Transcriptional Networks in Liver and Intestinal Development

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SUMMARY

The development of the gastrointestinal tract is a complex process that integrates signaling processes with downstream transcriptional responses. Here, we discuss the regionalization of the primitive gut and formation of the intestine and liver. Anterior–posterior position in the primitive gut is important for establishing regions that will become functional organs. Coordination of signaling between the epithelium and mesenchyme and downstream transcriptional responses is required for intestinal development and homeostasis. Liver development uses a complex transcriptional network that controls the establishment of organ domains, cell differentiation, and adult function. Discussion of these transcriptional mechanisms gives us insight into how the primitive gut, composed of simple endodermal cells, develops into multiple diverse cell types that are organized into complex mature organs.

Outline

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1 INTRODUCTION

The development of the gastrointestinal tract is a complex process. The gut is composed of multiple specialized cell types with contributions from all three primordial germ layers. The endoderm forms the epithelium of the stomach, intestine, lung, liver, and pancreas. The mesoderm forms both striated (in the esophagus) and smooth muscle that is responsible for peristaltic movements. The neural crest, derived from the neurectoderm, is critical for the enteric nervous system, which controls peristalsis and which is absolutely essential for the proper functioning of the digestive system.

During gastrulation, when the endoderm, mesoderm, and ectoderm are specified, the primitive gut becomes divided into regions with distinct gene expression patterns along the anterior–posterior (AP) axis. These regions set up the domains that give rise to each derivative endodermal organ, leading on to the diverse developmental programs required for each to achieve its unique adult function. The primitive gut is divided into the foregut, midgut, and hindgut. The foregut forms the esophagus, lungs, thyroid, stomach, liver, and pancreas. As the foregut organs are being specified, the gut tube is further regionalized by epithelial–mesenchymal interactions that establish gene expression throughout the gastrointestinal tract. The midgut and hindgut form the small and large intestine (colon), respectively. Even though the small and large intestine form a continuous tube and share similar developmental origins, their morphology and final functions are unique. As tissues grow, correct cell positioning within the gut is required for signaling across the germ layer derivatives. The interplay between multiple signals, acting between the endoderm and surrounding mesenchyme, is critical in both time and space for correct development. Close association with the mesoderm is required for both development and adult function.

There are three different mechanisms that are continuously deployed throughout gut development to maintain regional identity (Fig. 1). The first is the use of combinations of transcription factors for the coordination of gene expression in both time and space (an example is given in Fig. 1A). Key transcriptional regulators are required for both the initial specification of the endoderm as well as the appropriate coordination of downstream factors important for different stages of differentiation. Often, combinations of several, rather than individual, transcription factors are required to activate the appropriate downstream gene expression programs. This combinational control fine-tunes the activity of selected sets of genes that execute the function of the derivative organs. Second, extracellular signaling factors are required at various times throughout development (Fig. 1B,C). Interestingly, the same signaling

Figure 1. Molecular mechanisms guiding digestive tract development. (A) Transcription factors are required both in specification of the endoderm as well as for expression of genes important in formation of the pancreas. Sox17 is required to activate transcriptional programs for both initial formation of the endoderm through FoxA2 and also later during pancreas specification through Pdx1. (B) Extracellular signaling differentially activates the appropriate developmental programs during organ formation. During organ specification at the 7–8-somite stage, FGF signaling from the STM promotes liver specification and suppresses the pancreas gene program in the proximal endoderm. Ventral endoderm escapes the inhibitory signal, and the pancreas gene expression is initiated. (Adapted, with permission, from Zaret 2002 © Macmillan.) (C) Interactions between endoderm (yellow) and mesoderm (black) are required for morphological changes during intestinal development. At embryonic day 8.0 (E8.0), tight association of these tissues allows for gradients of Wnt, Bmp, Fgf, and RA signaling to direct proper A-P positioning along the gut tube. (Adapted, with permission, from Spence et al. 2011 © Wiley.)

factor may cause different and even opposing downstream effects at different times in ontogeny. For example, the fibroblast growth factors (FGFs) and bone morphogenetic proteins (BMPs), which repress liver-specific genes in the foregut, are subsequently required in the liver primordium

for differentiation (Zaret 2002; Zorn and Wells 2009). Third, cell positioning and morphogenetic processes are critical for appropriate signaling between neighboring tissues during development and homeostasis (Fig. 1C). Establishment of the subdivisions of the primitive gut requires the activation of specific transcription factors within the endoderm. Activation of many of these factors relies on signaling input from neighboring tissues, especially the mesoderm.

