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REGULATION OF GENE EXPRESSION IN THE INTESTINAL EPITHELIUM

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Abstract

Regulation of gene expression within the intestinal epithelium is complex and controlled by various signaling pathways that regulate the balance between proliferation and differentiation. Proliferation is required both to grow and to replace cells lost through apoptosis and attrition, yet in all but a few cells, differentiation must take place to prevent uncontrolled growth (cancer) and to provide essential functions. In this chapter, we will review the major signaling pathways underlying regulation of gene expression within the intestinal epithelium, based primarily on data from mouse models, as well as specific morphogens and transcription factor families that have a major role in regulating intestinal gene expression, including: the Hedgehog family, Forkhead Box (FOX) factors, Homeobox (HOX) genes, ParaHox genes, GATA transcription factors, canonical Wnt/ β -catenin signaling, EPH/Ephrins, Sox9, BMP signaling, PTEN/PI3K, LKB1, K-RAS, Notch pathway, HNF and MATH1. We will also briefly highlight important emerging areas of gene regulation including microRNA and epigenetic regulation.

Keywords

Intestine; gene expression; epithelium; Wnt; Notch; Hedgehog; forkhead; parahox; BMP; ephrins

I. INTRODUCTION

The small intestinal epithelium is a dynamic tissue in which short lived (2–3 days) non-proliferating, differentiated cells covering the villi are continuously replaced from the proliferating cell compartment in the crypts. Although crypt cells carry out some differentiated functions, much of the physiological role of the intestine is carried out in the villus cells, while most of the crypt cells fulfill a proliferative role. A fundamental question in understanding gene expression in the small intestine is how this distinct separation is established and maintained.

The endoderm, formed at gastrulation, gives rise to the epithelial layer of the gastrointestinal tract. Expression of key regulators, such as Foxa2, is already detectable in the early simple endoderm, well before the formation of the mature crypt/villus structure[1]. Cells of the endoderm undergo rapid proliferation as the embryo grows, giving rise to a pseudo-stratified epithelium. The proliferating cells become restricted to the intervillus regions late in gestation and villi begin to form in mice around embryonic day 15, while crypts do not

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develop until after birth. The mechanism of crypt/villus development is a complex one and is still incompletely understood. Crypt formation occurs in the developing intestine in the period immediately after birth and it is thought to be driven by areas of endodermal cells, likely containing intestinal stem cells, in the intervillus regions of the early gut tube [2]. Proliferation becomes restricted to these regions that then invaginate into the surrounding mesenchyme to form crypts. Unlike mammals, zebrafish never develop crypts but their stem cells instead remain clustered in focused areas in the intervillus regions [3].

Epithelial homeostasis is maintained by several mechanisms. Within the crypt epithelium reside intestinal stem cells and transit-amplifying cells, supported by a specialized niche, which together produce up to 200 cells per crypt per day [4]. The regular shedding of apoptotic cells from the villus tip balances this enormous cell production. Post-mitotic cells exit the crypt and begin to differentiate as they progress up the villus. Based on classical label retention experiments utilizing tritiated thymidine or BrdU (bromodeoxyuridine) to mark a cohort of cycling cells, it is well established that it takes between two and seven days for cells to make the journey from crypt to lumen [5]. Paneth cells are the exception, migrating downwards, past the traditional stem cell niche at the +4 position to the base of the crypt where they survive an average of 20 days. Each crypt has been postulated to contain between four and six stem cells, while the remaining 200 cells are their transit-amplifying progeny [6, 7]. Significant advances have recently established that crypt base columnar cells (CBCs), marked by *Lgr5* and *Ascl2* expression, represent a population of rapidly cycling stem cells, while cells marked by *Bmi-1* and telomerase mark separate stem cell populations. The transit-amplifying (TA) population is poorly understood and must contain progenitor cells at various stages of differentiation that then undergo terminal differentiation into enterocytes, goblet cells, and enteroendocrine cells upon exiting the crypt onto the villus surface or into Paneth cells upon reaching the crypt base [8]. Although some morphological differentiation occurs as cells divide and move upward in the crypt, it is unclear how this sequence of gene expression is controlled.

Regulation of gene expression within the intestinal epithelium is complex and controlled by various signaling pathways that regulate the balance between proliferation and differentiation [9]. For example, genetic studies have provided evidence that Wnt/ β -catenin signaling is important for proliferation [10], that Notch signaling is involved in intestinal cell fate determination [11–13], and that BMP (bone morphogenetic protein) signaling negatively regulates proliferation by suppression of Wnt/ β -catenin signaling [14]. Furthermore, communication within the niche is complex; Wnt signaling emanates from both mesenchymal and epithelial cells [15], BMP secretion comes primarily from mesenchymal cells adjacent to the epithelium [16], and additional regulatory factors are secreted by myofibroblasts [17] and enteric neurons [18].

