Review

Regional expression and regulation of intestinal sucrase-isomaltase

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Regional expression of intestinal sucrase-isomaltase, an abundant brush-border membrane disaccharidase, differs throughout the adult gastrointestinal tract, being highest in villus regions and the proximal small intestine. Its expression along the vertical axis (crypt-to-villus) appears to be regulated at the transcriptional level and increases with cellular maturation. However, along the proximal-to-distal small intestinal axis, sucrase-isomaltase expression appears to be determined by post-transcriptional factors, possibly at the level of sucrase-isomaltase mRNA translation. Total and specific activities of sucrase-isomaltase are also modulated by developmental, hormonal, differentiation, and nutritional factors and by disease states such as severe insulin-dependent diabetes. The following discussion will review the known cellular and molecular mechanisms that appear to determine sucrase-isomaltase expression and highlight new developments in this area that may provide insight into regulatory mechanisms of protein expression in intestinal epithelial cells.

Background

Sucrase—structure and function

Sucrase-isomaltase (SI), an abundant intestinal enzyme complex, comprises over 10% of brush border membrane (BBM) proteins.[1] The sucrase component accounts for 100% of the intestinal hydrolysis of sucrose into glucose and fructose, while isomaltase is responsible for the hydrolysis of isomaltose into two glucose subunits. SI also accounts for 80% of intestinal maltase activity.[2]

Sucrase-isomaltase can be divided into five structural domains[2]: the cytoplasmic domain, transmembrane segment, connecting segment (stalk), sucrase, and isomaltase (each containing a catalytic domain) *(Figure 1)*. The cytoplasmic tail contains 12 amino acids located at the N-terminal domain of isomaltase and has no known signal transduction function. The transmembrane segment, amino acids 13-32, is a highly hydrophobic and helical region[3] that spans the cell membrane only once.[4] This region is important for anchoring SI to the cell membrane and contains the signal sequence for SI translation.[5] The connecting segment, also known as the serine/threonine "stalk," is located between amino acids 32 and 64 and contains more than 17 serine or threonine residues. It is thought to be heavily glycosylated,[6] making it a highly hydrophilic segment of SI. These post-translational modifications may play a role in protecting the "stalk" region from degradation by luminal protease degradation and may help direct the catalytic domains into the intestinal lumen.[6] The serine/threonine "stalk," transmembrane segment, and cytoplasmic tail, all located at the N-terminal region of isomaltase, have no homology with any region in the sucrase subunit.[2]

In contrast, sucrase and isomaltase contain 100% amino acid homology at the site of catalytic activity, i.e., amino acid sequence Asp-Gly-Leu-Trp-Ile-Asp-Met-Asn-Glu.[2] Hydrolysis of sucrose and isomaltase is thought to occur in a similar manner. The initial step appears to involve protonation of the glycosyl oxygen, responsible for the linkage of the two monosaccharides, by an unidentified acid.[7] Next, the glucose subunit (containing an oxocarbonium ion) is stabilized by the carboxyl group of an aspartic acid, located in the mid-region of the catalytic site (amino acid 505 and 1393 of isomaltase and sucrase, respectively).[6] Final stabilization occurs with reformation of the alpha configuration of glucose.[7]

Homology of sucrase and isomaltase nucleotide and amino acid sequences

Within the last decade, significant progress has been made in the sequencing and cloning of SI. Hunziker, et al.[6] first sequenced the rabbit SI cDNA, which was found to contain 26 nucleotides prior to the initiation

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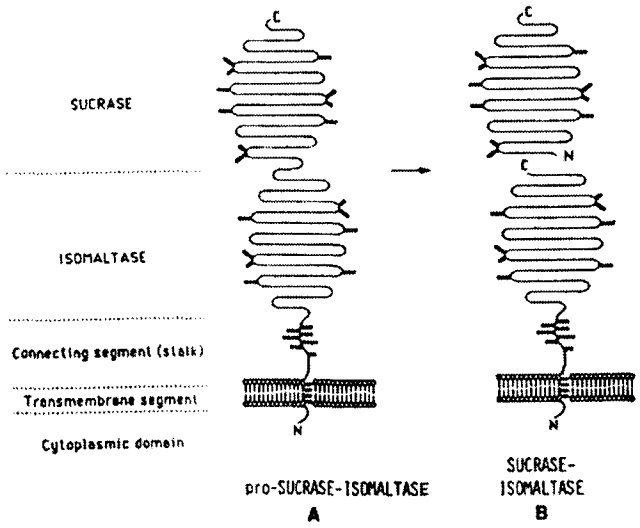

Figure 1 Model of sucrase-isomaltase structure and domains before (panel A) and after (panel B) exposure to pancreatic proteases (see review by Semenza[2]; reproduced with permission, from Annual Review of Cell Biology, Vol. 2, © 1986 by Annual Reviews Inc.).

codon and 5964 nucleotides thereafter. Isomaltase (amino acids 1–931) is located at the 5′ end of the gene, while sucrase (amino acids 932–1827) is located downstream. SI exhibits significant species homology in nucleotide and amino acid sequence. A partial cDNA to rat SI, cloned by Broyart et al.,[8] was found to be 78% homologous to corresponding regions of the rabbit cDNA (nucleotides 2207–4153). At the amino acid level, rat and rabbit sucrase N-terminal sequences are 78% homologous, while isomaltase sequences are 64% homologous.[8] In addition, the 5′ region (2 Kb) portion of the human SI cDNA is 85% homologous to corresponding regions of the rabbit gene and 82% homologous at the amino acid level.[9]

Significant homology (>50%) also exists between the sucrase and isomaltase portions of the SI gene,[6] suggesting that sucrase may be a duplicate gene of isomaltase. At the amino acid level, the homology appears to be in clusters of less than 12 identical amino acids; 41% of the amino acids are identical if properly aligned and another 40% of nonidentical residues represent conservative changes.[6] Semenza[2] hypothesized that an ancestral gene coding for a brush border membrane protein with maltase and isomaltase activity was partially duplicated over 300 million years ago. Over time, point mutations and/or deletions resulted in a gene encoding an enzyme with distinct sucrase and isomaltase activities.

Intracellular processing

Both sucrase and isomaltase are translated from a single mRNA transcript *(Figure 2)*, being approximately 6 kb in size in rabbit.[6] Translation of the signal sequence in the cytosol results in relocation and further translation at the endoplasmic reticulum (ER). However, unlike most proteins, the signal sequence is not cleaved and remains part of the membrane spanning portion of sucrase-isomaltase.[3,5]

Although there has been some debate as to whether sucrase and isomaltase were translated into a separate or a single-chain peptide, the latter case is now generally accepted. Sjostrom et al.[10] and Montgomery et al.[11] demonstrated that a single-chained SI polypeptide from porcine and rat intestinal brush border membranes, respectively, could be isolated in the absence of pancreatic proteases. Furthermore, Alpers et al.[12] and Wacker et al.[13] showed, by in vitro translation studies, that sucrase and isomaltase are translated into a single polypeptide chain.

