLT1 confirm previous observations of Moran et al. (29) and also demonstrate that its regulation occurs at the level of mRNA. Moreover, in the absence of glucose, SGLT1 expression is suppressed whereas GLUT-1 expression is enhanced.

MATERIALS AND METHODS

Cells and cell culture. LLC-PK_1 cells were provided by D. Ausiello (Renal Unit, Massachusetts General Hospital, Boston) and were grown in Dulbecco modified Eagle medium containing 25 mM glucose (GIBCO) supplemented with 10% fetal bovine serum. Cells were maintained in an atmosphere of 5% CO_2–95% air at 37°C. For experiments, LLC-PK_1 cells were used 4 to 6 days after confluence when domes had fully developed. For experiments with various concentrations of glucose or other monosaccharides, glucose-free medium (32) containing dialyzed fetal bovine serum was prepared.

Nucleic acid probes. From the sequence of the rabbit SGLT1 cDNA reported by Hediger et al. (18), the following complementary oligonucleotides were synthesized as probes: TCGGCCGCATTGCGGATGCGCTCATAGGACT CAAG (nucleotides [nt] 72 to 106), CCAATCTTACTGGCA AACAGAGAGGCTCCGATCG (nt 229 to 262), and AGAG CAACAGGGACAGAATGGAAAGGTAGATCTGG (nt 446 to 480). For GLUT-1 expression analyses, an *Eco*RI-*Sac*I fragment of the rat GLUT-1 cDNA (5), containing most of coding region, was used as a probe. Oligonucleotides were end labeled with ^{32}P (38), and cDNA probes were labeled by the random hexamer method (Pharmacia).

Isolation of glucose transporter cDNAs and sequencing. A rabbit small intestine cDNA library was screened with synthetic oligonucleotide probes complementary to rabbit SGLT1 (see above). The longest cDNA clone had the same nucleotide sequence as that reported (18), with the addition of TT at the 5′ terminus. Using rabbit SGLT1 as a probe, 10^6 phages from two independent LLC-PK_1 cDNA libraries (one each using cDNA synthesis kits obtained from Pharmacia and Invitrogen) were screened with low-stringency hybridization (42°C for 16 h; 30% formamide, 5× SSC [1× SSC is 0.15 M NaCl plus 15 mM sodium citrate, pH 7.0], 5× Denhardt solution [34], 20 mM sodium phosphate [pH 7.0], 0.1% sodium dodecyl sulfate, 1 mM EDTA, 10% dextran sulfate, 100 μg of sheared salmon testis DNA per ml) and washing (42°C for 1 h with 0.1× SSC–0.1% sodium dodecyl sulfate). After subcloning into pBSK (Stratagene), isolated cDNAs were sequenced with Sequenase (U.S. Biochemical) according to the supplier's instructions. Oligonucleotides primers were synthesized (DuPont Coder 300) to extend sequencing over the cDNA inserts.

In a similar fashion, using a cDNA library prepared from an Invitrogen kit and probed with the rat GLUT-1 cDNA, several homologous partial cDNAs were isolated and sequenced.

RNA blot analysis. Cells were lysed with 5 M guanidine thiocyanate–10 mM Tris chloride (pH 7.5)–10 mM EDTA. Total RNA was extracted (7), separated in 1% formaldehyde-agarose gels, and transferred to nitrocellulose filters. Equal amounts of RNA (30 μg) were loaded and probed with a rat α-tubulin probe as confirmation. Filters were hybridized with cDNA probes as described above except with 50% formamide. Blots were washed as described above except at 55°C and were exposed on Kodak XAR-5 film at −80°C with intensifying screens. In some cases, the hybridization signals on the autoradiograms were quantitated by densitometry.

For the porcine kidney sample, kidneys from a 21-day-old pig were removed and frozen immediately in a methanol-dry ice bath. Regions of the tissue were roughly separated by chipping while still frozen and extracted as noted above.

AMG uptake. LLC-PK_1 grown in 35-mm-diameter Falcon culture dishes were washed three times with prewarmed Earle balanced salt solution, pH 7.4 (EBSS), and then incubated for 60 min at 37°C in 0.5 ml of EBSS containing 0.1 mM methyl α-D-glucopyranoside (AMG) and 0.2 μCi of methyl α-D-[U-^{14}C]glucopyranoside per ml with or without 0.1 mM phlorizin, under an atmosphere of 5% CO_2–95% air. At the end of the incubation, cells were washed three times with ice-cold EBSS containing 0.1 mM phlorizin and solubilized with 0.3 N NaOH, and the cell-associated radioactivity was measured by liquid scintillation counting. Each point represents the average of duplicate determinations from which the phlorizin-insensitive uptake was subtracted. In all cases, phlorizin-insensitive uptake was <5% of the peak uptake observed. Protein content was determined as described by Lowry et al. (26).

