Genetic regulation of enterocyte function: a quantitative in situ hybridisation study of lactase-phlorizin hydrolase and Na\(^+\)-glucose cotransporter mRNAs in rabbit small intestine

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Abstract. The enterocyte undergoes sequential changes in its structure and function as it migrates rapidly from the small intestinal crypts to the villus tip. The mechanisms by which these changes are regulated “in tune” with ontogenic and dietary changes in the luminal environment are currently under investigation. This study has employed oligonucleotide probes to follow the expression of the lactase-phlorizin hydrolase (LPH) and Na\(^+\)-glucose cotransporter (SGLT1) genes in rabbit small intestine using quantitative in situ hybridisation histochemistry. The profiles of LPH mRNA and SGLT1 mRNA accumulation along the crypt-villus axis were found to be very similar. Although mRNA was undetectable in the crypt, LPH and SGLT1 mRNA levels rose rapidly at the crypt-villus junction, reaching a maximum between 210 \(\mu\)m and 330 \(\mu\)m above this point. Further up the villus the level of mRNAs declined. SGLT1 mRNA was present in all small intestinal segments (duodenum, jejunum and ileum), whereas LPH mRNA was absent from the ileum. LPH activity rose and fell in conjunction with mRNA, but SGLT1 activity was greatest at the villus tip where mRNA levels were considerably reduced. These data have been used to discuss the genetic regulation of enterocyte differentiation and function.

Key words: Differentiation – Brush border – Crypt – Villus – Absorption – Digestion – Glucose transport

Introduction

Enterocytes are the predominant cell type of the small-intestinal epithelium and are primarily responsible for nutrient digestion and acquisition. They originate from anchored pluripotent stem cells near the base of the crypts of Lieberkühn (for review see [25]) and thereafter migrate towards the villus tip where they are extruded into the intestinal lumen. Cell renewal occurs throughout the life of the animal; the epithelial cell layer is completely replaced every 2–5 days [9]. When the intestine is sectioned along the crypt-villus axis the enterocytes can be shown to undergo progressive modification to their structure and function [28]. Perhaps the most significant feature in this process is the change in phenotype observed at the junction of crypt and villus. The spatial and chronological organisation of the enterocyte life-cycle make this cell population a unique model with which to study the processes of eukaryotic cell differentiation.

The brush-border membrane, situated at the apical surface of the enterocyte, forms a continuous semi-permeable barrier stretching from the pylorus to the ileocecal junction. Embedded in its surface are numerous proteins which execute many of the absorptive and digestive functions of the gut. For the sequential processing of luminal nutrients the synthesis of digestive enzymes and transporters must be regulated according to the cells’ position along the length of the intestine (the longitudinal axis), as well as along the crypt-villus (vertical) axis. In addition, selective changes in the composition of this membrane take place during postnatal development and dietary manipulation [15, 18]. The way in which regulatory factors controlling the longitudinal and vertical differentiation of these cells interact with developmental and adaptive changes in the luminal environment remains poorly understood.

The synthesis of mRNA might be considered as the primary control point in the regulation of protein synthesis, and hence cell function. We have therefore developed molecular probes complementary to the mRNAs encoding for two enterocyte brush-border proteins: lactase-phlorizin hydrolase (LPH) (EC 3.2.1.23-62) [22], which hydrolysates the milk sugar lactose into its constituent monosaccharides D-glucose and D-galactose, and the Na\(^+\)-glucose cotransporter (SGLT1) [13, 14], which absorbs these sugars. These proteins are of particular inter-
Materials and methods

Materials were obtained from BDH Ltd, Poole, Dorset, UK, unless otherwise stated.

Collection of tissue. Rabbits (10 weeks old, n = 6) that had been maintained on laboratory chow until used were killed by an intravenous injection of a lethal dose of pentobarbitone (0.7 ml/kg, 20% w/w, Lethobarb, Duphar Veterinary Ltd., Southampton, UK). The abdomen was opened and the small intestine promptly removed. Samples of duodenum (10 cm from the pylorus), jejunum (50% of small intestinal length) and ileum (10 cm from the ileocecal junction) were washed with ice-cold phosphate-buffered saline containing 0.5 mM dithiothreitol and 1 U/ml RNase inhibitor (Sigma Ltd., Poole, Dorset, UK). The intestine was then either opened out, embedded in pig liver and rapidly frozen in isopentane cooled with solid carbon dioxide, or wrapped in foil and frozen in liquid N2. Samples were stored under liquid N2 until use.

