MOLECULAR BIOLOGY OF INTESTINAL GLUCOSE TRANSPORT

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INTRODUCTION

Glucose is an important nutrient for mammalian cells. It is a major source of energy, as well as a substrate for macromolecules including glycoproteins, proteoglycans, glycolipids and nucleic acids. Therefore glucose plays a central role in cellular homeostasis and metabolism. The cellular uptake of this nutrient is accomplished by carrier proteins located in the plasma membrane that bind glucose and transfer it across the lipid bilayer. Two classes of glucose carriers have been described in mammalian cells, the Na^+-coupled glucose cotransporters (SGLT) and the facilitative glucose transporters (GLUT).

SGLT1 is present on the luminal membrane of intestinal and kidney proximal tubule absorptive epithelial cells. This protein transports glucose from the lumen of the intestine or nephron against its concentration gradient by coupling glucose uptake with that of Na^+ and electrochemical gradients. The Na^+-gradient is maintained by the active transport of Na^+ out of the cell, across the basolateral membrane, by Na^+/K^+-ATPase (EC 3.6.1.3). SGLT1 has also been recently identified on the basolateral membrane of the secretory ovine parotid acinar cells (Tarpey *et al.* 1994).

Facilitative glucose transporters have been found in virtually all mammalian cells (for recent reviews see Baldwin, 1993; Gould & Holman 1993; Mueckler, 1994). The genes encoding these proteins are named GLUT 1–7. GLUT 1–4 glucose transporter isoforms have a well characterized tissue distribution and facilitate the movement of glucose across the plasma membrane down its chemical gradient either into or out of the cells. A fifth isoform, GLUT 5, is now known to be a fructose transporter (Burant *et al.* 1994) and GLUT 6 is a pseudogene that is not expressed at the protein level (Kayano *et al.* 1990). It has been proposed that GLUT 7 may serve as the glucose transporter within the endoplasmic reticulum of hepatocytes (Waddell *et al.* 1992; James, 1995).

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In the intestine D-glucose and D-galactose, the products of carbohydrate digestion, are transported from the lumen of the intestine across the brush border membrane by SGLT1, and are accumulated within the enterocytes. These sugars pass across the basolateral membrane, down their concentration gradient into the systemic system, *via* the facilitative sugar transporter, GLUT 2 (Thorens, 1993).

It is well established that, in most species, dietary carbohydrates can modulate the levels and activities of the intestinal brush border membrane Na^+/glucose cotransporter. Advances in molecular and cellular biology of intestinal sugar transport, and their application to an appropriate animal model, have increased our understanding of the molecular mechanisms involved in the regulation of SGLT1 by the sugar component of the diet. The role of luminal sugars in regulating the activity and the expression of SGLT1 represents the major discussion point of this review.

THE INTESTINAL Na^+/GLUCOSE COTRANSPORTER (SGLT1)

SGLT1 is found in the small intestine of most mammalian species. It has been cloned from the intestinal tissues of rabbit, human and lamb (Hediger *et al.* 1987, 1989; Shirazi-Beechey *et al.* 1994). The functional characteristics of SGLT1 have been shown to be similar in all these animals. The transporter prefers hexose sugars with an equatorial hydroxyl at position C2. These include the natural dietary sugars D-glucose and D-galactose and non-metabolized sugars, 3-*O*-methyl-α,D-glucopyranoside and methyl-α,D-glucopyranoside (Semenza *et al.* 1984). It is notable that 2-deoxy-D-glucose is not transported by SGLT1. The coupling cation, Na^+, is required for transport; cations such as K^+ and Li^+ do not support glucose transport. The transporter is competitively inhibited by phlorizin, with a lower inhibition constant (Ki = 5–10 μM) than the Michaelis constant (50–100 μM) for glucose (Wright *et al.* 1994).

The rabbit intestinal brush border SGLT1 was the first to be cloned by a novel expression-cloning technique (Hediger *et al.* 1987). Subsequently the human clone was isolated from an intestinal Lambda gt10 library, using the rabbit cDNA. The rabbit and human intestinal SGLT1 consist of 662 and 664 amino acids respectively (see Table 1). There are 84% identity and 94% similarity between the sequences of these proteins.

The proposed secondary structure model for SGLT1 indicates that the protein spans the plasma membrane 12 times (Fig. 1). Each hydrophobic transmembrane region (M1-M12) is composed of 21 amino acid residues arranged in an α-helix. Both the amino and carboxy termini are on the cytoplasmic face of the membrane, and the single N-linked glycosylation site, at asparagine 248, is on the hydrophilic domain between M5 and M6. Core glycosylation occurs in the endoplasmic reticulum, and further processing between the endoplasmic reticulum and the brush border membrane produces complex glycosylation of the tri- or tetra-antennary type. Glycosylation increases the mass by about 15 kDa, and the mature protein runs on SDS-PAGE with an apparent mass of approximately 75 kDa (Wright *et al.* 1994).

Antibodies have been raised to synthetic peptides, derived from defined regions of the rabbit SGLT1 amino acid sequence, and have been used to study the distribution of these epitopes in different species (Hirayama *et al.* 1991; Pajor *et al.* 1992). Similarly, the cDNA encoding the transporter has been used directly as a probe to screen libraries for the isolation of related cDNA, and in Northern analysis to examine the distribution of related mRNA in different species (Lescale-Matys *et al.* 1993; Vazquez *et al.* 1993). The results have indicated that the SGLT1 DNA and protein sequences are highly conserved throughout evolution.

