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Antony W. Burgess

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Growth control mechanisms in normal and transformed intestinal cells

Antony W. Burgess

Ludwig Institute for Cancer Research, Melbourne 3050, Australia (burgess@ludwig.edu.au)

The cells populating the intestinal crypts are part of a dynamic tissue system which involves the self-renewal of stem cells, a commitment to proliferation, lineage-specific differentiation, movement and cell death. Our knowledge of these processes is limited, but even now there are important clues to the nature of the regulatory systems, and these clues are leading to a better understanding of intestinal cancers. Few intestinal-specific markers have been described; however, homeobox genes such as *cdx-2* appear to be important for morphogenic events in the intestine. There are several intestinal cell surface proteins such as the A33 antigen which have been used as targets for immunotherapy. Many regulatory cytokines (lymphokines or growth factors) influence intestinal development: enteroglucagon, IL-2, FGF, EGF family members. In conjunction with cell–cell contact and/or ECM, these cytokines lead to specific differentiation signals. Although the tissue distribution of mitogens such as EGF, TGFα, amphiregulin, betacellulin, HB-EGF and cripto have been studied in detail, the physiological roles of these proteins have been difficult to determine. Clearly, these mitogens and the corresponding receptors are involved in the maintenance and progression of the tumorigenic state. The interactions between mitogenic, tumour suppressor and oncogenic systems are complex, but the tumorigenic effects of multiple lesions in intestinal carcinomas involve synergistic actions from lesions in these different systems. Together, the truncation of *apc* and activation of the *ras* oncogene are sufficient to induce colon tumorigenesis. If we are to improve cancer therapy, it is imperative that we discover the biological significance of these interactions, in particular the effects on cell division, movement and survival.

Keywords: growth factors; intestinal biology; colon cancer; oncogenes

1. INTRODUCTION

The intestinal mucosal cells are produced continuously at a rapid rate (Altmann & Enesco 1967) and all of the cells come from the proliferative/differentiative units, the individual crypts. In the small intestine, cells are produced in the crypt and migrate to the villus before dying and shedding into the lumen. In the large intestine (colon), cells at the top of the crypt die and shed directly into the lumen. There are four major intestinal cell types: columnar cells (almost 90% of the villus population), mucous (goblet) cells, enteroendocrine cells and Paneth cells (at the base of the crypt) (Le Blond & Cheng 1976). Each crypt arises from a single, embryonic, intestinal stem cell, and both mutational and genetic analysis indicate that a single, multipotential, 'self-renewing' stem cell can give rise to all cell types in the crypt or villus (Gordon *et al.* 1992).

There is a large turnover of cells in each crypt; the transit time from 'stem' cell to differentiated villus (or colonic) cell is only 2–3 days (Wright & Irwin 1982; Schmidt *et al.* 1985). A single stem cell generates a set of *ca.* 150 transitional cells that mature and divide to form *ca.* 600 functional cells at the top of the crypt and the villus. Differentiation, proliferation and death are topographically constrained; thus, at the base of the crypt few cells divide, whereas the bottom third contains the majority of dividing cells (the proliferative zone), and dying cells are usually only found at the top of the crypt or the tip of the villus. Migration of cells within the crypt is bi-directional; while most cells migrate from the bottom to the top of the crypt, Paneth cells migrate from the proliferative zone towards the base of the crypt (Cheng & Le Blond 1974). Paneth cells remain at the base of the crypt for up to four weeks.

The factors determining proliferation, differentiation, migration and the direction of migration are understood poorly. However, this process is undoubtedly influenced by several biochemical systems, including cell surface adhesion proteins (e.g. cadherins and integrins), chemotactic (morphogenetic) gradients, extracellular matrix (ECM) and growth factors/cytokines and their receptors. All of these systems are extraordinarily complex and undoubtedly interact strongly. If we are to understand either normal intestinal cell production or the processes of colonic tumorigenesis, it is important to improve our understanding of the role of each system in the intestinal mucosa. At present our knowledge and ability to analyse the molecules or biology of intestinal cells is limited.

One of the major limitations associated with a study of intestinal cell production is our inability to culture either intestinal stem cells or committed cells so that they will produce mature progeny on a medium-term (from 24 h to 4 days) or long-term (from 4 days to 14 days) basis. As soon as intestinal cells are removed from the animal, apoptosis is initiated (Friedman *et al.* 1984; Whitehead & Van Eeden 1991). At present this cell death is difficult to

control, and there are no known ECM components or growth factors that can be assigned confidently to a role in the physiology of intestinal cell production. Of course, there are many factors that impinge on intestinal cellularity, the rate of cell production and/or the rate of cell death in the intestinal mucosa of an animal (Potten 1997). Dietary changes (i.e. cellulose content) can induce profound hyperplastic responses (Konishi *et al.* 1984); indeed, the movement of faecal matter through the intestine is important for maintaining mucosal cellularity (Foster & Whitehead 1990). It is still not clear whether lumenal nutrition, physical distention or lymphoid antigen interactions are primary determinants of crypt or villus cellularity. Hormonal changes have occasionally been reported which suggest particular roles for peptide hormones such as enteroglucon (Gleeson *et al.* 1971). Cytokines such as epidermal growth factor (EGF) and other small peptides can influence intestinal cell production (Dignass *et al.* 1996; Park *et al.* 1997), but determining whether these effects are direct or indirect (via another hormonal cell system) has proved to be a difficult problem. There are several physiological and 'pathological' situations that lead to profound changes in the cellularity of the intestinal mucosa—for example, lactation (Prieto *et al.* 1994), adaption after surgery and colitis—but still no causative agent(s) has been identified which is responsible for the changes in cell production. In situations where there is intestinal damage, unusual differentiation and special cell types can arise owing to the local release of peptide hormones (Otto *et al.* 1996). Similarly, when intestinal lymphocytes are activated by lumen antigens (or bacteria), cells such as intestinal M-cells can be induced by lymphokines (Kernéis *et al.* 1997). However, our understanding of cell production in the normal intestine is far from satisfactory, and until this situation improves we are unlikely to make significant progress with the biological treatment of intestinal proliferative diseases. Many of the difficulties may be associated with the complex processes occurring in each crypt. In particular, the relationship between cell adhesion to matrix (or other cells) and the proliferative/differentiative potential of the cells requires further analysis. Even colonic tumour cell lines require growth factors, adhesion and cell–cell contact to survive and/or differentiate.

