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Genetic regulation of enterocyte differentiation

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Partly digested food entering the small intestine is finally broken down into a number of simple components by a variety of hydrolytic enzymes present on the surface of intestinal villi. These products of hydrolysis are then transported across the intestine by different carrier molecules present on the microvillus surface of villus enterocytes. Microvilli expand considerably the surface area over which nutrient digestion and absorption can take place. Genetic defects affecting any one of these three important components of enterocyte differentiation can lead to increasing morbidity and death (Milne, 1974; Freiburghaus *et al*. 1976; Phillips & Schmitz, 1992). Other factors affecting differentiation include interactions taking place between enterocytes and the underlying mesodermal tissue (Haffen *et al*. 1989) and between enterocytes and the gut-associated immune system (Smith, 1991). Change of diet and the proliferative state of the gut further affect the way in which enterocytes express microvillus structure and digestive and absorptive functions (Smith, 1992). All these factors should be considered by anyone wishing to understand how diet and health affect intestinal function.

Use of appropriate experimental models has been important in allowing specific aspects of enterocyte differentiation to be studied separately. Tissue recombination experiments were needed, for instance, to positively identify mesodermal effects on enterocyte development (Kedinger *et al*. 1981); immunocyte effects on enterocyte differentiation are best studied in germ-free animals challenged by a single organism (Savidge *et al*. 1991), and sugar induction of disaccharidase expression is best interpreted in the absence of any effect on crypt cell proliferation (Collins *et al*. 1989). However, studying normal enterocyte differentiation in culture has not proved successful as yet and the use of transgenic animals has only recently begun to muddy the waters (Hauft *et al*. 1992). Dealing intelligently with known variables seems on the whole to be preferable to ignoring them or artificially increasing their number.

PAST HISTORIC TO PRESENT CONTINUOUS

The original finding that enterocytes born in intestinal crypts subsequently migrated in a more or less linear fashion over the surface of villi to be later shed into the intestinal

lumen can now be seen to have been extremely important, both in creating an entirely new vision of how an intestine might operate and in directing research into a new area of intestinal cell biology (see Leblond, 1965). Subsequent measurement of brush-border membrane length (Brown, 1962), transport of nutrients (Kinter & Wilson, 1965; Stirling & Kinter, 1967) and digestive enzyme activities (Nordström *et al.* 1968; Weiser, 1973) showed all to increase as part of a process of enterocyte differentiation taking place on intestinal villi. Further research used new techniques of quantitative analysis to describe how this development took place in villus-attached enterocytes (King *et al.* 1983; Menge *et al.* 1983; Smith *et al.* 1984; Cheeseman, 1986; Cremaschi *et al.* 1986; Collins *et al.* 1989). Simple equations relating variables affecting enterocyte expression of microvillus structure and disaccharidase induction have recently emerged from this type of analysis (Collins *et al.* 1989; Smith & Brown, 1989). Enterocyte differentiation takes place sequentially, full expression of microvillus structure and hydrolase activities preceding that for absorptive function (Kinter & Wilson, 1965). Part of this change involves a significant increase in intracellular K^+ activity (Cremaschi *et al.* 1986). Subsequent cloning and sequencing of genes affecting different functions during development now makes it possible to study this aspect of enterocyte development in further detail. The following description refers mainly to findings obtained for genes coding for a vitamin D-dependent Ca-binding protein (CaBP gene) and a Na–glucose-linked transporter (SGLT1 gene).

CaBP EXPRESSION IN CHICKEN ENTEROCYTES

Intestinal CaBP mRNA has been shown by dot blot analysis to increase rapidly in vitamin D-deficient chicks injected with dihydroxyvitamin D (Mayel-Afshar *et al.* 1988). Recent experiments determine the cellular origin of this response; results obtained from these experiments are summarized in Fig. 1. CaBP mRNA is expressed maximally in basal villus enterocytes. This is also the site for maximal expression of mRNA coding for villin (Boller *et al.* 1988), fatty acid-binding protein (Iseki & Kondo, 1990), amino-peptidase N (*EC* 3.4.11.2; Norén *et al.* 1989), sucrase (*EC* 3.2.1.48)–isomaltase (*EC* 3.2.1.10; Traber, 1990), lactase (Freeman *et al.* 1992) and the Na–glucose-linked transporter (Smith *et al.* 1992). It is concluded from this that a common factor is probably responsible for inducing maximal expression of a number of mRNA species in enterocytes shortly after they leave the intestinal crypt.

