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Expression of GLUT8 in Mouse Intestine: Identification of Alternative Spliced Variants

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ABSTRACT

GLUT8 is a facilitative glucose transporter composed of 10 exons coding for a 477 amino acids protein. It is mainly expressed in the testis, but it has also been studied in a number of tissues such as brain, adipose tissue, and liver. In this work, we have characterized the expression of GLUT8 in the small and large intestine under normal physiological conditions. Protein assay revealed low GLUT8 protein levels in the intestine compared to the testis, with higher levels in the colon than in the small intestine. Immunohistochemistry studies showed an intracellular localization of GLUT8 in enterocytes and colonocytes with a supranuclear distribution next to the apical membrane. GLUT8 immunoreactivity was also detected in the crypt cells. Interestingly, we have identified three additional transcriptional variants in mouse intestine (mGLUT-SP1, mGLUT8-SP2, and mGLUT8-SP3) produced by the deletion of one, two, and four exons, respectively, whereas only the entire mRNA was detected in the testis. Expression of these alternative variants did not have an effect on glucose consumption in 3T3-L1 cells. Although the specific function of GLUT8 in intestine remains unclear, the alternative splicing of GLUT8 could reflect a mechanism for the regulation of the gene expression in a tissue-specific manner by targeting GLUT8 mRNA for nonsense-mediated decay. J. Cell. Biochem. 106: 1068–1078, 2009. © 2009 Wiley-Liss, Inc.

KEY WORDS: GLUT8; GUT; EXPRESSION; ALTERNATIVE SPLICING; GLUCOSE

In mammals, sugar transport across the cell membrane is mediated by sodium-dependent glucose co-transporters (SGLT) and facilitative glucose carriers (GLUT) proteins [Uldry and Thorens, 2004; Wright and Turk, 2004]. The GLUT family currently comprises 14 members that differ in their tissue distribution, kinetic properties, and substrate specificity. However, all GLUT transporter proteins have common structural features: the presence of 12 transmembrane helices with NH_2 and COOH termini facing the cytoplasm; a N-linked glycosylation site, and a number of conserved motifs for sugar transport such as PESPR/PETK located after helices 6 and 12, GRR/GRK in loops 2 and 8, and QQLSG in helix 7 [Joost and Thorens, 2001]. Based on their sequence alignment, structural and functional characteristics, GLUT family members have been classified into three subclasses [Joost and Thorens, 2001; Wood and Trayhurn, 2003]. GLUT1-5 was cloned between 1985 and 1990 and, in the last 8 years nine novel GLUT members (GLUT6–14) have been identified and cloned from different tissues [Manolescu et al., 2007].

Beyond the classic accepted model where SGLT1, GLUT2, and GLUT5 play a key role in intestinal sugar absorption [Wright, 1993; Wright et al., 2003], additional mechanisms for glucose transport have been described [Kellett, 2001; Kellett and Brot-Laroche, 2005]. However, the existence of novel GLUT proteins in intestinal tissue and their possible contribution within the absorption process is an unexplored issue. GLUT8 has been extensively studied since it was cloned in 2000 by three different laboratories [Carayannopoulos et al., 2000; Doege et al., 2000; Ibberson et al., 2000]. Its properties as a high glucose affinity transporter (K_m 2.4 mM), the ability to transport glucose and fructose, plus the fact that it is expressed in glucose and/or insulin-sensitive tissues, make it a good candidate to be involved in sugar uptake. Nevertheless, the functional role of GLUT8 remains "undefined" to date.

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In this work, we questioned the expression of GLUT8 in enterocytic and colonocytic cell types. Therefore, we studied the GLUT8 protein and mRNA expression along the intestinal tract. We found that the GLUT8 protein is expressed at low levels in the large and small intestine compared to the testis, and is intracellularly located in the apical part of both enterocytes and colonocytes. Interestingly, and in contrast with the testis, we found that GLUT8 mRNA is alternatively spliced specifically in the intestine, generating three additional transcriptional variants being a possible cellular strategy to regulate intestinal GLUT8 gene expression under normal physiological conditions.

MATERIALS AND METHODS

ANIMAL PROTOCOLS

Adult male Balb/cAnNHsd mice were obtained from Harlan Iberica S.L. (Barcelona, Spain). All animals were maintained on a 12 h day–night cycle at constant room temperature (22°C), and with free access to water and standard mouse fodder. Handling procedures, the equipment used, and the sacrifice of animals were all in accordance with the European Council Legislation 86/609/EEC concerning experimental animal protection. All experimental protocols were approved by the Ethical Committee of the University CEU – Cardenal Herrera. Since the expression of nutrient transporters could be regulated by nutrients presented in intestinal lumen [Ferraris and Carey, 2000], all experiments done for studying the expression of GLUT8 under normal conditions were performed with nonfasted animals.

MATERIALS

All generic reagents were obtained from either Sigma (Madrid, Spain), Roche Diagnostics (Barcelona, Spain), or Invitrogen (Carlsbad, CA).

Rabbit polyclonal antibody against a synthetic peptide corresponding to the 11 C-terminal residues (466–477) of mouse GLUT8 was prepared by Q-Biogene (Illkrich, France) as previously described [Gomez et al., 2006]. Rabbit polyclonal antibody against actin was obtained from Sigma (A5060). Hybond membranes, enhanced chemiluminescence (ECL) detection system, and anti-rabbit IgG secondary antibody were obtained from GE Healthcare (Chalfont St. Giles, UK). Bradford and Western blotting reagents were obtained from Bio-Rad (Hercules, CA).

TISSUE PREPARATION

Mice of 8–10 weeks old were sacrificed by cervical dislocation. The intestinal tract was perfused directly with 4% cold paraformaldehyde in phosphate-buffered saline (PBS) and then fixed for immunohistochemical studies. Following dissection, brain, testis, small and large intestine, liver, heart, adipose tissue, and kidney samples were quickly frozen at −80°C until its subsequent use for protein and RNA extraction.

IMMUNOHISTOCHEMISTRY

The intestinal tissue samples were placed in Bouin's fixative for 4–5 h. After fixation, samples were dehydrated in increasing concentrations of ethanol, embedded in paraffin, sectioned (3 μm) with a HM 310 Microm microtome and collected on polysine-coated slides. Sections were deparaffined, rehydrated, incubated with 3% H_2O_2 in methanol for 20 min to quench endogenous peroxidase activity, and processed for immunohistochemical analysis following the Vectastain Elite ABC Kit (Vector Laboratories, Burlingame, CA) manufacture's instructions. Briefly, nonspecific antibody binding was blocked with 10% normal goat serum for 20 min. Then, sections were incubated overnight at 4°C with rabbit polyclonal anti-GLUT8 antibody (dilution 1:500) [Gomez et al., 2006] or with preimmune serum from rabbit in PBS supplemented with 3% BSA (Sigma), rinsed twice in PBS for 10 min, incubated with Avidin–Biotin–Peroxidase secondary antibody complex for 40 min, and detected with diaminobenzidine tetrahydrochloride (DAB-4HCl) substrate. Finally, sections were rinsed for 5 min in distilled water, dehydrated, and prepared for optic image analysis. Some sections were stained with hematoxylin and eosin to identify cell morphology.

