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Review

Wnt, stem cells and cancer in the intestine

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The intestinal epithelium is a self-renewing tissue which represents a unique model for studying interconnected cellular processes such as proliferation, differentiation, cell migration and carcinogenesis. Although the stem cells of the intestine have not yet been physically characterized or isolated, data over the past decade have strongly implicated the Wnt/β-catenin signalling pathway in their maintenance and progression to cancer. This review will (i) describe the distinctive features of the intestinal epithelium in relation to stem-cell function, (ii) illustrate the major genetic alterations that can lead to cancer, and (iii) show how Wnt/β-catenin signalling controls homoeostasis in this tissue.

Introduction: self-renewal, epithelial lineages and stem cells of the intestinal epithelium

The mammalian intestinal tube, which comprises from head to tail the small intestine (divided into duodenum, jejunum, and ileum) and the large intestine (or colon), is lined by a single layer of epithelium cells (or mucosa). The epithelium of the small intestine is organized into two morphologically and functionally distinct compartments: flask-shaped submucosal invaginations known as crypts of Lieberkühn, and finger-shaped luminal protrusions termed villi (Figure 1). The villi surround the orifice of each crypt. This general architecture increases the exchange interface with the intestinal lumen, enabling adapted absorption of nutrients. In the colonic epithelium, the crypts are larger than in the small intestine. There are no villi in the colon; rather, a flat surface epithelium faces the lumen. The crypt is the proliferative compartment of the intestinal epithelium. It is monoclonal and maintained by multipotent stem cells. The villus represents the differentiated compartment, which is polyclonal as its cells derive from several crypts (Figure 1).

Much of our current understanding of intestinal self-renewal is grounded in classical studies performed by Potten and colleagues using the adult mouse small intestine as a model system (Potten and Loeffler, 1990); their conclusions are readily transposable to the colonic epithelium. These studies showed that every villus (covered with approx. 7000 epithelial cells in the duodenum and approx. 2000 cells in the ileum) is surrounded by several crypts (approx. 15), each containing from 350 to 550 cells (Wright and Irwin, 1982). There are about one million crypts in an adult mouse small intestine. Slowly dividing multipotent stem cells (with a cell cycle period of 24 h) are postulated to be anchored at the base of each crypt (more precisely in the lower third). In turn, stem cells give rise to an intermediate cell population of immediate descendants referred to as transit amplifying (TA) cells. The TA population undergoes rapid proliferation (approx. 150 cells dividing every 12 h; thus generating 300 new cells per crypt every day) and expands into a population of non-proliferating daughter cells which accumulates in the medium third of the crypt (Potten and Loeffler, 1990). Next these daughter cells gradually differentiate into four principal epithelial lineages: absorptive cells or enterocytes (90% of the villus-associated cells),

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Key words: colorectal cancer (CRC), intestinal epithelium, Wnt/β-catenin signalling.

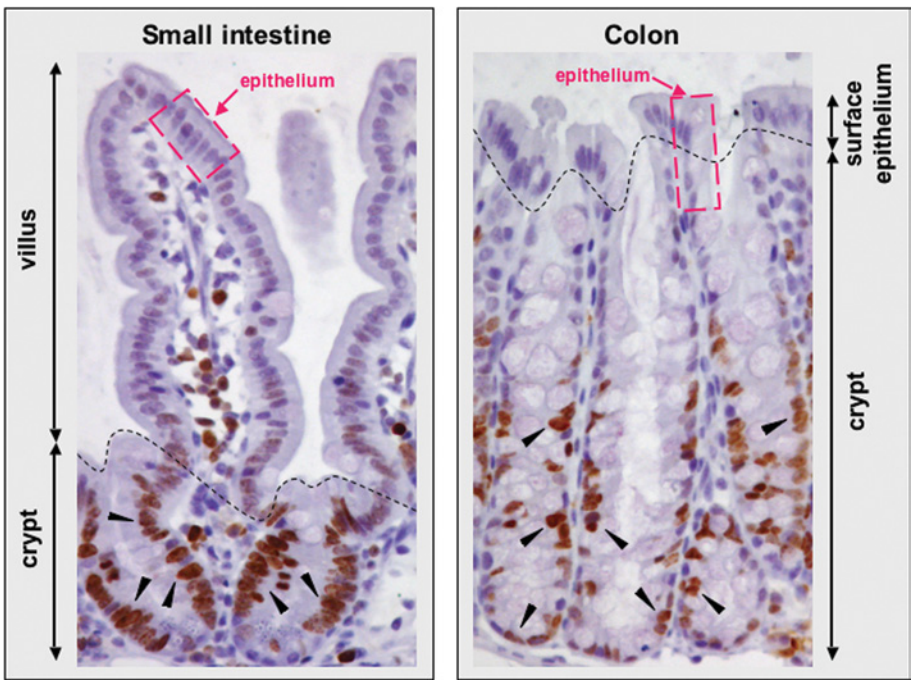
Abbreviations used: APC, adenomatous polyposis coli; CRC, colorectal cancer; CKI, casein kinase I; Dkk-1, Dickkopf-1; Dsh, Dishevelled; FAP, familial adenomatous polyposis; Fz, frizzled; GSK3β, glycogen synthase kinase 3β; LEF, lymphoid enhancing factor; LRP5/6, low-density lipoprotein receptor-related proteins 5 and 6; Min, multiple intestinal neoplasia; TA, transit amplifying; TCF, T-cell factor; Wnt, Wingless-type MMTV integration site family.

