Epigenetic Control of Gut Development

In addition to the hardwired program of gut development discussed above, it is clear that environmental influences, such as diet (*27*) and the microbial community of the gut (*28*), play important roles in the differentiation and function of the gut. For example, in mammals, the changing dietary input in the pre- and postnatal periods imposes different demands on the intestine and influences its morphology, enzymatic diversity, and transport. In addition, the diet contains substances such as polyamines and epidermal growth factor that do not play a nutritional role but appear to directly stimulate the growth of the intestinal epithelium (*27*). Similarly, *C. elegans* worms adjust the size of their pharynx, intestine, and intestinal microvilli depending on their nutritional state (*29*), and such plasticity is also seen in the willow ptarmigan, in which the winter consumption of fibrous food is associated with relatively long caecae. Investigations into the cellular and molecular underpinnings of such phenomena are just beginning.

Molecular Basis of Human Gut Malformations

In addition to the few disorders mentioned earlier in this article, naturally occurring human gut malformations are not rare; in fact, they are a major cause of perinatal morbidity and mortality. Also, because the endoderm extends along most of the AP axis of the embryo, it plays critical roles in many developmental events. For example, the anterior or pharyngeal endoderm contributes substantially to craniofacial structures and lies in close proximity to the developing heart, and thus defects in this tissue are likely to play a major role in the formation of these organs. It is clear, however, that although we can make educated guesses about which signaling pathway is likely to be defective in a select subset of gut malformations (*30*), much work remains to be done to establish molecular causality. In this regard, human genetic studies of gut malformations have certainly lagged behind those of the cardiovascular system, for instance. And as has already been illustrated regarding other organ systems such as the cardiovascular system, more subtle mutations in key developmental genes are likely to predispose one to gut disease and/or functional defects. Closer interactions between clinical and basic scientists interested in the gastrointestinal tract will greatly accelerate the pace of discovery in these areas.

Summary

Compared to readily accessible organs such as the limb, or to organs such as the heart and pancreas that are the focus of resourceful charities, the gut has been left behind. However, it is clear from the few vignettes presented here that the many fascinating developmental, evolutionary, and medical aspects of the gut will continue to attract much attention and generate pertinent information.

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32. I would like to apologize to the many authors whose research papers relevant to this review I was not able to reference because of space constraints. Gastrointestinal tract work in my lab is funded by NIH (National Institute of Diabetes and Digestive and Kidney Diseases), the Juvenile Diabetes Research Foundation, and the Packard Foundation. Thanks to M. Santoro (University of California, San Franscisco) for contributing the figure.

10.1126/science.1108709

REVIEW

Self-Renewal and Cancer of the Gut: Two Sides of a Coin

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The intestinal epithelium follows the paradigms of stem cell biology established for other self-renewing tissues. With a unique topology, it constitutes a two-dimensional structure folded into valleys and hills: the proliferative crypts and the differentiated villi. Its unprecedented self-renewal rate appears reflected in a high susceptibility to malignant transformation. The molecular mechanisms that control homeostatic self-renewal and those that underlie colorectal cancer are remarkably symmetrical. Here, we discuss the biology of the intestinal epithelium, emphasizing the roles played by Wnt, bone morphogenic protein, and Notch signaling cascades in epithelial self-renewal and cancer.

The intestinal epithelium represents an exquisite model for the study of stem cell biology and lineage specification. It is likely the simplest mammalian study model for tissue self-renewal, yet it features multipotent stem cells, transit-amplifying compartments, several binary lineage decisions, as well as programmed cell death. The function of the mammalian intestinal epithelium poses formidable challenges that evolution has met, often very elegantly. Like the epidermis of the skin, the intestinal epithelium constitutes a barrier between the body and the outside world (of which the intestinal lumen may be considered a particularly threatening version). Whereas the epi-

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dermis consists of multiple layers of cells and cell remnants that are harnessed by robust internal meshes of keratins, the intestinal epithelium consists of a single layer of fragile epithelial cells. These cells digest food and absorb the resulting mix of biological building blocks while keeping indigestible bulk and associated microflora inside the lumen. For this, the intestinal epithelium has distributed divergent biological tasks over only four differentiated cell types.

The basic tissue architecture of the mouse intestine is established during mid- to late gestation (*1*). At first, a pseudostratified intestinal epithelium of endodermal origin proliferates vigorously. In mice, conversion of this pseudostratified epithelium into a single-layered epithelium occurs around embryonic day 14.5 (E14.5) in a wave that progresses from stomach to colon. The epithelium of the small intestine forms protrusions, the prospective villi, and cell cycling becomes restricted to shallow pockets positioned between these villi. Simultaneously, the surrounding splanchnic mesoderm differentiates into smooth muscle and connective tissue. These processes are completed at E18.5. Around the third week of life, the proliferative pockets invade into the submucosa to form mature crypts of Lieberkühn (*2*).

