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Biochimica et Biophysica Acta 1561 (2002) 180–187

BIOCHIMICA ET BIOPHYSICA ACTA
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Expression and promoter analysis of SLC19A2 in the human intestine

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Received 30 October 2001; received in revised form 5 December 2001; accepted 13 December 2001

Abstract

The molecular mechanism and regulation of the intestinal uptake process of dietary thiamine is not well understood. Previous studies have established the involvement of a carrier-mediated system for thiamine uptake in the human intestine. Recently a human thiamine transporter, SLC19A2, was cloned from a number of human tissues. Little, however, is known about expression of the SLC19A2 message along the native human gastrointestinal tract, and no analysis of its promoter in intestinal tissue is available. Therefore, the current studies were aimed at investigating the expression of SLC19A2 in the human gastrointestinal tract and at analyzing the promoter of this potential intestinal thiamine transporter. First we cloned SLC19A2 cDNA from a human intestinal cell line (Caco-2) by reverse transcriptase–polymerase chain reaction, then used this cDNA as a probe in Northern blot analysis. SLC19A2 message was found to be expressed in all gastrointestinal tissues in the following order: liver > stomach > duodenum > jejunum > colon > cecum > rectum > ileum. SLC19A2 was also expressed at the protein level in Caco-2 cells and in native human small intestine by Western blot analysis. We also cloned the 5′-regulatory region of the SLC19A2 gene and confirmed activity of its promoter following transfection into intestinal epithelial Caco-2 cells. Furthermore, we identified the minimal promoter region required for basal activity of SLC19A2 in these cells which was found to be mainly encoded in a sequence between −356 and −36, and included multiple *cis*-regulatory elements. Transcription initiation sites of the SLC19A2 gene in intestinal epithelial Caco-2 cells were also identified by 5′-rapid amplification of cDNA ends. These results demonstrate that SLC19A2 is expressed in various regions of the human gastrointestinal tract. In addition, the results provide the first characterization of the SLC19A2 promoter. These findings raise the possibility that SLC19A2 may play a role in the normal intestinal thiamine absorption process.

Keywords: Caco-2 cell; Thiamine uptake; Promoter activity

1. Introduction

The water-soluble vitamin thiamine (B_1) is an essential micronutrient for normal cellular function, growth, and development. Thiamine pyrophosphate, the coenzyme form of the vitamin, plays an important role in carbohydrate metabolism [1]. Nutritional deficiencies of thiamine can lead to cardiovascular and neurological disorders [1–3]. Cardiovascular dis-

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0005-2736/02/$ – see front matter
PII: S0005-2736(02)00341-3

orders include peripheral vasodilation, biventricular myocardial failure, edema, and potentially acute fulminant cardiovascular collapse. Neurological disorders that have been observed are confusion, disordered ocular motility, neuropathy, and ataxia of gait. Thiamine deficiency also occurs in alcoholics [3–6], diabetics [7], coeliac and renal disease patients [8], the elderly [9], and individuals on long term diuretic medications [10]. Furthermore, patients with thiamine-responsive megaloblastic anemia (TRMA), an autosomal recessive disorder characterized by manifestations that include sensorineural deafness, and diabetes mellitus, respond in varying degrees to thiamine treatment [11,12].

Humans and other mammals have lost their ability to synthesize thiamine, and thus, must obtain the vitamin from exogenous sources. Intestinal absorption is the initial site of uptake and therefore must play a critical role in regulating thiamine body homeostasis. Thus, understanding the molecular mechanism(s) and regulation of thiamine uptake in the intestine is of significant importance. Previous studies using a variety of human and animal intestinal preparations have established the involvement of a specialized carrier-mediated mechanism for thiamine uptake ([13,14] and references therein). Our laboratory has shown that purified human brush border membrane vesicles and cultured human intestinal epithelial cells also posses a carrier-mediated system for thiamine uptake [15–17]. Several groups have recently identified a human thiamine transporter, SLC19A2, which is predicted to encode a multitransmembrane vitamin transport protein [18–21]. A mouse orthologue (Slc19a2) to the human thiamine transporter has also been recently identified [22]. When the SLC19A2 cDNA is expressed in mammalian cells, it specifically induced thiamine uptake [19]. Little, however, is known about expression of this gene in the different regions of the human gastrointestinal tract, and no information is available about its 5′-regulatory region. The results of our current study demonstrate that the SLC19A2 message is expressed in different regions of the human gastrointestinal tract. In addition, the results provide the first characterization of the 5′-regulatory region of the human SLC19A2 gene, and identify the minimal region required for its basal activity.