Clearly, there are spatial (morphogenic) gradients along the intestinal axis (e.g. villus height diminishes as the distance from the duodenum increases) (Hermiston & Gordon 1993). The establishment of these morphogenic gradients appear to occur during embryogenesis, and they provide an extraordinary opportunity for studying the genes responsible for these gradients (Hermiston & Gordon 1993). Similarly, the epithelial differentiation is dependent on interactions with lymphoid (Kernéis *et al.* 1997), fibroblastic and endothelial cells. Indeed, the interactions between the different cell types hold the key to many of the intestinal pathologies (including cancer). Therefore, it is critical that the molecular basis for cell–cell communication is determined.

We are now in an era of transgenesis, which allows the delivery and control of growth factors, receptors, cadherins, signalling proteins, apoptotic inhibitors and cell cycle regulators to specific intestinal cells. Unfortunately, the number of promoter systems suitable for intestinal specific delivery is limited. Studies have already commenced with the promoters from the fatty acid binding protein (Cohen *et al.* 1992) and ileal lipid binding protein genes. These intestinal promoters are usually expressed in mature intestinal cells but the genes need to be delivered to the proliferative (or stem cell) compartment, otherwise expression will be lost quickly. Some intestinal cell lines have been isolated by expressing the SV40 large T-antigen under an interferon-inducible promoter (Moyer & Aust 1984; Jat *et al.* 1991; Whitehead *et al.* 1993), but transgenic models of intestinal neoplasia have been elusive (Hermiston & Gordon 1993).

Fortunately, a spontaneous mutation to the mouse *apc* gene (the *min*) has allowed a number of interesting studies on the evolution of tumorigenic changes associated with intestinal polyposis (Moser *et al.* 1992). The identification of *apc*, and the high frequency of mutations of this gene in colon cancer (Gordon *et al.* 1991; Laken *et al.* 1997), has provided an important opportunity to probe the biological processes essential to the development of intestinal cancer. The apc molecule has a number of functional domains that, via adapter proteins such as β-catenin, link it to the EGF receptor (Takahasi *et al.* 1997), the adhesion protein system (Barth *et al.* 1997), the cytoskeleton (Ben-Ze-ev 1997), and transcriptional control systems (Anon 1997). Normally, *apc* controls the levels of β-catenin in cells (Munemitsu *et al.* 1995). In $apc^{-/-}$ cells, β-catenin levels rise and, consequently, several signalling systems including cell adhesion/motility are affected. *Apc* localizes at the leading edge of migrating cells (Nathke *et al.* 1996) and, once mutated, $apc^{-/-}$ cells appear to accumulate in the upper portion of the crypt rather than moving on to the villus (Polakis 1997).

The first mutations associated with familial colon cancer were the *apc* defects, but more recently, mutations in DNA repair enzymes have been identified as causes of inherited non-polyposis colon cancer (Van de Water *et al.* 1994; Marra & Boland 1995; Kolodner 1996; Liu *et al.* 1996). These latter defects allow other mutations to accumulate, which eventually result in the malignant phenotype. In both diseases, the progression from normal mucosa to carcinoma is associated with early dysplastic changes of the crypt morphology. Hyperplastic changes occur at a later point in the progression. The dysplasia results from changes in cell shape, attachment and internal architectural rather than increased cellularity or rates of mitosis.

Oncogenic activation of the ras protein occurs with high frequency in colon cancer (Bos *et al.* 1987). Although ras mutations can lead to growth factor secretion, there are also changes in the cell architecture, adhesion, motility and survival characteristics of the cells. It may well be that the primary, causative, oncogenic events are more to do with cell movement, positioning and survival than the stimulation of the proliferative state. In the normal mucosa, mitosis occurs at a rapid rate; this rate of cell cycling is hardly changed in dysplasia, adenomas or carcinomas. More cells in the upper portion of the crypts in carcinomas appear to be capable of undergoing mitosis; however, this may be related to changes and increase in the ability of these cells to survive, rather than a change in the stimulation or responsiveness of the cells.

If we are to improve our ability to control cancer cells, it is essential that we not only identify the genetic lesions, but that we also understand the biological/biochemical

implications of these mutations. Changes in autocrine growth factor production, receptor activation, signalling and transcription impinge on multiple biological processes. We need to identify the molecular/cellular changes that are critical for movement, location, survival and proliferation and attempt to manipulate these processes separately. Once we are confident of the biological processes critical for oncogenesis, selective therapy (whether by drugs, peptides, signalling inhibitors or genes) can be devised and delivered to the cancer cells.