The epithelium of the adult small intestine forms a contiguous two-dimensional sheet (Fig. 1). New cells are added in the crypts and removed by apoptosis upon reaching the villus tips a few days later (*3*). Stem cells and Paneth cells at the crypt bottom escape this flow (*4*). Although intestinal stem cells cannot currently be studied ex vivo and no unique in vivo markers are available, they can be labeled with ^{3}H-thymidine or bromodeoxyuridine during early postnatal life or after irradiation (*5*). Long-term label retention and the study of mouse chimeras (*6*, *7*) and postinjury regeneration have allowed an operational definition of stem cell characteristics. Stem cells of the small intestine occupy position +4, the fourth cell position counting from the crypt bottom [positions 1 to 3 being taken by the Paneth cells (Fig. 1)]. In the colon, Paneth cells are absent, and the stem cells reside directly at the crypt bottom (*8*). The epithelial stem cells self-renew throughout life, cycling infrequently to produce vigorously proliferating transit-amplifying cells that fill the remainder of the crypts. Transit-amplifying cells have a very limited self-renewal capacity; after three to four cell divisions, their offspring inevitably differentiates into one of the mature cell lineages. Whereas the shallow neonatal intervillus pockets are polyclonal, all cells in an adult crypt derive from one stem cell (*7*). Committed progenitors differentiate upon reaching the crypt-villus junction and migrate in colinear bands toward the tips of the villi. Thus, each villus receives inputs from several crypts and is essentially polyclonal (*7*, *9*). The surface epithelium of the colon lacks villi: Proliferative cells occupy the bottom two-thirds of colonic crypts, whereas differentiated cells constitute the top third and the surface epithelium. The crypt architecture of the small intestine is compared to that of the colon in Fig. 2.

A sheath of specialized fibroblasts is directly apposed to the epithelial crypt cells, separated only by the basal lamina (*10*). These so-called myo-epithelial fibroblasts form a syncytium that extends along the intestinal tract. It is believed that these cells are critical to the establishment of the crypt niche, which as discussed above harbors a few stem cells as well as a much larger number of transit-amplifying cells and Paneth cells. The crypt epithelium shapes the underlying myo-epithelial meshwork through secreted Hedgehog signals (*11*). Very little is known about putative reverse signals emanating from the myo-epithelial fibroblasts. Moreover, although a specialized stem cell niche is likely to exist within crypts, such a niche has not been identified yet by either morphological or molecular criteria.

Two main lineages of differentiated cell types are discerned within the intestinal epithelium: the enterocyte or absorbtive lineage and the secretory lineage. The latter lineage encompasses goblet cells, the enteroendocrine lineage, and Paneth cells (Fig. 1). Enterocytes are abundant in the small intestine, secreting hydrolases and absorbing nutrients. Goblet cells, secreting protective mucins, increase in numbers from duodenum to the colon. Enteroendocrine lineage cells can be further subdivided on the basis of the hormones they secrete, e.g., serotonine, substance P, or secretin (*12*, *13*), and these represent a small proportion (less than 1%) of all cells. Paneth cells occur only in the small intestine and reside at the crypt bottom. Paneth cells secrete antimicrobial agents such as cryptdins and defensins and lysozyme to control the microbial content of the intestine (*14*, *15*).

Colorectal Cancer (CRC)

About 50% of the Western population develops an adenomatous polyp by the age of 70. Some of these will progress to cancer, and the lifetime cancer risk is estimated to be 5% (*16*). From a molecular-genetic perspective, CRC is likely the best-understood solid malignancy, which relates to the accessibility of the tumors and the fact that different stages of the same malignancy can coexist within one patient. Histopathological and molecular analyses have led to the definition of the seminal adenoma-carcinoma sequence of tumor progression (*17*). The model integrates the notions that (i) colorectal tumors result from mutational activation of oncogenes combined with the inactivation of tumor-suppressor genes, (ii) multiple gene mutations