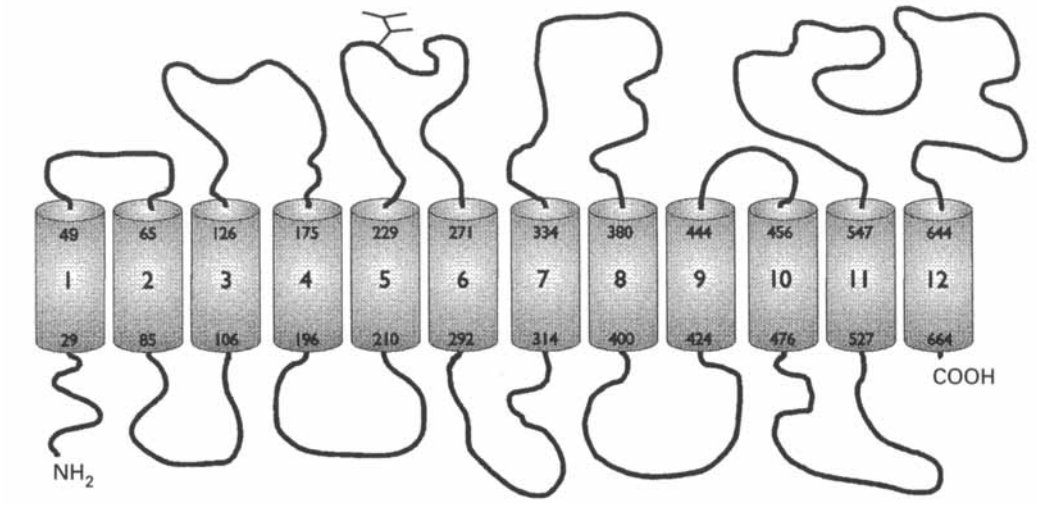

Fig. 1. The proposed secondary structural model of the ovine intestinal Na^+/glucose cotransporter. The transporter has a single N-linked glycosylation site at Asn-248, which adds approximately 15 kDa to the relative molecular weight on SDS-PAGE.

Ovine intestinal SGLT1 is the first ruminant intestinal SGLT1 to be cloned (Wood *et al.* 1994). Initially, the rabbit intestinal SGLT1 cDNA was used as a probe for Northern blot analysis of ovine intestinal (jejunum) mRNA. The cDNA probe showed 5 transcripts ranging in size from 2·4 to 5·5 kb. The reason for this multiple transcript expression of SGLT1 is unclear. The abundance of these transcripts declined from the preruminant lamb to the ruminant sheep, corresponding to the decline in abundance and activity of the protein. The rabbit intestinal SGLT1 cDNA was therefore used as a probe to screen a lamb intestinal cDNA library. The library did not yield a full length cDNA, and the 5′ end of the cDNA was isolated by a strategy based on a polymerase chain reaction.

The composite cDNA sequence, derived from overlapping clones, encodes a protein of 664 amino acids which exhibits homologies of 85% identity and 93% similarity to the rabbit intestinal SGLT1 sequence (Fig. 2 and Table 1). The SGLT1 related proteins isolated thus far share an extraordinary degree of similarity, and are structurally distinct from the GLUT family.

It is notable that the asparagine at position 248, thought to be the glycosylation site in rabbit SGLT1 (Hirayama & Wright, 1991), and the aspartate at position 28, which is changed to asparagine in the condition of glucose/galactose malabsorption (Turk *et al.* 1991), are conserved. Furthermore the sequence of the nonadecapeptide, amino acids 402–420, of the ovine and rabbit intestinal SGLT1 is identical (Fig. 2). This peptide sequence has been used as antigen for the production of an antibody; the antibody identifies the SGLT1 immunoreactive protein on Western blots in intestinal brush border membranes possessing Na^+/glucose cotransporter activity (Hirayama *et al.* 1991; Lescale-Matys *et al.* 1993; Vazquez *et al.* 1993).

DIETARY REGULATION OF INTESTINAL GLUCOSE TRANSPORT

It is well established that intestinal nutrient transporters are adaptively regulated by the type and the amount of nutrients entering the intestinal lumen. Intestinal sugar transport, for example, has been shown to be modulated, both *in vivo* (Hahn & Koldovský, 1966;