During translation in the ER, pro-SI is glycosylated to produce the high mannose form of sucrase-isomaltase termed SI_h, approximately 210 kD in size.[14,15] The SI_h form can be distinguished from other SI forms by its sensitivity to endo-beta-N-acetylglucosaminidase H (endo H), which cleaves high mannose glycans.[5] In addition, Sjostrom et al.[16] have shown that this form of SI has significantly less specific enzyme activity than the more mature forms.

Upon entry into the golgi, approximately 30 minutes after synthesis,[17,18] SI_h is further processed into a complexly glycosylated SI form, SI_c (M_r 217 kD). This form contains full enzymatic activity,[10,11,15] is resistant to endo H treatment, and has a sugar content greater than 15% in the rat and rabbit.[19] Naim et al.[14] determined by successive digestion with increasing amounts of endo-beta-N-acetylglucosaminidase F, which cleaves N-linked complex carbohydrate moieties, that SI_c contains eight N-linked glycan moieties. Sensitivity to treatment with trifluormethane-sulfonic acid, which cleaves O-linked glycans, suggests that SI_c contains O-linked carbohydrate moieties, as well.[14] Furthermore, tyrosine residues of SI appear to be post-translationally sulfated.[20]

Following the completion of post-translational modifications, SI_c is transported to the brush border membrane where it is anchored to the apical membrane by the hydrophobic N-terminal domain of the isomaltase

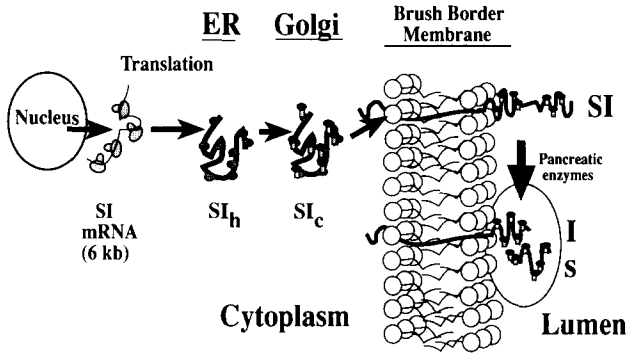

Figure 2 Sucrase-isomaltase (SI) intracellular processing. SI is cotranslationally glycosylated in the endoplasmic reticulum (ER) to produce SI_h, high mannose form. In the golgi, SI is further processed into the complexly glycosylated form, SI_c. Once at the brush border membrane (BBM), sucrase (S) and isomaltase (I) are cleaved apart by luminal proteases.

subunit.[2] Cleavage of the single-chain peptide into two disaccharidases *(Figure 1)* results from exposure to various luminal pancreatic proteases, elastase in rat[18] and trypsin in humans.[14] Isomaltase (M_r approximately 140 kD) remains attached to the brush border membrane while sucrase (M_r approximately 120 kD) stays in close association with the C-terminal domain of isomaltase through strong non-covalent interactions.[2,6] Tertiary structure is maintained, in part, by disulfide bonds[19]; however, disulfide bond formation does not play a role in the linkage between sucrase and isomaltase.[21] As for SI topological and quaternary structure, analyses conducted by Cowell et al.[22] suggest that both single-chained and cleaved SI forms are present predominantly in dimers regardless of membrane SI protein concentration. Although most brush border enzymes are thought to exist this way, there is still some debate concerning this issue.[15]

To compare the time course of SI processing with other BBM proteins, Danielsen[23] pulse labeled intestinal explants and examined labeled amino acid incorporation into SI and aminopeptidase N (APN) forms. Label appeared in complexly glycosylated APN significantly earlier than in SI_c (at 20–40 and 60–90 minutes post-injection, respectively) suggesting a slower rate of SI processing compared with APN.[24] Similar findings were obtained in differentiated Caco-2 cells (a spontaneously differentiating human colon carcinoma cell line), where Stieger et al.[25] reported a delay in appearance of newly synthesized SI compared with dipeptidylpeptidase IV (DPPIV) in the BBM. This difference appeared to result from a significantly slower ER-to-golgi transit rate resulting from slower SI_h conversion to SI_c and the existence of an intra-golgi pool of SI_c. In contrast, golgi-to-membrane transit rates were similar between SI and DPPIV (40–60 and 60–75 minutes, respectively). These data indicate that the rate-limiting steps for processing of SI probably occur at a pre- and intra-golgi stage and not during post-golgi transit. Matter and Matter[26] reported that differences in protein folding can account for different rates of pre- and intra-golgi processing, and that proper tertiary structure of proteins may be required to exit the golgi. Such a mechanism could explain the significantly slower rate of SI processing compared with other brush border hydrolases.

Sucrase-isomaltase expression in the intestine

Expression along the crypt to villus axis of the small intestine

Investigations of the crypt-to-villus axis of the small intestine have provided unique opportunities for studying aspects of epithelial cell differentiation. Four basic cell types—goblet, enteroendocrine, columnar, and paneth cells—originated from immature, dividing stem cells, anchored near the crypt base.[27] The most abundant and perhaps best-studied intestinal cell type is the columnar epithelial cell which comprises over 90% of the intestinal epithelial population.[28] As these cells mature and migrate toward villus regions, they change from a cuboidal configuration to a columnar shape and acquire a complex apical microvillar membrane, increasing mitochondria, rough ER, and cell-cell interdigitations.[29] Mature columnar cells also gain the ability to efficiently absorb and digest nutrients as evidenced by increased activities of disaccharidase,[30,31] peptidase,[31,32] and hexose transport.[33] However, at the upper villus tip regions, cells begin to show signs of atrophy, apoptosis, and cell death, reflecting continuous mucosal cell turnover occurring over 48–72 hours.[28,29]

SI activity along the villus-to-crypt axis, first determined in horizontal intestinal sections obtained by microdissection techniques,[34] is highest in villus regions where epithelial cells are differentiated, with levels declining toward crypt regions. As shown in *Figure 3*, sucrase activities, measured in intestinal cells sequentially isolated from villus-to-crypt regions, were also found to be elevated in villus compared with crypt regions.[35,36] In contrast, thymidine incorporation, a feature of immature dividing crypt cells, is highest in fractions corresponding to crypt regions *(Figure 3)*. Immunofluorescence studies conducted by Quaroni and Isselbacher[37] have also demonstrated sucrase and other brush border enzymes such as lactase, aminopeptidase, and alkaline phosphatase beginning at mid- to upper-crypt regions, with little or no expression at the crypt base. These findings suggest that SI expression is differentiation dependent, appearing only in more mature intestinal cells. Similar findings have been seen in HT-29 and Caco-2 intestinal culture cells where levels of SI protein only become detectable in differentiated cells.[38] Contrary to these findings, immunolabeling studies conducted by Beaulieu et al.[39] and Gorvel et al.[40] demonstrated SI expression in undifferentiated crypt cells. Similarly, although SI protein was not detectable in undifferentiated HT-29 cells, it was found to be translated in both differentiated and undifferentiated cells following pulse-chase.[8] In differentiated cells, SI_h is quickly converted into SI_c, whereas in undifferentiated cells SI_h is degraded with little being converted to SI_c. Thus, lack of SI expression in the membrane of undifferentiated HT-29 (and perhaps in undifferentiated crypt cells) could be a consequence of altered post-translational processing. Alternatively, differences in antibody affinity and specificity to newly synthesized SI forms could account for the apparent differences reported by various investigators. In support of this, Beaulieu et al.[39] found significant differences in the immunofluorescence patterns of SI along the villus-to-crypt axis in human jejunum using 12 different antibodies. When immunoprecipitation studies in Caco-2 cells were performed with these antibodies, other immunologically distinct forms of SI differing from "villus" SI forms were found. Using radiolabel pulse studies, an immature SI form was shown to precede the formation of SI_h and was termed "initial SI high mannose form." Beaulieu et al.[39] hypothesized that this initial immature SI form is converted to an alternate form of SI_c by proliferating and immature cells, whereas in differentiated cells it is directly con-

