Modelling molecular mechanisms controlling sequential gene expression in differentiating mammalian enterocytes

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Abstract. The gut epithelium represents a continuous developmental system in which cell proliferation in intestinal crypts is followed by the sequential expression of digestive and absorptive functions as enterocytes migrate out of crypts to the tips of intestinal villi. We have developed a mathematical model in the present work to mimic these sequential aspects of enterocyte differentiation. Using this model allows the characteristics of lactase expression to be ascribed to transcriptional control. In the case of a glucose transporter, however, it became necessary to assume an additional translational control that decreased exponentially as enterocytes migrated along villi. The suggestion that this type of modelling is useful in predicting which set of enterocytes is likely to use translation or transcription to control gene expression is also discussed.

INTRODUCTION

The fact that stem cell division in intestinal crypts leads to the eventual appearance of terminally differentiated cells on intestinal villi has long been established (see Leblond 1981). Opinion on how this process operates at the molecular level, however, remains largely speculative. This lack of knowledge arises partly from the complexity of the system being analysed. It is, for instance, sometimes difficult to recognize newly formed cell types and no area in the crypt is solely committed to proliferation, lineage specification or differentiation. Testing informed assumptions about events taking place in crypts can prove useful in suggesting new areas for research. Examples of this approach include the recent suggestion that crypt cells might mature without losing the capacity to divide (Potten & Loeffler 1990) and that genes regulating gut development in lower eucaryotes may be present as mammalian homologues (Hermiston *et al.* 1994).

Cells migrating out of crypts already exhibit morphological features allowing them to be classified as enterocytes, goblet cells and enteroendocrine cells. There are, however, many aspects of terminal differentiation still to be completed as these cells continue to migrate towards the tips of villi and some of these events can be measured directly in sectioned villus-attached enterocytes (Kiyama *et al.* 1991, Collins *et al.* 1993, Dong *et al.* 1997). It appears from this and earlier work that gene expression takes place sequentially during enterocyte

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differentiation (Smith 1991) but it is still not known how this process might be organized at the molecular level. It has, however, recently become possible to complete measurements of mRNA, protein and biological activities for both an early and late expressed gene in differentiating enterocytes (Collins 1994, Dong *et al.* 1997).

The present work uses these data to construct a mathematical model which allows predictions on how mechanisms controlling gene expression change during enterocyte development. This type of analysis could prove useful in suggesting new areas of research able to identify the molecular basis of enterocyte differentiation. Part of this work has already been published in abstract form (Brown & Smith 1998).

METHODS

Source and use of experimental data

Genes expressing lactase (LPH) and a sodium-dependent glucose transporter (SGLT1) were chosen to represent early and late events taking place during enterocyte differentiation. Results of measurements of LPH and SGLT1 mRNA, proteins and biological activities came from previous work carried out on 6-week-old pigs (LPH; Collins 1994) and 9–10-week-old rats (SGLT1; Debnam *et al.* 1995, Dong *et al.* 1997). Data obtained from this work involved the use of a microdensitometer to obtain sequential measurements of mRNA, protein and biological activities from base to tip of sectioned villi. Individual values of optical absorbance, taken directly from the microdensitometer and plotted as arbitrary units, allowed valid comparisons to be made between the expression of LPH and SGLT1 along jejunal villi.

In order to model the above data it was also necessary to know both the rate at which enterocytes migrated along villi (v) and the *in vivo* half-lives for LPH and SGLT1 proteins ($t_{1/2}$). These values were obtained or derived for jejunal tissue from the following publications: values of v for rat and pig of 8.5 and 8.7 $\mu\text{m h}^{-1}$ (Dauncey *et al.* 1983, Collins 1994, Thomson *et al.* 1994); values of $t_{1/2}$ for LPH and SGLT1 of 11 and 16 h (Castillo *et al.* 1990, Tsuboi *et al.* 1992, Karasov *et al.* 1983, Diamond & Karasov 1984). The above values can only be determined over a 2-day period and it is assumed that any change in cell proliferation taking place over this period will have no effect on these constants. It is, however, preferable to confine these types of measurements to the jejunum since this tissue shows little or no diurnal variation in crypt cell proliferation (Pilgrim *et al.* 1963).

The mathematical model

Notation

The initial assumption made when fitting the model to experimental data was that gene expression was only subject to transcriptional regulation. This implied that all the mRNA detected in each cell was capable of being fully translated. Later it became necessary to incorporate a measure of translational control. The notation used to describe various features of the model is shown in Figure 1.