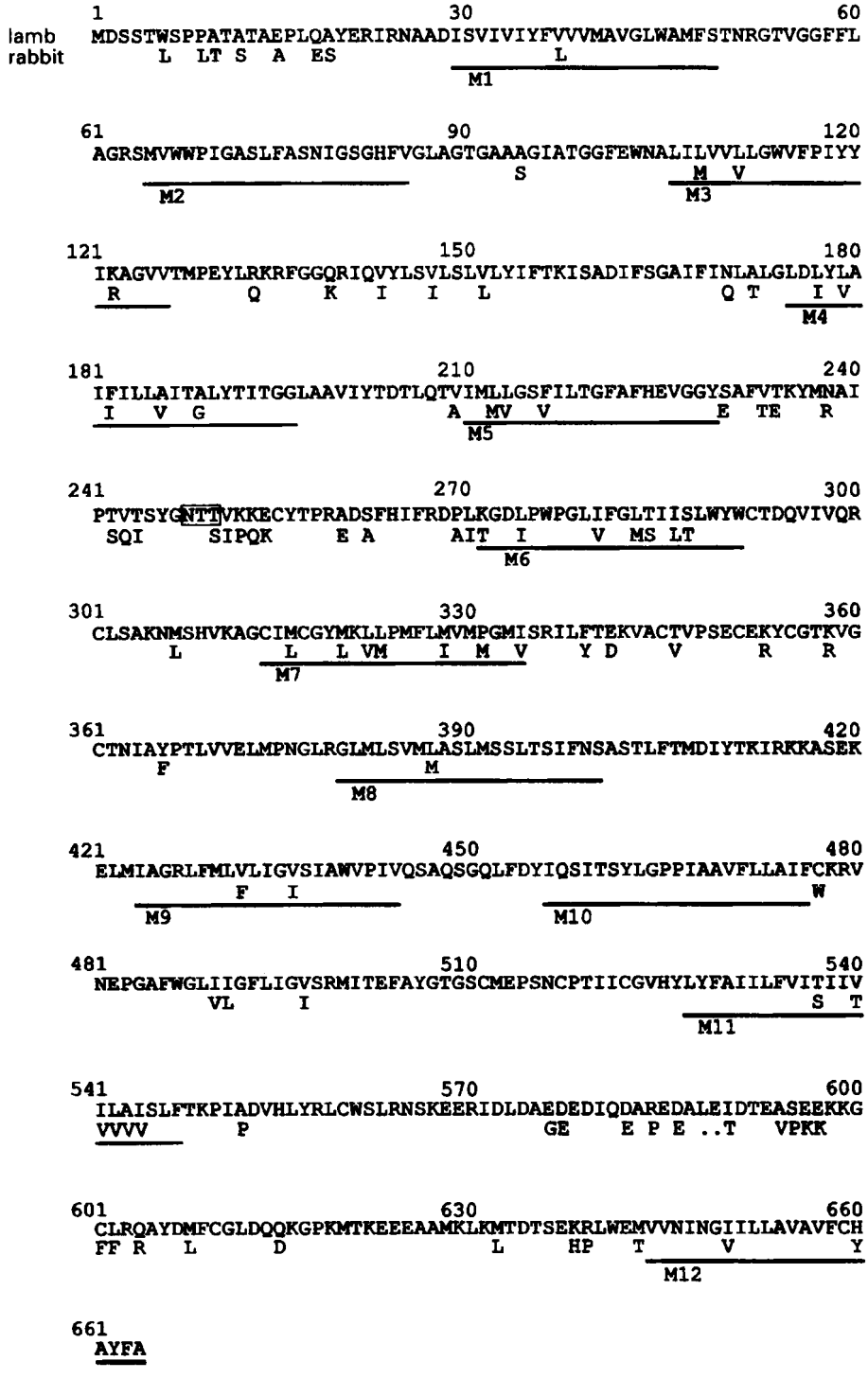

Fig. 2. Aligned amino acid sequences of lamb and rabbit intestinal Na^+/glucose cotransporter (SGLT1). The putative membrane spanning regions are labelled M1-M12. Single-letter abbreviations for amino acids are: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H,

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Table 1. *Comparison of amino acid sequences of SGLT1 proteins*

Tissue source	Identity (%)	Similarity (%)	Number of amino acids	Reference
Ovine intestine	100	100	664	Shirazi-Beechey *et al.* 1994, 1995
Ovine parotid	100	100	664	Tarpey *et al.* (in press)
Rabbit intestine	85	93	662	Hediger *et al.* 1987
Rabbit kidney	85	93	662	Coady *et al.* 1990
Rat kidney	88	94	665	Lee *et al.* 1994
Human intestine	86	94	664	Hediger *et al.* 1989
Pig kidney	89	95	662	Wright *et al.* 1994

The sequence comparisons are relative to the ovine intestinal sequence.

Ferraris & Diamond, 1989; Shirazi-Beechey *et al.* 1991; Lescale-Matys *et al.* 1993) and *in vitro* (Diamond & Karasov, 1984; Ferraris & Diamond, 1989) with changes in dietary carbohydrate levels.

In their pioneering work in this area, Diamond and various colleagues (Diamond & Karasov, 1987; Karasov & Diamond, 1987; Solberg & Diamond, 1987; Buddington & Diamond, 1989; Ferraris & Diamond, 1989; Ferraris *et al.* 1992) have shown that by increasing the carbohydrate content of diets fed to rats and mice, the rate at which D-glucose can be transported across the small intestine is also increased (Ferraris & Diamond, 1989). The increase was modest, 2–3-fold, and appeared to be reversible.

Ferraris & Diamond (1986) showed that when mice consuming a carbohydrate free ration are subsequently switched to a high carbohydrate ration, Na^+-dependent D-glucose uptake almost doubles within 1 d. When the mice on the high carbohydrate ration are switched to the carbohydrate free ration, it takes 1–3 d before brush border glucose uptake decreases significantly from control values (Karasov & Diamond, 1982; Diamond & Karasov, 1984).

To explore the basis of this dietary regulation, specific glucose protectable phlorizin binding was used to measure site density of intestinal Na^+-dependent D-glucose cotransporter in intact intestinal sleeves. It was shown that diet induced changes in the V_{max} of brush border D-glucose uptake are directly proportional to diet induced changes in the amount of specific phlorizin binding. No other mechanisms, such as changes in intestinal mass, solvent drag, passive permeability, or Na^+ gradient, appeared to be involved. It was concluded that the increased intestinal absorption of D-glucose is due solely to specific induction of Na^+-dependent D-glucose cotransporter (Diamond & Karasov, 1984; Ferraris & Diamond, 1986; Ferraris *et al.* 1990).