Materials. Methyl α-D-[U-^{14}C]glucopyranoside and ^{32}P-labeled nucleotides were obtained from New England Nuclear Corp. Phlorizin and monosaccharides were obtained from Sigma Chemical Co. Other chemicals were of the highest purity available from commercial sources.

Nucleotide sequence accession number. The DNA sequence reported is in the GenBank data base under accession number M34044.

RESULTS

Characterization of LLC-PK_1 SGLT1 cDNAs. In preliminary experiments probing with the rabbit SGLT1 cDNA, we consistently detected two transcripts of 2.2 and 3.9 kb in LLC-PK_1 cells (Fig. 1A). Both transcripts were also de-

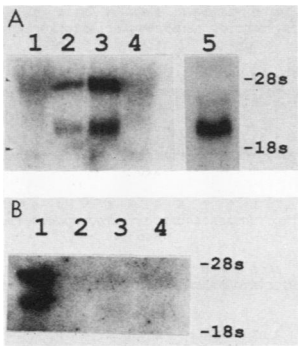


FIG. 1. RNA blot analysis of LLC-PK$_1$ and porcine kidney tissue, using a rabbit intestinal SGLT1 cDNA probe. (A) RNA from LLC-PK$_1$ cells, confluent for 5 days, was fractionated by chromatography through oligo(dT)-cellulose. For the final 2 days, the culture medium glucose was varied. Lanes: 1 and 2, 25 μg of unbound RNA (lane 1) and 5 μg of poly(A)$^+$ RNA (lane 2) from a culture containing 25 mM glucose; 3 and 4, 25 μg of poly(A)$^+$ RNA (lane 3) and 25 μg of unbound RNA (lane 4) from a culture containing 5 mM glucose; 5, 5 μg of poly(A)$^+$ RNA from rabbit small intestine (control). Lanes 1 to 4 were exposed for 18 h, and lane 5 was exposed for 2 h. (B) Total RNA (30 μg) from the kidney of a 21-day-old pig. Lanes: 1, LLC-PK$_1$ cells; 2, cortex; 3, outer medulla; 4, renal papillae.

tected in porcine kidney tissue (Fig. 1B), localized mainly in the outer medulla and papillae. Since the kidney sample used was frozen and no attempt was made to separate individual papillae, the signal in lane 4 may also be derived from outer medulla. To identify these two transcripts, we characterized several cDNA clones isolated from two independent LLC-PK$_1$ cDNA libraries. Although we did not obtain any full-length clones, the cDNAs fell into two major classes (Fig. 2), differing in the length of the 3′ untranslated region. The nucleotide sequence assembled from the two classes of