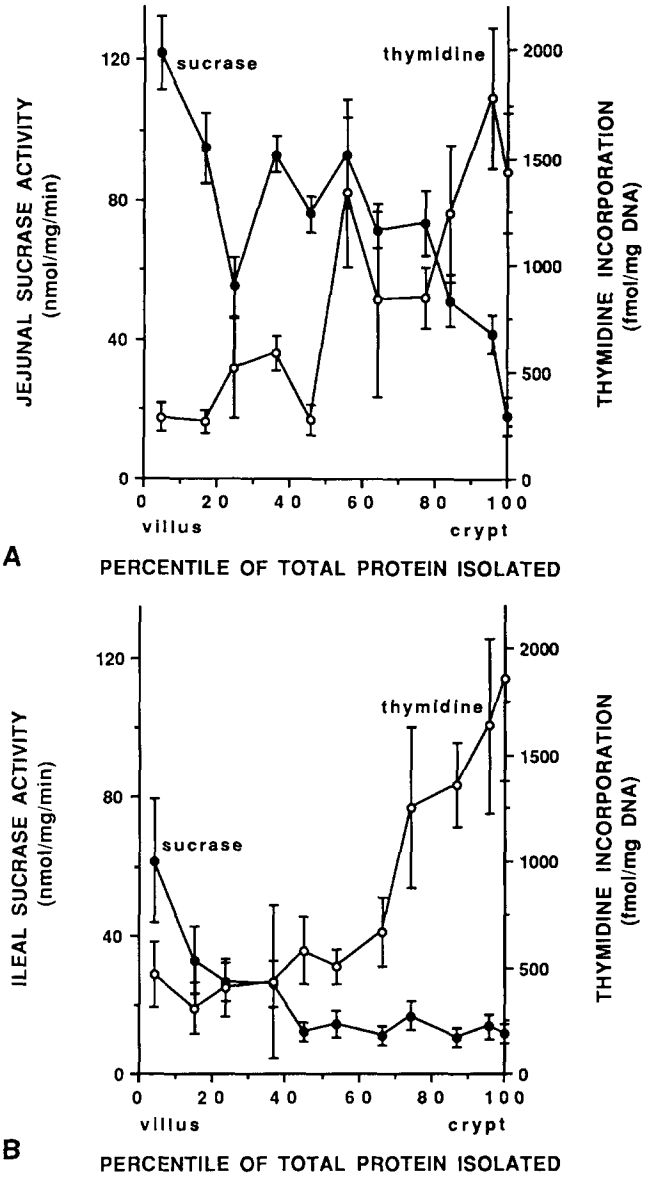

Figure 3 Verification of sequential isolation of intestinal epithelial cells from villus to crypt in jejunum (panel A) and ileum (panel B). Sucrase activity per mg of protein, protein concentration, and [^{3}H]thymidine incorporation into DNA were measured for each enterocyte fraction isolated along the jejunal and ileal vertical axis. The relative position of each fraction along the villus-to-crypt axis was expressed as a percentage of the total protein isolated with 1% representing upper villus and 100% crypt base regions. Measurements were pooled into groups corresponding to each 10th percentile of protein isolated. $n \geq 3$ for each point. Values are means ± SE.

verted into the SI_h and SI_c forms normally expressed by mature epithelial cells in situ.

In further support of a role for post-translational regulation of protein expression during cell differentiation, Oliger-Denis et al.[41] found a higher percentage of cellular proteins containing only partially trimmed high mannose chains in undifferentiated compared with differentiated HT-29 cells. The investigators suggest that a partial blockade in high mannose trimming exists in undifferentiated cells. Perhaps this alteration in post-translational processing of proteins leads to the unique immature form of SI described by Beaulieu,[39] which may be more susceptible to proteases as described by Trugnan et al.[38]

Differences in SI mRNA abundance along the villus-to-crypt axis are also thought to exist. Freund et al.[42] were the first to report an increase in SI mRNA abundance in the upper half compared with the bottom half of villus regions. To examine villus-to-crypt gradients, Hoffman and Chang,[35] Chandrasena et al.,[43] and Traber et al.,[44] measured SI mRNA abundance in sequentially isolated enterocyte fractions along the villus-to-crypt axis. In all cases, Northern blot analysis demonstrated steady-state levels of SI mRNA progressively decreasing from villus to crypt. The rate of declining SI mRNA abundance approximates that of sucrase activity in both jejunum and ileum *(Figure 4)*. In contrast, by in situ hybridization analysis, jejunal SI mRNA levels appear to be highest at the crypt-villus junction, decline at mid-villus regions, and are undetectable in crypt regions.[44] Differences in SI mRNA labeling availability in Northern and in situ hybridization may explain these contrasting findings. However, "admixtures" of villus and crypt cells in isolated epithelial cell fractions could also account for the Northern blot results.

Regulation of SI mRNA abundance along the villus-to-crypt axis probably occurs at the transcriptional level. Recently, Traber et al.[45] transfected spontaneously differentiating Caco-2 intestinal cells with reporter gene constructs of the SI promoter and showed expression correlated with increasing maturation. Furthermore, this group has identified (by transfection, deletion, and footprint analyses) regions within the 5′-flanking region of the human SI gene that have nuclear extract binding activity or transcriptional enhancement or silencing effects.[46,47] Several of these sites appear to induce transcription in Caco-2 cells, with little expression seen in non-intestinal cells. These results suggest a role for tissue-specific transcriptional factors in determining SI mRNA expression during differentiation. Studies using transgenic mice models are now under way to determine if this is also true in vivo.

In summary, the regulation of SI expression along the vertical axis of the small intestine is most likely determined by differences in transcription rates, although stabilization of SI mRNA cannot be ruled out. However, differences in post-translational regulation of SI expression may also determine SI expression along the villus-to-crypt axis. Extracellular signals, such as changes in dietary composition or hormonal factors, may modify these processes, thus providing the mucosa the means to adapt to changes in diet or metabolic states.