The concentration of LPH and SGLT1 proteins (ρ) expressed at any distance x along the villus is given as $\rho(x)$. The flow (or flux) of protein, $J(x + \delta x)$, after cells migrate a further distance (δx) from B to C along the villus is equal to the flow, $J(x)$, found at B plus that newly synthesized, minus that lost by degradation. The degradation rate for each protein (k) is determined from the half-life of the protein ($t_{1/2}$) where $t_{1/2} = \log_e(2)/k$. The rates of cell migration along villi (v) are also incorporated into model equations in order to predict the final concentrations of LPH and SGLT1 proteins present along the villus.

Figure 1. A schematic diagram illustrating the main variables in the mathematical model.

The cross-sectional circumference, referred to in the model (A) to represent the three-dimensional nature of the villus was later shown to have no specific effect upon the model. The concentration of message coding for these proteins at any point x along the villus is given as $m(x)$. The translation rate per unit amount of mRNA into protein is referred to as c .

It was necessary in the modelling to assume that enterocytes migrate linearly along villi and that the rate of cell migration remains constant. Villus shape resembles a flattened cylinder that narrows towards the top to form a closed tip, however, and there are different opinions as to how this might affect the paths of migration and cell kinetics (Tsubouchi 1983; Totafurno *et al.* 1990, see also Brown *et al.* 1993 for references). This problem was avoided in the present work by restricting the modelling of gene expression to the lower two thirds of villi. Having taken this precaution it then became possible to accept the above assumptions and derive equations describing the *expected* characteristics for LPH and SGLT1 expression for comparison with *actual* measurements of LPH and SGLT1 proteins determined in villus-attached enterocytes. Steps leading to the derivation of model equations are described below.

Modelling for transcriptional control

To derive a mathematical model, we first use the conservation principle as outlined above (i.e. in a steady state, output = input + creation – loss within specified region):

i.e. flux at C = flux at B – loss in slice + production in slice which can be written in symbols as

$$J(x + \delta x) = J(x) - \rho Ak \delta x + cmA \delta x$$

$$\frac{J(x + \delta x) - J(x)}{\delta x} = -\rho Ak + cmA$$

Letting $\delta x \rightarrow 0$, and writing $J(x)$ as $vA\rho$, and cancelling out A from the resulting expressions, we obtain

$$\frac{d(v\rho)}{dx} = -\rho k + cm$$

If we now consider the case when cell velocity v is constant along the length of the villus, we can divide through by v to obtain

$$\frac{d\rho}{dx} + \frac{k\rho}{v} = \frac{cm}{v} \quad (1)$$

This can be integrated by first multiplying through by $\exp(kx/v)$ to obtain the general expression

$$\rho(x) = \frac{ce^{-kx/v}}{v} \int_0^x e^{kz/v} m(z) dz$$

The cases that occurred in the present study involved messages which declined or rose exponentially along the villus, i.e. $m = m_0 \exp(\lambda x)$, in which case

$$\rho(x) = \frac{cm_0}{k + \lambda v} \left(e^{\lambda x} - e^{-kx/v} \right) \quad (2)$$

This equation can also be applied to obtain an expression for product density when the message cannot be written as a single exponential expression in distance along the villus, x , but as a sum of two or more exponentials, by treating each exponential term separately and adding the results.

Modelling for translational control

The general differential equation for the density of product at each point along the villus was given by equation (1)

$$\frac{d\rho}{dx} + \frac{k\rho}{v} = \frac{cm}{v}$$

If we now consider that the translation rate, c , changes along the villus, the above equation can be integrated using the method above to obtain

$$\rho(x) = \frac{e^{-kx/v}}{v} \int_0^x e^{kz/v} c(z)m(z) dz \quad (3)$$

where the $c(z)$ is now inside the integral sign, since this varies along the villus. If we model $m(z)$ as before by an exponential $m(z) = m_0 \exp(\lambda z)$ and $c(z)$ also by a similar exponential $c(z) = c_0 \exp(\beta z)$, this becomes

$$\rho(x) = \frac{c_0 m_0 e^{-kx/v}}{v} \int_0^x e^{kz/v + \lambda z + \beta z} dz$$

which is integrated to obtain

$$\rho(x) = \frac{c_0 m_0}{k + (\lambda + \beta)v} \left(e^{(\lambda + \beta)x} - e^{-kx/v} \right) \quad (4)$$

