REVIEW

The gastrointestinal stem cell

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Abstract. The longevity of adult stem cells, and their potential for vast tissue regeneration, makes them a focal point of current research and debate, with future aspirations for the use of stem cells in the treatment of a number of human pathological conditions. Due to the rapid rate of cell turnover in the gastrointestinal tract, the stem cells of this tissue are amongst the most assiduous in the body, although they remain unidentified to this day due to their immature, undifferentiated phenotype. However, our knowledge of the mechanisms regulating gastrointestinal stem cell function is evolving, with the identification of putative cellular markers and the elucidation of signalling pathways which regulate cell behaviour in the normal and neoplastic gastrointestinal tract. This review describes the fundamental properties of the gastrointestinal stem cell including: (i) their number, location and origins, (ii) their primary function of deriving gastrointestinal cell lineages and maintaining tissue homeostasis, (iii) the acquisition of gastrointestinal cell lineages from adult stem cells of extraneous tissues and the consequences of this in a therapeutic context, and (iv) the genetic and morphological phenomena surrounding neoplastic transformation in the gastrointestinal tract.

INTRODUCTION

The cells of the gastrointestinal epithelial mucosa are constantly under high regenerative pressure, due to the expeditious rate of cell turnover in this tissue. To regulate homeostasis in the gastrointestinal tract, a vital balance between cell apoptosis, senescence and the proliferation and differentiation of new cells must be maintained. This role is attributed to the gastrointestinal stem cell. Despite its significance as the most important regulatory element of gastrointestinal function, limited evidence exists to definitively substantiate the location, quantity, regulatory pathways or function of the ever-elusive gastrointestinal epithelial stem cell, albeit a number of contrasting hypotheses attempt to define these properties. Stem cells have the capacity to undergo replicative divisions and replace themselves with an identical daughter cell, and produce daughter cells committed to terminal differentiation, which will differentiate to create the

entire adult cell repertoire within a tissue. Studies of clonality in the normal and neoplastic gastrointestinal tract have given an insight into the numbers and location of the gastrointestinal stem cell, although results have proved ambiguous and the debate continues. The epithelial : mesenchymal cell signalling pathways that regulate cell proliferation are emerging, as are putative gastrointestinal epithelial stem cell markers such as Musashi-1 and integrin subunits, both increasing our understanding of the fundamental properties of the gastrointestinal stem cell and the stages of genetic change that occur with the onset of neoplasia. The origin of adult cell lineages within many tissues appears to be less specific than previously assumed, with the discovery that adult stem cells can engraft within foreign tissues and contribute to functional cell populations outwith their native tissue. Indeed, adult cells in the bone marrow have been shown to contribute to multiple cell populations in the mouse and human gastrointestinal tract. However, the mechanisms of this supposed adult stem cell 'plasticity' are hotly debated and poorly understood, and there is currently no substantial evidence that gastrointestinal stem cells, whether mesenchymal or epithelial, originate from extraneous tissue stem cell populations.

THE GASTROINTESTINAL STEM CELL AND THE STEM CELL NICHE

Stem cells are undifferentiated, primitive cells that exist within an tissue throughout the lifetime of an organism, due to their ability to divide asymmetrically and undergo self-replication, and also to produce committed daughter cells that can differentiate to form all the adult cell lineages within a tissue. Although morphologically indistinct, adult epithelial stem cells can be defined functionally by this potential for asymmetrical division and are characterized by their residence within a stem cell compartment or 'niche', a specialized micro-environment that provides an optimal milieu for stem cell survival and function, believed to be created and maintained by the mesenchymal cells of the underlying lamina propria. Mesenchymal : epithelial cell interactions occurring between cells of the gastrointestinal mucosa and the subjacent lamina propria are vital for the maintenance of normal epithelial cell function, and the ongoing elucidation of the signalling pathways which maintain steady-state epithelial cell turnover, discussed below, bring us ever closer to characterization and understanding of the gastrointestinal epithelial stem cell population. For all adult stem cells, the 'conceptual' stem cell niche should possess three standard constituents; the supporting cells and their secreted extracellular matrices to regulate stem cell behaviour via mesenchymal : epithelial crosstalk, the target cell range covered by the signalling molecules, and the stem cells themselves (Lin 2002). Functionally, a niche is characterized by its persistence upon removal of the stem cells and, conversely, if stem cells are extracted from their niche, they cease to retain their stem cell potential, or 'stemness', and become committed to differentiation (Spradling *et al.* 2001).

CELL LINEAGES OF THE GASTROINTESTINAL TRACT

Epithelial cells

The gastrointestinal tract is composed of several specialized histologically distinct organs: the oral cavity, pharynx, oesophagus, stomach, small intestine and colon. Throughout the gastrointestinal tract, the internal luminal surface is lined by a simple, columnar epithelial mucosa with

glandular invaginations, and has an underlying vascular lamina propria and muscularis mucosa. The nature and distribution of each cell type varies throughout the different functional regions of the gastrointestinal tract, although each cell is believed to derive from a stem cell, originating in the endoderm during embryogenesis (Maunoury *et al.* 1988). In the stomach, the mucosal lining is folded to form structures called gastric glands, which are further subdivided into foveolus, isthmus, neck, and base regions. In the fundus, the gastric glands contain 'surface mucous' cells and 'mucous neck' cells, acid-secreting 'parietal' (oxyntic) cells, pepsinogen-secreting 'zymogen' (chief or peptic) cells and numerous endocrine cell families including the histamine-producing enterochromaffin-like (ECL) cells, and gastrin-producing 'G-cells'. In the pylorus, the gastric glands contain many more mucinous cells, no peptic cells and few parietal cells. The epithelial mucosa of the small intestine and colon has an increased absorptive surface area due to the presence of frequent concave indentations known as 'crypts' and, in the small intestine, finger-like structures known as 'villi' project into the lumen and expand the surface area further. The fully differentiated cells of the intestinal crypts and villi are situated towards the luminal surface, and are continually being shed into the lumen and replaced by a constant stream of proliferating progenitor cells in a hierarchical migratory fashion. The columnar cells are the most abundant epithelial cell type, termed 'enterocytes' in the small intestine and 'colonocytes' in the colon, specialized for absorption by the presence of apical microvilli. The 'goblet' cells are dispersed throughout the colonic epithelium and secrete mucus into the intestinal lumen to trap and expel micro-organisms. The gastrointestinal tract is the largest endocrine organ in the body, and hence the 'endocrine', 'neuroendocrine' or 'enteroendocrine' cells are abundant throughout the epithelium, although in the small intestine are more common to the crypts than the villi. These cells are specialized in endocrine or paracrine secretion of peptide hormones from their dense core, neurosecretory granules. The 'paneth' cells are almost exclusive to the crypt base of the small intestine and ascending colon, and contain large apical secretory granules and express various proteins including lysozyme and anti-bacterial peptides called defensins or cryptdins (Ouellette *et al.* 1994; Darmoul *et al.* 1997; Ouellette *et al.* 2000). Other less common cell lineages are also present, such as caveolated cells (Nabeyama & Leblond 1974), and M- (membranous- or microfold-cells) which function in antigen transport, and are found in the small intestine within specialized lymphoid follicles, known as 'Peyers patches' (reviewed in Kerneis & Pringault 1999).

The lamina propria

The subepithelial mesenchymal cells and their secreted basement membrane factors comprise the lamina propria which provides a supporting network for the epithelial cells, and regulates epithelial cell function (reviewed in Kedinger *et al.* 1998 and Powell *et al.* 1999). The lamina propria contains two intestinal myofibroblast populations: the intestinal subepithelial myofibroblasts (ISEMF), and the interstitial cells of Cajal (ICC). The ISEMFs form a vascular, fenestrated sheath subjacent to the epithelial cells, innervated by the enteric nervous system, and connected in a cellular syncytium via gap and adherens junctions (Joyce *et al.* 1987). ISEMFs have a broad range of functions, including regulation of epithelial cell proliferation and differentiation, mucosal protection and wound healing, and water and electrolyte transport (reviewed in Powell *et al.* 1999). The ICC are pacemaker cells located in an intramuscular space between the submucosa and muscularis propria, which regulate gastrointestinal smooth muscle motility, facilitate the propagation of electrical events, and regulate neurotransmission (Sanders 1996). Other cell lineages are also present in the lamina propria, including: fibroblasts and fibrocytes, vascular endothelial and smooth muscle cells, and blood cell lineages such as granulocytes, mast cells, macrophages, T and B lymphocytes, plasma cells, verifying the role of the lamina propria in

immune responses (Hunyady *et al.* 2000). The number and distribution of cell types in the lamina propria, and their secretion of regulatory factors, varies in accordance to fluctuating physiological conditions in the GI tract (Hunyady *et al.* 2000).

