Cell Prolif. 2006, **39**, 403–414

An enteroendocrine cell-based model for a quiescent intestinal stem cell niche

I. R. Radford* and P. N. Lobachevsky†

**South Yarra, Victoria, Australia, and †Trescowthick Research Laboratories, Peter MacCallum Cancer Centre, St. Andrews Place, East Melbourne, Victoria, Australia*

Received 28 *November* 2005*; revision accepted* 2 *May* 2006

Abstract. We have shown that the kinetics of conversion of intestinal crypt cell populations to a partially or wholly mutant phenotype are consistent with a model in which each crypt contains an infrequently dividing 'deep' stem cell that is the progenitor of several more frequently dividing 'proximate' stem cells. An assumption of our model is that each deep stem cell exists in a growth inhibitory niche. We have used information from the literature to develop a model for a quiescent intestinal stem cell niche. This niche is postulated to be primarily defined by an enteroendocrine cell type that maintains stem cell quiescence by secretion of growth inhibitory peptides such as somatostatin and guanylin/uroguanylin. Consistent with this model, there is evidence that the proteins postulated as defining a growth-inhibitory stem cell niche can act as intestinal tumour suppressors. Confirmation that a growth-inhibitory niche does exist would have important implications for our understanding of intestinal homeostasis and tumorigenesis.

INTRODUCTION

Adult mammalian stem cells are thought to exist in specific niches that provide a microenvironment necessary for their maintenance and regulation. Recently, significant progress has been made in defining niches such as those occupied by haemopoietic and neural stem cells (Doetsch 2003; Zhang *et al*. 2003). A common feature of such niches is that the stem cells exist in close association with a differentiated cell type that acts as a source of regulatory signals.

The extensive proliferation that occurs in the intestinal epithelium is thought to be ultimately dependent on stem cells located in the crypts of Lieberkuhn (Marshman *et al*. 2002). Comparatively, little is known about the intestinal epithelium stem cell niche, although it has been postulated to be defined by the mesenchymal cells of the underlying lamina propria and by the intervening basement membrane (by Brittan & Wright 2004).

In the accompanying paper (Lobachevsky & Radford 2006), we have demonstrated that the kinetics of conversion of intestinal crypt cell populations to a partially or wholly mutant phenotype are consistent with the presence in each crypt of an infrequently dividing 'deep' stem cell that is the progenitor of several more frequently dividing 'proximate' stem cells. An assumption of our model is that each deep stem cell exists in a growth inhibitory niche. Quiescent niches for

Correspondence: Dr Ian Radford, 20 Kensington Road, South Yarra, Melbourne, Victoria 3141, Australia.
Tel.: +61-3-9826-3519. E-mail: ianrradford@optusnet.com.au

haemopoietic (Arai *et al*. 2004) and skin (Blanpain *et al*. 2004) stem cells have been identified; however, their existence in the intestinal epithelium has not been conclusively demonstrated. Indeed, it has generally been assumed that all intestinal epithelium stem cells are continuously in a multiplicative state (Potten *et al*. 2003). As we have discussed previously, such a state of continuous replication would result in intestinal stem cells greatly exceeding the limits normally imposed on the number of divisions that can be completed by adult mammalian cells (Lobachevsky & Radford 2006). In addition, it has been shown that the progeny of embryonic stem cells that over-express a LEF-1/β-catenin fusion gene (β-catenin can combine with a member of the TCF/LEF protein family to form a transcriptional activator that is an important mediator of proliferation in intestinal epithelium) are eliminated from the intestine of chimeric mice during development (Wong *et al*. 2002), supporting an absolute requirement for intestinal stem cells to be convertible to a quiescent state.

Here we present a model for a quiescent intestinal stem cell niche along with supportive evidence. We have not tried to consider/review all possible aspects of intestinal stem cell niche regulation, as this is not the purpose of this study; rather, we have examined those aspects that we believe are relevant to our model.

Searching for a niche

The intestinal crypt epithelium is a single layer of cells resting on a basement membrane and surrounded by a lamina propria that contains myofibroblasts, inflammatory cells, capillaries, lymphatics and neural processes (Madara & Trier 1987). Although some differences in basement membrane composition between crypt regions have been described (Teller & Beaulieu 2001), such differences do not appear relevant to defining the location of stem cells (Simon-Assmann *et al*. 1998), and it is not obvious why one region of the crypt should be preferred to another as the locale for stem cells. Indeed, there appear to be region-dependent differences in their placement along the rodent colon, with stem cells found at the base of crypts in the mid and distal regions but towards the centre of crypts in the proximal colon (Sato & Ahnen 1992; Potten & Grant 1998). Based on our hypothesis of a quiescent intestinal stem cell niche and on the deduction that the niche was unlikely to be solely or largely defined by elements external to the epithelium (that is, we were unable to identify components of the lamina propria that might be responsible for a highly localized growth inhibitory signal), we became interested in the distribution of cell types within the crypt epithelium.

The progeny of intestinal stem cells include both enterocytes and a variety of enteroendocrine cell types. The latter is characterized by differences in peptide hormone and biogenic amine output (Jenny *et al*. 2002; Rindi *et al*. 2004). The production of these differentiated cell types is generally assumed to follow a hierarchical model involving cell division and ultimate restriction of differentiatative and replicative ability (Jenny *et al*. 2002; Marshman *et al*. 2002). Whilst these cells are dividing and differentiating, they move up the crypt wall and are ultimately discarded (Kaur & Potten 1986). This suggests that an additional property of the intestinal stem cell niche is inhibition of migration and/or increased cellular adhesion. Indeed, studies with model systems have demonstrated the essential role of niche adhesion in stem cell maintenance and function (Song & Xie 2002).

Various studies have described the presence of enteroendocrine cells at the base of crypts from human or rodent large and small intestine (Cheng & Leblond 1974; Tsubouchi & Leblond 1979; Evans & Potten 1988; Satoh *et al*. 1988; Krantis *et al*. 1994; Nakajima *et al*. 1997). The presence of enteroendocrine cells in crypt bases led Evans & Potten (1988) to comment that these cells are ‘an obvious candidate of interest’ regarding niche formation. We have gone a step further and now suggest that a type of enteroendocrine cell is the critical definer of the quiescent

intestinal stem cell niche and, in the remainder of this study, we present a model for how it may perform this role.

Functions of a quiescent niche

Important functions of a stem cell niche include regulation of proliferation and adhesion/migration, as well as suppression of differentiation and facilitation of asymmetric division (Spradling *et al*. 2001). In this section, we examine whether there is evidence to support the possible performance of these functions by an enteroendocrine cell-defined niche.

