PART 1

Anatomy and development

CHAPTER 1

Development and differentiation of the gastrointestinal system

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Chapter menu

Developmental biology seeks to understand how organisms are formed. Central to the field are questions about differentiation, morphogenesis, and growth – the processes that give rise to our physical appearance, physiology, and (when perturbed) diseases. Despite many years of intensive research, our understanding of the molecular mechanisms that guide normal vertebrate development remains incomplete. Perturbations in these processes, resulting in congenital malformations or functional diseases, are difficult to study because developmental insults may occur weeks or months before a defect is detectable.

While an understanding of how the body is formed is intrinsically important, it is also clinically relevant. Exploiting developmental processes offers the promise of creating "cell therapies" – growing tissues ex vivo for use in tissue transplantation and augmentation, or coaxing cells in vivo to acquire characteristics that restore function. Fulfilling this promise will undoubtedly require a more complete delineation of developmental mechanisms.

The chapter has been divided into several sections to facilitate an appreciation for the complexity of the development of the gastrointestinal system. *Early development* outlines the basic mechanisms by which the embryo achieves a spatial "pattern," setting the stage for further developmental steps. *Organogenesis* focuses on the known molecular mechanisms that guide development of the liver, the pancreas, and the lumenal gastrointestinal tract. *Developmental physiology* samples important events during the functional maturation of the gastrointestinal tract. *Disorders of development*, the fourth and final section, focuses on specific diseases that highlight the relationship between molecular events and clinical consequences. The embryology of the human gastrointestinal tract involves many temporally and spatially regulated tissue interactions and the creation of many varied structures. The ensuing discussion focuses on the mechanisms of gastrointestinal development. What hurdles must be surmounted to create a gastrointestinal tract with normal form and function and how can these processes be controlled for therapeutic benefit?

Early development

The complex anatomy of adult mammals has its origins in a single fertilized egg. The transformation from egg to newborn occurs in many steps marked by discrete milestones (Figure 1.1). The fertilized egg initially grows in cell number through cleavage divisions into a blastocyst – an asymmetrical collection of cells containing the precursors of both embryo and placenta – which implants in the uterine wall. After implantation, the

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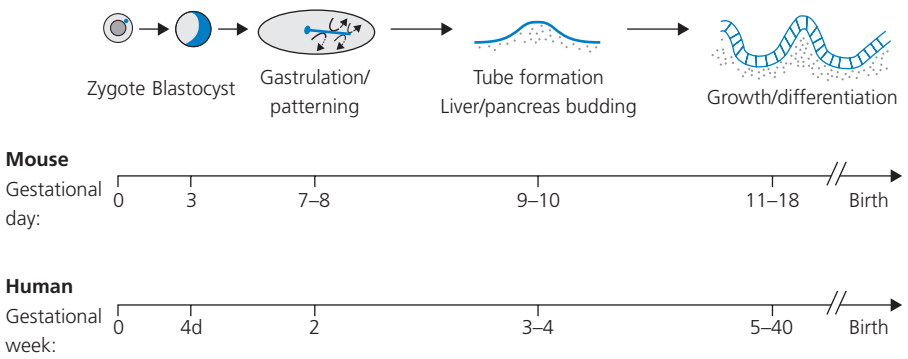


Figure 1.1 Overview of gastrointestinal development. Timelines for milestones of mouse (18-day gestation) and human (40-week gestation) embryogenesis. See text for details of individual steps.

endoderm – the cell layer from which the epithelium of all gastrointestinal organs is derived – is formed through the process of *gastrulation*. Subsequently, the endoderm is segmented (*patterned*) into domains that become *committed* to give rise to specific organs. Finally, solid organ buds emerge from the gut tube, and organogenesis proceeds with the processes of *differentiation* and *morphogenesis*.

Many studies of gastrointestinal development have been performed in model organisms, including fruit flies (*Drosophila melanogaster*), frogs (*Xenopus laevis*), zebrafish (*Danio rerio*), and mice (*Mus musculus*). Despite differences in anatomy and timing of development (Figure 1.1), most studies suggest that many developmental mechanisms in the mouse are comparable to those in the human. Information gained from model organisms can therefore be reasonably extrapolated to humans because of evolutionary conservation of mechanism.

Gastrulation and tube formation

To understand gastrointestinal form and function, it is necessary to recognize the steps that precede organogenesis. The most important of these is *gastrulation*, the process by which three distinct "germ layers" – ecotoderm, mesoderm, and endoderm – are formed. After implantation in the uterus, the embryo exists as a disc of cells called the epiblast. Two structures – the node and the primitive streak – appear in the posterior half of the epiblast layer, and cells migrate caudally toward, and down through, the primitive streak, giving rise to new layers of cells – the embryonic mesoderm and embryonic endoderm (Figure 1.2). As a consequence of gastrulation, the three axes of the embryo are also established: the anterior–posterior (or rostral–caudal) axis is defined by the location of the primitive streak (posterior); the dorsal–ventral axis is defined by the ectoderm (dorsal) and endoderm (ventral); and the left–right axis is defined by the other two axes.

How the cells that migrate through the primitive streak are instructed to become mesoderm or endoderm is incompletely understood. Phylogenetic analyses of organisms including fish, frogs, and mice point to a conserved pathway for endoderm development that involves the transforming growth factor-β (TGF-β)-related nodal pathway and several classes of DNA-binding transcription factors that belong to the homeobox, forkhead (winged helix), zinc finger, and high mobility group (HMG) families [1].

The tubular structure of the gut arises from two ventral invaginations that form at the anterior (proximal) and posterior (distal) ends of the embryo after gastrulation (see Figure 1.2). These will eventually form the structures of the foregut and hindgut, respectively. The anterior fold, or anterior intestinal portal, and the caudal fold, or caudal intestinal portal, move towards each other and meet in the midline of the embryo at the level of the yolk sac. As a result, ventral structures close to the midline (e.g., lung, liver, and ventral pancreas) derive from endoderm that is distinct and distant from the endoderm that gives rise to dorsal structures (e.g., dorsal pancreas). This arrangement means that the dorsal and ventral portions of the pancreas are independently induced, although these tissues eventually combine to form one functioning organ.