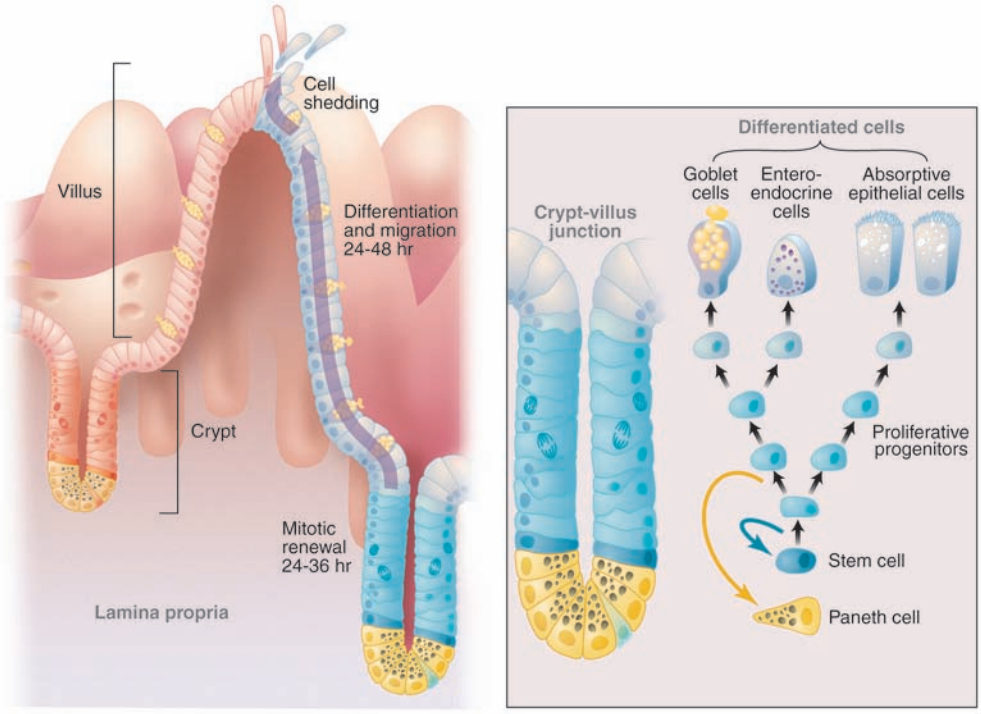

Fig. 1. The anatomy of the small intestinal epithelium. The epithelium is shaped into crypts and villi (**left**). The lineage scheme (**right**) depicts the stem cell, the transit-amplifying cells, and the two differentiated branches. The right branch constitutes the enterocyte lineage; the left is the secretory lineage. Relative positions along the crypt-villus axis correspond to the schematic graph of the crypt in the center.

are required to produce malignancies, and (iii) genetic alterations may occur in a preferred sequence, yet the accumulation of changes rather than their chronologic order determines histopathological and clinical characteristics of the colorectal tumor.

In the adenoma-carcinoma sequence, the earliest identifiable lesion is an aberrant crypt focus, a small dysplastic lesion in the colonic epithelium. Two different models propose the origin and growth of dysplastic aberrant crypt foci. Vogelstein and colleagues suggested a top-down morphogenesis model in which mutant cells at the surface epithelium of the colon spread laterally and downward to form new crypts (*18*). This view was challenged with an alternative model proposing that adenomas grow initially in a bottom-up pattern (*19*). Aberrant crypt foci expand over time to form macroscopically visible adenomatous polyps. The transition from benign (adenoma) to malignant growth (carcinoma) is believed to be progressive. Adenomas first advance to the carcinoma in situ stage. Overtly invasive carcinomas often represent the first clinical presentation of colorectal tumors. Little is known about the genetic alterations and precise mechanisms driving progression from early stage in situ carcinomas through the successive clinically defined stages of regional invasion and distant metastasis.

Our current molecular insights into CRC rely heavily on studies on hereditary forms of this disease. Five to 10% of CRC cases are inherited in an autosomal-dominant fashion (*20*). Hereditary cancer syndromes are often divided into two categories on the basis of absence or presence of polyposis, best exemplified by the two major forms of hereditary colorectal cancer: hereditary nonpolyposis CRC and familial adenomatous polyposis (FAP). The hallmark of tumors in hereditary nonpolyposis CRC is instability of microsatellites (repeats of short DNA sequences) (*21*, *22*). Indeed, hereditary nonpolyposis CRC can be caused by mutations in mismatch repair genes, such as *MSH2* and *MLH1* (*23*, *24*), collectively termed the caretakers of genome integrity (*25*). It is believed that these DNA repair defects lead to mutations in cancer-causing genes such as adenomatous polyposis coli (APC) (*26*), which in turn transform cells. For further reading on the subject of microsatellite instability, we refer the reader to the above references; the polyposis syndrome FAP is more extensively discussed below.