Expression along the proximal-to-distal axis of the small intestine

Regions of the small intestine are phenotypically and functionally distinct. However, no distinct anatomical

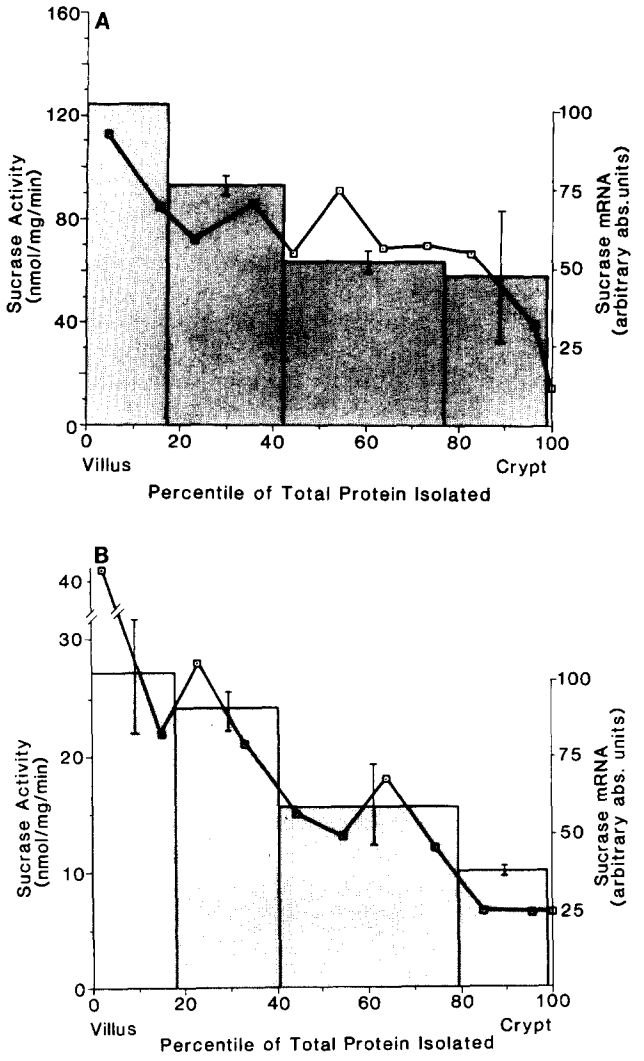

Figure 4 Comparison of sucrase activities to steady state mRNA levels along the villus to crypt axis in jejunum (panel A) and ileum (panel B). Autoradiographs of radiolabeled SI cDNA hybridized to slot-blots containing RNA from cells isolated from villus to crypt of jejunum and ileum were quantitated by laser densitometry. Measurements, represented by the columns, were normalized to amount of 18S rRNA and expressed as means ± SE (n = 3). The value obtained for the first jejunal fraction was defined as 100% sucrase mRNA abundance. The average percentile of total protein isolated for each fraction determined its location along the x-axis where 1% and 100% total protein isolated represent upper villus and crypt base regions, respectively. The line graph represents measured jejunal (A) and ileal (B) sucrase activities.

marker exists that can easily separate duodenum and jejunum from ileum. Several intestinal enzymes, including lactase,[48] Na^+-dependent glucose transporter,[49] biotin transporter,[50] cytosolic glycyl-leucine and proline dipeptidases,[51] and endopeptidase 24.11 are highly expressed in jejunum compared with ileum.[52] However, other brush border membrane proteins, such as glucoamylase,[53] acid aminopeptidase and dipeptidyl peptidase,[51] and aspartate aminopeptidase,[48] are highly expressed in distal ileum compared with jejunum. In addition, another group of intestinal proteins, including neutral aminopeptidase and maltase, appear not be expressed in a gradient fashion at all.[48,53]

Studies of SI expression along the longitudinal (proximal-to-distal) axis have largely been confined to the jejunum, as activities have previously been reported to be highest in this region.[48,53] In ileum, sucrase activities along the entire villus-to-crypt axis are in general three to five fold less than in jejunum. However, as shown in *Figure 3*, sucrase activity is highest in villus regions and declines toward crypt regions in both ileum and jejunum.

In contrast to sucrase activity, no major differences in SI mRNA levels can be found between corresponding fractions of enterocytes isolated along the vertical axis from jejunum and ileum[35] *(Figure 5)*. These findings suggest that differences in regional regulation of sucrase activity along the longitudinal axis are caused by differences in translational or post-translational processing of SI.

Regional differences in post-translational processing

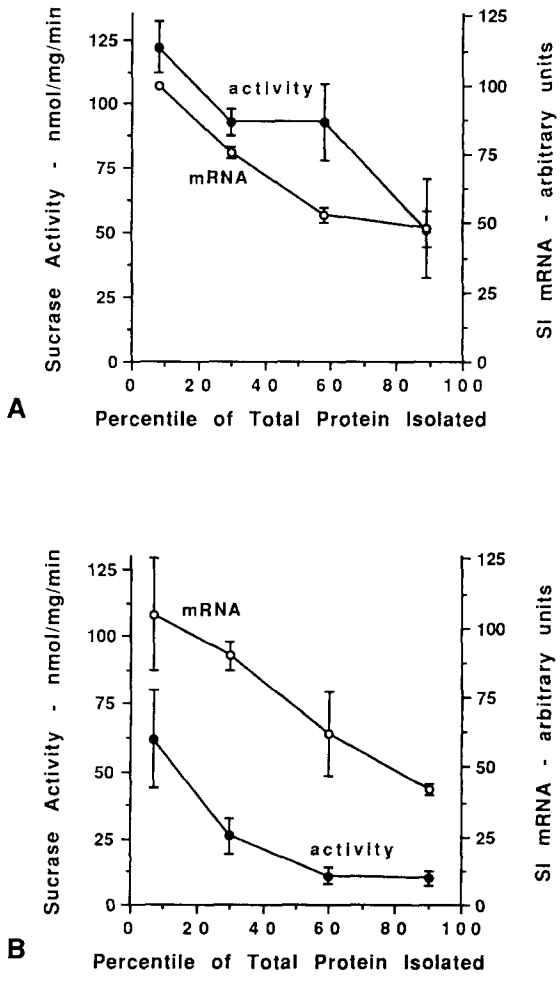

Figure 5 Comparison of jejunal (panel A) and ileal (panel B) steady state SI mRNA abundance in relation to sucrase activities along the villus-to-crypt axis. As described in *Figure 4*, SI mRNA levels, as determined by laser densitometry of hybridized slot blots and normalized to amounts of 18S rRNA, are compared with sucrase activities along the vertical axis.

of SI forms do appear to exist. Although jejunal and ileal SI_h forms appear to be similar, significant differences are seen in the gel mobilities of SI_c and mature sucrase (S) and isomaltase (I) forms, with ileal forms having a higher apparent molecular weight *(Figure 6)*.[35] These findings suggest that the differences in regional SI protein processing occur at the level of golgi. Despite these differences, however, functional activities of the mature ileal and jejunal sucrase and isomaltase forms do not appear to be different.[35] Regional differences in enzyme processing have also been reported for lactase in suckling rats,[54] apparently due to differences in protein sialylation, a processing step known to occur in the golgi. However, it is not clear whether these differences determine lactase expression in these regions.