2. INTESTINAL CELL BIOLOGY

There are now several interesting mouse models for studying cell production and transformation in mice. First, as mentioned earlier, the $min^{+/-}$ mouse carries a mutation in the *apc* gene, which results in adenomatous polyposis. Second, the transgenic promoter of fatty acid binding protein (*Fabpi*) has been used to express both reporter and functional genes in the intestinal mucosa. Third, a targeted disruption of one copy of the *Cdx-2* homeobox gene induces polyp formation (Chawengsaksophak *et al.* 1997). Finally, and most recently, a cre-lox system has been developed which allows disruption of the *apc* gene in adult mice (Shibata *et al.* 1997).

3. INTESTINAL-SPECIFIC PROMOTERS—*Fabpi*

The studies with the *Fabpi* reporter promoter have allowed more detailed examination of crypt formation and interactions. In particular, the patterns of expression of *Fabpi* transgenes indicate that although individual crypts are monoclonal, crypts within a particular vicinity of the intestine are derived from the descendants of single active stem cells (Cohn *et al.* 1991), whether by seeding of individual crypts with the progenitor cells or through multiple crypt fission. It appears that in particular patches of the intestine, the stem cells may have local characteristics that dictate their differentiation and/or replacement patterns. In the colons of the *Fabpi* transgenic mice, the expression of the transgene occurs in these local patches, but after 2–3 months the expression is extinguished, presumably owing to the coordinated replacement of 'extinguished' stem cells. It is not clear how these stem cells are 'replaced': by changes in stem cell gene expression or replacement of the stem cells from a quiescent pool or from the proliferative zone.

The *Fabpi* promoter has been used to express the SV40 large T-antigen (*large TAg*) (Hauft *et al.* 1992). This antigen binds to *p53* and, as a consequence, usually leads to uncontrolled proliferation. When *large TAg* is expressed on villus-associated intestinal cells, it is possible to stimulate these mature cells back into a cycling state. However, this transgene does not appear to predispose the mice to adenocarcinoma. An interesting variation on this transgenic system has been developed with a temperature-sensitive version of the *large TAg* (TAg_{ts}) (Jat *et al.* 1991; Whitehead *et al.* 1993). This gene has been expressed under the influence of an *H2* promoter and, consequently, production can be induced using interferon. In mice, the TAg_{ts} is inactive (and/or degraded) and therefore does not influence cell production, differentiation or survival. However, when tissues are removed and placed in culture at 35 °C in the presence of interferon, the TAg_{ts} is expressed and activated: cell lines grow out from most epithelial tissues, including the colon. The cell lines proliferate unless the temperature is raised above 39 °C, where the TAg_{ts} is inactivated. Under these conditions the cells stop proliferating and undergo a rapid apoptotic cell death; consequently, it has been difficult to induce these cells to differentiate. The cells express low levels of brush-border peptidases that can be modulated by sodium butyrate. The cells express cytokeratins and, in low levels of serum, EGF or bFGF, stimulate DNA synthesis (Whitehead *et al.* 1993).

4. 'CAUDAL-LIKE'

Studies in *Drosophila* revealed the expression of the caudal homeobox gene product in the posterior midgut and hind gut (Mlodzik *et al.* 1985). The first mouse homologue of caudal was called *Cdx-1* (Duprey *et al.* 1988) and its expression appeared to be restricted to the intestine. Disruption of the *Cdx-1* gene leads to skeletal abnormalities (Subramanian *et al.* 1995). Another caudal homologue, *Cdx-2* (James *et al.* 1994), is expressed in the colon and the rectum. *Cdx-2* is expressed in most cells of the colon, but the *Cdx-2* mRNA is most abundant at the bottom of crypts. When *Cdx-2* is expressed in the undifferentiated intestinal cell line (IEC-6), columnar cells with distinct morphological characteristics (including microvilli) are induced (Suh & Traber 1996).

$Cdx\text{-}2^{-/-}$ mice die early in embryogenesis. However, heterozygote $Cdx\text{-}2^{+/-}$ mice are born with a homeotic shift of vertebrae and malformation of ribs (Chawengsaksophak *et al.* 1997). Within 12 weeks of birth, the heterozygotes develop intestinal polyps that occasionally contain areas of metaplasia. The polyps appear to have lost all *Cdx-2* expression.

The expression of *Cdx-2* is reduced in advanced colonic carcinomas (Mallo *et al.* 1997), and it will be interesting to determine whether mutations at this locus are associated with any forms of human polyposis. There are sequences in the *Cdx-2* promoter which suggest that its expression may be regulated by the β-catenin/Tcf-4 complex. Thus, when *apc* is mutated, *Cdx-2* could be induced, leading to profound morphological effects on the intestinal mucosa. Mesenchymal–epithelial interactions, which induce colonic cell differentiation, also reduce the levels of *Cdx-2*. The role of *Cdx-2* in determining cell migration and location during the development of the intestine deserves detailed analysis. Furthermore, the tissue specificity of the *Cdx-2* promoter should be useful for targeting gene expression to the intestine.

5. LYMPHOKINES

Lymphoid cells influence cell production of intestinal cells. In animals, the mucosal-associated lymphoid tissue is separated from the lumen by the epithelial cells. One specialized cell (the M-cell) transports microorganisms to Peyer's patches (Kernéis *et al.* 1997). It is now possible to convert human Caco-2 cells to M-cells by culturing in the presence of mouse lymphoid cells. The induction of differentiation appears to involve both soluble factors and cell contact. Although the lymphoid factors responsible for this differentiation induction have yet to be identified,

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lymphokines such as IL-2, IL-4, IL-10 and IL-11 are also known to influence mucosal cellularity (Nielsen *et al.* 1996; Potten 1996; Fritsch *et al.* 1997; Ludviksson *et al.* 1997).