WESTERN BLOT ANALYSIS

To obtain intestinal proteins, tissues were homogenized in TES (20 mM Tris, 1 mM EDTA, 255 mM sucrose, pH 7.1) plus an inhibitory cocktail protease (Roche Diagnostics) and 10 mM PMSF (Roche Diagnostics) with an Ultra Turrax T25 basic (IKA Labortechnik, Staufen, Germany). The homogenized was then sonicated, and kept on ice. Then, samples were centrifuged 16,000*g* for 20 min at 4°C. The pellet was resuspended in 2 mM Tris/50 mM mannitol pH 7.1 buffer. Western blots were performed with 20–50 μg of total protein. Protein samples were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) in 10% acrylamide gels, the proteins transferred to Hybond-ECL membranes (GE Healthcare), which were blocked in 5% nonfat milk in PBS–T (PBS, 0.1% Tween-20) for 1 h. Then, the nitrocellulose membrane was incubated with a polyclonal antibody against the C-terminal GLUT8 protein [Gomez et al., 2006], diluted 1:1,000 in blocking solution for 2 h at room temperature with gentle shaking. After several washes with PBS–T, the membrane was incubated with a secondary anti-rabbit IgG antibody coupled to horseradish peroxidase (dilution 1:3,000) for 1 h. Protein detection was performed using the ECL Plus system (GE Healthcare). Blots were reprobed with an anti-actin antibody diluted 1:300 after stripping in 200 mM glycine, pH 2.5, 0.4% SDS for 30 min, washed in PBS–T, and 1 h blocking. Optical density analysis of GLUT8 and actin was performed with the QuantityOne software (Bio-Rad).

RNA ISOLATION AND REVERSE TRANSCRIPTION–POLYMERASE CHAIN REACTION (RT-PCR)

Total RNA was extracted from isolated tissues using an Ultra-Turrax T25 basic for tissue homogenization and the RNeasy Mini Kit and QIAshredder columns (Qiagen, Hilden, Germany). To analyze the mRNA expression in the mucous layer, this layer was removed by scraping from the rest of duodenum, jejunum, ileum, and colon tissues, and the total RNA was isolated as previously described [Mesonero et al., 2000].

Reverse transcription was performed from 2 μg of total RNA. After treatment with DNaseI-RNase free (Roche Diagnostics), cDNA was synthesized with the SuperScript First-Strand Synthesis System

(Invitrogen), using the oligo(dT) primers. Based on the GLUT8 murine nucleotide sequence with GenBank accession number NM_019488, we designed specific primers to amplify the entire GLUT8 transcript (mGLUT8-L) and/or the transcriptional spliced variants (mGLUT8-SP1, mGLUT8-SP2, mGLUT8-SP3) (Table I). Primers used to determine the expression of mRNA GLUT8 in different tissues were GLUT8-exon4-f and GLUT8-exon9-r (Table I). The primers used to amplify and clone GLUT8 from testis and intestine were GLUT8-exon1-f and GLUT8-exon10-r (Table I). PCR reactions were performed using a thermocycler GeneAmp® PCR2700 (Applied Biosystems, Foster City, CA) and Platinum Taq DNA polymerase (Invitrogen). Control reactions were performed with the primers in the absence of cDNA. All PCR products were analyzed by agarose 1% gel electrophoresis and visualized by ethidium bromide staining. PCR reactions were performed twice in three independent experiments.

PCR PRODUCTS CLONING AND DNA SEQUENCING

The PCR-amplified products were cloned in pCR2.1-TOPO plasmid (Invitrogen) following the manufacturer's instructions and used for bacterial transformation. The cloned nucleotide sequence was tested by the dideoxy chain termination method, using an ABl 377 model automatic sequencer (Applied Biosystems) at the IBMCP-UPV's DNA Sequencing Service (Valencia, Spain). For eukaryotic expression, the PCR fragment was subcloned in pcDNA3.1(+) (Invitrogen). Double DNA sequencing was carried out using specific primers, corresponding to the flanking region of the plasmid pCR2.1-TOPO or pcDNA3.1(+) cloning sites. Sequence homologies were compared in the GenBank database using the BLAST 2.0 program [Altschul et al., 1997] at the NCBI server. The cDNA sequences reported in this article have been deposited in the GenBank nucleotide sequence database under the following accession numbers: mGLUT8-L as AY856043; mGLUT8-SP1 as AY856044; mGLUT8-SP2 as AY856045; and mGLUT8-SP3 as AY856046.

SOUTHERN BLOT ANALYSIS

RT-PCR products amplified from the testis and intestine using GLUT8-exon1-f and GLUT8-exon10-r were diluted 1:20 and 1:10, respectively, in TE (10 mM Tris–HCl, 0.1 mM EDTA, pH 8). Samples were treated with 40 U nuclease S1 (Roche Diagnostics) 5 min at 37°C to avoid nonspecific DNA heteroduplex, and were electrophoresed for 3 h at 70 V in 2.4% agarose gel. Then, the gel was washed with bidistilled water, shaken 15 min in 0.25 M HCl solution, washed again, and incubated in 0.5 M NaOH denaturized solution for 20 min with constant shaking. Denaturalized DNA was transferred to a nitrocellulose membrane overnight, washed in 2× SSC (0.3 M NaCl, 34.1 mM sodium citrate, pH 7), and prehybridized in hybridization solution (50% formamide, 5× SSC, 2% Blocking Reagent, 0.1% *N*-laurosylsarcosine, 0.02% SDS) at 37°C for 1 h with shaking. Detection of GLUT8 DNA fragments was performed by hybridizing the membrane with a Digoxigenin (DIG)-labeled ~1,000-bp DNA probe prepared by PCR amplification using the PCR-DIG DNA-labeling kit (Roche Diagnostics) and GLUT8-exon4-f and GLUT8-exon10-r primers (Table I). Chemiluminescence was detected using the ECL detection system (GE Healthcare), and visualized by different exposure times to autoradiography films.

CELL CULTURE AND TRANSIENT TRANSFECTIONS

3T3-L1 mouse embryonic fibroblast-adipose like cell line from passages 3–9 were used in all studies. Cells were cultured as previously described [Moreno-Aliaga and Matsumura, 1999; Moreno-Aliaga et al., 2001] in Dulbecco's modified Eagle's medium (DMEM) containing 25 mM glucose and 10% calf bovine serum. Confluent cells were induced to differentiate by incubation with 1 μM dexamethasone, 0.5 mM isobutylmethylxanthine (IBMX), and 10% fetal bovine serum (FBS) in DMEM for 72 h. Cells were then maintained in DMEM containing 10% FBS but without dexamethasone or IBMX (post-differentiation medium) 48 h prior transfection [Moreno-Aliaga et al., 2001]. 3T3-L1 cells differentiated on adipocytes were transiently transfected with 2.5 μg of pcDNA3.1(+) empty plasmid or constructs containing the corresponding splicing variant (mGLUT8-SP1; mGLUT8-SP2; and/or mGLUT8-SP3), by the calcium phosphate method as previously described [Mesonero et al., 1998].

COS-7 cells, cultured in DMEM and 10% FBS, were also transfected by the calcium phosphate method [Mesonero et al., 1998], with 2.5 μg of pcDNA3.1(+) empty plasmid or constructs containing the corresponding splicing variant (mGLUT8-L; mGLUT8-SP1; mGLUT8-SP2; and/or mGLUT8-SP3).

BIOCHEMICAL ASSAYS: GLUCOSE CONSUMPTION AND PROTEIN CONCENTRATION

Glucose consumption rates were determined at 24, 48, and 72 h after transient transfection by measuring the concentration of glucose in the culture medium using the Glucose Oxidase/Peroxidase Kit (Sigma) according to the manufacturer's instructions. Protein concentration was determined by the method of Bradford [1976].

TABLE I. PCR Primers Used for Amplification and Cloning the Glucose Transporter Gene GLUT8 (mGLUT8-L) and Splice Variants (mGLUT8-SP1, mGLUT8-SP2, mGLUT8-SP3) in Mouse Tissues

Primer nomenclature	Primer sequence (5′–3′)	Sequences recognized
GLUT8-exon1-f	TGACATGTCTCCCGAGGACC	mGLUT8-L, -SP1, -SP2, and -SP3
GLUT8-exon4-f	ATCTCGGAAATCGCCTACCC	mGLUT8-L, -SP1, and -SP2
GLUT8-exons6-9-f	GGAGGCCAAGTTCAAGGCT	mGLUT8-SP2
GLUT8-exon7-r	ATTATGCCCACAGTGACC	mGLUT8-L
GLUT8-exons6-8-r	GAACACCATGATCACACCTT	mGLUT8-SP1 and -SP3
GLUT8-exon9-r	TTGGTGAGGACACAGATGCC	mGLUT8-L, -SP1, -SP2, and -SP3
GLUT8-exon10-r	AAAGGGTCTGTCATCGTCC	mGLUT8-L, -SP1, -SP2, and -SP3

STATISTICAL ANALYSES

The results are expressed as means ± SEM. Statistical comparisons were performed by the unpaired Student's *t*-test. $P < 0.01$ was considered to be significant.