The quiescence of haemopoietic and skin stem cell niches has been associated with the production of growth inhibitory ligands by niche-forming cells (Arai *et al*. 2004; Blanpain *et al*. 2004). Hence, if an enteroendocrine cell type is primarily responsible for the creation of a quiescent intestinal stem cell niche, it might be expected to similarly produce growth inhibitory molecules. Consistent with this prediction, enteroendocrine cells can produce potent growth-inhibitory peptides such as somatostatin (Patel 1999) and guanylin and its close relative, uroguanylin (Beltowski 2001). Indeed, somatostatin-positive enteroendocrine cells were found predominantly at the base of human colonic crypts (Krantis *et al*. 1998), and costorage of guanylin and somatostatin has been demonstrated in enteroendocrine cells (Ieda *et al*. 1998; Magert *et al*. 1998). Accordingly, we suggest that somatostatin and guanylin/uroguanylin are responsible for maintaining intestinal stem cell quiescence. Such a postulate is consistent with the finding that multiple signalling pathways are commonly used to regulate important cellular activities (e.g. Oe *et al*. 2004).

The actions of guanylin/uroguanylin and somatostatin on target cells result from the activation of membrane-bound receptors. Somatostatin receptor activation can lead to a variety of growth-inhibitory events including: increased levels of the cyclin-dependent kinase inhibitors 1 A (p21, Cip1) and 1B (p27, Kip1) (Alderton *et al*. 2001; Charland *et al*. 2001) and of the hypophosphorylated form of retinoblastoma-1 protein (Sharma *et al*. 1999); increased production of somatostatin potentially resulting in a negative autocrine loop (Delesque *et al*. 1997); and activation of the tyrosine phosphatase SHP-1, which is a negative regulator of various activated growth factor tyrosine kinase receptors, an inhibitor of β-catenin/TCF transcriptional activity (Duchesne *et al*. 2003), and an activator of neuronal nitric oxide synthase, which in turn can elevate cGMP levels (via production of nitric oxide and activation of cytoplasmic guanylate cyclase) leading to inhibition of cell growth (Lopez *et al*. 2001). Guanylin and uroguanylin bind and activate guanylate cyclase 2C resulting in increased intracellular cGMP levels (Beltowski 2001). Increased cGMP levels may have various growth inhibitory effects including activation of a cyclic nucleotide-gated channel that allows entry of calcium ions (Pitari *et al*. 2003).

Mice that are homozygous for loss of the somatostatin, guanylate cyclase 2C, uroguanylin or guanylin gene develop normally, although guanylin loss leads to an increase in colonic crypt depth that reflects an increase in crypt cell number (Schulz *et al*. 1997; Low *et al*. 2001; Steinbrecher *et al*. 2002; Lorenz *et al*. 2003). These nullizygous mouse results do not invalidate our postulate that somatostatin and guanylin/uroguanylin are mediators of niche quiescence for the following reasons: gunaylin and uroguanylin are both expressed in the intestine and have similar functions (Beltowski 2001); in addition to guanylate cyclase 2C, there appear to be other uncharacterized receptors for guanylin and uroguanylin (Carrithers *et al*. 1999); there may be an uncharacterized somatostatin-related gene that is up-regulated in intestinal tissue of mice lacking somatostatin (Ramirez *et al*. 2002); and, as already evidenced, the growth inhibitory effects of somatostatin and guanylin/uroguanylin may partially overlap.

As well as having growth inhibitory actions, somatostatin has been shown to increase cellular adhesion to basement membrane components (Levite *et al*. 1998; Talme *et al*. 2001) and to

inhibit cell migration (Pola *et al*. 2003). Guanylin also appears to have a role in inhibiting intestinal crypt cell movement (Steinbrecher *et al*. 2002). Hence, guanylin and somatostatin can potentially perform at least two important quiescent-niche-defining functions (that is, inhibition of both cell proliferation and migration). γ-Aminobutyric acid (GABA) has been found in enteroendocrine cells near the base of rat colonic crypts (Krantis *et al*. 1994), and has been shown to inhibit the migration of colonic carcinoma cells (Joseph *et al*. 2002). Hence, GABA might also contribute to the postulated retention of the deep stem cell within its niche.

An important role of the stem cell niche is to prevent differentiation. In other stem cell systems, cell contact-dependent activation of a member of the Notch family of transmembrane receptors by a delta-like or a jagged ligand, present on a neighbouring differentiated cell, has been associated with inhibition or induction of differentiation. Examples of notch signalling apparently facilitating proliferation and inhibiting differentiation ('lateral inhibition') include the haemopoietic stem cell niche, where niche-defining osteoblasts express high levels of jagged1 (Calvi *et al*. 2003), and the neural stem cell niche where endothelial cells appear to perform the same function (Shen *et al*. 2004). We suggest that enteroendocrine cells play a similar role in the putative intestinal stem cell niche. Schonhoff *et al*. (2004) have previously suggested that endocrine cells can mediate lateral inhibition. In support of a role for notch signalling in the intestinal stem cell niche, it has been shown that members of the notch and jagged families are expressed in the proliferative region of intestinal crypts (Sander & Powell 2004), that Hes-1 (a transcriptional repressor activated by notch signalling) is expressed in proliferating intestinal crypt cells but is absent from enteroendocrine cells, and that delta-like ligands are up-regulated in the intestine of mice lacking Hes-1 (Jensen *et al*. 2000).

Another important niche property is the facilitation of asymmetric division in which a stem cell reproduces itself and also generates a second cell capable of producing differentiated progeny. An unequal distribution in daughter cells of notch signalling pathway components may, in some stem cell systems, be an important determinant of this type of division (Roegiers & January 2004). We suggest, however, that asymmetric division and the postulated presence of one deep stem cell per intestinal crypt may reflect an inability of the niche-defining enteroendocrine cell to interact with more than one stem cell.

Regulating and maintaining a quiescent niche

The postulated existence of a quiescent intestinal niche in turn raises questions of niche regulation and maintenance, such as, how is peptide hormone release from the niche-defining enteroendocrine cell regulated and directed to the deep stem cell? How is the deep stem cell stimulated to divide? What happens when the niche-defining enteroendocrine cell dies or is displaced? Possible answers to these questions are presented in succeeding discussions.

Interactions between neural processes and enteroendocrine cells control crucial intestinal functions such as the peristaltic reflex (Wade *et al*. 1996). In addition, the results of chemical or surgical denervation suggest that the enteric nervous system has an inhibitory effect on intestinal epithelium proliferation, although the basis for this action is unclear (See *et al*. 1990; Hadzijahic *et al*. 1993). Consistent with such functional interactions, morphological studies have shown that intestinal crypts are surrounded by a network of nerve fibres (Bjerknes & Cheng 2001) and that there are close contacts between enteroendocrine cells and neural processes (Wade & Westfall 1985). Accordingly, we postulate that the niche-defining enteroendocrine cell be regulated by interaction with neural processes.