In this chapter, we will review the major signaling pathways underlying regulation of gene expression within the intestinal epithelium, based primarily on data from mouse models, as well as specific morphogens and transcription factor families that have a major role in regulating intestinal gene expression, including: the Hedgehog family, Forkhead Box (FOX) factors, Homeobox (HOX) genes, ParaHox genes, GATA transcription factors, canonical Wnt/ β -catenin signaling, EPH/Ephrins, Sox9, BMP signaling, PTEN/PI3K, LKB1, K-RAS, Notch pathway, HNF and MATH1. We will also briefly highlight important emerging areas of gene regulation including microRNA and epigenetic regulation.

II. HEDGEHOG SIGNALING

Sonic hedgehog (*Shh*), and the related Indian hedgehog (*Ihh*), binding initiates a signaling pathway involved in the molecular control of early endodermal/mesodermal patterning

events. Both ligands are restricted to the epithelial layer of the gut at the base of the crypts and are progressively concentrated in the intervillus region during early development. *Shh* and *Ihh* bind to the receptors Smoothened, Patched, and Hedgehog interacting protein (HHIP), which are transmembrane proteins restricted to the local mesenchyme [19]. This epithelial/mesenchymal interaction is necessary for the appropriate development and differentiation of the epithelium. *Shh* appears to play an important role in crypt formation given that it is only expressed in undifferentiated epithelial cells in the crypt (terminally differentiated Paneth cells do not express *Shh* despite their crypt base position)[20]. Diffusion of *Shh* and *Ihh* within the crypt is thought to inhibit additional crypt formation locally, thereby guaranteeing orderly crypt spacing, and promoting regular villus development. Early in development, *Shh* induces BMP4 expression in the splanchnic mesoderm where it negatively regulates both gut smooth muscle and neuron formation as part of its global role in intestinal patterning [21]. In models where *Shh* is blocked by HHIP over-expression, no villi form and the epithelium remains pseudostratified and poorly differentiated with unfettered proliferation [19]. In contrast, *Shh* mutant mice display distorted foreguts, with absent adjacent submesoderm, suggesting that hedgehog signaling between the epithelium and mesenchyme is required for both formation of the villi as well as the associated delimitation of proliferation to the intervillus regions [22].

III. FORKHEAD BOX (FOX) TRANSCRIPTION FACTORS

The forkhead box (Fox) family of transcription factors includes more than 100 genes and is characterized by its highly conserved winged helix DNA-binding domain [23, 24]. The Foxa subfamily of genes, which includes Foxa1, Foxa2 and Foxa3, have been found to play important roles in organ development as well as in the regulation of key metabolic functions later in life [25, 26]. Mice null for Foxa2 are embryonic lethal with significant effects on gut development as well as notochord formation, likely due to failure to activate *Shh* [27]. Foxa3 knockout mice, in contrast, develop normally and have average lifespans, although they do develop significant hypoglycemia after prolonged fast, suggesting the Foxa family plays an important role in hepatic gluconeogenesis [28]. In addition, Foxa1 and a2 are required for hepatic specification; in double knockout mice, the embryonic liver bud does not form [29]. Finally, Foxa1 and Foxa2 contribute to enteroendocrine and goblet cell differentiation in the intestine[30].

Foxa family members regulate such diverse functions across a range of tissues. They have been likened to “pioneer” factors, which serve to establish endodermal “competence” rather than functioning as transcription factors per se [31]. Given the structural similarity of Foxa proteins to the DNA linker histone, interactions with chromatin are thought to provide access to and facilitate binding of other transcription factors to previously inaccessible chromatin [26, 32].

IV. HOMEBOX GENES

The homeobox (hox) genes contain a highly conserved homeodomain sequence that forms their DNA binding region. Their primary function is to regulate axial patterning, providing the organism with both spatial and temporal co-linearity (both the sites along the body axis and timing of gene expression echo chromosomal order). In mammals, the Hox system consists of four groups, Hox a–d, which arose from a two-step duplication of the ancestral complex [33, 34]. Hox expression begins in the posterior primitive streak in early development and then spreads as a wave towards the anterior, creating a gradient of positional information that is then clonally transmitted to future progeny of cells from the streak [35]. Several hox genes have been shown to regulate patterning of the GI tract, principally in the formation of sphincters. The Nkx group of dispersed homeobox genes are

also differentially expressed during development, and inactivation of *Nkx2.3* leads to delayed villus development in the jejunum and associated crypt hyperplasia [36] while knockout of *Nkx2.2*, a transcription factor essential for pancreatic endocrine differentiation and expressed during intestinal enteroendocrine cell differentiation, leads to dramatic down-regulation or absence of most intestinal hormones (serotonin, CCK, gastrin) and loss of many types of enteroendocrine cells [37].