2 THE PRIMITIVE GUT: ESTABLISHMENT OF ANTERIOR–POSTERIOR PATTERNING AND REGIONALIZATION

2.1 Initial Establishment of Regional Identities

The primitive streak is the most evident morphological sign of anterior–posterior (AP) positioning in the vertebrate embryo. The process of the establishment of the primitive streak has been reviewed in detail elsewhere (Rivera-Perez and Magnuson 2005; Lee and Anderson 2008). Primitive streak cells form the progenitors for the three germ layers—endoderm, mesoderm, and ectoderm. Early in the process, *Mixl1*, a member of the *Mix/Bix* family of paired-like homeodomain proteins, is essential for the establishment of Nodal signaling within the primitive streak (Hart et al. 2002). Subsequently, Nodal, a transforming growth factor β (TGF- β) family member, is required for the activation of multiple transcription factors that function in endoderm specification such as *Sox17*, *FoxA2*, and *Hhex* (Shen 2007; Zorn and Wells 2007).

During gastrulation, movement of definitive endoderm progenitors out of the primitive streak is associated with early anterior–posterior regionalization of the gut. Endodermal cells have been traced from gastrulation to early organogenesis using fluorescent markers, which were introduced into pregastrulation embryos by electroporation (Tam and Beddington 1992; Franklin et al. 2008). Cells that leave the primitive streak first are specified as anterior endoderm, whereas cells migrating later form the posterior endoderm (Lawson et al. 1986; Lawson and Pedersen 1987; Tam and Beddington 1992). These different groups of cells form anterior and posterior pockets of endoderm, also termed the anterior and posterior intestinal portals, respectively. These pockets then elongate toward both ends of the embryo, while the intervening sheet of endoderm closes ventrally to form a connected tube. This process requires Wnt signaling in many organisms, although the situation is still unclear in mice. Work in zebrafish and frog shows that convergence and extension during gastrulation require the redundant actions of several noncanonical Wnt ligands including *Wnt4a* and *Wnt11* (Matsui et al. 2005; Zerbe et al. 2008). Only *Wnt5a* has been shown to be important

in mice during midgut elongation; however, many Wnt genes are expressed throughout gut tube formation, and it is hard to discern if lack of phenotypes is due to redundancy (Lickert et al. 2001; Cervantes et al. 2009). Several transcription factors such as *Sox17*, *Foxa2*, *Hhex*, and *Cdx2* are critical for the establishment of regional identity (see below).

Initial specification of the definitive endoderm and morphogenesis requires the transcription factor *Sox17* (an SRY-related HMG factor) in multiple species (Hudson et al. 1997; Alexander and Stainier 1999; Clements and Woodland 2000; Kanai-Azuma et al. 2002). *Sox17* expression is high in all definitive endoderm cells early on. *Sox17* was shown to cooperate with Wnt signaling and to activate *Foxa2* (a member of the Forkhead transcription factor family) (Sinner et al. 2004). Subsequently, expression of *Sox17* is restricted to the posterior end of the embryo, and *Sox17*-null cells are incapable of forming midgut and hindgut (Kanai-Azuma et al. 2002). Later in gestation, *Sox17* interacts with another transcription factor, *Pdx1* (pancreatic and duodenal homeobox 1), which is required for the specification of the pancreas (Spence et al. 2009). Using both loss-of-function and gain-of-function techniques in the mouse embryo, *Sox17* was shown to repress *Pdx1* expression in the liver primordium, a process that is critical for establishing organ domain boundaries between liver and pancreas (Spence et al. 2009).

The anterior endodermal region requires two major transcription factors, *FoxA2* and *Hhex*. *FoxA2* is the master regulator of the anterior primitive gut. *FoxA2* mutant mice show defects in cell migration after endodermal specification and thus loss of all foregut and midgut structures; however, hindgut development is unaffected (Weinstein et al. 1994; Dufort et al. 1998). Using tetraploid embryo complementation, it was subsequently shown that *Foxa2*-null cells can form the hindgut but are never incorporated into the developing foregut or midgut (Dufort et al. 1998). *Hhex* expression is required for anterior endoderm development and activated by both Nodal and Wnt signaling (Martinez-Barbera et al. 2000; Smithers and Jones 2002). The promoter of *Hhex* has been shown to have both activation and repression domains that are responsive to multiple signaling pathways including Nodal, Wnt, and BMP (Rodriguez et al. 2001; Rankin et al. 2011). *Sox2* is also required in a dose-dependent manner in the developing foregut (Que et al. 2007). All of these factors are important throughout the morphogenesis of the anterior part of the gut.