6. EGF-LIKE MITOGENS

Several growth factors and cytokines have been shown to influence both normal and neoplastic intestinal tissue. Small intestinal hyperplasia has been reported for patients with tumours that express enteroglucagon (Gleeson *et al.* 1971); however, a direct effect on the intestinal cells has been difficult to prove. A similar problem confounds the analysis of the action of members of the epidermal growth factor (EGF) family (Dignass *et al.* 1996; Perk *et al.* 1997). Clearly, when EGF is injected into rodents or humans the intestinal mucosal cellularity increases. There is no effect when EGF is delivered through the lumen. There are EGF receptors on the basolateral surface of intestinal cells; however, indirect effects on other organs have made it difficult to determine that EGF is influencing the intestinal cells directly. The presence of transforming growth factor-α (TGFα) throughout the intestinal tract (Cartlidge & Elder 1989) (and the absence of EGF) led to suggestions that TGFα was a normal physiological regulator of intestinal cell production. However, mice lacking TGFα have normal intestinal mucosa (Mann *et al.* 1993). There are a number of EGF-like mitogens in the gut: amphiregulin, heparin binding EGF, cripto, heregulins and betacellulin. Although there is some evidence of tissue preferences (e.g. betacellulin is present in the pancreas (Seno *et al.* 1996) and cripto is associated with the colon (Saeki *et al.* 1992)), we still do not know whether any (or all) of these EGF family members regulate specific aspects of normal intestinal cell production, differentiation, movement or survival.

As well as multiple EGF-like mitogens, there are at least four EGF receptor family members: EGFR, erbB2 (also called neu or her-2), erbB3 and erbB4 (Riese *et al.* 1996). Because these family members heterodimerize, ten different dimers can form and are likely to bind distinctly different EGF-like molecules and to deliver different signalling outcomes when activated. Betacellulin binds to both EGFR and erbB4 homodimers and activates a different pattern of receptor phosphorylation to EGF. Although the three-dimensional structure of the mitogenic domain of cripto is similar to that of EGF, cripto does not compete with EGF for binding to the EGFR (Brandt *et al.* 1994). Clearly, cripto is a mitogen for a number of epithelial cell lines, suggesting that it binds to a distinct cell surface receptor. Its interactions with the EGFR family members and its effects *in vivo* need to be examined in more detail.

Attempts to identify physiological regulators of normal intestinal cell production have been constrained by the difficulties in keeping intestinal stem or progenitor cells alive in culture. As a consequence, cell lines have been used as assay systems for putative normal regulators of colonic growth or for the detection of autocrine growth factors that might contribute the malignant properties of the neoplastic cells. We have used a cell line derived from a human colonic tumour, LIM1215, to characterize the mitogens in mucosal or pituitary extracts (Whitehead *et al.* 1990). Under the appropriate assay conditions, LIM1215 cells respond to EGF or TGFα. We identified an extended form of basic fibroblast growth factor (bFGF), which stimulated LIM1215 proliferation (Nice *et al.* 1991). Although the extracts contain other mitogens, we have not been able to identify these as yet. FGF has been shown to stimulate crypt hyperplasia when applied to the apical surface of the colonic mucosa (Edwards & Whitehead 1995).

Although TGFα is not essential for cell production in the normal intestine, most carcinoma cell lines produce autocrine TGFα. At high cell density this TGFα stimulates proliferation, and either antisense TGFα oligonucleotides and/or EGFR antibodies will inhibit the proliferation of LIM1215 cells in culture (Sizeland & Burgess 1992). These results are typical of dozens of mammalian carcinoma cell lines and suggest that neoplastic, but not normal epithelial progenitor cells, require TGFα for their survival and/or proliferation. Consequently, TGFα antagonists or EGFR inhibitors might prove to be highly effective anti-cancer agents. At low density, LIM1215 cells will not respond to TGFα, but they do proliferate in the presence of conditioned medium (CM) from high-density LIM1215 cells. Fractionation of the CM revealed two other factors important for the stimulation of the low-density LIM1215 cells: a survival factor (low molecular mass) and a spreading factor that acted in conjunction with EGF to induce the spreading of LIM1215 cells. This latter factor appears to be an extracellular matrix component, as it can be applied to the culture dishes before the cells are plated or it can be included in the culture medium at the time of the assay (N. Pouliot and A. W. Burgess, unpublished data). Neither the survival factor nor the spreading factor has been fully characterized as yet, but there is clearly a synergy between the EGF/EGFR system and cell adhesion activation.

Others have also observed apoptotic cell death when adenomas are cultured at low cell density. If cell–cell contact is maintained, the cells are more resistant to death; however, single cell death is rapid and appears to be associated with a decrease in c-myc production (Hague *et al.* 1997). In this culture system, neither *p53* nor *apc* mutations influenced apoptosis when the cell–cell contacts were removed. Insulin-like growth factor and EGF are able to protect the cells from apoptosis, but the role of integrins and ECM components in promoting cell survival have yet to be investigated.

7. APC AND TUMOUR PROGRESSION

Genetic analysis of colonic tumours has identified a common pathway for progression from normal to neoplastic tissue. One of the earliest events is associated with the inactivation of the *apc* gene; this is often followed by the activation of *ras* and the loss of *p53*. In the absence of *apc* defects, *p53* and *ras* appear to induce intestinal hyperplasia, but no adenomas. Thus mutation of the apc protein is an important, common step in the development of adenomatous polyps and, presumably, cancer. apc is a large protein (more than 2800 amino acids) with many distinct domains. However, most cancerous mutations involve premature chain termination and loss of the C-terminal half of the apc protein. Familial *apc* defects are inherited from heterozygous parents; homozygous *apc* mutants are lethal and die very early in the gestation period. The intestinal mucosa of individuals heterozygous at the *apc* locus appears susceptible to further genetic

mutations. Polyps appear in the intestines of mice heterozygous for the apc$^{+/-}$ protein after 40–60 days (Moser *et al.* 1992). In all cases of adenomatous polyp formation in the *min* mice the second *apc* allele is lost. It appears that the truncated *apc* increases the susceptibility for cells to the loss of heterozygosity. Once this occurs, polyp formation follows within a few weeks. Crossing the *min* (*apc*$^{+/-}$) mice with *p53*$^{-/-}$ only leads to a modest increase in intestinal adenomas, although these mice are now more susceptible to pancreatic cancers.