RESULTS

GLUT8 PROTEIN EXPRESSION IN INTESTINAL TISSUE

To study the expression of GLUT8 protein along the small and large intestine, we first performed a Western blot using an antibody against the GLUT8 C-terminal amino acid sequence. In Figure 1, a 42-kDa band corresponding to GLUT8 is observed in all samples including the testis, used as a positive control. The molecular size was similar to that reported by different research groups [Doege et al., 2000; Augustin et al., 2005; Gomez et al., 2006]. Compared with the testis, the protein levels of GLUT8 were ~4-fold lower in small intestine and ~3-fold lower in colon. Within the intestinal tract, we observed higher levels in the colon than in the small intestine.

Then, we performed immunohistochemistry studies to further examine the cellular localization of GLUT8 in enterocytes and colonocytes. Immunohistochemical detection of GLUT8 in the ileum and colon is shown in Figure 2 as representative of the small and large intestine, respectively. Immunostaining in the ileum revealed a clear intracellular and supranuclear localization in the apical region of the enterocytes (Fig. 2A). In the same section, we also found staining in the crypt cells (Fig. 2B). In the colon, the immunostaining is remarkable in intracellular compartments in a supranuclear location, close to the apical membrane (Fig. 2C). No signal was obtained when preimmune serum was used instead of the primary antibody (Fig. 2D–F), thereby confirming the specificity of the antibody reaction.

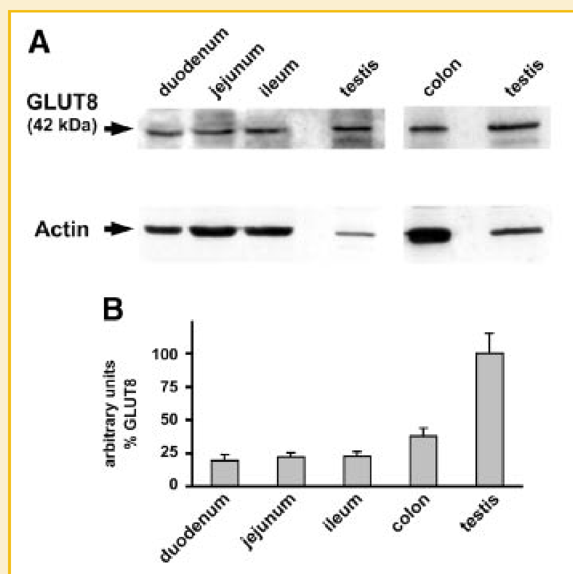

Fig. 1. GLUT8 protein expression along the small and large intestine. A: 20–50 μg of total protein from testis, duodenum, jejunum, ileum, and colon were SDS–PAGE electrophoresed. Immunodetection of GLUT8 (~42 kDa) and actin proteins was performed using an anti-C-terminal GLUT8 and commercial anti-actin antibodies. Representative gels from n = 4 independent experiments are shown. B: Histogram represents the densitometric quantification of GLUT8 from four blots derived from different membrane preparations (arbitrary units ± SEM relative to testis = 100).

GLUT8 mRNA EXPRESSION IN INTESTINAL TISSUE

Previous reports by Northern blot have shown that GLUT8 is widely expressed in many tissues with the highest levels in the testis [Doege et al., 2000]. In this work, RT-PCR analyses from testis, brain, heart, liver, heart, adipose tissue, kidney, and intestine tissues were performed, as shown in Figure 3A. GLUT8 mRNA was expressed in all the tissues, corroborating the initial published data about the distribution of mRNA GLUT8. Figure 3A shows that an 818-bp PCR product resulted from the GLUT8 amplification using GLUT8-exon4-f and GLUT8-exon9-r primers (Table I). A lower fragment of ~550 bp was observed in the intestine with slight intensity. This fragment was not visible in the other tissues. Similar results were obtained by increasing annealing temperature to improve the PCR reaction specificity (data not shown). In addition, we examined the expression of GLUT8 in small (duodenum, jejunum, and ileum) and large (colon) intestine tissues and in the isolated mucous layer. As Figure 3B shows, GLUT8 mRNA is also expressed in the mucous layer, as well as in the whole tissue. In this experiment, we used a different set of primers (GLUT8-exon1-f and GLUT8-exon10-r) (Table I) for PCR amplification to obtain the GLUT8 full-length fragment (1,448 bp). In this case, we observed a lower fragment again which was slightly visible in some samples (Fig. 3B). These observations suggested the existence of GLUT8 alternative mRNA transcripts in mouse intestine.

IDENTIFICATION AND CHARACTERIZATION OF MULTIPLE mRNA GLUT8 TRANSCRIPTS DERIVED FROM ALTERNATIVE SPLICING

We confirmed by Southern blot analysis that the lower PCR-detected DNA fragments were GLUT8 transcriptional variants. GLUT8 mRNA expression was analyzed in intestinal tissue and compared with the testis. Primers used to amplify the full-length mGLUT8 sequence were GLUT8-exon1-f and GLUT8-exon10-r (Table I). Figure 4 shows the results of GLUT8 hybridization with a ~1,000-bp probe with and without S1 previous nuclease treatment. In addition to the full-length GLUT8 mRNA (1,448 bp), we detected two additional bands in the intestine, whereas only the full-length GLUT8 fragment was detected in the testis (Fig. 4).

Then, to study the multiple mRNA GLUT8 transcripts in mouse intestine, we cloned the PCR products from mouse intestine and testis into the pCR2.1-TOPO vector, as described in the Materials and Methods Section. Analysis of the clones by restrictive *Eco*RI digestion showed that GLUT8 PCR amplification from testis only generated a single product of 1,448 bp according to the Southern blot results. However, the amplification from mouse intestine generated several additional products that differed from the 1,448 bp product (Fig. 5A). The sequence analysis revealed the presence of four different GLUT8 transcripts in intestine tissue (mGLUT8-L, mGLUT8-SP1, mGLUT8-SP2, and mGLUT8-SP3) (Fig. 5B). mGLUT8-L (1,448 bp) matches the cDNA sequence of GLUT8 gene and corresponds to the full-length known GLUT8;