Several genes have been identified that are required for tube formation of the gut (Table 1.1). One of these genes encodes GATA4, a zinc finger-containing, DNA-binding protein. Although endoderm is able to develop in *Gata4* mutant mice, formation of the anterior intestinal portal is faulty and results in failure to form a foregut [2–4]. Other genes that are required for tube formation or closure include those encoding the forkhead-winged helix DNA-binding transcription factor FOXA2 (previously HNF3B), which has additional roles in foregut and midgut development, and the FURIN protease, which may be necessary to process TGF-β signals [5–7]. A critical and conserved role for two other families – the HMG domain-containing SOX factors and the homeodomain-containing MIX factors – has been demonstrated [8,9]. GATA4-like and FOXA-like factors are involved in gut development in organisms as distantly related to mammals as the fruit fly *Drosophila* and the nematode *Caenorhabditis elegans* [10], whereas the involvement of SOX and MIX factors appears to become important only in "higher" vertebrates, including zebrafish, *Xenopus*, and mammals.

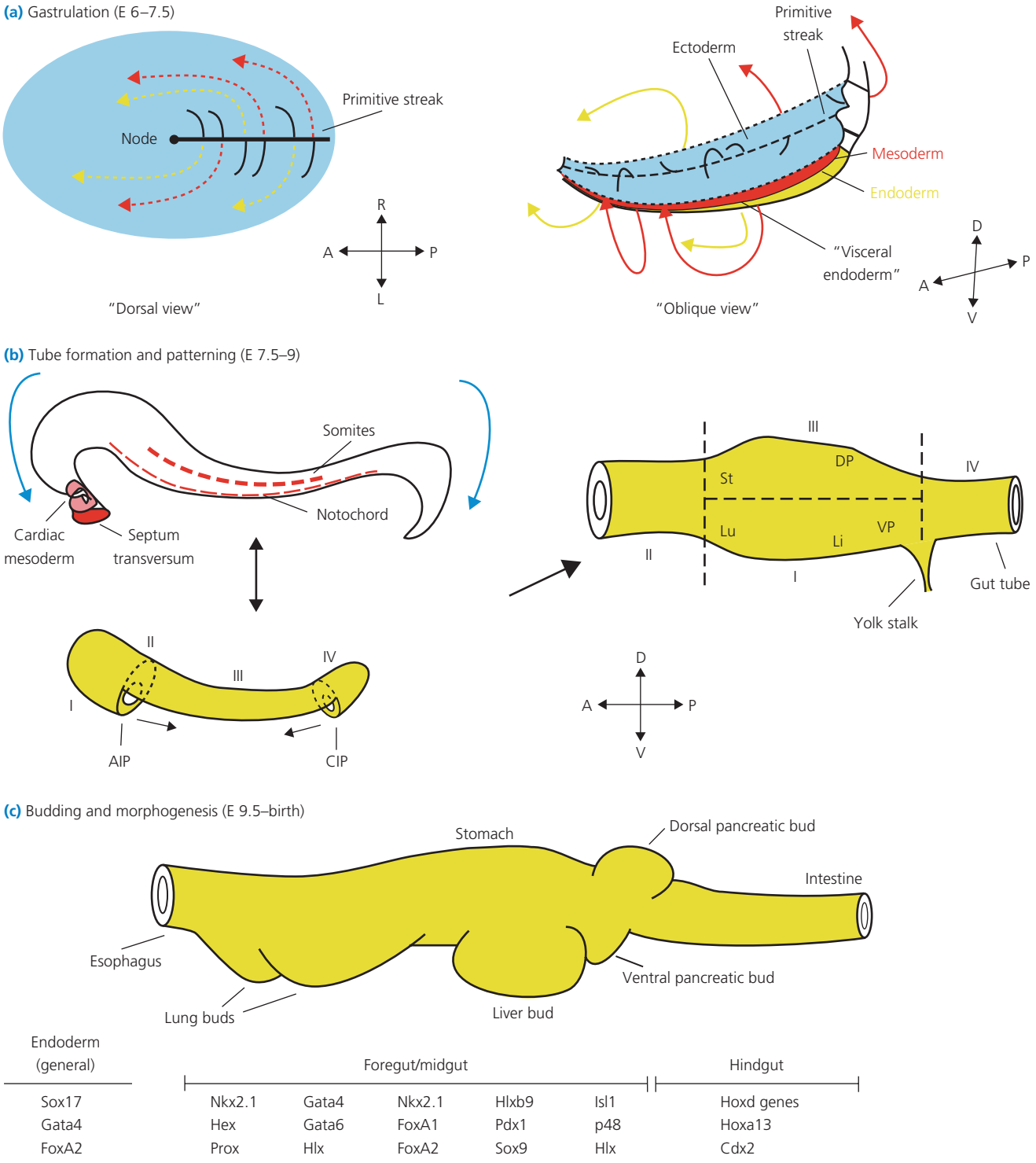


Figure 1.2 Major events in early mammalian endoderm development. **(a)** Gastrulation. (*left*) The embryonic epiblast (blue) viewed from above. Epiblast cells (solid black) migrate down through the primitive streak, becoming mesoderm (dashed red) or endoderm (dashed yellow) cells. (*right*) Oblique view of migrating epiblast cells, in which formation of the new mesoderm and endoderm is visible. **(b)** Tube formation and patterning. (*left*) The mesoderm and ectoderm have been pulled back to reveal the endoderm below. At this stage, the anterior endoderm is adjacent to the cardiac mesoderm and septum transversum (which mediate liver induction) whereas more dorsal portions of the endoderm are in contact with the notochord (which mediates pancreas induction). The folds of the anterior intestinal portal (AIP) and caudal intestinal portal (CIP) form the gut tube as they migrate towards each other at the midline. Blue arrows indicate the process of turning, by which the embryo switches from a convex to a concave shape, with the endoderm on the "inside". The roman numeral designations are derived from fate-mapping studies and indicate the prospective regions of endoderm that will give rise to later endoderm derivatives. (*right*) The relative position of endoderm domains changes with the completion of gut tube folding; the region that previously constituted the most anterior portion of endoderm (I) shifts to the ventral midline and gives rise to lung (Lu), liver (Li), and ventral pancreas (VP). **(c)** Budding and morphogenesis. Budding of endoderm derivatives begins shortly after the gut tube is formed and involves many transcription factors. DP, dorsal pancreas; E, embryonic day; St, stomach. Source: Adapted from Stanger and Melton 2003 [227]. Reproduced with permission of Oxford University Press.

Table 1.1 Transcription factors in gastrointestinal development.

Gene class	Region	Function
HMG-box genes		
SOX17	Endoderm	Formation of definitive endoderm
SOX10	Enteric nervous system	Development of neural crest derivatives
SOX9	Intestine	Formation of pyloric sphincter
GATA genes		
GATA4	Endoderm	Anterior intestinal portal and foregut development
GATA6	Liver	Liver bud outgrowth, regulates HNF4
FOXA genes		
FOXA1 (*HNF3A*)	Endoderm, liver	FOXA1/A2 cooperate to specify the liver
FOXA2 (*HNF3B*)	Endoderm, liver	FOXA2 required for foregut and midgut development
FOXA3 (*HNF3G*)	Endoderm, liver	Liver gene transcription
Onecut factors		
HNF6 (*OC1*)	Liver, pancreas	Bile duct, pancreatic duct, and islet development
OC2	Liver	Bile duct development
bHLH genes		