In situ hybridisation using oligonucleotide probes. The basic method used for in situ hybridisation has been described previously [27]. Briefly, cryostat sections (10μm) were thaw-mounted onto poly-L-lysine-coated slides and then either fixed in 4% paraformaldehyde and stored in 95% ethanol at 4°C or stored unfixed at -20°C for in situ hybridisation and cytochemical analysis, respectively. Complementary “sense” and “anti-sense” oligodeoxyribonucleotide probes were synthesised on a Biosearch 8700 DNA synthesiser and purified on 8% polyacrylamide/8 M urea preparative sequencing gel (three probes corresponding to sequences of the rabbit or human LPH cDNA [EC 3.2.1.23-62] [22] and one to the rabbit/human SGLT1 [13, 14] sequence, see Table 1). The sequences of all the probes were unique to LPH or SGLT1 when examined on the GenBank and EMBL databases [2, 41], releases 70 and 30 respectively. The probes were labelled with [35S]dATP (130 Ci/mM, NEN, Stevenage, Herts., UK) using terminal deoxynucleotidyl transferase (Pharmacia, Milton Keynes, Bucks., UK) at 37°C for 1 h. Probes were applied to the sections in hybridisation buffer containing 4X standard sodium citrate (SSC), 50% deionised formamide, 25 mM sodium phosphate (pH 7.0), 5X Denhardt’s solution, 200 μg/ml hydrolysed salmon sperm DNA, 120 μg/ml heparin, 100 μg/ml polyadenylic acid, 10% dextran sulphate and 40 μM dithiothreitol. The slides were incubated overnight at 42°C before being washed in 1X SSC containing 20 mM mercaptoethanol for 1 h at 55°C and dehydrated through alcohol. Slides were exposed to X-ray film (Kodak XAR.5) for 5 days and then dipped in Ilford K5 emulsion and stored desiccated at 4°C for 2 weeks. After development, sections were stained with eosin and mounted with a coverslip.

Quantification of in situ hybridisation. The procedure used to quantify the in situ hybridisation signal was similar to that used by other workers for the quantification of calbindin mRNA in chicken jejunum [21]. In the present study every attempt was made to minimise variation due to localised differences in the intestine and photographic emulsion. When preparing slides at least 50 μm was allowed between consecutive sections, and three to five sections were placed on each slide. All in situ hybridisation was carried out in a single assay and slides were treated identically throughout.

Sections were examined using a MPV-3 microdensitometer (Leitz, Milton Keynes, Bucks., UK) at a magnification of ×400. The wavelength of the spectrophotometer was set at 640 nm. The measuring window (15X60 μm) was placed over the lamina propria near to the villus to be measured and the absorbance reading was set at zero. Consecutive absorbance readings were then taken from over the enterocyte population from the crypt base to the villus tip on five separate villi per slide.

LPH cytochemistry. Cytochemical determination of LPH activity was performed using 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside at pH 6.0 for 50 min at 37°C according to the method of Gutschmidt and Emslie [10]. After incubation, sections were fixed in 4% formaldehyde and mounted in glycerine jelly. LPH activity was quantified in a manner similar to that described for the in situ hybridisation, using a MPV3 microdensitometer, with the exceptions that the wavelength was set at 660 nm and a measuring window of 3.2X3.2 μm was used.

Northern blot. Total tissue RNA was prepared using the hot phenol method from samples of duodenum, jejunum and ileum, pooled from three rabbits [26]. Poly(A)-rich RNA was purified by oligo(dT)-cellulose chromatography [26] and precipitated overnight at -20°C in 70% alcohol. The poly(A)-rich RNA was then separated on a formaldehyde denaturing 1.2% agarose gel by electrophoresis and blotted onto a Hybond N membrane (Amersham International, Amersham, Bucks., UK). RNA was fixed to the membrane by UV irradiation and molecular mass markers (BRL, Uxbridge, Middx., UK) were made visible by ethidium bromide staining. After prehybridisation at 42°C for 4 h the blot was hybridised overnight at 42°C with the 32P-labelled SGLT1 anti-sense probe in hybridisation buffer under the same conditions as those described for in situ hybridisation. After washing in 1XSSC/0.1% sodium dodecyl sulphate at room temperature for 30 min and then at 55°C for 30 min, the blot was exposed to Kodak XAR5 X-ray film overnight at -80°C in X-ray cassettes containing DuPont Cronex Lightning Plus intensifying screens. Blots were evaluated on a Chromoscan 3 (Joyce Loeb, Gateshead, Tyne & Wear, UK) gel scanner.

Analysis of data. The mean level of mRNA or activity (absorbance) at each point along the crypt-villus axis was calculated from the mean of five readings from five separate villi selected from each sampling position. Since there were variations in the length of individual villi, mean absorbance values along the crypt-villus axis were included in the results only when over half the villi being measured still gave readings. The mean villus absorbance was calculated for each animal and a paired t-test used to compare enterocyte LPH and SGLT1 mRNA levels in the various intestinal positions. To examine the relationship between the level of LPH mRNA and LPH activity, mean villus LPH mRNA and activity values were related using regression analysis.

Results and discussion

The quantitative in situ analysis of enterocyte LPH and SGLT1 mRNA levels, and LPH activity, along the crypt-villus axis as well as along the length of the small intestine illustrates some of the fundamental processes that regulate enterocyte gene expression and therefore absorptive and digestive function.

Gene expression along the crypt-villus (vertical) axis

In situ hybridisation of the 35S-labelled oligonucleotide probes to mRNAs encoding for LPH and SGLT1 was carried out on sections of rabbit duodenum, jejunum and