Recently, studies have shown that differences in expression of SI along the proximal-to-distal axis of the small intestine may be due to translational events. Within 15 minutes of pulse labeling, radiolabel incorporation appears predominantly in SI_h in both ileum and jejunum. By 50 min label is apparent in SI_c, and by 120 and 180 min, increasing amounts of radiolabel can be seen in mature S and I of both regions *(Figure 6)*. Thus, no major difference in the rates of intracellular SI processing could be detected between jejunum and ileum. However, the most striking finding of these studies is that radiolabel incorporation into corresponding SI forms are consistently three to five fold greater in jejunum than in ileum at all time points *(Figure 7)*. This ratio corresponds to observed differences in regional sucrase activities, thus suggesting that regulation of SI expression along the proximal-to-distal axis occurs at the level of translation. In support of this, the distribution of SI mRNA between active and inactive translational pools in ileum and jejunum was found to be different, i.e., a greater proportion of jejunal SI mRNA compared to ileal transcripts co-migrated with membrane-bound polyribosomes.[35] Although the degree of association of ribosomal units to endoplasmic reticulum was slightly greater in jejunum (~1.5 fold greater than that in ileum), this could not entirely account for the differences in intracellular SI mRNA distribution in these regions. Thus, differences in initiation rates of translation or in rates of SI mRNA transit and docking to the ER membrane could play a role in SI expression along the proximal-to-distal small intestinal axis. If the latter, factors such as signal recognition particles (SRPs) and SRP receptors might be involved in SI regulation. If differences in initiation rates of SI translation exist, then distribution between "free" and RNP-bound (non-translatable) pools could account for some of the regional differences in SI expression. In either case, it will be important to study the effects of region-specific cytosolic and nuclear factors on SI translation.

Collectively, these data suggest that proximal and distal regions of the small intestine are distinct. SI expression along the longitudinal axis appears to be regulated by differences in translational mechanisms.

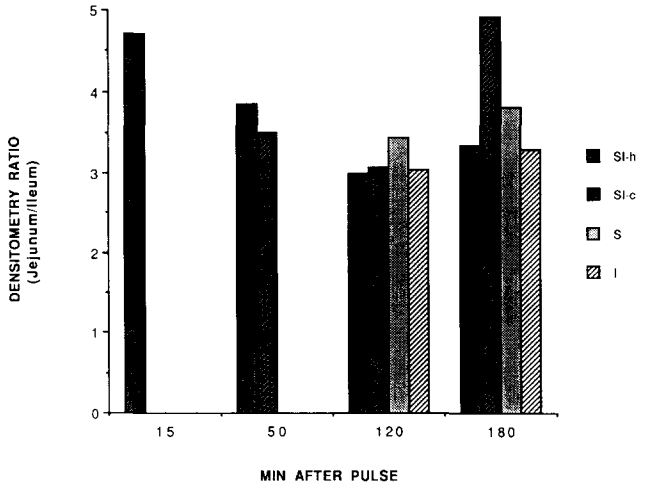

Figure 7 Ratio of jejunal-to-ileal radiolabel incorporation into SI forms. Autoradiographs of jejunal and ileal SI immunoprecipitates were quantified by laser densitometry and the ratios determined for corresponding forms of jejunal and ileal SI. Only SI_h was detected within 15 minutes, both SI_h and SI_c were present at 30 minutes, and by 120 minutes all forms were apparent.

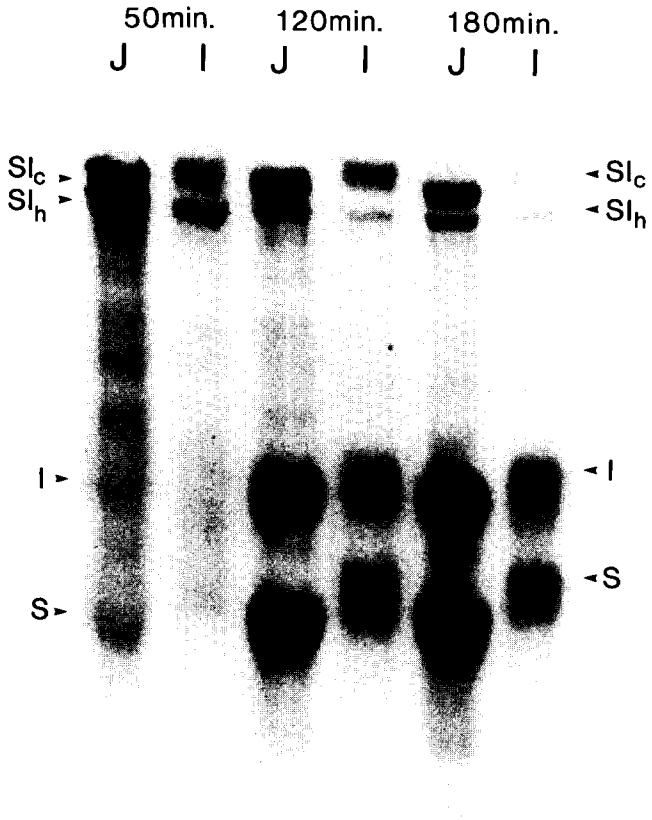

Figure 6 Autoradiograph of [^{35}S]methionine-labeled SI immunoprecipitants. Rats were given an intravenous dose of [^{35}S]methionine and sacrificed at 50, 120, and 180 minutes post-injection. SI was immunoprecipitated from jejunal (J) and ileal (I) mucosal homogenates. Samples were resolved by 5% SDS-PAGE and autoradiography performed. Jejunal and ileal SI forms are marked to the left and right of the lanes, respectively.

Similar findings were reported by Danielsen et al.[55] for aminopeptidase N, an intestinal brush border enzyme, which appears to be translationally regulated during pig intestinal development. [^{35}S]Methionine pulse studies demonstrated a 30-fold difference between adult and fetal intestinal aminopeptidase-N radiolabeled incorporation corresponding to differences in aminopeptidase activities at these stages. In contrast, aminopeptidase N mRNA abundance did not change during pig intestinal development and was similar in adult and fetal animals. Cell free translation rates of adult and fetal aminopeptidase mRNA were similar. However, upon the addition of microsomal membranes only, adult aminopeptidase-N translation was stimulated. Furthermore, adult newly synthesized aminopeptidase N was shown to be processed to a high mannose form more efficiently than fetal primary transcripts.