2. Materials and methods

2.1. Materials

Restriction enzymes were purchased from New England Biolabs (Beverly, MA). DNA polymerase was purchased from Clontech (Palo Alto, CA). Routine biochemicals were all of molecular biology quality, and purchased from Fisher Scientific Corp. (Tustin, CA). Cell culture reagents were purchased from Sigma Chemical Co. (St. Louis, MO), and Life Technologies (Rockville, MD). Fetal bovine serum (FBS) was obtained from Omega Scientific (Tarzana, CA). DNA oligonucleotide primers were purchased from Sigma Genosys (Woodlands, TX).

2.2. Cloning of the SLC19A2 cDNA from Caco-2 cells

The SLC19A2 cDNA was cloned from Caco-2 cells by reverse transcriptase–polymerase chain reaction (RT–PCR). Briefly, poly(A)$^+$ RNA isolated from Caco-2 cells was primed with a gene specific primer 5′-GCTGCTGTGAAGTCAAGAAAT-3′ to generate first-strand cDNA using an RT–PCR kit (Life Technologies). The RT–PCR product was subsequently used in a PCR with the same reverse primer, and a gene-specific forward primer 5′-CGCGCCCCGGATGGATGT-3′ (see Table 2 for the position of the primers). The nucleotide sequence of the resulting PCR product was determined by sequencing using a commercial vendor (Seqwright, Houston, TX) and found to be identical to the open reading frame (ORF) of SLC19A2 [18–21].

2.3. Northern and Western blot analysis

Northern blot analysis was performed as previously described [16]. The full-length ORF SLC19A2 clone from Caco-2 cells was randomly labeled with [^{32}P]dCTP, and used as a probe on a commercially available blot that contained poly(A)$^+$ RNA (2 μg) from different regions of the human digestive tract (Clontech). The probe was simultaneously used on a blot containing an equivalent amount of poly(A)$^+$ RNA (2 μg) isolated from Caco-2 cells. The blots were stripped and reprobed with a labeled human β-actin cDNA. SLC19A2 message level was normal-

Table 1
Preparation of the promoter and promoter deletion constructs

Construct name	Enzyme/primers	Position	Size
pGL3-P	*Kpn*I	−2250 to −36	2214
pGL3-PΔ1	*Nru*I	−2179 to −36	2143
pGL3-PΔ2	*Nde*I	−1645 to −36	1609
pGL3-PΔ3	*Pvu*II	−450 to −36	414
pGL3-PΔ4	F7/GSP9	−429 to −36	393
pGlL3-PΔ5	*Aat*II	−356 to −36	320
pGL3-PΔ6	F6/GSP9	−317 to −36	281
pGL3-PΔ7	F3/GSP9	−275 to −36	239
pGL3-PΔ8	F4/GSP9	−139 to −36	103
pGL3-PΔ9	F5/GSP9	−108 to −36	72

The relative position of the SLC19A2 gene sequence is based on numbering the A of the translation initiation codon as 1 with upstream sequences indicated as negative numbers.

ized relative to the β-actin signal using the Eagle Eye II densitometer (Stratagene). Western blot analysis was performed as previously described [16] using a specific antibody to the SLC19A2 protein on Caco-2 cells and purified human intestinal brush border membranes. Brush border membranes were prepared from the jejunum of organ donors as previously described [15]).