Enteroendocrine cells share some features with neural cells, such as the uptake, synthesis, storage and release of various neurotransmitters and neuromodulators, and the presence of neural synapse-like storage vesicles (Rindi *et al*. 2004). The neural cell products, GABA and nitric

oxide, are potential regulators of the release from enteroendocrine cells of growth-inhibitory peptides. GABA is an activator of cell membrane ion channels, and through $GABA_A$ receptors can produce cell type-dependent membrane depolarization or hyperpolarization (Glassmeier *et al*. 1998). The depolarization of enteroendocrine cells has been shown to be coupled to the release of peptides such as somatostatin (Glassmeier *et al*. 1998; Patel 1999). Nitric oxide has also been linked to the release of somatostatin by endocrine cells (Burrell *et al*. 1996; Arebi *et al*. 2002). The nitric oxide may derive from nitrergic processes, which are abundant in the mucosal lamina propria (Krantis *et al*. 1998; Chino *et al*. 2002). Alternatively, nitric oxide may be produced within the enteroendocrine cell by activation of neuronal nitric oxide synthase following an increase in the intracellular calcium level (Mayer *et al*. 1992) produced by GABA-induced depolarization resulting in the opening of voltage-gated calcium channels (Mantelas *et al*. 2003) or by the binding of a Wnt protein of the 5a class produced by a neighbouring myofibroblast (Lickert *et al*. 2001). (The functioning of intestinal myofibroblasts is known to be regulated by interaction with neural processes (Powell *et al*. 1999)). In support of this regulatory scheme, it has been shown that a GABAergic fibre network underlies the base of rat colonic crypts (Krantis & Clark 1991), that enteroendocrine cells at the base of rat colonic crypts take up GABA (Krantis *et al*. 1994) and that neuronal nitric oxide synthase is found in close association with the secretory granules of somatostatin-producing endocrine cells (Burrell *et al*. 1996). In view of the possible involvement of nitric oxide in both the release of growth inhibitory peptides from the niche-forming cell and the quiescence of the putative intestinal deep stem cell, it is of interest that studies using inhibitors of nitric oxide synthase suggested that nitric oxide is a negative regulator of haemopoietic stem cell division (Michurina *et al*. 2004).

The postulated existence of a niche containing a single quiescent deep stem cell, which must exist in close proximity to actively dividing daughter cells, suggests a requirement for a mechanism of directed communication between the niche-forming enteroendocrine cell and the deep stem cell. Various studies have described highly polarized somatostatin-containing enteroendocrine cells with basal axon-like processes that contain secretory granules and extend along the basement membrane and beneath neighbouring cells (Larsson *et al*. 1979; Tsubouchi & Leblond 1979; Magney *et al*. 1986; Satoh *et al*. 1988; Krantis *et al*. 1994). Although there is disagreement between these reports as regards the frequency in different regions of the gastrointestinal tract of enteroendocrine cells with cytoplasmic processes, it is, nevertheless, clear that such cells can be found close to the presumed location of intestinal stem cells (Satoh *et al*. 1988; Krantis *et al*. 1994). It has been suggested that differences in the length of basal processes reflect the distance between the enteroendocrine cell and its target, and that this distance may increase with the age of the cell (Satoh *et al*. 1988). Hence, cytoplasmic processes, or other methods of directed communication, may provide the means for paracrine signalling by the niche-forming enteroendocrine cell.

Studies of crypt lifespan (Bjerknes 1986; Li *et al*. 1994) and recovery after irradiation (Martin *et al*. 1998) suggest that the postulated intestinal crypt niche must be both resilient and stable. How then to explain the niche's regeneration after possible loss of the niche-forming enteroendocrine cell through death or displacement? The presence of enteroendocrine cells close to the presumed location of intestinal stem cells is difficult to explain based on classical hierarchical models of stem cell differentiation and given the continual upward movement of most differentiating crypt cells. We suggest that the niche-forming enteroendocrine cell can, when necessary, be derived by direct differentiation of a stem cell or its daughter. Indeed, recent studies of stem cell differentiation suggest the lack of true hierarchies and that there can be considerable plasticity in stem cell differentiatative ability (Colvin *et al*. 2004; Zipori 2005).

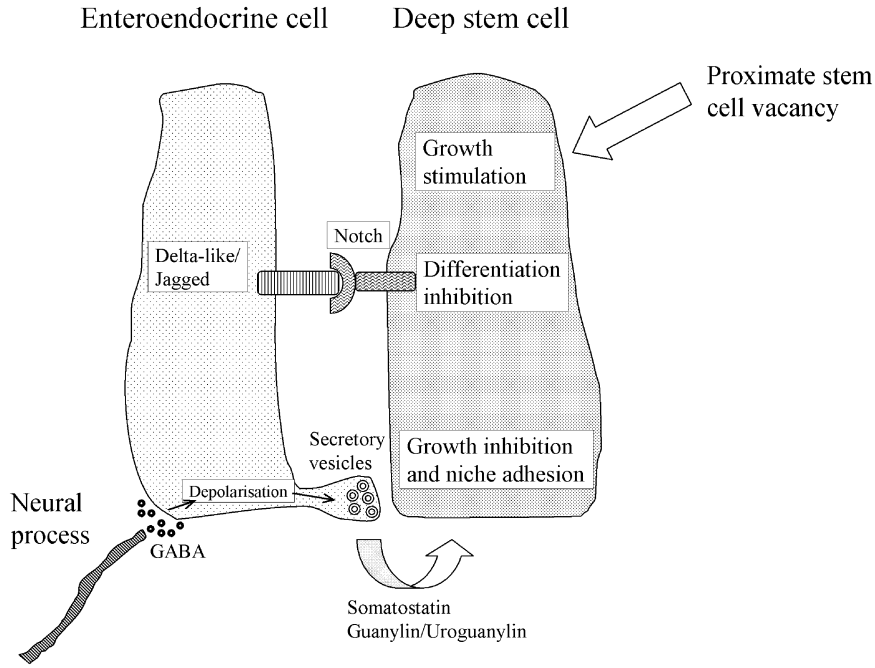

Figure 1. Basic features of the proposed intestinal deep stem cell niche.