Maximal expression of CaBP protein also occurs in basal villus enterocytes and this is the site where many other gene products first appear in appreciable amounts. Special to CaBP, however, is the fact that no protein can be detected in any enterocyte in the absence of vitamin D. This means that CaBP appearance can be directly related to levels of CaBP mRNA. Relative excess of message over product in basal villus enterocytes then indicates a decreased ability of message to be translated into protein (Fig. 1). Further insight into message control over CaBP synthesis can be obtained by studying the time-course of induction following injection of dihydroxycholecalciferol into vitamin D-deficient chickens. Results from these experiments are summarized in Fig. 2.

Trace amounts of CaBP mRNA detected around the crypt–villus junction increase to maximal levels 8 h after injection of dihydroxycholecalciferol into vitamin D-deficient chicks. There is also a decreasing gradient of mRNA concentration along villi taken 3–24 h after injection of dihydroxycholecalciferol. CaBP detected in trace amounts 3 h after
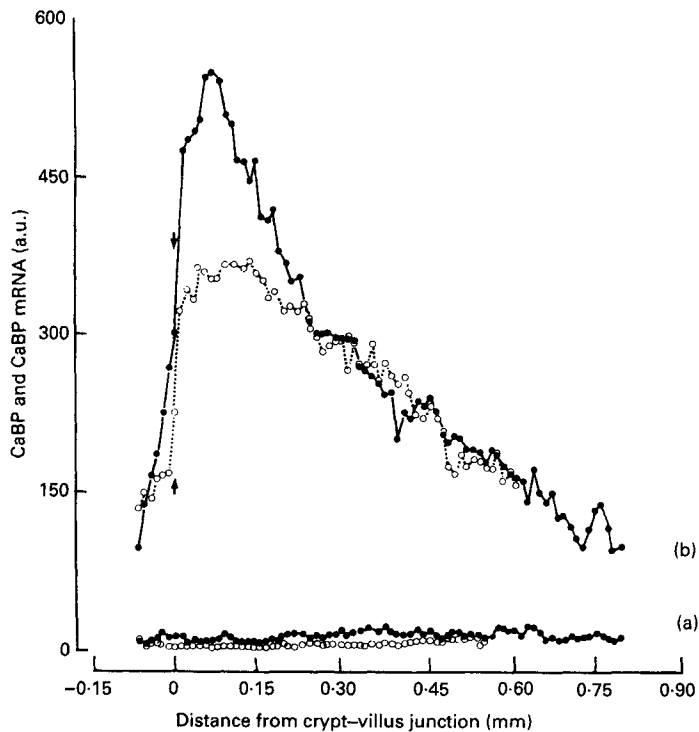
Fig. 1. Vitamin D effects on calcium-binding protein (CaBP) gene expression and CaBP mRNA production by chicken enterocytes. Tissues taken from vitamin D-deficient chickens before (a) or 48 h after injection of vitamin D (b) were processed to determine CaBP (O) and CaBP mRNA levels (●). ↑, Position of the crypt–villus junction. a.u., arbitrary units (Kiyama *et al.* 1991).

injection reaches maximal levels after 24 h. This is at a time when mRNA levels are already beginning to decrease. There is always an excess of mRNA over CaBP in crypt and basal villus enterocytes. This discrepancy is greatest 8 h after injection of dihydroxy-cholecalciferol. Previous work carried out on intestinal homogenates has suggested either a transcriptional (Spencer *et al.* 1978; Siebert *et al.* 1982; Theofan *et al.* 1986) or mainly post-transcriptional control over CaBP synthesis (Dupret *et al.* 1987; Mayel-Afshar *et al.* 1988). Present work suggests that mechanisms controlling CaBP synthesis change during enterocyte differentiation. It is finally interesting to compare these results with those found under vitamin D-replete conditions. Results obtained using tissue taken from immature and mature egg-laying chickens are shown in Fig. 3.