GASTROINTESTINAL STEM CELL NUMBER AND LOCATION

As the hierarchical migratory pattern of cell proliferation and differentiation in the gastric glands and intestinal crypts are well characterized, it is presumed that a pool of progenitor cells, located at the origin of cellular migration, are responsible for this continual cellular flux. With the exception of the paneth cells in the small intestine, epithelial cells originate in the base of the crypt and undergo proliferation and differentiation as they travel upwards to be shed into the intestinal lumen. The epithelial stem cell compartment is therefore proposed to be in the crypt base of the colon, and at approximately cell position 4–5 in the small intestinal crypts, just superior to the paneth cells (Potten *et al.* 1997). In the stomach, epithelial cell migration is bi-directional, with cells originating in a putative stem cell compartment in the neck/isthmus region of the gastric gland and migrating both upwards towards the lumen, and down into lower regions of the gland (Karam 1999). Albeit fundamentally based upon circumstantial evidence, it is generally accepted that the gastrointestinal epithelial stem cells are located and maintained within a mesenchymal niche, situated towards the centre of the gastric gland, and near the base of the intestinal crypts. Investigations into the clonal origins of gastric glands and intestinal crypts have attempted to deduce whether they are monoclonal populations derived from a single stem cell, or whether multiple stem cells proliferate to produce polyclonal glands and crypts. The 'Unitarian hypothesis' states that the gastrointestinal stem cell is multipotential, with the capacity to undergo clonal expansion and produce the entire, gastrointestinal adult cell repertoire in the intestinal crypts (Cheng & Leblond 1974). Other reports state that between four and six stem cells located at cell position 4–5 from the bottom of the crypt, just superior to the paneth cells, comprise the small intestinal stem cell population (Cai *et al.* 1997; Potten *et al.* 1997; Potten 1998), and others claim that up to 16 or more stem cells can exist in a single intestinal crypt (Roberts *et al.* 1995). In a series of reports based upon microscopic morphometry and autoradiography-labelling studies, the 'stem cell zone' hypothesis was proposed. This describes the location of stem cells at cell positions 1–4 in the crypt base of the small intestine, wherein the stem cells are induced to proliferate but do not undergo differentiation until they have migrated to position 5 (Bjerknes & Cheng 1981b,c,d,e). In this hypothesis, the paneth cells also appear initially at cell position 5, and migrate downwards to the crypt base (Bjerknes & Cheng 1981a). It is possible that the stem cell number fluctuates throughout the crypt cycle, with a threshold number of stem cells being the signal for crypt replication, or 'crypt fission' to occur (Loeffler *et al.* 1997), and stem cell number may vary throughout different regions of the gastrointestinal tract (Cai *et al.* 1997). Monoclonal intestinal crypts have been demonstrated following irradiation damage, showing that a single surviving stem cell can regenerate an entire crypt (Ponder *et al.* 1985), and the single cell-cloned human colorectal carcinoma cell line, HRA19, can form each of the major epithelial cell types *in vivo* (Kirkland 1988), although these reports of a single, multipotent stem cell in damaged or diseased epithelium cannot confirm that this process occurs under normal circumstances. Early studies of lineage commitment in the normal and neoplastic gastrointestinal epithelium have amassed evidence to both confirm and refute claims that intestinal crypts and gastric glands are monoclonal structures (reviewed in Brittan 2003b).

Clonal origins of the gastric glands

In studies using chimaeric mice, where cells of the two founder populations can readily be distinguished, adult gastric glands have been shown to be monoclonal structures, derived from a single stem cell (Thompson *et al.* 1990; Tatematsu *et al.* 1994). However, a subsequent study showed that gastric glands are polyclonal for the first 6 weeks of life, with an estimated 3–4 stem cells per gland, and gradually become monoclonal by an unresolved ‘purification’ process, wherein a single stem cell may become predominant or, alternatively, the gastric glands divide by ‘fission’ to produce clonal offspring. Fission of gastric glands and intestinal crypts is the mechanism by which these structures divide and replicate themselves during the ‘crypt cycle’, in development and following damage caused by irradiation or carcinogenic stimuli (Cairnie & Millen 1975; Maskens & Dujardin-Loits 1981). Intestinal crypts and gastric glands undergo basal bifurcation and budding, leading to longitudinal division and formation of identical daughter glands and crypts. Crypt fission is believed to occur in the intestine when a maximal crypt size is reached (Park *et al.* 1997), possibly when a threshold number of stem cells becomes exceeded (Loeffler & Grossmann 1991). In humans, the random methylation and subsequent transcriptional inactivation of CpG islands on X-chromosome-linked genes during embryogenesis, including phosphoglycerate kinase (*PGK*), the androgen receptor gene (*HUMARA*) and glucose-6-phosphate dehydrogenase (*G6PD*), provides a mode of distinction between the two female X-chromosomes, and allows studies of tissue clonality. In the female human gastric mucosa, the pyloric glands are homotypic for the *PGK* or *HUMARA* locus and thus are monoclonal, although approximately 50% of the fundic glands are heterotypic for these loci and thus appear to be polyclonal, indicating that the mode of human gastric mucosal development is more complex than in the mouse, with regional variations (Nomura *et al.* 1996).

Clonal origins of the intestinal crypts

Similar to the gastric glands in the mouse stomach, studies of chimaeric mice have shown that the intestinal crypts are polyclonal for the first 2 weeks of life, eventually becoming monoclonal possibly due to the positive selection of a single dominant clone, or by crypt fission (Park *et al.* 1995). The epithelial cells in the crypts remain monoclonal in the adult mouse intestine, thereby substantiating the Unitarian hypothesis and the existence of a single, sustainable stem cell within each crypt (Ponder *et al.* 1985; Winton & Ponder 1990; Bjerknes & Cheng 1999). In female mice with a heterozygous polymorphism of the X-linked gene, *G6PD*, the individual X chromosomes are distinguished by their natural ‘mosaic’ pattern, and this model eliminates any artefact that may arise in the aforementioned chimaeric mouse models. The clonal origin of murine intestinal crypts was confirmed following histochemical analyses in these mice, although the small intestinal villi in these mice showed a polyclonal derivation, and are presumably formed by the upwards migration of epithelial cells from multiple crypts (Thomas *et al.* 1988). This is concordant with observations that crypts, although smaller than villi, are 7-fold more numerous in the mouse duodenum, and 4-fold in the ileum (Wright & Alison 1984). In the human, the intestinal crypts appear to be monoclonal, and the small intestinal villi are polyclonal, analogous to the situation in the mouse. This was shown in a rare XO/XY patient with familial adenomatous polyposis (FAP), where Y chromosome-containing cells were detected using non-isotopic *in situ* hybridization, to show that the colonic crypts were clonal populations composed almost entirely of Y chromosome-positive or Y chromosome-negative cells, and small intestinal villi were a mixture of XO and XY cells (Novelli *et al.* 1996). Studies of random sequence variations in methylation tags of three neutral loci in cells of human colonic crypts can be utilized in phylogenetic analyses to predict stem cell division histories and to map cell fate. Cells in separate, unrelated crypts show variations in these methylation patterns, as expected, and cells closely

opposed within one crypt display identical or closely related, sequential methylation tags. This indicates that human colonic crypts contain multiple stem cells, which are constantly lost and replaced, eventually leading to a 'bottleneck' effect wherein all cells are related to the closest stem cell ascendant and are monoclonally derived (Yatabe *et al.* 2001). Both inherited and spontaneous mutations occur frequently in the human mitochondrial genome, and are implicated in the progression of many different diseases, with a role in ageing and carcinogenesis (Brierley *et al.* 1998; Michikawa *et al.* 1999; Collier *et al.* 2001). Mathematical models indicate that the high frequency of mitochondrial DNA (mtDNA) mutations, and their accumulation within individual cells result in a defect in oxidative phosphorylation (Sciaccò *et al.* 1994), and is a result of clonal expansion by genetic drift (Chinnery & Samuels 1999; Collier *et al.* 2001). Cells demonstrating > 50% cytochrome-*c* oxidase enzyme function, and normal succinate dehydrogenase (SDH) activity, generally show increased mtDNA mutations (Johnson *et al.* 1993). The biochemical function of samples of both normal and cancerous human colonic mucosa was deduced by sequencing their mtDNA, and by histological analysis of cytochrome-*c* oxidase and SDH function. Results showed a mixture of crypts that were either composed entirely of cells with mtDNA mutations, suggesting clonal expansion from a single, mutated stem cell, or containing ribbons of cytochrome-*c* oxidase-deficient cells occupying only partial sections of the crypts, compatible with the presence of multiple stem cells in a single crypt, with varying levels of mtDNA mutations. Mitochondrial DNA mutations accumulate in stem cells in the colonic crypts in an age-dependent fashion, and can result in a biochemical deficiency dependent on the site and severity of the mutation. This study has implications for the role of mtDNA mutations in the onset of cancer, and presents a link between mutations in mtDNA and the resultant aberrant oxidative phosphorylation in cancer cells, as accumulating mutations occur within crypt stem cells which then undergo clonal expansion.