An important issue is determining how a normally quiescent deep stem cell might occasionally be stimulated to divide. In the accompanying paper, we have suggested that intestinal deep stem cells have a finite replicative ability and that, in order to both provide sufficient cells to populate the crypt over a lifetime and to stay within their cell division limit, they only divide in response to loss (following apoptosis or differentiation) of a proximate stem cell (Lobachevsky & Radford 2006). That is, we are suggesting that a vacancy amongst the neighbouring proximate stem cells in some way stimulates division of the deep stem cell. Consistent with this postulate, studies of radiation effects have suggested that mouse intestinal crypt stem cells undergoing apoptosis release signals (the molecular nature of which is obscure) that initiate the replication of surviving stem cells (Tsubouchi & Potten 1985; Potten & Grant 1998).

A schematic outline of our postulated intestinal deep stem cell niche is shown in Fig. 1.

Predictions of the model and further supportive evidence

The model presented in previous discussions makes a number of testable predictions. Some of these predictions are outlined in succeeding paragraphs and, where possible, compared with the available evidence.

It is commonly assumed that tumour-initiating genetic alterations arise in stem cells (Cairns 1975). Accordingly, changes in the regulation of stem cells could potentially alter the risk of tumorigenesis. Indeed, there is evidence that the proteins postulated previously as defining a growth-inhibitory stem cell niche can act as intestinal tumour suppressors. Several studies have linked loss of somatostatin- or guanylin/uroguanylin-initiated cellular pathways to intestinal tumorigenesis. Lack of expression of somatostatin receptor 2 (an important cytostatic receptor that is expressed in colonic crypts (Warhurst *et al*. 1996; Pages *et al*. 1999)) was found to be

common in human colorectal tumours (Buscail *et al*. 1996), and it is perhaps also relevant that the transforming growth factor-β signalling pathway (elements of which are often mutated in human colorectal tumours (Woodford-Richens *et al*. 2001)) mediates transcriptional up-regulation of the somatostatin receptor 2 gene (Puente *et al*. 2001). Similarly, a frequent lack of expression of guanylin and uroguanylin in intestinal tumours has been reported (Zhang *et al*. 1997; Cohen *et al*. 1998; Shailubhai *et al*. 2000; Steinbrecher *et al*. 2000), and it is of interest that guanylate cyclase 2F receptor (which is expressed in colonic epithelium (Zhang *et al*. 1997), but whose ligand is currently undetermined) shows a significant frequency of mutations in human colorectal tumours (Bardelli *et al*. 2003).

An inverse correlation between the incidences of infectious diarrhoea and colorectal cancer, led to the suggestion that bacterial heat-stable enterotoxin protects against gastrointestinal tumours (Pitari *et al*. 2003). Heat-stable enterotoxin is a structural and functional homologue of guanylin/uroguanylin (Beltowski 2001), and its putative anticancer action has been linked to potent stimulation of the growth inhibitory guanylate cyclase 2C receptor on intestinal tumour cells (Pitari *et al*. 2003). An additional/alternative explanation for the link between diarrhoea and suppression of colorectal cancer, based on the intestinal stem cell niche model and the role of guanylin in intestinal cell adhesion is that chronic exposure to heat-stable enterotoxin increases cell adhesion and thereby helps to retain precancerous stem cells within the niche, where their multiplication is more easily regulated. Two pieces of evidence are supportive of the latter suggestion. First, oral administration of uroguanylin was shown to approximately halve the incidence of intestinal polyps in genetically predisposed mice (Shailubhai *et al*. 2000), suggesting that uroguanylin suppresses the formation and not just the growth of polyps. Second, inactivating mutations of the adenomatous polyposis coli gene are a common early event in the development of human colorectal cancer (Rowan *et al*. 2000), and loss of the encoded protein decreases cell adhesion (Bienz & Hamada 2004; Faux *et al*. 2004). We suggest that mutation of the adenomatous polyposis coli gene be such a frequent and central event in colorectal tumorigenesis because, in addition to its well-described effects on β-catenin transcriptional signalling, it can lead to a decrease in cell adhesion that allows the precancerous stem cell to escape its quiescent niche.

We acknowledge that different explanations of the link between intestinal tumorigenesis and the previously mentioned growth-suppressive molecules can be offered. Nevertheless, we suggest that our stem cell niche model provides an integrated explanation for a variety of observations.

During development and following injury, intestinal crypts have the capacity to increase their number by fission (Park *et al*. 1995; Cheng *et al*. 2000). Our model predicts that a necessary precursor to crypt fission be a doubling of the number of niche-forming enteroendocrine cells.

A further prediction of our stem cell niche model is that there is a defined sequence of developmental events, including interaction between the enteric nervous system and a stem cell, resulting in the appearance of a niche-forming enteroendocrine cell and culminating in the production of a monoclonal intestinal crypt. That is, we are suggesting that the stem cell selection process derives from the formation of a quiescent niche and that monoclonality reflects the size of this niche. The available evidence is limited but appears broadly consistent with this prediction. Enteroendocrine cells first appear in the mouse foetal colon at around day 15.5, with somatostatin-containing cells found at day 18.5 (Upchurch *et al*. 1996). During this same period of foetal development, neuroblasts of various ultimate phenotype populate the enteric submucosal ganglia (Pham *et al*. 1991). Mouse intestinal crypts begin to form at around the time of birth and assume adult appearance around 14 days later (Vidrich *et al*. 2003), by which time the cells of most crypts are monoclonal in origin (Schmidt *et al*. 1988).

The ultimate defining characteristic of a niche is its ability to support stem cell activity of competent exogenous stem cells (Spradling *et al*. 2001). Accordingly, we predict that a proximate intestinal stem cell would function as a deep stem cell when placed in an enteroendocrine cell-defined niche. We are not aware of evidence that supports such a prediction.

Studies of intestinal radiation response are potentially supportive of an interrelationship between enteroendocrine cells and crypt stem cells. Prostaglandin E_2, synthesized via cyclooxygenase-1 or -2, is known to be an important radioprotector of mouse intestinal crypt clonogens (Houchen *et al*. 2000; Anant *et al*. 2004) and has been shown to up-regulate anti-apoptotic proteins in target cells (Lin *et al*. 2001; Tessner *et al*. 2004). It has been reported that cyclooxygenase-1 (Muller-Decker *et al*. 1999) and -2 (Nakajima *et al*. 1997; Soslow *et al*. 2000) are expressed in enteroendocrine cells at or near the base of human colonic crypts, and it has been shown that mouse colonic crypt clonogenic cells are relatively radioresistant and appear to express elevated levels of the anti-apoptotic Bcl-2 protein (Merritt *et al*. 1995).