Determinants of SI expression

Diet

Luminal nutrients have been shown in a variety of studies to affect mucosal growth and the expression of intestinal epithelial enzymes,[56,57] including SI activities. Deren et al.[58] reported that after a 3-day fast, high maltose (65%) and high sucrose (65%) feedings can increase sucrase activity by nearly two fold within 5 hours after refeeding. Rosamond et al.[59] and Riby and Kretchmer[60] demonstrated a correlation between sucrase specific activities and carbohydrate content of diets. However, the type of carbohydrate also appeared to be significant. At high carbohydrate concentrations (60%), both sucrose and starch induced sucrase activity to the same extent, but at low concentrations (30%), sucrose rather than starch was a more potent inducer of sucrase activity. Yamada et al.[61] and Goda et al.[62] reported similar results, with sucrase and maltase activities being increased two to four fold in rats fed a high sucrose (70%) diet compared with rats fed a high diet (73% fat, 5% carbohydrate). Goda et al.[62] also showed that sucrase immunoreactivity paralleled increases in sucrase activity following sucrose feeding. Diets high in sucrose were also found to increase the V_{max}, but not K_m of SI, corroborating induction of sucrase protein without a change in functional activity.[63]

Increased disaccharidase activities induced by high sucrose diets largely occur in proximal regions of the intestine, with only moderate increases seen distally.[56] This increase is also evident along the entire villus-to-crypt axis where a two to four fold increase in sucrase, lactase, and maltase activities has been shown by horizontal microdissection[61] and by sequential cell isolation techniques.[63,64] Pulse-chase studies performed by Riby and Kretchmer[60] further suggested increased synthesis of SI at the crypt-villus junction, and decreased degradation along the entire vertical axis in response to rats fed a high sucrose compared with a carbohydrate-free diet.

Carbohydrates may also increase SI expression by inducing increases in SI mRNA abundance. Raul et al.,[65] for example, demonstrated that sucrose-induced increases in SI activity could be inhibited by pretreatment with actinomycin D, an inhibitor of transcription. In addition, Broyart et al.[8] showed that in rats fed a high cellulose (55%) diet for 3 days, sucrose refeeding resulted in a marked increase in sucrase mRNA levels (2.5 fold) and a gradual increase in sucrase activity within 1.5 hours of feeding.

In contrast to carbohydrate feedings, starvation down regulates SI activities and reduces mucosa weight, protein, and DNA levels throughout the entire intestine.[66] Kawamura and Kimura,[67] Holt et al.,[68] and Gorostiza et al.[69] have shown that sucrase-specific activities are decreased during starvation, in contrast to lactase-specific activities, which are increased.[70,71] Along the longitudinal axis of starved animals, sucrase activities per mg protein or μg DNA are decreased (1.5 fold) in proximal and mid-segments only, whereas lactase activity is increased (two fold) along the entire jejunum-to-ileum axis.[66] Along the vertical axis, sucrase-specific activity is proportionately decreased from villus to crypt, whereas lactase activity appears to be induced mainly in mid- and upper villus regions.[66] Thus, fasting appears to affect disaccharidases differently throughout the gut.

These studies suggest that luminal nutrients are important trophic factors for the intestinal mucosa. After 3 days of total parenteral nutrition (TPN), mucosal mass in rats significantly decreases, even though caloric requirements have been met.[72] Enterally fed animals have significantly higher intestinal protein and DNA content than TPN-fed or fasted (72 hours) rats.[73] Glucose absorption per cm was also found to be greater in enterally fed rats than TPN-fed or fasted rats.[74] As for disaccharidase expression, Schedl et al.[75] demonstrated that 4 days of TNP decreases sucrase and maltase specific and total activities by two to three fold. These studies further suggest a role for luminal nutrients in regulating sucrase expression.

Circadian rhythm

Circadian rhythms may have some effects on SI activities. Sucrase activity,[76] as well as immunoreactivity,[77] appear to increase approximately 1 hour before the onset of feeding and decrease within 1–3 hours after feeding. The rhythm can be shifted by altering the time of feeding as shown by Saito,[78] but is not affected by light/dark cycles.[79] Differences in SI expression during the course of a day appear to result from altered sucrase-isomaltase degradation rates, as determined by pulse-chase studies.[77] Thus, the half life of sucrase-isomaltase protein before and after feeding corresponds to 38 and 6 hours, respectively. The onset of feeding appears to result in a rapid increase in the rate of sucrase-isomaltase degradation that plateaus 3 hours after feeding has begun. Fasting did not inhibit increases in SI degradation rates normally seen at the time of past feedings. Starvation for several days, however, could eliminate daily fluctuations in sucrase ac-

tivity.[77] These results suggest that luminal nutrients are not necessary to continue short-term circadian fluctuations, but are important in the long-term maintenance of diurnal variations in SI activity.

Developmental regulation

During development a complex pattern of intestinal protein expression occurs. In humans, these changes occur before birth.[80] In most mammals, however, developmental changes in intestinal protein expression can be seen after birth.[80,81] For example, lactase expression peaks soon after birth in rats and begins to decline by day 17. Sucrase, as well as isomaltase and maltase, activities, however, begin to increase at this time.[82] These changes coincide with the time when the diet of the rat changes from one containing mostly lactose to one that has other disaccharides. Cessation of suckling and altered nutrient intake, however, do not affect disaccharidase activities during development. Animals that are prevented from weaning still exhibit the same pattern of SI expression.[80] Furthermore, isografts of fetal rat intestine grown in adult hosts are still able to develop SI expression without exposure to luminal nutrients.[83,84]

Several studies have suggested that pre-translational events are probably responsible for developmental regulation of SI expression. Leeper and Henning[85] detected increasing levels of SI mRNA and activity in rat jejunum from 18–24-day-old rats, with both parameters being higher in jejunum compared with ileum (unlike findings in adult rats). After day 24, sucrase mRNA abundance in jejunum plateaued while activity declined. Sebastio et al.[86] also found sucrase activity and mRNA to change in parallel in developing rabbit intestine. Sucrase activity and mRNA were detectable in tissue from 15-day-old animals and increased to adult levels by day 35. However, unlike findings in rat, neither sucrase activity nor mRNA abundance plateaued or declined until adult levels were reached. From these studies, it appears that developmental expression of sucrase activity is primarily determined at the level of transcriptional or post-transcriptional events. However, the discrepancy between SI mRNA and activity levels in rats greater than 24 days old[85] suggests that translational or post-translational factors may also play a role in regulating sucrase expression.