Monoclonal: Derived from a single starting cell.

Polyclonal: Derived from genetically distinct cells.

Figure 1 | Morphology of the intestinal and the colonic mucosa of an adult mouse

The small intestine epithelium is organized into crypts and villi (delimited by dashed lines). In the colon, the crypts are larger, and there are no villi but a flat surface epithelium. The villus and surface epithelium of the colon consist of differentiated epithelial cells, while the crypts are proliferative units as shown by expression of the cell cycle marker Ki-67 (arrows).

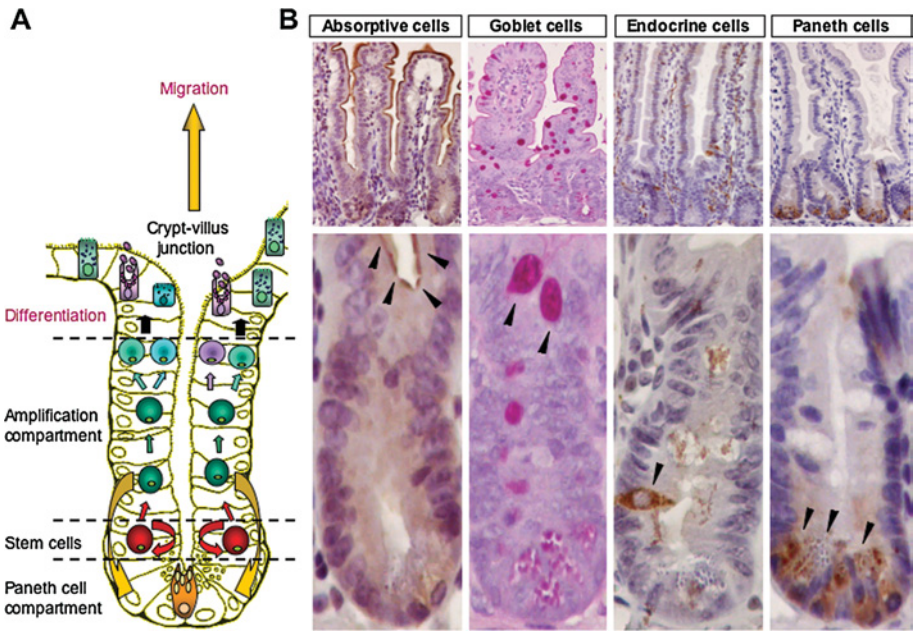


mucus-producing goblet cells (providing protection to the epithelium), enteroendocrine cells [secreting hormones such as 5-hydroxytryptamine (serotonin) or secretin (Hocker and Wiedenmann, 1998)], and Paneth cells [secreting antimicrobial peptides and enzymes such as cryptidins, defensins and lysozyme (Porter et al., 2002)]. The differentiation process is completed while cells migrate upwards (Figure 2). Characteristically, Paneth cells migrate to the bottom of the crypt where they reside for about 20 days (Bry et al., 1994; Garabedian et al., 1997), while the three other differentiated cell types migrate upward from the upper third of the crypt to the apex of the villus. At the top of the villus, the differentiated epithelial cells become apoptotic and are exfoliated into the intestinal lumen (Hall et al., 1994). The entire intestinal epithelium is renewed every 3 days in the mouse (5 days in man) (Wright and Irwin, 1982), which corresponds to the time needed for a differentiated cell to cover the distance between the base and the top of a villus, where about 1400 cells are exfoliated every day (Potten and Loeffler, 1990). This linked cell replenishment and migration process along the crypt/villus axis guarantees a continuous and rapid renewal of the intestinal mucosa, ensuring maintenance of the barrier and absorptive functions, which defines the term homoeostasis in this tissue.

A lack of specific markers for intestinal stem cells has impeded accurate identification. Recently *musashi-1* has been tentatively considered as a putative candidate (Kayahara et al., 2003; Potten et al., 2003). Nevertheless, studies employing wild-type (Marshman et al., 2002), chimeric (Winton and Ponder, 1990; Bjerknes and Cheng, 1999) and transgenic mice (Gordon et al., 1992) have allowed inferences to be drawn regarding the location of crypt stem cells, their numbers, kinetics of proliferation, and mode of lineage commitment. One successful approach has been to label the stem cells with [^{3}H]thymidine, following irradiation or during neonatal life, to identify a population of cells that permanently resides in crypts (i.e. that retains radiolabel indefinitely) (Potten et al., 2002). Label retention is considered a hallmark of stem cells: protective mechanisms against DNA-replication-induced errors ensure that the stem cells selectively retain old (i.e.