V. PARAHOX GENES

The ParaHox gene cluster, which includes *Cdx2* and *Pdx1*, is evolutionarily related to the Hox gene cluster, having arisen from duplication of an ancestral “protohox” gene cluster [38].

a. CDX GENES

The mammalian *Cdx* genes, homologues of the *Drosophila* gene *Caudal*, play an important role in epithelial gut patterning by regulating cranio-caudal differentiation. *Cdx1*, *Cdx2*, and *Cdx4* each regulate unique topographical domains, although there is significant functional overlap based on single gene knockout studies. They are expressed both during development as well as in the adult animal and their actions are not limited to the gut.

Cdx1 is expressed early in development at the post-somite stages during the transition from a pseudostratified epithelium to a monolayer epithelium [39]. In adult mice, its expression is restricted to the intestinal crypts, where it has been proposed to play a role in stem cell maintenance [40]. The available data, however, including a lack of a phenotype for null mice, do not support this role. *Cdx4* acts in a posterior-to-anterior distribution during hindgut invagination in early development, but less is known about its functions thereafter [41].

Cdx2 expression is initially required in the trophoblast for blastocyst implantation [40]. Its expression is then limited to the intestinal epithelium by day 12 of mouse gestation in a distribution from the hepatic diverticulum to the distal colon. It is expressed throughout adult life, although levels are highest in the proximal colon with a decreasing gradient of expression both cranially and caudally [40, 42, 43]. Mice heterozygous for a *Cdx2* null allele go on to develop multiple ileal and proximal colon polyps with histology consistent with normal gastric epithelium, suggesting a loss of anterior-posterior positioning [44]. These lesions show loss of the remaining *Cdx2* allele, causing isolated gastric adenomas. Interestingly though, there is histologic recapitulation of normal epithelium at the edge of the gastric mucosal “lesions,” with an orderly cranio-caudal progression of cardiac, corpus, antral and small intestinal epithelium [45]. In contrast, ectopic expression of *Cdx2* (driven by *Foxa3*) in the stomach where it is normally not expressed, led to the development of both secretory and absorptive lineages of the small intestine [46, 47]. These experiments have led to the notion that gastric epithelium represents the “default” epithelial type in areas where *Cdx2* is not expressed [48]. However, when *Cdx2* was conditionally ablated in the intestine at the onset of gut formation, the ileum was replaced by a flat, squamous epithelium resembling the esophagus [49].

b. PDX1

Pdx1, a homeodomain transcription factor, is required for normal pancreatic development, ongoing islet maintenance, and is maximally expressed in the gut in the villus epithelium of the proximal duodenum [50]. Murine knockout models are lethal in the neonatal period due to lack of pancreatic formation but they are also noted to have reduced numbers of enteroendocrine cells, no Brunner’s glands, cystic malformations of the gut, and lack of villi in the most proximal duodenum [51]. In contrast, conditional inactivation of *Pdx1* in the

intestine alone using a *Pdx1^{flox/flox} VilCre* system shows an unchanged number of enteroendocrine cells from baseline; however, levels of mRNA for the enteroendocrine cell products somatostatin, gastric inhibitory polypeptide (Gip) as well as the enterocyte-specific intestinal alkaline phosphatase are dramatically decreased [52]. These findings indicate that Pdx1 is necessary for patterning of enterocyte and enteroendocrine gene expression in the proximal intestine.

VI. GATA TRANSCRIPTION FACTORS

The GATA family of transcription factors is known for its zinc-finger DNA binding motif. GATA-1/-2/-3 are involved in hematopoietic differentiation and development, while GATA-4/-5/-6 are involved in meso-endodermal development. GATA-4/-5/-6 are expressed in the adult intestinal epithelium but their role in intestinal development is less clear given that GATA-4 and GATA-6 null mice die on embryonic day 8 and 5, respectively, prior to the initiation of intestinal development [53, 54]. GATA-4 is expressed in a proximal to distal gradient in the small bowel, while GATA-5 and -6 are more highly expressed in the jejunum and ileum where they have been shown to repress or activate intestine-specific genes encoding lactase, sucrase and apical sodium-dependent bile acid transporter (ASBT) [55, 56].

Interestingly, GATA factors may play a role in lineage specific differentiation given their expression is restricted to specific cell types. GATA-4 is exclusively expressed by enterocytes in the adult mouse while GATA-5 is found in cells from the secretory lineage, and GATA-6 is primarily limited to enteroendocrine cells [57]. Additional regulation results from members of the Friend of GATA (FOG) cofactor family, which contain multi-zinc finger domains. For example, FOG1 modifies GATA-4-regulated repression in the proximal intestine of specific intestinal genes [56].

VII. WNT SIGNALING

Wnt signaling can be divided into the canonical (β -catenin/T cell factor [TCF] dependent) and noncanonical (β -catenin/TCF independent) pathways [58, 59]. In this chapter, we will focus on canonical Wnt signaling, which plays an important role in maintaining cellular proliferation within intestinal crypts, differentiation of Paneth cells, and in directing the migration of epithelial cells along the villus axis.

