Chapter 1

Endoderm Development: From Progenitors to Organ Buds

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Introduction

Gut development in mammalian embryos begins with the recruitment of precursor cells to the endoderm layer during gastrulation (Figure 1.1A). This event occurs 6–7 days after fertilization in the mouse and 2–2.5 weeks after conception in humans. The endoderm undergoes a series of morphogenetic movements to first form the foregut and hindgut invaginations, separated by an open midgut region (Figure 1.1B). Following the closure of the lateral body wall, the endoderm forms an epithelial tube (Figure 1.1C), which constitutes the embryonic gut. Localized proliferation, multilayering, and folding of the epithelium result in the formation of the pharyngeal pouches and outgrowth of the organ buds such as the thyroid, lung, liver, gall bladder, and pancreas. Formation of embryonic gut tube is completed by E9-10 in the mouse (~6 weeks of gestation in humans). The endoderm of the embryonic gut contributes to the epithelial lining of the digestive tract, which later acquires organ-specific architecture, such as the stratified epithelium of the pharynx and esophagus, the glandular epithelium of the stomach, and the villous epithelium of the intestine. The endoderm also gives rise to cells lining the ducts that connect the tubular gut with the associated organs, such as the tracheobronchial tree of the lung, the bile duct of the gall bladder, and the pancreatic duct, as well as the epithelial cells within the associated organs (e.g., hepatocytes and biliary cells of the liver and pancreatic acinar, ductular, and islet cells). The gut and its organs also contain neural tissues derived from ectoderm (see Chapters 16 and 17), as well as tissues derived from the mesoderm, including the serosa (see Chapter 11), musculature, fibroblasts, vasculature, and lymphoid tissues.

In this chapter, we review our current knowledge of the early stages of endoderm development and its organ derivatives, focusing on the information gleaned from studying early postimplantation development in the mouse. We discuss the allocation of progenitor cells

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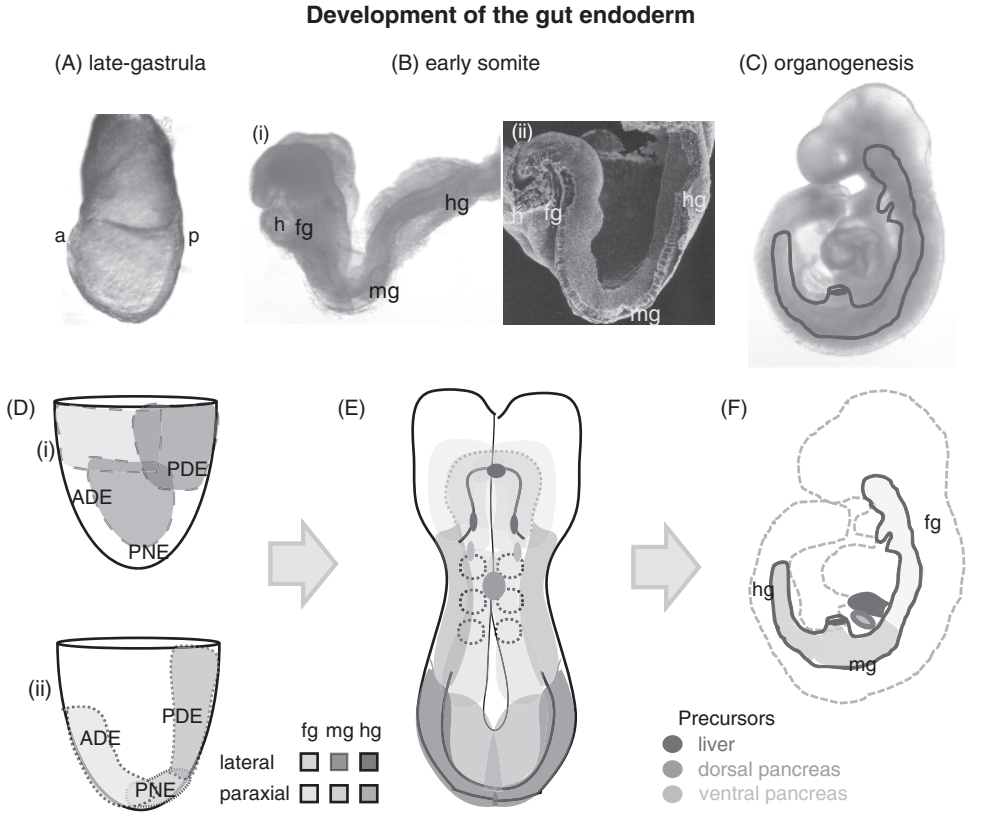