A recent study of the mechanisms of stem cell division in the mouse small intestine has revealed that intestinal stem cells undergo asymmetrical division, and are therefore functionally typical of other tissue stem cells, and also that these cells retain an innate mechanism of genome protection. By labelling DNA template strands of the intestinal stem cells with tritiated thymidine ($[^3\text{H}]\text{TdR}$) during development or in tissue regeneration, and by bromodeoxyuridine (BrdUrd)-labelling the newly synthesized daughter strands, both DNA strands can be visualized during cell division. Results showed that the original, template DNA is retained within the stem cell, and the newly synthesized BrdUrd-labelled strands are passed on to the daughter cells that leave the stem cell niche, and relinquish their stem cell potential, and differentiate to adult intestinal lineages. Newly synthesized DNA is more prone to replication-induced mutation, and by discarding this strand during asymmetrical cell division, the intestinal stem cell utilizes an inherent mechanism of genome protection (Potten *et al.* 2002). This study is in accordance with the hypothesis of Cairns, who suggested that selective retention of the template DNA strand during stem cell division, provides a means of protection against DNA replication errors (Cairns 1975).

ADULT STEM CELL PLASTICITY IN THE GASTROINTESTINAL TRACT

It is generally accepted that gastrointestinal epithelial stem cells undergo multilineage differentiation to form the entire gastrointestinal epithelial cell repertoire. However, a novel science has recently emerged, based upon the tenet that adult stem cells from several tissues, in addition

to their fundamental role of formation of all adult cell lineages within their native tissue, can extricate their niche and engraft within foreign tissues and transform to contribute to adult cell lineages within these tissues. This phenomenon is commonly termed 'plasticity', or 'transdifferentiation'. Adult bone marrow cells can contribute to adult cell lineages within several non-haematopoietic tissues, and the gastrointestinal tract is no exception, with reports of bone marrow-derived epithelial and mesenchymal lineages in both mouse (Fig. 1) (Krause *et al.* 2001; Brittan *et al.* 2002; Jiang *et al.* 2002) and humans (Brittan *et al.* 2002; Korbliing *et al.* 2002). It is important to demonstrate that bone marrow-derived adult cells are functional with a capacity to restore diseased or damaged tissues, and thus convey a possible clinical relevance. In a mouse model of the metabolic liver disease, Type 1 tyrosinaemia, transplantation of as few as 50 bone marrow stem cells can rescue mice from the fatal enzyme deficiency by fusing with Fah-negative hepatocytes and turning them into functionally normal hepatocytes and restoring enzyme levels to normal (Lagasse *et al.* 2000). In the gastrointestinal tract, bone marrow stem cells have not yet been shown to cure a lethal condition, although following bone marrow transplantation and subsequent induction of colitis by intrarectal administration of trinitrobenzene sulphonic acid (TNBS), bone marrow cells increase their engraftment into the diseased colon and form myofibroblasts, fibroblasts, epithelial cells, smooth muscle cells and endothelial cell lineages (Fig. 1) (Brittan 2003a). The contribution of bone marrow to multiple adult lineages in the gastrointestinal tract has an increased propensity in diseased tissue, and suggests a possible therapeutic potential of bone marrow in the treatment of gastrointestinal disease, including inflammatory bowel disease. So, what gravity do these observations have on our understanding of gastrointestinal stem cells? Is it possible that a superfluous stem cell population exists in the bone marrow that contributes to intestinal epithelial lineages when regenerative demand exceeds the limits of the endogenous gastrointestinal epithelial stem cell? It is unlikely that this is the case, as bone marrow-derived epithelial cells are rare, and appear as single entities, suggesting that these cells do not undergo proliferation (Brittan 2003a). Bone marrow may play a more important role in mucosal regulation via the formation of mesenchymal cell types, as bone marrow has been shown to contribute to over 70% of intestinal myofibroblasts in mice with chemically induced colitis that have received a lethal dose of irradiation and a bone marrow transplant (Brittan 2003a). Many morphological and functional parallels can be drawn to the bone marrow-derived myofibroblasts and the indigenous intestinal myofibroblast. Donor-derived myofibroblasts are frequently observed as cellular columns spanning from the crypt base to the apical lumen, which is typical of myofibroblast morphology (Powell *et al.* 1999), and suggestive of their proliferation from a common transdifferentiated progenitor cell (Brittan *et al.* 2002; Brittan 2003a). Indeed, the proliferative and migratory capacity of the intestinal myofibroblast is well validated (Kaye *et al.* 1968; Pascal *et al.* 1968; Parker *et al.* 1974), and these cells are thought to originate from a mesenchymal stem cell located in the lamina propria towards the crypt base (Pascal *et al.* 1968; Marsh & Trier 1974a,b; Sappino *et al.* 1989). Therefore, though there is little evidence that bone marrow cells can form intestinal epithelial stem cells (Korbliing *et al.* 2002; Krause *et al.* 2001), it is possible that bone marrow cells form a mesenchymal stem cell in the lamina propria, although further investigation into the proliferative capacity and clonal derivations of bone marrow-derived myofibroblasts is necessary before this conjecture can be made. Bone marrow-derived myofibroblasts surround the base of the intestinal crypt, subjacent to the epithelial mucosa – the proposed location of the epithelial stem cell niche. As previously discussed, cells in the lamina propria, including the myofibroblasts, are believed to form and maintain the epithelial stem cell niche (Powell *et al.* 1999). It is therefore important to deduce the contribution of bone marrow to the epithelial stem cell niche in their ability to undergo paracrine secretion of the specific growth factors and cytokines that regulate epithelial cell proliferation.

Figure 1. Transplanted bone marrow cells contribute to myofibroblasts in the normal and diseased colon. Bone marrow cell-derived intestinal myofibroblasts in the lamina propria of the mouse colon are detected by the presence of a Y chromosome (dark brown nuclear density), and expression of SMA antigen (red cytoplasm). In the normal colon 6 weeks post-transplant, these cells are frequently present as cellular columns, spanning from the base of the crypt to the intestinal lumen (a, b) (arrows). Bone marrow cell-derived myofibroblasts are located in the lamina propria, surrounding the epithelial mucosa of the intestinal crypts, and possibly form and maintain the epithelial stem cell niche (seen in cross section in c, and high power in d) (arrows). Following the induction of experimental colitis, bone marrow cell contribution to intestinal myofibroblasts is significantly up-regulated in regions of fibrosis, implicating that these cells have an important role in tissue regeneration in inflammatory bowel disease (e) (arrows) (from M. Brittan).

Stem cell plasticity: heterokaryon formation or transdifferentiation?

The mechanisms of stem cell plasticity are currently under close scrutinization based upon emerging evidence that stem cells merely fuse with pre-existing cells within a tissue to form a cell with multiple, genetically variant nuclei, a heterokaryon. In the FAH^{-/-} mouse mentioned above, cells are rescued from their fatal metabolic disease by transplantation of bone marrow stem cells (Krause *et al.* 2001). However, subsequent studies showed that almost all the newly formed, FAH-synthesizing hepatocytes in these transplanted mice are products of fusion of transplanted bone marrow cells with host hepatocytes (Vassilopoulos *et al.* 2003; Wang *et al.* 2003). Heterokaryon formation by transplanted adult stem cells has also recently been shown to occur in a murine model of myocardial infarction, where heterokaryon formation occurred between indigenous cardiomyocytes and a transplanted population of isolated cardiac stem cells (Oh *et al.* 2003), and transplanted stem cells from the bone marrow can fuse with Purkinje cells in brains of both mice (Weimann *et al.* 2003b), and humans (Weimann *et al.* 2003a). Evidence of heterokaryon formation following stem cell transplantation in the gastrointestinal tract has never been reported and, paradoxically, gastrointestinal epithelial cells derived from transplanted mobilized peripheral blood stem cells were reported to contain a normal chromosomal component (Korbling *et al.* 2002). This poses a number of important questions as to whether adult stem cells, although potentially therapeutic and easily accessible from tissues such as the bone marrow, have a future in the treatment of human pathological conditions. Do adult stem cells transdifferentiate to form foreign adult cell lineages in a manner equivalent to the native stem cells in this tissue, or are stem cells merely homing to a tissue and fusing with an indigenous cell nucleus to form a tetraploid hybrid cell? In the reported incidences of spontaneous cell fusion, the original stem cell markers are repressed and the transplanted cells appear to reprogramme their new 'host' nuclei to produce a sustainable, functional cell, and the incidence of these heterokaryons increases with increasing age, suggesting that they can divide (Weimann *et al.* 2003b). It remains to say that, before we overlook, or indeed overstate, the capacity of adult stem cells to regenerate damaged or diseased tissue, further clarification of the mechanisms involved in this apparent stem cell plasticity is essential.