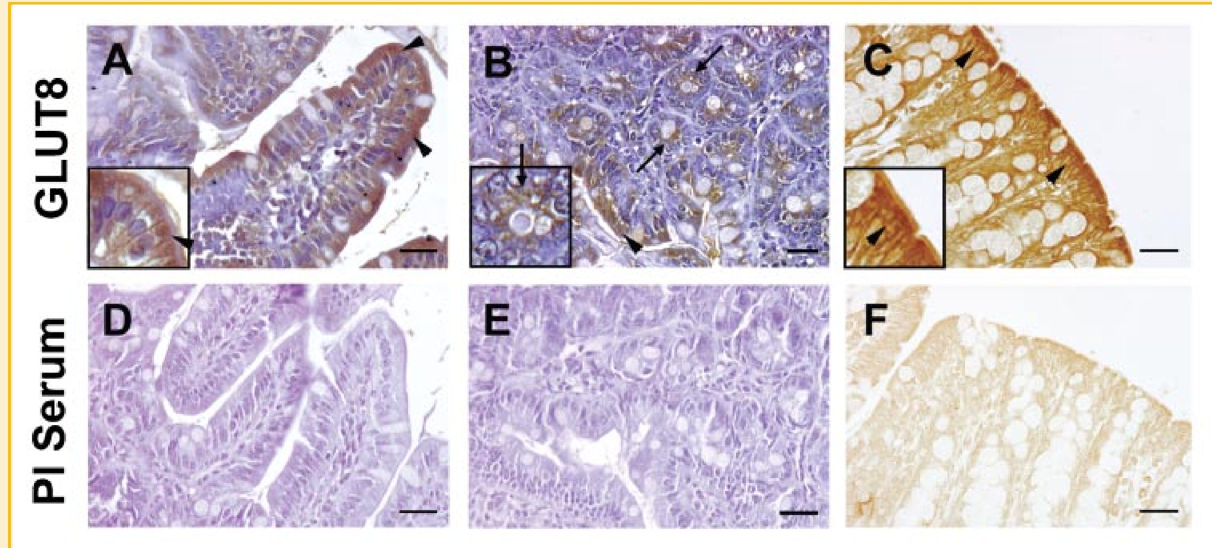

Fig. 2. Immunohistochemical localization of GLUT8 protein in intestine. Ileum (A,B,D,E) and colon (C,F) sections were incubated with a polyclonal anti-GLUT8 (A–C) or preimmune (PI) serum (D–F) and counterstained with hematoxylin. GLUT8 shows an intracellular immunostaining mainly with supranuclear localization in the enterocytes (A, arrowheads) and colonocytes (C, arrowheads) and also in crypt cells (B, arrows). Insets (A–C) show a higher magnification of GLUT8 immunostaining. The scale bar is 20 μm. The results shown are representative of each tissue analyzed. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

mGLUT8-SP1 (1,339 bp) lacks exon 7; mGLUT8-SP2 (1,168 bp) misses exons 7 and 8; and mGLUT8-SP3 (869 bp) lacks exons 2, 3, 4, and 7 (Fig. 5B). mGLUT8-SP1 and mGLUT8-SP2 correspond to the lower bands detected by Southern blot analysis (1,339 and 1,168 bp, respectively) (Fig. 4). However, we did not detect mGLUT8-SP3 by Southern blot, likely due to the low hybridization efficiency. The mouse cDNA transcripts sequences reported in this article have been deposited in the GenBank nucleotide sequence database under the following accession numbers: AY856043 for mGLUT8-L; AY856044 for mGLUT8-SP1; AY856045 for mGLUT8-SP2; and AY856046 for mGLUT8-SP3. Predicted protein analysis of spliced variants using the SosuiTM, DAS, and TMpred servers indicate that mGLUT8-SP1 and mGLUT8-SP2 encode, respectively, for 291 and 329 amino acids putative integral membrane proteins with seven membrane-

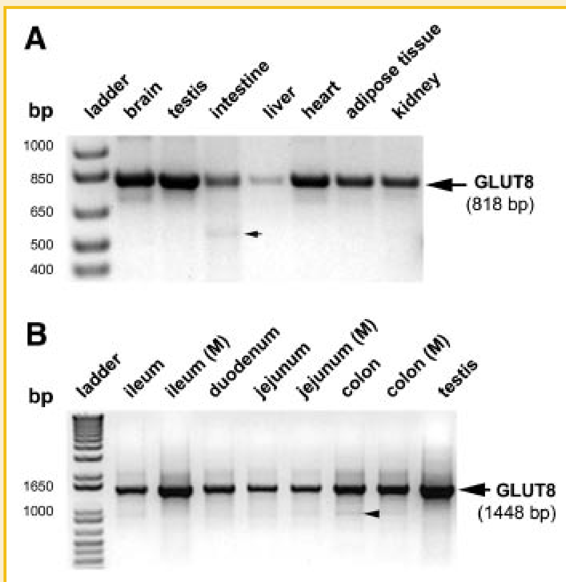

Fig. 3. GLUT8 mRNA expression in small and large intestine tissue. A: RT-PCR detection of GLUT8 in mouse tissues. Two micrograms of total RNA from brain, testis, intestine, liver, heart, adipose tissue, and kidney were used as templates for reverse transcription. GLUT8-specific primers used for cDNA PCR amplification (818 bp) were GLUT8-exon4-f and GLUT8-exon9-r. B: RT-PCR detection of GLUT8 in mouse small and large intestine. "M" represents the mucous layer isolated. Primers used for full-length GLUT8 amplification (1,448 bp) were GLUT8-exon1-f and GLUT8-exon10-r. Arrowheads indicate the presence of a lower band amplified in intestinal tissue.

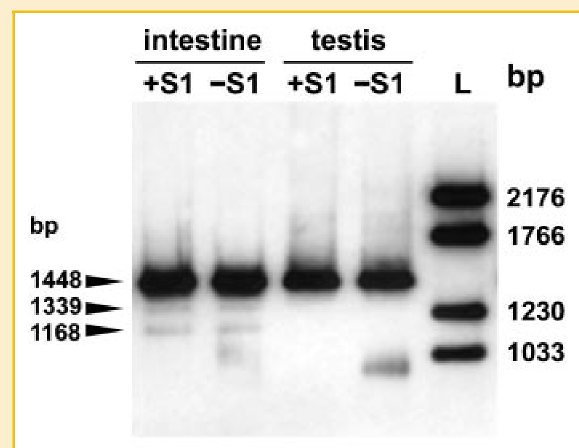

Fig. 4. Detection of GLUT8 transcriptional variants by Southern blot analysis. Intestine and testis RT-PCR products were Southern blotted and hybridized with a mouse GLUT8 cDNA probe, before (−S1) and after (+S1) S1 nuclease treatment (a representative result from n = 3 is shown). After 20 min of exposure, additional 1,339 and 1,168 bp bands appeared in intestinal tissue while only the full-length GLUT8 (1,448 bp) was detectable in testis.

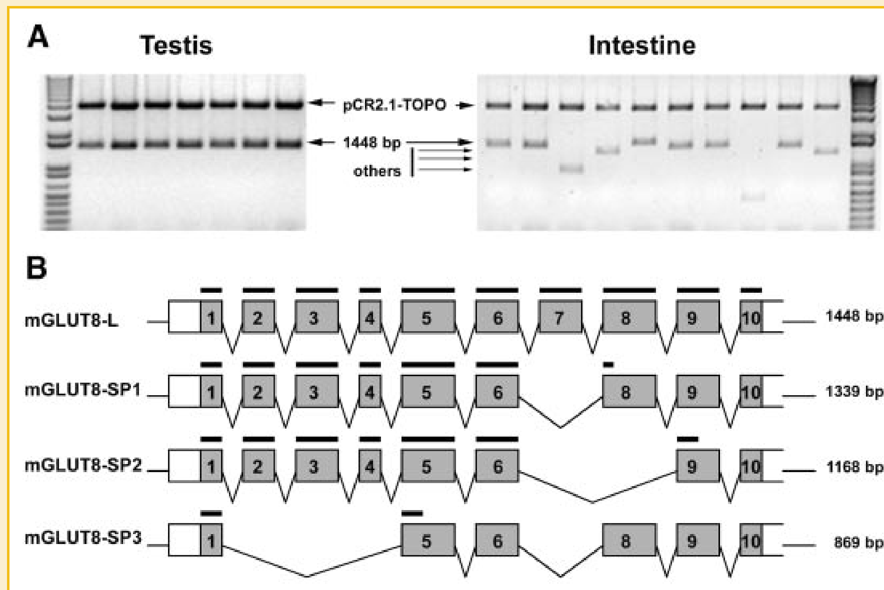

Fig. 5. Identification of alternative splicing of GLUT8 in intestine tissue by cloning studies. A: Testis and intestine RT-PCR products, obtained using the specific primers that amplify the full-length GLUT8, were cloned in pCR2.1-TOPO vector. More than 100 individual colonies were analyzed and digested with *Eco*RI. Small fragments, including the 1,339, 1,168, and 869 bp fragments, were cloned from intestinal tissue (right) whereas only one fragment corresponding to full-length GLUT8 was cloned from testis tissue (left). A representative film is shown. B: Genomic organization and alternative splicing of the mouse GLUT8 gene. GLUT8 gene consists of 10 exons. Boxes indicate the length and position of each exon. Deletion of one or more complete exon(s) from mGLUT8-L originates a 1,339 bp mGLUT8-SP1 (deletion of exon 7), an 1,168 bp mGLUT8-SP2 (deletion exons 7 and 8), and an 869 bp mGLUT8-SP3 (deletion of exons 2, 3, 4, and 7) spliced variants. Horizontal bars (above the exons) represent the translated amino acid sequence until the codon stop in each transcript.

spanning domains identical to GLUT8, except for the C-terminal portion (TMH8–TMH12) (Fig. 6). On the other hand, mGLUT8-SP3 encodes for a putative 71 amino acids soluble protein, identical to GLUT8, only in the N-terminal region (Fig. 6). Therefore, all these hypothetical proteins lack the GLUT8 C-terminal region that is critical for sugar transport activity.