Figure 1.1. *Morphogenesis of the embryonic gut*: (A) Precursor cells of the embryonic gut are localized in the outer cell layer (the endoderm) of the late gastrula (anterior, a; posterior, p). (B) This endoderm cell layer contributes to the foregut (fg) adjacent to the heart (h), and lines the prospective mid- (mg) and hindgut (hg) of the early-somite-stage embryo; (i) whole embryo, (ii) bisected embryo. (C) The embryonic gut (outlined) in the organogenesis stage embryo. (D–F) Developmental fates of the gut endoderm. (D) The precursor cells of the gut endoderm are regionalized as the anterior definitive endoderm (ADE, underneath the head folds), the perinodal endoderm (PNE, in the vicinity of the node and somites) and posterior definitive endoderm (PDE, associated with the PS). (i) endoderm of the lateral part of the embryonic gut, (ii) endoderm of the paraxial part of the embryonic gut. (E) Descendants of cells at different locations in the ADE, PNE, and PDE (color-coded sites) of the early-head-fold-stage embryo have been mapped to specific parts of the foregut, midgut, and the hindgut of the early-somite-stage embryo. The distribution of the progeny of lateral and paraxial endoderm cell populations in (D) the late-gastrula-stage embryo is shown in a schematic diagram of the embryonic gut of (E) the early-somite-stage embryo. Liver (magenta) and pancreas (blue) are formed by convergence of multiple distinct precursor cell populations into a composite organ that buds off from the embryonic gut. (F) The three major segments of the embryonic gut of the early-somite-stage embryo constitute the corresponding (color-coded) foregut (fg), midgut (mg), and hindgut (hg) of the organogenesis-stage embryo. Source: Tam PP, Khoo PL, *et al*. 2004. Regionalization of cell fates and cell movement in the endoderm of the mouse gastrula and the impact of loss of Lhx1(Lim1) function, *Dev Biol* 274(1): 171–87. Copyright 2004, with permission from Elsevier. (*See insert for color representation of this figure*.)

to the endoderm lineage, the morphogenesis of the gut tube including the cellular changes that accompany the formation of the organ primordia, and the signals, tissue interactions, and transcriptional regulation that control regionalization, tissue patterning, and formation of gut-derived organs.

Definitions

Gastrulation: Developmental stage (commencing at around embryonic day 6.5 in the mouse) in which the primary germ layers (ectoderm, mesoderm, and definitive endoderm) are formed. From these, all tissues will develop.
Epiblast: Cells derived from the inner cell mass of the embryo that will give rise to endoderm, mesoderm, and ectoderm of the embryo during gastrulation.
Primitive streak: The conduit through which epiblast cells pass and emerge as precursor cells of either the gut endoderm or the mesoderm.
Epithelial–mesenchymal transition: Process by which epithelial cells, typically forming a barrier on the surface of a structure and *characterized by tight junctions and polarized morphology*, lose these characteristics and adopt a less regular, morphology and become migratory.
Mesenchymal–epithelial transition: Reverse process in which mesenchymal cells gain characteristics of polarized epithelial cells.
Visceral endoderm: Population of endodermal cells derived directly from the primitive endoderm of the blastocyst, which covers the embryo before the formation of the definitive endoderm during gastrulation.
Organogenesis: Stage of development following gastrulation during which the single epithelial layer of endoderm begins to develop organ-specific characteristics.

Emergence of the Progenitor Population

Allocation of the Endoderm Progenitors

Gastrulation commences at E6.5 in the mouse with the formation of the primitive streak (PS). Early studies suggested that definitive endodermal cells emerging from the PS replace the preexisting population of visceral endoderm, which therefore does not contribute to the embryonic gut (1,2). However, recent lineage-tracing studies reveal that some descendants of the visceral endoderm remain, predominantly in the hindgut of early-organogenesis-stage embryos (3). The fate of these visceral endoderm-derived cells in the adult gut is not known, but this finding suggests that the gut endoderm may have two different embryological origins.

Regionalization of Cell Fates

In the mouse, progenitors of the foregut endoderm are first identified in the anterior definitive endoderm (ADE) that underlies the head folds of late-gastrula-stage embryos (Figure 1.1D) (4). Cells overlying the posterior part of the embryo contribute to the hindgut (Figure 1.1D–F), whereas progenitors of the midgut endoderm emerge

later and are intercalated between the expanding ADE and the posterior endoderm. Active recruitment of the cells from the epiblast to the gut endoderm ceases at the completion of gastrulation (5–7). Consequently, all the endodermal derivatives of the digestive tract and the associated organs are generated from a pool of about 6000 cells in the late-gastrula-stage embryo.

Establishment and Maintenance of the Epithelial Endoderm Layer

During gastrulation, incorporation of the gut endoderm precursors into the visceral endoderm layer occurs at multiple sites, resulting in the widespread intercalation of the nascent population. Intercalation is not simultaneous and some prospective gut endoderm cells are incorporated after a period of retention in the mesoderm (3). Initially, the incoming cells can be distinguished by the expression of markers such as *Sox17* and *Foxa2*, which are not expressed by the visceral endoderm at this stage. However, by late gastrulation, all cells within the endoderm layer display a uniform definitive endoderm phenotype irrespective of their origin.