MOLECULAR REGULATION OF GASTROINTESTINAL STEM CELL FUNCTION

Elucidation of the molecules and receptors expressed by gastrointestinal epithelial stem cells, and indeed the regulatory molecules secreted by mesenchymal cells in the stem cell niche, will provide a clearer perspective of the clonal architecture of the gastrointestinal mucosa and an insight into the behaviour of the gastrointestinal stem cell. The signalling pathways such as Wnt, and the genetic mutations that occur in these pathways often leading to tumourigenesis, are vital in our understanding of how cells in the gastrointestinal mucosa divide and differentiate, and undergo neoplastic transformation.

Putative molecular markers of the GI stem cell

The mammalian neural stem cell marker, Musashi-1 (Msi-1) (Sakakibara *et al.* 1996) up-regulates expression of the transcriptional repressor molecule, Hairy and Enhancer of Split homologue-1 (Hes-1) (Imai *et al.* 2001), which is essential for neural stem cell self-renewal and suppression of neural stem cell differentiation (Akazawa *et al.* 1992; Sasai *et al.* 1992; Nakamura *et al.* 2000). Msi-1 and Hes-1 proteins are co-expressed in cells just superior to the paneth cells

in the small intestine, the postulated stem cell region (Potten *et al.* 1997), and thus co-localization of Msi-1 and Hes-1 may denote the epithelial stem cell population in the mouse small intestine (Kayahara *et al.* 2003). Hes-1 is expressed in reduced levels, in epithelial cells migrating toward the villus tip, and it is possible that Hes-1 expression alone represents proliferating cells committed to differentiation that have migrated outwith the stem cell niche (Kayahara *et al.* 2003). Musashi-1 mRNA and protein expression has also been confirmed in putative stem cells in the neonatal and adult murine intestinal crypts (Potten *et al.* 2003), and is expressed in epithelial cells at position 1–10 in human colonic crypts (Nishimura *et al.* 2003) – further evidence of Musashi-1 as a possible gastrointestinal stem cell marker.

The integrin superfamily of heterodimeric transmembrane glycoproteins, and their receptors, define basement membrane function and activate the cellular signalling pathways controlling epithelial cell survival, proliferation and differentiation (Juliano & Varner 1993). Integrin subunits have been identified as stem cell markers in the epidermis (Jones & Watt 1993; Hotchin *et al.* 1995; Li *et al.* 1998), and testes (Shinohara *et al.* 1999), and have recently been suggested as markers of intestinal clonogenic cells, based upon observations of a restricted expression of the β 1-integrin subunit in proliferating epithelial cells in the lower third of the human colonic crypts, and the expression of the α 2 β 1 integrin in epithelial cells in the base of the crypts in the human small intestine (Beaulieu 1992). Interestingly, the expression of communal markers by stem cells of different tissues, e.g. Msi-1 expression by both neural and intestinal stem cells, lends support to the belief that adult stem cells may repopulate adult lineages outwith their tissue of origin. It may be possible to identify other gastrointestinal stem cell markers by investigating expression patterns of known stem cell markers of adult tissues, such as those found in the brain and epidermis, in the cells of the gastrointestinal tract.

The Wnt/ β -catenin/Tcf pathway of intestinal epithelial cell transdifferentiation

The Wnt signalling proteins and their many receptors and ligands are important in embryogenesis and are known to drive adult intestinal epithelial cell proliferation and become activated in colorectal carcinogenesis. During development of the gastrointestinal tract, Wnts 2a, 4, 5a, 5b, 6 and 11 display regional expression patterns throughout embryonic days 12.5–16.5 (Lickert *et al.* 2001). In the human adult colon, Wnt5a is expressed in the crypt base, with reduced expression in the small intestinal villi, and is slightly up-regulated in colon cancer. Wnt2 is absent in the normal human colon, but is expressed in colon cancer. Wnts 1, 4, 5b, 6, 7b and 10b are ubiquitously and strongly expressed in the normal colon and in colon cancer, and Wnt 7a has a low level of expression in both the normal and neoplastic colon (Holcombe *et al.* 2002). The Wnt receptors ‘frizzled’ (Fz) are also differentially expressed in the normal and neoplastic colon, with no expression of Fz receptors-1 and -2 mRNA in the normal colon and in well-differentiated tumours, although both receptors are strongly expressed in poorly differentiated tumours (Holcombe *et al.* 2002). These results suggest an involvement of Wnt2 and Wnt5a and the Fz1/2 receptors in the progression of colon cancer (Holcombe *et al.* 2002).

Under normal circumstances, when Wnt signalling pathways are inactive, the tumour suppressor protein, adenomatous polyposis coli (APC), forms a subcellular, trimeric complex with glycogen synthase kinase-3 β (GSK3 β) and axin/conductin. This so-called ‘destruction complex’ interacts with β -catenin, causing its serine phosphorylation and degradation by the ubiquitin-proteasome pathway, thereby maintaining low levels of nuclear and cytosolic β -catenin. In the canonical Wnt pathway, binding of a Wnt ligand to its Fz receptor, inhibits GSK3 β phosphorylation of β -catenin to produce an accumulation of cytosolic β -catenin, a key signal transducer downstream in the Wnt signalling pathway (Kinzler & Vogelstein 1996). β -catenin then interacts with a member of the Tcf/LEF (T-cell factor/lymphocyte enhancer factor) family of DNA-binding

Figure 2. The canonical Wnt signalling pathway. A model of the canonical Wnt signalling cascade, in the non-signalling cell (a), and its signalling counterpart (b) [from (Van Noort & Clevers 2002), with permission].

proteins, to produce a transcriptionally active bipartite complex, which, in turn, translocates to the nucleus and activates downstream target genes to increase cell proliferation (Fig. 2) (reviewed in Polakis 1999; Benez & Clevers 2000). Tcf-4 is the most commonly expressed member of the Tcf/LEF family in epithelial cells of the small intestine and colon (Korinek *et al.* 1997). Mice with a targeted deletion of Tcf-4 lack a stem cell compartment in the small intestine, although cells in the underlying lamina propria are not affected. Therefore, Tcf-4 transcription, activated by Wnt signalling from cells in the underlying mesenchyme, is vital for establishment of a stem cell population in the developing small intestine (Korinek *et al.* 1998; Wielenga *et al.* 1999). Upon removal of the Wnt signal, β -catenin is removed from the nucleus by APC, and the transcriptional repressor function of the Tcf is reinstated by its interaction with the transcriptional co-repressor TLE-1, the human homologue of the *Drosophila* protein, Groucho (Cavallo *et al.* 1998; Levanon *et al.* 1998; Roose *et al.* 1998; Benez 2002), or by the transcriptional co-activator, CREB-binding protein (CBP) (Waltzer & Benez 1998). A direct role of Wnt proteins in controlling epithelial cell proliferation is shown in mice with a targeted deletion of a known canonical Wnt pathway inhibitor, Dickkopf-1 (Dkk1). These mice have reduced epithelial cell proliferation and display a patchy loss of crypts in the small intestine (Pinto *et al.* 2003). The epithelial cells of these mice were devoid of nuclear β -catenin nuclear, and expression of the known oncogene, *c-Myc*, as expected (Pinto *et al.* 2003).

The Wnt signalling cascade in neoplastic transformation

The multifunctional *APC* gene is mutated in the cancer predisposition syndrome, familial adenomatous polyposis (FAP), which precedes multiple adenoma formation, and carcinoma in the human colon. *APC* is also mutated in 80% of all sporadic colorectal tumours, and its inactivation is believed to be one of the first, if not the initiating step in colon carcinogenesis (Kinzler & Vogelstein 1996). Mutation of *APC* renders the GSK3 β /Axin/APC complex unable to degrade β -catenin, resulting in increased β -catenin/Tcf transcriptional complexes in the nucleus, and an accompanying over-transcription of target genes (Mei *et al.* 1999; Wielenga *et al.* 1999). In 50% of colon cancers that express wild-type *APC*, β -catenin is mutated in a phosphorylation residue in one of its regulatory domains, and does not undergo proteasomal degradation by GSK3 β (Sparks *et al.* 1998). However, small adenomas showing a loss of β -catenin are less likely to

progress to larger adenomas and invasive carcinomas, as adenomas with mutations in the *APC* allele, indicating that inhibition of an additional function(s) of APC, above its regulation of β -catenin levels via the Wnt signalling cascade, are conducive to the malignant progression of colorectal adenomas (Samowitz *et al.* 1999). Mutations in *APC* and *β -catenin* arise independently in colon cancer, and it is important to note that some colon cancers have wild-type copies of both *β -catenin* and *APC* genes, indicating that other members of the Wnt pathway may also be important in neoplastic change in the colon (Sparks *et al.* 1998). Identification of downstream target genes in the Wnt signalling cascade has proven difficult, although c-Myc and cyclin D1 are targets of β -catenin/Tcf-4 transcriptional complexes in human colon cancer, and have known roles in the regulation of cell proliferation, apoptosis and the cell cycle (Ahmed *et al.* 1998; He *et al.* 1998; Wong *et al.* 1998; Shtutman *et al.* 1999; Tetsu & McCormick 1999).