The Caudal-related homeobox transcription factor *Cdx2* is required for posterior gut development. *Cdx2* expression is highest at E8.5 in the hindgut, and its expression in the intestinal epithelium defines a clear-cut boundary at the foregut–midgut junction (Silberg et al. 2000; Beck and Stringer 2010). *Cdx2* is absolutely required in the midgut

and hindgut for the formation of the intestine (Gao et al. 2009). Studies in Caco-2 cells, a colon cancer cell line that is used as a model for the transition of cells from progenitor to differentiation in the intestine, have shown the Cdx2 regulates expression of both progenitor- and differentiation-specific genes (Gao et al. 2009; Verzi et al. 2010b). Cdx2 may play a role in mediating chromatin accessibility at these loci (Verzi et al. 2011). Cdx2 is also required for homeostasis of mature intestinal epithelial cells, which is discussed in detail in the section below entitled “Regional Specification and Morphogenesis of the Intestine.”

Canonical Hox genes are also expressed in specific AP domains in the intestine, the so-called enteric Hox code (Pitera et al. 1999; Kawazoe et al. 2002). Misexpression of HoxA4 in transgenic mice led to the formation of megacolon (Wolgemuth et al. 1989). Early studies in the chicken hindgut showed that ectopic expression of Sonic hedgehog in the endoderm was sufficient to induced expression of BMP4 and Hoxd13 in the mesoderm (Roberts et al. 1995; Roberts et al. 1998). In fact, when Hoxd13 was misexpressed in the primitive midgut mesoderm, this was sufficient to cause transformation of the midgut into a structure resembling the hindgut (Roberts et al. 1998). Loss-of-function mouse models of various Hox genes have also established their importance in intestinal maturation. For instance, ablation of the Hoxa5 gene leads to abnormal stomach development (Aubin et al. 2002). Importantly, the formation of the ileocecal valve and the anal sphincter is dependent on the Hoxd cluster (Zakany and Duboule 1999).

2.2 Signaling across Tissues Modulates Regional Transcription Factor Activity

Signaling from the mesoderm maintains hindgut fates and actively represses foregut development in the posterior endoderm, at least in *Xenopus* development (Zorn and Wells 2007). Wnt signaling, well known for its role in establishing the anterior–posterior axis of the embryo (Huelsen et al. 2000), is highly active in the hindgut and represses foregut identity (McLin et al. 2007). Similarly, bone morphogenetic protein (BMP) signaling is required for hindgut development, and the naturally occurring BMP antagonists noggin and chordin are required to allow foregut development (Sasai et al. 1996; Zorn et al. 1999; Tiso et al. 2002). In addition, BMP signaling has been shown to be important in determining cell fates in the foregut during organogenesis, which is discussed in more detail below. Fibroblast growth factor (FGF) seems to be expressed in a gradient, with highest expression in the posterior gut (Fig. 1C), and it represses anterior markers (Serls et al. 2005; Dessimoz et al. 2006). However, varying concentrations of FGF are also required

for different lineages that arise from the ventral foregut, such as the liver (Jung et al. 1999; Zaret 2001; Calmont et al. 2006).

Retinoic acid signaling has multiple roles in establishing anterior–posterior regional identity. Mice deficient for retinoic acid signaling in the foregut, through deletion of the retinaldehyde dehydrogenase 2 (Raldh2) gene or treatment with a pan-retinoic acid receptor (RAR) antagonist, show failure to develop multiple anterior organs (Wendling et al. 2000; Molotkova et al. 2005; Wang et al. 2006). Retinoic acid seems to act through regulation of transcription factors with retinoic acid–responsive enhancers including Hoxb1 and Hoxa5 (Huang et al. 1998; Niederreither et al. 2000; Matt et al. 2003; Grapin-Botton 2005). However, there may be multiple additional effects of retinoic acid signaling including activation of other signaling factors such as FGF10 and Sonic hedgehog (Shh) (Ivins et al. 2003; Wang et al. 2006).

2.3 Combinations of Transcription Factors Determine Organ Domains in the Primitive Gut Tube

Extensive investigation of the expression of transcription factors has been performed in an effort to understand the establishment of the multiple organ domains in the gastrointestinal system. For example, a study of 15 transcription factors expressed within the developing mouse foregut identified more than a dozen unique domains that roughly correspond with particular organs (Sherwood et al. 2009). Endodermal organ domains were isolated during embryonic development, and gene expression was analyzed by microarray. Sherwood et al. (2009) found that each organ domain contained a unique combination of transcription factors. For instance, the dorsal pancreas domain at embryonic day 9.5 (E9.5) shows expression of Pdx1, Prox1, and Hlxb9. Using whole-mount immunofluorescence of the dorsal pancreas, subregions were identified with different patterns of coexpression of these factors. The mechanism behind the complex combinatorial control is organ specific and is discussed in the following sections.