The integration of the gut endoderm precursor cells into the epithelial layer requires a mesenchymal to epithelial transition (MET). MET is driven by *Foxa2* (Table 1.1), a transcription factor that regulates genes involved in cell polarity and cell–cell junctions (8,9). After incorporation, *Sox17* activity becomes essential for the further maintenance and expansion of the endoderm (Table 1.1). In *Sox17*-null embryos, apoptosis is elevated

Table 1.1. Gene activity associated with the formation and maintenance of definitive endoderm and the loss-of-function phenotype.

Gene	Phenotype	Reference
Generation of progenitors		
Nodal	No endoderm formed in null embryos	(71)
Porcn	No endoderm formed, prolonged Pou5f1 expression	(72)
Ctnnb1	No endoderm formed	(73,74)
Lrp5/6	No endoderm formed	(29)
Wnt3	No endoderm formed, prolonged POU5f1 expression	(75)
Drap1	Excessive mesoderm formed at the expanse of endoderm	(76)
Formation of definitive endoderm		
Mixl1	Lack definitive endoderm but excess T and nodal expressing tissue	(77)
Nodal	Hypomorphic mutants form mesoderm but not definitive endoderm	(20)
Foxa2	Lack anterior definitive endoderm; null cells cannot intercalate into preexisting endoderm layer	(9,78,79)
Maintenance and proliferation of definite endoderm		
Sox17	Deficiency in gut due to decreased proliferation and increased apoptosis Abnormal morphogenesis of hindgut	(10,80)
Ctnnb1	Transdifferentiation of endoderm to cardiac mesoderm	(81)

in the foregut, resulting in loss of cells, while the hindgut endoderm does not proliferate properly (10) and *Sox17*-null cells are less able to contribute to the gut endoderm than wild-type cells (11). As a result, the gut tube contains a larger proportion of visceral endoderm-derived cells than wild-type embryos (10). *Sox17* expression in the gut is regulated by Wnt signaling. Loss of β-catenin function in the gut leads to cellular deficiency in the mid- and hindgut, which phenocopies the loss of *Sox17* function (12).

Morphogenesis of the Embryonic Gut

Concurrent with the formation of head folds in early-somite-stage embryos (E7.5-8.0), the cuplike endodermal sheet (Figure 1.1A) begins to transform into a tube. The first step is the formation of the foregut invagination, beginning as a shallow concavity and crescent-shaped fold in the ADE. Further folding of the lateral margins generates an endoderm-lined pocket (Figure 1.1B). In *Sox17*-null embryos, aberrant cell movements result in a wider and shallower foregut (11). It is from the foregut that several organs, including the thyroid, liver, lung, pancreas, and stomach (1) will develop. The precursors of the thyroid diverticulum and the lung bud are localized to the ventral midline of the foregut, whereas the precursor cells of the liver and pancreas reside at multiple sites within the foregut endoderm, as discussed subsequently (13–15). The morphogenetic movements that form the foregut pocket are instrumental in bringing together these precursor cells for assembly into an organ rudiment.

The formation of the hindgut invagination follows shortly after the initiation of foregut development via similar morphogenetic tissue movements (16). The open midgut region is progressively reduced as the foregut and hindgut pockets extend toward one another. A closed gut tube is formed by the fusion of the lateral body folds along the ventral midline and pursing around the umbilical connection with the yolk sac and placenta. Completion of gut closure and internalization of the tube occurs at around E9.0, as the body of the embryo assumes the fetal shape. This marks the initiation of organogenesis.

Signaling Activities Controlling Endoderm Formation

The acquisition of endoderm cell fate is influenced by an evolutionally conserved molecular network of molecules: T-box factors, Nodal/Smads, Gata, Mix, Fox, and Sox activity (Table 1.1) (17,18).

There is strong evidence for a critical role of the Tgfβ family member, Nodal, in the establishment of endoderm (18) (see also Chapters 18–24). Analysis of mutant embryos suggests that a high Nodal level facilitates the specification of the endoderm, while lower activity impedes endoderm differentiation (Table 1.1) (19–21). In the absence of Nodal signaling, no germ layers are formed (Table 1.1) (22). This suggests that there is a requirement for correct regulation of the level of Nodal signaling for endoderm formation. Binding of Nodal to receptors and coreceptors activates an intracellular cascade, promoting endoderm differentiation (23–26), and the level of signaling activity is modulated by Nodal antagonists Cer1 and Lefty1 secreted from anterior visceral endoderm as well as BMP4 from the extraembryonic tissues (27,28). Formation of the

PS, which enables endoderm formation, requires Wnt and Fgf signaling (Table 1.1). Wnt/β-catenin signaling activity acts independently of Fgf signaling, but both pathways are critical for cell movement during the formation of the endoderm (29).