CELLULAR MECHANISMS OF NEOPLASTIC TRANSFORMATION IN COLON CARCINOGENESIS

In addition to identifying the molecular and genetic targets that regulate cell proliferation in the normal mucosa, and go awry in carcinogenesis, elucidation of the morphogenetic events occurring in neoplastic transformation is important in our understanding of gastrointestinal stem cell function. It is widely accepted that colorectal carcinoma develops sequentially in the adenoma–carcinoma pathway, where a spontaneous microadenoma forms from an initial aberrant crypt focus (ACF), and may further progress to a macroscopic adenoma, and colorectal carcinoma. Loss of function of the tumour suppressor gene *APC* is one of the originating genetic mutations in adenoma development, at least in FAP (Morson 1974), and is believed to be central to both the initiation and promotion of colorectal cancer (Fodde 2002). FAP patients carry an autosomally inherited germ line mutation of the *APC* locus on chromosome 5q21 (Bodmer *et al.* 1987), and are susceptible to spontaneous development of colorectal adenomas if loss of heterozygosity (LOH) of the remaining *APC* allele occurs, in the ‘two-hit’ loss of tumour suppressor gene hypothesis (Knudson 1993).

Two main hypotheses attempt to explain the cellular mechanisms of spontaneous adenomatous morphogenesis, both of which describe the initial mutation in an intestinal stem cell in the crypt. In the ‘bottom-up’ hypothesis, an adenomatous clone of cells is produced by stochastic expansion of a mutated stem cell in the base of the crypt, and the dysplastic epithelium colonizes an entire crypt to form a monocryptal adenoma, wherein the lesion is clonal (Novelli *et al.* 1996). These dysplastic monoclonal crypts expand and replicate by crypt fission, which is rarely observed in the normal mucosa, although the *crypt fission index* (the proportion of crypts in fission) is up-regulated in adenomatous epithelia, and in non-adenomatous crypts in FAP, with unusual asymmetrical budding from the superficial and mid-crypts (Araki *et al.* 1995; Preston *et al.* 2003). The bottom-up hypothesis was propounded following immunohistochemical analyses of serial sections from a number of small tubular adenomas, wherein a distinct cut-off was observed between dysplastic cells lower in the crypt which expressed nuclear β -catenin, and the normal cells toward the luminal surface in the top of the crypts which did not (Fig. 3a and b). In the normal colonic epithelium, β -catenin expression is highest in the proliferative compartment, and is decreased in the upper two-thirds of the crypt, in accordance with its role in controlling stem cell proliferation and differentiation (Korinek *et al.* 1998) (Fig. 3c). In the adenomas, β -catenin staining was localized in the nuclei of cells in crypts undergoing fission, and was marked in the budding processes in the dividing crypts (Preston *et al.* 2003) (Fig. 3d). This

Figure 3. Opposing hypotheses for the role of stem cells in adenomatous morphogenesis. (a) Junction between early adenomatous crypts, showing a sharp junction on the surface with accumulation of nuclear b-catenin, giving way sharply to membranous staining in the normal surface cells. (b) The next serial section to (a) demonstrating a sharp junction between the nuclear staining in adenomatous cells and membrane staining in normal surface cells. (c) Nuclear b-catenin extends to the bottom of crypts in early adenomas, including the very bases of the crypts. (d) b-catenin staining in nuclei of budding crypts. (e) Surface continuity between crypts showing nuclear b-catenin staining. (f) Crypts from a larger adenoma stained for b-catenin showing invasion of adjacent crypt territories in a top-down fashion. (a–f) from (<109>Preston *et al.* 2003), with permission. (g) Hematoxylin and eosin stained sections of a small tubular adenoma. Dysplastic epithelium is superficial within the crypts, with histologically normal underlying epithelium. (h) Abrupt transition between dysplastic and normal-appearing epithelial cells at the mid-point of this crypt. Proliferative activity assessed with the Ki-67 antibody, distributed throughout the dysplastic epithelium at the top of the crypts. (i) Nuclear b-catenin is highly expressed and distributed throughout the dysplastic epithelium at the top of the crypts, but not in the crypt bases. (j) b-catenin in the nuclei of adenomatous crypts from a tiny tubular adenoma. (g–j) from (<114>Shih *et al.* 2001), with permission.

β -catenin staining pattern indicates that Wnt signalling may be instrumental in the process of crypt replication. Adenomatous cells in early sporadic lesions appear to migrate upwards in the crypts, similar to cells in normal crypts (Wong *et al.* 2002; Preston *et al.* 2003). However, in older adenomas, cell migration kinetics appear to be reversed, and proliferation is restricted to cells nearer the top of the crypts (Fig. 3f) (Moss *et al.* 1996; Shiff & Rigas 1997), with an increased rate of apoptosis in cells in the crypt base (Sinicrope *et al.* 1996). This redistribution of proliferating cells in late adenomas advocates the second theory of adenomatous spread, the 'top-down' hypothesis, wherein a mutated stem cell located in an 'intercryptal zone' near the luminal surface proliferates and migrates laterally and downwards into adjacent crypts replacing the normal epithelium, and similar to the bottom-up hypothesis, creates a monoclonal lesion (Shih *et al.* 2001). The top-down hypothesis is based on results of studies of early non-FAP adenomas, where dysplastic cells were seen exclusively at the orifices and luminal surface of colonic crypts (Fig. 3h-j) (Shih *et al.* 2001). LOH for *APC* and nucleotide sequence analysis of the mutation cluster region of the *APC* gene showed that 50% of these adenomas had LOH in the upper regions of the crypts, most with truncating *APC* mutations. A restricted nuclear localization of β -catenin was seen in cells located superficially in the crypt, indicating LOH of a gene regulated by Wnt signalling, most likely *APC* and, furthermore, proliferative activity was limited to the same population of dysplastic cells (Shih *et al.* 2001). Earlier studies have reported morphologically similar adenomas located near the mucosal surface of the colon, although it was suggested that these endophytic lesions originate in the proliferative compartment of the crypt, and sprout outwards through the lamina propria, migrating upwards in synchrony with the crypt epithelial cells, to form single-gland adenomas near the mucosal surface (Nakamura & Kino 1984). However, the rapid rate of epithelial cell turnover in the gastrointestinal tract means that the lifespan of a cell is shorter than the time required for the genetic pathways of neoplastic transformation to occur, and therefore the long-lived intestinal stem cells are the proposed target cells for the onset of carcinogenesis, and it is unlikely that an initial mutation in an epithelial cell committed to differentiation in the proliferative compartment of a crypt would lead to adenoma morphogenesis. The 'top-down' expansion of adenomatous clones contradicts the widely accepted location of the intestinal stem cell in the crypt base, as it suggests that an intracryptal stem cell zone exists. Analyses of the methylation histories of colorectal adenomas have revealed a stem cell architecture supporting the 'bottom-up' spread of adenomatous growth, and mitotic events appear to be evenly distributed throughout the cells of an adenomatous crypt, and not concentrated in the cells located superficially in the crypts, as suggested by the 'top-down' hypothesis (Tsao *et al.* 1998). It has alternatively been suggested that a stem cell in the crypt base acquires a mutation and then migrates into the intracryptal zone before undergoing adenomatous spread (Wright 2000). However, there is evidence substantiating both the 'top-down' and the 'bottom-up' mechanisms of monoclonal adenomatous morphogenesis, and it is conceivable that both mechanisms exist (Wright & Poulson 2002).

CONCLUSION

The gastrointestinal stem cell is an empowering force, regulating homeostasis throughout the entire gastrointestinal tract, with the capacity to alter the rate of cell proliferation and differentiation as regenerative demand dictates it. The stem cell also has the capacity to undergo self-renewal and programmed cell death, and maintains a constant stem cell number for optimal gastrointestinal function. However, this cell does not act alone, and we are beginning to define

the complex relationship between the gastrointestinal epithelial cells and the underlying mesenchymal cells in the stem cell niche. Due to its primitive nature, and the lack of any histological or morphological markers, the gastrointestinal epithelial stem cell remains unidentified. However, the vast amount of investigation undertaken to define the characteristics of this single cell are broadening our understanding of the genetic, molecular and cellular mechanisms involved in normal gastrointestinal regulation and the erroneous events leading to neoplastic transformation.