Wnt signaling maintains crypt progenitor compartments. A crypt and its associated villus can be viewed as the smallest self-renewing unit from which the epithelium is built. Position along the crypt-villus axis defines the various stages in the life of an epithelial cell (Fig. 1). Current evidence indicates that the Wnt cascade is the dominant force in controlling cell fate along the crypt-villus axis. Wnt genes encode secreted signaling proteins and are found in the genomes of all animals. Signaling is initiated when Wnt ligands engage a complex consisting of a serpentine receptor of the frizzled family and a member of the low-density lipid receptor family, Lrp5 or Lrp6 (*27*) (Fig. 3A). The key molecule in the cascade is a cytoplasmic protein termed β-catenin, whose stability is regulated by the so-called destruction complex. When Wnt receptors are not engaged, two scaffolding proteins in the destruction complex, the tumor suppressors adenomatous polyposis coli (APC) and axin, bind newly synthesized β-catenin. CKI and GSK3, two kinases residing in the destruction complex, then sequentially phosphorylate a set of conserved Ser and Thr residues in the N terminus of β-catenin. The resulting phosphorylated footprint recruits a β-TrCP–containing E3 ubiquitin ligase, which targets β-catenin for proteasomal degradation. When Wnts bind the receptor complex, the activity of the destruction complex is inhibited. As a consequence, β-catenin accumulates and binds to nuclear DNA binding proteins of the Tcf/Lef family (*27*). In the absence of a Wnt signal, Tcf/Lef proteins repress target genes through association with corepressors such as groucho. The interaction with β-catenin transiently converts Tcf/Lef factors into transcriptional activators, a process that also involves legless/Bcl9 and pygopus, two additional partner proteins of β-catenin. In sum, the canonical pathway translates a Wnt signal into transient transcription of a Tcf/Lef target gene program (*27*).

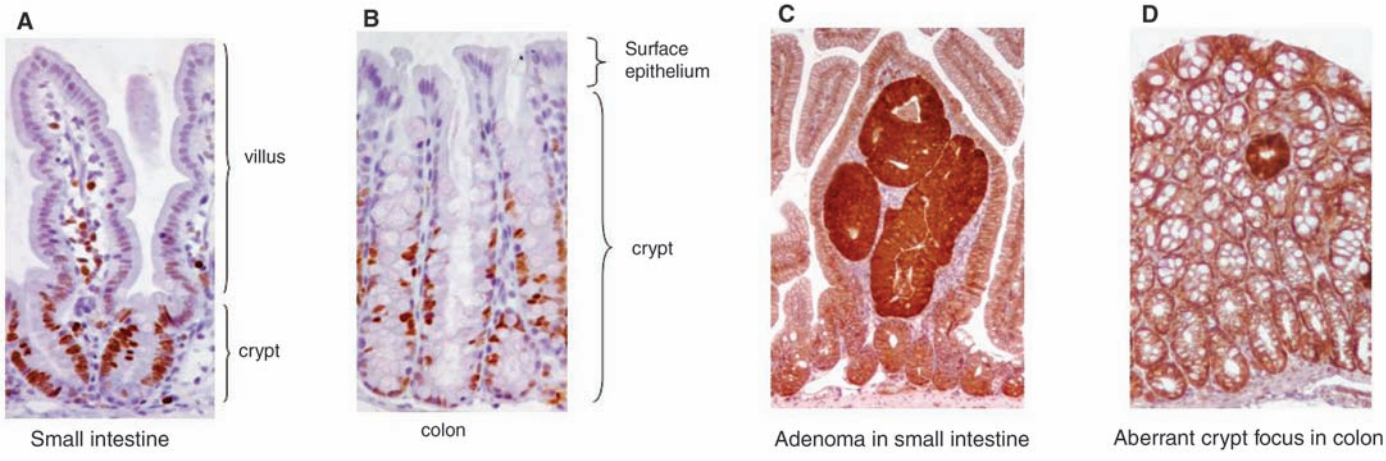

Fig. 2. Comparison of normal epithelium and adenomas in murine small intestine and colon. (**A**) Small intestinal crypt and villus. (**B**) Colonic crypt and surface epithelium. Proliferative cells are stained for the cell cycle marker Ki67 (brown nuclei) in (A) and (B). (**C**) An adenoma residing inside a villus of the small intestine of a *min* mouse. (**D**) A small aberrant crypt focus in the colon of a *min* mouse. (C) and (D) are stained for β-catenin. Note the presence of β-catenin (in brown) in the cell boundaries of all nondiseased epithelial cells and the accumulation of β-catenin throughout the cells in the adenoma and aberrant crypt focus.