HES1	Liver, pancreas, intestine	Notch signaling; numerous roles in differentiation
NGN3, *NEUROD*	Pancreas, intestine	Pancreatic, gut endocrine cell specification
PTF1/p48	Pancreas	Early development of pancreas; exocrine transcription
MATH1	Intestine	Secretory vs enterocyte cell fate specification
Homeobox genes		
HEX	Liver	Growth of early liver bud
PROX1	Liver, pancreas	Growth of early liver bud, endocrine differentiation
HNF1B	Liver	Cholangiocyte formation
PDX1	Pancreas	Growth of pancreatic progenitor cells
HLXB9	Pancreas	Budding of dorsal pancreas, β-cell development
ISL1	Pancreas	Budding of dorsal pancreas, islet development
NKX2.2	Pancreas	β-cell development
NKX6.1	Pancreas	β-cell development
PAX4	Pancreas	β-cell development
PAX6	Pancreas	Islet development (α cells > β cells)
ARX	Pancreas	α-cell development
BARX1	Stomach	Patterning of the stomach
HLX	Intestine, liver	Early growth of liver and intestine
NKX2.5	Intestine	Formation of pyloric sphincter
CDX2	Intestine	Anterior–posterior patterning of intestine
HOXA/*HOXD* clusters	Intestine	Anterior–posterior patterning
Other		
HNF4	Liver	Terminal differentiation of hepatocyte
SMAD2	Endoderm	Endoderm development

HMG, high mobility group; bHLH, basic helix-loop-helix.

Pattern formation

The process of "pattern formation" ensures that the esophagus and lung are positioned in the anterior or rostral part of the gastrointestinal tract, while the colon is always positioned in the posterior or caudal region. Pattern formation also enables the embryo to "know" where along the newly formed gut tube each of these organs should sprout, ensuring that new tubes (e.g., the pancreatobiliary system) form at the appropriate location.

Patterning refers to the stereotypical commitment of cells to certain *fates*, constituting in its most overarching form the establishment of the "body plan" – the spatial arrangement of all tissue types in three-dimensional space. In the endoderm, patterning establishes the correct relationship of domains that will give rise to the respiratory tract and the gastrointestinal organs along the anterior–posterior axis. Our understanding of how embryos are patterned comes largely from classical studies in *Drosophila*, in which homeobox-containing transcription factors (*Hox* genes, in particular) were identified as the major determinants of the body pattern [11]. Subsequent studies have confirmed the critical role that *HOX* genes (and related homeobox-containing genes) also play in establishing the body plan of all higher organisms, including humans, a testament to the remarkable conservation of biological mechanisms across evolution.

HOX genes, of which there are 39 in humans, have a distinct organization in the genome. Specifically, *HOX* genes are arranged sequentially within each of four distinct "clusters" (A, B, C, and D). This chromosomal organization of the *HOX* genes within the DNA sequence mirrors their spatial expression in the embryo, so-called "colinearity" of expression. For example, the mouse *Hoxa* cluster consists of 12 genes; *Hoxa1* is expressed more anteriorly in the embryo than *Hoxa2*, which in turn is expressed more anteriorly than *Hoxa3*, and so forth. Loss-of-function analyses – also known as "gene knockout" studies – have shown that these carefully regulated expression boundaries dictate the *pattern* of the ectoderm and mesoderm. Mutations in *Hoxa2* therefore cause more anterior malformations affecting the head, while mutations in *Hoxa3* affect the neck and chest; this property holds true for all *Hox* clusters and genes. Conversely, ectopic expression of a *Hox* gene in a particular segment can cause it to turn into a more anterior (or posterior) segment. This respecification of fate is referred to as a *homeotic transformation*.

