Circadian Periodicity of Intestinal Na\(^+\)/Glucose Cotransporter 1 mRNA Levels Is Transcriptionally Regulated*

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Intestinal expression of the high affinity Na\(^+\)/glucose cotransporter 1 (SGLT1), which absorbs dietary glucose and galactose, exhibits both circadian periodicity in its activity and induction by dietary carbohydrate. Because the daily variation in SGLT1 activity is established by the feeding schedule (whether ad libitum or imposed) and persists in the absence of food, this variation has been described as anticipatory. To delineate the mechanisms regulating SGLT1, its expression was examined in rats maintained in a 12-h photoperiod with free access to chow. SGLT1 mRNA levels varied significantly, with the maximum abundance occurring near the onset of dark and the minimum near the onset of light. The SGLT1 transcription rate was 7-fold higher in the morning (1000–1100 h) than in the afternoon (1600–1700 h). An element for hepatocyte nuclear factor 1 (HNF-1) was identified in the SGLT1 promoter that formed different complexes with small intestinal nuclear extracts, depending on the time when the source animal was killed. Serological tests indicated that HNF-1α was present in complexes throughout the day, while HNF-1β binding exhibited circadian periodicity. We propose that exchange of HNF-1 dimerization partners contributes to circadian changes in SGLT1 transcription. Because SGLT1 mRNA levels also varied in rhesus monkeys (offset by approximately one-half day from rats), a similar mechanism appears to be present in primates.

The central role of glucose in cellular metabolism has led to the evolution of two gene families of eukaryotic glucose transporters, each with a specific cell and tissue expression pattern to subserve its particular functions (for review, see Hediger and Rhoads (1)). Na\(^+\)/coupled glucose transporters (SGLTs), which couple glucose uptake to the inwardly directed electrochemical Na\(^+\) gradient (2), are expressed at the luminal brush-border (apical) surface to absorb glucose. Facilitated glucose transporters (GLUTs), which permit passive movement of glucose across plasma membranes down its concentration gradient, are generally expressed on serosal (basolateral) surfaces to release absorbed glucose into the bloodstream. Identification of several glucose transporters by cDNA cloning over the last several years has permitted characterization of the expression pattern and regulatory behavior of the individual glucose transporters. The high affinity SGLT1 is expressed in the apical membranes of the renal proximal tubule and the intestinal mucosa to (re)absorb glucose, which is released into the bloodstream via basolaterally expressed GLUT2- and GLUT1-facilitated glucose transporters (3–6). The intestine also expresses GLUT5 (7), a facilitated fructose transporter localized to the brush-border membrane (4).

Intestinal glucose transporter expression in rodents exhibits circadian periodicity, induction by carbohydrate intake, and dysregulation in experimental diabetes. Glucose uptake activity in rats fed ad libitum peaks late in the dark phase (8) or early in the light phase (9) and depends on the imposed feeding schedule rather than the light cycle (8, 10). Because the food consumption pattern in rats fed ad libitum is established by the light cycle (11), the circadian rhythmicity of glucose transport activity is “cued by feeding” (10) rather than the result of an inherent oscillatory behavior of the small intestine. Persistence of this periodicity in animals deprived of food has led to the concept that this behavior is anticipatory, i.e. dependent on prior rather than current food intake (12). The recent demonstration of diurnal periodicities in the mRNA levels of SGLT1, GLUT2, and GLUT5 (13) suggests that transcriptional changes may underlie this anticipation, but this hypothesis is as yet untested.

Responsiveness of intestinal glucose transporter expression to carbohydrate intake has been explored by Ferraris et al. (14–17). These authors reported that long term adaptation of mice to a high carbohydrate diet resulted in significantly more intestinal glucose transporters as assayed by the binding of phloridzin (a specific inhibitor of Na\(^+\)/glucose cotransporters) (14). Moreover, from the kinetics of change in phloridzin binding along the crypt-villus axis in response to shifting from a no carbohydrate to a high carbohydrate diet (or vice versa), these authors proposed that enterocytes can alter glucose transporter expression only in response to carbohydrate availability as they exit the crypt (15, 17). However, whether changes occurred at the transcriptional or posttranscriptional level was not addressed. Moreover, the modulation of SGLT1 expression in the absence of dietary shifts was not examined. In rats, a shift from a no carbohydrate to a high carbohydrate diet resulted in increased SGLT1 transcription over a 5-day period (18), but only one time point per day was examined.