The Wnts are secreted ligands which bind to the Wnt receptor complex consisting of a Frizzled receptor and a lipoprotein-related protein co-receptor (LRP-5 or 6). In the absence of Wnt ligands, β -catenin, a cytoplasmic protein, is rapidly degraded by its destruction complex, made up of the scaffold protein Axin, the tumor suppressor gene Adenomatous Polyposis Coli (APC), and Glycogen Synthase Kinase 3- β . In the presence of a Wnt ligand, the receptor complex is activated, which allows β -catenin to separate from its destruction complex and translocate to the nucleus where it binds TCF/LEF (lymphoid enhancing factor) transcription factors. These displace the Wnt repressor Groucho, thereby promoting Wnt target gene expression (e.g., cMyc, cyclin D) [60]. Therefore, nuclear β -catenin is a marker for activated Wnt signaling that is negatively regulated by APC. Of note, almost 80% of all spontaneous colorectal cancers are due to mutations in the APC gene [61].

In the small bowel, the major Wnt ligands are Wnt3, Wnt6, and Wnt9B, which are primarily expressed in crypt epithelial cells [15]. Interestingly, the corresponding receptors, Frizzled 5, 6, 7 and the LRP5 and 6 co-receptors, are *also* found in the epithelium, in contrast to BMP signals, which emanate primarily from the mesenchyme to the epithelium [see below][15].

Multiple studies examining the effects of deletion or inactivation of down-stream effectors or targets of the Wnt pathway have demonstrated the importance of this key signaling cascade. Deletion of TCF4, one of the primary effectors of the Wnt pathway, results in the loss of the proliferative epithelial compartment and death shortly after birth, suggesting that TCF4 is required for maintenance of the crypt [10]. Conditional ablation of β -catenin within the intestinal epithelium also results in loss of the proliferative capacity within the crypts and death from intestinal failure [62, 63]. Conditional ablation of the Wnt target gene c-Myc results in decreased crypt cell size and number without an increase in apoptosis (see Prochownik 2008 [64] for an excellent review on the role of c-Myc)[65]. Conditional deletion of the Wnt transmembrane receptor Frizzled 5 (Fz5) results in the production of immature Paneth cells that are no longer localized to the crypt base but rather are scattered throughout the crypt-villus axis, while deletion of Sox9 (see below), a Wnt dependent transcription factor, shows a complete lack of Paneth cells as well as a 40% reduction in goblet cells [66–68].

Using a screen for Wnt-responsive genes, the Clevers group identified Lgr5 and Ascl2 as markers for rapidly cycling crypt base columnar intestinal stem cells [69]. These cells are intercalated between the Paneth cells at the crypt base and differ from previously defined long lived and multipotent stem cell populations based on their rapid cell cycling.

Up-regulation of the Wnt pathway via homozygous deletion of the APC gene or other inactivating mutations leads to dramatically enlarged crypts and villus stunting, which progress to large adenomatous polyps composed of multiple aberrant crypts, suggesting a shift towards intestinal cell proliferation at the expense of differentiation [70, 71]. The Min (APC^{min/+}) mouse and zebrafish with APC mutations are important experimental models of colorectal cancer and have an increased incidence of intestinal adenomatous tumors due to loss of heterozygosity [16, 72].

Additional regulation is also mediated through diffusible Wnt inhibitors, such as Dickkopf-1 (Dkk1), which allow for potent and specific negative regulation of the pathway. Consistent with this, over-expression of Dkk1 in adult mice results in a smaller number of shorter villi and crypts as well as a decrease in cells of the secretory lineage [62, 73]. Together, these results suggest that while Wnt signaling has a primary role in mediating cellular proliferation, it also has a role in differentiation of the secretory lineage.

VIII. EPH/EPHRINS

Eph/Ephrin molecules, membrane-associated proteins that mediate adjacent cell-cell interactions, whose expression is regulated via the β -catenin/TCF transcription complex, act to help segregate cells along the crypt/villus axis [74, 75]. Like Wnt signals, Eph (receptors) and Ephrin (transmembrane ligands) are expressed in a gradient along the villus. EphB2 is expressed at high levels in Lgr5⁺ columnar base cells (rapidly cycling stem cells)—although not in Paneth cells—and decreases in expression towards the top of the crypt [75]. EphrinB1 and EphrinB2 are also expressed in a gradient, highest in terminally differentiated cells at the crypt-villus junction and decreasing toward the crypt base. Working through lateral inhibition, the changing gradients of expression of EphB-EphrinB guide cells up the crypt and prevent downwards migration as the cells mature. High levels of Wnt signaling in the crypt activate EphB2 and EphB3 and inhibit EphrinB1 expression, which is increasingly activated as cells move away from the Wnt stimulus deep in the crypt [75, 76]. Because Paneth cells only express EphB3, they are forced to remain at the crypt base.

Deletion of EphB2 in adult mice results in misdistribution of putative progenitor cells from their location above the Paneth cells to more random positions throughout the crypt [73, 75]. Loss of EphB2 expression is associated with human adenocarcinoma, is a poor prognostic

factor, and is correlated with invasive behavior [77, 78]. Loss of EphB3 prevents Paneth cells from migrating downwards causing them to be found throughout the crypt-villus axis [62].