On the basis of the key role that *Hox* genes play in establishing the anterior–posterior pattern of the ectoderm and mesoderm, it would be logical to assume that these genes function similarly in the endoderm. Indeed, there are rare cases of homeotic transformations resulting from the misexpression of homeobox-containing proteins; for example, *Cdx2* is expressed in the early preimplantation embryo and its expression is maintained in the endoderm throughout development [12]. Although *Cdx2*-deficient embryos die before implantation, animals heterozygous for *Cdx2* develop colonic lesions that exhibit an anterior histology [13,14]. Conversely, misexpression of *Cdx2* in the stomach causes intestinal metaplasia [15,16], a more posterior phenotype. Thus, *Cdx2* seems to pattern the endoderm by directing cells to adopt a more posterior fate. Nevertheless, *Cdx2* seems to be the exception rather than the rule, and the rarity of homeotic transformations in the endoderm suggests that homeobox-containing genes are for the most part indirectly responsible for regulating endoderm pattern. Although many *Hox* mutations result in intestinal malformations [17–20], these phenotypes are not specific to the endoderm despite the fact that boundaries of *Hox* expression in the endoderm correlate with organ boundaries [21–23].

If this elegant system of *Hox* gene expression does not give the endoderm its pattern, what does? The answer involves one of the most important principles of development: the process of *epithelial–mesenchymal crosstalk*. After gastrulation, the developing gut tube is surrounded by mesoderm from the so-called lateral plate. It has long been appreciated that patterning is normally influenced by interactions between mesoderm derivatives (mesenchyme) and endoderm derivatives (epithelia). Epithelial–mesenchymal interactions can be demonstrated by transplantation experiments in which pieces of endoderm and mesoderm from different regions are recombined [24–27]. When tissues from postgastrulation embryos are recombined in this way, the fate of the endoderm is largely dependent on the type of mesoderm with which it is cultured; thus, anterior endoderm becomes "posteriorized" when recombined with posterior mesoderm, and posterior endoderm becomes "anteriorized" when recombined with anterior mesoderm [28]. Importantly, the mesoderm may be capable of providing the endoderm with a pattern because it has already been patterned by *Hox* gene activity.

Finally, other factors may participate in endoderm patterning, among them the vitamin A derivative retinoic acid. Embryos exposed to excess doses of retinoic acid exhibit congenital malformations resulting from the transformation of anterior embryonic structures to more posterior fates, a "posteriorization" phenotype that also involves the endoderm [29,30]. The mechanism by which retinoic acid influences patterning in such a global fashion remains unclear, but almost certainly involves the corruption of regulated retinoic acid-related signaling that occurs in normal development.

Fate and potential

The role of epithelial–mesenchymal crosstalk in endoderm patterning makes it clear that the fate of endodermal epithelial cells is strongly influenced by adjacent mesoderm/mesenchyme. Yet even before gastrulation, cells in the epiblast contain information about their future identity and position. This has been shown through the construction of *fate maps*, in which individual cells are marked and their progress is traced during development. Fate maps of the epiblast illustrate a stereotyped pattern of development, in which the endoderm is largely derived from cells that surround the anterior primitive streak before gastrulation [31,32]. However, assignment of cell *fate*

may not be irreversible, and cells may remain capable of adopting identities other than their assigned fates. This capacity to change fate in response to environmental cues is referred to as *potential*, and it confers the cell with a certain amount of plasticity. Fate and potential represent important and complementary properties of a cell during development, and they provide the embryo with the means to correct errors that may occur in the course of embryogenesis.

It is generally accepted that a loss in potential accompanies gastrulation. The ability of cells within the very early embryo to become any cell type (*totipotency*) is therefore reduced within each germ layer to a more limited set of possibilities after gastrulation. This progressive *commitment* means that the parenchymal cells of the gastrointestinal organs are derived exclusively from endoderm, and, at later stages, different organs are derived only from specific portions of the endoderm. This classical notion of progressive commitment has been challenged by studies in which cells appear to be capable of traversing germ layer boundaries, a process known as "transdifferentiation." As will be discussed later, it remains unclear whether such cellular behavior contributes significantly to normal tissue homeostasis.

Attention has focused on the importance of *chromatin* in the regulation of tissue competence. Chromatin defines the structural state of DNA–protein complexes, determining whether a given DNA sequence is "open," or accessible, for transcription factors to bind. A model in which competence and commitment are achieved through sequential changes in chromatin has been suggested by studies of the regulatory region of the liver-specific albumin gene [33]. In these studies, the binding of the endodermal transcription factors GATA and FOXA to the albumin gene was assessed in several different cell types. In neural tube cells, which lack GATA and FOXA, the albumin enhancer is empty. In dorsal endoderm, the albumin enhancer is bound by GATA and FOXA, even though albumin is not transcribed in these cells, whereas in embryonic liver cells, the albumin promoter is bound by these and other factors and is transcriptionally active. This suggests that it is the chromatin state of a cell that confers a given set of potential fates. Consistent with this model, FOXA factors are themselves capable of modifying chromatin [34], and the ability to form a liver is lost in *Foxa1*/*Foxa2* mutant murine embryos [35].

Signaling in development

The assignment of cell fate in the endoderm is achieved through cell–cell signaling between neighboring cells or between adjacent cell layers. Such signals can be divided into two classes: *permissive* signals, which allow a tissue to progress to a fate that has already been assigned, and *instructive* or *inductive* signals, which divert a tissue to a new fate that would not otherwise have been followed. Instructive signals play an important role in regulating patterning by assigning cells that have not yet become committed (i.e., multipotent cells) to specific lineages.