Finally, the up-regulation of the glucose transporter expres-
sion in streptozotocin-induced diabetic rats (19) appears to be due to a premature induction of SGLT1 mRNA and protein along the crypt-villus axis (20). However, the molecular mechanism(s) underlying this induction are not known.

To elucidate the mechanisms underlying SGLT1 regulation, we began by examining the influence of nutritional status (dietary carbohydrate content and fasting-refeeding) on intestinal SGLT1 mRNA levels. Remarkably, a distinct circadian rhythmicity in SGLT1 mRNA levels and transcription were observed in the absence of dietary manipulation. These findings place a specific interpretation on the anticipatory expression of digestive functions (12). Furthermore, these data suggest that dietary influences regulating the SGLT1 gene and its pathological dysregulation are more complex than previously appreciated.

EXPERIMENTAL PROCEDURES

Chemicals—[32P]-Labeled nucleotides were obtained from NEN Life Science Products. Antiserum recognizing all three rat C/EBP isoforms was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). All other chemicals were of the highest purity commercially available.

Animals—Rats were maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were housed in wire-bottomed cages within an AAALAC-accredited animal facility under an approved research protocol. The room was maintained at 21–22 °C with a 12-h photoperiod (0700–1900 h). Six-week-old female Sprague-Dawley rats (Taconic Farms, Taconic, NY) were adapted to the animal facility for at least 5 days prior to the initiation of any study. Animals were allowed ad libitum access to water and standard laboratory chow (Rodent Laboratory Chow, Purina 5001) throughout each study.

Duodenal biopsies were obtained from three young adult female rhesus monkeys housed at the New England Regional Primate Research Center (Southborough, MA) under a protocol approved by Harvard Medical Area Standing Committee on Animals. Monkeys were fasted overnight before biopsy under ketamine-general anesthesia and were not provided with food overnight. Spleen and muscle samples were obtained from each animal under ketamine-general anesthesia and were not provided with food overnight.

Nucleic Acid Probes—The following cDNA probes were used for Northern blot and transcription rate assays: rat SGLT1 (21), human SGLT1 (22), rat GLUT2 (23), rat GLUT5 (24), rat slc2a10 (25), rat liver-type pyruvate kinase (26), human c-myc (provided by E. Schmidt, Massachusetts General Hospital), and c-myc (provided by A. Rustgi, Massachusetts General Hospital). Probes were radiolabeled with [α-32P]dCTP using MegaPrime (Amersham Corp.). For nuclear run on blot transcription assay, plasmids were linearized and immobilized on Hybond-N membrane. pBSK+ (Stratagene, La Jolla, CA) was used as a negative control. For genomic cloning, a 127-nt BstXI fragment from the rat SGLT1 cDNA (GenBank accession no. U03120) was labeled with [α-32P]dCTP using MegaPrime and a synthetic noncomplementary to the 3′-terminus of the upper strand instead of the random primers included in the kit.

Northern Blot Analysis—Under anesthesia, jejunal segments were removed, flushed with cold phosphate-buffered saline, and everted, and the mucosa was scraped. One portion of mucosa was frozen in liquid nitrogen for later extraction of RNA (27), while the other was used to prepare postnuclear membranes (see below). Equal amounts (20 μg) of RNA were loaded per lane on 1% formaldehyde-agarose gels. After electrophoresis, the RNA integrity was confirmed by ethidium bromide staining, and the RNA was transferred to Hybond-N filters (Amersham Corp.). Following hybridization in Rapid-Hybe buffer (Amersham Corp.), blots were washed three times in 2× SSC, 0.1% SDS (1× SSC is 0.15 μM NaCl, 15 mM sodium citrate, pH 7.0) for 10 min at room temperature, once in 1× SSC, 0.1% SDS for 15 min at 65 °C, three times in 0.1× SSC, 0.1% SDS for 20 min at 65 °C, and exposed on Kodak XAR-5 film at −80 °C with double intensifying screens. To detect different mRNAs, probes were stripped from filters by boiling in 0.1% SDS. Filters were successfully probed four times without loss of sensitivity. Densitometry was performed using NIH Image software.