IX. SOX9

The transcription factor Sox9 is a downstream target of Wnt signaling thought to be involved in maintenance of Wnt-dependent intestinal progenitors. Sox9 acts to repress genes associated with differentiation, including Cdx2 and the mucin-encoding gene Muc2 [79]. Over-expression of Sox9 decreases the expression of both Cdx2 and Muc2, via an unidentified intermediate repressor gene. Sox9 is expressed in an increasing gradient from the duodenum to the distal colon and is limited to the proliferative zone in the lower half of the crypt in adults and in the proliferative intervillus region in neonates.

Immunohistochemical staining for Sox9 shows consistent overlap with that of Ki67, a marker of active cell cycling [79]. In general, immature, undifferentiated cells stain for Sox9, with the exception of Paneth cells at the base of the crypt, which are terminally differentiated [79]. Given that Sox9 is expressed in both undifferentiated and terminally differentiated cell types, it is unlikely to be a specific marker for intestinal stem cells, as recently argued [80]. Conditional inactivation of Sox9 further support its Wnt-dependence by showing increased crypt cell proliferation as well as increased expression of the Wnt target genes, cMYC and Cyclin D1 [67].

X. BMP Signaling

Cellular proliferation in the intestinal crypt is regulated in part by signaling between the epithelium and mesenchyme. Among the factors playing a major role are Bone Morphogenic Proteins (BMPs), which belong to the transforming growth factor (TGF) β family. BMPs are expressed primarily in the mesenchyme and signal through epithelial receptors. Recent data, however, suggest that BMP signaling might be more complex, with the possibility for bi-directional signaling [81]. BMP signaling is balanced by the antagonist, Noggin, which is epithelially expressed in crypts at and below the “+4 position,” as well as in the submucosa adjacent to the crypts, where BMP signaling seems to be most active [14, 16]. BMP acts by binding to the complex of BMP receptor type 1 and 2, which leads to downstream phosphorylation of SMAD proteins 1, 4, 5, and 8. Once phosphorylated, SMAD 1, 5 or 8 heterodimerize with SMAD4 and translocate to the nucleus where they initiate transcription important for both intestinal development and adult organ homeostasis [60].

Mice homozygous for null alleles of BMPR1A, BMP2, and BMP4 are embryonic lethal. In addition, mutations in BMPR1A and SMAD4 have been recognized in humans with juvenile polyposis syndrome (JPS) and are emerging markers of the invasive propensity of colorectal carcinomas [82–85]. Analysis of conditional BMPR1A mutants within intestinal crypts and stroma reveal increases in the number of crypts and hamartomatous polyp formation with histology reminiscent of JPS polyps [14, 86]. Curiously, while increased crypt number and fission is observed when deletion is limited to the epithelium, polyp formation is *not* observed, suggesting an important role for the stroma in this process [86]. In addition, epithelial-specific deletion of BMP signaling also revealed a role in the maturation of cells from the secretory lineage [86]. BMP signaling also helps to localize new crypts to the areas directly overlying adjacent muscularis [16]. Over-expression of the antagonist noggin also leads to an increased number of crypts in ectopic locations, likely via increased nuclear β -catenin in the stem cell compartment from cross-talk between the BMP and Wnt pathways [14]. Noggin may act locally around the crypt base to neutralize the effects of BMP and thereby ensure a niche of permissive proliferation within the crypt. In adult mice, BMP4 is localized in the mesenchyme near the +4 position while BMPR1A is expressed in the

epithelial cells located at this position, suggesting that BMP/Noggin interaction is important in the activation and proliferation of putative stem cells in this location [14]. BMP signaling may therefore be a mechanism for Wnt-independent modulation of the stem cell compartment.

XI. PTEN/PI3K

The PTEN/PI3K pathway also plays an important role in the regulation of intestinal proliferation and growth. Human intestinal cancers provide *in vivo* opportunities to observe the over-expression of this pathway and the up-regulation of cell survival, growth and proliferation. Mutations in the PI3K pathway have been recognized in hematopoietic, intestinal, renal, breast and neurologic tumors [87, 88]. In colorectal cancer, almost 40% of tumors demonstrate PI3K alterations leading to decreased expression of PTEN, a lipid-protein phosphatase at the center of the pathway [89]. Composed of two subunits, p85 and p110, PI3K is a lipid kinase located in the cytoplasm and is inactive until the ligand (growth factor) binds, initiating receptor tyrosine kinase autophosphorylation. The regulatory subunit p85 typically inhibits the catalytic subunit p110 until it is phosphorylated, at which point the inhibition is lifted. The pathway is modulated by PTEN, active while unphosphorylated and inhibited after phosphorylation, which serves to slow the proliferative process [90].