Human deficiency

Although not well known, there are many documented cases of human sucrase-isomaltase deficiency. In Greenland[87] and in North America,[88] sucrase malabsorption is relatively common and may be a result of a genetic defect, although the exact mechanism(s) remains unknown. Sucrase deficiency appears to arise from several types of aberrations in SI processing. Concomitant with the absence of sucrase activity, isomaltase and maltase activities are severely reduced; this pattern corresponds to the known contributions of SI to these activities.[80] At least two categories of SI deficiency have been described. The first includes those disorders in which decreases in SI activity are paralleled by decreased immunoreactivity, most likely because of defects in intracellular SI processing.[89,90] Naim et al.[91] documented two patients with an over expression of a slightly larger than normal SI_h, which appears to be blocked from transport to the golgi. Hauri et al.[92] and Lloyd and Olsen[93] reported another type of SI deficiency with normal processing of SI in ER, but not golgi. Although immunoreactive SI is found in golgi, it is not found at the brush border membrane. Western analysis demonstrated the presence of SI degradation products in the golgi rather than the normal presence of SI_c.[92,93] Thus, conversion of SI_h to SI_c appears to be inefficient or incomplete in these patients, resulting in significant degradation of SI. In contrast, in other SI-deficient patients, SI immunoreactive protein is present, but dysfunctional in the brush border membrane.[89,91] Thus, a variety of defects in SI processing, reminiscent of aberrations seen in cell culture studies, can lead to human sucrase-isomaltase deficiency. Understanding mechanisms responsible for altered expression of SI in experimental models, animals, and cell culture systems could further our understanding of SI processing in humans and perhaps lead to a cure for or amelioration of SI deficiency.

Diabetes mellitus

Diabetes mellitus is a commonly found disorder, affecting millions of people.[94] This disease has dramatic effects in organs throughout the body, including the intestine where marked changes in physiology and function are known to occur. Vinnik et al.[95] was one of the first groups to report that insulin-deficient diabetic patients had significantly enhanced glucose absorption when compared with nondiabetic control patients. Subsequent studies[96,97] failed to corroborate these in vivo findings, but for the most part these studies were not controlled and no attempts were made to correlate hexose absorption with the severity or duration of diabetes.

Subsequent studies of diabetic intestinal adaptation have largely been conducted in experimental animal models. Within a week following the induction of diabetes, hypertrophy of rat intestinal mucosa and extraintestinal tissue occurs,[75,98,99] possibly as a consequence of stimulated cellular proliferation.[98] Five days after induction of diabetes, lysine, glucose, and 3-O-methylglucose uptake are increased nearly two to three fold in diabetic rat jejunum compared with controls.[100] By 90 days post-induction of diabetes, elevated hexose uptake increases to levels nearly seven fold greater than those in normal ileum.[101] Furthermore, specific binding of [^{3}H]phlorizin is greater in diabetic tissue and extends further into mid-villus regions when compared with normal tissue. This finding suggests that a larger fraction of villus epithelial cells participate in glucose absorption.[101]

One of the most prominent adaptive changes observed in diabetic intestine is the upregulation of sucrase activity. Therefore, diabetes mellitus is of particular

interest in the understanding of mechanisms resulting in abnormal regulation of SI expression. In acutely diabetic rats, sucrase activities measured in mucosal scrapings from proximal, mid, and distal sections are significantly increased (two to three fold), but the normal proximal-to-distal (high-to-low) gradient remained intact.[75,102] Furthermore, jejunal sucrase specific activities along the entire villus-to-crypt axis are increased within 4 days of the induction of diabetes.[103] Similar results were found in chronically diabetic (greater than 40 days) rats.[104] In these animals, sucrase specific activities were increased by at least two to four fold along the entire villus-to-crypt axis of both jejunum and ileum *(Figure 8)*. This increase in sucrase activities cannot be explained by mucosal hypertrophy, as intestinal mass increases by only 17% in diabetic animals.[102] Kinetic data suggest that the observed increase in sucrase activity is due to an increase in the rate of maximal hydrolysis, V_{max}, rather than an alteration in function, K_m, of jejunal mucosal sucrase.[105] Correspondingly, jejunal brush border membrane isolates have higher sucrase activities in diabetic compared with control rats, suggestive of an increase in the amount of sucrase protein per cell.[105] Quantitative immunoprecipitation reactions further demonstrate greater levels of sucrase protein in diabetic compared with normal jejunum.[106]

To determine if increases in diabetic rat sucrase activity and sucrase protein correlated with increases in SI mRNA abundance, Northern and slot-blot analyses were performed on total RNA extracted from fractions of enterocytes sequentially isolated from villus-to-crypt regions of normal and diabetic rat jejunum and ileum.[104] A single band of approximately 6 kb size was found in all jejunal and ileal fractions, corresponding to the known size of SI mRNA. When quantified by laser densitometry and normalized to 18S ribosomal RNA abundance *(Figure 9)*, a two to three-fold increase in diabetic enterocyte SI mRNA abundance was observed when compared with corresponding villus-to-crypt fractions from ileum and jejunum of normal rats. These values approximate observed increases in measured gradients of SI activity. In vitro transcription assays suggest that increases in SI mRNA associated with diabetes do not primarily result from increased SI transcriptional rates.[104] Thus, increases in intestinal SI activity in diabetic animals may be a result of increased stabilization of SI mRNA.

As previously reported for normal rats,[35] SI mRNA levels are similar between corresponding jejunal and ileal enterocyte fractions obtained from diabetic rats. Furthermore, ileal sucrase, isomaltase, and SI_c (complexly glycosylated SI form) from diabetic rats have significantly higher apparent molecular weights than corresponding jejunal forms, as reported for normal rats.[35] Sucrase functional activities (sucrase activity per unit of immunoreactive SI) in diabetic ileum and jejunum do not appear to differ and were similar to those seen in normal rats.[104] These findings correspond with those of Olsen and Korosmo[106] who demonstrated similar catalytic efficiency of sucrase isolated from normal and acutely diabetic rat jejunum. These data suggest that the functional activity of sucrase and differences in post-translational processing of sucrase along the longitudinal axis of rat intestine are not altered by diabetes.