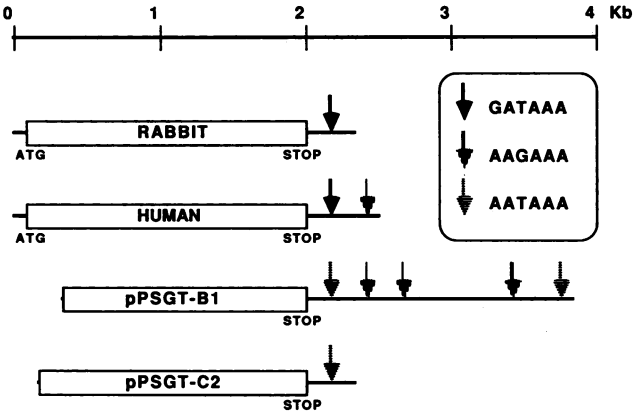


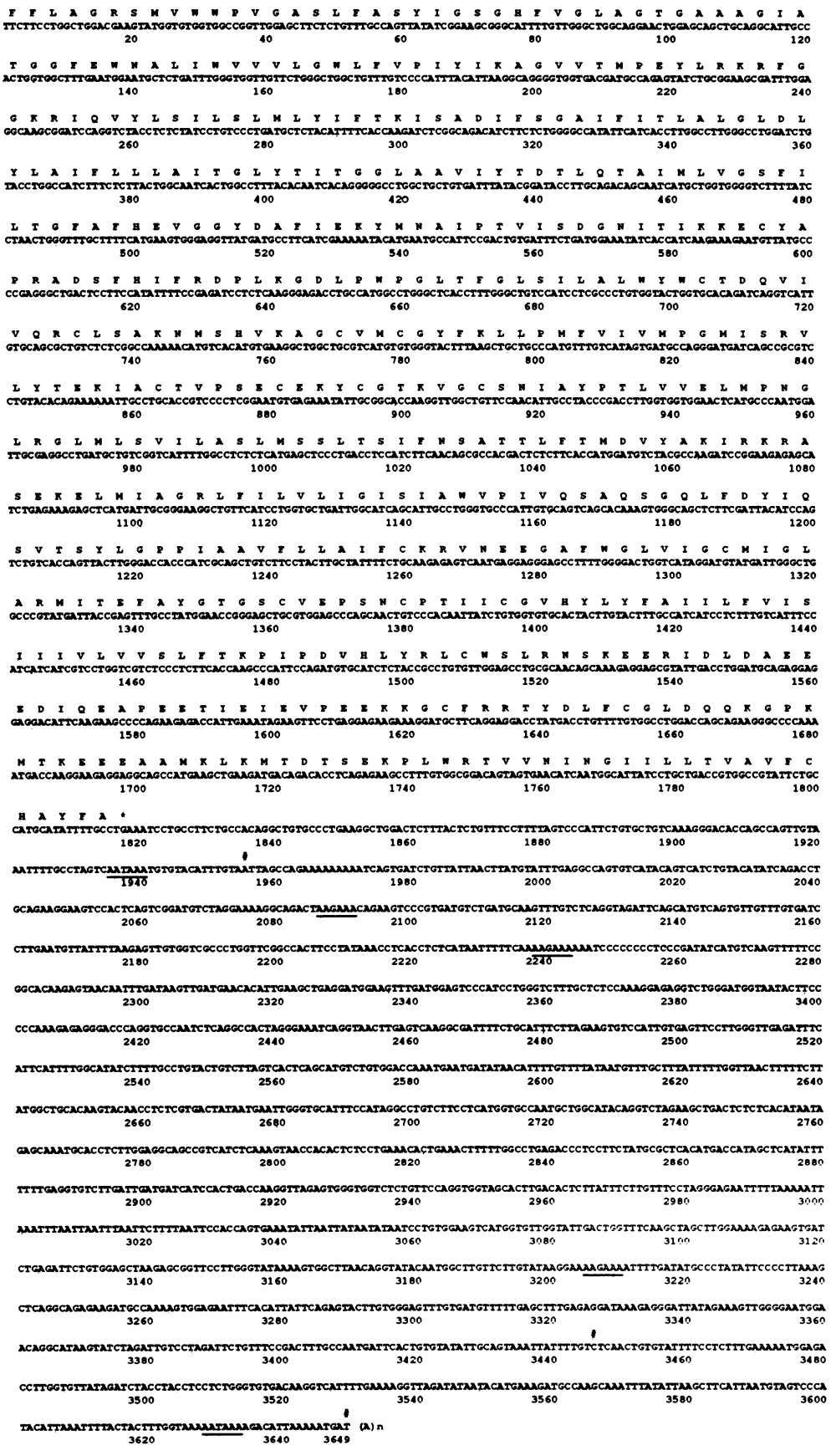
FIG. 2. Structures of the SGLT1 mRNAs from human small intestine, rabbit small intestine, and LLC-PK$_1$ (porcine kidney) cells. Schematics of the human and rabbit mRNAs were drawn from sequences reported by Hediger et al. (18, 19). The porcine clones, pPSGT-B1 and pPSGT-C2, were the longest obtained from the two sizes of transcripts detected in LLC-PK$_1$. Open boxes represent the single open reading frame in each sequence. Only cDNAs from LLC-PK$_1$ transcripts polyadenylated following an AATAAA signal were obtained in our studies, with the single exception of clone pPSGT-F1 (see Fig. 3). The human transcript is polyadenylated at both sites indicated as well as at a third site corresponding to the second AATAAA in LLC-PK$_1$ cells (19).

clones, 3,649 nt, and its deduced amino acid sequence are shown in Fig. 3. Clone pPSGT-C2 was polyadenylated at nt 1958. The sequence contains an open reading frame over the first 1,818 nt homologous to the rabbit and human SGLT1 cDNAs (84 and 87%, respectively) beginning at amino acid residue 58. Primer extension indicated that the longest clone, pPSGT-B1, lacked approximately 300 nt from the 5′ terminus (data not shown), consistent with the structures of the 5′ regions of the rabbit and human SGLT1 cDNAs. Therefore, full-length cDNAs should be 2.2 and 3.9 kb, consistent with the transcript sizes observed on RNA blots. The two cDNA classes contained identical coding regions and differed only in the length of the 3′ untranslated region. Clone pPSGT-B1 was polyadenylated after nt 3649 (Fig. 3). Some of the longer clones (5 of 12) were lacking T at nt 3649.