3 FORMATION OF THE INTESTINE: COORDINATION OF TRANSCRIPTION FACTOR NETWORKS ACROSS MIDGUT AND HINDGUT DOMAINS

3.1 Regional Specification and Morphogenesis of the Intestine

Cdx2 is one of the earliest transcription factors expressed in the primitive gut tube and is required for defining both midgut and hindgut regions that contribute to the entire intestine. Cdx2 is expressed most highly in the hindgut, but its expression extends all the way to the foregut–midgut

boundary (Silberg et al. 2000). In fact, although the very first duodenal epithelial cell is Cdx2-positive, all cells of the stomach and esophagus lack Cdx2. Cdx2 expression is frequently used as a marker of intestinal metaplasia, a precursor to cancer (Bai et al. 2002). Specification of the colon and expression of many intestine-specific genes require Cdx2 (Gao et al. 2009; Gao and Kaestner 2010; Verzi et al. 2010a, 2011). Regulation of Hox gene expression along the A–P axis further specifies the intestine and defines areas of major anatomical constrictions (Kawazoe et al. 2002; Grapin-Botton 2005; Hanamura et al. 2006). A subset of the posterior enteric Hox code is dependent on the presence of Cdx2, indicative of a transcriptional network in the establishment of regional identity in the gut (Gao et al. 2009).

Multiple signaling pathways converge on Cdx2 to regulate intestinal development. Persistent Wnt signaling in the hindgut regulates the expression of Cdx2. In mice null for the transcriptional effectors of Wnt signaling Tcf1 and Tcf4, the severely posteriorly truncated embryos lack a hindgut altogether, similar to what is seen in Cdx2-null mice (Gregorieff et al. 2004). Importantly, expression of Cdx2 in the remaining gut tube is severely decreased, with apparent transformation into gastric epithelia (Gregorieff et al. 2004; Cervantes et al. 2009). Interestingly, partial ablation of Wnt signaling in Wnt5a-null mice, although resulting in a dramatically shortened gut tube, did not affect Cdx2 expression (Cervantes et al. 2009). Wnt signaling is required transiently between E7.5 and E8.5, and dosage activates different intestinal programs through

Cdx2 (Sherwood et al. 2011). FGF signaling also plays a role in the establishment of the Cdx boundary at the duodenal–pyloric junction (Dessimoz et al. 2006; Rubin 2007; Benahmed et al. 2008).

Hedgehog signaling from the endoderm is also important for interactions between the mesenchyme and endoderm for intestinal specification and regionalization. Mutants in both Sonic hedgehog (Shh) and Indian hedgehog (Ihh) show defects in the gastrointestinal tract, including intestinal transformation of the stomach (Ramalho-Santos et al. 2000; van den Brink 2007; Saqui-Salces and Merchant 2010). In addition, ectopic expression of Shh causes transformation of pancreas into intestine (Apelqvist et al. 1999).

At the closure of the midgut on day 9.5 of gestation in the mouse, the gut tube consists of a central lumen surrounded by the polarized epithelium derived from endoderm. During the next few days, the epithelium increases in thickness, until there is a dramatic remodeling, or “epithelial transition,” during which the smooth luminal surface acquires villi, or finger-like projections, with mesenchymal cores (Fig. 2). A recent careful morphometric analysis by Grosse and colleagues showed that the intestinal epithelium on day 12.5 is, indeed, pseudostratified (Grosse et al. 2011). In this epithelium, proliferation is accompanied by interkinetic nuclear migration, which is the movement of nuclei from the basal side of the cell in S phase to the apical surface in M phase (Fig. 2A). Villi are projections into the lumen that are morphologically different but contiguous

Figure 2. Intestinal morphology changes during development. (A) At E10.5, the pseudostratified epithelium (yellow) and mesoderm (black) are tightly associated. (B) Polarized folds of epithelium form at E15 because of signaling between the prospective villus regions through BMP and Hh. (C) At E16.5, villi have been fully formed through BMP signaling. (D) Prospective crypt regions require Foxl1 and Wnt signaling from the mesoderm to maintain proper proliferative areas. (Adapted, with permission, from Spence et al. 2011 © Wiley.)

with the crypts, which are invaginations into the supporting mesenchyme. Villi are formed from initially polyclonal polarized folds of endoderm that are the beginnings of crypt formation (Fig. 2B–D) (Abud and Heath 2004; Abud et al. 2005). Genetic lineage tracing has recently been used to show that although initially polyclonal, intestinal crypts resolve into monoclonality through a process called “neutral drift” (Lopez-Garcia et al. 2010). In this process, random loss of a stem cell within the crypt is compensated by increased replication of another stem cell, until eventually the entire crypt is dependent on a single clone of stem cells.