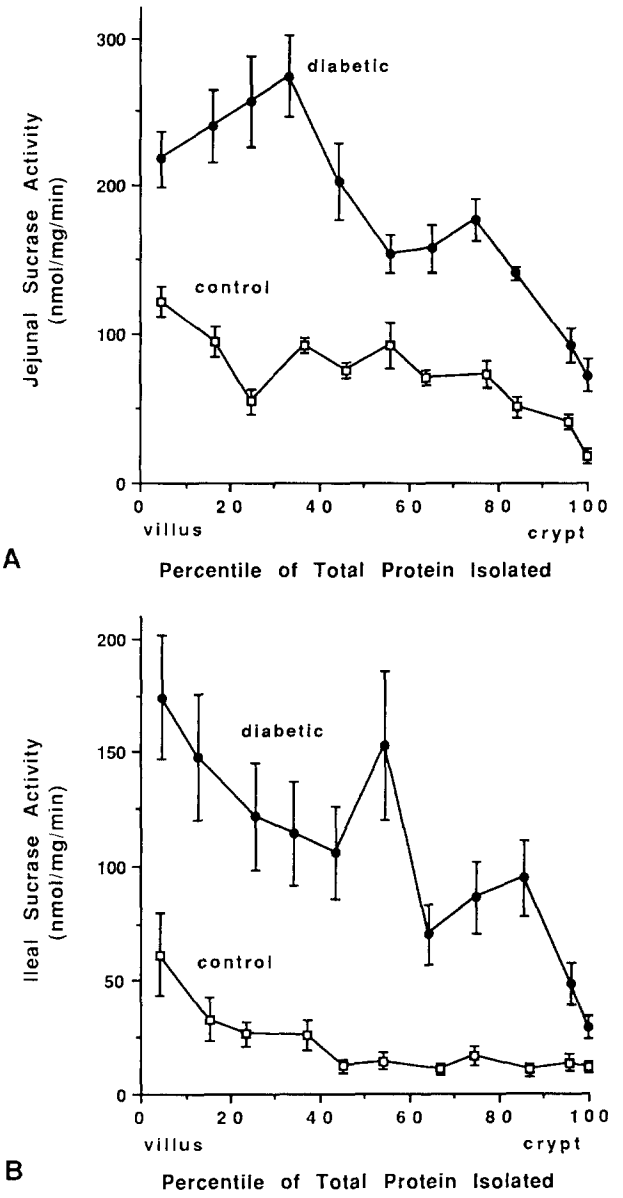

Figure 8 Measurement of regional sucrase activities. Gradients of sucrase-specific activities in enterocyte fractions sequentially isolated from villus-to-crypt regions in diabetic and nondiabetic rat jejunum (panel A) and ileum (panel B). Fractions were pooled by each 10th percentile and are represented by the group's average percent of total protein isolated. Values are means ± SE ($n \geq 3$ for each point), * $P \leq 0.05$.

As of yet, little is known about factors responsible for diabetes-induced increases in SI expression or alterations in any other intestinal brush border membrane enzyme. Because diabetic rats are hyperphagic, the role of increased luminal nutrient exposure in causing diabetic intestinal hypertrophy and upregulation of

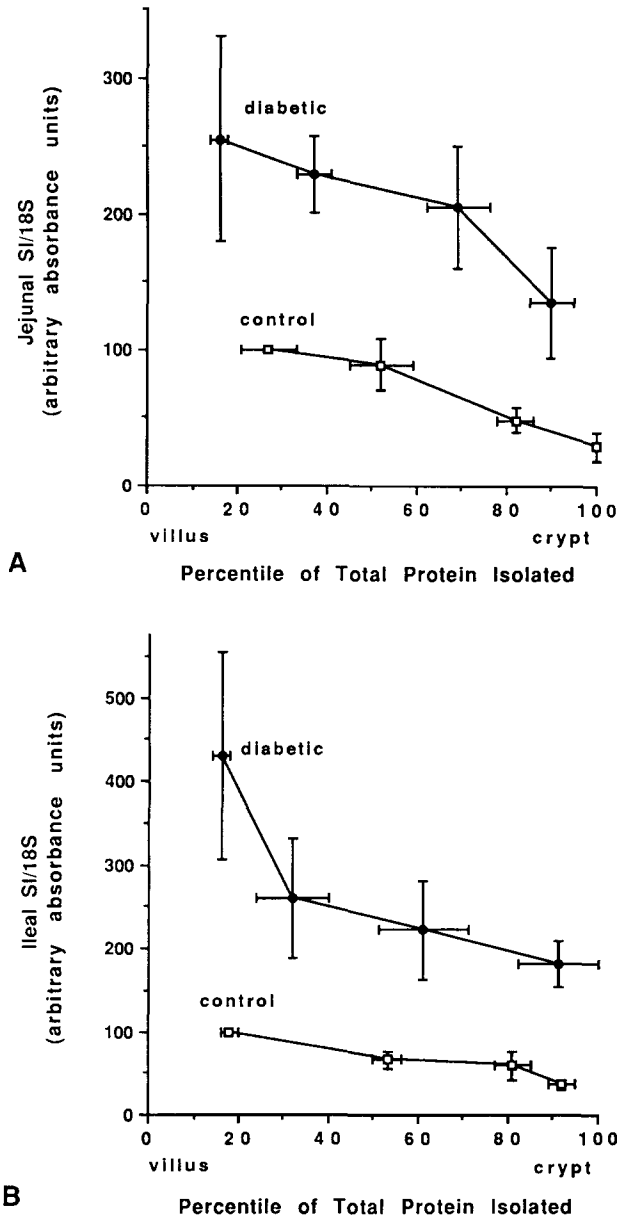

Figure 9 Quantitation of SI mRNA abundance in jejunal (panel A) and ileal (panel B) enterocyte fractions from control and diabetic rats. Densitometry measurements from individual blots were standardized to the first control fraction from jejunum (panel A) or ileum (panel B) and normalized to 18S rRNA abundance. Values were pooled and expressed as means ± SE (n = 4 for each point).

sucrase activity has been studied. Studies by Schedl et al.[75] in acutely diabetic rats suggest that the route of feeding, whether oral, enteral, or parenteral, has little effect on the increased specific activity of sucrase or maltase. Furthermore, these studies suggest that hyperphagia had no effect on diabetes-induced sucrase activities. Similarly, in chronically diabetic animals,[107] restriction of food intake to normal levels (two fold less than ad libitum-fed diabetic animals) did not affect increases in sucrase activities seen in ad libitum-fed animals.

As previously mentioned, intestinal hypertrophy alone cannot account for most diabetes-induced intestinal adaptive changes.[102,108,109] In support of this, increased hexose and amino acid transport are evident before any changes in mucosal mass can be detected.[106] Furthermore, adaptation in protein expression appears to differ for various intestinal brush border membrane proteins. For example it is greatest for Na-dependent glucose absorption, less for Na-dependent amino acid transport, and non-existent for passive absorption of certain amino acids and Na-dependent sulfate transport.[106,109]

Insulin, on the other hand, has been shown to reverse acute diabetes-induced increases in SI activity.[96] In chronically diabetic rats, insulin treatment can also restore sucrase activities along the villus-to-crypt axis of jejunum and ileum to normal levels.[107] However, these findings do not necessarily support a role for insulin in the regulation of sucrase activity. In diabetic rat pups in which pancreatic insulin is decreased by 95% but blood insulin levels are still normal, increased sucrase activities are still found.[110] Furthermore, when rats are food deprived (for 5 days) and exhibit low plasma insulin levels nearly equal to diabetic animals, sucrase activities in the small intestine are not increased.[107] These studies suggest that insulin state alone is not responsible for increased sucrase activity in diabetic rat small intestine. Other hormones, such as glucocorticoids,[111] have been shown to stimulate intestinal sucrase expression. However, their role in diabetes-induced alterations in sucrase activity has not been established.

The possibility that hyperglycemia stimulates sucrase expression has not been adequately explored. However, in preliminary studies using adult nondiabetic rats, continuous intravenous glucose infusion sufficient to make animals hyperglycemic for 48 hours produced no increase in total or specific activities of mucosal sucrase (unpublished observations).

Conclusions

The cellular processes involved in the regulation of SI expression in small intestine and in cultured intestinal epithelium are diverse and complex. They appear to have different roles and relative importance in various regions of the gut. The diversity of these mechanisms may be essential for region-specific adaptive responses by the gut mucosa during development, in various disease states, and with changes in nutrient requirement, load, or composition.

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