By crossing the *min* (*apc*$^{+/-}$) mice with immortomice (*large T Ag*$_{ts}$) it has been possible to derive colonic cell lines which carry the *apc* mutation (Whitehead & Joseph 1994). The *in vitro* growth kinetics and tumorigenicity of the *min* cell lines (called IMCE) are identical to the parental colonic cell lines (called YAMC). Neither IMCE nor YAMC cells are tumorigenic in nude mice. Interestingly, when IMCE cells are transfected with oncogenic *ras* they become tumorigenic (D'Abaco *et al.* 1996), whereas YAMC cells expressing oncogenic *ras* do not transform to malignant cells. Thus, it appears that despite the modest changes to adherent cell growth, a reduction in *apc* levels or the presence of the mutated (truncated) form of *apc* allows oncogenic *ras* to induce the tumorigenic state. This is manifested by the ability of IMCE-*ras* cells to grow as large colonies in soft agar and by the formation of tumours in nude mice.

Adenoma formation in *min* mice is associated with the loss of the wild-type allele (Luongo *et al.* 1994). This usually occurs at a significant rate after 8–10 weeks, i.e. when polyps can be detected. In humans who carry *apc* mutations, the first polyps do not usually appear until the late teenage years. Does this mean that other genetic changes must also occur before the adenomas can develop? An elegant mouse model of the FAP syndrome has been developed by Tetsuo Noda and his colleagues (Shibata *et al.* 1997) to study the relationship between polyp formation and the loss of wild-type *apc*. A conditional targeting system was designed which allowed the *apc*$^{-/-}$ mutations to be produced specifically in the colorectal mucosa. Lox P sites were inserted into the introns around *Apc* exon 14. Embryonic stem (ES) cells carrying the mutant form of the *Apc* gene (*Apc*^{580S}) were introduced into mouse blastocysts to produce mice carrying the *Apc*^{580S} mutation. The chimeric mice were bred to produce a strain homozygous for *Apc*^{580S}. These mice were normal and showed no propensity for the development of intestinal tumours.

A *Cre* recombinase gene was introduced into an adenoviral vector (AxSRαCre). This vector was able to infect the *Apc*^{580S} cells and to remove exon 14 from the mutant *Apc* gene. These mutant ES cells give rise to mice that develop multiple intestinal neoplasias. A similar Cre adenovirus was introduced into the colorectum of *Apc*^{580S} homozygote mice. Ademonas developed in 80% of the mice infected with the Cre virus. These adenomas appeared within 14 weeks of introducing the virus. The adenomas were highly vascular, but did not immediately invade the submucosa. Some of the *Apc*^{580S} mice infected with the Cre virus were observed for 12 months: none died of their adenomas, but 50% of the tumours had developed into adenocarcinomas that were invading the submucosal layer. It would appear that other mutations must occur before the adenomas progress to a malignant phenotype.

It is interesting to consider whether polyp formation also requires other mutations. The adeno-Cre virus infects 10–20% of the cells within the region of the injection, but relatively few adenomas develop. Either another mutation is required, or only a small proportion of stem cells is infected and/or able to express *Cre* from the serum response promoter. Given the low frequency of stem cells, it is likely that few are infected and that most *apc*$^{-/-}$ stem cells lead directly to polyps. The availability of this system will allow detailed investigations of colonic stem cell biology *in vivo*.

The details of biochemical and biological studies of the apc protein will provide important information on both cell signalling and carcinogenesis. ES cells with an *apc*$^{-/-}$ genotype appear to have adhesion defects during blastocyst development. A major feature of the apc protein involves its ability to bind and stimulate the degradation of β-catenin. There are two series of β-catenin binding sites and the C-terminal set is associated with both binding and degradation of β-catenin. When these sequences are missing in apc, the degradation of β-catenin is impaired and the concentrations increase (Anon 1997; Polakis 1997). Normally, β-catenin is confined to cell–cell junctions, but when the levels increase it is present in both the cytoplasm and the nucleus, where it is known to stimulate gene expression. Although other disruptions occur when the C-terminus of apc is missing, it is interesting that stabilizing mutations at the N-terminus of β-catenin are also associated with colonic tumour formation (Morin *et al.* 1997; Rubinfeld *et al.* 1997).

Analysis of the distribution of apc in epithelial cells shows that there are concentrated clusters of the protein at the inner edge of the migrating regions of the cell membrane. Microtubules appear to be attached to the apc clusters, and if the microtubular system is disrupted (e.g. by nocodozole) the apc clusters disperse and the protein appears to diffuse into the cytoplasm (Nathke *et al.* 1996). When cell migration is stimulated by wound healing or hepatocyte growth factor, again the apc protein accumulates at the migratory edge of the cell. The direct participation of apc in cell migration is consistent with a disruption of cell movement at the top of the colonic crypts. When adenomas form, the cells accumulate rather than moving to the lumenal surface and dying.