Although the pPSGT-B1 clone has five possible polyadenylation signals (two AATAAA signals and three AAGAAA signals) in its 3′ untranslated region (Fig. 2 and 3), we detected usage of only the two AATAAA signals in LLC-PK$_1$. The downstream signal appeared to be the preferential polyadenylation site. The relative abundance of the two transcripts was approximately 1.8:1, as determined by densitometry of the signals obtained from RNA blots, and did not vary over the course of our experiments (see Fig. 1A and 5A). Unlike the porcine SGLT1 mRNA, both human and rabbit transcripts contained the sequence GATAAA as the upstream polyadenylation site (Fig. 2). We obtained one clone, pPSGT-F1, polyadenylated at a site not following any of the noted polyadenylation signals (Fig. 3). Since we did not detect an obvious transcript on RNA blots corresponding to the size of pPSGT-F1, it is possible that this clone represents an aberrantly polyadenylated transcript.

The deduced amino acid sequences are well conserved among the porcine SGLT1 cDNA sequence and those of rabbit and human small intestine (Fig. 4 and Table 1). Hydropathy profiles are nearly identical (data not shown). Furthermore, two potential N-linked glycosylation sites (Asn-X-Ser/Thr) are conserved (amino acids 248 and 306). The human sequence contains two more amino acids than either the rabbit or pig sequence. In Fig. 4, we have aligned the sequences to indicate that the amino acids absent in the porcine and rabbit sequences correspond to human amino acids 592 and 593. This assignment differs from that made by Hediger et al. (19) for the rabbit sequence (human amino acids 590 and 591) but retains the homology as well as aligns the gap with that most likely in the porcine sequence. These data are consistent with the similarities previously described among the three SGLT1 proteins: affinity for glucose and substrate specificity, 2:1 Na:glucose stoichiometry, and inhibition by phlorizin (21, 27, 34). Significant amino acid sequence divergence of the three transporters occurs in the sixth cytoplasmic domain (amino acids 585 to the 11th membrane-spanning domain). A less significant divergence is seen in the third extracellular domain (amino acids 233 to 271). The sequences in these domains are therefore less likely to be essential for function and represent species-specific regions.

Isolation of GLUT-1 cDNAs. Several partial cDNAs homologous to a rat GLUT-1 cDNA probe were isolated. The longest clone, pPGT-B1, contained a 1.7-kb insert that had 89% homology to the corresponding region of the rat cDNA and was identical to the 3′ 1,715 nt of a porcine glucose transporter cDNA clone isolated from brain microvessels (44), an abundant source of GLUT-1 in rat (11). GLUT-1 appears to be the only, or at least the major, GLUT in this cell line, since we were unable to detect any additional



transcripts by low-stringency hybridization with GLUT-1 or GLUT-2 probes (data not shown).

Effect of medium glucose concentration on expression of glucose transporters. Medium D-glucose levels affect the activities of both SGLT1 (29) and GLUT-1 (31, 40) in LLC-PK$_1$ cells. We have previously shown that GLUT-1 in this cell line during D-glucose limitation is regulated at the level of mRNA (40), but it has not been established at which step SGLT1 is regulated. Furthermore, there is no information available on the relationship between SGLT1 and GLUT-1 expression.

The effect of various concentrations of D-glucose on phlorizin-sensitive AMG uptake activity of LLC-PK$_1$ cells is shown in Fig. 5. AMG uptake was linear and sodium dependent under the assay conditions used. In the experiments shown, the highest activities were obtained at 5 mM D-glucose. Both higher and lower D-glucose concentrations, especially the former, decreased AMG uptake activity. Figures 6A and B show the effect of medium D-glucose on SGLT1 mRNA, using the shorter cDNA clone pPSGT-C2 (1.9 kb) as a probe. In the RNA blot shown in Fig. 6A, highest SGLT1 RNA levels were observed at 5 and 10 mM D-glucose. Figure 6B is a densitometric representation of the film shown in Fig. 6A as well an independent RNA blot analysis. The optimum D-glucose concentration for SGLT1 expression was narrow, with repression observed even at 2 or 15 mM D-glucose. The parallel response of mRNA levels and AMG uptake activity provides strong evidence that SGLT1 activity is regulated at the level of message. The responses of GLUT-1 and SGLT1 mRNA levels differed in that the lower the D-glucose concentration in which cells were cultured, the higher the expression of GLUT-1 mRNA observed (Fig. 6C). The maximum level of expression was observed even in the absence of glucose. Although expression of both SGLT1 and GLUT-1 was affected by the D-glucose concentration in the culture medium, the distinctly different patterns suggest that the two genes are independently regulated.

Effect of various monosaccharides on SGLT1 and GLUT-1 expression. Monosaccharides other than D-glucose affect glucose transport activity expressed by LLC-PK$_1$ cells during growth (29). To determine whether this regulation occurs at the level of mRNA, we studied the effects of several monosaccharides on AMG uptake activity and expression of SGLT1 and GLUT-1.