Figure 2 | Self-renewal along the crypt-villus axis of the adult mouse small intestine

(**A**) Schematic representation of the crypt illustrates stem cells residing at the crypt bottom immediately above the Paneth cell compartment (courtesy of E. Sancho). The (red) slowly-dividing immature stem cells divide asymmetrically to yield a (green) population of TA cells and replacement stem cells (red arrows). TA cells gradually commit to differentiation during bipolar migration (yellow arrows). The fully differentiated epithelial cells are represented in green, purple and blue at the crypt-villus junction, and in orange at the base of the crypt. (**B**) Chemical and immunohistochemical detection of the four principal cell lineages of the small intestine (arrowheads): villus-associated absorptive cells (alkaline phosphatase), Goblet mucus-secreting cells (periodic acid/Schiff), enteroendocrine cells (synaptophysin), and Paneth cells (lysozyme) (reproduced from Pinto et al., 2003, with permission). The upper small panels show the entire crypt-villus axis while the lower large panels show magnifications of the crypts.



[^{3}H]thymidine-labelled) DNA template strands while newly synthesized strands labelled with a different marker (bromodeoxyuridine) segregate to the TA cells and, therefore, are not retained (Potten et al., 2002). In this way the position of stem cells near the base of crypts was revealed. As an alternative approach, experiments with mouse embryo aggregation chimeras, combined with the use of cell lineage markers (such as the polymorphic *Dbl-1* membrane receptor gene), allowed investigators to demonstrate the clonal nature of crypt populations (Ponder et al., 1985).

According to these studies, stem cells in intestinal crypts of adult mice are characterized by:

A monoclonal origin. Crypts which form during neonatal life are polyclonal and only acquire a monoclonal identity after a poorly understood process of crypt clonal 'purification' (Schmidt et al., 1988).

The retention of an undifferentiated phenotype.

Ability to divide asymmetrically. Thus one daughter remains a stem cell, and the other becomes a TA progenitor, progressively committing to differentiated lineages.

Multipotency. Stem cells are able to produce all epithelial cell types populating the mucosa.

A high proliferative potential throughout life. During the lifespan of a laboratory mouse, stem cells divide about a thousand times (Potten and Loeffler, 1990).

The ability to repopulate entire intestinal crypts upon injury (e.g. irradiation). This occurs by clonal

Aggregation chimera: An organism composed of cells of two different genotypes (or two parental types) as a result of embryo aggregation.

expansion, thus re-establishing the entire stem cell lineage (from a single surviving stem cell, the post-irradiation process of regeneration takes 2 to 3 days).

Anchorage at the crypt base (see Figure 2). This does not strictly imply physical linkage but rather a notion of permanent residency in a particular region of the crypt, referred to as a niche conferring 'stemness' (i.e. the niche determines the stem potential of the cells within). Thus, only crypt cells residing in the niche or its immediate vicinity receive instructions to proliferate; proliferative potential in the intestine is not cell-autonomous (Hermiston et al., 1996). Observations based on [^{3}H]thymidine incorporation suggest that 4–6 stem cells might be located at the fourth or fifth cell position from the bottom of the crypt immediately above the Paneth cell compartment (Potten, 1998). More recent studies suggest that stem cells may also be intermingled with Paneth cells at the bottom-most positions of the crypts (Kayahara et al., 2003; Potten et al., 2003; Stappenbeck et al., 2003). In the colon, stem cells are located at the crypt base (Potten, 1992).

A question which still remains subject to debate is whether a crypt is kept populated from a single stem cell only, or via contributions from multiple stem cells (Loeffler et al., 1993). The 'unitarian' hypothesis of Cheng and Leblond (1974), states that all differentiated cell lineages from the intestinal epithelium share a common stem cell origin. Certainly, if one takes a snap shot of a crypt population, then it appears monoclonal in origin; however, as alluded to above, [^{3}H]thymidine incorporation suggests that the crypt niche contains several (4 to 6) stem cells morphologically indistinguishable from each other. Multiple stem cells could still generate the appearance of clonality if all were derived from the same embryonic progenitor (i.e. were themselves clonal). Alternatively, one stem cell, from a population of clonally distinct stem cells, may come to dominate a crypt, with the caveat that supremacy might be lost in time to a competing stem cell. A recent analysis of inheritance of methylated DNA within individual crypts suggests that a heterogeneous stem cell population goes through a selection process (Yatabe et al., 2001).

Genetic alterations leading to cancer: the role of APC (adenomatous polyposis coli) and β-catenin

As mentioned above, the normal intestinal epithelium is capable of establishing a very tight balance between proliferation, differentiation, migration and cell death, to ensure its perpetual renewal. Tumorigenesis occurs when these mechanisms become uncoupled resulting in hyperproliferation at the cost of differentiation. The main pathological manifestation of this in man is found in patients suffering from colorectal cancers (CRCs).