The role of Nodal-related signaling has been demonstrated in mouse and human embryonic stem cells (ESCs) (see Chapter 24). Activin A, another TGFβ family member, was reported to drive mouse and human ESCs to differentiate into *Sox17*-expressing endoderm cells (30,31). However, there are differences in the response of the progenitor cells to these signaling factors, as Nodal-induced ES-derived endoderm cells are more able to contribute to the foregut endoderm *in vivo* and to form pancreatic tissues than Activin A- stimulated cells (32).

Regionalization of the Embryonic Gut

Patterning in the Anterior-Posterior Axis

At the early organogenesis stage, the gut tube is broadly divided into three domains: the foregut, midgut, and hindgut (Figure 1.1F). The foregut region is defined by *Sox2* expression and the mid- and hindgut are marked by expression of *Cdx1* and *2* (Figure 1.2A). In the absence of *Cdx2*, the colon and rectum do not develop. Instead, the gut is closed caudally and the morphology of the small intestinal epithelium resembles that of the esophagus (33). Loss of *Cdx2* expression results in diminished expression of genes that mark the posterior endoderm and upregulation of anterior genes, such as *Sox2*. When *Sox2* is ectopically expressed in the posterior endoderm, the ability of *Cdx2* to activate transcriptional targets is impaired and the posterior endoderm adopts a more anterior fate (34). Therefore, a balance of *Sox2* and *Cdx2* activities is required to direct anterior–posterior (A–P) patterning of the endoderm. More details about the molecular cross talk involved in patterning the early gut tube can be found in Chapters 2, 4, and 7, while additional information about the clinical consequences of aberrant patterning of the gut is presented in Chapters 3, 5 and 8.

The identity of the endoderm along the A–P axis is also defined by instructive signals from the surrounding tissues, including retinoic acid (RA), Wnt, and Fgf signals. RA specifies the posterior pharyngeal endoderm in mice and is required for dorsal pancreas development (35–37). Wnt signaling is also required to establish the A–P axis, and is most active in posterior tissues. Deletion of *Tcf1* and *Tcf4*, transcription factors that activate Wnt signaling target genes, results in embryos with a posterior truncation and a complete absence of the hindgut as early as E8.5, apparently due to an anterior transformation of gut segments. Tissues fated for the duodenum are transformed into the stomach and posterior gut tissues fail to develop (38). Ectopic activation of Wnt signaling by expressing constitutively active (CA) β-catenin in the foregut endoderm induces *Cdx2* expression and represses *Sox2*, and CA β-catenin-expressing cells tend to localize to the posterior endoderm that gives rise to the intestinal epithelial precursors (39). The Fgf pathway also affects the A–P identity of the endoderm: a high concentration of FGF4 induces genes normally expressed in the posterior, whereas lower concentrations induce more anterior genes (40). The potent instructive effects of these soluble signals are being used to direct