The morphological changes that accompany the epithelial transition require tight interactions between the epithelium and underlying mesenchyme. This was shown, for instance, by a delay in epithelialization in the absence of the mesenchymal transcription factor FoxL1 (Kaestner et al. 1997). Reciprocal communication between these tissues requires Hh, Wnt, and BMP signaling to establish the crypt–villus axis (Fig. 2D) (Li et al. 2007; Madison et al. 2009). Intervillus regions require active Wnt signaling in the epithelium to initiate proliferation and subsequent invasion of the submucosa to form crypts (Korinek et al. 1998; Kim et al. 2007). Hh expression in the epithelium and BMP expression in the underlying mesenchyme suppress Wnt signaling in villus regions and are required for proper villi formation (Karlsson et al. 2000; Ramalho-Santos et al. 2000; Sukegawa et al. 2000; He et al. 2004). Transgenic mice that express Hh-interacting protein (Hhip), a pan-Hedgehog inhibitor, in the developing intestinal endoderm show mislocalization of myofibroblast cells underlying the epithelium that are a source of Wnt and regulate crypt size and location (Madison et al. 2005). Misexpression of the BMP antagonist Noggin causes ectopic crypt formation due to activation of Wnt signaling (Haramis et al. 2004; Batts et al. 2006). Several studies have suggested that BMP expression is directly induced by Hh signaling; however, this has not been shown *in vivo* in the mammalian intestine (Sukegawa et al. 2000; Ishizuya-Oka et al. 2006; Ishizuya-Oka and Hasebe 2008).

4 FORMATION OF ORGANS ASSOCIATED WITH THE GUT: A CASCADE OF TRANSCRIPTIONAL REGULATORS

All gut-associated organs (also called the para-alimentary tract) use signals from adjacent tissues to invade the local mesenchyme adjacent to the primitive gut tube to form an organ bud. At this point, all gut-associated organs follow unique programs that allow for proliferation and differentiation. The lung depends on endoderm–mesenchymal interactions to direct its branching structure and generation of

several functional cell types, with major contributions from FGF and TGF- β signaling (Maeda et al. 2007). The pancreas forms polarized microlumina that eventually coalesce to form the final ductal tree (Gittes 2009; Villasenor et al. 2010). The liver has a close association with the vasculature and generates bipotential progenitors that differentiate into a homogeneous population of functional cells (Zaret and Grompe 2008; Nagaoka and Duncan 2010). Here we outline the development of the liver as an example of the transcriptional regulation of endoderm organ formation.

4.1 Setting Up Transcription Factor Networks in the Hepatic Primordium

As mentioned above, transcription factors are required not only for initial specification of the endoderm at gastrulation, but also are continually involved throughout liver development. FoxA1 and FoxA2, which also play important roles in gastrulation, act in concert to enable the subsequent induction of the hepatic gene program (Lee et al. 2005). These transcription factors are thought to function as pioneer factors by facilitating the opening of chromatin at several important liver-specific genes, including albumin and α -fetoprotein (Gualdi et al. 1996; Zaret 1996; Cirillo et al. 1998; Crowe et al. 1999). The GATA family of zinc finger transcription factors, GATA 4 and 6, also acts together in hepatic gene induction, including the activation of the albumin locus (Bossard and Zaret 1998; Cirillo et al. 1998), and subsequent liver development requires the presence of at least one of them (Holtzinger and Evans 2005; Zhao et al. 2005).

The choice of hepatic cell fate is further influenced by signals from the surrounding mesenchyme. FGF signaling from the cardiac mesoderm activates MAPK signaling that induces hepatic gene induction (Rossi et al. 2001; Chen et al. 2003; Zhang et al. 2004; Serls et al. 2005; Calmont et al. 2006; Shin et al. 2007). BMP 2 and 4 signals from the septum transversum mesoderm enhance the hepatic competence of the endoderm (Jones et al. 1991; Smith and Harland 1992; Furuta et al. 1997). TGF- β acts as a developmental timer to maintain hepatocyte competency while restricting differentiation until endodermal cells are positioned correctly (Wandzioch and Zaret 2009). Wnt signaling is required for liver bud development and hepatic growth through the activation of transcription factors such as Hhex (Finley et al. 2003; Monga et al. 2003; Suksaweang et al. 2004).

4.2 Tissue Patterning and Morphogenesis

Liver bud formation takes place on the ventral wall of the foregut endoderm and requires several morphological

changes. Cells positioned near the developing heart receive signals that are critical for epithelial thickening and formation of the liver bud outgrowth from the endoderm (Douarin 1975). This signal is mediated, at least in part, by FGF, as shown through in vitro culture studies (Gualdi et al. 1996). These endodermal cells then delaminate and invade the septum transversum mesenchyme (STM) to begin the formation of the final organ. Hepatoblasts subsequently differentiate fully into two functional cell types that are discussed below.