CRCs have been extensively studied in terms of histopathology and cytogenetics (Morson, 1984): to summarize, tumorigenesis occurs incrementally. The process initiates in the colon or the rectum with epithelial hyperplasia that becomes increasingly dysplastic resulting in aberrant crypt foci (Cheng and Lai, 2003). These progress to benign tumours termed adenomas or adenomatous polyps, that can eventually develop into malignant tumour stages termed carcinomas. Controversy still smoulders over the origin of the cells from which tumours arise. Some propose that tumours arise from neoplastically transformed stem cells at the base of the crypt, progress to adenomas which next expand by crypt fission to the surface epithelium in a 'bottom-up' fashion (Preston et al., 2003). Alternatively, others suggest that adenomas arise from dysplastic cells located on the surface epithelium, next expand by lateral migration, and grow down into the crypt through a 'top-down' mechanism (Shih et al., 2001).

CRCs are amongst the most common causes of cancer death (after smoking-related cancers) in Western countries, and by the age of 70 years approximately half of this population will have developed one or more adenomas. Approx. 15% of CRCs occur in the context of a familial (i.e. inherited) predisposition, e.g. familial adenomatous polyposis (FAP), whereas the remainder (85%) arise sporadically. FAP individuals develop hundreds to thousands of adenomatous polyps in the colon and rectum at an early age, of which a subset invariably progresses to malignant cancers if not surgically removed (Lynch and de la Chapelle, 2003). Germline (loss-of-function) mutations in the *APC* gene were found to be the essential

Hyperplasia: An abnormal increase in cell proliferation.

genetic event responsible for FAP. Subsequently, somatic mutations in the same gene were associated with the majority (up to 85%) of sporadic CRCs and benign intestinal neoplasms. In 90% of these cases, mutation results in truncation of the APC protein (Fodde and Khan, 1995). The APC protein comprises 2843 amino acids and encompasses numerous domains like conserved regions, such as the Armadillo repeats, and regions that interact with at least 10 protein partners, including tubulin, the microtubule-associated protein EB-1, the human discs large protein, and β-catenin (Fodde et al., 2001). The most severe and common inactivating mutations in the *APC* gene are located between codons 450 and 1578, and represent the earliest genetic alterations so far detected in the genesis of CRCs (Powell et al., 1992; Polakis, 1997). Thus, mutation of the *APC* gene is linked to the initiation of intestinal tumour formation and might therefore represent a prerequisite for entry into this process. *APC* is considered a classic tumour suppressor gene as both alleles must be inactivated for loss of tumour suppressing activity. For FAP and CRC patients, the molecular mechanisms underlying the lack of APC protein can be a second truncating mutation or, more typically, an allelic loss of the second allele (termed loss-of-heterozygosity). The second APC mutation represents the limiting step for tumour initiation (Ichii et al., 1992; Levy et al., 1994; Rowan et al., 2000).

It is worth noting that heterozygous activating mutations in the gene encoding β-catenin are found in approx. 10% of the remaining cases of sporadic CRCs. *APC* and β-catenin mutations are mutually exclusive (Sparks et al., 1998), and both result in the stabilization and accumulation of the β-catenin protein in the nucleus of a cell (see below for further discussion of the relevance of this event to cellular transformation). Nuclear β-catenin is detectable in even the smallest neoplastic lesions of the colonic adenoma-carcinoma sequence, such as aberrant crypt foci (Hao et al., 2001). However, small adenomas with β-catenin mutations do not appear to be as likely to progress to larger adenomas and invasive carcinomas as observed for *APC* mutations, and β-catenin mutations are only rarely seen in invasive cancers (Inomata et al., 1996; Ilyas et al., 1997; Samowitz et al., 1999). This suggests that *APC* and β-catenin mutations are not entirely equivalent. Taken together, these studies indicate that, as for the inactivating mutations in the *APC* gene, activating mutations in the β-catenin gene can be an early, perhaps initiating, event in colorectal tumorigenesis, and define β-catenin as an oncogene (Peifer, 1997). Moreover, it appears that any mutation leading to stabilized nuclear β-catenin is sufficient to trigger neoplastic transformation in colonic mucosa (Brabletz et al., 2002).

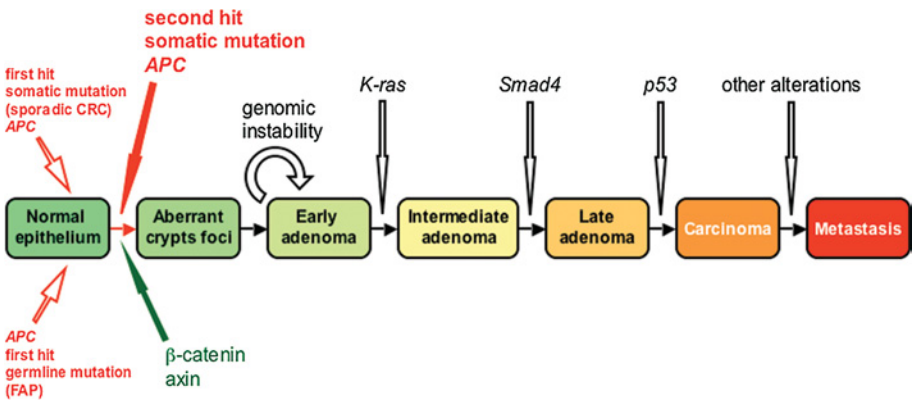
Although initiation of colorectal tumorigenesis is principally associated with mutations in the *APC* gene and, to a lesser extent, in the β-catenin-encoding gene, other mutational alterations (affecting oncogenes like *K-ras* and tumour suppressor genes like *p53*) and epigenetic events (conferring genomic instability) are needed for stepwise progression (Fodde et al., 2001). Several studies lead to the conclusion that tumour progression in the intestine (as in fact elsewhere) is propelled by the selection of specific genetic (and epigenetic) alterations that accumulate in strict order. Indeed, while mutation events are stochastic, the order in which they accumulate is non-random, supporting strongly the argument that only certain mutations confer a selective advantage at a given stage of a tumour's natural history, allowing clones of tumour cells to pass through a series of 'bottlenecks'. Fearon and Vogelstein (1990) were the first to propose such a sequential model for CRC genesis (Figure 3).