Low but detectable levels of CaBP mRNA present in tissue taken from 11- and 17-week-old non-laying chickens are not associated with any detectable expression of CaBP. High levels of CaBP mRNA measured in 25-week-old laying birds produce a profile similar to that seen after vitamin D injection into vitamin D-deficient chickens (Fig. 1). However, expression of CaBP protein in these older birds is increasing throughout enterocyte migration to the villus tip. This change in profile results from the fact that CaBP is a stable protein having a half-life of approximately 29 h (Norman *et al.* 1981). Most of the CaBP synthesized during differentiation will remain, therefore, in each cell until it becomes extruded from the villus tip. This situation does not apply in
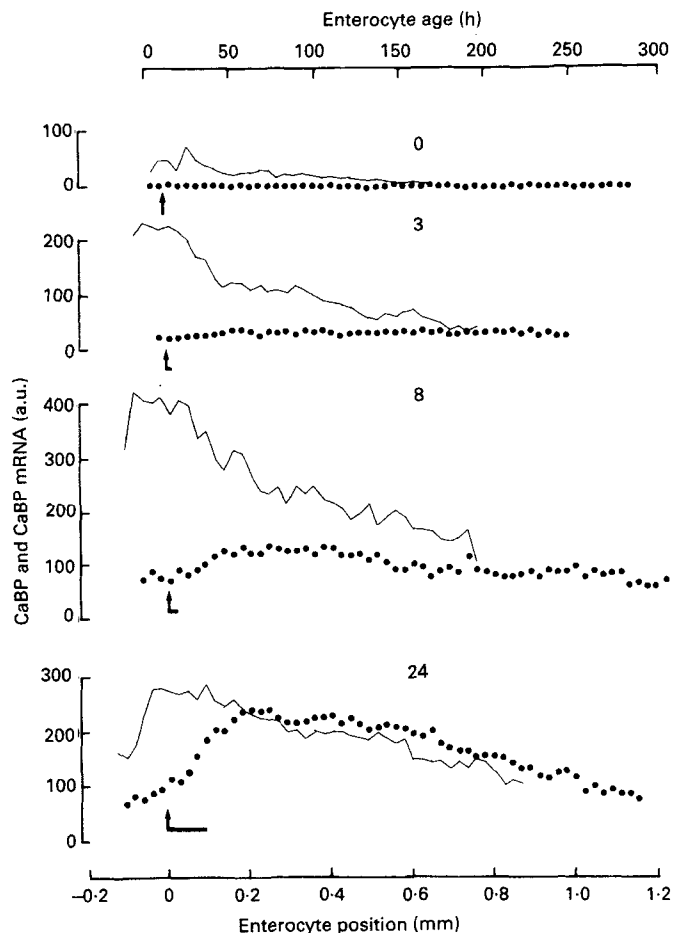
Fig. 2. Effect of dihydroxycholecalciferol on calcium-binding protein (CaBP) mRNA and CaBP expression by chicken enterocytes. Tissues taken from vitamin D-deficient chickens before and 3, 8 and 24 h after intracardiac injection of 125 ng dihydroxycholecalciferol were used to determine CaBP (●) and CaBP mRNA levels (–). ↑, Position of the crypt–villus junction. ↑ Distances travelled by enterocytes 3, 8 and 24 h after dihydroxy-cholecalciferol injection (Wu *et al.* 1992). a.u., arbitrary units.

vitamin D-deficient birds where enterocytes at different positions on the villus are being induced to synthesize CaBP for the first time following the injection of vitamin D (Kiyama *et al.* 1991). Failure to recognize this type of difference could lead to erroneous interpretation of results when studying mechanisms controlling the biosynthesis of other markers of enterocyte differentiation.

SGLT1 EXPRESSION IN RABBIT ENTEROCYTES

The preceding section dealt with gene expression during an early stage of enterocyte differentiation. Work with the SGLT1 gene considers the later expression of sugar transport and relates this to the level of SGLT1 mRNA expression in individual villus-attached enterocytes (Smith *et al.* 1992). Results obtained from this work are summarized in Fig. 4.
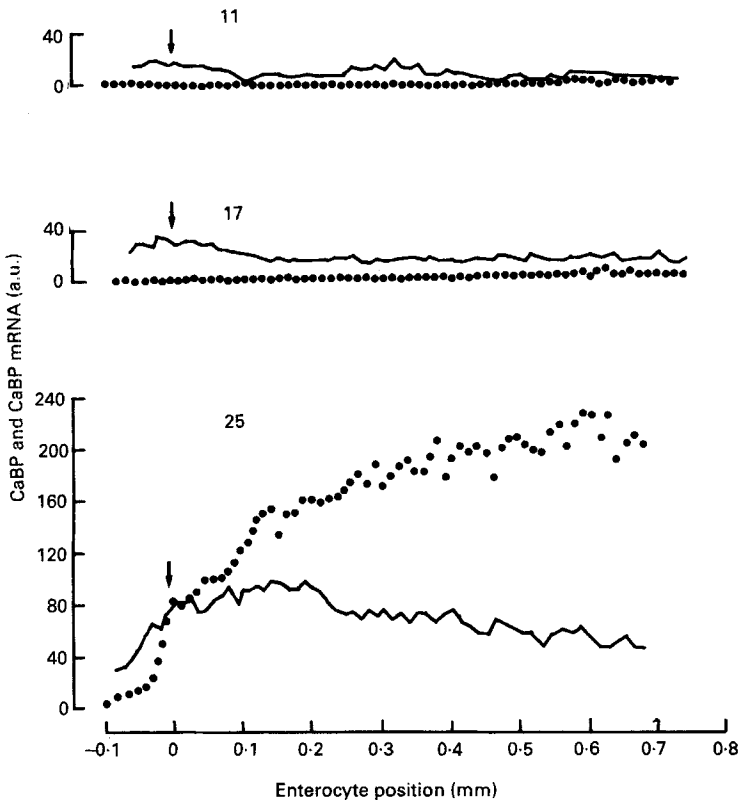
Fig. 3. Calcium-binding protein (CaBP) mRNA and CaBP expression measured in enterocytes taken from immature, mature non-laying and mature laying chickens. Jejunal tissue taken from 11-, 17- and 25-week-old chickens was used to determine CaBP (●) and CaBP mRNA (–). ↓ , Position of the crypt–villus junction (Wu *et al*. 1993). a.u., arbitrary units.