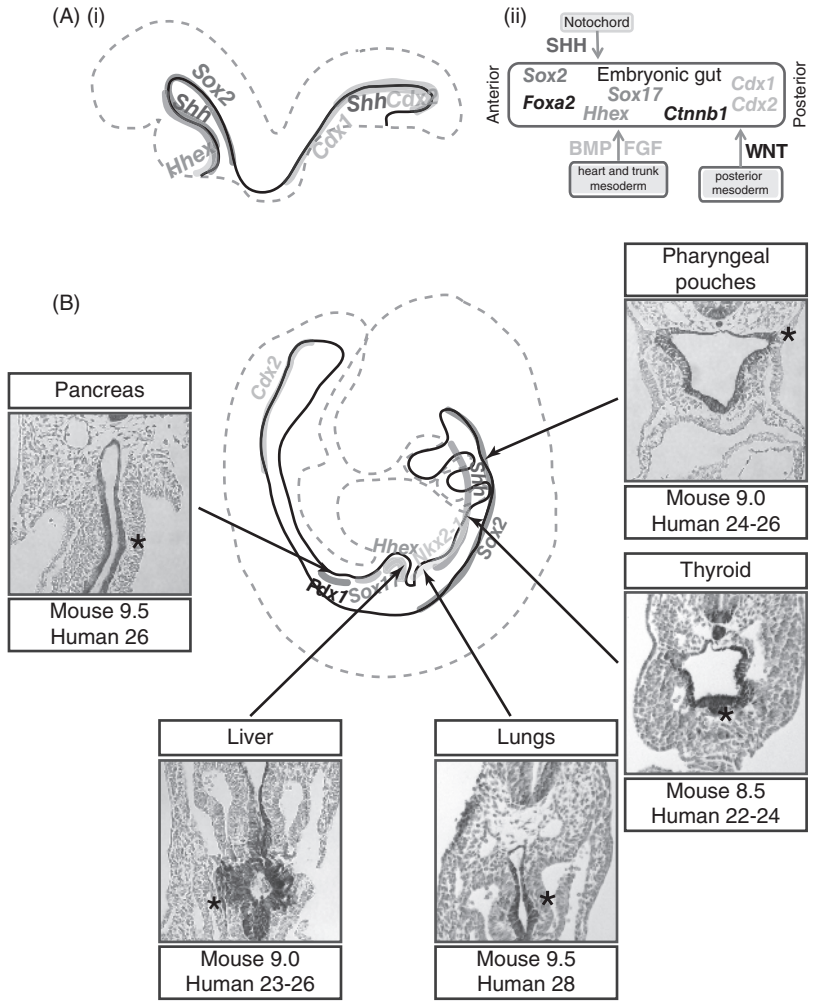


Figure 1.2. *Regionalization of the gut tube and initiation of organ budding*. Regionalized gene expression in the endoderm at E8.5 (A) and E9.5 (B). Anterior–posterior (A–P) and dorsal–ventral (D–V) patterning of the endoderm are effected by factors expressed in the endoderm that respond to signals from the adjacent germ layers (e.g., Shh from the notochord and FGF and BMP from the mesoderm in A (i) and (ii), which demarcate broad A–P (e.g., *Cdx2*, *Sox2*) or D–V (*Nkx2-1*, *Sox2*, *Shh*) domains. These expression domains herald the initiation of organ budding at specific sites (eg: *Hhex*, *Pdx1*, *Sox17*). (B) The early organ buds (asterisks) are shown in histological sections, with the endoderm indicated by the dark staining for β-galactosidase reporter activity, which has been activated by endoderm-specific *Foxa2*-Cre recombinase activity (70). The domains of gene expression associated with the specific organ buds are marked. The time of development (in days after conception) when each organ bud first appears in mice and humans is indicated below each image. (*See insert for color representation of this figure*.)

ESCs or induced pluripotent stem cells to fates characteristic of different gut regions (see Chapter 23).

Patterning in the Dorsal–Ventral Dimension

In addition to the demarcation of domains along the A–P axis of the gut tube, the endoderm acquires dorsal–ventral (D–V) positional information. The liver, lungs, trachea, and thyroid develop from the ventral endoderm, whereas the esophagus forms dorsally. The subdivision of the foregut into esophageal (dorsal) and tracheal (ventral) tubes is enabled by Bmp, Wnt, and Hedgehog signaling. Inactivation of the Bmp receptor genes *Bmpr1a* and *Bmpr1b* results in failure to partition the gut tube and causes the arrest of tracheal development (41). *Nkx2-1*, a transcription factor required for separation of the esophagus and trachea, requires Bmp signaling for its expression in the tracheal region (41,42). Conversely, *Sox2* is directly repressed by Bmp signaling via Smad factors, and is ectopically activated in the ventral regions when Bmp signaling is disrupted (41). WNT2 and WNT2b signaling via *Ctnnb1* is involved in D–V patterning of the endoderm. Loss of *Wnt2/2b* or *Ctnnb1* results in a lack of lung development in the ventral endoderm; and ectopic activation of the *Ctnnb1*-dependent Wnt signaling pathway biases fates of the foregut endoderm to lung epithelium, with loss of SOX2 expression that typically marks dorsal endoderm (43,44). Finally, *Shh* expression in the dorsal foregut is required for the division of the esophagus and trachea. Impaired SHH signaling results in esophageal atresia and close juxtaposition of the esophageal and tracheal tubes (45,46). Separation of the esophagus and trachea is considered in more detail in Chapter 2 and the clinical consequences of abnormal patterning of these structures are discussed in Chapter 3.

Laterality of the Gut Tube

Although the gut tube initially develops as a bilaterally symmetrical structure, the gut and associated organs acquire left–right (L–R) asymmetries during subsequent development. The stomach forms by unequal growth, predominantly on the left side of the gut tube, while branching of the lung buds occurs asymmetrically with multiple lobes forming on the right side. Looping and rotation of the gut tube also occurs in a stereotypic manner; gut malrotation is seen in *Shh* mutants and is a significant clinical problem (see Chapters 7 and 8). Looping of the gut tube is preceded by the separation of the tube from the body wall by the formation of the dorsal mesentery. This process is influenced by the activity of Robo, a cell surface receptor for Slit signaling (47). Subsequently, differential rates of cell proliferation in the left and right mesenchyme investing the embryonic gut leads to the asymmetrical looping and rotation of the gut (48).