In non-ruminant species, the level of sugars reaching the intestinal lumen is maintained during adult life through the digestion of dietary carbohydrates, such as starch and various mixtures of maltodextrins, sucrose, lactose and fructose. Complex carbohydrates are digested in the gut through the action of pancreatic α-amylase (EC 3.2.1.1), and brush border hydrolases. The resultant monosaccharides D-glucose, D-galactose and D-fructose are absorbed across the brush border membrane by SGLT1 and GLUT 5 respectively (Burant *et al.* 1992; Davidson *et al.* 1992).

Similarly, in preruminant lambs the milk sugar lactose is hydrolysed by intestinal lactase (EC 3.2.1.23) into D-glucose and D-galactose. These sugars are transported by SGLT1, and the rate of absorption is very high (Shirazi-Beechey *et al.* 1989, 1991).

histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; and Y, tyrosine.

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Lambs are normally weaned at 3–8 weeks, and as their diet changes from milk to grass the rumen develops. However, in ruminants such as sheep the ingested carbohydrates are fermented to volatile fatty acids in the rumen and as a consequence hardly any monosaccharides reach the small intestine (Bassett, 1975). This is coordinated with the loss of both SGLT1 function and protein from the brush border membrane of enterocytes, and a significant decline in SGLT1 mRNA levels (Lescale-Matys *et al.* 1993; Shirazi-Beechey *et al.* 1994).

Rumen development provides a natural and efficient way of depriving the small intestine of available monosaccharides. Therefore, the intestinal tract of ruminant animals such as sheep is a unique model for studying the dietary regulation of sugar absorption.

The antibody raised against the nonadecapeptide, amino acids 402–420, of either the ovine or rabbit intestinal SGLT1 amino acid sequence has identified an abundant 75 kDa immunoreactive protein in the brush border membrane vesicles isolated from the intestinal tissues of preruminant lambs. This immunoreactive band was specifically blocked by pre-absorbing the antibody with the immunizing peptide (Shirazi-Beechey *et al.* 1991). There was a good quantitative correlation between the abundance of SGLT1 protein in the membrane and SGLT1 transport activity measured in the brush border membrane vesicles.

Both Na^+-dependent D-glucose transporter activity and the abundance of SGLT1 protein decreased 100–500-fold during normal postnatal development, and became negligible in the intestinal brush border membrane of ruminant sheep. Kinetic studies indicated that the decline is in the V_{max} of the transport rather than the affinity of the cotransporter for glucose.

The reduction in the capacity to transport D-glucose and D-galactose during postnatal development was correlated with the diminution of monosaccharides reaching the intestine. In contrast, the transport of L-lysine (Scharrer *et al.* 1979) and L-proline (Shirazi-Beechey *et al.* 1989) remained constant. These changes cannot be attributed to modifications in the structure of the absorptive surface (Scharrer *et al.* 1979; Shirazi-Beechey *et al.* 1991), neither can they be explained in terms of an age related process, since prolongation of the period of milk feeding retards the regression of Na^+-dependent glucose transport activity and coordinated SGLT1 protein abundance (Shirazi-Beechey *et al.* 1991).

Infusing D-glucose or methyl-α,D-glucopyranoside (30 mM) into the intestinal lumen of the ruminant sheep through a duodenal cannula increased the rate of Na^+-dependent D-glucose transport, and the abundance of SGLT1 protein, to levels close to those seen in the preruminant state. Kinetic studies also indicated that the induced transporter is operationally identical to that determined under normal conditions (Shirazi-Beechey *et al.* 1991; Lescale-Matys *et al.* 1993). It was concluded that the ontogenic change in intestinal glucose transport is due to a decline in the level of monosoccharides reaching the intestine.

The molecular basis for these diet induced changes in Na^+-dependent D-glucose transport activity were examined by measuring the abundance of brush border SGLT1 protein by Western blotting, and mucosal SGLT1 mRNA by Northern hybridization analysis (Lescale-Matys *et al.* 1993). The diet induced changes in Na^+/glucose cotransporter activity were proportional to the brush border SGLT1 protein abundance (Shirazi-Beechey *et al.* 1991), but were not matched by corresponding changes in intestinal mRNA (Freeman *et al.* 1993; Lescale-Matys *et al.* 1993). It was therefore concluded that the principal level of SGLT1 regulation by luminal sugars is post-transcriptional.

EXPRESSION OF SGLT1 PROTEIN AND SGLT1 mRNA ALONG THE CRYPT–VILLUS AXIS OF THE INTESTINE

Epithelial tissue, carrying out sugar absorption in the small intestine, is in a continuous state of cell renewal. Enterocytes produced in the intestinal crypt migrate to the tips of villi where they are shed into the intestinal lumen (Cheng & Leblond, 1974). This leads to complete cell renewal every 2–3 d in rodents (Ferraris & Diamond, 1993), 3–4 d in ovine (Attaix & Meslin, 1991) and 5–6 d in human small intestine (Traber, 1990). Enterocyte differentiation of structure and function occurs during this process of migration from crypt base to the tip of the villus (Cheng & Leblond, 1974).

The distribution of SGLT1 protein and mRNA from crypt to villus has been examined using immunocytochemical and *in situ* hybridization techniques. Immunochemical studies, using peptide antibodies against SGLT1, indicate that SGLT1 protein is located on the brush border lining the enterocytes of the upper villus in rabbit and mouse (Hwang *et al.* 1991; Ferraris *et al.* 1992). Similar studies carried out on rat intestine have shown the location of the SGLT1 to be on the brush border of the entire villus (Takata *et al.* 1992).