Concluding comments

We have only considered the possible form of the niche occupied by a putative intestinal deep stem cell; however, such a niche is presumed to include or exist in close inter-relationship with the proximate stem cells. A variety of growth stimulatory proteins, which may act on proximate stem cells, has been identified, and includes members of the epidermal growth factor and Wnt families (Dignass & Sturm 2001; Brittan & Wright 2004). Recently, an important role for bone morphogenetic protein 4 (produced by mesenchymal cells in the intestinal lamina propria) in inhibiting Wnt-β-catenin signalling-induced proliferation of stem cells in mouse small intestine has been identified (Haramis *et al*. 2004; He *et al*. 2004). We suggest that the latter effect acts as a brake on proximate stem cell division (for which β-catenin signalling is a crucial initiator), rather than defining the quiescence of the deep stem cell niche. Experimental confirmation that a growth-inhibitory niche containing a deep stem cell does exist would be an important starting point for a deeper understanding of crypt cell proliferation and its relationship to intestinal tumorigenesis.

REFERENCES

Alderton F, Humphrey PPA, Sellers LA (2001) High-intensity p38 kinase activity is critical for $p21^{cip1}$ induction and the antiproliferative function of G_i protein-coupled receptors. *Mol. Pharmacol*. **59**, 1119–1128.

Anant S, Murmu N, Houchen CW, Mukhopadhyay D, Riehl TE, Young SG, Morrison AR, Stenson WF, Davidson NO (2004) Apobec-1 protects intestine from radiation injury through posttranscriptional regulation of cyclooxygenase-2 expression. *Gastroenterology* **127**, 1139–1149.

Arai F, Hirao A, Ohmura M, Sato H, Matsuoka S, Takubo K, Ito K, Koh GY, Suda T (2004) Tie2/Angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. *Cell* **118**, 149–161.

Arebi N, Healey ZV, Bliss PW, Ghatei M, Van Noorden S, Playford RJ, Calam J (2002) Nitric oxide regulates the release of somatostatin from cultured gastric rabbit primary D-cells. *Gastroenterology* **123**, 566–576.

Bardelli A, Parsons DW, Silliman N, Ptak J, Szabo S, Saha S, Markowitz. S, Willson JK, Parmigiani G, Kinzler KW, Vogelstein B, Velculescu VE (2003) Mutational analysis of the tyrosine kinome in colorectal cancers. *Science* **300**, 949.

Beltowski J (2001) Guanylin and related peptides. *J. Physiol. Pharmacol*. **52**, 351–375.

Bienz M, Hamada F (2004) Adenomatous polyposis coli proteins and cell adhesion. *Curr. Opin. Cell Biol*. **16**, 528–535.

Bjerknes M (1986) A test of the stochastic theory of stem cell differentiation. *Biophys. J*. **49**, 1223–1227.

Bjerknes M, Cheng H (2001) Modulation of specific intestinal epithelial progenitors by enteric neurons. *Proc. Natl. Acad. Sci. USA* **98**, 12497–12502.

Blanpain C, Lowry WE, Geoghegan A, Polak L, Fuchs E (2004) Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. *Cell* **118**, 635–648.

Brittan M, Wright NA (2004) The gastrointestinal stem cell. *Cell Prolif*. **37**, 35–53.
Burrell MA, Montuenga LM, Garcia M, Villaro AC (1996) Detection of nitric oxide synthase (NOS) in somatostatin-producing cells of human and murine stomach and pancreas. *J. Histochem. Cytochem*. **44**, 339–346.
Buscail L, Saint-Laurent N, Chastre E, Vaillant JC, Gespach C, Capella G, Kalthoff H, Lluis F, Vaysse N, Susini C (1996) Loss of sst2 somatostatin receptor gene expression in human pancreatic and colorectal cancer. *Cancer Res*. **56**, 1823–1827.
Cairns J (1975) Mutation selection and the natural history of cancer. *Nature* **255**, 197–200.
Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC, Martin RP, Schipani E, Divieti P, Bringhurst FR, Milner LA, Kronenberg HM, Scadden DT (2003) Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* **425**, 841–846.
Carrithers SL, Hill MJ, Johnson BR, O'Hara SM, Jackson BA, Ott CE, Lorenz. J, Mann EA, Giannella RA, Forte LR, Greenberg RN (1999) Renal effects of uroguanylin and guanylin *in vivo*. *Braz. J. Med. Biol. Res*. **32**, 1337–1344.
Charland S, Boucher MJ, Houde M, Rivard N (2001) Somatostatin inhibits Akt phosphorylation and cell cycle entry, but not p42/p44 mitogen-activated protein (MAP) kinase activation in normal and tumoral pancreatic acinar cells. *Endocrinology* **142**, 121–128.
Cheng H, Leblond CP (1974) Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. III. Enteroendocrine cells. *Am. J. Anat*. **141**, 503–519.
Cheng L, Araki K, Furuya Y, Matsuoka T, Mashima K, Kobayashi M, Matsuura K (2000) Morphological study of the regeneration mechanism of acetic acid-injured colon crypts in the rat. *Med. Electron Microsc*. **33**, 165–171.
Chino Y, Fujimura M, Kitahama K, Fujimiya M (2002) Colocalization of NO and VIP in neurons of the submucous plexus in the rat intestine. *Peptides* **23**, 2245–2250.
Cohen MB, Hawkins JA, Witte DP (1998) Guanylin mRNA expression in human intestine and colorectal adenocarcinoma. *Laboratory Invest*. **78**, 101–108.
Colvin GA, Lambert JF, Abedi M, Dooner MS, Demers D, Moore BE, Greer D, Aliotta JM, Pimentel J, Cerny J, Lum LG, Quesenberry PJ (2004) Differentiation hotspots: the deterioration of hierarchy and stochasm. *Blood Cells Mol. Dis*. **32**, 34–41.
Delesque N, Buscail L, Esteve JP, Saint-Laurent N, Muller C, Weckbecker G, Bruns C, Vaysse N, Susini C (1997) sst2 Somatostatin receptor expression reverses tumorigenicity of human pancreatic cancer cells. *Cancer Res*. **57**, 956–962.
Dignass AU, Sturm A (2001) Peptide growth factors in the intestine. *Eur. J. Gastroenterol. Hepatol*. **13**, 763–770.
Doetsch F (2003) A niche for adult neural stem cells. *Curr. Opin. Genet. Dev*. **13**, 543–550.
Duchesne C, Charland S, Asselin C, Nahmias C, Rivard N (2003) Negative regulation of β-catenin signaling by tyrosine phosphatase SHP-1 in intestinal epithelial cells. *J. Biol. Chem*. **278**, 14274–14283.
Evans GS, Potten CS (1988) The distribution of endocrine cells along the mouse intestine: a quantitative immunocytochemical study. *Virchows Arch. B Cell Pathol*. **56**, 191–199.
Faux MC, Ross JL, Meeker C, Johns T, Ji H, Simpson RJ, Layton MJ, Burgess AW (2004) Restoration of full-length adenomatous polyposis coli (APC) protein in a colon cancer cell line enhances cell adhesion. *J. Cell Sci*. **117**, 427–439.
Glassmeier G, Herzig KH, Hopfner M, Lemmer K, Jansen A, Scherubl H (1998) Expression of functional $GABA_A$ receptors in cholecystokinin-secreting gut neuroendocrine murine STC-1 cells. *J. Physiol*. **510**, 805–814.
Hadzijahic N, Renehan WE, Ma CK, Zhang X, Fogel R (1993) Myenteric plexus destruction alters morphology of rat intestine. *Gastroenterology* **105**, 1017–1028.
Haramis APG, Begthel H, van den Born M, van Es J, Jonkheer S, Offerhaus GJA, Clevers H (2004) De novo crypt formation and juvenile polyposis on BMP inhibition in mouse intestine. *Science* **303**, 1684–1686.
He XC, Zhang J, Tong WG, Tawfik O, Ross J, Scoville DH, Tian Q, Zeng X, He X, Wiedemann LM, Mishina Y, Li L (2004) BMP signaling inhibits intestinal stem cell self-renewal through suppression of Wnt-β-catenin signaling. *Nat. Genet*. **36**, 1117–1121.
Houchen CW, Stenson WF, Cohn SM (2000) Disruption of cyclooxygenase-1 gene results in an impaired response to radiation injury. *Am. J. Physiol. Gastrointest. Liver Physiol*. **279**, G858–G865.
Ieda H, Naruse S, Furuya S, Ozaki T, Ando E, Nokihara K, Hori S, Kitagawa M, Hayakawa T (1998) Coexistence of proguanylin (1–15) and somatostatin in the gastrointestinal tract. *J. Gastroenterol. Hepatol*. **13**, 1225–1233.
Jenny M, Uhl C, Roche C, Duluc I, Guillermin V, Guillemot F, Jensen J, Kedinger M, Gradwohl G (2002) Neurogenin3 is differentially required for endocrine cell fate specification in the intestinal and gastric epithelium. *EMBO J*. **21**, 6338–6347.
Jensen J, Pedersen EE, Galante P, Hald J, Heller RS, Ishibashi M, Kageyama R, Guillemot F, Serup P, Madsen OD (2000) Control of endodermal endocrine development by Hes-1. *Nat. Genet*. **24**, 36–44.
Joseph J, Niggemann B, Zaenker KS, Entschladen F (2002) The neurotransmitter γ-aminobutyric acid is an inhibitory regulator for the migration of SW 480 colon carcinoma cells. *Cancer Res*. **62**, 6467–6469.