AMG uptake activity in cells cultured in 20 mM monosaccharide in addition to 5 mM D-glucose, the concentration at which we observed the highest level of SGLT1 activity, is shown in Table 2. As noted above, total 25 mM D-glucose (addition of 20 mM D-glucose) strongly suppressed AMG uptake activity compared with that observed in 5 mM D-glucose. L-Glucose, as expected, had no effect on AMG uptake. D-Galactose, a substrate for SGLT1, partially reversed AMG uptake. D-Fructose and D-mannose, neither of which are transported by SGLT1, each suppressed AMG uptake activity more strongly than that did D-galactose. AMG uptake was only slightly reduced when uridine was included in the culture medium.

We also determined the effects of these monosaccharides on SGLT1 and GLUT-1 mRNA levels of LLC-PK$_1$ cells. D-Glucose markedly suppressed both SGLT1 and GLUT-1 expression (Table 2). D-Fructose suppressed expression of both transporters to the same extent as did D-glucose. As mentioned above, although D-galactose partially suppressed AMG uptake activity of the cells, it slightly enhanced the SGLT1 mRNA level while partially reducing the GLUT-1 mRNA level. D-Mannose also suppressed the SGLT1 mRNA level, but it had even stronger suppressive effect on GLUT-1 mRNA level than did D-glucose. Uridine had little effect on the SGLT1 mRNA level; however, it suppressed the GLUT-1 mRNA level as well as D-glucose did. Since the patterns of mRNA levels observed for the two transporters differed in the various conditions tested, SGLT1 and GLUT-1 appear to be regulated by independent mechanisms.

DISCUSSION

The LLC-PK$_1$ cell line provides a useful model for studying regulation of glucose transport in epithelial cells of the kidney proximal tubule. Moran et al. (29) originally observed that sodium-coupled glucose transport activity in this cell line is enhanced by limiting D-glucose in the culture medium to 5 mM. We have extended these studies by demonstrating that (i) the sodium-coupled glucose transporter is SGLT1 by sequencing several partial cDNA clones and (ii) SGLT1 activity is regulated at least in part at the level of mRNA and is actually diminished at D-glucose concentrations below 5 mM. While we cannot exclude the possibility that an additional SGLT exists in LLC-PK$_1$ cells, studies on the stoichiometry of phlorizin binding in this cell line indicate that only a single component is present (28). Moreover, by cloning and hybridization studies, we have demonstrated the presence of GLUT-1 in this cell line. Expression of GLUT-1 is also sensitive to D-glucose concentration, exhibiting an optimum expression at $\leq$2 mM D-glucose. Moreover, the metabolism of other monosaccharides in the presence of 5 mM D-glucose, optimal for SGLT1 expression, led to altered mRNA levels for both transporters, although expression patterns differed somewhat between the two.

The several SGLT1 cDNAs cloned fell into two classes differing in the length of the 3′ untranslated regions. Lengths of the complete cDNAs were estimated to be 2.2 and 3.9 kb by primer extension analysis. Comparison of the deduced amino acid sequences of SGLT1 from rabbit (18), human (19), and LLC-PK$_1$ (porcine) cells showed that the SGLT1 proteins, including the locations of the predicted membrane-spanning domains, were well conserved among these species. Significant variations were observed only in the third extracellular loop and the sixth cytoplasmic loop. Therefore, other domains might be more essential to SGLT1 function. Porcine SGLT1 would appear to have a 5′ structure similar to that of human and rabbit SGLT1 based on transcript size and primer extension studies, but confirmation of this conclusion must await isolation of full-length porcine SGLT1 clones.

Positions of the polyadenylation signals were also conserved among human, rabbit, and pig sequences. The longest

FIG. 3. Partial composite sequence of LLC-PK$_1$ SGLT1. The deduced amino acid sequence appears above the nucleotide sequence determined for the strands of clones pPSGT-B1 and pPSGT-C2. Putative polyadenylation signals are underlined, and polyadenylation sites are marked with #. Two clones were polyadenylated at nt 1958, and 12 clones were polyadenylated at about nt at 3649. A single clone, pPSGT-F1, was polyadenylated at nt 3449. The apparent polyadenylation signal at nt 3647 is due to addition of A residues at nt 3650 to 3652, since the genomic sequence in this region is GATAAC (unpublished data).