2.4. 5′-RACE

The rapid amplification of cDNA ends (RACE) was performed using the 5′-RACE Version 2.0 kit purchased from Life Technologies. The manufacturer's protocols were followed for all reactions. Two micrograms of RNA isolated from Caco-2 cells was used with the gene specific reverse primer GSP8R in the initial RT–PCR. The first-strand cDNA was isolated and tailed. The PCR of tailed cDNA was then performed using the manufacturer's Abridged Anchor Primer and the gene-specific reverse primer GSP9R. A subsequent nested amplification was performed using the manufacturer's AUAP primer and the gene-specific reverse primer R4. PCR products were analyzed on a 3% agarose gel in TAE buffer, extracted from the gel, purified using the gene-clean II kit (Bio101, Carlsbad, CA), subcloned into the pGEM-T Easy vector (Promega, Madison, WI) and

Table 2
Sequence of primers used in PCR

Primer name	Sequence (5′–3′)	Position
ORF F	CGCGCCCCGGATGGATGT	−14 to 4
ORF R	GCTGCTGTGAAGTCAAGAAAT	1594 to 1615
GSP8 R	GGCAAGAACCAGCATTCG	76 to 93
GSP9 R	ACGTAAAGCTT CCCCTTCCTTCTCCTCCTC	−54 to −36
R4	ACGTAAAGCTT CGCCGCCTCCGGCTACA	−152 to −135
GSP6 F	TATTACCACATCAGCTTAGCTCACA	−2922 to −2898
GSP7 F	ACGTA*CTCGAG*TATGTAGCCCCCTCCAACA	−2669 to −2650
F7	TATCGGTACCAGAGAGCTCCGCCGCAAG	−429 to −412
F6	TATCGGTACCTGTGACCCACGACCAATG	−317 to −300
F3	TATCGGTACCGTCCGCTGTGATTGGTTC	−275 to −258
F4	TATCGGTACCCGGCGGCGAGTCCAGAAC	−139 to −125
F5	TATCGGTACCAGGGAGAAGGCGTCACTC	−108 to −91

The *Hin*dIII site is underlined, the *Xho*I site is italic, and the *Kpn*I site is double underlined. The position number of the primers only represents the matching sequence in the 5′-regulatory region of the *SLC19A2* gene not including the added restriction sites, and is therefore not reflective of primer size. The relative position of the *SLC19A2* gene sequence is based on numbering the A of the translation initiation codon as 1 with upstream sequences indicated as negative numbers.

the sequence was verified by the University of California, Irvine (UCI) core DNA sequencing facility. See Table 2 for the sequence and position of the primers.

2.5. Cloning of the 5′-regulatory region for the SLC19A2 gene

To obtain the genomic DNA fragment that contained the 5′-regulatory region of the *SLC19A2* gene we utilized the sequence information deposited in GenBank (accession number AL021068) for the *SLC19A2* gene and flanking sequence. A PCR was then performed using two gene-specific primers (GSP6F and GSP8R, Table 2) and 100 ng of human genomic DNA (Invitrogen). A reaction buffer and polymerase specially developed to allow amplification through GC-rich regions in the DNA sequence was used (Advantage GC Genomic PCR kit; Clontech). PCR conditions were denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 4 min, then a final extension at 72°C for 15 min. The 3005 basepair (bp) product was run on a 0.7% agarose gel and purified. The purified DNA was then used in a second PCR with nested primers (GSP7F and GSP9R, Table 2) and the same reaction conditions described above. The resulting product of size 2635 bp was purified as described above, subcloned into the pGEM-T Easy vector (Promega) and the DNA sequence was verified (see below).