Evidence regarding the correlation of crypt-villus distribution of SGLT1 protein and SGLT1 mRNA measured in rat and rabbit is equally conflicting. Hwang *et al.* (1991) used the *in situ* hybridization technique to map the distribution of SGLT1 mRNA in rabbit intestine, from crypt to villus tip. They reported that SGLT1 mRNA is 6 times more abundant in rabbit upper villus cells than in crypt cells, and is thus coordinated with the crypt–villus gradient of SGLT1 protein. In contrast, Smith *et al.* (1992) reported phlorizin sensitive glucose uptake to be confined to upper villus cells of rabbit intestine, while SGLT1 mRNA was detected on the entire villus.

Immunofluorescence localization of SGLT1, using antipeptide antibody to the synthetic peptide of the ovine/rabbit intestinal SGLT1 sequence, showed labelling over the entire brush border surface, including the lower region of the villus of preruminant lamb intestine (Fig. 3*a*, *c*). The intensity of labelling decreased toward the villus tips, and was undetectable at the bottom of crypts. The labelling was blocked when the primary antibody was pre-incubated with the peptide antigen, indicating the specific labelling of SGLT1 protein (Fig. 3*b*, *d*). A similar distribution pattern for SGLT1 protein along the crypt–villus axis of rat intestine has been observed by Takata *et al.* (1992).

These results indicate that SGLT1 protein is expressed in the brush border membrane of enterocytes lining the entire villus, and the onset of glucose transport takes place near the crypt–villus junction (see Fig. 3). This conclusion is supported by findings that brush border membrane vesicles prepared from cell populations isolated from upper and lower villus regions of lamb intestine could transport glucose in a Na^+-dependent manner.

The profile of expression of SGLT1 mRNA, along the crypt–villus axis of lamb intestine, was determined by quantitative *in situ* hybridization histochemistry (Freeman *et al.* 1993). The SGLT1 mRNA is first detected in crypt cells, ~ 60 μm below the crypt–villus junction, rising rapidly to a peak ~ 150 μm above this point. After reaching a maximum, the amount of message gradually declines towards the villus tip (Fig. 4*a*, *b*).

It is notable that the distribution of SGLT1 mRNA along the crypt–villus axis correlates with the expression of functional SGLT1 protein (see Figs 3, 4, 5). This implies that SGLT1 gene is transcribed, the SGLT1 mRNA is translated and the functional protein is inserted into the brush border membrane of enterocytes located near the crypt–villus junction.

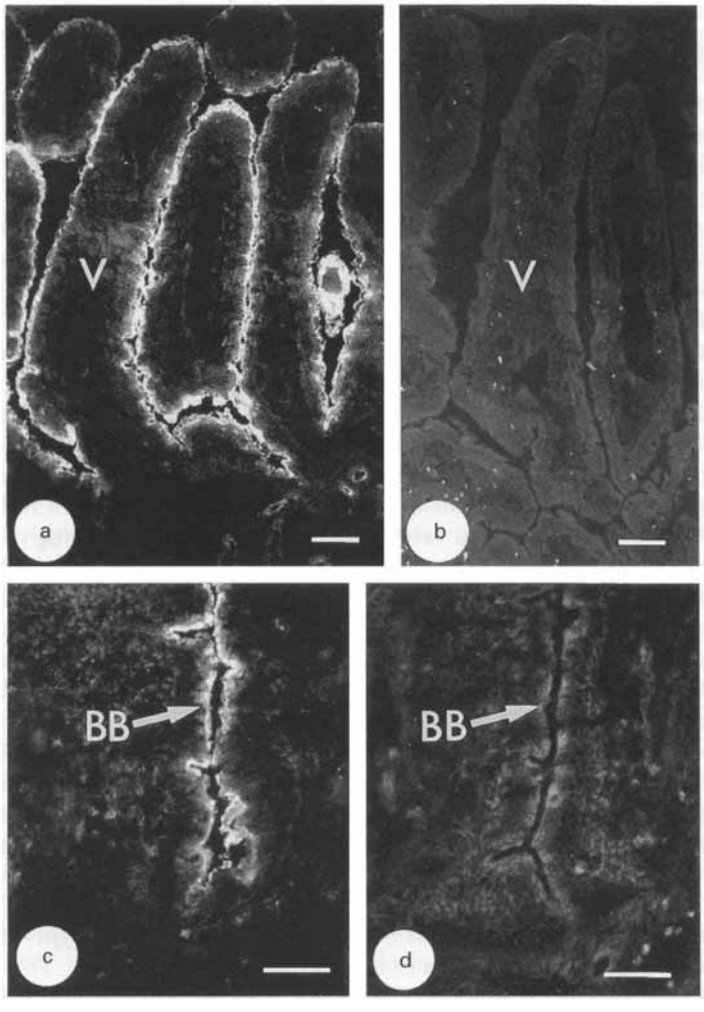

Fig. 3. Immunofluorescence localization of SGLT1 protein along the crypt–villus axis. Typical immunofluorescence images showing localization of SGLT1 on jejunal villi of 14-day-old lamb. **(a)** Labelling over the entire villus brush border membrane **(V)** surface. **(c)** Brush border **(BB)** labelling in the lower regions of two adjacent villi. **(b)** and **(d)** Similar fields to those depicted in **(a)** and **(c)** showing the absence of labelling when primary antibody is pre-incubated with, and used in the presence of, the peptide antigen. Scale bars **(a)** and **(b)** = 100 μm, **(c)** and **(d)** 50 μm.