Wnt signaling is intimately linked with the biology of the intestinal epithelium. This first became clear when APC, originally identified as an intestinal tumor suppressor gene, was recognized as a key negative regulator of the cascade (see below). Later evidence implied a role for the Wnt cascade in controlling epithelial physiology. Nuclear β-catenin, the indicator of active Wnt signaling, was observed in crypts (*28*). In Tcf4$^{-/-}$ neonatal mice, the proliferative compartment was entirely absent, indicating that Wnt is required for maintenance of progenitors (*29*). Concordantly, inhibition of Wnt receptors by transgenic dickkopf-1 leads to loss of proliferative crypts in adult mice (*30*). Expression analysis of all mouse Wnt genes has revealed that at least four are expressed by the crypt cells proper, whereas no Wnt expression was detected in the directly

apposed mesenchyme, implying the existence of an autocrine mechanism (*31*).

Wnt Signaling, FAP, and CRC

FAP patients carry in their colon numerous florid adenomatous polyps, which are defined as benign epithelial neoplasia (polyps) built from glandular elements (adenomatous) (*32*). Because of the polyp burden, FAP patients invariably develop colorectal cancer around the age of 40. FAP patients carry one mutant copy of the tumor suppressor gene *adenomatous polyposis coli* (*APC*) in their genome (*33*, *34*). *APC* is not only mutated in the hereditary FAP syndrome, but somatic *APC* mutations also occur in the vast majority of sporadic adenomas and adenocarcinomas (*35*–*37*). The earliest identifiable lesion in the adenoma-carcinoma sequence (*17*), the aberrant crypt focus, already bears detectable *APC* mutations (*38*), implying that loss of APC function represents the initiating event in sporadic CRC. Taking these observations together, *APC* appears to be the key gene, the gatekeeper, in the molecular pathogenesis of most sporadic and hereditary forms of CRC.

The *APC* mutations in intestinal polyps and adenocarcinomas typically truncate the protein, suggesting a critical tumor-suppressive role for the C-terminal domain. This domain binds to and causes the degradation of cytoplasmic β-catenin (*39*–*42*). Indeed, stable β-catenin accumulates to high concentrations in the cytoplasm and nucleus of epithelial cells transformed upon loss of APC function. The discovery of the partnership of β-catenin and Tcf proteins as the ultimate effectors of Wnt signaling (*43*, *44*) led to the realization that the increased amounts of β-catenin in *APC* mutant cancer cells inappropriately activate transcriptional activity of the intestinal Tcf family member TCF4 (*45*). The recognition of nuclear β-catenin/Tcf4 complexes as the key effectors in cancer initiation was confirmed by rare mutations in other WNT pathway components, i.e., in β-catenin (*46*, *47*) and in axin2/conductin (*48*). Ultimately, malignant transformation of intestinal epithelial cells is thus caused by the inappropriate, constitutive transcription of Tcf target genes.

On the basis of the determination of the Tcf4 target gene program in human CRC cell lines, we have proposed the following scenario (*49*). In crypts, Wnt signaling drives the formation of β-catenin/Tcf4 complexes, thus imposing a proliferative phenotype onto crypt epithelial cells. The strict compartmentalization along the crypt-villus axis itself is controlled at least in part by the Wnt source at the crypt bottom and translated through Wnt-controlled expression of the EphB sorting receptors (*28*). The absence of Wnt signaling in the villus compartment results in rapid cell cycle arrest and differentiation. Thus, Tcf4 constitutes the dominant switch between the proliferative progenitor and the differentiated epithelial cell. At all stages of CRC this switch is permanently on, because Tcf4 is constitutively activated by mutations in the Wnt cascade. This overrides the physiological signals leading to cell cycle arrest and induces the slow formation of long-lived adenomatous polyps.

Several *Apc*-mutant mouse models have been generated (*37*). *Min* (*multiple intestinal neoplasia*) mice carry a nonsense mutation at codon 850 of *Apc* (*50*). Heterozygous Apc^{min} mice develop as many as 100 intestinal tumors, which have typically inactivated the second *Apc* allele. Although most *Min* tumors develop in the small intestine rather than the colon, these mice are used extensively as animal models for FAP and CRC. Because homozygosity for *Apc* mutations invariably results in embryonic lethality (*37*), conditional *Apc* mutant mice have also been generated. In these mice, focal deletion of both alleles of *Apc* rapidly induced colorectal adenomas, proving that loss of APC is the only requirement for adenoma formation (*51*). When deletion of the *Apc* gene was induced throughout the adult intestine, all epithelial cells displayed a proliferative crypt progenitor phenotype within days (*52*). DNA array analysis revealed upregulation of a Tcf4-driven crypt progenitor program, resembling that of human CRC cell lines (*49*). The induced transgenic expression of an activated β-catenin gene has independently confirmed that persistent Wnt signaling is sufficient to induce adenomas (*53*). Taken together, mutational activation of the Wnt signaling pathway now appears strongly to represent the key initiating event of intestinal tumorigenesis in human and mouse.