Fitting the model

In the simple case, where a single exponential was adequate to describe the message profile along the villus, the expression $m(x) = m_0 \exp(\lambda x)$ was fitted by nonlinear least squares to the message profile, and then expression (2) or (3) was fitted as appropriate to the product using unweighed least squares, but constraining the parameters m_0 and λ to take the values obtained in the original fit to the message. The Genstat package (Payne *et al.* 1993) was used throughout for model fitting. In the simple case when translational rate is constant and equation (2) was fitted, the only parameter to be fitted is the constant c . In the case of translational control, the parameters to be fitted are c_0 and β .

RESULTS

Initial control experiments

The fact that LPH protein can sometimes remain inactive (Maiuri *et al.* 1994) and that SGLT1-mediated transport can sometimes become activated (Koopsall & Spangenberg 1994) raises questions about whether protein or activity should be used as a marker of gene expression. It was therefore initially decided to compare proteins and biological activities expressed by these two genes along the villus to test whether any differences observed were operationally significant. The results obtained are summarized in Figure 2. LPH protein and enzyme activity increased steadily to a plateau reached at about 250 μm from the crypt–villus junction. SGLT1 protein and transport activity increased slowly and then more rapidly to reach a plateau 320–350 μm from the crypt–villus junction. No significant difference could be detected at any point along the villus between measurements of protein and activity for LPH or SGLT1. It was therefore decided to calculate mean values of protein/activity along the villus and use these as markers of enterocyte differentiation.

Curve fitting for transcriptional control

Curve fitting to data relating to LPH expression is shown in Figure 3. There is good agreement between experimental findings for LPH mRNA and the exponential curve chosen to fit the data (Figure 3a). Constants describing this curve were then used to generate a second theoretical curve predicting the positional profile for the expression of LPH product assuming $t_{1/2}$ for LPH to be 11 h and v to be 8.7 $\mu\text{m h}^{-1}$. Experimentally determined values for LPH product fit closely around this theoretically derived curve (Figure 3b).

A range of values for $t_{1/2}$ and v was next used to determine which combination of constants would produce theoretical curves of best fit to the experimental data. The results obtained from this exercise are summarized in Figure 4. Curves obtained when changing $t_{1/2}$ from 2 to 20 h, with v constant at 8.7 $\mu\text{m h}^{-1}$, showed minimal residual deviance when $t_{1/2}$ was 6 h (Figure 4a). Curves obtained when changing v from 2 to 20 $\mu\text{m h}^{-1}$, with $t_{1/2}$ constant at 11 h, showed minimal residual deviance when v was 5 $\mu\text{m h}^{-1}$ (Figure 4b). These revised values for $t_{1/2}$ and v are, however, themselves subject to error arising from the assumption that v was 87.5 $\mu\text{m h}^{-1}$ when varying $t_{1/2}$ and that $t_{1/2}$ was 11 h when varying v . Further modelling of different combinations of these constants produced a final curve of best fit having a $t_{1/2}$ value

Figure 2. Positional dependence of LPH and SGLT1 gene expression of protein and biological activity in proximal jejunal villus-attached enterocytes. Values of LPH (a) and SGLT1 (b) protein (O) and biological activities (+) are plotted in arbitrary units (au) against the distance enterocytes migrate from the crypt/villus junction (c/v).

of 11 h and a v of $5 \mu\text{m h}^{-1}$. These values are similar to those found by experiment. Varying $t_{\frac{1}{2}}$ and v over a wide range produces only small changes in residual deviance.

Similar curve fitting routines were used to test for transcriptional control over SGLT1 expression. The results obtained are summarized in Figure 5. The initial fitting of an exponential curve to experimental values of SGLT1 mRNA was again good (Figure 5a). Further use of this curve to predict a profile for SGLT1 product, however, produced a theoretical curve bearing no resemblance to the actual distribution pattern of SGLT1 product measured by experiment (Figure 5b). The amount of product predicted to be present in the lower half of the villus was considerably greater than that found experimentally. This situation was reversed in the upper part of the villus.

Figure 3. Positional dependence of LPH expression along pig intestinal villi. (a) Experimental values for LPH mRNA fitted by an exponential curve; and (b) a model curve generated on the assumption that there is only transcriptional control over the expression of LPH product. O; values of LPH product measured experimentally.