Postnuclear membranes were prepared from isolated intestinal mucosal cells (see below) according to the method described by Burant et al. (20). Equal amounts of membrane protein (10 μg) as estimated by the Bradford method (Bio-Rad) were resolved by SDS-polyacrylamide gel electrophoresis (12% gel), and electrophoretically transferred to PVDF-Plus membrane filter (Micron Separations Inc., Westboro, MA). SGLT1 protein was detected using a 1:7000 dilution of antiserum raised against the rabbit SGLT1 peptide sequence (amino acids 604–615) (28) and the Western Light Chemiluminescence detection kit (Tropix, Bedford, MA).

Preparation of Nuclei from Isolated Intestinal Mucosal Cells and Transcription Rate Assay—Intestinal mucosal cells were isolated from small villi at 4 °C (29). The three villus tip fractions (V1, V2, and V3), consisting of Na-Hepes, pH 7.4, 3 mM MgCl2, 10 mM NaCl, 0.5 mM dithiothreitol, and 0.5% Nonidet P-40 with 10 gentle strokes in a Dounce homogenizer using the loose (B) pestle. Nuclei were recovered by centrifugation for 10 min at 500 × g, carefully suspended in 1 volume of storage buffer (Tris-CL, 50 mM, pH 8.3, 5 mM MgCl2, 40% glycerol, and 0.1 mM EDTA), and stored at −80 °C in 200-μl aliquots. Transcription rate assays were performed by a modification of the method of Greenberg (30). Following quantitation of incorporation, an equal number of disintegrations/min from each sample were precipitated for 30 min with 0.4 M LiCl and 2 volumes of ethanol at −80 °C, then recovered by centrifugation at 12,000 × g for 30 min at 4 °C. The RNA samples were dissolved in 1.0 ml of Rapid-Hybe buffer, transferred to siliconized glass scintillation vials containing the filter-bound plasmids (5 μg each), and hybridized for 20 h at 65 °C. Following washing, the filters were exposed to Reflection 32 film (NEN Life Science Products).

Preparation of Nuclear Extracts—Extracts were prepared from isolated intestinal epithelial cell nuclei by a modification of the method of Dignam et al. (31). Extraction was performed with 2 volumes of buffer C (25 mM Na-Hepes, pH 7.4, 0.4 mM NaCl, 0.2 mM EDTA, 0.5 mM dithiothreitol) containing 0.4 mM phenylmethylsulfonyl fluoride and 0.5 μg/ml each aprotinin, leupeptin, and antipain. Debris was removed by centrifugation at 11,000 × g for 15 min, dialyzed against buffer D (20 mM Na-Hepes, pH 7.9, 0.1 mM EDTA, 0.2 mM EGTA, 0.5 mM dithiothreitol, and 20% glycerol) for 3–4 h, and centrifuged again prior to storage at −80 °C.

Genomic Cloning—A rat genomic library (500,000 plaques; CLONTECH, Palo Alto, CA) was screened with the SGLT1 BstXI fragment using protocols provided by the supplier. Of approximately 20 positive clones identified, sequence analysis (Sequenase, U. S. Biochemicals, Cleveland, OH) and general analysis indicated that clone λ-H2 contained a 17-kb insert that included Exon 1.