Kaur P, Potten CS (1986) Cell migration velocities in the crypts of the small intestine after cytotoxic insult are not dependent on mitotic activity. *Cell Tissue Kinet*. **19**, 601–610.

Krantis A, Clark D (1991) Localization of [^{3}H]GABA-labelled nerve fibre networks in the rat intestinal mucosa. *J. Auton. Nerv. Syst*. **34**, 195–200.

Krantis A, Tufts K, Nichols K, Morris GP (1994) [^{3}H]GABA uptake and GABA localization in mucosal endocrine cells of the rat stomach and colon. *J. Auton. Nerv. Syst*. **47**, 225–232.

Krantis A, Nichols K, Staines W (1998) Neurochemical characterization and distribution of enteric GABAergic neurons and nerve fibres in the human colon. *J. Auton. Nerv. Syst*. **68**, 33–42.

Larsson LI, Goltermann N, de Magistris L, Rehfeld JF, Schwartz. TW (1979) Somatostatin cell processes as pathways for paracrine secretion. *Science* **205**, 1393–1395.

Levite M, Cahalon L, Hershkoviz. R, Steinman L, Lider O (1998) Neuropeptides, via specific receptors, regulate T cell adhesion to fibronectin. *J. Immunol*. **160**, 993–1000.

Li YQ, Roberts SA, Paulus U, Loeffler M, Potten CS (1994) The crypt cycle in mouse small intestinal epithelium. *J. Cell Sci*. **107**, 3271–3279.

Lickert H, Kispert A, Kutsch S, Kemler R (2001) Expression patterns of Wnt genes in mouse gut development. *Mech. Dev*. **105**, 181–184.

Lin MT, Lee RC, Yang PC, Ho FM, Kuo ML (2001) Cyclooxygenase-2 inducing Mcl-1-dependent survival mechanism in human lung adenocarcinoma CL1.0 cells. Involvement of phosphatidylinositol 3-kinase/Akt pathway. *J. Biol. Chem*. **276**, 48997–49002.

Lobachevsky PN, Radford IR (2006) Intestinal crypt properties fit a model that incorporates replicative ageing and deep and proximate stem cells. *Cell Prolif* submitted.

Lopez. F, Ferjoux G, Cordelier P, Saint-Laurent N, Esteve JP, Vaysse N, Buscail L, Susini C (2001) Neuronal nitric oxide synthase: a substrate for SHP-1 involved in sst2 somatostatin receptor growth inhibitory signaling. *FASEB J*. **15**, 2300–2302.

Lorenz. JN, Nieman M, Sabo J, Sanford LP, Hawkins JA, Elitsur N, Gawenis LR, Clarke LL, Cohen MB (2003) Uroguanylin knockout mice have increased blood pressure and impaired natriuretic response to enteral NaCl load. *J. Clin. Invest*. **112**, 1244–1254.

Low MJ, Otero-Corchon V, Parlow AF, Ramirez. JL, Kumar U, Patel YC, Rubinstein M (2001) Somatostatin is required for masculinization of growth hormone-regulated hepatic gene expression but not of somatic growth. *J. Clin. Invest*. **107**, 1571–1580.

Madara JL, Trier JS (1987) Functional morphology of the mucosa of the small intestine. In: Johnson, LR, ed. *Physiology of the Gastrointestinal Tract*, 2nd edn. New York: Raven Press, 1209–1249.

Magert HJ, Reinecke M, David I, Raab HR, Adermann K, Zucht HD, Hill O, Hess R, Forssmann WG (1998) Uroguanylin: gene structure, expression, processing as a peptide hormone, and co-storage with somatostatin in gastrointestinal D-cells. *Regul. Pept*. **73**, 165–176.