The profile describing SGLT1 mRNA in rabbit is similar to that found recently for many other markers of an early phase in enterocyte differentiation. Glucose transport only appears in the upper half of the villus. This latter finding confirms previous work carried out in hamster intestine (Kinter & Wilson, 1965). The question of whether expression of SGLT1 transporter matches that for mRNA or glucose transport is now being studied. Unpublished findings showing phloridzin binding present in all regions of the villus suggests that the SGLT1 protein is expressed early in development. This would agree with other work showing the protein to be expressed all along the villus in rat intestinal tissue (Haase *et al*. 1990; Takata *et al*. 1992).

New methods used to examine the process of enterocyte differentiation taking place *in situ* raise several questions that now need further attention. What precipitates a general switching on of mRNA expression as cells leave crypts? Why does one sometimes see mRNA which is not being translated? Can late activation of the glucose transporter explain results obtained in the rabbit? These questions give some examples of subjects suitable for future research. Of equal importance is the realization that each enterocyte carries its own history of events taking place within it since its time of birth in an
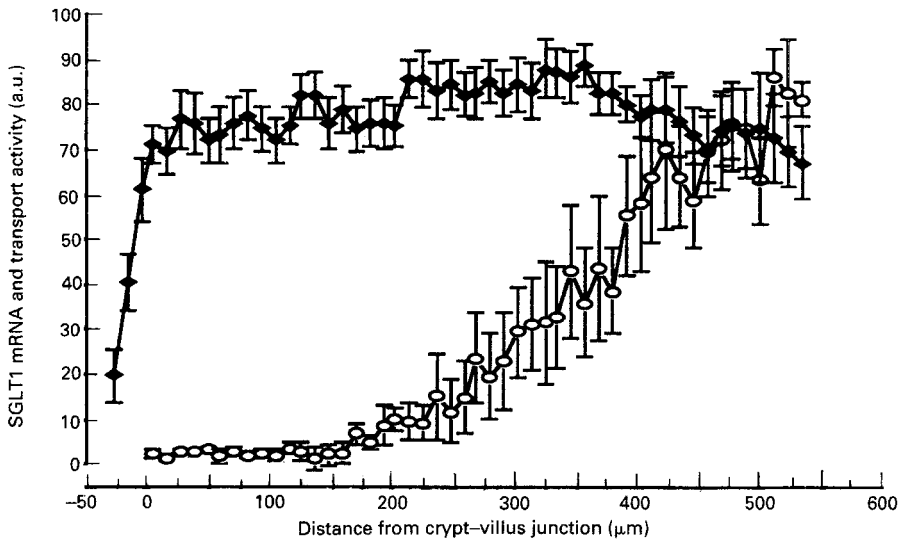
Fig. 4. Relationship between enterocyte expression of SGLT1 mRNA (◆) and phloridzin-sensitive SGLT1 glucose uptake (○) (Smith *et al.* 1992). a.u., arbitrary units.

intestinal crypt. The message can in this case be regarded as an engine driving a process leading to formation of a protein product. The amount of that product in turn depends on the amount of message and the stability of the product. Trying to relate simple measurements of total mRNA to total protein formed in a tissue as complicated as the intestine is clearly not going to advance knowledge in the future.

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