PTEN/PI3K (the active form) is principally expressed in epithelial cells at the +4 position in the crypt where it is positively regulated by BMP signaling and antagonized by Noggin, suggesting a potential role for the PI3K pathway in modulating stem cell self-renewal cycles [14]. Conditional ablation of PTEN leads to polyp formation, histologically similar to those in the human congenital PTEN mutation disease Cowden's syndrome, whose phenotype is similar to that of JPS [91]. Isolated epithelial loss of PTEN, however, is not sufficient for polyp formation, again supporting the notion that stromal cells are a source of additional signals that ultimately facilitate the development of polyps [60]

XII. LKB1 SIGNALING

Two separate groups simultaneously cloned the LKB1 gene in 1998 using linkage analysis from families with Peutz Jeghers syndrome (PJS), an autosomal-dominant hamartomatous polyposis syndrome, thereby establishing its role in intestinal homeostasis [92, 93]. Patients with PJS have a dramatically increased risk of both intra- and extra-intestinal carcinoma, and more than 80% of PJS cases are due to inactivating mutations in the LKB pathway. LKB1 is a serine-threonine kinase implicated in RAS-induced cell metaplasia, p53-regulated apoptosis, and control of cell cycle arrest, and is coupled to both Wnt and TGF- β signaling [94]. Invertebrate orthologs of the gene in *C. elegans* and *Drosophila* (PAR-4 and dLKB1, respectively) act as essential mediators of early anterior/posterior patterning and establishment of epithelial polarity during development [95, 96]. Gene deletion in mice results in significant neural tube and vascularization defects with embryonic lethality, while mice with only one functional copy of the gene develop gastric and small bowel hamartomatous polyps in adult life, resembling those found in PJS [97, 98].

LKB1 forms a heterotrimeric cytoplasmic complex with STRAD and MO25, which stabilize LKB1 and increase its kinase activity [99]. Recent studies demonstrate that the LKB1-STRAD-MO25 complex plays an important role in metabolism through direct activation of AMPK [100]. In addition, LKB1 regulates cellular polarization in intestinal cells, directing the distribution of proteins to their respective apical and basolateral locations [4].

XIII. K-RAS

Another system regulating proliferation in the intestine is the K-RAS family of GTPase proteins. This system is coupled with multiple signaling pathways that include the receptor tyrosine kinases RAF, MEK, and MAPK. The signaling cascade ultimately results in activated MAPKs, which translocate to the nucleus allowing phosphorylation of a series of transcription factors promoting cell growth and survival [101].

XIV. NOTCH SIGNALING

The Notch pathway works in concert with Wnt signaling to effect spatial patterning and cell fate decisions of adjacent cells. The Notch ligands Jagged 1 and 2, and Delta-like 1, 3 and 4 bind the transmembrane protein receptors, Notch1–4, to facilitate communication between physically adjacent cells. Ligand binding initiates proteolytic cleavage of the receptor by γ -secretase allowing the cleaved receptor to translocate to the nucleus where it combines with RBP-Jk. Activation of the Notch pathway induces proteins such as Hes1 (hairy/enhancer of split), a basic helix-loop-helix transcription factor, which acts on target genes to regulate proliferation and differentiation [60]. Notch and its receptors are expressed almost exclusively in the adult intestinal crypt epithelium, suggesting that their primary site of action is in the proliferative crypt [102].

Continuous activation of Notch signaling results in increased proliferation and decreased differentiation [11]. Given that Hes1 is upregulated in undifferentiated cells in intestinal adenomas, the Notch pathway may represent a context-dependent mechanism to promote proliferative Wnt signaling [12, 103]. In contrast, deletion of Notch pathway components reveals an important role in cell fate determination. When RBP-Jk is conditionally ablated, secretory cells dramatically increase in number, with a corresponding reduction in the absorptive lineage [103]. The same is true in the zebrafish mutant mindbomb (mib), where inactivation of the Notch pathway results in a secretory epithelium with exclusion of absorptive cells [104, 105]. Partial suppression of Hes1 in mice, or mutation of its zebrafish ortholog deltaD, leads to large numbers of goblet and enteroendocrine cells, while Hes1 knockout mice are embryonic lethal [105, 106]. In contrast, up-regulation of Notch signaling shows a severe reduction of all epithelial cells in the secretory lineage [11]. Together, these data underscore the essential role of Notch signaling in specification of the secretory lineage.

XV. HNF

Hepatocyte nuclear factors 1 α and 1 β were initially described in the liver, although they are expressed in other organs, specifically in the pancreas, kidney and intestine. Both Hnf1 α and 1 β can directly activate the Notch pathway via action of Jagged1, thereby upregulating the downstream Notch effectors Hes1, Hes5, and Hes6 [107]. The HNF1 α and HNF1 β genes encode for related dimeric homeobox proteins, which can form homo- or heterodimers. Together, they play important roles in terminal differentiation and cell fate commitment in the intestine. Hnf1 β is expressed throughout development from the primitive gut stage through organogenesis, while Hnf1 α is expressed later during organogenesis [108].