The link between loss of APC function and CRC formation has been confirmed in experimental mouse models, where chemical mutagenesis or genetic engineering has resulted in ablation of APC. The first and still most widely used model is the ethylnitrosourea-induced Min mouse (for multiple intestinal neoplasia) (Moser et al., 1990; Su et al., 1992). *Min* is a mutant allele of murine *APC*, carrying a nonsense mutation at codon 850 and stably expressing truncated APC protein. Mice homozygous for this mutation die *in utero* approx. 8 days post coitus (Moser et al., 1995). Heterozygous *Min* mice, which rarely live longer than 3–4 months, phenotypically reflect the intestinal manifestations observed in FAP

Epigenetic events: Events not associated with genetic mutations.

Neoplasia: The presence or formation of new, abnormal growth of tissue.

Figure 3 | Genetic model of colorectal carcinogenesis [adapted from Fearon and Vogelstein (1990)]
Mutations in *APC*, β-catenin or axin genes are required for tumour initiation. Subsequent progression towards malignancy is accompanied by genomic instability and sequential mutations in K-*ras*, *Smad4*, *p53* genes, as well as in other unknown genes.



patients: they develop numerous (up to 100) adenomas (adenomatous polyps) depending on the genetic background, predominantly in the small intestine rather than the colon. All of these adenomas harbour allelic loss of the wild-type *APC* allele (complete deletion of the chromosome 18 carrying *APC*), and only the *APC* allele carrying *Min* remains in the intestinal adenomas (Levy et al., 1994; Luongo et al., 1994). These findings suggest strongly that the induction of the tumour process in mouse requires a bi-allelic inactivation of the gene, as observed in human patients developing CRCs (Powell et al., 1992). Moreover, Min mouse neoplasms are each composed of a complex population of undifferentiated and differentiated cells (all four epithelial lineages) which implies that these adenomas derive from the mutation of a single multipotent stem cell (Moser et al., 1992).

To elucidate APC's mode of action, additional *APC*-deficient mouse models have been generated by conventional gene targeting. To date, homologous recombination has been used to engineer four recombinant mouse lines that express APC protein truncated at codons 1638, 716, 1309 and 474 respectively (Fodde et al., 1994; Oshima et al., 1995; Quesada et al., 1998; Sasai et al., 2000). As for the Min mouse, homozygosity for all the above APC mutations invariably results in early embryonic lethality. Mice heterozygous for these mutations share phenotypes with the Min mouse, with some variability concerning the lifespan of the animals (some strains can live up to 16 months) and the numbers of adenomas throughout the intestinal tract. Again, genotyping even the smallest tumours reveals loss of the wild-type *APC* allele due to the complete deletion of chromosome 18, as for Min mice, but surprisingly none of the tumours carry K-*ras* or *p53* mutations, as previously observed in man (Smits et al., 1997; Oshima et al., 1997). All these findings confirm finally that inactivation of the *APC* gene is a requisite step for initiating intestinal tumorigenesis in mouse as in man, but also emphasize that tumour growth and progression might be linked to different mutational pathways in these two species. Furthermore, a morphogenetic analysis of adenomatous polyps in the $APC^{\Delta 716}$ knockout mice illustrates that the initial event in the formation of an adenoma is an outpocketing of proliferating cells from a single crypt, which then extends into the interior of an adjoining villus (Oshima et al., 1997).

However, all the aforementioned APC mouse models present certain limitations regarding the study of CRC progression. Firstly, the lifespan of the animals is frequently too short to permit significant accumulation of somatic mutations, thus reducing the likelihood of observing advanced malignancies. Secondly, the tumours preferentially arise in the small intestine rather the colon, contrary to FAP and sporadic CRCs in man. To circumvent these limitations, as well as the embryonic lethality of *APC* deficiency, a conditional gene targeting system has been used which directs *APC* bi-allelic inactivation specifically to the colorectal epithelium of adult mice, resulting in APC truncation at codon 580 (Shibata

et al., 1997). These mice develop colorectal adenomas within 4 weeks, again implying that inactivation of the *APC* gene is sufficient to drive polyp formation.