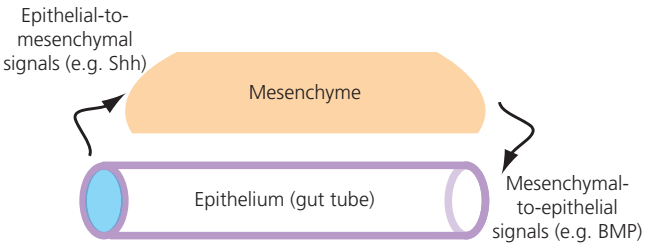


Figure 1.3 Epithelial–mesenchymal signaling. The gut tube, derived from the endoderm and comprised of epithelial cells, produces soluble signals (e.g., Sonic hedgehog [Shh]) that diffuse outward to the surrounding mesoderm-derived mesenchyme. Receptors on mesenchymal cells receive this signal, causing them produce a reciprocal signal (e.g., bone morphogenetic proteins [BMPs]), which diffuse back to the epithelial cells. This kind of epithelial–mesenchymal crosstalk is important for patterning, tissue outgrowth, and morphogenesis (and is also critical in carcinogenesis where it takes the form of "tumor–stroma interactions").

Developmental signals have traditionally been identified through transplantation studies, in which different embryonic structures (e.g., epithelium and mesenchyme) are cocultured. The resulting fate (or absence thereof) indicates whether signals are present or absent, and if present, whether the signals are permissive or instructive. Several features of development complicate the study of the specific ligands that mediate this intercellular communication. As development is a highly dynamic process, cells and cell layers are in constant movement relative to each other. Cell or tissue interactions may exist only transiently – long enough for a signal to be received, but not long enough to be easily characterized experimentally. Furthermore, signaling often occurs in a *reciprocal* manner (Figure 1.3). For example, the epithelium may respond to signal "A" from the mesenchyme by supplying signal "B", which in turn prompts the mesenchyme to secrete signal "C", and so forth. The number of secreted factors encoded in the genome is vast, further precluding straightforward analysis of epithelial–mesenchymal signaling.

While additional layers of complexity will undoubtedly be discovered, it appears that a limited repertoire of signals controls development. At least four signaling *modules*, each consisting of a family of ligands, receptors, and signal-modifying factors, are used iteratively during development: the fibroblast growth factor (FGF), hedgehog (Hh), bone morphogenetic protein (BMP), and BMP-related tumor growth factor (TGF) families (Figure 1.4, Table 1.2). In addition, two other classes of signaling modules, Wnt and Notch, act predominantly in regulating differentiation within established organs. Crosstalk between signaling modules active in specific tissue layers (in particular epithelium-derived Hh and mesenchyme-derived FGF and BMP) exemplifies the reciprocal nature of epithelial–mesenchymal signaling.

Fibroblast growth factors

The FGFs comprise a large family of ligands that are capable of binding to one of four FGF receptors. As both ligands and