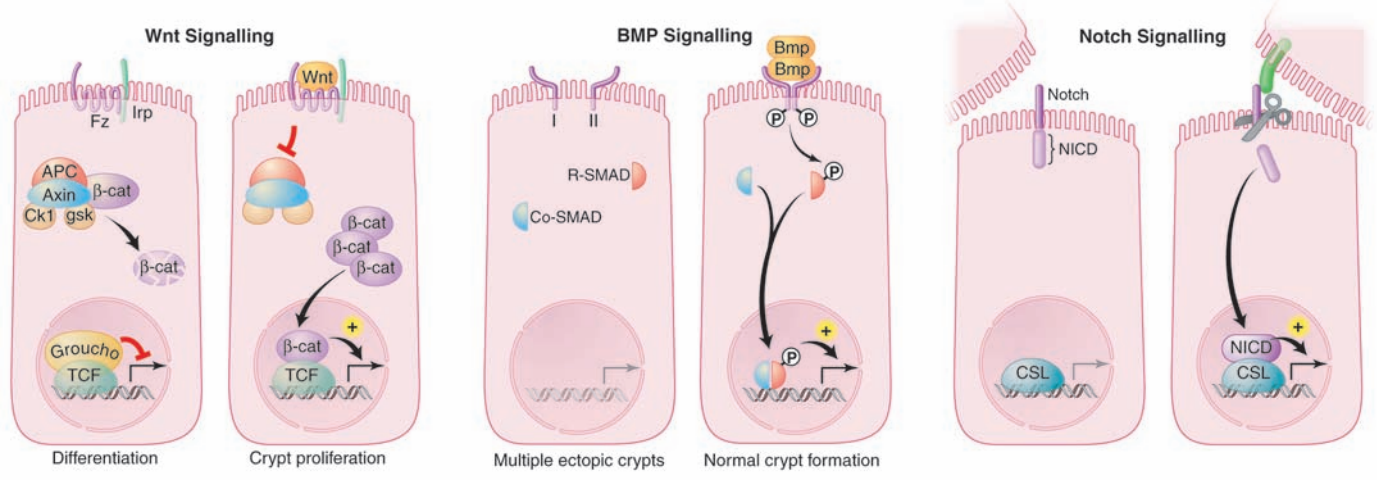

Fig. 3. Wnt, BMP, and Notch pathways control target gene transcription. **(Left)** Wnt-responsive cells carry a receptor complex consiting of a frizzled seven-transmembrane receptor (Fz) and Lrp5 or Lrp6. In the absence of secreted Wnt factor (left), the destruction complex (APC, axin, and the kinases CK1 and GSK3 β) induces degradation of cytoplasmic β-catenin. Tcf complexed to corepressors such as groucho represses specific Wnt target genes. Receptor engagement (right) blocks the destruction complex; β-catenin accumulates and binds to Tcf in the nucleus to activate transcription of Wnt target genes. **(Center)** Type I and type II BMP receptors are not complexed in the absence of signal. Secreted BMP factors bring the two receptors together, ultimately leading to the phosphorylation of R-SMADs, their association with co-SMAD, translocation to the nucleus, and subsequent activation of BMP target genes in the nucleus. **(Right)** When Notch receptor meets its cell-bound ligand (jagged or delta), sequential proteolytic steps lead to the release of its intracellular domain (NICD), which travels to the nucleus, where it complexes with the transcription factor CSL to activate Notch target gene transcription.

Bone Marrow Protein Signaling, Juvenile Polyposis Syndrome, and the Induction of Crypt Formation

Not all polyposis syndromes are manifested as adenomatous lesions. Hamartomatous polyposis syndromes are characterized by an overgrowth of tissues native to the organ area, which are usually of mesenchymal origin (*54*). The juvenile polyposis syndrome (JPS) is one

such autosomal hamartoma syndrome, and in this condition patients bear between 50 and 200 polyps, usually in the rectosigmoid region of the gut. Polyps display gross chronic inflammation and contain cystic spaces lined by a columnar epithelium and surrounded by abundant stroma (*55*). JPS patients have an increased risk of developing gastrointestinal polyps and colorectal cancer. Inactivating mutations in *Smad4* (*56*) and *bone morphogenic protein* (*BMP*) *receptor type 1A* (*57*, *58*) account for up to 50% of JPS cases, implying that loss of intestinal BMP signaling is the primary cause of JPS. This in turn suggests a role for the BMP pathway in epithelial homeostasis.