2.6. Preparation of promoter–luciferase and deletion constructs of the SLC19A2 promoter

The 2635 bp PCR product obtained above was digested with the restriction enzymes *Xho*I and *Hin*dIII (these sites were designed into the nested primers used in the final PCR, Table 2) and subcloned into the pGL3-Basic vector (Promega) digested with the same restriction enzymes. This places the PCR product upstream of the promoter-less luciferase gene for promoter activity analysis. Several deletions were then made using restriction sites common to both the subcloned PCR product and the pGL3-Basic vector multiple cloning site or using gene-specific forward primers and the GSP9R primer (Tables 1 and 2). To generate the deletions using restriction enzymes, the subcloned pGL3-Basic construct was digested with a specific restriction enzyme, agarose gel purified as described above and re-ligated using the Rapid Ligation Kit (Roche, Indianapolis, IN). To generate more deletion constructs gene-specific primers (Tables 1 and 2) were used in a PCR with the 2635 bp subclone-pGL3-Basic construct described above as a template. The PCR products were purified and subsequently subcloned into the *Kpn*I and *Hin*dIII sites of the pGL3-Basic vector. All constructs were verified with DNA sequencing using the UCI core sequencing facility. See Table 1 for restriction sites, primers, and sizes of deletions. See Table 2 for the sequence and position of the primers.

2.7. Cell culture, transfection, and luciferase assay

Four μg of each of the constructs in Table 1 were transfected separately into human intestinal Caco-2 cells using the Lipofectamine reagent (Life Technologies, Gaithersburg, MD) and the manufacturer's procedure. To normalize for transfection efficiency, the cells were co-transfected with 100 ng of pRL-TK (Promega) plasmid along with the promoter constructs. Total cell lysate was prepared from cells 24 h post transfection and firefly luciferase activity was assayed using the Dual Luciferase Kit (Promega) and a Turner Design 20/20 Luminometer (Sunnyvale, CA). The activity was normalized to the Renilla luciferase activity from pRL-TK in the same extract. Data presented are mean ± S.E.M. of at least three independent experiments and given as fold expression over pGL3-Basic expression set arbitrarily at 1. Statistical analysis was performed using Student's *t*-test.

3. Results

3.1. Cloning of the SLC19A2 cDNA from human intestinal epithelial Caco-2 cells and expression in different regions of the gastrointestinal tract

In this study, we first cloned the SLC19A2 cDNA from Caco-2 cells, then used it as a probe to determine the relative distribution of the message along the native human gastrointestinal tract. cDNA was cloned by RT–PCR using gene-specific primers de-

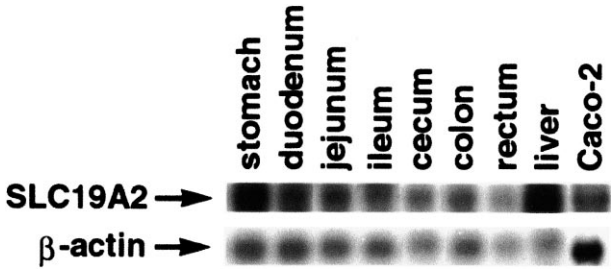

Fig. 1. Expression of SLC19A2 along the native human gastrointestinal tract, liver, and Caco-2. Northern blots containing 2 μg/lane of poly(A)$^+$ RNA isolated from the listed native tissues of the human digestive system (Clontech) and 2 μg poly (A)$^+$ RNA from Caco-2 cells simultaneously probed with randomly labeled SLC19A2 cDNA. The blots were stripped and reprobed with a labeled human β-actin cDNA. SLC19A2 message level was normalized relative to the β-actin signal using the Eagle Eye II densitometer (Stratagene).

signed to detect the ORF of the published SLC19A2 sequence [21]. The identity of the obtained cDNA was confirmed by sequencing and found to match the published sequence of the SLC19A2 predicted ORF. Distribution of the SLC19A2 message was assessed by Northern blot analysis using the cloned SLC19A2 cDNA as a probe and a commercially available blot that contained poly(A)$^+$ RNA (2 μg) from different regions of the human digestive tract (Clontech). A blot containing an equivalent amount of Caco-2 poly(A)$^+$ RNA (2 μg) was simultaneously probed. The results showed that the SLC19A2 message was expressed in all gastrointestinal regions examined as well as in Caco-2 cells (Fig. 1); expression level was as follows: liver > stomach > duodenum > jejunum > colon > cecum > Caco-2 = rectum -> ileum. Not only SLC19A2 RNA, but also the corresponding protein was found to be expressed in Caco-2 cells and in native human small intestine (jejunal brush border membranes were isolated from organ donors as described previously, [15]) as shown by Western blot analysis using polyclonal antibodies raised against a specific peptide of the SLC19A2 protein [16] (Fig. 2).