LUMINAL SUGARS AS DIETARY SIGNALS

Investigating the regulatory effect of various dietary monosaccharides, Solberg & Diamond (1987) reported the effect of rations containing D-glucose, D-galactose, 3-*O*-methyl-α,D-glucopyranoside, D-fructose and D-maltose on intestinal glucose transport. Maltose would be hydrolysed by intestinal maltase (EC 3.2.1.20) to glucose. They showed that all these sugars induce the Na^+/glucose cotransporter, with dietary galactose and fructose being the most effective (Solberg & Diamond, 1987). It was concluded that regulatory sugar need not be a substrate of SGLT1 or be metabolized (Solberg & Diamond, 1987). In recent work, Miyamoto *et al.* (1993) showed that a high glucose diet, as well as diets containing fructose, 3-*O*-methyl-α,D-glucopyranoside, mannose and xylose, fed to rats increased the levels of

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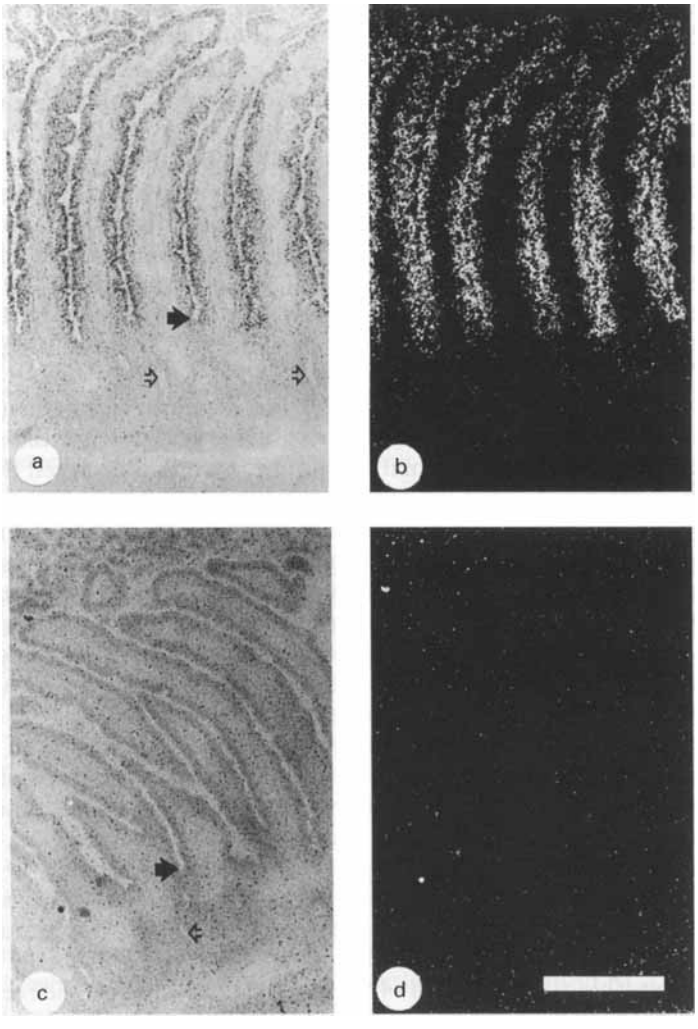

Fig. 4. Distribution of SGLT1 mRNA along the crypt–villus axis. Bright field (**a**) and dark field (**b**) photomicrographs of the hybridization of the ^{35}S-labelled 'anti-sense' oligonucleotide probe to SGLT1 mRNA in frozen fixed sections (10 μm) of 14-day-old lamb jejunum. Fig. (**c**) and (**d**) show similar pictures of the same intestinal tissue after incubation with ^{35}S-labelled 'sense' probe performed under identical conditions as used for hybridization of the 'anti-sense' probe. Sections were exposed to photographic emulsion for 14 d and counterstained with eosin. Solid arrows indicate the crypt–villus junction and open arrows crypts. Scale bar = 250 μm.

jejunal SGLT1 mRNA and the rates of the transepithelial transport of glucose. Their results confirmed earlier studies that the inducing sugars need not be substrates of SGLT1 or be metabolized (Solberg & Diamond, 1987; Dyer *et al.* 1994).

Various sugar analogues have also been tested for their ability to induce functional SGLT1 in the intestinal brush border of ruminant sheep. Solutions (30 mM, on dilution with intestinal digesta) of D-glucose, D-galactose, methyl-α,D-glucopyranoside, 3-*O*-methyl-α,D-glucopyranoside, D-fructose, 2-deoxy-D-glucose, D-sorbitol and D-mannitol were infused through duodenal cannulae into the intestine for 4 d, while the animals were maintained on a roughage diet. The brush border membrane vesicles isolated from the

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intestine of these animals were screened for Na^+/glucose cotransporter activity and the abundance of SGLT1 protein (Dyer *et al.* 1994).

Infusing the intestine with either D-glucose or D-galactose resulted in the induction of functional SGLT1, while infusion with D-mannitol and D-sorbitol (non-transported, non-metabolizable alditols) did not. The induction of functional SGLT1 in the brush border membrane by infusion of methyl-α,D-glucopyranoside or 3-*O*-methyl-α,D-glucopyranoside, non-metabolizable substrates of SGLT1, indicates that there is no prerequisite for the substrate to be metabolized by the enterocytes. Methyl-α,D-glucopyranoside cannot be transported out of the cell via GLUT 2 into the systemic system; this excludes any possible systemic effects. Induction of functional SGLT1 by D-fructose and 2-deoxy-D-glucose implies that the inducing sugar need not necessarily be a substrate of SGLT1.