BMP Signaling and JPS

BMPs form a large subgroup of signaling cytokines within the TGF-β superfamily. Originally identified as molecules inducing cartilage and bone formation, they now rival the Wnts as general regulators of development (*59*). TGF-β superfamily ligands signal through a common mechanism by bringing together type I and type II serine–threonine kinase receptors. This results in the phosphorylation of the type I receptor by the type II receptor and activation of the intracellular SMAD transcriptional regulators (*59*). These fall into three classes: receptor-regulated SMADs (R-SMADs), common SMAD (co-SMADs), and inhibitory SMADs (I-SMADs). Upon receptor activation, R-SMADs become phosphorylated by type I receptors, resulting in association with the co-SMAD SMAD4 and in translocation of the complex to the nucleus (Fig. 3B). Once in the nucleus, the SMAD complex can interact with either co-activators or co-repressors of transcription to control target gene expression. BMP signaling is mediated through SMAD1, 5, and 8 (*59*).

BMPs are expressed in the stroma of villi, whereas phosphorylated SMAD1, 5, and 8, indicative of active BMP signaling, are observed in the nuclei of the differentiated villus epithelial cells. This implies that constitutive BMP signaling occurs from the villus mesenchyme to the epithelium (*60*). Most mutations in BMP pathway components are embryonic-lethal in mice, precluding a genetic assessment of a role for the BMP pathway in the gut (*2*). However, inhibition of BMP signaling in the villus through the transgenic expression of the secreted BMP inhibitor noggin resulted in a JPS-like phenotype, including the late development of adenomatous polyps (*60*). The stromal BMP signal apparently confines the postnatal formation of crypts to regions directly opposed to the intestinal wall. When BMP signaling is blocked, crypts appear de novo anywhere in the epithelium, leading to the characteristic JPS histology and ultimately to adenomas. A similar phenotype was observed in mice mutant for the *Bmpr1a* gene (*61*). Vogelstein has coined the term ''landscaper'' for the molecular defect in JPS (*25*), and although much remains to be learned about crypt initiation, it appears appropriate that the JPS-landscaper genes control spatial positioning of crypts in the intestinal epithelium.

Notch/bHLH Signaling

The Notch cascade represents a third developmental signaling pathway that plays a controlling role in intestinal homeostasis. *Notch* genes encode evolutionarily conserved single-transmembrane receptors, which regulate many cell fate decisions and differentiation processes. Mice and humans possess four Notch receptors, and these can be activated by transmembrane ligands of the delta and jagged families expressed on neighboring cells (*62*). This initiates a cascade of proteolytic cleavages of the receptor close to and within the transmembrane domain. Ultimately, the intramembrane γ-secretase protease liberates NICD (Notch intracellular domain), which translocates to the nucleus and engages the transcription factor CSL {CBF1/RBPjk in vertebrates, suppressor of hairless [Su(H)] in *Drosophila*, and Lag-1 in *C. elegans*}, thereby activating transcription (*62*). Some of the best-characterized Notch target genes encode members of the hairy-enhancer of split (Hes) family of transcriptional repressors, which are nuclear basic helix-loop-helix (bHLH) proteins. These HES family members regulate downstream genes, involved (at least in *Drosophila*) in the segregation of neuronal and epidermal lineages (*63*, *64*).

Notch/bHLH Signaling in the Intestinal Epithelium

Genetic evidence implies a role for several putative Notch target genes in the control of intestinal homeostasis. For example, *Hes1* mutant mice die perinatally because of severe neurological defects, whereas the intestines of *Hes1*$^{-/-}$ fetuses contain increased numbers of secretory cells at the expense of adsorptive enterocytes (*65*). The transcription factor gene *Math1* is repressed by Hes1 in multiple organs, including the intestine (*65*, *66*). The intestines of *Math1*-deficient mice show a relatively normal crypt-villus architecture that is populated entirely by enterocytes, indicating that Math1 is required for all secretory cell lineages (*66*). This phenotype is somewhat reciprocal to the phenotype observed in *Hes1*$^{-/-}$ mice. Taken together, these results suggest that Notch-mediated *Hes1* expression regulates a binary decision between adsorptive and secretory cell fates whereas other bHLH proteins may refine these fate decisions. For example, mice deficient in the bHLH factor neurogenin-3 specifically lack enteroendocrine precursors (*67*). BETA2/neuroD, a bHLH protein best known for its role in pancreatic differentiation, appears to further refine the enteroendocrine fate by controlling terminal differentiation of the enteroendocrine secretin- and cholecystokinin (CKK)-producing cells (*68*). Additional indirect support for the control of intestinal cell fate by Notch stems from the use of γ-secretase inhibitors developed for the treatment of Alzheimer's disease (*69*). The generation of NICD from Notch requires γ-secretase (*70*). Rodents treated with γ-secretase inhibitors display increases in goblet cell numbers in the gut (viewed as an undesirable side effect), most probably through the inhibition of Notch signaling (*69*, *71*).