STUDY OF GLUT8 SPLICED VARIANTS IN SMALL AND LARGE MOUSE INTESTINE

To further examine the presence of transcriptional variants along the small and large intestine, we designed a set of primers (Table I) that specifically amplified the spliced transcripts (Fig. 7). We analyzed the expression of mGLUT8-L, mGLUT8-SP1, -SP2, and -SP3 from jejunum, ileum, and colon intestinal tissues by RT-PCR. The first set of primers (GLUT8-exon4-f and GLUT8-exon7-r) amplified mGLUT8-L (473 bp). The second set of primers (GLUT8-exon4-f and GLUT8-exons6-8-r) amplified mGLUT8-SP1 (455 bp). For mGLUT8-SP2 amplification (310 bp), we used GLUT8-exons6-9-f and GLUT8-exon10-r primers, and we amplified mGLUT8-SP3 (421 bp) using GLUT8-exon1-f and GLUT8-exons6-8-r. The results show that the alternative splicing process is a common feature in all the intestinal fragments analyzed, indicating that GLUT8 is not differentially expressed along the intestine.

FUNCTIONAL ANALYSIS OF GLUT8 SPLICED VARIANTS

It has previously shown that an overexpression of glucose/fructose transporters at the plasma membrane is correlated to an increase of the sugar consumption rates of the cells [Mesonero et al., 1995]. In order to examine if mGLUT8-SP1, -SP2, and -SP3 transcriptional variants could have a role in glucose transport activity across the plasma membrane, we transfected 3T3-L1 cells, which endogenously express mouse GLUT8 protein, with each transcriptional variant alone or with the three variants together. Expression of the mRNA spliced variants was confirmed by RT-PCR (data not shown) and then, the glucose consumption was determined at 48 and 72 h after transfection. Table II shows that neither the three splicings alone nor the three transcripts together had an effect on glucose consumption compared to cells containing the pcDNA3.1(+) empty vector. Similar results were obtained when we transfected COS-7 cells: neither transfection with mGLUT8-L nor with the splice variants modified glucose consumption (results not shown). These results indicate that the spliced variants did not produce functional glucose transporter proteins at the plasma membrane.

DISCUSSION

In this study, we show that the GLUT8 facilitative sugar transporter is expressed along the small and large intestine. Intestinal sugar absorption, as the imperative entry of glucose into the organism, makes the study of possible additional mechanisms involved in this process interesting. To date, it is known that SGLT1 and GLUT5, located in the brush-border membrane of the enterocyte [Davidson et al., 1992; Wright, 1993], transport glucose and fructose, respectively, when the sugar concentration in the lumen is low. After a sugary meal when the glucose concentration in the lumen is

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hGLUT8       MTPEDPEETQPLLGPPGGSAPRGRRVFLAAFAAALGPLSFGFALGYSSPAIPSLQRAAPP  60
mGLUT8-L     MSPEDPQETQPLLRPPEARTPRGRRVFLASFAAALGPLSFGFALGYSSPAIPSLRRTAPP  60
mGLUT8-SP1   MSPEDPQETQPLLRPPEARTPRGRRVFLASFAAALGPLSFGFALGYSSPAIPSLRRTAPP  60
mGLUT8-SP2   MSPEDPQETQPLLRPPEARTPRGRRVFLASFAAALGPLSFGFALGYSSPAIPSLRRTAPP  60
mGLUT8-SP3   MSPEDPQETQPLLRPPEARL--GPRVALAGRAGLCAPHPHAAAHVLHARNPTFSPHSTPV  58
             ************************************************************

hGLUT8       APRLDDAAASWFGAVVTLGAAAGGVLGGWLVDRAGRKLSLLLCSVPFVAGFAVITAAQDV 120
mGLUT8-L     ALRLGDNAASWFGAVVTLGAAAGGILGGWLLDRAGRKLSLLLCTVPFVTGFAVITAARDV 120
mGLUT8-SP1   ALRLGDNAASWFGAVVTLGAAAGGILGGWLLDRAGRKLSLLLCTVPFVTGFAVITAARDV 120
mGLUT8-SP2   ALRLGDNAASWFGAVVTLGAAAGGILGGWLLDRAGRKLSLLLCTVPFVTGFAVITAARDV 120
mGLUT8-SP3   PGGHGCLALPVGL                                                 71
             ************************************************************

hGLUT8       WMLLGGRLLTGLACGVASLVAPVYISEIAYPAVRGLLGSCVQLMVVVGILLAYLAGWVLE 180
mGLUT8-L     WMLLGGRLLTGLACGVASLVAPVYISEIAYPAVRGLLGSCVQLMVVTGILLAYVAGWVLE 180
mGLUT8-SP1   WMLLGGRLLTGLACGVASLVAPVYISEIAYPAVRGLLGSCVQLMVVTGILLAYVAGWVLE 180
mGLUT8-SP2   WMLLGGRLLTGLACGVASLVAPVYISEIAYPAVRGLLGSCVQLMVVTGILLAYVAGWVLE 180
             ************************************************************

hGLUT8       WRWLAVLGCVPPSLMLLLMCFMPETPRFLLTQHRRQEAMAALRFLWGSEQGWEDPPIGAE 240
mGLUT8-L     WRWLAVLGCVPPTLMLLLMCYMPETPRFLLTQHQYQEAMAALRFLWGSEEGWEEPPVGAE 240
mGLUT8-SP1   WRWLAVLGCVPPTLMLLLMCYMPETPRFLLTQHQYQEAMAALRFLWGSEEGWEEPPVGAE 240
mGLUT8-SP2   WRWLAVLGCVPPTLMLLLMCYMPETPRFLLTQHQYQEAMAALRFLWGSEEGWEEPPVGAE 240
             ************************************************************

hGLUT8       -QSFHLALLRQPGIYKPFIIGVSLMAFQQLSGVNAVMFYAETIFEEAKFKDSSLASVVVG 300
mGLUT8-L     HQGFQLALLRRPGIYKPLIIGISLMVFQQLSGVNAIMFYANSIFEEAKFKDSSLASVTVG 300
mGLUT8-SP1   HQGFQLALLRRPGIYKPLIIGISLMVFQQLSGVNAIMFYANSIFEEAKFKV          291
mGLUT8-SP2   HQGFQLALLRRPGIYKPLIIGISLMVFQQLSGVNAIMFYANSIFEEAKFKALRWAGDPSP 300
             **************************************************

hGLUT8       VIQVLFTAVAALIMDRAGRRLLLVLSGVVMVFSTSAFGAYFKLTQGGPGNSSHVAISAPV 360
mGLUT8-L     IIQVLFTAVAALIMDRAGRRLLLALSGVIMVFSMSAFGTYFKLTQSLPSNSSHVG-LVPI 360
mGLUT8-SP2   GSSCQRSSLCMSRVWLPASVSSPTGSWPF                                329

hGLUT8       SAQPVDASVGLAWLAVGSMCLFIAGFAVGWGPIPWLLMSEIFPLHVKGVATGICVLTNWL
mGLUT8-L     AAEPVDVQVGLAWLAVGSMCLFIAGFAVGWGPIPWLLMSEIFPLHVKGVATGICVLTNWF 420

hGLUT8       MAFLVTKEFSSLMEVLRPYGAFWLASAFCIFSVLFTFSCVPETKGKTLEQITAHFEGR   477
mGLUT8-L     MAFLVTKEFSSVMEMLRPYGAFWLTAAFCALSVLFTLTVVPETKGRTLEQVTAHFEGR   477
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Fig. 6. Amino acid sequence alignment of GLUT8 and its spliced variants. Human (hGLUT8) and mouse (mGLUT8-L) protein sequence alignment with the spliced variants mGLUT8-SP1, mGLUT8-SP2, and mGLUT8-SP3 was performed with ClustalX program. Deletion of one or more exons from the original GLUT8 mRNA bears premature termination codons and changes in the lecture frameshifts originating a hypothetical 291, 329, and 71 amino acids proteins, respectively. Horizontal lines on the amino acid sequences depict transmembrane domains in GLUT8 protein. * indicates identical homology in at least three of the sequences compared.