3.2. *Determination of the transcription initiation site for SLC19A2 in intestinal Caco-2 cells using 5′-RACE*

Transcription initiation site(s) for *SLC19A2* in intestinal Caco-2 cells was identified using 5′-RACE as described in Section 2. Two clones for each products isolated from independent 5′ RACE reactions were sequenced. The results showed the potential existence of three transcription initiation sites for SLC19A2 in Caco-2 cells, one begins at position −183, another at −192 and the last at −220 using the A in the initiator ATG sequence as position 1.

3.3. *Cloning of the 5′-regulatory region of the human SLC19A2 gene and determining promoter activity in intestinal epithelial Caco-2 cells*

Using primers designed to the published sequence for the *SLC19A2* gene and flanking sequence, we isolated a 2635 bp DNA fragment from the 5′-regulatory region. The identity of the genomic DNA fragment was established by sequencing. To verify the functionality of the putative human promoter, we prepared a construct placing the DNA fragment in the promoter-less pGL3-Basic vector for use in the dual luciferase system (Promega), described previously [23]. The promoter activity was determined by analysis of the luciferase activity in transiently transfected Caco-2 cells. The 2635 bp fragment was determined to have significant promoter activity using the luciferase system. To determine the minimal region required for basal activity of the promoter, we generated a series of deletion fragments fused to the firefly luciferase reporter gene (see Tables 1 and 2) and found that activity was confined mainly to a sequence between −356 and −36, using the A of the initiation codon as position 1 (see Fig. 3,

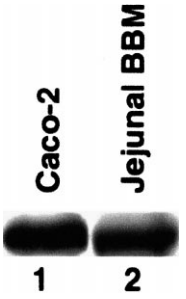

Fig. 2. Expression of the SLC19A2 protein in Caco-2 cells and in native human intestinal tissue. Western blot analysis containing 150 μg of protein from a membrane fraction isolate of Caco-2 cells and 150 μg of protein from human jejunal brush border membranes. The blot was probed with a polyclonal antibody directed against a specific peptide corresponding to the SLC19A2 protein and detected using the ECL system.