Concluding Remarks

The crypt-villus unit represents one of the simplest self-renewing entities in mammalian biology. Although a molecular understanding is still in its infancy, several principles are taking shape: First, regulatory signaling pathways that have emerged from the study of embryonic development of model organisms play key roles in this adult mammalian structure. Second, the malignant transformation of the intestinal epithelial cells does not rely on mutational changes in generic oncogenes or tumor suppressors. Rather, the transformation process appears to specifically subvert the physiological regulators of the epithelium. Third, structure and function of the crypt-villus unit are intricately linked. The epithelium is therefore preferably studied in its histological context, allowing marker analysis while preserving the positional information of individual cells. By contrast, the best-understood self-renewing tissue, the bone marrow, is usually treated as an unordered suspension of cells studied by flow cytometry outside the natural context. Nevertheless, this reductionist approach has yielded spectacular results. Elaborate cell culture systems have been established to study the hemopoietic system, often at the level of single primary cells (*72*). Unfortunately, no such culture system exists yet for primary epithelial cells of the intestine.

The insights that are emerging from the studies discussed in this review are painting a growing picture of the factors that control intestinal cell behavior in physiology as well as in cancer. Apparently, the transformation process uses crucial physiological regulators of normal intestinal epithelium as a strategy to open the most efficient road to cancer. Malignancies that arise in other self-renewing tissues may exploit similar strategies. In-depth insights into the molecular physiology of the self-renewal process of gut epithelium should ultimately allow the design of sophisticated modes of therapeutic interference in the diseased intestine.

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10.1126/science.1104815

SPECIAL SECTION

REVIEW

The Gut and Energy Balance: Visceral Allies in the Obesity Wars

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In addition to digesting and assimilating nutrients, the intestine and associated visceral organs play a key sensing and signaling role in the physiology of energy homeostasis. The gut, the pancreatic islets of Langerhans, elements in the portal vasculature, and even visceral adipose tissue communicate with the controllers of energy balance in the brain by means of neural and endocrine pathways. Signals reflecting energy stores, recent nutritional state, and other parameters are integrated in the central nervous system, particularly in the hypothalamus, to coordinate energy intake and expenditure. Our understanding of regulatory neural circuits and the signaling molecules that influence them has progressed rapidly, particularly after the discovery of the adipocyte hormone leptin. These discoveries have led to exploration of novel routes for obesity control, some of which involve gut-derived pathways.

In addition to the obvious role of the gut in the digestion and absorption of nutrients, the intestine and associated visceral organs, including the pancreas, liver, and visceral adipose depots, have important sensing and signaling roles in the regulation of energy homeostasis. To accomplish this role, the gut uses neural and endocrine pathways to communicate with controllers of energy balance in the hypothalamus and hindbrain. In this Review, we examine the role of the gut in energy balance and assess the possibility that insights into gut-derived signals will stimulate previously unexplored therapeutics for obesity and other disorders of energy balance.

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Integration of Peripheral Signals of Energy Balance

There is no doubt that food intake in humans is influenced by emotional factors, social cues, and learned behavior. These influences overlay highly conserved systems within the brain that sense and integrate signals reflecting overall energy stores, recent energy intake, and presence of specific classes of nutrients (Fig. 1). The hypothalamus, especially the arcuate nucleus, is relatively accessible to circulating factors and also receives inputs from other areas of the brain. Here, signals are received that relate to total energy stores in fat and to immediate changes in energy availability, including nutrients within the gut. These two categories of signals are not exclusive, because signals relating to long-term energy stores, including insulin and leptin, can modulate responses to short-term nutritional inputs. The hypothalamus integrates these peripheral and central signals and exerts homeostatic control over food intake, levels of physical activity, basal energy expenditure, and endocrine systems, including those that determine reproductive competence (Fig. 2).

Short-term eating behavior is also controlled by the hindbrain. The nucleus of the tractus solitarius (NTS) receives input from vagus nerve afferents, whereas the area postrema is a target for circulating factors such as amylin and glucagon-like peptide 1 (GLP-1) (*1*). Classical studies show that when higher inputs are surgically interrupted, the hindbrain can regulate food intake in response to peripheral signals (*2*).

Signals of Long-Term Energy Balance

Insulin, produced by pancreatic β cells, is vital for regulating the storage of absorbed nutrients and also acts as an adiposity signal to the

Self-Renewal and Cancer of the Gut: Two Sides of a Coin

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Science **307** (5717), 1904-1909.
DOI: 10.1126/science.1104815