REFERENCES

- AHMED Y, HAYASHI S, LEVINE A, WIESCHAUS E (1998) Regulation of armadillo by a *Drosophila* APC inhibits neuronal apoptosis during retinal development. *Cell* **93**, 1171.
- AKAZAWA C, SASAI Y, NAKANISHI S, KAGEYAMA R (1992) Molecular characterization of a rat negative regulator with a basic helix-loop-helix structure predominantly expressed in the developing nervous system. *J. Biol. Chem.* **267**, 21879.
- ARAKI K, OGATA T, KOBAYASHI M, YATANI R (1995) A morphological study on the histogenesis of human colorectal hyperplastic polyps. *Gastroenterology* **109**, 1468.
- BEAULIEU JF (1992) Differential expression of the VLA family of integrins along the crypt-villus axis in the human small intestine. *J. Cell Sci.* **102**, 427.
- BIENZ M (2002) The subcellular destinations of APC proteins. *Nat. Rev. Mol. Cell Biol.* **3**, 328.
- BIENZ M, CLEVERS H (2000) Linking colorectal cancer to Wnt signaling. *Cell* **103**, 311.
- BJERKNES M, CHENG H (1981a) The stem-cell zone of the small intestinal epithelium. I. Evidence from paneth cells in the adult mouse. *Am. J. Anat.* **160**, 51.
- BJERKNES M, CHENG H (1981b) The stem-cell zone of the small intestinal epithelium. II. Evidence from paneth cells in the newborn mouse. *Am. J. Anat.* **160**, 65.
- BJERKNES M, CHENG H (1981c) The stem-cell zone of the small intestinal epithelium. III. Evidence from columnar, enteroendocrine, and mucous cells in the adult mouse. *Am. J. Anat.* **160**, 77.
- BJERKNES M, CHENG H (1981d) The stem-cell zone of the small intestinal epithelium. IV. Effects of resecting 30% of the small intestine. *Am. J. Anat.* **160**, 93.
- BJERKNES M, CHENG H (1981e) The stem-cell zone of the small intestinal epithelium. V. Evidence for controls over orientation of boundaries between the stem-cell zone, proliferative zone, and the maturation zone. *Am. J. Anat.* **160**, 105.
- BJERKNES M, CHENG H (1999) Clonal analysis of mouse intestinal epithelial progenitors. *Gastroenterology* **116**, 7.
- BODMER WF, BAILEY CJ, BODMER J, BUSSEY HJ, ELLIS A, GORMAN P, LUCIBELLO FC, MURDAY VA, RIDER SH, SCAMBLER P (1987) Localization of the gene for familial adenomatous polyposis on chromosome 5. *Nature* **328**, 614.
- BRIERLEY EJ, JOHNSON MA, LIGHTOWLERS RN, JAMES OF, TURNBULL DM (1998) Role of mitochondrial DNA mutations in human aging: implications for the central nervous system and muscle. *Ann. Neurol.* **43**, 217.
- BRITTAN M (2003a) Bone marrow-derived stem cells contribute to multiple cell lineages in experimental colitis. *J. Pathol.* **201**(Supp): 1a.
- BRITTAN M (2003b) *The Stem Cell Origin of Cell Lineages, Proliferative Units Cancer Gastrointestinal Tract*. Gut 2004, in press.
- BRITTAN M, HUNT T, JEFFERY R, POULSON R, FORBES SJ, HODIVALA-DILKE K, GOLDMAN J, ALISON MR, WRIGHT NA (2002) Bone marrow derivation of pericryptal myofibroblasts in the mouse and human small intestine and colon. *Gut* **50**, 752.
- CAI WB, ROBERTS SA, POTTEN CS (1997) The number of clonogenic cells in crypts in three regions of murine large intestine. *Int. J. Radiat. Biol.* **71**, 573.
- CAIRNIE AB, MILLEN BH (1975) Fission of crypts in the small intestine of the irradiated mouse. *Cell Tissue Kinet.* **8**, 189.
- CAIRNS J (1975) Mutation selection and the natural history of cancer. *Nature* **255**, 197.
- CAVALLO RA, COX RT, MOLINE MM, ROOSE J, POLEVOY GA, CLEVERS H, PEIFER M, BEJSOVEC A (1998) *Drosophila* Tcf and Groucho interact to repress Wingless signalling activity. *Nature* **395**, 604.
- CHENG H, LEBLOND CP (1974) Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. III. Entero-endocrine cells. *Am. J. Anat.* **141**, 503.
- CHINNERY PF, SAMUELS DC (1999) Relaxed replication of mtDNA: a model with implications for the expression of disease. *Am. J. Hum. Genet.* **64**, 1158.
- COLLER HA, KHRAPKO K, BODYAK ND, NEKHAIEVA E, HERRERO-JIMENEZ P, THILLY WG (2001) High frequency of homoplasmic mitochondrial DNA mutations in human tumors can be explained without selection. *Nat. Genet.* **28**, 147.

- DARMOUL D, BROWN D, SELSTED ME, OUELLETTE AJ (1997) Cryptdin gene expression in developing mouse small intestine. *Am. J. Physiol.* **272**, G197.
- FODDE R (2002) The APC gene in colorectal cancer. *Eur. J. Cancer* **38**, 867.
- HE TC, SPARKS AB, RAGO C, HERMEKING H, ZAWEL L, DA COSTA LT, MORIN PJ, VOGELSTEIN B, KINZLER KW (1998) Identification of c-MYC as a target of the APC pathway. *Science* **281**, 1509.
- HOLCOMBE RF, MARSH JL, WATERMAN ML, LIN F, MILOVANOVIC T, TRUONG T (2002) Expression of Wnt ligands and frizzled receptors in colonic mucosa and in colon carcinoma. *Mol. Pathol.* **55**, 220.
- HOTCHIN NA, GANDARILLAS A, WATT FM (1995) Regulation of cell surface $\beta 1$ integrin levels during keratinocyte terminal differentiation. *J. Cell Biol.* **128**, 1209.
- HUNYADY B, MEZEY E, PALKOVITS M (2000) Gastrointestinal immunology: cell types in the lamina propria – a morphological review. *Acta Physiol. Hung.* **87**, 305.
- IMAI T, TOKUNAGA A, YOSHIDA T, HASHIMOTO M, MIKOSHIBA K, WEINMASTER G, NAKAFUKU M, OKANO H (2001) The neural RNA-binding protein Musashi1 translationally regulates mammalian numb gene expression by interacting with its mRNA. *Mol. Cell Biol.* **21**, 3888.
- JIANG Y, JAHAGIRDAR BN, REINHARDT RL, SCHWARTZ. RE, KEENE CD, ORTIZ-GONZALEZ. XR, REYES M, LENVIK T, LUND T, BLACKSTAD M, DU J, ALDRICH S, LISBERG A, LOW WC, LARGAESPADA DA, VERFAILLIE CM (2002) Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* **418**, 41.
- JOHNSON MA, BINDOFF LA, TURNBULL DM (1993) Cytochrome c oxidase activity in single muscle fibers: assay techniques and diagnostic applications. *Ann. Neurol.* **33**, 28.
- JONES PH, WATT FM (1993) Separation of human epidermal stem cells from transit amplifying cells on the basis of differences in integrin function and expression. *Cell* **73**, 713.
- JOYCE NC, HAIRE MF, PALADE GE (1987) Morphologic and biochemical evidence for a contractile cell network within the rat intestinal mucosa. *Gastroenterology* **92**, 68.
- JULIANO RL, VARNER JA (1993) Adhesion molecules in cancer: the role of integrins. *Curr. Opin. Cell Biol.* **5**, 812.
- KARAM SM (1999) Lineage commitment and maturation of epithelial cells in the gut. *Front. Biosci.* **4**, D286.
- KAYAHARA T, SAWADA M, TAKAISHI S, FUKUI H, SENO H, FUKUZAWA H, SUZUKI K, HIAI H, KAGEYAMA R, OKANO H, CHIBA T (2003) Candidate markers for stem and early progenitor cells, Musashi-1 and Hes1, are expressed in crypt base columnar cells of mouse small intestine. *FEBS Lett.* **535**, 131.
- KAYE GI, LANE N, PASCAL RR (1968) Colonic pericryptal fibroblast sheath: replication, migration, and cytodifferentiation of a mesenchymal cell system in adult tissue. II. Fine structural aspects of normal rabbit and human colon. *Gastroenterology* **54**, 852.
- KEDINGER M, DULUC I, FRITSCH C, LORENTZ. O, PLATEROTI M, FREUND JN (1998) Intestinal epithelial–mesenchymal cell interactions. *Ann. NY Acad. Sci.* **859**, 1.
- KERNEIS S, PRINGAULT E (1999) Plasticity of the gastrointestinal epithelium: the M cell paradigm and opportunism of pathogenic microorganisms. *Semin. Immunol.* **11**, 205.
- KINZLER KW, VOGELSTEIN B (1996) Lessons from hereditary colorectal cancer. *Cell* **87**, 159.
- KIRKLAND SC (1988) Clonal origin of columnar, mucous, and endocrine cell lineages in human colorectal epithelium. *Cancer* **61**, 1359.
- KNUDSON AG (1993) Antioncogenes and human cancer. *Proc. Natl Acad. Sci. USA* **90**, 10914.
- KORBLING M, KATZ RL, KHANNA A, RUIFROK AC, RONDON G, ALBITAR M, CHAMPLIN RE, ESTROV Z (2002) Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. *N. Engl. J. Med.* **346**, 738.
- KORINEK V, BARKER N, MOERER P, VAN DONSELAAR E, HULS G, PETERS PJ, CLEVERS H (1998) Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nat. Genet.* **19**, 379.
- KORINEK V, BARKER N, MORIN PJ, VAN WICHEN D, DE WEGER R, KINZLER KW, VOGELSTEIN B, CLEVERS H (1997) Constitutive transcriptional activation by a β -catenin-Tcf complex in APC^{-/-} colon carcinoma. *Science* **275**, 1784.
- KRAUSE DS, THEISE ND, COLLECTOR MI, HENEGARIU O, HWANG S, GARDNER R, NEUTZEL S, SHARKIS SJ (2001) Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* **105**, 369.
- LAGASSE E, CONNORS H, AL-DHALIMY M, REITSMA M, DOHSE M, OSBORNE L, WANG X, FINEGOLD M, WEISSMAN IL, GROMPE M (2000) Purified hematopoietic stem cells can differentiate into hepatocytes *in vivo*. *Nat. Med.* **6**, 1229.
- LEVANON D, GOLDSTEIN RE, BERNSTEIN Y, TANG H, GOLDENBERG D, STIFANI S, PAROUSH Z, GRONER Y (1998) Transcriptional repression by AML1 and LEF-1 is mediated by the TLE/Groucho corepressors. *Proc. Natl Acad. Sci. USA* **95**, 11590.
- LI A, SIMMONS PJ, KAUR P (1998) Identification and isolation of candidate human keratinocyte stem cells based on cell surface phenotype. *Proc. Natl Acad. Sci. USA* **95**, 3902.
- LICKERT H, KISPERT A, KUTSCH S, KEMLER R (2001) Expression patterns of Wnt genes in mouse gut development. *Mech. Dev.* **105**, 181.
- LIN H (2002) The stem-cell niche theory: lessons from flies. *Nat. Rev. Genet* **3**, 931.