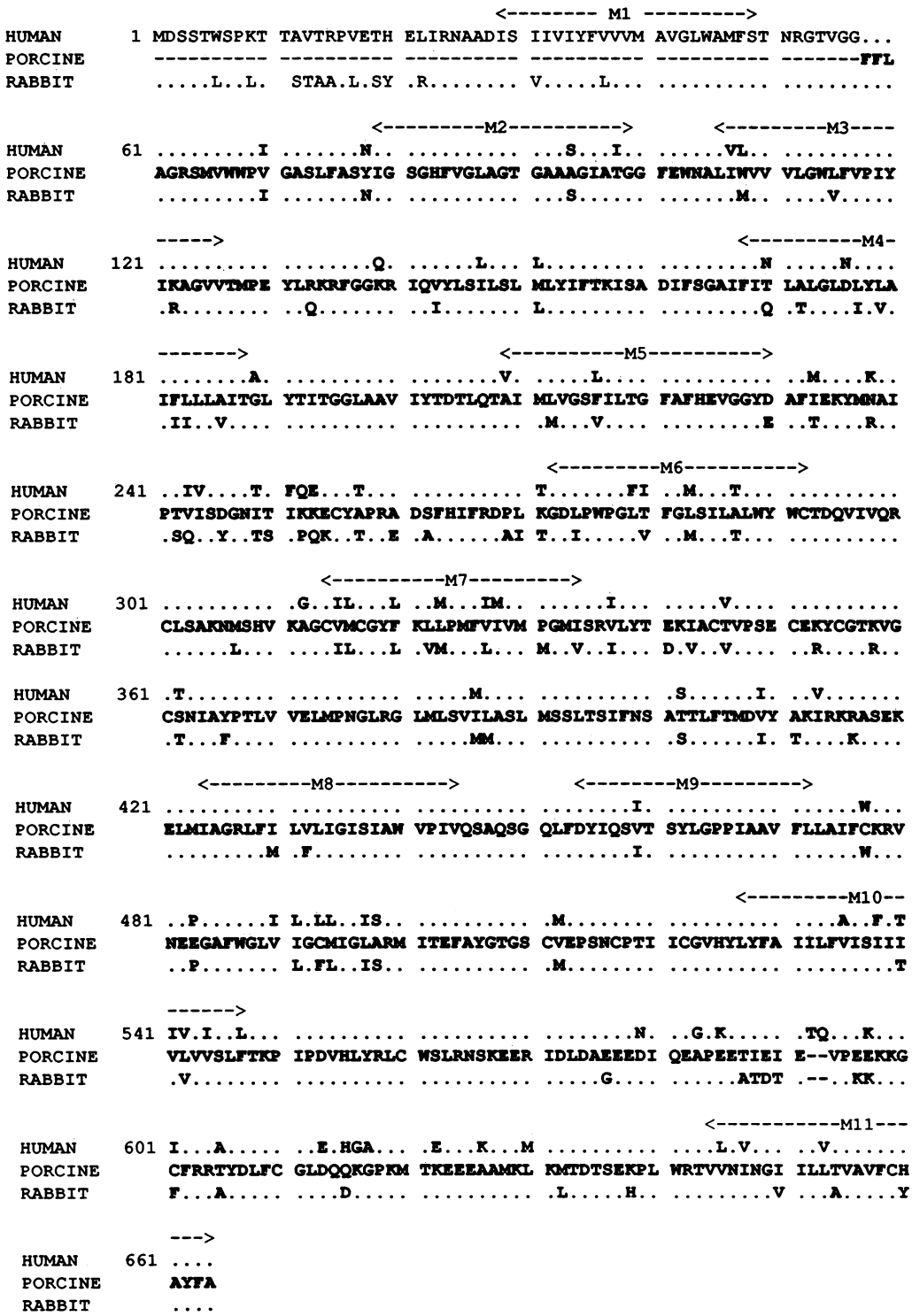


FIG. 4. Alignment of the deduced amino acid sequence for LLC-PK$_1$ SGLT1 with those for human and rabbit proteins. Only differences from the porcine sequence are noted except for the first 57 amino acids, which are shown from the human sequence since our longest clone did not contain nucleotides corresponding to the 5′ region of the porcine transcript. Human and rabbit sequences and putative membrane-spanning domains (M1 to M11) are taken from Hediger et al. (19). Gaps are indicated by dashes. Assignment of the gap in the rabbit sequence to the two amino acids following amino acid 591 is discussed in the text.

porcine cDNA clone, pPSGT-B1, had five possible polyadenylation signals, although we detected utilization of only the first and last. In rabbit small intestine, a single transcript of 2.3 kb was reported (18). On the other hand, three transcripts, of 2.2, 2.6, and 4.8 kb, have been reported in the human small intestine (19). It is likely that rabbit small intestine utilizes the polyadenylation signal corresponding to the first one of LLC-PK$_1$, whereas the human small intestine

TABLE 1. Comparison of SGLT1 amino acid sequences

		% Similarity		
		Porcine	Rabbit	Human
% Identity	Porcine		92	95
	Rabbit	84		94
	Human	87	84	

utilizes three polyadenylation signals (19). Although the complete sequence of the 3′ untranslated region of human SGLT1 is not available, the first and second signals correspond to the first two of the LLC-PK$_1$ SGLT1; the longest human transcript is probably polyadenylated at a site homologous to the last one in LLC-PK$_1$. The mechanism and physiological significance of the transcript number and polyadenylation site(s) in the different species are unknown. It is unlikely that the position of polyadenylation is related to the stability of mRNA, since the ratio of the two transcripts did not vary significantly even though total transcript levels varied over a 10-fold range. The position of polyadenylation might regulate its translatability.