Magney JE, Erlandsen SL, Bjerknes ML, Cheng H (1986) Scanning electron microscopy of isolated epithelium of the murine gastrointestinal tract: morphology of the basal surface and evidence for paracrine-like cells. *Am. J. Anat*. **177**, 43–53.

Mantelas A, Stamatakis A, Kazanis I, Philippidis H, Stylianopoulou F (2003) Control of neuronal nitric oxide synthase and brain-derived neurotrophic factor levels by GABA-A receptors in the developing rat cortex. *Brain Res. Dev. Brain Res*. **145**, 185–195.

Marshman E, Booth C, Potten CS (2002) The intestinal epithelial stem cell. *Bioessays* **24**, 91–98.

Martin K, Potten CS, Roberts SA, Kirkwood TBL (1998) Altered stem cell regeneration in irradiated intestinal crypts of senescent mice. *J. Cell Sci*. **111**, 2297–2303.

Mayer B, Klatt P, Bohme E, Schmidt K (1992) Regulation of neuronal nitric oxide and cyclic GMP formation by Ca^{2+}. *J. Neurochem*. **59**, 2024–2029.

Merritt AJ, Potten CS, Watson AJM, Loh DY, Nakayama KI, Nakayama K, Hickman JA (1995) Differential expression of bcl-2 in intestinal epithelia. Correlation with attenuation of apoptosis in colonic crypts and the incidence of colonic neoplasia. *J. Cell Sci*. **108**, 2261–2271.

Michurina T, Krasnov P, Balazs A, Nakaya N, Vasilieva T, Kuzin B, Khrushchov N, Mulligan RC, Enikolopov G (2004) Nitric oxide is a regulator of hematopoietic stem cell activity. *Mol. Ther*. **10**, 241–248.

Muller-Decker K, Albert C, Lukanov T, Winde G, Marks F, Furstenberger G (1999) Cellular localization of cyclo-oxygenase isozymes in Crohn's disease and colorectal cancer. *Int. J. Colorectal Dis*. **14**, 212–218.

Nakajima T, Hamanaka K, Fukuda T, Oyama T, Kashiwabara K, Sano T (1997) Why is cyclooxygenase-2 expressed in neuroendocrine cells of the human alimentary tract? *Pathol. Int*. **47**, 889–891.

Oe S, Lemmer ER, Conner EA, Factor VM, Leveen P, Larsson J, Karlsson S, Thorgeirsson SS (2004) Intact signaling by transforming growth factor β is not required for termination of liver regeneration in mice. *Hepatology* **40**, 1098–1105.

Pages P, Benali N, Saint-Laurent N, Esteve JP, Schally AV, Tkaczuk J, Vaysse N, Susini C, Buscail L (1999) sst2 Somatostatin receptor mediates cell cycle arrest and induction of $p27^{Kip1}$. Evidence for the role of SHP-1. *J. Biol. Chem.* **274**, 15186–15193.

Park HS, Goodlad RA, Wright NA (1995) Crypt fission in the small intestine and colon. A mechanism for the emergence of G6PD locus-mutated crypts after treatment with mutagens. *Am. J. Pathol.* **147**, 1416–1427.

Patel YC (1999) Somatostatin and its receptor family. *Front. Neuroendocrinol.* **20**, 157–198.

Pham TD, Gershon MD, Rothman TP (1991) Time of origin of neurons in the murine enteric nervous system: sequence in relation to phenotype. *J. Comp. Neurol.* **314**, 789–798.

Pitari GM, Zingman LV, Hodgson DM, Alekseev AE, Kazerounian S, Bienengraeber M, Hajnoczky G, Terzic A, Waldman SA (2003) Bacterial enterotoxins are associated with resistance to colon cancer. *Proc. Natl. Acad. Sci. USA* **100**, 2695–2699.

Pola S, Cattaneo MG, Vicentini LM (2003) Anti-migratory and anti-invasive effect of somatostatin in human neuroblastoma cells: involvement of Rac and MAP kinase activity. *J. Biol. Chem.* **278**, 40601–40606.

Potten CS, Grant HK (1998) The relationship between ionizing radiation-induced apoptosis and stem cells in the small and large intestine. *Br. J. Cancer* **78**, 993–1003.

Potten CS, Booth C, Hargreaves D (2003) The small intestine as a model for evaluating adult tissue stem cell drug targets. *Cell Prolif.* **36**, 115–129.

Powell DW, Mifflin RC, Valentich JD, Crowe SE, Saada JI, West AB (1999) Myofibroblasts. II. Intestinal subepithelial myofibroblasts. *Am. J. Physiol. Cell Physiol.* **277**, C183–C201.

Puente E, Saint-Laurent N, Torrisani J, Furet C, Schally AV, Vaysse N, Buscail L, Susini C (2001) Transcriptional activation of mouse sst2 somatostatin receptor promoter by transforming growth factor-β. Involvement of Smad4. *J. Biol. Chem.* **276**, 13461–13468.

Ramirez. JL, Mouchantaf R, Kumar U, Otero Corchon V, Rubinstein M, Low MJ, Patel YC (2002) Brain somatostatin receptors are up-regulated in somatostatin-deficient mice. *Mol. Endocrinol.* **16**, 1951–1963.

Rindi G, Leiter AB, Kopin AS, Bordi C, Solcia E (2004) The 'normal' endocrine cell of the gut: changing concepts and new evidences. *Ann. N. Y. Acad. Sci.* **1014**, 1–12.

Roegiers F, Jan YN (2004) Asymmetric cell division. *Curr. Opin. Cell Biol.* **16**, 195–205.

Rowan AJ, Lamlum H, Ilyas M, Wheeler J, Straub J, Papadopoulou A, Bicknell D, Bodmer WF, Tomlinson IPM (2000) *APC* mutations in sporadic colorectal tumors: a mutational 'hotspot' and interdependence of the 'two hits'. *Proc. Natl. Acad. Sci. USA* **97**, 3352–3357.

Sander GR, Powell BC (2004) Expression of notch receptors and ligands in the adult gut. *J. Histochem. Cytochem.* **52**, 509–516.

Sato M, Ahnen DJ (1992) Regional variability of colonocyte growth and differentiation in the rat. *Anat. Record* **233**, 409–414.

Satoh Y, Oomori Y, Ishikawa K, Satoh T, Ono K (1988) Application of immunohistochemistry to the isolated mucosa of the mouse gastrointestinal tract, with special reference to somatostatin cells. *Acta Anat.* **133**, 229–233.

Schmidt GH, Winton DJ, Ponder BA (1988) Development of the pattern of cell renewal in the crypt-villus unit of chimaeric mouse small intestine. *Development* **103**, 785–790.