- LOEFFLER M, GROSSMANN B (1991) A stochastic branching model with formation of subunits applied to the growth of intestinal crypts. *J. Theor. Biol.* **150**, 175.
- LOEFFLER M, BRATKE T, PAULUS U, LI YQ, POTTEN CS (1997) Clonality and life cycles of intestinal crypts explained by a state dependent stochastic model of epithelial stem cell organization. *J. Theor. Biol.* **186**, 41.
- MARSH MN, TRIER JS (1974a) Morphology and cell proliferation of subepithelial fibroblasts in adult mouse jejunum. I. Structural features. *Gastroenterology* **67**, 622.
- MARSH MN, TRIER JS (1974b) Morphology and cell proliferation of subepithelial fibroblasts in adult mouse jejunum. II. Radioautographic studies. *Gastroenterology* **67**, 636.
- MASKENS AP, DUJARDIN-LOITS RM (1981) Kinetics of tissue proliferation in colorectal mucosa during post-natal growth. *Cell Tissue Kinet.* **14**, 467.
- MAUNOURY R, ROBINE S, PRINGAULT E, HUET C, GUENET JL, GAILLARD JA, LOUVAUD D (1988) Villin expression in the visceral endoderm and in the gut anlage during early mouse embryogenesis. *EMBO J.* **7**, 3321.
- MEI JM, HORD NG, WINTERSTEIN DF, DONALD SP, PHANG JM (1999) Differential expression of prostaglandin endoperoxide H synthase-2 and formation of activated β -catenin-LEF-1 transcription complex in mouse colonic epithelial cells contrasting in Apc. *Carcinogenesis* **20**, 737.
- MICHIKAWA Y, MAZZUCHELLI F, BRESOLIN N, SCARLATO G, ATTARDI G (1999) Aging-dependent large accumulation of point mutations in the human mtDNA control region for replication. *Science* **286**, 774.
- MORSON BC (1974) Evolution of cancer of the colon and rectum. *Cancer* **34** (Suppl.), 845.
- MOSS SF, LIU TC, PETROTOS A, HSU TM, GOLD LI, HOLT PR (1996) Inward growth of colonic adenomatous polyps. *Gastroenterology* **111**, 1425.
- NABEYAMA A, LEBLOND CP (1974) 'Caveolated cells' characterized by deep surface invaginations and abundant filaments in mouse gastro-intestinal epithelia. *Am. J. Anat.* **140**, 147.
- NAKAMURA S, KINO I (1984) Morphogenesis of minute adenomas in familial polyposis coli. *J. Natl Cancer Inst.* **73**, 41.
- NAKAMURA Y, SAKAKIBARA S, MIYATA T, OGAWA M, SHIMAZAKI T, WEISS S, KAGEYAMA R, OKANO H (2000) The bHLH gene *hes1* as a repressor of the neuronal commitment of CNS stem cells. *J. Neurosci.* **20**, 283.
- NISHIMURA S, WAKABAYASHI N, TOYODA K, KASHIMA K, MITSUFUJI S (2003) Expression of Musashi-1 in human normal colon crypt cells: a possible stem cell marker of human colon epithelium. *Dig. Dis. Sci.* **48**, 1523.
- NOMURA S, KAMINISHI M, SUGIYAMA K, OOHARA T, ESUMI H (1996) Clonal analysis of isolated single fundic and pyloric gland of stomach using X-linked polymorphism. *Biochem. Biophys. Res. Commun.* **226**, 385.
- NOVELLI MR, WILLIAMSON JA, TOMLINSON IP, ELIA G, HODGSON SV, TALBOT IC, BODMER WF, WRIGHT NA (1996) Polyclonal origin of colonic adenomas in an XO/XY patient with FAP. *Science* **272**, 1187.
- OH H, BRADFUTE SB, GALLARDO TD, NAKAMURA T, GAUSSIN V, MISHINA Y, POCIUS J, MICHAEL LH, BEHRINGER RR, GARRY DJ, ENTMAN ML, SCHNEIDER MD (2003) Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proc. Natl Acad. Sci. USA* **100**, 12313.
- OUELLETTE AJ, HSIEH MM, NOSEK MT, CANO-GAUCI DF, HUTTNER KM, BUICK RN, SELSTED ME (1994) Mouse paneth cell defensins: primary structures and antibacterial activities of numerous cryptdin isoforms. *Infect. Immun.* **62**, 5040.
- OUELLETTE AJ, SATCHELL DP, HSIEH MM, HAGEN SJ, SELSTED ME (2000) Characterization of luminal paneth cell α -defensins in mouse small intestine. Attenuated antimicrobial activities of peptides with truncated amino termini. *J. Biol. Chem.* **275**, 33969.
- 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.
- PARK HS, GOODLAD RA, AHNEN DJ, WINNETT A, SASIENI P, LEE CY, WRIGHT NA (1997) Effects of epidermal growth factor and dimethylhydrazine on crypt size, cell proliferation, and crypt fission in the rat colon. Cell proliferation and crypt fission are controlled independently. *Am. J. Pathol.* **151**, 843.
- PARKER FG, BARNES EN, KAYE GI (1974) The pericryptal fibroblast sheath. IV. Replication, migration, and differentiation of the subepithelial fibroblasts of the crypt and villus of the rabbit jejunum. *Gastroenterology* **67**, 607.
- PASCAL RR, KAYE GI, LANE N (1968) Colonic pericryptal fibroblast sheath: replication, migration, and cytodifferentiation of a mesenchymal cell system in adult tissue. I. Autoradiographic studies of normal rabbit colon. *Gastroenterology* **54**, 835.
- PINTO D, GREGORIEFF A, BEGTHEL H, CLEVERS H (2003) Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. *Genes Dev.* **17**, 1709.
- POLAKIS P (1999) The oncogenic activation of β -catenin. *Curr. Opin. Genet. Dev.* **9**, 15.
- PONDER BA, SCHMIDT GH, WILKINSON MM, WOOD MJ, MONK M, REID A (1985) Derivation of mouse intestinal crypts from single progenitor cells. *Nature* **313**, 689.
- POTTEN CS (1998) Stem cells in gastrointestinal epithelium: numbers, characteristics and death. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **353**, 821.