over 50 mM, the capacity of glucose absorption by SGLT1 saturates, and GLUT2, that was initially associated with the basolateral membrane [Cheeseman, 1993], is rapidly translocated to the brush-border membrane, facilitating glucose and fructose uptake [Kellett and Helliwell, 2000; Au et al., 2002; Affleck et al., 2003]. Although apical GLUT2 has been reported in several animal species, the appropriate conditions to detect apical GLUT2 in human small intestine remain to be reported. Despite of this fact, the contribution of GLUT2 to the classic model does not exclude the possible expression of other sugar transporter proteins involved in the intestinal sugar traffic under different physiological conditions. In fact, other than the existence of GLUT8, a novel glucose and fructose transporter GLUT7 has been recently described in the intestinal tissue. GLUT7 is mainly expressed in the ileal brush border, but its biological role remains unknown [Li et al., 2004; Manolescu et al., 2005]. GLUT8 immunodetection under normal conditions reveals an intracellular localization close to the apical membrane in both enterocytes and colonocytes (Fig. 2). Therefore, GLUT8 cellular

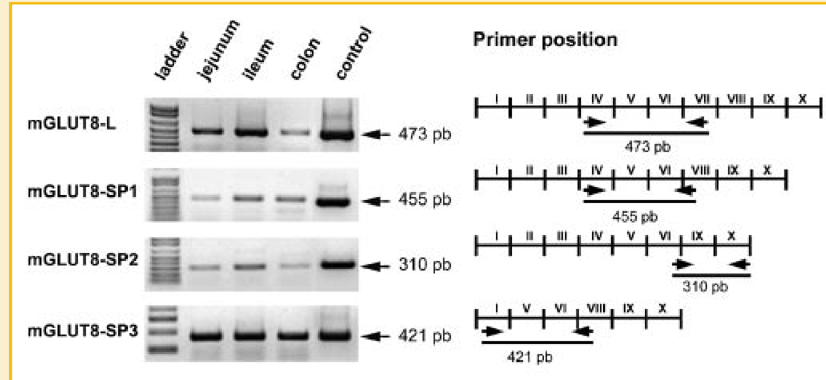

Fig. 7. Analysis of GLUT8 transcriptional variants along the small and large intestine. Left panel: RT-PCR analysis of spliced variants in jejunum, ileum, and colon was performed using GLUT8-exon4-f and GLUT8-exon7-r primers for mGLUT8-L transcript amplification (473 bp); GLUT8-exon4-f and GLUT8-exons6-8-r primers for mGLUT8-SP1 (455 bp); GLUT8-exons6-9-f and GLUT8-exon10-r primers for mGLUT8-SP2 (310 bp); and GLUT8-exon1-f and GLUT8-exons6-8-r for mGLUT8-SP3 amplification (421 bp). Plasmid constructions with pcDNA3.1(+) containing mGLUT8-L, mGLUT8-SP1, mGLUT8-SP2, and mGLUT8-SP3 were used as internal controls. Right panel: Schematic representation of specific primers localization and its amplification products. Arrows depict the primers position, and lines depict the sequence amplified.

distribution in the intestine seems to be unusual compared to the SGLT1, GLUT5, GLUT2, and GLUT7 transporters. GLUT8 cellular localization has been analyzed in other tissues. Previous studies demonstrated that GLUT8 in the brain is associated with intracellular membranes [Piroli et al., 2002, 2004]. It is also associated with the acrosomal region of sperm [Gomez et al., 2006; Kim and Moley, 2007]. In hepatocytes, GLUT8 presents a cytoplasmic localization [Gorovits et al., 2003], and a perinuclear expression pattern of GLUT8 has been described in mice kidney cells [Schiffer et al., 2005]. Our results in the intestine are similar to those obtained in other tissues and suggest that the functional role of GLUT8 could not be directly associated with plasma membrane transport. Therefore, GLUT8 may act mainly as a sugar facilitative transporter within intracellular compartments, and might possibly be involved in glycosylation processes as different authors have previously proposed [Piroli et al., 2002; Augustin et al., 2005; Gomez et al., 2006], rather than in intestinal sugar absorption. It is known that the N-terminal dileucine motif ($LL^{12/13}$) is responsible for the intracellular recruitment of the protein in a variety of different cell types. However, the presence of GLUT8 in the plasma membrane is still a matter of debate. Augustin et al. [2005] showed that GLUT8 does not recycle to the plasma membrane in cell cultures in contrast with the observations made by Lisinski et al. [2001]. Moreover, the stimulus that could mediate the translocation of GLUT8 to the plasma membrane is still unknown. In blastocysts, only insulin provokes GLUT8 translocation to the plasma membrane [Carayannopoulos et al., 2000; Pinto et al., 2002]. A more extensive analysis will be necessary to elucidate the possible traffic of GLUT8 to the apical membrane in intestinal cells, associated or not to specific metabolic conditions.

TABLE II. Glucose Consumption in 3T3-L1 Cells Transfected With GLUT8 Transcriptional Variants

Constructs	Time after cell transfection (h) 48	72
pcDNA3.1(+) plasmid	101.9 ± 2.0 (11)	82.0 ± 2.7 (11)
mGLUT8-SP1	103.4 ± 2.6 (12)	81.9 ± 3.0 (12)
mGLUT8-SP2	102.9 ± 2.8 (12)	81.1 ± 3.3 (12)
mGLUT8-SP3	106.0 ± 2.9 (12)	82.9 ± 3.7 (12)
mGLUT8-SP1-3	102.8 ± 3.6 (9)	82.4 ± 4.7 (9)

The cells were transfected with the pcDNA3.1(+) empty plasmid and the pcDNA3.1(+)-mGLUT8-SP1, -SP2, and -SP3 constructs. Transfected cells were cultured in standard conditions as described in the Materials and Methods Section, and medium samples were taken 48 and 72 h after transfection. Glucose consumption rates are given in μg/plate/h. Data represent means ± SEM for (n) independent cultures. No significant differences were observed. Similar results (not shown) were obtained at 24 h after cell transfection.