Schonhoff SE, Giel-Moloney M, Leiter AB (2004) Minireview: development and differentiation of gut endocrine cells. *Endocrinology* **145**, 2639–2644.

Schulz. S, Lopez. MJ, Kuhn M, Garbers DL (1997) Disruption of the guanylyl cyclase-C gene leads to a paradoxical phenotype of viable but heat-stable enterotoxin-resistant mice. *J. Clin. Invest.* **100**, 1590–1595.

See NA, Epstein ML, Dahl JL, Bass P (1990) The myenteric plexus regulates cell growth in rat jejunum. *J. Auton. Nerv. Syst.* **31**, 219–229.

Shailubhai K, Yu HH, Karunanandaa K, Wang JY, Eber SL, Wang Y, Joo NS, Kim HD, Miedema BW, Abbas SZ, Boddupalli SS, Currie MG, Forte LR (2000) Uroguanylin treatment suppresses polyp formation in the $Apc^{Min/+}$ mouse and induces apoptosis in human colon adenocarcinoma cells via cyclic GMP. *Cancer Res.* **60**, 5151–5157.

Sharma K, Patel YC, Srikant CB (1999) C-terminal region of human somatostatin receptor 5 is required for induction of Rb and G1 cell cycle arrest. *Mol. Endocrinol.* **13**, 82–90.

Shen Q, Goderie SK, Jin L, Karanth N, Sun Y, Abramova N, Vincent P, Pumiglia K, Temple S (2004) Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. *Science* **304**, 1338–1340.

Simon-Assmann P, Lefebvre O, Bellissent-Waydelich A, Olsen J, Orian-Rousseau V, De Arcangelis A (1998) The laminins: role in intestinal morphogenesis and differentiation. *Ann. N. Y. Acad. Sci.* **859**, 46–64.

Song X, Xie T (2002) DE-cadherin-mediated cell adhesion is essential for maintaining somatic stem cells in the *Drosophila* ovary. *Proc. Natl. Acad. Sci. USA* **99**, 14813–14818.

Soslow RA, Dannenberg AJ, Rush D, Woerner BM, Khan KN, Masferrer J, Koki AT (2000) COX-2 is expressed in human pulmonary, colonic, and mammary tumors. *Cancer* **89**, 2637.

Spradling A, Drummond-Barbosa D, Kai T (2001) Stem cells find their niche. *Nature* **414**, 98–104.

Steinbrecher KA, Tuohy TM, Heppner Goss K, Scott MC, Witte DP, Groden J, Cohen MB (2000) Expression of guanylin is downregulated in mouse and human intestinal adenomas. *Biochem. Biophys. Res. Commun.* **273**, 225–230.

Steinbrecher KA, Wowk SA, Rudolph JA, Witte DP, Cohen MB (2002) Targeted inactivation of the mouse guanylin gene results in altered dynamics of colonic epithelial proliferation. *Am. J. Pathol.* **161**, 2169–2178.

Talme T, Ivanoff J, Hagglund M, Van Neerven RJJ, Ivanoff A, Sundqvist KG (2001) Somatostatin receptor (SSTR) expression and function in normal and leukaemic T-cells. Evidence for selective effects on adhesion to extracellular matrix components via SSTR2 and/or 3. *Clin. Exp. Immunol.* **125**, 71–79.

Teller IC, Beaulieu JF (2001) Interactions between laminin and epithelial cells in intestinal health and disease. *Expert Rev. Mol. Medical* 28 September, http://www-ermm.cbcu.cam.ac.uk/01003623h.htm.

Tessner TG, Muhale F, Riehl TE, Anant S, Stenson WF (2004) Prostaglandin E_2 reduces radiation-induced epithelial apoptosis through a mechanism involving AKT activation and bax translocation. *J. Clin. Invest.* **114**, 1676–1685.

Tsubouchi S, Leblond CP (1979) Migration and turnover of enteroendocrine and caveolated cells in the epithelium of the descending colon, as shown by radioautography after continuous infusion of ^{3}H-thymidine into mice. *Am. J. Anat.* **156**, 431–451.

Tsubouchi S, Potten CS (1985) Recruitment of cells in the small intestine into rapid cell cycle by small doses of external gamma or internal beta-radiation. *Int. J. Radiat. Biol.* **48**, 361–369.

Upchurch BH, Fung BP, Rindi G, Ronco A, Leiter AB (1996) Peptide YY expression is an early event in colonic endocrine cell differentiation: evidence from normal and transgenic mice. *Development* **122**, 1157–1163.

Vidrich A, Buzan JM, Cohn SM (2003) Intestinal stem cells and mucosal gut development. *Curr. Opin. Gastroenterol.* **19**, 583–590.

Wade PR, Westfall JA (1985) Ultrastructure of enterochromaffin cells and associated neural and vascular elements in the mouse duodenum. *Cell Tissue Res.* **241**, 557–563.

Wade PR, Chen J, Jaffe B, Kassem IS, Blakely RD, Gershon MD (1996) Localization and function of a 5-HT transporter in crypt epithelia of the gastrointestinal tract. *J. Neurosci.* **16**, 2352–2364.

Warhurst G, Higgs NB, Fakhoury H, Warhurst AC, Garde J, Coy DH (1996) Somatostatin receptor subtype 2 mediates somatostatin inhibition of ion secretion in rat distal colon. *Gastroenterology* **111**, 325–333.

Wong MH, Huelsken J, Birchmeier W, Gordon JI (2002) Selection of multipotent stem cells during morphogenesis of small intestinal crypts of Lieberkuhn is perturbed by stimulation of Lef-1/β-catenin signaling. *J. Biol. Chem.* **277**, 15843–15850.

Woodford-Richens KL, Rowan AJ, Gorman P, Halford S, Bicknell DC, Wasan HS, Roylance RR, Bodmer WF, Tomlinson IP (2001) SMAD4 mutations in colorectal cancer probably occur before chromosomal instability, but after divergence of the microsatellite instability pathway. *Proc. Natl. Acad. Sci. USA* **98**, 9719–9723.

Zhang L, Zhou W, Velculescu VE, Kern SE, Hruban RH, Hamilton SR, Vogelstein B, Kinzler KW (1997) Gene expression profiles in normal and cancer cells. *Science* **276**, 1268–1272.

Zhang J, Niu C, Ye L, Huang H, He X, Tong WG, Ross J, Haug J, Johnson T, Feng JQ, Harris S, Wiedemann LM, Mishina Y, Li L (2003) Identification of the haematopoietic stem cell niche and control of the niche size. *Nature* **425**, 836–841.

Zipori D (2005) The stem state: plasticity is essential, whereas self-renewal and hierarchy are optional. *Stem Cells* **23**, 719–726.