- POTTEN CS, BOOTH C, PRITCHARD DM (1997) The intestinal epithelial stem cell: the mucosal governor. *Int. J. Exp. Pathol* **78**, 219.
- POTTEN CS, OWEN G, BOOTH D (2002) Intestinal stem cells protect their genome by selective segregation of template DNA strands. *J. Cell Sci.* **115**, 2381.
- POTTEN CS, BOOTH C, TUDOR GL, BOOTH D, BRADY G, HURLEY P, ASHTON G, CLARKE R, SAKAKIBARA S, OKANO H (2003) Identification of a putative intestinal stem cell and early lineage marker; musashi-1. *Differentiation* **71**, 28.
- POWELL DW, MIFFLIN RC, VALENTICH JD, CROWE SE, SAADA JI, WEST AB (1999) Myofibroblasts. II. Intestinal sub-epithelial myofibroblasts. *Am. J. Physiol.* **277**, C183.
- PRESTON SL, WONG WM, CHAN AO, POULSON R, JEFFERY R, GOODLAD RA, MANDIR N, ELIA G, NOVELLI M, BODMER WF, TOMLINSON IP, WRIGHT NA (2003) Bottom-up histogenesis of colorectal adenomas: origin in the monocryptal adenoma and initial expansion by crypt fission. *Cancer Res.* **63**, 3819.
- ROBERTS SA, HENDRY JH, POTTEN CS (1995) Deduction of the clonogen content of intestinal crypts: a direct comparison of two-dose and multiple-dose methodologies. *Radiat. Res.* **141**, 303.
- ROOSE J, MOLENAAR M, PETERSON J, HURENKAMP J, BRANTJES H, MOERER P, VAN DE WETERING M, DESTREE O, CLEVERS H (1998) The Xenopus Wnt effector XTcf-3 interacts with Groucho-related transcriptional repressors. *Nature* **395**, 608.
- SAKAKIBARA S, IMAI T, HAMAGUCHI K, OKABE M, ARUGA J, NAKAJIMA K, YASUTOMI D, NAGATA T, KURIHARA Y, UESUGI S, MIYATA T, OGAWA M, MIKOSHIBA K, OKANO H (1996) Mouse-Musashi-1, a neural RNA-binding protein highly enriched in the mammalian CNS stem cell. *Dev. Biol.* **176**, 230.
- SAMOWITZ WS, POWERS MD, SPIRIO LN, NOLLET F, VAN ROY F, SLATTERY ML (1999) β -Catenin mutations are more frequent in small colorectal adenomas than in larger adenomas and invasive carcinomas. *Cancer Res.* **59**, 1442.
- SANDERS KM (1996) A case for interstitial cells of Cajal as pacemakers and mediators of neurotransmission in the gastrointestinal tract. *Gastroenterology* **111**, 492.
- SAPPINO AP, DIETRICH PY, SKALLI O, WIDGREN S, GABBIANI G (1989) Colonic pericryptal fibroblasts. Differentiation pattern in embryogenesis and phenotypic modulation in epithelial proliferative lesions. *Virchows Arch. A Pathol. Anat. Histopathol.* **415**, 551.
- SASAI Y, KAGEYAMA R, TAGAWA Y, SHIGEMOTO R, NAKANISHI S (1992) Two mammalian helix-loop-helix factors structurally related to *Drosophila* hairy and Enhancer of split. *Genes Dev.* **6**, 2620.
- SCIACCO M, BONILLA E, SCHON EA, DIMAURO S, MORAES CT (1994) Distribution of wild-type and common deletion forms of mtDNA in normal and respiration-deficient muscle fibers from patients with mitochondrial myopathy. *Hum. Mol. Genet.* **3**, 13.
- SHIFF SJ, RIGAS B (1997) Colon adenomatous polyps – do they grow inward? *Lancet* **349**, 1853.
- SHIH IM, WANG TL, TRAVERSO G, ROMANS K, HAMILTON SR, BEN-SASSON S, KINZLER KW, VOGELSTEIN B (2001) Top-down morphogenesis of colorectal tumors. *Proc. Natl Acad. Sci. USA* **98**, 2640.
- SHINOHARA T, AVARBOCK MR, BRINSTER RL (1999) β 1- and α 6-integrin are surface markers on mouse spermatogonial stem cells. *Proc. Natl Acad. Sci. USA* **96**, 5504.
- SHTUTMAN M, ZHURINSKY J, SIMCHA I, ALBANESE C, D'AMICO M, PESTELL R, BEN-ZE'EV A (1999) The cyclin D1 gene is a target of the β -catenin/LEF-1 pathway. *Proc. Natl Acad. Sci. USA* **96**, 5522.
- SINICROPE FA, RODDEY G, McDONNELL TJ, SHEN Y, CLEARY KR, STEPHENS LC (1996) Increased apoptosis accompanies neoplastic development in the human colorectum. *Clin. Cancer Res.* **2**, 1999.
- SPARKS AB, MORIN PJ, VOGELSTEIN B, KINZLER KW (1998) Mutational analysis of the APC/ β -catenin/Tcf pathway in colorectal cancer. *Cancer Res.* **58**, 1130.
- SPRADLING A, DRUMMOND-BARBOSA D, KAI T (2001) Stem cells find their niche. *Nature* **414**, 98.
- TATEMATSU M, FUKAMI H, YAMAMOTO M, NAKANISHI H, MASUI T, KUSAKABE N, SAKAKURA T (1994) Clonal analysis of glandular stomach carcinogenesis in C3H/HeN $\leftrightarrow$ BALB/c chimeric mice treated with N-methyl-N-nitrosourea. *Cancer Lett.* **83**, 37.
- TETSU O, MCCORMICK F (1999) β -Catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature* **398**, 422.
- THOMAS GA, WILLIAMS D, WILLIAMS ED (1988) The demonstration of tissue clonality by X-linked enzyme histochemistry. *J. Pathol.* **155**, 101.
- THOMPSON M, FLEMING KA, EVANS DJ, FUNDELE R, SURANI MA, WRIGHT NA (1990) Gastric endocrine cells share a clonal origin with other gut cell lineages. *Development* **110**, 477.
- TSAO JL, ZHANG J, SALOVAARA R, LI ZH, JARVINEN HJ, MECKLIN JP, AALTONEN LA, SHIBATA D (1998) Tracing cell fates in human colorectal tumors from somatic microsatellite mutations: evidence of adenomas with stem cell architecture. *Am. J. Pathol.* **153**, 1189.
- VAN NOORT M, CLEVERS H (2002) TCF transcription factors, mediators of Wnt-signaling in development and cancer. *Dev. Biol.* **244**, 1.

- VASSILOPOULOS G, WANG PR, RUSSELL DW (2003) Transplanted bone marrow regenerates liver by cell fusion. *Nature* **422**, 901.
- WALTZER L, BIENZ M (1998) *Drosophila* CBP represses the transcription factor TCF to antagonize Wingless signalling. *Nature* **395**, 521.
- WANG X, WILLENBRING H, AKKARI Y, TORIMARU Y, FOSTER M, AL-DHALIMY M, LAGASSE E, FINEGOLD M, OLSON S, GROMPE M (2003) Cell fusion is the principal source of bone-marrow-derived hepatocytes. *Nature* **422**, 897.
- WEIMANN JM, CHARLTON CA, BRAZELTON TR, HACKMAN RC, BLAU HM (2003a) Contribution of transplanted bone marrow cells to Purkinje neurons in human adult brains. *Proc. Natl Acad. Sci. USA* **100**, 2088.
- WEIMANN JM, JOHANSSON CB, TREJO A, BLAU HM (2003b) Stable reprogrammed heterokaryons form spontaneously in Purkinje neurons after bone marrow transplant. *Nat. Cell Biol.* **5**, 959.
- WIELENGA VJ, SMITS R, KORINEK V, SMIT L, KIELMAN M, FODDE R, CLEVERS H, PALS ST (1999) Expression of CD44 in Apc and Tcf mutant mice implies regulation by the WNT pathway. *Am. J. Pathol.* **154**, 515.
- WINTON DJ, PONDER BA (1990) Stem-cell organization in mouse small intestine. *Proc. R. Soc. Lond. B Biol. Sci.* **241**, 13.
- WONG MH, RUBINFELD B, GORDON JI (1998) Effects of forced expression of an NH2-terminal truncated β -catenin on mouse intestinal epithelial homeostasis. *J. Cell Biol.* **141**, 765.
- WONG WM, MANDIR N, GOODLAD RA, WONG BC, GARCIA SB, LAM SK, WRIGHT NA (2002) Histogenesis of human colorectal adenomas and hyperplastic polyps: the role of cell proliferation and crypt fission. *Gut* **50**, 212.
- WRIGHT NA (2000) Epithelial stem cell repertoire in the gut: clues to the origin of cell lineages, proliferative units and cancer. *Int. J. Exp. Pathol.* **81**, 117.
- WRIGHT NA, ALISON MR (1984) *The Biology of Epithelial Populations*. Oxford: Oxford University Press.
- WRIGHT NA, POULSOM R (2002) Top down or bottom up? Competing management structures in the morphogenesis of colorectal neoplasms. *Gut* **51**, 306.
- YATABE Y, TAVARE S, SHIBATA D (2001) Investigating stem cells in human colon by using methylation patterns. *Proc. Natl Acad. Sci. USA* **98**, 10839.