SGLT1 in LLC-PK$_1$ is regulated by glucose at the level of mRNA. We are now determining the relative contribution of transcription rate and message stability to the alteration in mRNA levels. However, it is interesting that the D-glucose concentration yielding both the highest SGLT1 activity and the highest mRNA level is within the physiological range. Suppression of SGLT1 at least at higher D-glucose concentrations may have relevance to modulations of sodium-coupled glucose transport activity previously observed by others: (i) intestinal mucosa in response to hyperglycemia (for a review, see reference 24) and (ii) hyperglycemic suppression of sodium-coupled glucose transport activity in the kidneys of streptozocin-treated rats (15, 45).

Regulation of GLUT-1 in LLC-PK$_1$ cells is consistent with the behavior of this gene observed in other cell lines, e.g., D-glucose limitation enhances glucose transport activity (16, 25) as well as message levels (43). Kidney cells express at least GLUT-1, GLUT-2, and GLUT-3 (2), and proximal tubule cells have been shown to possess either GLUT-1 or GLUT-2 (40a). In the case of LLC-PK$_1$ cells, we have cloned several partial cDNAs showing distinct homology to rat GLUT-1 and identity to porcine brain GLUT-1 (44). These results, along with our inability to detect additional GLUT-1 (or GLUT-2) hybridizing transcripts in these cells, lead us to conclude that LLC-PK$_1$ cells express GLUT-1 as their facilitated glucose transporter. Consistent with their derivation from the late proximal tubule, LLC-PK$_1$ cells express the high-affinity isoform of GLUT as well as SGLT (40a, 41). Interestingly, at limiting glucose concentrations, LLC-PK$_1$ down-regulates SGLT1 while expressing the highest observed levels of GLUT-1. Mullin et al. (31) also observed enhanced sodium-independent glucose transport after 16 h of D-glucose deprivation, although to a lesser extent than the increase in mRNA level that we observed after 48 h. It is tempting to speculate that this behavior represents an adaptive mechanism whereby cells reduce energy expenditures, synthesizing SGLT1 in the presence of

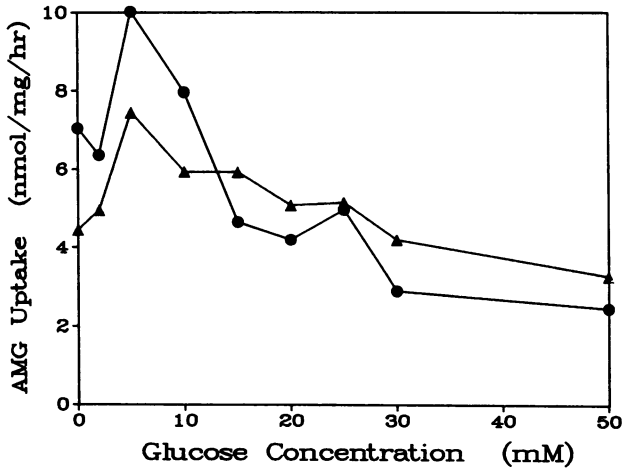


FIG. 5. Effect of glucose concentration in LLC-PK$_1$ culture medium on sodium-coupled AMG uptake. Duplicate confluent monolayers of LLC-PK$_1$ cells, cultured for 48 h in media containing the indicated D-glucose concentrations, were assayed for phlorizin-sensitive AMG uptake. Values obtained were averaged for each of two independent experiments, shown as separate curves. Phlorizin-insensitive AMG uptake represented <5% of the maximum uptake observed.