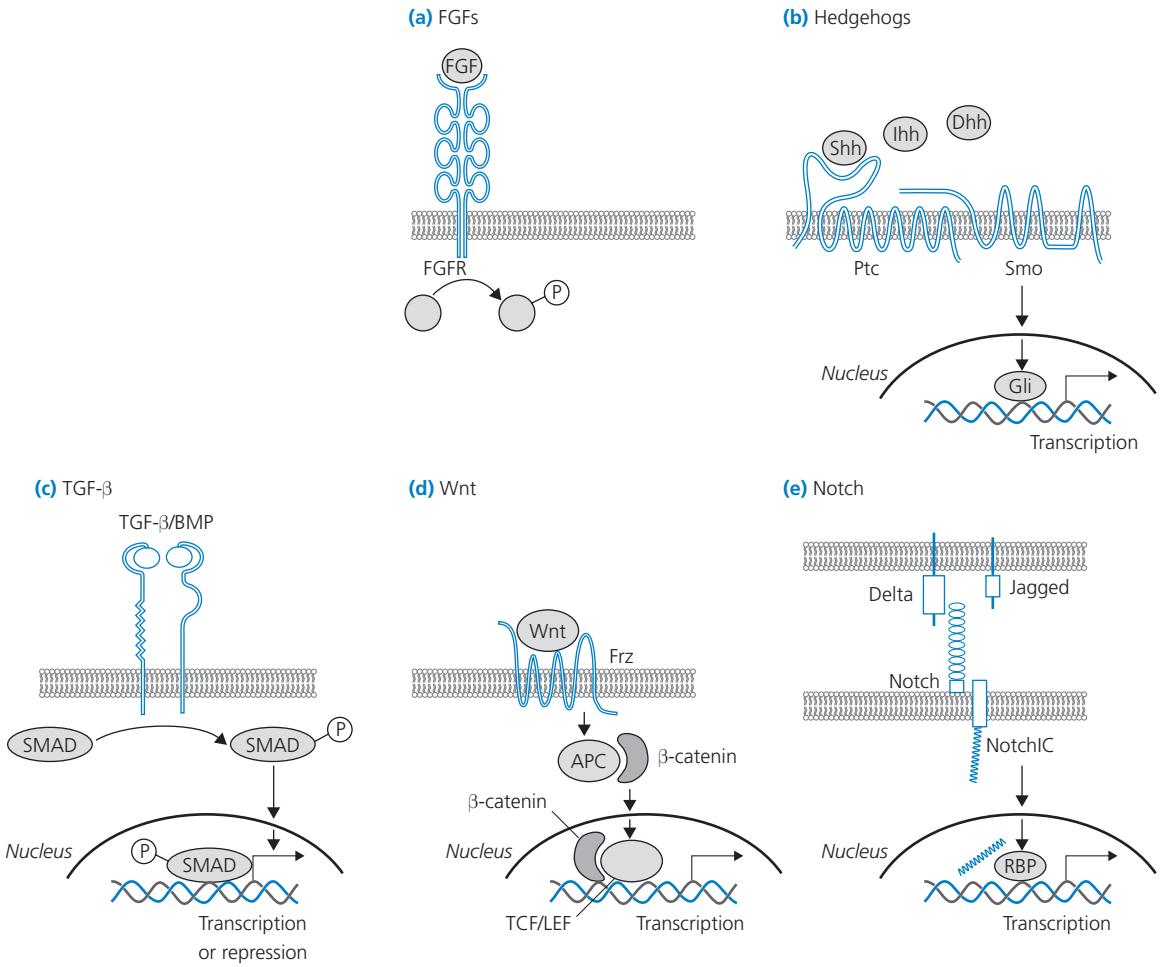


Figure 1.4 Ligand–receptor pairs in gastrointestinal development. **(a)** Fibroblast growth factor (FGF) signaling. Binding of an FGF ligand to one of four FGF receptors (FGFRs) leads to receptor dimerization and activation of FGFR tyrosine kinase activity. Phosphorylation of target proteins leads to the activation of multiple pathways, including Ras, phosphatidylinositol 3-kinase, phospholipase C, and STAT pathways. **(b)** Hedgehog signaling. All three hedgehog ligands – Sonic hedgehog (Shh), Indian hedgehog (Ihh), and Desert hedgehog (Dhh) – are capable of binding to the Patched receptor (Ptc). Ligand binding causes the membrane-bound signaling mediator Smoothened (Smo) to activate downstream transcription factors in the Gli family. These factors migrate to the nucleus and activate transcription. **(c)** Transforming growth factor (TGF)-β/bone morphogenetic protein (BMP) signaling. TGF-β family members bind to a heterodimeric membrane receptor complex consisting of a type I receptor and a type II receptor. The activated receptor complex phosphorylates SMAD transcription factors, which migrate to the nucleus where they mediate or repress transcriptional activation. **(d)** Wnt signaling. Binding of a soluble Wnt ligand to one of the seven transmembrane Frizzled (Frz) receptors results in the activation of the canonical Wnt pathway, in which adenomatous polyposis coli (APC) dissociates from β-catenin, allowing the latter to migrate to the nucleus where it becomes part of a transcriptionally active complex that includes T-cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors. **(e)** Notch signaling. Cell–cell signaling is mediated by an interaction between one of the membrane-associated Notch ligands, Delta or Jagged, and one of the four Notch receptors on an adjacent cell. Ligand binding causes the intracellular portion of the Notch receptor (NotchIC) to migrate to the nucleus where it activates the retinol-binding protein (RBP) transcription factor.

receptors are subject to a significant degree of regulation of splicing, the combinatorial ligand–receptor repertoire is vast and subject to complex variability in binding specificity and tissue-specific expression. FGF receptor signaling is largely mediated by the tyrosine kinase activity of the receptor, acting through Ras and phospholipase C pathways [36]. FGFs are expressed in the primitive streak, mesodermal structures of the postgastrulation embryo, and in developing organs, and they have important roles in endoderm patterning (possibly through a concentration gradient) and in organogenesis of the liver, pancreas, and intestine (see Section Organogenesis) [28,37,38].

Hedgehogs

The hedgehog genes were first identified through studies of *Drosophila*, and their names, like those of other developmental genes (e.g., *Notch*), arise from the hedgehog-like appearance of mutant flies. There are three mammalian hedgehogs – Indian, Sonic, and Desert – all of which bind to the membrane receptor

Table 1.2 Soluble signals in gastrointestinal development

Tissue	Signal	Source	Function
Liver	FGF	Cardiac mesoderm	FGF 1, 2, and/or 8 specify prehepatic endoderm, promote liver bud outgrowth
	BMP	Septum transversum mesenchyme	BMP4 (and other BMPs?) cooperates with FGFs in specification, outgrowth
	HGF	Septum transversum mesenchyme	Mediate hepatoblast growth and suppress apoptosis
	Unknown	Blood vessels	Expansion of liver bud into septum transversum mesenchyme
	Jagged 1	Portal mesenchyme	Specification or survival of cholangiocyte precursors (ductal plate)
Pancreas	Shh	[Endoderm]	Shh repression signals pancreatic specification
	FGF/activin	Notochord	Candidate mediators of Shh repression
	FGF10	Pancreatic mesenchyme	Outgrowth of pancreatic bud, pancreatic epithelium
	Delta/Jagged	Unknown	Notch-mediated inhibition of pancreatic progenitor cell differentiation
	TGF-β family	Unknown	Regulation of endocrine vs exocrine fate decisions
Intestine	Shh	Epithelium	Epithelial–mesenchymal crosstalk (Shh–BMP) regulates intestinal pattern
	BMP	Mesenchyme	Shh mediates radial pattern of gut. BMP regulates intestinal stem cell niche
	GDNF	Mesenchyme	Migration and/or survival of enteric neurons
	Endothelins	Mesenchyme	Migration and/or survival of enteric neurons
	Frizzled	Mesenchyme and Paneth cells	Ligands for Wnt regulation of intestinal stem/progenitor cells
	Delta/Jagged	Mesenchyme and Paneth cells	Ligands for Notch regulation of intestinal stem/progenitor cells

Patched (Ptc). In the absence of ligand, Ptc acts as a repressor of the signaling mediator Smoothened (Smo); after hedgehog ligand binding to Ptc, Smo is derepressed and activates Gli transcription factors. Importantly, cells are able to distinguish different concentrations of hedgehog ligand, allowing hedgehog to create patterns through a "gradient effect" in which cell fate depends on whether a high, intermediate, or low concentration of ligand is sensed. Sonic hedgehog (Shh) is particularly important in gastrointestinal development. Shh is expressed in the endoderm at the time of formation of the gut tube (in the anterior and caudal intestinal portals) and participates in the specification of the pancreas and regionalization/morphogenesis of the gut.

Bone morphogenetic proteins and the TGF-β superfamily

BMPs are members of the TGF-β superfamily of secreted proteins, a family that also includes the activins. Receptors for TGF-β family members are serine–threonine kinases that modulate the activity of TGF-β-responsive transcription factors (termed SMADs) through phosphorylation. The relevance of BMPs to gut development was also first suggested by studies in *Drosophila*, which showed that the BMP orthologue *decapentaplegic* responds to hedgehog signaling and is necessary for midgut development. This specific example of reciprocal signaling between TGF-β and hedgehog family members is conserved in mammals, where Shh is expressed in the epithelium of the developing gut, and induces expression of particular BMPs in the adjacent mesenchyme.