In contrast to SGLT1, GLUT2, GLUT5, and GLUT7, which are mainly expressed in the small intestine, GLUT8 is also detected in the colon (Figs. 1 and 2). In fact, within the intestine, the expression of GLUT8 is higher in the colon than in the small intestine (Fig. 1). The presence of the GLUT8 protein in the colon could be physiologically important since no previous reports exist of any facilitative sugar transporter in the large intestine. Usually, the colonic flora has the ability to ferment carbohydrates malabsorbed in the small bowel to the short-chain fatty acids, which are absorbed across the colonic mucosa resulting in a carbohydrate energy source [Wong et al., 2006]. Although the major sugar absorption is produced in the jejunum and ileum, it has been suggested that the colon could be capable of glucose absorption [Taha et al., 2004]. Thus, GLUT8 may assume an important role in sugar traffic in the colon where alternative systems are absent. Under normal conditions, however, we speculate that the particular environment of colonic cells favors the expression of GLUT8 probably due to different metabolic phenotypes. Indeed, studies with isolated enterocytes and colonocytes have shown that short-chain fatty acids and glucose oxidation rates were greater in cells taken from the colon than from the jejunum [Fleming et al., 1991]. Moreover, we have also observed a clear immunostaining in intestinal crypts (Fig. 2B). Since there are undifferentiated progenitor cells in the crypts that are constantly

differentiating to form the variety of epithelial intestinal cells, this result supports the idea that the primary role of GLUT8 could be associated with specific cellular processes that require high glucose or energy demands. For example, the formation of the acrosome during spermatogenesis, or the cellular proliferation in tumor cells [Goldman et al., 2006], and undifferentiated cells with constant proliferation capacity. Furthermore, in human intestinal Caco-2 cells, the mRNA GLUT8 expression is related to high glucose metabolism rates and to actively dividing cells [Romero et al., 2007].

In contrast to the testis, the gene expression analysis shows that GLUT8 mRNA processing produces multiple splice variants in the intestine. From the original GLUT8 gene sequence, we have found four transcriptional variants originated (mGLUT8-L, mGLUT8-SP1, mGLUT8-SP2, and mGLUT8-SP3) as a result of one exon or more removed (Fig. 5B). Transcriptional variants in the GLUT protein family have been described in other members such as GLUT9 [Augustin et al., 2004; Keembiyehetty et al., 2006] and GLUT11 [Sasaki et al., 2001; Wu et al., 2002; Scheepers et al., 2005]. Human GLUT9 displays an alternative form in the kidney by the deletion of one exon in the 5′-extreme [Augustin et al., 2004]. Moreover, homologous GLUT9 spliced variants with different start sites have been cloned from mouse adult liver, showing a differential tissue distribution and regulation in diabetes [Keembiyehetty et al., 2006]. Several transcripts have been described for GLUT11 [Sasaki et al., 2001; Wu et al., 2002] and have been classified into three types of variants [Sasaki et al., 2001]. The first type implies the existence of three different exons 1 with different promoters that originate three proteins with different tissue expressions. The second type describes a variant originated by the deletion of exon 6, which provokes the loss of five transmembrane domains. The third type includes a stop codon located between exons 8 and 9, generating a new truncated protein. Collectively, all these studies strongly suggest a role of the GLUT spliced variants in the regulation of sugar transport mechanisms in mammalian cells.

The low mRNA expression of the transcriptional variants compared to the full-length GLUT8 mRNA detected by RT-PCR and Southern blot (Figs. 3 and 4) suggests that this type of transcriptional variants may target the RNA for nonsense-mediated decay (NMD). NMD is an mRNA surveillance mechanism that has been described from yeast to humans and ensures mRNA quality by selectively targeting mRNAs that harbors premature termination codons (PTCs) for rapid degradation. PTCs (stop codons >50 nucleotides upstream of the final splice junction) that are introduced as a consequence of DNA rearrangements, frameshifts, or are caused by errors during transcription or splicing, can lead to nonfunctional or deleterious proteins [Cartegni et al., 2002]. There is growing evidence that the mechanistic coupling of alternative splicing and NMD provides an often-used means of regulating gene expression [Lewis et al., 2003]. The skipping of exon 7 and exons 2–3–4–7 entails PTC, respectively, for the mGLUT8-SP1 and mGLUT8-SP3 transcripts (Figs. 5B and 6). Missing exons 7 and 8 in mGLUT8-SP2 lead to a stop codon that, although it cannot be classified as a PTC, also results in an altered reading frame. Nowadays, the alternative splicing process is considered to be a cellular mechanism that not only allows individual genes to produce multiple protein isoforms, but it also has a hidden function in quantitative gene control by targeting RNAs for NMD [Lewis et al., 2003; Matlin et al., 2005]. Since we observed a low expression of the GLUT8 protein compared with the testis under physiological conditions, we believe that the alternative spliced process coupled with NMD in the intestine may play an important role in regulating the intestinal GLUT8 gene expression. It has been suggested that NMD can play a role in cancer development [Scholzova et al., 2007]. It will be interesting to investigate the regulation of the different transcripts of GLUT8 in tumors of epithelial intestinal cells since, as we have previously shown, both metabolism and differentiation processes could be implicated in the control of GLUT8 expression in intestinal epithelial cells [Romero et al., 2007].

Furthermore, the data presented in this work cannot exclude the existence of similar transcriptional variants in other tissues where GLUT8 is expressed at low levels. Another possibility is that truncated proteins generated as a result of the GLUT8 alternative splicing process could be easily degraded or may play a role that remains unknown.

At least, it is evident that the hypothetical predicted proteins mGLUT8-SP1, -SP2, and -SP3 lack glucose transport activity due to the lack of several conserved motifs for sugar transport at the C-terminus region level. Moreover, no sequence homology with other protein families was found.

In conclusion, we demonstrate that GLUT8 is expressed along the small and large mouse intestine. Its protein expression is low compared with the testis under normal physiological conditions; however, we have found four different transcriptional variants of GLUT8 in the intestine. Although the potential role of this sugar transporter in the intestine is still unknown, the presence of alternative splicing is a new finding that will contribute to understand the possible mechanisms that regulate the GLUT8 gene expression.

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REFERENCES

Affleck JA, Helliwell PA, Kellett GL. 2003. Immunocytochemical detection of GLUT2 at the rat intestinal brush-border membrane. J Histochem Cytochem 51:1567–1574.

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res 25:3389–3402.

Au A, Gupta A, Schembri P, Cheeseman CI. 2002. Rapid insertion of GLUT2 into the rat jejunal brush-border membrane promoted by glucagon-like peptide 2. Biochem J 367:247–254.

Augustin R, Carayannopoulos MO, Dowd LO, Phay JE, Moley JF, Moley KH. 2004. Identification and characterization of human glucose transporter-like protein-9 (GLUT9): Alternative splicing alters trafficking. J Biol Chem 279:16229–16236.

Augustin R, Riley J, Moley KH. 2005. GLUT8 contains a [DE]XXXL[LI] sorting motif and localizes to a late endosomal/lysosomal compartment. Traffic 6:1196–1212.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254.

Carayannopoulos MO, Chi MM, Cui Y, Pingsterhaus JM, McKnight RA, Mueckler M, Devaskar SU, Moley KH. 2000. GLUT8 is a glucose transporter responsible for insulin-stimulated glucose uptake in the blastocyst. Proc Natl Acad Sci USA 97:7313–7318.

Cartegni L, Chew SL, Krainer AR. 2002. Listening to silence and understanding nonsense: Exonic mutations that affect splicing. Nat Rev Genet 3:285–298.

Cheeseman CI. 1993. GLUT2 is the transporter for fructose across the rat intestinal basolateral membrane. Gastroenterology 105:1050–1056.

Davidson NO, Hausman AM, Ifkovits CA, Buse JB, Gould GW, Burant CF, Bell GI. 1992. Human intestinal glucose transporter expression and localization of GLUT5. Am J Physiol 262:C795–C800.

Doege H, Schurmann A, Bahrenberg G, Brauers A, Joost HG. 2000. GLUT8, a novel member of the sugar transport facilitator family with glucose transport activity. J Biol Chem 275:16275–16280.

Ferraris RP, Carey HV. 2000. Intestinal transport during fasting and malnutrition. Annu Rev Nutr 20:195–219.

Fleming SE, Fitch MD, DeVries S, Liu ML, Kight C. 1991. Nutrient utilization by cells isolated from rat jejunum, cecum and colon. J Nutr 121:869–878.

Goldman NA, Katz EB, Glenn AS, Weldon RH, Jones JG, Lynch U, Fezzari MJ, Runowicz CD, Goldberg GL, Charron MJ. 2006. GLUT1 and GLUT8 in endometrium and endometrial adenocarcinoma. Mod Pathol 19:1429–1436.