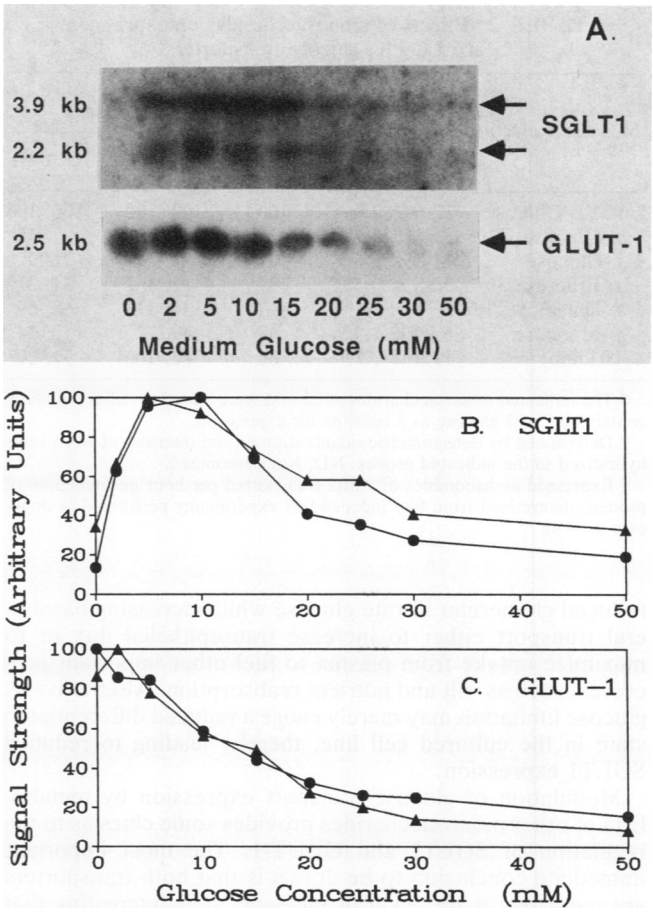


FIG. 6. Effect of glucose concentration in LLC-PK$_1$ culture medium on mRNA levels of SGLT1 and GLUT-1. (A) RNA blot analysis of LLC-PK$_1$ cells, cultured for 48 h in media containing the indicated D-glucose concentrations, before RNA was extracted. The blot was probed sequentially for SGLT1 with a pPSGT-C2 cDNA probe and for GLUT-1 with a rat GLUT-1 cDNA probe. D-Glucose concentrations are indicated below the lanes. The film shown as well as the film from an independent RNA blot analysis were quantitated densitometrically. Relative signal intensities are shown graphically for SGLT1 (B) and GLUT-1 (C). Symbols: ●, experiment 1 (same as in panel A); ▲, experiment 2.

TABLE 2. Effects of monosaccharides on expression of LLC-PK$_1$ glucose transporters

Monosaccharide[a]	SGLT1 activity		mRNA relative intensity[b]	
	Rate[c]	% of control	SGLT1	GLUT-1
5 mM D-Glucose	12.6 ± 2.0	100	100, 100	100, 100
+ D-Glucose	3.83 ± 0.72	30	34, 42	37, 35
+ L-Glucose	11.8 ± 1.6	94	ND	ND
+ D-Fructose	4.87 ± 0.58	39	31, 46	31, 27
+ D-Galactose	7.14 ± 1.1	57	134, 133	68, 69
+ D-Mannose	5.99 ± 1.5	48	49, 72	17, 14
+ Uridine	10.9 ± 1.7	86	82, 100	31, 29

[a] The indicated monosaccharides at 20 mM were added to culture medium containing 5 mM glucose as a baseline for expression.

[b] Determined by densitometric quantitation of film intensity of RNA blots hybridized to the indicated probes. ND, Not determined.

[c] Expressed as nanomoles of AMG transported per hour per milligram of protein, determined from two independent experiments performed in duplicate (n = 4).

reduced glomerular filtrate glucose while increasing basolateral transport either to increase transepithelial flux or to maximize uptake from plasma to fuel other important processes, such as salt and nutrient reabsorption. Alternatively, glucose limitation may merely cause a reduced differentiated state in the cultured cell line, thereby leading to reduced SGLT1 expression.

Modulation of glucose transport expression by metabolism of other monosaccharides provides some clues as to the regulation of SGLT1 and GLUT-1. The most important immediate conclusion to be drawn is that both transporters are regulated at the level of message. It is interesting that cultivation of LLC-PK$_1$ cells in galactose partially suppressed SGLT1 activity while leading to a slight increase in mRNA. While there could be other explanations, it is possible that the excess galactose caused altered glycosylation of the SGLT1 protein to a form that was either less active or less able to be transported to the plasma membrane. Further studies are required to decide among the possibilities.

Our demonstration of the presence of transcripts in the porcine kidney cell line LLC-PK$_1$ similar in structure (length and polyadenylation sites) and nearly identical in coding sequence to human small intestine SGLT1 provides strong evidence that kidney also expresses SGLT1. Isolation of an SGLT1 cDNA from rabbit kidney (7a) confirms this conclusion. While the presence of additional SGLT(s) in kidney and intestine appears to be well established, their identity and characterization by cloning remain to be determined. Identification of SGLT1 in LLC-PK$_1$ cells enables numerous studies related to the regulation of this important transporter. The experiments presented in this report are consistent with regulation of both SGLT1 and GLUT-1 at the level of transcription. Further studies will be required to determine whether this hypothesis is correct. It will also be of interest to determine what monosaccharide metabolites are responsible for modulation of glucose transport in LLC-PK$_1$ cells, particularly the modulation of SGLT1.

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