Hnf1 α null mice survive to birth but have significant impairment of renal, pancreatic, and hepatic function [109]. In contrast, complete ablation of Hnf1 β is embryonic lethal due to defective differentiation of extra-embryonic visceral endoderm [110]. Both factors are able to activate the expression of enterocyte-specific markers of differentiation, suggesting a role for them in the differentiation program of the gut. Analysis of conditional single- and double-gene inactivation in adult mice demonstrated that while isolated loss of Hnf1 β shows

no significant changes, loss of Hnf1 β in the context of Hnf1 inactivation leads to death within a few weeks [107]. Inactivation leads to death within a few weeks [107]. Double mutant mice display a dramatic downregulation of Cdx2 expression and an increased number of goblet cells throughout the small bowel, although enteroendocrine cell number appeared unchanged [107]. In contrast, mature Paneth cells were completely lost from the crypt base, despite normal activation of the Wnt pathway, suggesting that Hnf1 α and β play crucial roles in cell fate commitment of the secretory lineage [107].

XVI. MATH 1

MATH1/Atoh1 (Hath1 in humans), a basic helix-loop-helix transcription factor, appears to mark the precursor cell that gives rise to the three main cell types of the secretory lineage. In addition, Hes1 directly represses MATH1/Atoh1, providing a link to the Notch signaling pathway. Mice deficient in MATH1 lack all secretory cell types but have normal absorptive cells [111]. Based on this finding, it has been proposed that MATH1 expression signifies an early secretory cell fate decision. Its expression in mature secretory cells also suggests an additional role to maintain the lineage in adulthood.

XVII. INTEGRATION OF NOTCH AND WNT SIGNALING

Both Notch and Wnt signaling seem to be necessary for the maintenance of cellular proliferation within intestinal crypts. When Notch is inhibited, secretory cells abound but proliferation also seems to stop [13]. A combination of Wnt up-regulation and Notch inhibition via treatment of Apc Min mice with a γ -secretase inhibitor is inadequate to overcome the slowed proliferation; despite the fact that tumors develop, local proliferation remains blocked [13]. Furthermore, over-activation of the Notch pathway and inactivation of canonical Wnt signaling is unable to drive cellular proliferation, suggesting that both Notch and Wnt signaling must be balanced in order to maintain a fully proliferative state [112].

Because Notch signaling requires cell communication between adjacent cells, such signals can be used for lateral inhibition, that is, to prevent neighboring cells from adopting a similar cell fate. When this concept is combined with the fact that Wnt signaling is spatially restricted to the crypt due to the limits of diffusion, the following model of Wnt and Notch inter-regulation may be plausible. After Wnt signaling activates the Notch pathway, Notch signaling in turn mediates lateral inhibition on adjacent Wnt⁺ cells so that some express Notch ligands (Delta/Jagged) and remain Notch-negative while others, without Delta or Jagged, become Notch⁺ [13]. The Wnt⁺, Notch⁻ cells are committed to a secretory lineage early on and stop proliferating, while the Wnt⁺, Notch⁺ cells continue undifferentiated proliferation until the daughter cells leave the crypt base and move far enough away to no longer receive Wnt signals. Once freed of Wnt activation, these cells can proceed to differentiate into absorptive (Notch⁺) or secretory (Notch⁻) cells [113].

XVIII. EMERGING MECHANISMS OF GENE REGULATION

Our understanding of the mechanisms involved in the regulation of gene expression is expanding at a rapid pace. With advances in information technology, the discovery of microRNA and our ever increasing understanding of epigenetic regulation, it is becoming increasingly clear how much still remains to be learned.

a. NEW TECHNOLOGIES

New tools such as computational data mining and large format unbiased gene expression studies can help to identify transcription factors expressed in a limited distribution or time frame in development. Using this approach, the intestine-specific homeobox (Isx) gene was

discovered by screening available databases using computational algorithms [114]. *Isx* is limited to the epithelium in both fetal and adult intestine and is initially expressed at the time of villus growth during development [114]. Homozygous *Isx*-null mice are phenotypically and clinically normal but do show upregulated expression of the HDL receptor and lipid transporter *Scarb1* [114]. This discovery demonstrates the power of these new tools to further elucidate mechanisms of gene expression in the intestine.

b. MICRORNA

MicroRNAs (miRNA) are noncoding RNAs that serve an important regulatory role by binding to mRNA transcripts. First discovered in 1993, miRNAs typically act to prevent translation or promote degradation of their mRNA targets. One such miRNA, miR-145, is strongly expressed in the developing intestine in zebrafish and acts to dampen endogenous GATA6 expression [115]. Conditional suppression of miR-145, using a morpholino antisense oligonucleotide approach, showed a significantly underdeveloped gut at 96 hours post-fertilization, which lacked villi and showed altered epithelial cell morphology [115]. miR-194 is another newly described microRNA, which is regulated by HNF1 α and is highly expressed in terminally differentiated epithelial cells and therefore may work with HNF1 α to modulate intestinal cell maturation and differentiation [116]. Recently, a complete compendium of microRNAs, obtained by ultra-high throughput sequencing, was reported for the large and small intestine [117].