Gomez O, Romero A, Terrado J, Mesonero JE. 2006. Differential expression of glucose transporter GLUT8 during mouse spermatogenesis. Reproduction 131:63–70.

Gorovits N, Cui L, Busik JV, Ranalletta M, Hauguel de-Mouzon S, Charron MJ. 2003. Regulation of hepatic GLUT8 expression in normal and diabetic models. Endocrinology 144:1703–1711.

Ibberson M, Uldry M, Thorens B. 2000. GLUTX1, a novel mammalian glucose transporter expressed in the central nervous system and insulin-sensitive tissues. J Biol Chem 275:4607–4612.

Joost HG, Thorens B. 2001. The extended GLUT-family of sugar/polyol transport facilitators: Nomenclature, sequence characteristics, and potential function of its novel members (review). Mol Membr Biol 18:247–256.

Keembiyehetty C, Augustin R, Carayannopoulos MO, Steer S, Manolescu A, Cheeseman CI, Moley KH. 2006. Mouse glucose transporter 9 splice variants are expressed in adult liver and kidney and are up-regulated in diabetes. Mol Endocrinol 20:686–697.

Kellett GL. 2001. The facilitated component of intestinal glucose absorption. J Physiol 531:585–595.

Kellett GL, Brot-Laroche E. 2005. Apical GLUT2: A major pathway of intestinal sugar absorption. Diabetes 54:3056–3062.

Kellett GL, Helliwell PA. 2000. The diffusive component of intestinal glucose absorption is mediated by the glucose-induced recruitment of GLUT2 to the brush-border membrane. Biochem J 350:155–162.

Kim ST, Moley KH. 2007. The expression of GLUT8, GLUT9a, and GLUT9b in the mouse testis and sperm. Reprod Sci 14:445–455.

Lewis BP, Green RE, Brenner SE. 2003. Evidence for the widespread coupling of alternative splicing and nonsense-mediated mRNA decay in humans. Proc Natl Acad Sci USA 100:189–192.

Li Q, Manolescu A, Ritzel M, Yao S, Slugoski M, Young JD, Chen XZ, Cheeseman CI. 2004. Cloning and functional characterization of the human GLUT7 isoform SLC2A7 from the small intestine. Am J Physiol Gastrointest Liver Physiol 287:G236–G242.

Lisinski I, Schurmann A, Joost HG, Cushman SW, Al-Hasani H. 2001. Targeting of GLUT6 (formerly GLUT9) and GLUT8 in rat adipose cells. Biochem J 358:517–522.

Manolescu A, Salas-Burgos AM, Fischbarg J, Cheeseman CI. 2005. Identification of a hydrophobic residue as a key determinant of fructose transport by the facilitative hexose transporter SLC2A7 (GLUT7). J Biol Chem 280: 42978–42983.

Manolescu AR, Witkowska K, Kinnaird A, Cessford T, Cheeseman C. 2007. Facilitated hexose transporters: New perspectives on form and function. Physiology 22:234–240.

Matlin AJ, Clark F, Smith CW. 2005. Understanding alternative splicing: towards a cellular code. Nat Rev Mol Cell Biol 6:386–398.

Mesonero J, Matosin M, Cambier D, Rodriguez-Yoldi MJ, Brot-Laroche E. 1995. Sugar-dependent expression of the fructose transporter GLUT5 in Caco-2 cells. Biochem J 312:757–762.

Mesonero JE, Gloor SM, Semenza G. 1998. Processing of human intestinal prolactase to an intermediate form by furin or by a furin-like proprotein convertase. J Biol Chem 273:29430–29436.

Mesonero JE, Tanfın Z, Hilly M, Colosetti P, Mauger JP, Harbon S. 2000. Differential expression of inositol 1,4,5-trisphosphate receptor types 1, 2 and 3 in rat myometrium and endometrium during gestation. Biol Reprod 63: 532–537.

Moreno-Aliaga MJ, Matsumura F. 1999. Endrin inhibits adipocyte differentiation by selectively altering expression pattern of CCAAT/enhancer binding protein-alpha in 3T3-L1 cells. Mol Pharmacol 56:91–101.

Moreno-Aliaga MJ, Stanhope KL, Havel PJ. 2001. Transcriptional regulation of the leptin promoter by insulin-stimulated glucose metabolism in 3t3-l1 adipocytes. Biochem Biophys Res Commun 283:544–548.

Pinto AB, Carayannopoulos MO, Hoehn A, Dowd L, Moley KH. 2002. Glucose transporter 8 expression and translocation are critical for murine blastocyst survival. Biol Reprod 66:1729–1733.

Piroli GG, Grillo CA, Hoskin EK, Znamensky V, Katz EB, Milner TA, McEwen BS, Charron MJ, Reagan LP. 2002. Peripheral glucose administration stimulates the translocation of GLUT8 glucose transporter to the endoplasmic reticulum in the rat hippocampus. J Comp Neurol 452:103–114.

Piroli GG, Grillo CA, Charron MJ, McEwen BS, Reagan LP. 2004. Biphasic effects of stress upon GLUT8 glucose transporter expression and trafficking in the diabetic rat hippocampus. Brain Res 1006:28–35.

Romero A, Terrado J, Brot-Laroche E, Mesonero JE. 2007. Glucose transporter GLUT8 mRNA expression in intestinal Caco-2 cells is regulated by growth and metabolism. Horm Metab Res 39:62–64.

Sasaki T, Minoshima S, Shiohama A, Shintani A, Shimizu A, Asakawa S, Kawasaki K, Shimizu N. 2001. Molecular cloning of a member of the facilitative glucose transporter gene family GLUT11 (SLC2A11) and identification of transcription variants. Biochem Biophys Res Commun 289:1218–1224.

Scheepers A, Schmidt S, Manolescu A, Cheeseman CI, Bell A, Zahn C, Joost HG, Schurmann A. 2005. Characterization of the human SLC2A11 (GLUT11) gene: Alternative promoter usage, function, expression, and subcellular distribution of three isoforms, and lack of mouse orthologue. Mol Membr Biol 22:339–351.

Schiffer M, Susztak K, Ranalletta M, Raff AC, Bottinger EP, Charron MJ. 2005. Localization of the GLUT8 glucose transporter in murine kidney and regulation in vivo in nondiabetic and diabetic conditions. Am J Physiol Renal Physiol 289:F186–F193.

Scholzova E, Malik R, Sevcik J, Kleibl Z. 2007. RNA regulation and cancer development. Cancer Lett 246:12–23.

Taha MO, Ribeiro EB, Sardinha FL, Fraga MM, Fagundes DJ, Caricati-Neto A. 2004. Interposition of a distal colon segment following 80% jejunum-ileal resection: Evaluation of glucose absorption in rats. Transplant Proc 36:370–372.

Uldry M, Thorens B. 2004. The SLC2 family of facilitated hexose and polyol transporters. Pflugers Arch 447:480–489.

Wong JM, de Souza R, Kendall CW, Emam A, Jenkins DJ. 2006. Colonic health: Fermentation and short chain fatty acids. J Clin Gastroenterol 40: 235–243.

Wood IS, Trayhurn P. 2003. Glucose transporters (GLUT and SGLT): Expanded families of sugar transport proteins. Br J Nutr 89:3–9.

Wright EM. 1993. The intestinal Na+/glucose cotransporter. Annu Rev Physiol 55:575–589.

Wright EM, Turk E. 2004. The sodium/glucose cotransport family SLC5. Pflugers Arch 447:510–518.

Wright EM, Martin MG, Turk E. 2003. Intestinal absorption in health and disease—Sugars. Best Pract Res Clin Gastroenterol 17:943–956.

Wu X, Li W, Sharma V, Godzik A, Freeze HH. 2002. Cloning and characterization of glucose transporter 11, a novel sugar transporter that is alternatively spliced in various tissues. Mol Genet Metab 76:37–45.