Normal development of the endoderm is required to establish L–R asymmetry and *Sox17* plays an important role in this process. Not only is asymmetric endoderm cell displacement lost in *Sox17*-null embryos, demonstrating a specific role in laterality of the endoderm (11), but defective gap junction formation in the endoderm disrupts the propagation of signaling activities that are instrumental for global L–R tissue patterning (49,50).

Formation of the Organ Primordia

Initiation of Organ Budding

Organ budding is accompanied by localized remodeling of the single-layered endodermal epithelium, coincident with cytoskeletal changes that may be regulated by the action of Rho GTPases (51). Budding of the liver primordium commences with the acquisition of a thickened pseudostratified epithelial morphology, before the expansion of a multilayered bud into the surrounding mesenchyme. This process requires the transcription factor Hhex (52), a target of BMP signaling (53). The thyroid and pancreas primordia similarly develop by multilayering of the epithelium, but the thyroid becomes detached from the gut tube as it develops (54) and the pancreatic endoderm reverts to a simple epithelial layer as branching commences (55). During budding, the organ primordia retain expression of epithelial markers such as E-cadherin, indicating that they do not undergo complete epithelial–mesenchymal transition (EMT) (55,56).

Budding from the primitive gut tube requires inductive interactions with the surrounding mesenchyme to promote proliferation and differentiation (Figure 1.2B) (57,58). The critical role of endoderm–mesoderm interactions is demonstrated in the development of the pancreas (59,60) and liver (37,61), as detailed subsequently and in Chapters 12 and 14.

Organ-Specific Developmental Features

Liver Bud and Bile Duct

The liver bud is formed at E9-9.5 following rapid hepatoblast proliferation, delamination, and invasion into the mesenchyme of the septum transversum (Figure 1.2C) (52). Specification of hepatic cell fate requires Fgf signals emanating from the heart mesoderm and Bmp from the septum transversum. Blocking these signals or removal of the cardiogenic mesoderm impairs the induction of liver development (58,62,63). A gradient of Fgf activity patterns the foregut endoderm, with highest activity inducing lung-specific gene expression and lung bud formation (Figure 1.2C) and moderate levels favoring liver development (61). *Hhex* regulates foregut endodermal cell proliferation and movement toward the Fgf-producing cardiac mesoderm (64); and loss of *Hhex* function results in failure of the liver bud to extend into the septum transversum (65). While the liver and intrahepatic ducts originate from the anterior hepatic diverticulum, the gall bladder and the extrahepatic bile ducts share a common origin with the ventral pancreas and are not derived from the hepatic diverticulum (66). *Sox17* activity is not essential for liver formation (*Sox17* represses liver-specific gene expression (11)), but is sufficient to induce biliary cell fates and is required to enable the development of the bile duct and gall bladder (66,67).

Pancreatic Buds

Pancreas morphogenesis starts with the formation of the dorsal and ventral pancreatic buds. Although both buds arise from the endoderm epithelium, and both display similar *Hlbx9* and *Pdx1* activity, these two buds are induced by different signals from different

mesodermal tissues. In the mouse embryo, the dorsal bud arises from the medial endoderm at the level of somites 2–4 and requires RA signalling (37) as well as secreted factors (e.g., Activin and Fgf) from the notochord and dorsal aorta that repress *Shh* expression in the dorsal pancreatic bud (68). In the dorsal and ventral buds, the epithelium proliferates and undergoes branching morphogenesis to form a tubular network. These tubules will eventually give rise to the exocrine acinar cells, tubular ducts, and cells that delaminate from the tubular epithelium to form the hormone-producing islets (69).

Relevance of the Embryology of the Gut to Clinical Conditions

Our understanding of early development in the gastrointestinal organs and the tissues that give rise to them has pointed the way to the elucidation of the pathologies in these organs. Defects in the early phases of development are likely to have the most drastic impact on the morphogenesis and function of organs, which could impact on postnatal survival and morbidity.

There are many examples of clinical conditions affecting the gastrointestinal system that may be traced to errors in early development (Table 1.2). Dysplasias, atresias, and hypoplasias of the organs are likely to be the consequence of deficiencies in the formation and the initial growth of the organ primordia. Esophageal atresia and stenosis and tracheoesphageal fistula may result from errors in the D–V patterning of the embryonic foregut, leading to abnormal partitioning of the foregut into oesphagus and trachea. Improper rotation of the intestine can lead to volvulus and to several congenital disorders including gastroschisis, intestinal atresias, and omphalocele. Fistulas are common in anorectal malformations, and can be secondary to anal atresia or stenosis, the cause of which may be traced to abnormal patterning and morphogenesis of the hindgut.

Table 1.2. Congenital malformations potentially associated with errors of early development of endoderm derivatives.