Electrophoretic Mobility Shift Assays (EMSA)—EMSA were performed using a modification of the method of Bernard (32). Briefly, binding reactions with 10 μg of protein, 125 ng of salmon testis DNA (Sigma), and 10,000 cpm double-stranded oligonucleotide probe were prepared on ice, incubated at room temperature for 15 min, and electrophoresed for 45 min in a 4% polyacrylamide gel at 4 °C in a Mini-Protein II gel electrophoresis apparatus (Bio-Rad). Binding buffer consisted of Na-Hepes, pH 7.4, 100 mM KCl, 5 mM MgCl2, 0.5 mM EDTA, 1 mM dithiothreitol, and 5% glycerol. Electrophoresis buffer was 0.5× TBE (1× TBE is 89 mM Tris, 89 mM boric acid, pH 8.3, and 2 mM EDTA). The probe was prepared by annealing complementary synthetic oligonucleotides, 5′-end-labeled with T4 polynucleotide kinase (New England Biolabs, Beverly, MA), and purified by nondenaturing polyacrylamide gel electrophoresis. Antisera to HNF-1 isoforms α and β were provided by Dr. G. R. Crabtree (33), and 0.5 μl was added to each indicated binding reaction.

RESULTS

To examine the regulation of the SGLT1 gene, we initially tested two protocols to assess the influence of dietary carbohydrate on intestinal SGLT1 mRNA abundance: varying the carbohydrate content in the chow and fasting/refeeding. While both interventions altered mRNA levels, temporal changes in mRNA abundance in control animals were clearly greater than those induced by experimental manipulations. Furthermore, these changes were reproducible from day to day, following a circadian periodicity. To examine the basis for the rhythmicity in SGLT1 mRNA levels occurring in animals maintained under standard conditions (12-h photoperiod with free access to food), we compared the experimental conditions below. For rats, both activity and food consumption are greater during the night than the day. Thus, the observed changes in SGLT1 expression are likely in response to the feeding pattern established by the photoperiod and not intrinsic clock signals per se.

Intestinal SGLT1 Expression in the Rat Varies in a Circadian Manner at Both the mRNA and Protein Levels—To follow
FIG. 1. Daily variation in intestinal SGLT1 expression. Northern and Western blot analysis of small intestinal mucosal cells. See “Experimental Procedures” for details. A, Northern blot analysis. Female Sprague-Dawley rats were killed at the indicated times, and their jejunal mucosa were scraped for preparation of RNA. Total jejunal RNA (20 μg/lane) was sequentially hybridized with 32P-labeled cDNA probes for SGLT1, sucrase, liver-type pyruvate kinase (L-PK), c-myc, and α-tubulin (α-Tub). B, Western blot analysis. Female Sprague-Dawley rats were killed at the indicated times and postnuclear membranes were prepared from isolated villus mucosal cells. Membrane proteins (10 μg/lane) were probed with rabbit polyclonal antibodies against SGLT1, c-myc, and α-tubulin (α-Tub). C, densitometric analysis of rat jejunal SGLT1 mRNA and protein levels. Film intensity signals of SGLT1 mRNA (n = 6 per time point) and protein (n = 4 per time point) from rats killed at 6-h intervals were quantitated by scanning densitometry and indexed to the respective the abundance of intestinal SGLT1 mRNA over the course of a day. Northern blots were prepared from RNA extracted from jejunal mucosal scrapings of rats killed at 6-h intervals. In a typical experiment (Fig. 1A), significantly more SGLT1 mRNA was present in the jejunal mucosa of rats killed at 1600 and 2200 h than those killed at the other times. In six sets of animals examined (age range, 7–9 weeks), densitometry indicated that the level at 2200 h was 3.7 ± 0.1 times (p < 0.001) greater than at 0400 h (Fig. 1C). Sucrase-isomaltase and liver-type pyruvate kinase (L-PK) showed similar circadian variations (Fig. 1A). In another experiment in which rats were sacrificed at 3-h intervals around the beginning of the dark phase, the peak SGLT1 mRNA level occurred at 1900 h, the time of lights out (not shown).

Western blotting revealed that SGLT1 protein levels were higher at both 1600 and 2200 h, consistent with the increased mRNA levels at those times. (Fig. 1, B and C). Thus, the increased transporter activity observed by other investigators (8, 9) appears to be due to increased SGLT1 protein synthesis from the increased mRNA levels.