It is essential that experimental systems are developed that allow the interactions between the growth factor, cytoskeletal, cadherin and apc systems to be studied in detail. Only then will we be in a position to understand the critical processes during the progression from normal mucosa → dysplastic crypts → adenoma → invasive carcinoma. At present, the emphasis on alterations to proliferative regulators dominates much of cancer aetiology. However, evidence is mounting which suggests more emphasis should be placed on understanding how tumour suppressors or oncogenic mutations influence cell movement and/or location.

REFERENCES

Altmann, G. G. & Enesco, M. 1967 Cell number as a measure of distribution and renewal of epithelial cells in the small intestine of growing and adult rats. *Am. J. Anat.* **121**, 319–336.

Anon 1997 (Editorial) Minireviews, minidogmas and mythinformation. *BioEssays* **19**, 537–539.

Barth, A. I., Pollack, A. L., Altschuler, Y., Mostov, K. E. & Nelson, W. J. 1997 NH2-terminal deletion of beta-catenin results in stable colocalization of mutant beta-catenin with adenomatous polyposis coli protein and altered MDCK cell adhesion. *J. Cell Biol.* **136**, 693–706.

Ben-Ze-ev, A. 1997 Cytoskeletal and adhesion proteins as tumour suppressors. *Curr. Opin. Cell Biol.* **9**, 99–108.

Bos, J. L., Fearon, E. R., Hamilton, S. R., Verlaan-de Vries, M., van Boom, J. H., van der Eb, J. A. & Vogelstein, B. 1987 Prevalence of ras gene mutations in human colorectal cancers. *Nature* **327**, 293–297.

Brandt, R. (and 11 others) 1994 Identification and biological characterization of an epidermal growth factor-related protein: Cripto-1. *J. Biol. Chem.* **25**, 17 320–17 328.

Cartlidge, S. A. & Elder, J. B. 1989 Transforming growth factor alpha and epidermal growth factor levels in normal human gastrointestinal mucosa. *Br. J. Cancer* **60**, 657–660.

Chawengsaksophak, K., James, R., Hammond, V. E., Köntgen, F. & Beck, F. 1997 Homeosis and intestinal tumours in *Cdx2* mutant mice. *Nature* **386**, 84–87.

Cheng, H. & Le Blond, C. P. 1974 Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. IV. Paneth cells. *Am. J. Anat.* **141**, 521–536.

Cohen, S. M., Simon, T. C., Roth, K. A., Birkenmeier, E. H. & Gordon, J. I. 1992 Use of transgenic mice to map *cis*-acting elements in the intestinal fatty acid binding protein gene (*Fabpi*) that control its cell lineage-specific and regional patterns of expression along the duodenal–colonic crypt–villus axes of the gut epithelium. *J. Cell Biol.* **119**, 27–44.

Cohn, S. M., Roth, K. A., Birkenmeier, E. H. & Gordon, J. I. 1991 Temporal and spatial patterns of transgene expression in aging adult mice provide insights about the origins, organization and differentiation of the intestinal epithelium. *Proc. Natn. Acad. Sci. USA* **88**, 1034–1038.

D'Abaco, G. M., Whitehead, R. H. & Burgess, A. W. 1996 Synergy between Apc^{min} and an activated *ras* mutation is sufficient to induce colon carcinomas. *Molec. Cell Biol.* **16**, 884–891.

Dignass, A. U., Stow, J. L. & Babyatsky, M. W. 1996 Acute epithelial injury in the rat small intestine *in vivo* is associated with expanded expression of transforming growth factor alpha and beta. *Gut* **38**, 687–693.

Duprey, P., Chowdhury, K., Dressler, G. R., Balling, R., Simon, D., Guenet, J. L. & Gruss, P. 1988 A mouse gene homologous to the *Drosophila* gene *caudal* is expressed in epithelial cells from the embryonic intestine. *Genes Dev.* **2**, 1647–1654.

Edwards, W. H. & Whitehead, R. H. 1995 Effects of vesicocolic anastomosis on the proliferation of colonic mucosa in a rat model. *Aust. NZ J. Surg.* **65**, 667–671.

Foster, H. M. & Whitehead, R. H. 1990 Intravenous but not intracolonic epidermal growth factor maintains colonocyte proliferation in defunctional rat colorectum. *Gastroenterology* **99**, 1710–1714.

Friedman, E. A., Gillin, S. & Lipkin, M. 1984 12-O- tetradecanoylphorbol-13-acetate stimulation of DNA synthesis in cultured preneoplastic familial polyposis colonic epithelial cells but not in normal colonic epithelial cells. *Cancer Res.* **44**, 4078–4086.

Fritsch, C., Simon-Assmann, P., Kedinger, M. & Evans, G. S. 1997 Cytokines modulate fibroblast phenotype and epithelial–stroma interactions in rat intestine. *Gastroenterology* **112**, 826–838.

Gleeson, M. H., Bloom, S. R., Polak, J. M., Henry, R. & Dowling, R. H. 1971 Endocrine tumours in kidney affecting small bowel structure, motility and absorptive function. *Gut* **12**, 773–782.

Gordon, J. (and 22 others) 1991 Identification and characterization of the familial adenomatous polyposis coli gene. *Cell* **66**, 589–600.

Gordon, J. I., Schmidt, G. M. & Roth, K. A. 1992 Studies of intestinal stem cells using normal, chimeric, and transgenic mice. *FASEB J.* **6**, 3039–3050.

Hague, A., Hicks, D. J., Bracey, T. S. & Paraskeva, C. 1997 Cell–cell contact and specific cytokines inhibit apoptosis of colonic epithelial cells: growth factors protect against c-myc-independent apoptosis. *Br. J. Cancer* **75**, 960–968.