Morphogenetic process disrupted	Examples of developmental defects
Organ bud formation	Agenesis: thyroid, liver, pancreas
Growth and morphogenesis of the organ primordium	Dysgenesis/hypoplasia: thyroid, pancreas Extrahepatic biliary duct: artresia, anatomical anomalies Annular pancreas Anomalies of liver lobulation
Partitioning of the foregut	Esophageal artresia and stenosis Tracheoesophageal fistula
Closure of the midgut/lateral body folds	Gastroschisis Omphalocoele, umbilical hernia Persistent Meckel's diverticulum
Morphogenesis of the gut tube	Atresia, stenosis, fistula: intestine, colon, rectum Malposition of stomach Malrotation, fixation of intestine

The knowledge gleaned from the studies of experimental models of endoderm development and gut malformation therefore provides useful insights into the pathogenesis of birth defects of the digestive tract and accompanying organs. In addition, the genetic information collated from the investigation of mutant mouse models will help focus our attention on the most plausible candidate – genetic determinants – in future clinical investigations of the congenital malformations of the gastrointestinal organs. Finally, we are already seeing that decades of intense research in gut developmental biology is leading to improved ability to grow and manipulate gastrointestinal tissues *in vitro* (see Chapters 23 and 24), providing opportunities never before available for the study of human disease.

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References

1. Lawson KA, Meneses JJ, *et al*. 1986. *Dev Biol* 115: 325–39.
2. Tam PP, Beddington RS. 1987. *Development* 99: 109–26.
3. Kwon GS, Viotti M, *et al*. 2008. *Dev Cell* 15: 509–20.
4. Tam PP, Khoo PL, *et al*. 2004. *Dev Biol* 274: 171–87.
5. Tam PP. 1984. *J Embryol Exp Morphol* 82: 253–66.
6. Wilson V, Beddington RS. 1996. *Mech Dev* 55: 79–89.
7. Cambray N, Wilson V. 2007. *Development* 134: 2829–40.
8. Tamplin OJ, Kinzel D, *et al*. 2008. *BMC Genomics* 9: 511.
9. Burtscher I, Lickert H. 2009. *Development* 136: 1029–38.
10. Kanai-Azuma M, Kanai Y, *et al*. 2002. *Development* 129: 2367–79.
11. Pfister S, Jones VJ, *et al*. 2011. *Int J Dev Biol* 55: 45–58.
12. Engert S, Burtscher I, *et al*. 2013. *Development* 140: 3128–38.
13. Tremblay KD, Zaret KS. 2005. *Dev Biol* 280: 87–99.
14. Angelo JR, Guerrero-Zayas MI, *et al*. 2012. *PLoS One* 7: e40707.
15. Miki R, Yoshida T, *et al*. 2012. *Mech Dev* 128: 597–609.
16. Franklin V, Khoo PL, *et al*. 2008. *Mech Dev* 125: 587–600.
17. Tam PP, Kanai-Azuma M, *et al*. 2003. *Curr Opin Genet Dev* 13: 393–400.
18. Tam PP, Loebel DA. 2007. *Nat Rev Genet* 8: 368–81.
19. Dunn NR, Vincent SD, *et al*. 2004. *Development* 131: 1717–28.
20. Lowe LA, Yamada S, *et al*. 2001. *Development* 128: 1831–43.
21. Vincent SD, Dunn NR, *et al*. 2003. *Genes Dev* 17: 1646–62.
22. Zhou X, Sasaki H, *et al*. 1993. *Nature* 361: 543–7.
23. Hart AH, Willson TA, *et al*. 2005. *Biochem Biophys Res Commun* 333: 1361–9.
24. Hoodless PA, Pye M, *et al*. 2001. *Genes Dev* 15: 1257–71.
25. Izzi L, Silvestri C, *et al*. 2007. *EMBO J* 26: 3132–43.
26. Sakuma R, Ohnishi Yi Y, *et al*. 2002. *Genes Cells* 7: 401–12.