Wnts

Wnt ligands play a critical role in the formation of differentiated cell types in the embryo, a process called *cell fate determination*. Wnts are a family of secreted factors (there are at least 19 known mammalian Wnts) that bind to "frizzled" receptors on the membrane. A complex series of events follow receptor binding. In the best characterized, or canonical, pathway, Wnt signaling leads to the release of β-catenin from the adenomatous polyposis coli (APC) protein, and the former then moves to the nucleus where it activates T-cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors.

Despite the involvement of Wnt signaling in multiple developmental systems, including the intestine, developmental disorders with prominent gastrointestinal tract manifestations have not yet been associated with perturbed Wnt signaling. Rather, alterations in Wnt signaling are predominantly associated with carcinogenesis, particularly in the colon (colon adenocarcinoma), liver (hepatoblastoma), and pancreas (pancreatoblastoma) (Box 1.1).

Notch

Like Wnt, Notch signals regulate the differentiation of cells within established tissues (Figure 1.5). Of note, a role for Notch in the formation of endoderm itself has also been postulated [39,40]. There are four mammalian Notch receptors, which are activated by two classes of ligands, Delta and Serrate/Jagged. In contrast to ligands from the other important signaling modules, including Wnts, FGFs, BMPs, and hedgehogs, Delta and Serrate/Jagged are transmembrane ligands. Hence, Notch mediates

Box 1.1 Cancer and its relationship to development: cancer stem cell hypothesis.

The observation that developmental signaling pathways are often activated in adult tumors has forged a bridge between the fields of developmental biology and cancer biology. The notion that cancer recapitulates development dates to the 19th century (reviewed by Sell [229]) and is embodied in the hypothesis that tumors arise from stem cells in adult tissues that retain an embryonic phenotype. Strong evidence for such a model exists in hemopoiesis but it remains to be determined whether stem cells represent a target for malignant transformation in solid organs.

Further evidence for a link between development and cancer comes from the "reemergence" of signals normally prominent in development during the course of tumor initiation and progression. Wnt signaling, normally important during embryogenesis, is commonly activated in pediatric hepatoblastomas and pancreatoblastomas. Mutations in the type 1A BMP receptor (BMPR1A) or the downstream signaling element SMAD4 are common in juvenile polyposis syndrome.

Links between developmental signals and tumorigenesis are not limited to cancers that occur in children or inherited cancers. Like their heritable counterparts, most sporadic colorectal cancers exhibit activated Wnt signaling. Many adult pancreatic adenocarcinomas exhibit a reactivation of PDX1, Sonic hedgehog, and Notch signaling, which are either completely absent or only present in a subset of cells in the adult pancreas. Furthermore, several gastrointestinal malignancies (esophageal and gastric, in particular) are preceded by metaplasia. This replacement of one tissue type with another may reflect the emergence of more primitive cells with a greater capacity for growth.

Furthermore, tumors are composed of both mutant cancer cells and nonmutant "stromal cells" that comprise the so-called "tumor microenvironment." The formation of the malignant stroma is reminiscent of the process of epithelial–mesenchymal crosstalk that occurs during normal organogenesis (Figure 1.3), as it arises through reciprocal signaling between cancer cells and mesenchyme-derived noncancer cells.

Similarly, the concept of "cancer stem cells" – special cells within a tumor that provide the tumor with an inexhaustible supply of new cancer cells – is based on this apparent link between development and cancer. The *cancer stem cell hypothesis* posits that most cells within a tumor have a limited capacity for division and are themselves generated from cells with an unlimited capacity for division. In several tumors (breast and brain, in particular), a small subset of tumor cells have been identified and shown to be uniquely capable of reconstituting the tumor [230,231].

The cancer stem cell hypothesis has significant implications for cancer therapy. Most cancer therapies are assessed by their effect on tumor mass, the easiest assay for antitumor activity. However, if the cancer stem cell hypothesis is true, these agents would primarily target a cell population with a limited self-renewal capacity – analogous to a "transient amplifying population" – but may only inefficiently kill the cancer stem cells that are actually fueling the growth of the tumor. Stem cells that normally reside in adult tissues seem to be more resistant to chemotherapy than other cells [232], giving additional plausibility to this model. If the cancer stem cell hypothesis is correct, then it would be highly desirable to have therapies that specifically target these cells, as they might provide more durable cures and simultaneously generate less toxicity.

signaling exclusively between cells that are in direct contact with each other. Ligand engagement leads to the detachment of the intracellular portion of the Notch receptor from the membrane, where it travels to the nucleus and alters the transcriptional program of the cell. Like Wnt signals, Notch signals are subject to complex regulatory inputs at all stages of the signal transduction pathway, from ligand binding to cytoplasmic and nuclear activation of downstream mediators.

The role of these signaling modules in adult homeostasis remains to be fully defined. However, it is known that some signals are necessary for function throughout life. For example, Notch and Wnt signals maintain the proper balance of cell types in both the embryonic and the adult intestine. It is not clear how developmental specificity is achieved when signals from a single family are used repeatedly. It is likely that signals are interpreted in the context of cellular identity, thereby causing the same signal to have different effects on different tissues (i.e., pancreas vs liver vs intestine).

Transdifferentiation and dedifferentiation

Several studies have challenged the notion that commitment imposes a nearly absolute boundary between different lineages. Investigators have reported that certain somatic cells, particularly the cells derived from bone marrow, have the capacity to give rise to many different tissues in vitro and in vivo, including the cells of skin, lung, kidney, muscle, and all of the gastrointestinal organs [41]. A significant fraction of this apparent plasticity may actually reflect the effect of cell fusion between the bone marrow-derived cells and other differentiated cells, giving rise to tetraploid cells with the characteristics of hepatocytes, cardiomyocytes, and neurons [42–44]. Although it is possible that bone marrow-derived cells can transdifferentiate, albeit with low efficiency, into other somatic cells, the physiological significance of such a rare event is unclear, and the paradigms of lineage commitment established early in the 20th century remain largely intact.