Similarly, transgenic mice inducibly expressing a constitutively active form of β-catenin in the small intestine exhibit intestinal adenomas resembling those of $APC^{\Delta 716}$ knockout mice (Harada et al., 1999). It was also shown previously, as in FAP individuals, that nuclear β-catenin accumulates in adenomas from Min and $APC^{\Delta 716}$ mice (Sheng et al., 1998); therefore, stabilization of β-catenin protein and its subsequent nuclear accumulation are involved in inducing intestinal tumours in mouse.

The Wnt/β-catenin signalling pathway controls the homoeostasis of the intestinal epithelium

In 1993, the groups of Paul Polakis and of Bert Vogelstein unveiled the physical interaction existing between β-catenin, a known adhesion and signalling protein, and the tumour suppressor APC (Rubinfeld et al., 1993; Su et al., 1993). Since this seminal discovery, the attention given to β-catenin (and to its related signalling pathway) in the field of colorectal carcinogenesis, and consequently in intestinal development and homoeostasis, has not abated (Giles et al., 2003; Sancho et al., 2003, 2004).

β-Catenin and APC proteins are components of the Wnt/β-catenin signalling pathway, also termed the canonical Wnt signalling pathway. This pathway is highly conserved among all metazoa. Indeed, genetic approaches principally in *Drosophila* and *Xenopus*, and later in mouse, zebrafish and nematode (Pires-daSilva and Sommer, 2003), combined with biochemical approaches, have underpinned our current detailed understanding. The pathway involves a complex relay of protein interactions that transmits a signal from the extracellular space, through the plasma membrane, amplifies the signal in the cytosol and ultimately reprogrammes gene expression in the nucleus. Various levels of positive and negative regulation have evolved to fine tune the response.

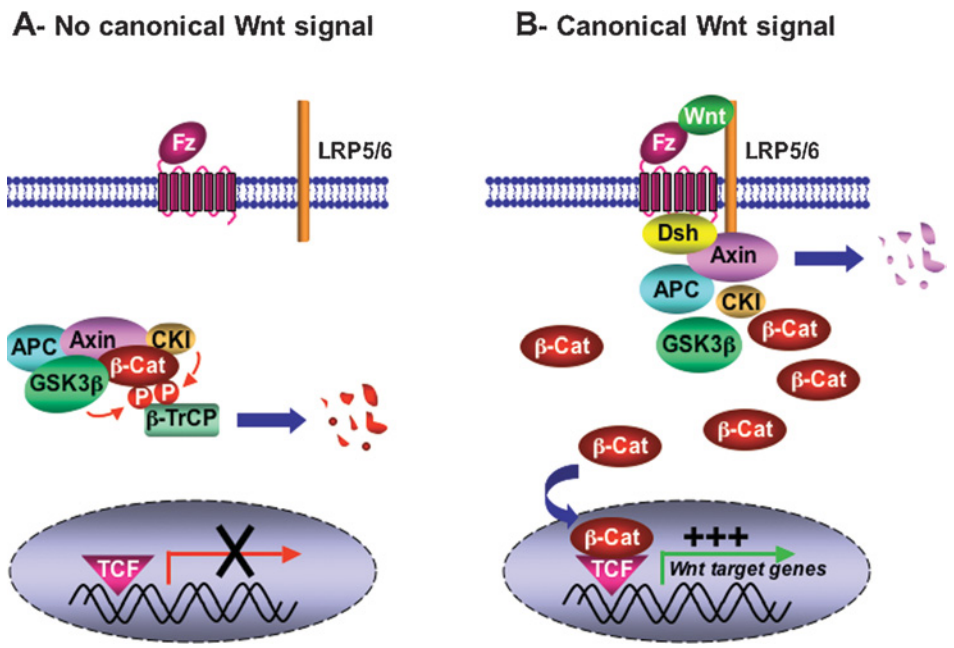
Other reviews have provided detailed descriptions of canonical Wnt pathway components (Giles et al., 2003; He et al., 2004). In the following passage, the crux of the pathway (which is most immediately relevant to cancer) is presented (Figure 4). The pathway is triggered by binding of secreted Wnt glycoproteins to the frizzled (Fz) seven-span transmembrane receptors together with the low-density lipoprotein receptor-related proteins 5 and 6 (LRP5/6) single-span transmembrane co-receptors, members of the low-density lipoprotein receptor related family. Various secreted factors, such as Cerberus and secreted frizzled-related proteins, bind to Wnt ligands and block the interaction with Fz proteins. Dickkopf-1 (Dkk-1) secreted protein potently and specifically antagonizes canonical Wnt action by blocking access to the LRP5/6 co-receptors. Dkk-1 can induce LRP5/6 internalization, and its subsequent removal from the plasma membrane, by cooperating with Kremen 1/2 transmembrane proteins (Kawano and Kypta, 2003).