The intestinal expression as determined by Northern blotting of several other carbohydrate responsive genes, GLUT2, GLUT5, and lactase-phloridzin hydrolase, exhibited similar behavior (not shown). These genes also varied in the ileum, but to a lesser extent. The mRNA levels of glyceraldehyde phosphate dehydrogenase (not shown), as well as that for α-tubulin (Fig. 1A), were constant. Also shown is that the mRNA level for c-myc, a marker of cell proliferation, does not change in intestinal mucosal cells over the course of the day (Fig. 1A).

Monkeys also Exhibit Circadian Variation in SGLT1 mRNA Levels—To determine whether the SGLT1 mRNA level also changes in primates, RNA was extracted from duodenal biopsies of rhesus monkeys obtained at either 0900 or 2000 h (taken 2 weeks apart). In the analysis of the monkey shown, the SGLT1 mRNA level was over 5-fold higher in the morning than in the evening (Fig. 1D). Monkeys express two SGLT1 mRNA species, probably corresponding to the 4.8- and 2.6-kb species found in humans (22). Two additional monkeys exhibited identical behavior (densitometric differences were 5.5-, 4.7-, and 6.1-fold higher in the morning for the three monkeys). Thus, a daily rhythmicity of SGLT1 mRNA levels appears to be a general phenomenon, with its occurrence in the diurnal primate shifted approximately half a day from that found in the nocturnal rat.

SGLT1 Transcription Varies in a Circadian Manner—To assess the involvement of transcription in the SGLT1 mRNA periodicity, nuclei were isolated from rat jejunal mucosal cells for a transcription rate assay. Cells can be sequentially eluted from the intestinal mucosa along the villus-crypt axis by incubating the intestine in buffer containing a chelating agent to remove divalent cations (34). To optimize preservation of transcriptional activity of nuclei during isolation, a low temperature modification employing citrate as the chelator in place of EDTA was used (29). In nuclei isolated from combined villus tip enterocytes prepared by this method, SGLT1 transcription was several times higher in nuclei from enterocytes obtained at 1100 versus 1630 h (Fig. 2A). As expected, the α-tubulin signal was similar at both times. In four experiments, the SGLT1 signal estimated densitometrically (normalized to the α-tubulin intensity obtained at 0400 h; *p < 0.02 versus 0400 h; **p < 0.001 versus 0400 h (log-transformed data). Error bars for SGLT1 protein at 1000 and 2200 h fell within the column borders. D SGLT1 mRNA levels in a young adult female rhesus monkey. Duodenal biopsies were obtained from the same monkey at 0900 h (AM) and 2000 h (PM) 2 weeks apart. Total RNA (5 μg) was hybridized with a 32P-labeled cDNA for human SGLT1.
lin signal) was 6.4 ± 1.0 times greater between 1000 and 1100 h than between 1600 and 1700 h \((p < 0.007, 2\text{-}tailed \text{ t test})\). A GLUT2 signal was not detected at either time examined while that of GLUT5 appeared similar at both times (Fig. 2A). These data indicate that the SGLT1 transcription rate is not constant, but also exhibits periodicity. In a study in which nuclei were isolated at 6-h intervals, the marked increase in SGLT1 transcription was observed only at 1000 h, but not at the other times examined (Fig. 2B). A modest signal was also detected for Sucrase and GLUT2 at 1000 h, but not at the other times. The rates of transcription of glyceraldehyde-3-phosphate dehydrogenase and \(\alpha\)-tubulin were constant.