Hauft, S. M. (and 12 others) 1992 Expression of SV-40 T antigen in the small intestinal epithelium of transgenic mice results in proliferative changes in the crypt and re-entry of villus-associated enterocytes into the cell cycle but has no apparent effect on cellular differentiation programs and does not cause neoplastic transformation. *J. Cell Biol.* **117**, 825–839.

Hermiston, M. L. & Gordon, J. I. 1993 Use of transgenic mice to characterize the multipotent intestinal stem cell and to analyze regulation of gene expression in various epithelial cell lineages as a function of their position along the cephalocaudal and crypt-to-villus (or crypt-to-surface epithelial cuff) axes of the gut. *Devl Biol.* **4**, 275–291.

James, R., Erler, T. & Kazenwadel, J. 1994 Structure of the murine homeobox gene *Cdx2*: expression in embryonic and adult intestinal epithelium. *J. Biol. Chem.* **269**, 15 229–15 237.

Jat, P. S., Noble, M. D., Ataliotis, P., Tanaka, Y., Yannoutsos, N., Larsen, L. & Kioussis, D. 1991 Direct derivation of conditionally immortal cell lines from an H-2Kb-tsA58 transgenic mouse. *Proc. Natn. Acad. Sci. USA* **88**, 5096–5100.

Kernéis, S., Bogdanova, A., Kraehenbuhl, J.-P. & Pringault, E. 1997 Conversion by Peyer's Patch lymphocytes of human enterocytes into M cells that transport bacteria. *Science* **277**, 949–952.

Kolodner, R. 1996 Biochemistry and genetics of eukaryotic mismatch repair. *Genes Dev.* **10**, 1433–1442.

Konishi, F., Oku, T. & Hosoya, N. 1984 Hypertrophic effect of unavailable carbohydrate on cecum and colon in rats. *J. Nutr. Sci. Vitaminol.* **30**, 373–379.

Laken, S. J. (and 15 others) 1997 Familial colorectal cancer in Ashkenazim due to a hypermutable tract in APC. *Nat. Genet.* **17**, 79–83.

Le Blond, C. P. & Cheng, H. 1976 Identification of stem cells in the small intestine of the mouse. In *Stem cells of renewing cell populations* (ed. A. B. Cairnie, P. K. Lala & D. G. Osmond), pp. 7–31. New York: Academic.

Liu, B. (and 13 others) 1996 Analysis of mismatch repair genes in hereditary non-polyposis colorectal cancer patients. *Nat. Med.* **2**, 169–174.

Ludviksson, B. R., Ehrhardt, R. O. & Strober, W. 1997 TGF-beta production regulates the development of the 2,4,6-trinitrophenol-conjugated keyhole limpet hemocyanin-induced colonic inflammation in Il-2-deficient mice. *J. Immunol.* **159**, 3622–3628.

Luongo, C., Moser, A. R., Gledhil, S. & Dove, W. F. 1994 Loss of Apc^{+} in intestinal adenomas from Min mice. *Cancer Res.* **54**, 5947–5952.

Mallo, G. V., Rechreche, H., Frigerio, J.-M., Rocha, D., Sweibaum, A., Lacasa, M., Jordan, B. R., Dusetti, N. J., Dagorn, J.-C. & Iovanna, J. L. 1997 Molecular cloning, sequencing and expression of the mRNA encoding human Cdx1 and Cdx2 homeobox. Down-regulation of Cdx1 and Cdx2 mRNA expression during colorectal carcinogenesis. *Int. J. Cancer* **74**, 35–44.

Mann, G. B., Fowler, K. J., Gabriel, A., Nice, E. C., Williams, R. L. & Runn, A. R. 1993 Mice with a null mutation of the TGF alpha gene have abnormal skin architecture, wavy hair, and curly whiskers and often develop corneal inflammation. *Cell* **73**, 249–261.

Marra, G. & Boland, C. R. 1995 Hereditary nonpolyposis colorectal cancer: the syndrome, the genes, and historical perspectives. *J. Natn. Cancer Inst. USA* **87**, 1114–1125.

Mlodzik, M., Fjose, A. & Gehring, W. J. 1985 Isolation of *caudal*, a *Drosophila* homeobox-containing gene with maternal expression whose transcripts form a concentration gradient at the preblastoderm stage. *EMBO J.* **4**, 2961–2969.

Morin, P. J., Sparks, A. B., Korinek, V., Barker, N., Clevers, H., Vogelstein, B. & Kinzler, K. W. 1997 Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. *Science* **275**, 1787–1790.

Moser, A. M., Dove, W. F., Roth, K. A. & Gordon, J. I. 1992 The *Min* (multiple intestinal neoplasia) mutation: its effect on gut epithelial cell differentiation and interaction with a modifier system. *J. Cell Biol.* **116**, 1517–1526.

Moyer, M. P. & Aust, J. B. 1984 Human colon cells: culture and *in vitro* transformation. *Science* **224**, 1445–1447.

Munemitsu, S., Albert, I., Souza, B., Rubinfeld, B. & Polakis, P. 1995 Regulation of intracellular beta-catenin levels by the adenomatous polyposis coli (APC) tumor-suppressor protein. *Proc. Natn. Acad. Sci. USA* **92**, 3046–3050.

Nathke, I. S., Adams, C. L., Polakis, P., Sellin, J. H. & Nelson, W. J. 1996 The adenomatous polyposis coli tumor suppressor protein localizes to plasma membrane sites involved in active cell migration. *J. Cell Biol.* **134**, 165–179.

Nice, E. C., Fabri, L., Whitehead, R. H., James, R., Simpson, R. J. & Burgess, A. W. 1991 The major colonic cell mitogen extractable from colonic mucosa is an N terminally extended form of basic fibroblast growth factor. *J. Biol. Chem.* **266**, 14 425–14 430.