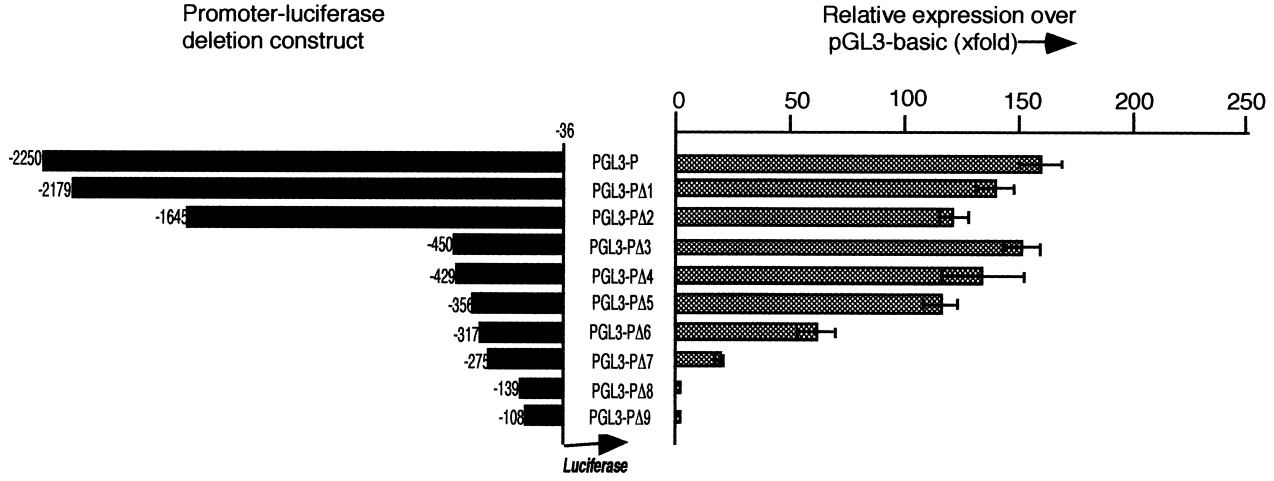

Fig. 3. Functional analysis and identification of the minimal region required for basal activity of the SLC19A2 putative promoter. The size and position of different promoter–luciferase constructs are shown on the left. The results of a luciferase assay for each construct following transient transfection into human intestinal Caco-2 cells is shown on the right. Firefly luciferase activity was normalized relative to the activity of simultaneously expressed *Renilla* luciferase. The results are expressed relative to the pGL3-Basic vector set at 1 and represent the average of at least three independent experiments.

pGL3-PΔ5). A further deletion of 39 bp resulted in an approximate 50% reduction in activity, pGL3-PΔ6 (Fig. 3), and a deletion of another 42 bp nearly abolished activity, pGL3-PΔ7 (Fig. 3). The identified putative promoter region was found to have a CAAT box at position −305 but no classical TATA elements at the expected positions. We analyzed the minimal region of the promoter (−356 to −36) for transcription factor binding sites using the TRANSFAC program [24] and found several putative regulatory elements including, Nuclear Factor-1 (NF1), Activator Protein-1 (AP1), Gut enriched Kruppel-like Factor (GKLF), and Stimulating Protein-1 (SP1) (Fig. 4). The roles of these putative *cis*-elements in the transcriptional regulation of SLC19A2 are currently under investigation in our laboratory.

AatII
-356 NF1
CCGCGCTTAGCCCTGGGACAGAGGCCTGGCGTGCTGCCACCGCCCCAGTTC

AP1 GKLF
ACGTCGTGTGACCCACGACCAATGGAAGAGCAGGCAAGTATTCCCGGCGTC
-317 F6 -305 -275

SP1
CGCTGTGATTGGTTCCCGGAGTGGAGGCGGTGGCAGAGGGTGGGCCTTTAGG
F3

ACGGGTCTCCCTTAAACTGGGCGATCAGGCAGCGACCCTAGAGGCGTCTGT
-220 -192 -183

AGGGTAAAGCTGGGGGTTCTGTAGCCGGAGGCGGCGGCGAGTCCAGAAC
-139 F4

Fig. 4. Diagrammatic representation of the minimal 5′-region of the SLC19A2 gene required for basal promoter activity. The nucleotide sequence of the minimal region required for basal activity in Fig. 3 is shown. Boxes indicate the positions of several identified putative regulatory elements. The nucleotides of the three transcription initiation sites are underlined. Numbers represent nucleotides relative to the translational start site A of ATG as number 1. Primers used in making deletion constructs are shown in bold (Tables 1 and 2).

4. Discussion

Despite the existence of a significant body of work on the physiological and biochemical aspects of the intestinal thiamine uptake process, little is known about the molecular characteristics of the absorption event. Recently a human thiamine transporter, SLC19A2, has been cloned from a number of human tissues [18–21], but very little is known about expression of this gene in native tissues of the human gastrointestinal tract, and no information is available about its 5′ regulatory region. In this study, we first cloned the SLC19A2 cDNA from the human derived intestinal epithelial Caco-2 cells by RT–PCR, then determined expression of the SLC19A2 message in different tissues of the native gastrointestinal tract and the liver. Our cloning effort produced a cDNA identical to that of SLC19A2 [18–21]. This finding clearly shows that SLC19A2 is expressed in this human intestinal epithelial cell line. Using this cDNA as a probe, we then determined the relative expression of the SLC19A2 message along the native hu-

man gastrointestinal tract and the liver by Northern blot analysis. The SLC19A2 message was found to be expressed in all regions of the digestive system examined in the following order: liver > stomach > duodenum > jejunum > colon > cecum > Caco-2 = rectum > ileum. We also confirmed the expression of *SLC19A2* in Caco-2 cells as well as native human small intestinal mucosa at the protein level using Western blot analysis with antibodies specific to the transporter. Functionality of the human intestinal cDNA was confirmed in our laboratory by expression in *Xenopus* oocytes and HeLa cells (unpublished observations) with results similar to those described previously [9].