From these results one can speculate on the presence of a sugar sensing system, which has a different sugar specificity from that of SGLT1. Since 2-deoxy-D-glucose is not transported by any known intestinal brush border membrane protein (Hopfer, 1987; Wright *et al.* 1994), the induction by 2-deoxy-D-glucose implies that the sugar sensor is located on the external face of the luminal membrane. It should be noted that infusing the systemic system of the ruminant sheep with D-glucose, methyl-α,D-glucopyranoside or 2-deoxy-D-glucose did not lead to the induction of luminally located SGLT1.

Experiments were designed to determine where, along the crypt–villus axis, the dietary sugar signal is perceived. In one set of experiments, daily biopsies were removed through duodenal cannulae from the intestines of sheep which were infused continuously for 4 d with D-glucose, while they were maintained on a roughage diet. The functional SGLT1 protein was first detected in brush border membranes isolated from mature enterocytes (upper villus enterocytes) after 3 d; it was maximal after 4 d.

In a wide ranging series of experiments, it was noted that an infusion of D-glucose into the intestinal lumen of ruminant sheep for 2 h had no effect on the ability of existing mature enterocytes to transport D-glucose. However, the presence of the functional SGLT1 was detected 4 d later in the newly formed mature enterocytes (Dyer *et al.* 1994; Shirazi-Beechey *et al.* 1994).

The simplest interpretation of these results is that the signal receiving site is localized within the crypt. The induction is rapid and the observed lag for the appearance of SGLT1 activity is correlated with cell migration time along the crypt–villus axis. However, one cannot exclude a possible villus location of the receptor. In this case the receptor could be linked to crypt events *via* a neural or paracrine mechanism.

Ferraris & Diamond (1993) have also observed that the time lag between switching a mouse on to a high carbohydrate diet and the first appearance of 2-fold enhanced sugar transport is between 12 and 24 h (Diamond & Karasov, 1984). When dietary carbohydrate is removed, it takes 3 d before the brush border glucose uptake decreases significantly from control values (Ferraris, 1994).

In order to determine the site of diet induced glucose transporter along the crypt–villus axis of the mouse intestine, glucose protectable phlorizin binding was measured in cells sequentially removed from along the intestinal crypt–villus axis of mice maintained on high carbohydrate and carbohydrate free diets. Two-fold stimulation of glucose-protectable phlorizin binding was clearly demonstrable all along the crypt–villus axis, and not just in the villus tips (Ferraris & Diamond, 1993). Surprisingly, immunofluorescent labelling, using an antibody against the Na^+/glucose cotransporter, only recognized a specific protein in the upper villus of the mouse intestine.

In order to determine diet induced transient variations in glucose transporter site density along the crypt–villus axis, mice were switched, at a series of short times, from a carbohydrate free diet to a high carbohydrate diet, and *vice versa*. For any increase or

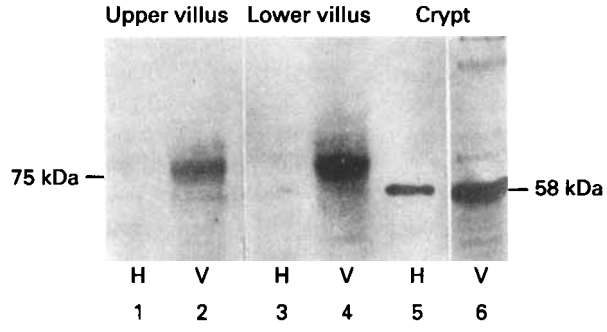

Fig. 5. The profile of D-glucose induced SGLT1 protein along the crypt–villus axis. Western blots of cell homogenates (**H**), and brush border membrane vesicles (**V**) isolated from the upper villus, lower villus and crypt cells from the intestine of ruminant sheep that were infused with glucose. The antibody to ovine SGLT1 identifies the 75 kDa immunoreactive protein in the upper and the lower villus cell fractions. The band is 25–30-fold enriched in the membrane vesicles over the cell homogenates. The same antibody recognizes a 58 kDa immunoreactive protein in the crypt fractions. In all cases, the immunoreactivity can be blocked by pre-incubating the antibody with the peptide antigen.

decrease in dietary carbohydrate, a corresponding change in phlorizin binding site density first appeared in the crypts and spread, over the course of several days, to the villus tips (Ferraris & Diamond, 1993; Ferraris, 1994). They concluded that the signal for glucose transporter regulation is perceived in the crypts, and the observed delay in uptake is attributable largely to cell migration times (Ferraris & Diamond, 1993).

THE PROFILE OF THE SUGAR INDUCED SGLT1 PROTEIN ALONG THE CRYPT–VILLUS AXIS

The profile of induction of SGLT1 was determined along the crypt–villus axis of ruminant sheep intestine infused with either D-glucose or 2-deoxy-D-glucose for 2 h, and subsequently maintained for a further 4 d on a roughage diet. Crude cellular homogenates and brush border membrane vesicles isolated from cells sequentially removed from along the crypt–villus axis were analysed by Western blotting using an anti-SGLT1 antibody. The abundance and enrichment of the mature 75 kDa SGLT1 protein in the vesicle fractions, over the cellular homogenate, can be seen in Fig. 5, lanes 1–4. There is 25–30-fold enrichment in the abundance of SGLT1 protein in the vesicle fraction over the homogenate, which indicates that SGLT1 is located mainly on the brush border membrane of the villus enterocytes. The same population of brush border membrane vesicles transported D-glucose in a Na^+-dependent manner, indicating the presence of functional SGLT1 along the length of the villus (Fig. 6). There is little mature 75 kDa SGLT1 protein and transport activity associated with the membranes isolated from crypt regions.