27. Perea-Gomez A, Vella FD, *et al*. 2002. *Dev Cell* 3: 745–56.
28. Pereira PN, Dobreva MP, *et al*. 2012. *Development* 139: 3343–54.
29. Kelly OG, Pinson KI, *et al*. 2004. *Development* 131: 2803–15.
30. Kubo A, Shinozaki K, *et al*. 2004. *Development* 131: 1651–62.
31. D'Amour KA, Agulnick AD, *et al*. 2005. *Nat Biotechnol* 23: 1534–41.
32. Chen AE, Borowiak M, *et al*. 2013. *Development* 140: 675–86.
33. Gao N, White P, *et al*. 2009. *Dev Cell* 16: 588–99.
34. Raghoebir L, Bakker ER, *et al*. 2012. *J Mol Cell Biol* 4: 377–85.
35. Wendling O, Dennefeld C, *et al*. 2000. *Development* 127: 1553–62.
36. Martin M, Gallego-Llamas J, *et al*. 2005. *Dev Biol* 284: 399–411.
37. Molotkov A, Molotkova N, *et al*. 2005. *Dev Dyn* 232: 950–7.
38. Gregorieff A, Grosschedl R, *et al*. 2004. *EMBO J* 23: 1825–33.
39. Sherwood RI, Maehr R, *et al*. 2011. *Mech Dev* 128: 387–400.
40. Wells JM, Melton DA. 2000. *Development* 127: 1563–72.
41. Domyan ET, Ferretti E, *et al*. 2011. *Development* 138: 971–81.
42. Yuan B, Li C, *et al*. 2000. *Dev Dyn* 217: 180–90.
43. Goss AM, Tian Y, *et al*. 2009. *Dev Cell* 17: 290–8.
44. Harris-Johnson KS, Domyan ET, *et al*. 2009. *Proc Natl Acad Sci* 106: 16287–92.
45. Ioannides AS, Henderson DJ, *et al*. 2003. *J Pediatr Surg* 38: 29–36.
46. Litingtung Y, Lei L, *et al*. 1998. *Nat Genet* 20: 58–61.
47. Domyan ET, Branchfield K, *et al*. 2013. *Dev Cell* 24: 52–63.
48. Davis NM, Kurpios NA, *et al*. 2008. *Dev Cell* 15: 134–45.
49. Viotti M, Niu L, *et al*. 2012. *PLoS Biol* 10: e1001276.
50. Saund RS, Kanai-Azuma M, *et al*. 2012. *Development* 139: 2426–35.
51. Loebel DA, Studdert JB, *et al*. 2011. *Development* 138: 4511–22.
52. Bort R, Signore M, *et al*. 2006. *Dev Biol* 290: 44–56.
53. Zhang W, Yatskievych TA, *et al*. 2002. *J Biol Chem* 277: 45435–41.
54. Fagman H, Nilsson M. 2010. *Mol Cell Endocrinol* 323: 35–54.
55. Villasenor A, Chong DC, *et al*. 2010. *Development* 137: 4295–305.
56. Fagman H, Grande M, *et al*. 2003. *Endocrinology* 144: 3618–24.
57. Shannon JM, Nielsen LD, *et al*. 1998. *Dev Dyn* 212: 482–94.
58. Gualdi R, Bossard P, *et al*. 1996. *Genes Dev* 10: 1670–82.
59. Kim SK, Hebrok M, *et al*. 1997. *Development* 124: 4243–52.
60. Ahlgren U, Pfaff SL, *et al*. 1997. *Nature* 385: 257–60.
61. Serls AE, Doherty S, *et al*. 2005. *Development* 132: 35–47.
62. Calmont A, Wandzioch E, *et al*. 2006. *Dev Cell* 11: 339–48.
63. Rossi JM, Dunn NR, *et al*. 2001. *Genes Dev* 15: 1998–2009.
64. Bort R, Martinez-Barbera JP, *et al*. 2004. *Development* 131: 797–806.
65. Martinez Barbera JP, Clements M, *et al*. 2000. *Development* 127: 2433–45.
66. Spence JR, Lange AW, *et al*. 2009. *Dev Cell* 17: 62–74.
67. Uemura M, Hara K, *et al*. 2010. *Biochem Biophys Res Commun* 391: 357–63.
68. Hebrok M, Kim SK, *et al*. 1998. *Genes Dev* 12: 1705–13.
69. Kesavan G, Sand FW, *et al*. 2009. *Cell* 139: 791–801.
70. Park EJ, Sun X, *et al*. 2008. *Dev Dyn* 237: 447–53.
71. Conlon FL, Lyons KM, *et al*. 1994. *Development* 120: 1919–28.
72. Biechele S, Cox BJ, *et al*. 2011. *Dev Biol* 355: 275–85.
73. Huelsken J, Vogel R, *et al*. 2000. *J Cell Biol* 148: 567–78.
74. Morkel M, Huelsken J, *et al*. 2003. *Development* 130: 6283–94.
75. Liu P, Wakamiya M, *et al*. 1999. *Nat Genet* 22: 361–5.

76. Iratni R, Yan YT, *et al*. 2002. *Science* 298: 1996–9.
77. Hart AH, Hartley L, *et al*. 2002. *Development* 129: 3597–608.
78. Ang SL, Rossant J. 1994. *Cell* 78: 561–74.
79. Weinstein DC, Altaba A, *et al*. 1994. *Cell* 78: 575–88.
80. Hara K, Kanai-Azuma M, *et al*. 2009. *Dev Biol* 330: 427–39.
81. Lickert H, Kutsch S, *et al*. 2002. *Dev Cell* 3: 171–81.