Curve fitting for translational control

The method for modelling translational control assumes that the efficiency with which SGLT1 mRNA can be translated increases exponentially along the villus. The characteristics of the curve describing this supposed effect can then be matched with an exponential fall in SGLT1 mRNA to produce a curve fitting the experimentally determined values of SGLT1 product. The values of v and $t_{\frac{1}{2}}$ used in this calculation were the same as those used to construct the curve in Figure 5b.

Translation of SGLT1 mRNA in this model is predicted to be about 5% efficient in cells leaving the crypt (Figure 6a). Fifty per cent translational efficiency is assumed to be reached as enterocytes pass into the upper third of the villus. Combining the characteristics of the

(a)

(b)

Figure 4. Effect of varying $t_{1/2}$ and v constants on model curve fitting to LPH results. The method of model curve fitting was as described in the legend to Figure 2. The error associated with curve fitting is given as residual deviance (sum of squares, SS) for $t_{1/2}$ values varying from 2 to 20 h (a) and v varying from 2 to 20 $\mu\text{m h}^{-1}$ (b). An experimentally determined value for v of 87.5 $\mu\text{m h}^{-1}$ was used when varying $t_{1/2}$. The corresponding value for $t_{1/2}$ was 11 h when varying v (b).

curve in Figure 6a with those described in Figure 5a produces a theoretical profile for the appearance of SGLT1 product that closely fits experimental findings (Figure 6b). A range of $t_{1/2}$ and v values was finally used to determine which theoretical curve would best fit the experimental data plotted for SGLT1 expression in Figure 6b. The results of this exercise are plotted in Figure 7.

The residual variance fell from 445 to 280 when increasing $t_{1/2}$ from 2 to 20 h. The greatest fall in residual variance occurred over the lower range of $t_{1/2}$ values (165 from 2 to 10 h; 30 from 10 to 20 h). A similar pattern of decreasing residual variance occurred when v was increased from 2 to 20 $\mu\text{m h}^{-1}$ (430–289 for values of v increasing from 2 to 10 $\mu\text{m h}^{-1}$; 289–270 when increasing v from 10 to 20 $\mu\text{m h}^{-1}$). No minimum of the residual variance could be

Figure 5. Positional dependence of SGLT1 expression along rat intestinal villi. The two curves in (a) and (b) were generated by the model as described in the legend to Figure 2.

demonstrated even when extending the range of t_1 and v to 40 h and $40 \mu\text{m h}^{-1}$, respectively (data not shown). The precision with which curve fitting took place for SGLT1 data appeared similar to that found for LPH. The general insensitivity of the model to variations in t_1 and v in regions containing values determined by experiment was also similar to that found for LPH.

DISCUSSION

Regulation of eucaryotic gene expression can involve changes taking place in transcription, RNA processing, translation and the selective activation or inhibition of protein products (Alberts *et al.* 1997). Each of these possibilities is normally studied in isolation, however, with

(a)

(b)

Figure 6. Modelling translational control over SGLT1 gene expression along rat intestinal villi. (a) Exponential curve describing a putative increase in translational efficiency taking place during enterocyte migration along the crypt-villus axis. (b) Exponential curve obtained by combining information coming from experimental measurements of SGLT1 mRNA (Figure 4a) with constants describing the theoretical curve described in (a). O; values of SGLT1 protein/activity determined experimentally.

no apparent need to postulate more than one mechanism affecting the expression of a single gene. The situation for SGLT1 and LPH in developing enterocytes appears to be more complex with evidence suggesting control over both transcription and translation (Dong *et al.* 1997, Rossi *et al.* 1997) as well as the activation and inhibition of the proteins produced (Sebastio *et al.* 1989, Koepsall & Spangenberg 1994). This complexity has been largely recognized through work carried out on intact sectioned villi, where patterns of differentiation can be readily identified according to the position and age of the cells concerned. Destroying this organization by homogenizing mixed populations of cells for analysis or attempting to create a similar organization under controlled conditions in

(a)

(b)

Figure 7. Effect of varying $t_{\frac{1}{2}}$ and v constants on model curve fitting to SGLT1 results. The error associated with curve fitting is given as residual deviance (sum of squares, SS). (a) varying $t_{\frac{1}{2}}$ with v equal to $87.5 \mu\text{m h}^{-1}$; and (b) varying v with $t_{\frac{1}{2}}$ equal to 16 h.

transformed carcinoma cells is unlikely to provide useful information as to how differentiation takes place in the intact animal. In order to progress further with this type of research it is therefore necessary to continue work on sectioned villi to identify events controlling the sequential expression of different genes during differentiation. There are, however, many ways to control gene expression. Mathematical modelling in this case provides a means to select between various possibilities and suggest which villus enterocytes should be used for future biochemical studies.