Intracellularly, the activation of canonical Wnt signalling leads to stabilization of β-catenin. In the absence of Wnt, β-catenin is phosphorylated by casein kinase I (CKI) at Ser-45. This in turn enables glycogen synthase kinase 3β (GSK3β) to phosphorylate the remaining regulatory sites: Thr-41, Ser-37 and Ser-33 (Polakis, 1997). Phosphorylation of these N-terminal residues elicits ubiquitination of β-catenin by the β-TrCP–E3-ligase complex, and its subsequent degradation by the proteasome. Phosphorylation of β-catenin occurs in a multiprotein complex containing the scaffold protein Axin, APC and Diversin (which recruits CKI to the complex). In the presence of Wnt, Dishevelled (Dsh) blocks β-catenin degradation possibly by recruiting GBP/Frat-1, which displaces GSK3β from Axin (Huelsken and Behrens, 2002; van Es et al., 2003). Moreover, Dsh might also participate in the docking of Axin at the cytoplasmic domain of LRP5/6 co-receptors, thus causing functional inhibition or degradation of Axin (He et al., 2004). In response to canonical Wnt signalling, Axin is no longer able to perform its central role of scaffold protein; this leads to stabilization of β-catenin.

Stabilized β-catenin accumulates in the cytoplasm and is translocated into the nucleus by a poorly understood mechanism. There, β-catenin interacts with transcription factors of the T-cell factor/lymphoid enhancing factor (TCF/LEF) family to form a bipartite complex (Behrens et al., 1996; Molenaar et al., 1996): TCF/LEF factors provide the DNA-binding specificity, while β-catenin provides transactivation domains. This bipartite complex leads then to the transcription of Wnt target genes, such as c-*myc* oncogene (He et al., 1998), well known for its role in cell

Figure 4 | Simplified overview of the canonical Wnt signalling pathway (adapted from He et al., 2003)

(**A**) In the absence of Wnt ligand, β-catenin is sequestered in a multiprotein degradation complex containing the scaffold protein Axin, the tumour suppressor gene product APC, as well as the kinases CKI and GSK3β, among others. Upon sequential phosphorylation, β-catenin is ubiquitinated by the β-TrCP–E3-ligase complex and subsequently degraded by the proteasome machinery. There is no transcription of *Wnt* target genes. (**B**) Wnt ligand associates with Fz and LRP5/6 co-receptors. This in turn can lead to translocation of Axin (and perhaps the whole multiprotein complex) to the plasma membrane through direct interaction with LRP5/6 and Dsh/Fz. Translocation results in Axin degradation and/or dissociation of the multiprotein complex. GSK3β also might be displaced from this complex through Dsh action. β-catenin is then released from the multiprotein complex, accumulates in the cytoplasm in a non-phosphorylated form, and subsequently translocates into the nucleus where by association with TCF/LEF factors it promotes transcription of Wnt target genes.



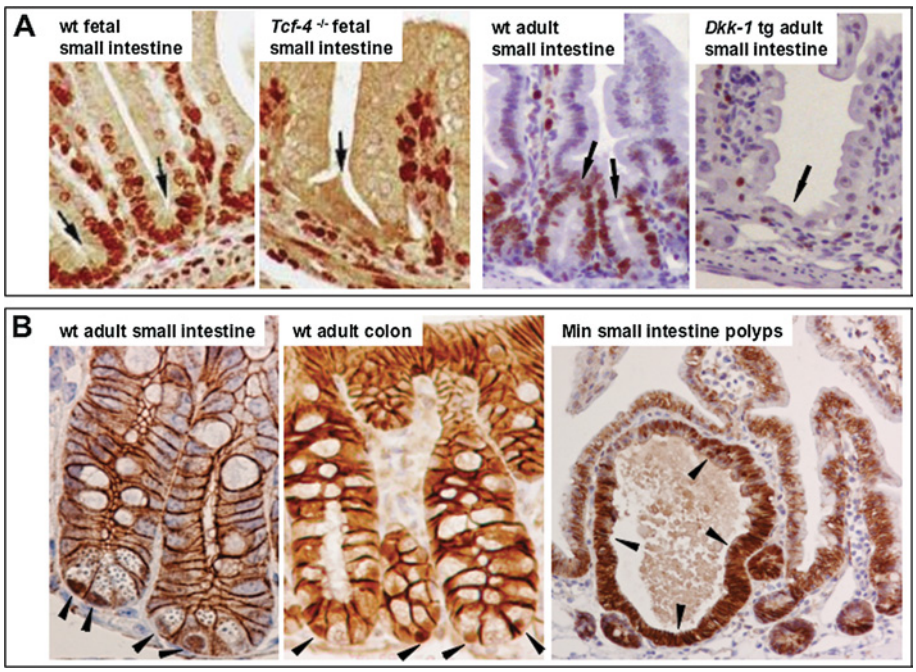
proliferation and oncogenesis. β-Catenin participates in transactivation by recruiting two further transcriptional co-factors, CBP/p300 acetyltransferase and the chromatin-remodelling protein Brg-1, to TCF target gene promoters (Hurlstone and Clevers, 2002). Transcriptional activation is also enhanced by the interaction of β-catenin with Legless/Bcl9 and its binding partner Pygopus, although the mechanism of their action is not presently understood (Townsley et al., 2004). When the source of Wnt is removed, β-catenin is exported from the nucleus by APC and subsequently degraded (Bienz, 2002).