Cell migration is dependent on two homeobox transcription factors, Hhex and Prox1. Hhex is a transcriptional repressor and required for hepatoblast proliferation, but not their initial specification (Keng et al. 2000; Martinez Barbera et al. 2000). Hhex is also required for liver induction by positioning the ventral endoderm within the cardiogenic field (Martinez Barbera et al. 2000; Bort et al. 2004; Hunter et al. 2007). Interestingly, loss of Prox1, a prospero-related homeobox transcription factor, has no effect on liver-specific gene expression; however, the levels of the cell adhesion molecule E-cadherin are increased dramatically in Prox1-mutant embryos, preventing hepatoblasts to delaminate from the ventral foregut (Sosa-Pineda et al. 2000). Thus, it is apparent that transient migratory behavior is required for liver development.

4.3 Diversification and Specification of Cell Types in the Liver

A complex transcriptional network is required for liver development. These factors include HNF-1 α , HNF-1 β , FoxA1, FoxA2, FoxA3, HNF-4, COUP-TFII, LRH-1, FXR α , PXR, C/EBP α , and HNF-6 (Cereghini 1996; Costa et al. 2003). Multiple transcription factors bind to hepatic gene promoters to induce robust expression (Fig. 3A). For instance, promoters of active hepatic genes that were bound with HNF-1 or HNF-6 are also often occupied by HNF-4 α

(Odom et al. 2004). These factors also have reciprocal regulation in which expression of one factor depends on another factor in the same cell (Fig. 3B) (Kuo et al. 1992; Bulla 1997; Bailly et al. 1998). Investigation of the promoter occupancy and expression patterns of these transcription factors during liver development revealed that an increased number of interactions are correlated with hepatocyte differentiation. In essence, although there are only a few connections between the different transcription factors early in liver development, later on multiple, often reciprocal activating interactions stabilize the transcriptional network (Kyrnizi et al. 2006).

Hepatoblasts are the earliest differentiated liver cell type. Expression of albumin (Alb), transthyretin (Ttr) and α -fetoprotein (Afp) is the earliest marker of hepatoblasts (Gualdi et al. 1996; Jung et al. 1999). Hepatoblasts are bipotential cells that further differentiate into mature epithelial cell types, the hepatocytes that form the main functional cell of the liver and the cholangiocytes that form the biliary tree. Interestingly, the liver is not homogeneous in function despite its appearance, displaying a distinct regional distribution, or “zonation,” of metabolic functions. Remarkably, this differential transcriptional program between the pericentral and periportal hepatocytes is dependent on pericentral Wnt/ β -catenin signaling, a striking example of the “reuse” of the same signaling system at various stages of ontogeny (Torre et al. 2010).

Hepatocytes make up ~78% of the total liver volume (Blouin et al. 1977). They are a polarized epithelial cell type that has many functions including controlling the levels of metabolites and serum proteins in the blood (Stamatoglou and Hughes 1994). HNF-4 α is critical for terminal hepatocyte differentiation and epithelialization of the liver, although it is not required for early liver specification (Spath and Weiss 1998; Li et al. 2000; Battle et al. 2006; Hayhurst et al. 2008). Direct activation of target transcription may be through regulation of chromatin accessibility by HNF-4 α

Figure 3. Regulation of liver-specific gene expression using transcriptional networks. (A) Activation of liver-specific genes is dependent on combinations of transcription factors. HNF1 α , HNF4 α , and HNF6 binding is required for expression of hepatocyte-specific genes such as HNMT, PLGL, C8B, and AMBP. (B) Coexpression of transcription factors is required for maintenance of expression levels for both factors. Binding of HNF1 α and HNF4 α proteins to the promoters of the HNF4 α and HNF1 α genes, respectively, is required for robust expression of both factors.

(Li et al. 2000; Soutoglou and Talianidis 2002). HNF-4 α also targets genes indirectly through the activation of the transcriptional regulators Hnf1a and PXR, which are crucial for expression of subsets of hepatocyte-specific genes (Tian and Schibler 1991; Kuo et al. 1992; Holewa et al. 1996). Differentiation of human embryonic stem cells into hepatocyte-like cells requires HNF-4 α for activation and maintenance of expression for several key transcription hepatic progenitor factors including FoxA2, GATA4, GATA6, HNF1B, and HNF1A (DeLaForest et al. 2011). These data show that HNF-4 α functions as a master regulator of hepatocyte differentiation through transcriptional regulation at multiple levels.