**Cloning the Rat SGLT1 Gene Promoter**—The temporal change in the SGLT1 transcription rate suggested that the SGLT1 promoter contains one or more elements responsible for its periodic activity. To obtain information on the rat SGLT1 promoter, a genomic library was probed with a 5'-terminal BstXI restriction fragment from the rat cDNA (21). One of the positive clones contained a 6-kb BamHI fragment that included the first exon and approximately 1 kb of 5'-flanking sequence of the rat SGLT1 gene. A restriction map of the upstream 2.3 kb of this fragment and the sequence of the first 971 nt are shown in Fig. 3. The first exon-intron boundary occurs within the same codon as in humans (35). The 300 nt upstream of the TATA box show 70% identity to the same region in the human gene (35). The putative mRNA start site (by homology to the human sequence) is 30 base pairs downstream of the TATA box. The reported sequence of the rat SGLT1 cDNA clone (21) contained 69 bp upstream of the TATA box (nt 867 in Fig. 3B). It is not clear whether these additional nucleotides arose from a cryptic upstream start site or were merely part of an aberrant transcript. Analysis of the sequence for transcription factor binding sites (Wisconsin Package, Genetics Computer Group, Madison, WI) using the TfdSites data base (36) revealed the presence of potential sites for MLTF/USF (major late transcription factor/upstream stimulatory factor) and HNF-1 (hepat...
cytomegalovirus nuclear factor 1) (Fig. 3B). Of note, the presence of the HNF-1 and the MLTF/USF sites is reminiscent of the carbohydrate response element found in liver-type pyruvate kinase, which in addition to these two sites also contains closely linked sites for NF1 and HNF-4 (37). These latter two sites may be responsible for the largely liver expression of this gene. However, liver type pyruvate kinase is also expressed as a minor pyruvate kinase isoform in the small intestine, but it shows a similar diet responsiveness in both tissues (38).

Nuclear Factor Binding to the SGLT1 Promoter Exhibits Circadian Periodicity—The observed circadian changes in SGLT1 transcription suggest the presence of concomitant circadian changes in the abundance or activity of transcription factor(s). Because the carbohydrate response element in the liver type pyruvate kinase gene is important in its regulation, we reasoned that the analogous region in the SGLT1 gene might account for its circadian periodicity. In preliminary experiments, EMSAs using a 53-bp probe containing both the MLTF/USF and the HNF-1 binding sites (Fig. 3B; nt 878–930), two different complexes were formed depending on the time intestinal mucosal cells were isolated as sources of nuclear extracts (not shown). Competition assays with a series of overlapping 21-mers indicated that the 3′-terminus (Fig. 3B; nt 910–930), which included the putative HNF-1 element, contained the element(s) able to form the two different complexes (not shown). Further experiments were performed with the 21-bp probe containing the HNF-1 site, referred to as the HNF probe. When the HNF probe was end-labeled, temporally dependent binding was also observed, a more rapidly migrating AM complex at 1000 h and again at 0400 h and a slower and broader PM complex at 1600 and 2200 h (Fig. 4A, left). Inclusion of a 500-fold excess of unlabeled competitor reduced the signal obtained for either complex to below detectable levels (Fig. 4A, right).

To determine whether HNF-1 isoforms were involved in either the AM or the PM complex, the effect of antisera to the α and β isoforms of HNF-1 (33) were examined in EMSAs. When antiserum to HNF-1β was included in the binding reaction, the PM complex was absent and a new complex co-migrating with the AM complex appeared (Fig. 4B). Thus, the AM and the PM complexes differed both in gel migration and in sensitivity to HNF-1β antisera. Addition of antiserum to HNF-1β to the binding reaction immediately prior to electrophoresis did not affect migration of either complex (data not shown). This behavior suggests that the HNF-1β antisera prevents the binding of HNF-1β.

On the other hand, when antiserum to HNF-1α was added to the binding reactions, two supershifted complexes could be observed in both the AM and PM complexes (Fig. 4C). A probe derived from the rat albumin HNF1 site (39) formed similar DNA-protein complexes with intestinal mucosal nuclear extracts, and an unlabeled albumin HNF probe competed with the SGLT1 HNF probe and vice versa (not shown). Thus, HNF-1α appears to be present in both the AM and PM complexes, while HNF-1β is present at least the PM complex. The presence of HNF-1β in the AM complex cannot be unambiguously established at this time. Although the HNF probe also overlaps with a potential C/EBP binding site, an antisense directed against all three isoforms of rat C/EBP failed to affect the migration of either the AM or the PM complex (not shown).