The framework used for the mathematical modelling, which is original, is based on a simple conservation principle. The model chosen is also robust and general in the sense that it is derived with minimal assumptions, relating mainly to the geometry of the villus, the mechanics of cell movement and the relationship of parameters of transcription and

translation to distance along the villus. The main assumption about the geometry of the villus is that it maintains the same circumference along its length. This means that cells move linearly along the villus and that it is reasonable to suppose cells retain a constant velocity and remain constant in size in the restricted length of the villus analysed. A contrasting geometry where this does not hold is the Peyer's patch where the area available to cells becomes smaller very rapidly as the peak of the patch is approached (see Brown *et al.* 1993 for a method of dealing with the resulting complications). It is therefore assumed that message, product and translation rate vary along the villus, but are constant within each plane perpendicular to this direction. A second assumption is that all processes are in a steady state so that the patterns observed do not change with time. This is a standard assumption underlying many measurements and mathematical analyses of dynamic biological processes (Shipley & Clark 1972). This latter assumption enables processes to be modelled using ordinary differential equations in x , the distance along the villus, rather than using partial differential equations in x and t , which would be needed if allowance had to be made for changes in time, t .

There are two main reasons for adopting the mathematical forms used to describe the relationship of message density and translation rate to distance along the villus. Firstly, the experimental data fitted these models well. Secondly, they are mathematically convenient, enabling a straightforward integration of the differential expression (1) to obtain forms such as expressions (2) and (4) which can then be easily fitted to, and tested against, experimental data on message and product. In summary, therefore we have developed and tested a framework for one of the simplest one-dimensional geometries and used it to make inferences about the stages at which protein expression can be controlled during enterocyte differentiation.

Use of the model has already shown that the small differences noted between protein content and biological activities for LPH and SGLT1 are operationally insignificant when considering the overall profiles for gene product expression along villi. This reduces the possible significance of earlier suggestions that LPH and SGLT1 proteins can be subsequently activated or inhibited (Sebastio *et al.* 1989, Koepsall & Spangenberg 1994). More importantly the model has also enabled the tentative identification and characterization of a second component to gene regulation which is assumed to involve the activation of translation of dormant SGLT1 mRNA. The analogy here may be with oocytes and spermatocytes which also 'choose' to translate stored mRNA at different stages in development (Richter 1991; Schäfer *et al.* 1990). The supposed activation of SGLT1 mRNA translation is postulated to increase exponentially as enterocytes migrate along villi and this gives rise to a corresponding exponential rise in SGLT1 product as measured by experiment. The real importance of this modelling is, however, to predict the location of enterocytes containing dormant or fully activated mRNA. This could give rise to experiments designed to identify the general cause of translational silencing. The role of transcription in affecting SGLT1 expression in this case is seen as a separate issue where a variety of diet-induced signals modify the amounts of SGLT1 mRNA expressed in crypts. It was, however, also assumed that the time at which SGLT1 mRNA is expressed can also be controlled with both mechanisms eventually determining the capacity of the villus to absorb glucose.

Obtaining complete sets of data suitable for mathematical modelling depends on the availability of molecular probes to identify different species of mRNA, proteins and biological activities. Acquiring or making such probes in the past has been difficult, but this will become easier in the future. Also, a single species should be used for this type of research to distinguish between species variation and changes directly affecting mechanisms controlling enterocyte

differentiation. The present work shows an early expression of LPH in pigs, and a late expression of SGLT1 in rats, but fortunately there is a long history confirming that this major difference is not species specific. The opportunity now exists to select further genes for study to establish the general characteristics of delayed gene expression and the mechanisms controlling this phenomenon. Selection in this case could arise from the large number of identified genes and transgenes already known to be expressed in different patterns in the small intestine (Hermiston *et al.* 1994). Mathematical modelling in this context can then be expected to lead to the eventual identification of molecules controlling gene expression taking place normally, and in cases where the intestine adapts to a variety of external stimuli.

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