Cholangiocytes are cells that line the bile ducts and function in synthesizing and secreting components of bile; they make up a small percentage of the liver. Hnf6, hepatic nuclear factor 6, is required for the formation of biliary ducts (Clotman et al. 2002). In the liver, Hnf6 trans-activates the promoter of another transcription factor, Hnf1b, which is required generally for the development of tubules during organogenesis (Clotman et al. 2002; Coffinier et al. 2002). The exact mechanism of this transcriptional cascade is still being studied but most likely is regulated by signals from the septum transversum mesenchyme (Kalinichenko et al. 2002).

5 HOMEOSTASIS IN THE INTESTINAL EPITHELIUM AND THE LIVER

5.1 Regulation of Cellular Turnover in the Intestine

The adult intestinal epithelium is one of several epithelial tissues in the body that maintain its function by constant production of several types of short-lived cells. The intestinal epithelium is composed of a single layer of cells that perform the essential role of digestion and absorption of nutrients into the bloodstream. Epithelial cells migrate from the crypt region to the villus, changing from a progenitor state to fully differentiated cell in the process. Once cells reach the tip of the villus, they are shed into the gut lumen. This entire process takes ~3–5 d in mice and human. Four major differentiated cell types are required to maintain small intestinal function. Ninety percent of all epithelial cells are enterocytes that function as absorptive cells. The remainder of the cells is composed of enteroendocrine, goblet, and Paneth cells, collectively referred to as the secretory cell lineage. These cells secrete hormones that regulate digestion and signal to the body, elaborate mucous that protects the epithelium, and produce defensins to protect against infections, respectively. The mature epithelial cell types must be maintained in the appropriate ratio or there are severe consequences for intestinal function. Interestingly,

the relative representation of the four differentiated cell types also varies across the anterior–posterior axis, with the duodenum, the most anterior section of the intestine, elaborating far fewer goblet cells than the colon, whereas Paneth cells are found in the small intestine but are missing from the large bowel. Thus, positional cues must maintain differences in progenitor cell differentiation even in the adult.

The intestinal epithelium maintains its self-renewal capacity by maintaining a multipotent stem cell niche (Fig. 4). The intestinal epithelium is constantly repopulated by the coordinated division of stem cells into faster cycling transit–amplifying cells that divide to produce all differentiated cells (Sancho et al. 2004). Intestinal stem cells are found in the bottom of the crypts and divide symmetrically to produce both stem cells and transit-amplifying cells (Snippert et al. 2010). These cells are bona fide stem cells and are sufficient to form new crypts in culture (Sato et al. 2009). Intestinal stem cells also express general stem cell markers such as Lgr5 (Barker et al. 2010), Bmi-1 (Sangiorgi and Capecchi 2008), Prominin/CD133 (Zhu et al. 2009; Snippert et al. 2010), DCMKL-1 (May et al. 2009), and HopX (Takeda et al. 2011); however, little is known concerning how or if these markers themselves contribute to maintenance of self-renewal. Intestinal stem cells may be maintained in two distinct pools with expression of distinct markers (Bmi-1/HopX vs. Lgr5) and different cycling dynamics (Takeda et al. 2011; Tian et al. 2011; Yan et al. 2012). Ablation of Lgr5-expressing stem cells shows little effect on maintenance of the epithelium because of expansion and compensation of the Bmi-1 population (Tian et al. 2011). However, the Lgr5 relative, Lgr4, is expressed in the crypt epithelium as well as the surrounding mesenchyme and was shown using ex vivo culture techniques to be required within the epithelium for maintenance of the crypts (Mustata et al. 2011). How these two populations of stem cells interact to maintain self-renewal is still under investigation.

Wnt signaling is required for maintenance of undifferentiated cells. There is a gradient of Wnt expression, with the highest levels found at the bottom of the crypt, that gradually decreases as cells transit up (van de Wetering et al. 2002; Pinto et al. 2003; Ireland et al. 2004; Sansom et al. 2004). Many Wnt-responsive genes also show highest activation in the crypt region (Van der Flier et al. 2007). Mutations that cause excessive activation of Wnt signaling in the intestinal epithelium, such as those found in the adenomatous polyposis coli (APC) gene, cause massive growth in the epithelium and proliferation into cancerous polyps (Fearon 2011).