DISCUSSION

A daily periodicity in the intestinal transport activity and several other digestive proteins were documented before the genes responsible were characterized at the molecular level. Nevertheless, the observed activity changes were clearly ascribed to the feeding pattern (whether ad libitum or scheduled) rather than to an inherent circadian signal. However, the specific mechanisms linking food intake to gene expression remained obscure. The cloning of cDNAs from various diet-responsive genes has now provided the opportunity to explore their molecular regulation. Using the rat SGLT1 cDNA as a probe, we have detected a periodicity in intestinal SGLT1 expression, both at the level of mRNA abundance and transcriptional activity. Moreover, analysis of the 5′-flanking region of the rat SGLT1 gene has revealed an HNF-1 element capable of forming different complexes with nuclear extracts depending on the time of day intestinal mucosal cells were isolated. These studies provide evidence for a periodicity in HNF-1 activity,
possibly occurring via exchanges in dimerization partners.

The Timing of SGLT1 Transcription—The marked changes in SGLT1 mRNA levels over a 24-h period likely result from modulations in both net mRNA synthesis and degradation. To understand the mechanisms underlying these changes, we initially focused on transcription. When transcription was examined in nuclei isolated at either 1100 and 1630 h, points near the lowest mRNA abundance and the midpoint of the mRNA increase, respectively, it was found that the SGLT1 transcription rate at the earlier time was 7-fold higher than at the later time. This observation raises two points. First, for nocturnal rats, transcription is high and mRNA levels increase during their inactive period in the light. Thus, the cue(s) to increase SGLT1 transcription must lie either in (i) a specific stage of the digestive process of the previous night’s meal or (ii) a more global habituation pattern. While the present data do not distinguish between these two possibilities, it remains clear that anticipatory expression of SGLT1 includes induction of SGLT1 gene transcription. Second, because mRNA levels are still increasing when SGLT1 transcription rate subsides in the afternoon, it is likely that there is a concomitant increase in mRNA stability. Likewise, the precipitous drop of mRNA levels during the night may be due to an increase in the mRNA degradation rate. This hypothesis remains to be tested directly.

Role of the HNF-1 Promoter Element—Isolation of the rat SGLT1 promoter region has permitted the identification of an HNF-1 binding site immediately upstream of the TATA box. HNF-1 proteins are a group of atypical homeodomain proteins (40) expressed in liver, kidney, and gut involved in the tissue-specific expression of several genes in these tissues (39). To date, α (33) and β (41) isoforms have been described, having similar target binding sequences but differing in relative abundance in expressing tissues. HNF-1 proteins form both homo- and heterodimers, with dimerization stabilized and transcriptional activity enhanced by the further binding of a dimer of a third partner named DCoH (for dimerization cofactor of HNF-1) (42). In the present study, we have found that the change in the SGLT1 transcription rate is accompanied by a change in the HNF-1 isoform complement at the HNF-1 site as detected by gel-shift assays. Specifically, early in the light phase when transcription is high (1000–1100 h), nuclear extracts form the AM complex with the HNF-1 probe containing HNF-1α. Later in the light phase when transcription is lower (1600–1630 h), the PM complexes form containing both HNF-1α and HNF-1β. Thus, it is tempting to speculate that SGLT1 transcription is in part regulated by the DCoH-mediated exchange of an HNF-1α for an HNF-1β partner during transcriptional activation. Further studies will be necessary to establish the composition of the HNF-1 dimers unambiguously as well as to determine the involvement of DCoH.

It is instructive to compare the HNF-1 site identified in the SGLT1 gene to HNF-1 sites in related genes (Scheme 1). First, the rat SGLT1 sequence shows an 11/13 match to the consensus HNF-1 target sequence (39). Moreover, the 13 nucleotides are identical between the rat and human and occur in the same position in relation to the TATA boxes. Also of note, the expression of two other carbohydrate-responsive genes, sucrase-isomaltase (43) and liver type pyruvate kinase (including its expression in the small intestine (38), are dependent on HNF-1. The HNF-1 elements in these two genes show 9/13 and 8/13 identity to the rat SGLT1 element, respectively. In particular, the terminal 5 nt, “TAAAC,” are present in all four genes. It will be interesting to examine the nature of the HNF-1 complexes formed using these two sequence elements as probes. Finally, similar HNF-1 binding sequences are not present in the proximal promoter regions of either GLUT2 (44) or GLUT5 (45).
SGLT1 Expression in the Small Intestine

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