A very characteristic 58 kDa immunoreactive protein was revealed by Western blotting when the crude cellular homogenate and membrane vesicles isolated from the crypt cell population were probed with anti-SGLT1 antibody (see Fig. 5). The abundance of this protein was 5-fold enriched in the membrane fraction, with respect to the cellular homogenate. As the enterocytes migrate out of the crypt, the abundance of the 58 kDa protein diminishes, while the level of 75 kDa SGLT1 increases. The 58 kDa protein detected was not glycosylated. The molecular mass of mature SGLT1 protein when deglycosylated is also 58 kDa (Dyer *et al.* 1994). From these data it may be postulated that the 58 kDa protein is potentially the precursor protein of mature SGLT1.

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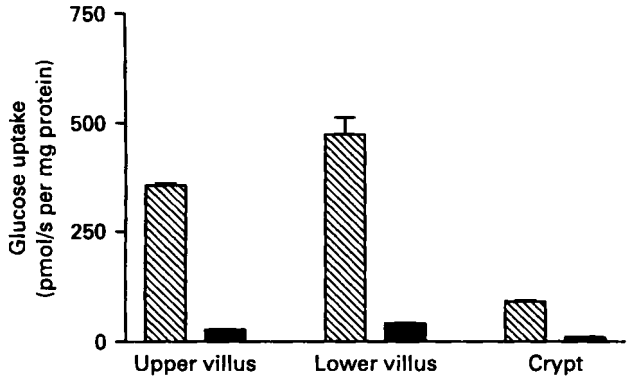

Fig. 6. Profile of SGLT1 activity along the crypt–villus axis. Brush border membrane vesicles were prepared from cells sequentially removed from along the crypt–villus axis of the intestine of ruminant sheep infused with D-glucose. The initial rate of transport was measured in the presence of Na^+ (■) and K^+ (■). D-glucose transport activity in the membrane vesicles isolated from the upper and lower villus regions indicates that the functional sugar induced SGLT1 protein is located along the entire villus. There is little SGLT1 transport activity associated with the membranes isolated from crypt cells. Results shown are the means ± S.E.M. (n = 3).

CONCLUSION AND FUTURE INVESTIGATIONS

The activity of the intestinal glucose transporter SGLT1 has been shown to be regulated by dietary carbohydrates in both non-ruminants and ruminants (Ferraris, 1994; Shirazi-Beechey *et al.* 1994). The intestinal tract of ruminant animals, such as sheep, provides a unique model for studying the dietary regulation of sugar absorption. In ruminants the ingested carbohydrates are fermented to volatile fatty acids in the rumen and, as a consequence, hardly any monosaccharides reach the small intestine. The development of the rumen is associated with a marked decrease, 100–500-fold, in both SGLT1 activity and protein in the brush border membrane of enterocytes (Shirazi-Beechey *et al.* 1991). There is also a significant decline in SGLT1 mRNA levels (Shirazi-Beechey *et al.* 1994). Introduction of D-glucose or derivatives into the intestinal lumen of the ruminant sheep increases the activity and abundance of SGLT1 to levels similar to those measured in the preruminant animal and produces a 3-fold increase in the levels of SGLT1 mRNA.

The experimental data suggest that the introduction of certain sugars into the luminal contents of the ovine intestine stimulates the synthesis of functional SGLT1. The signal, initiated by luminal sugar, is perceived by a receptor located on the external face of cells within the crypt. The molecular mechanisms involved in the intracellular signalling from this receptor, resulting in the synthesis of functional SGLT1, are unknown. The profile of the coordinated distribution of SGLT1 protein and mRNA along the crypt–villus axis of the intestine implies that the SGLT1 gene is transcribed, the mRNA is translated, and the functional protein is inserted into the brush border membrane of enterocytes located near the crypt–villus junction.

The sequencing of the promoter region of the ovine SGLT1 gene, the identification of the receptor, the signalling pathway and molecular mechanisms involved in the intracellular synthesis and processing of SGLT1 will provide valuable information on how dietary sugar regulates the intestinal sugar transporter. To assist in identifying the molecular events by which SGLT1 gene expression is regulated in response to dietary carbohydrates in the whole animal, it is useful to develop satisfactory models in suitable cell lines. Such cell lines could be manipulated in experiments which would be difficult or impossible with live animals or excised tissues. Work is currently under way in our laboratory to establish such

models for the ovine enterocyte which display sugar dependent expression, or constitutive expression, of a reporter gene which is transcriptionally linked to either SGLT1 upstream sequences, or to suitable constitutive promoter sequences.

Models such as these would be used to identify important regions of upstream sequence which are involved in the control of SGLT1 gene expression, and the transcription factors acting upon them; they could also be employed to determine the post-transcriptional and translational consequences of the differential RNA splicing which occurs during the processing of SGLT1 transcripts *in vivo*. Eventually cell-line models, in association with the sheep intestinal model, can facilitate the identification of a membrane located sugar receptor, and elucidation of the signal transduction pathways through which it operates.

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