Previous work in our laboratory and others has described the mechanism and regulation of thiamine uptake in human intestinal Caco-2 cells and other human intestinal preparations [15–17,25,26]. As mentioned above, the characteristics of thiamine transport in Caco-2 cells and other human intestinal preparations are very similar to those reported for HeLa cells transfected with a SLC19A2 cDNA ([19], and unpublished observations in our laboratory). These characteristics include similar apparent K_m, Na^+ independence, thiamine substrate specificity, pH profile, and a lack of affect of unrelated organic cations on thiamine uptake. In addition, we have recently been able to detect the expression of the SLC19A2 message in rat enterocytes (unpublished observations). Furthermore, a recent study by Fleming et al. [27] has also reported expression of the mouse orthologue (Slc19a2) of the thiamine transporter in the mouse intestine. Collectively, the above described findings raise the possibility that SLC19A2 may play a role in normal intestinal thiamine uptake process.

Further characterization of the SLC19A2 message in intestinal epithelial Caco-2 cells revealed multiple transcription initiation sites. This conclusion is based on results of the 5′-RACE analysis using RNA isolated from these cells. In a related study, we cloned the 5′-regulatory region for the *SLC19A2* gene and showed the region to have significant promoter activity following transfection into human intestinal Caco-2 cells. The identified promoter region does not appear to be interrupted with alternative exon sequences and our genomic clone included one continuous sequence containing all published 5′-untranslated region sequences [18–21]. We have also identified the minimal promoter region required for its basal activity and found it to be encoded in a sequence between −356 and −36. The identified promoter region was found to have a CAAT box at position −305 but no classical TATA elements at the expected position, typical for a so called housekeeping gene. The 320 bp region is GC-rich at 64% and has several putative *cis*-elements including NF1, AP1, GKLF, and SP1which may be involved in the regulation of transcription of the SLC19A2 gene. It is worth mentioning here that a recent study using computational analysis of the 5′-regulatory region of the mouse *Slc19a2* gene has also reported the existence of similar promoter *cis*-elements to those described above for the human gene [22].

The findings described above of significant promoter activity for *SLC19A2* in Caco-2 cells further demonstrate the ability of the human intestine to express this thiamine transporter. In summary, our study shows that *SLC19A2* is expressed in different regions of the native human gastrointestinal tract. The results also describe, for the first time, characterization of the 5′-regulatory region of this gene and identify the minimal promoter region required for its basal activity. This should serve as a basis for future investigation into the molecular regulation of this potential human intestinal thiamine transporter.

Acknowledgements

This study was supported by grants from the Department of Veterans Affairs and the National Institutes of Health (DK56061, DK58057, and DK54016). We would like to thank Drs. Ellis Neufeld and Judith Fleming for their advice and discussions during the course of these investigations.

References

[1] C.D. Berdanier, Advanced Nutrition Micronutrients, CRC, New York, 1998.
[2] V. Tanphaichirt, in: M.E. Shils, J.A. Olsen, M. Shike (Eds.), Modern Nutrition in Health and Disease, Lea and Febiger, New York, 1994 pp. 359–375.
[3] M. Victor, R.D. Adams, G.H. Collins, The Wernicke–Korsakoff Syndrome and Related Neurological Disorders due to