Nuclear accumulation of β-catenin is therefore a hallmark of activated canonical Wnt signalling. It is also clear from the above description that APC and Axin are critical for β-catenin degradation. It is then not surprising that CRCs from humans or from mouse models, which carry inactivating mutations in *APC* (that selectively disable binding to β-catenin), or activating mutations in β-catenin (that remove the N-terminus regulatory phosphorylation sites), display nuclear accumulation of β-catenin (Giles et al., 2003), nor that human CRC-derived cell lines have greatly enhanced levels of TCF/β-catenin-mediated transcription (Korinek et al., 1997; Morin et al., 1997). Inactivating mutations in axin have also been found in CRC cell lines and tumours (Webster et al., 2000; Jin et al., 2003). Taken together, it appears that any mutational event stabilizing nuclear β-catenin, which has the effect of rendering the canonical Wnt signalling pathway permanently active, leads to tumour initiation in the gut.

The next requirement for a better understanding of the molecular basis of gut tumorigenesis lay in defining the downstream genetic programme promoted by activation of canonical Wnt signalling.

Figure 5 | Canonical Wnt signalling in the mouse intestinal crypt

(**A**) Inactivation of the canonical Wnt pathway leads to depletion of proliferative compartments in *Tcf-4*$^{-/-}$ fetal mice (reproduced from Korinek et al., 1998, with permission) and loss of crypts in *Dkk-1* transgenic adult mice (reproduced from Pinto et al., 2003, with permission), as shown by expression of the cell cycle marker Ki-67 (arrowheads). (**B**) Nuclear β-catenin (arrowheads), the hallmark of activated canonical Wnt signalling, accumulates at the bottom of normal adult crypts in small intestine and colon, as well as in small intestine polyps of Min mice (reproduced from Batlle et al., 2002; van de Wetering et al., 2002, with permission). wt, wild-type.



With this aim in mind, our laboratory performed microarray analysis of human colon carcinoma cells where the endogenous TCF/β-catenin complex is inhibited by the inducible expression of a dominant-negative TCF protein (van de Wetering et al., 2002). This analysis revealed that TCF-4/β-catenin target genes in CRC cells are also expressed in normal proliferating cells of the crypt, while repressed genes are expressed in normal villus-associated differentiated cells. Thus TCF-4/β-catenin activity constitutes a crucial proliferation/differentiation switch along the crypt-villus axis of the intestinal epithelium. This is mirrored by the pattern of nuclear accumulation of β-catenin, which decreases in a gradient from the base of the crypt to the interface with the villus (Batlle et al., 2002; van de Wetering et al., 2002). By inference, constitutive signalling through the canonical Wnt pathway imposes a crypt progenitor phenotype on colon carcinoma cells. Moreover, some of these target genes, belonging to the EphB/Ephrin-B family, are implicated in restricting the intermingling of proliferating and differentiated cells in the crypt, revealing an independent role for the canonical Wnt signalling pathway in controlling cell positioning within crypts (Batlle et al., 2002). Interestingly, the data described above and obtained from cultured human CRC cells have been confirmed in mouse models. Thus, the same genetic program is switched off in *Tcf-4*-deficient and *Dkk-1* transgenic mice (Korinek et al., 1998; Pinto et al., 2003), and ectopically activated in a mouse model with inducible APC loss (Sansom et al., 2004), strongly suggesting that transcription of Wnt target genes constitutes the primary transforming event in CRC.

These *in vivo* models also illuminate the role played by canonical Wnt signalling in driving proliferation in normal intestinal epithelium through development and adulthood (Figure 5). The transgenic expression of Dkk-1 in the intestine of adult mice results in greatly reduced epithelial proliferation,

coincident with the loss of crypts, a phenotype largely reminiscent of the depletion of intestinal proliferative compartments observed in fetal *Tcf-4* knockout mice (Korinek et al., 1998; Pinto et al., 2003). Conversely, inducible inactivation of *APC* in the intestine of adult mice leads to enlarged crypt-like regions after 5 days (Sansom et al., 2004). As a further point of interest, the phenotype of *Dkk-1* transgenic mice confirmed the presence of a Wnt source in the crypt (previously undetermined), since Dkk-1 acts at the membrane level to block Wnt ligation (Zorn, 2001).

The studies described in this review clearly demonstrate a fundamental role for endogenous Wnt signalling in regulating intestinal homoeostasis. Aberrant signalling is also shown to be the primary cause of intestinal tumorigenesis. We propose that the most consistent explanation for these effects of Wnt signalling is that this pathway directly influences stem cell behaviour. In the context of neoplasia, initiated cells appear to be trapped in a primitive progenitor (possibly stem cell) mode of existence. The immediate challenges for the field appear to be defining the source of Wnt in the crypt and physically characterizing and isolating intestinal stem cells.

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*Articles of special interest

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