Multiple signaling pathways are critical regulators of differentiation that occurs in the crypt region. Active Notch and BMP signaling promotes differentiation into specific

Figure 4. Intestinal homeostasis: Stem cells are located in the bottom of the crypt interspersed with Paneth cells. Stem cells give rise to transit-amplifying (progenitor) cells that rapidly move up the villus and differentiate into functional absorptive, goblet, and enteroendocrine cells. Once cells reach the top of the villus, they are shed into the lumen. (Reprinted, with permission, from Crosnier et al. 2006 © Macmillan.)

intestinal cell types. Underlying the epithelium is mesenchymal tissue that serves as both structural support and signaling center. Expression of Hedgehog (epithelial) and BMP (mesenchymal) ligands serves as a way for mesenchyme and epithelium to communicate through reciprocal signaling, and disruption of either causes defects in

proliferation (Crosnier et al. 2006; Madison et al. 2009). These interactions between cells are important because support cells such as myofibroblasts enhance survival and growth of intestinal epithelium in vitro culture (Ootani et al. 2009). It is unclear how signaling pathways interact to regulate gene expression as cells transition from stem to

differentiated states within the epithelium, because the concentrations of the various ligands have to be modulated across the very small distances that separate stem, progenitor, and differentiated cells.

Positioning within the crypt/villus axis and cell migration is essential for regulation of proliferation and differentiation. EphB and Ephrin-B levels vary with position along the crypt/villus axis (Batlle et al. 2002). The migratory behavior of the cell is tightly correlated with its differentiation status (Wimmer-Kleikamp et al. 2004; Pasquale 2005; Vearing and Lackmann 2005). Components of the Eph–ephrin signaling pathway are targets of Wnt/ β -catenin signaling (Batlle et al. 2002). Recent evidence has shown that Eph–ephrin signaling is also dependent on Notch and TGF- β signaling; however, this may be due indirectly through modulation of Wnt/ β -catenin (Koo et al. 2009; Furukawa et al. 2011). Compound EphB2/EphB3 mutant mice show differentiated cells occupying positions in the proliferative zone and a reduced proliferative zone, suggesting that the EphB receptors play a role in repelling the downward migration of differentiated cells (Batlle et al. 2002). A comprehensive investigation of the transcriptional profile of Eph receptors by Genander et al. (2009) showed that EphB controls proliferation and cell positioning through Cyclin D1 and phosphatidylinositol 3-kinase (PI3K), respectively.

As mentioned above, intestinal epithelial cells are classified as either absorptive enterocytes (termed “colonocytes” in the colon) or secretory cells (goblet, Paneth, and enteroendocrine cells). Notch signaling activates Hes1, which antagonizes enterocyte fate. Notch works by lateral inhibition to prevent adjacent cells from adopting the same fate as the signal-emitting cell, thus controlling the final composition of differentiated cell types (Artavanis-Tsakonas et al. 1999; Gaiano et al. 2000). Secretory cells depend on Math1 expression in progenitor cells (Yang et al. 2001; Shroyer et al. 2007; VanDussen and Samuelson 2010). Math1 then activates the expression of Sox9, Klf4, and NeuroD/Ngn3 to direct full differentiation into Paneth, goblet, and enteroendocrine cells, respectively (Naya et al. 1997; Jenny et al. 2002; Katz et al. 2002; Lee et al. 2002; Mori-Akiyama et al. 2007). More detail on intestinal epithelial differentiation can be found in a recent review (May and Kaestner 2010).

5.2 Homeostasis in the Liver

Much work has been done to investigate the ability of the adult liver to regenerate, because this process is important in liver transplantation. The liver can fully regenerate after an acute injury, for instance, the surgical removal of 70% of the liver mass (partial hepatectomy). However, the ability

for the liver to recover after chronic injury is often compromised. Multiple signaling pathways including FGF, BMP, Wnt, and Notch have been shown to be involved in this recovery process of the liver (Bohm et al. 2010). This rapid growth process is largely due to hepatocyte replication (Evarts et al. 1987, 1989). However, in situations in which hepatocyte replication is blocked, facultative hepatic progenitors, often referred to as “oval cells,” are thought to contribute to liver repopulation. Recently, results of genetic lineage tracing experiments provided strong evidence for this epithelial bipotential adult progenitor cell. The *Foxl1-Cre* transgene, which is normally silent in the liver, was activated in cells near the portal triad following toxic liver injury. In *Foxl1-Cre, Rosa26R* double transgenic mice, blue cells appeared regardless of the nature of liver injury (Sackett et al. 2009). Many of these cells were proliferative and were shown over time to develop into cholangiocytes or hepatocytes. Thus, by these stringent *in vivo* criteria, *Foxl1-Cre* expression marks a bipotential progenitor of both epithelial lineages in the liver. In fact, when *Foxl1-Cre*-labeled cells were isolated and placed in culture, they proved to be clonogenic, and these clonal cell lines could be differentiated toward the hepatocyte and cholangiocyte lineage *in vitro* (Shin et al. 2011). A similar population of bipotential and clonogenic liver progenitor cells was also isolated based solely on the expression of specific cell surface antigens from biliary cells following liver injury (Dorrell et al. 2011). One can envision that in the future the isolation and *ex vivo* expansion and differentiation of these hepatic progenitors might be put to therapeutic use.

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