Alcoholism and Malnutrition, Davis, Philadelphia, PA, 1989.
[4] C.M. Leevy, H. Baker, Am. J. Clin. Nutr. 21 (1968) 1325–1328.
[5] C.M. Tallaksen, H. Bell, T. Bohmer, Alcohol Clin. Exp. Res. 17 (1993) 712–716.
[6] P.A. Tomasulo, R.M.H. Kater, F.L. Iber, Am. J. Clin. Nutr. 21 (1968) 1341–1344.
[7] N. Saito, M. Kimura, A. Kuchiba, Y. Itokawa, J. Nutr. Sci. Vitaminol. (Tokyo) 33 (1987) 421–430.
[8] A.D. Thompson, Clin. Sci. (Colch.) 31 (1996) 167–179.
[9] H.K. Nichols, T.K. Basu, J. Am. Coll. Nutr. 13 (1994) 57–61.
[10] H. Seligmann, H. Halkin, S. Rauchfleisch, N. Kaufmann, M. Motro, Z. Vered, D. Ezra, Am. J. Med. 91 (1991) 151–155.
[11] H. Mandel, M. Berant, A. Hazani, Y. Naveh, New Engl. J. Med. 311 (1984) 836–838.
[12] L.E. Rogers, F.S. Porter, J.B. Sidbury Jr., J. Pediatr. 74 (1969) 494–504.
[13] G. Rindi, Acta Vitaminol. Enzymol. 6 (1984) 47–55.
[14] H.M. Said, R. Rose, B. Seetharam, in: K.E. Barrett, M. Donowitz (Eds.), Current Topics in Membranes: Gastrointestinal Transport Molecular Physiology, Academic Press, San Diego, CA, 2000, pp. 35–76.
[15] P.K. Dudeja, S. Tyagi, R.J. Kavilaveettil, R. Gill, H.M. Said, Am. J. Physiol. 281 (3) (2001) C786–C792.
[16] H.M. Said, A. Ortiz, V.S. Subramanian, E.J. Neufeld, M.P. Moyer, P.K. Dudeja, Am. J. Physiol. Gastrointest. Liver Physiol. 281 (2001) G144–G150.
[17] H.M. Said, A. Ortiz, C. Kumar, N. Chatterjee, P. Dudeja, S. Rubin, Am. J. Physiol. Cell Physiol. 277 (1999) C645–C651.
[18] G.A. Diaz, M. Banikazemi, K. Oishi, R.J. Desnick, B.D. Gelb, Nat. Genet. 22 (1999) 309–312.
[19] B. Dutta, W. Huang, M. Molero, R. Kekuda, F.H. Leibach, L.D. Devoe, V. Ganapathy, P.D. Prasad, J. Biol. Chem. 274 (1999) 31925–31929.
[20] J.C. Fleming, E. Tartaglini, M. Steinkamp, D.F. Schorderit, N. Cohen, E.J. Neufeld, Nat. Genet. 22 (1999) 305–308.
[21] V. Labay, T. Raz, D. Baron, H. Mandel, H. Williams, T. Barrett, R. Szargel, L. McDonald, A. Shalata, K. Nosaka, S. Gregory, N. Cohen, Nat. Genet. 22 (1999) 300–304.
[22] K. Oishi, T. Hirai, B.D. Gelb, G.A. Diaz, Mol. Genet. Metab. 73 (2001) 149–159.
[23] N.S. Chatterjee, S.A. Rubin, H.M. Said, Am. J. Physiol. 280 (2001) C548–C55.
[24] E. Wingender, X. Chen, E. Fricke, R. Geffers, R. Hehl, I. Liebich, M. Krull, V. Matys, H. Michael, R. Ohnhäuser, M. Prüß, F. Schacherer, S. Thiele, S. Urbach, Nucleic Acids Res. 29 (2001) 281–283.
[25] A.M. Hoyumpa Jr., R. Strickland, J.J. Sheehan, G. Yarborough, S. Nichols, J. Lab. Clin. Med. 99 (5) (1982) 701–708.
[26] U. Laforenza, C. Patrini, C. Alvisi, A. Faelli, A. Licandro, G. Rindi, Am. J. Clin. Nutr. 66 (1997) 320–326.
[27] J.C. Fleming, M.P. Steinkamp, R. Kawatsuji, E. Tartaglini, J.L. Pinkus, G.S. Pinkus, M.D. Fleming, E.J. Neufeld, Mol. Genet. Metab. 74 (2001) 273–280.