c. EPIGENETIC REGULATION

The epigenetics of the intestine is a burgeoning field and is one where much remains to be learned. Broadly defined, epigenetics is the study of the dynamic processes that can modify gene expression or cellular phenotype without altering the underlying genetic code and yet can be perpetuated to the cell's progeny over multiple cell divisions. Imprinting, X chromosome inactivation, DNA methylation, regulation of histone modifications, and chromatin remodeling are all specific epigenetic mechanisms, although the process of cellular differentiation already discussed above is perhaps the best understood. Epigenetics and its implications for developmental plasticity and cellular reprogramming [118] suggest possible future targets of medical therapeutics and alternative mechanisms for understanding the underlying regulation of the intestinal epithelium.

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Table 1
Summary Of Factors Involved In Regulation Of Gene Expression In The Intestine.

Gene/Pathway	Major Effects	Regulators/Targets	Location	Proliferation/Differentiation	Role in Cancer	Ref
HEDGEHOG SIGNALING (Shh, Ihh)	Role in endodermal/mesodermal patterning, crypt formation and spacing	Positively regulated by Shh and Ihh, negatively regulated by HHIP, regulator of BMP signaling	epithelium (ligands); mesenchyme (receptors)			18, 20
FORKHEAD BOX (FOX) TRANSCRIPTION FACTORS (FOXa1/2/3)	"Pioneer" factors serve to establish endodermal competence, some family members with metabolic functions	Regulation of intestine specific genes and potentially hedgehog signaling	epithelium			23, 24, 29
HOMEODOMAIN GENES (Hoxa/b/c/d, Nkx2.3, Nkx2.2)	Regulation of axial patterning and differentiation	Regulation of intestine specific genes, endocrine cell differentiation	epithelium	Differentiation	Yes (Nkx2.3)	32, 33
PARAHOX GENES (Cdx2, Pdx1)	Regulation of cranio-caudal patterning and differentiation, pancreatic development	Regulation of intestine specific genes	epithelium	Differentiation	Yes (Cdx2)	37, 39, 45
GATA/FOG TRANSCRIPTION FACTORS (GATA 4/5/6, FOG1)	Role in mesoderm and endoderm development, possible role in cell specific differentiation	Regulate intestine specific genes, GATA 4 regulates regional specificity	epithelium	Differentiation		51, 52, 53
WNT SIGNALING (Wnt, B-catenin, Frizzled, Dkk1)	Role in maintaining cellular proliferation within crypts, secretory lineage development, migration along villus axis	Positively regulated by Wnt ligand binding, negatively regulated by APC and Dkk1, downstream targets include cMyc, cyclin D, Sox9	epithelium	Proliferation/Differentiation	Yes	14, 55, 56
BMP SIGNALING (Bmp, Noggin, SMAD4)	Negative regulation of crypt proliferation	Positively regulated by BMPs and antagonized by Noggin, downstream targets include SMAD and PTEN	epithelium (ligands); mesenchyme (receptors)	Differentiation	Yes	13, 82
EPH/EPHRINS (EphB2/3, EphrinB1/2)	Regulation of cellular position along the crypt/villus axis	Downstream targets of Wnt signaling	epithelium (ligand); mesenchyme (receptor)	Differentiation	Yes	70, 71
PTEN/PI3K SIGNALING	Negative regulation of intestinal proliferation and growth	Positively regulated by growth factors, mediates nuclear translocation of β -catenin	epithelium	Blocks Proliferation	Yes	83, 84
SOX9	Maintenance of Wnt-dependent intestinal progenitors, down-regulation	Downstream target of Wnt signaling, downstream targets include Cdx2, Muc2	epithelium	Blocks Proliferation		63, 75

Gene/Pathway	Major Effects	Regulators/Targets	Location	Proliferation/Differentiation	Role in Cancer	Ref
LKB1 SIGNALING	Role in control of proliferation, anterior/posterior axis formation, epithelial polarity	Regulator of both Wnt and TGF-beta signaling	epithelium	Blocks Proliferation	Yes	90
K-RAS (RAF, MEK, MAPK)	Regulation of cellular proliferation and survival	Coupled with receptor tyrosine kinases RAF, MEK, and MAPK activated MAPKs	epithelium	Proliferation	Yes	97
NOTCH SIGNALING (Jagged, Delta-like, Hes1)	Major regulator of secretory lineage development and crypt proliferation via direct cell-cell contacts	Activation induces transcription factors such as Hes1	epithelium	Proliferation/Differentiation	Yes	11, 98, 101, 108
HNF (HNF1a, HNF1b)	Roles in terminal secretory differentiation and cell fate commitment	Activate notch signaling, downstream targets include lactase, sucrase	epithelium	Differentiation		103, 105
MATH 1	Role in specifying secretory lineage	Negatively regulated by notch signaling (Hes1)	epithelium	Differentiation		107