Nielsen, O. H., Koppen, T., Rugider, N., Horn, T., Eriksen, J. & Kirman, I. 1996 Involvement of interleukin-4 and -10 in inflammatory bowel disease. *Digest. Dis. Sci.* **41**, 1786–1793.

Otto, W. R. (and 11 others) 1996 Effects of pancreatic spasmolytic polypeptide (PSP) on epithelial cell function. *Eur. J. Biochem.* **235**, 64–72.

Park, H. S., Goodlad, R. A., Ahnen, D. J., Winnett, A., Sasieni, P., Lee, C. Y. & Wright, N. A. 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**, 843–852.

Polakis, P. 1997 The adenomatous polyposis coli (APC) tumor suppressor. *Biochim. Biophys. Acta* **1332**, F127–F147.

Potten, C. S. 1996 Protection of the small intestinal clonogenic stem cells from radiation-induced damage by pretreatment with interleukin 11 also increases murine survival time. *Stem Cells* **14**, 452–459.

Potten, C. S. 1997 Epithelial cell growth and differentiation. II. Intestinal apoptosis. *Am. J. Physiol.* **273**, G253–G257.

Prieto, R. M., Ferrer, M., Fe, J. M., Rayo, J. M. & Tur, J. A. 1994 Morphological adaptive changes of small intestinal tract regions due to pregnancy and lactation in rats. *Ann. Nutr. Metab.* **38**, 295–300.

Riese, D. J. II, Bermingham, Y., van Raaij, T. M., Buckley, S., Plowman, G. D. & Stern, D. F. 1996 Betacellulin activates the epidermal growth factor receptor and erB-4, and induces cellular response patterns distinct from those stimulated by epidermal growth factor or neuregulin-β. *Oncogene* **12**, 345–353.

Rubinfeld, B., Robbins, P., El-Gamil, M., Albert, I., Porfiri, E. & Polakis, P. 1997 Stabilization of beta-catenin by genetic defects in melanoma cell lines. *Science* **275**, 1790–1792.

Saeki, T., Stromberg, K., Qi, C. F., Gullick, W. J., Tahara, E., Normanno, N., Ciardiello, F., Kenney, N., Johnson, G. R. & Salomon, D. S. 1992 Differential immunohistochemical detection of amphiregulin and cripto in human normal colon and colorectal tumours. *Cancer Res.* **52**, 3467–3473.

Schmidt, G. H., Wilkinson, M. M. & Ponder, B. A. J. 1985 Cell migration pathway in the intestinal epithelium: an *in situ* marker system using mouse aggregation chimeras. *Cell* **40**, 425–429.

Seno, M., Tada, H., Kosaka, M., Sasada, R., Igarishi, K., Shing, Y., Folkman, J., Ueda, M. & Yamada, H. 1996 Human beta-cellulin, a member of the EGF family dominantly expressed in pancreas and small intestine, is fully active in a monomeric form. *Growth Factors* **13**, 181–191.

Shibata, H. (and 15 others) 1997 Rapid colorectal ademona formation initiated by conditional targeting of the *Apc* gene. *Science* **278**, 120–123.

Sizeland, A. M. & Burgess, A. W. 1992 Anti-sense transforming growth factor α oligonucleotides inhibit autocrine stimulated proliferation of a colon carcinoma cell line. *Molec. Biol. Cell* **3**, 1235–1243.

Subramanian, V., Meyer, B. I. & Gruss, P. 1995 Disruption of the murine homeobox gene *Cdx1* affects axial skeletal identities by alerting the mesodermal expression domains of *Hox* genes. *Cell* **83**, 641–653.

Suh, E. & Traber, P. G. 1996 An intestine-specific homeobox gene regulates proliferation and differentiation. *Molec. Cell Biol.* **16**, 619–625.

Takahashi, K., Suzuki, K. & Tsukatani, Y. 1997 Induction of tyrosine phosphorylation and association of beta-catenin with EGF receptor upon tryptic digestion of quiescent cells at confluence. *Oncogene* **15**, 71–78.

Van de Water, N., Jeevaratnam, P., Browett, P. J., Stewart, S. M., Lane, M. R. & Jass, J. R. 1994 Direct mutational analysis in a family with hereditary non-polyposis colorectal cancer. *Aust. NZ J. Med.* **24**, 682–686.

Whitehead, R. H. & Joseph, J. L. 1994 Derivation of conditionally immortalised cell lines containing the min mutation from the normal colonic mucosa and other tissues of an 'immortomouse'/Min hybrid. *Epithelial Cell Biol.* **3**, 119–125.

Whitehead, R. H. & Van Eeden, P. M. 1991 A method for the prolonged culture of colonic epithelial calls. *J. Tissue Culture Meth.* **13**, 103–106.

Whitehead, R. H., Nice, E. C., Lloyd, C. J. & Burgess, A. W. 1990 Detection of colonic growth factors using a human colonic carcinoma cell line (LIM 1215). *Int. J. Cancer* **46**, 858–863.

Whitehead, R. H., Van Eeden, P. E., Noble, M. D., Ataliotis, P. & Jat, P. S. 1993 Establishment of conditionally immortalized epithelial cell lines from both colon and small intestine of adult *H-2K^b*-tsA58 transgenic mice. *Proc. Natn. Acad. Sci. USA* **90**, 587–591.

Wright, N. A. & Irwin, M. 1982 The kinetics of villus cell populations in the mouse small intestine: normal villi—the steady state requirement. *Cell Tissue Kinet.* **15**, 595–609.

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