A major exception to this rule of irreversible commitment from a less-differentiated state to a more-differentiated is the finding that under experimental conditions, a terminally differentiated cell can be induced into a pluripotent stem cell (iPSC) capable of giving rise to all differentiated cell types [45]. Known as "cellular reprogramming," this process can be used to generate pluripotent cells from an individual patient, which have the potential to generate cell types that are lost from injury or degenerative disease. In the future, the ability to manipulate the identity of adult cells – either through fusion or exploitation of developmental plasticity – may constitute a method for cell replacement in such disease states through an approach that is now being called *regenerative medicine*.

Conclusions

Early development of the gastrointestinal tract is characterized by gastrulation and endoderm formation, followed by midline migration of anterior and posterior invaginations (i.e., the

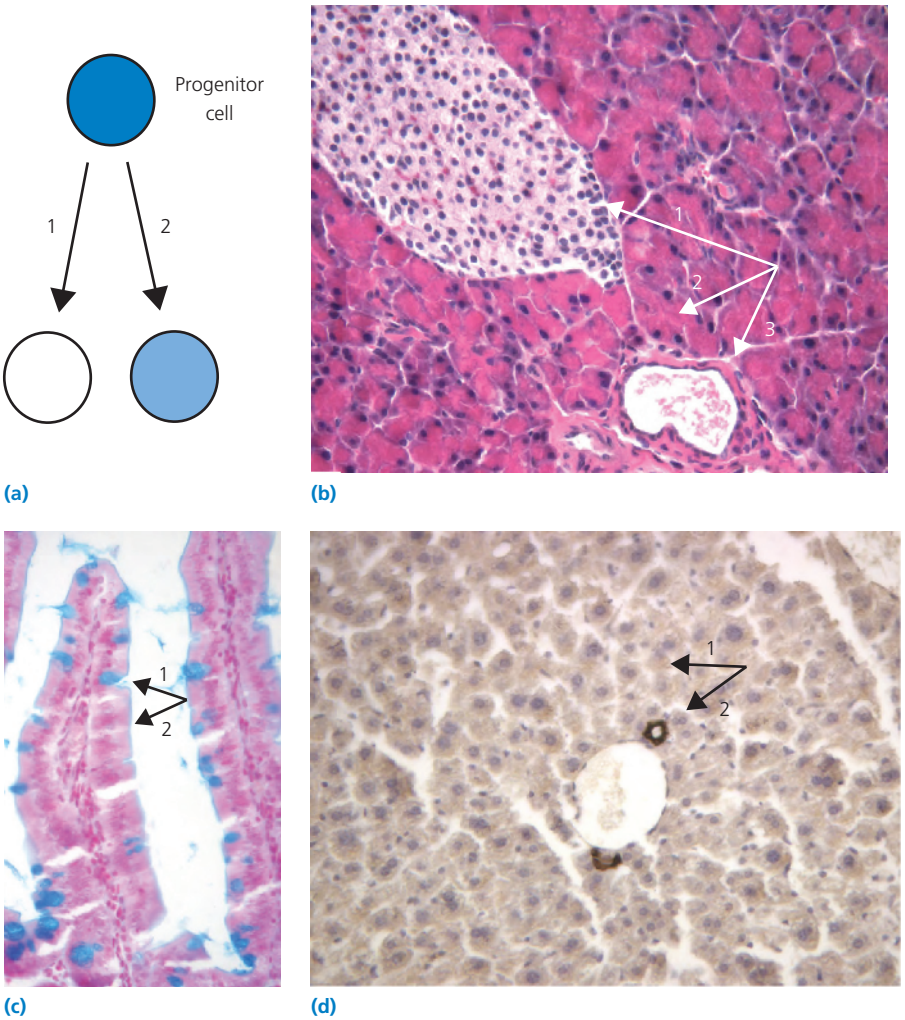


Figure 1.5 Control of gastrointestinal development by Notch. **(a)** The fate of a multipotent progenitor cell (dark blue) is influenced by whether it receives a signal from a Notch ligand (Delta or Jagged). In this example, active Notch signaling causes the cell to adopt fate 1 (white), whereas absence of signaling results in fate 2 (light blue). Evidence supports distinct roles for Notch in various gastrointestinal tissues. In the pancreas **(b)**, a Notch signal prevents the differentiation of the progenitor cell into any of the mature pancreatic cell types – islet (1), acinar (2), or ductal (3). In intestinal progenitor cells **(c)**, activation of Notch signaling promotes the formation of enterocytes (2). The formation of secretory cells (1), such as the goblet cells visualized here with Alcian blue staining, is inhibited by Notch signaling. In the liver **(d)**, Notch signaling is required for the proper formation of bile ducts (2), visualized by staining for cytokeratin 19.

anterior and caudal intestinal portals), resulting in a gut tube. The endoderm is patterned into organ domains along its anteroposterior axis through the activity of homeobox-containing transcription factors and epithelial–mesenchymal signaling. Cell fate remains plastic during the initial stages of development, with tissue identity depending on reciprocal signals that are refined until the commitment to a particular organ fate is made. Cells maintain their differentiated identity once commitment has occurred, although a new paradigm of "cellular reprogramming" may make it possible to convert cells from one identity into another (e.g., liver into pancreas).

Organogenesis

After gastrulation, the endoderm undergoes more easily recognizable changes of organogenesis. Studies delineating the development of the gastrointestinal tract were performed over the 20th century and the timing of most key biochemical, physiological, and morphological events during human development was established at least 30 years ago [46]. Accordingly, this section will focus primarily on the mechanisms that underlie these remarkably complex and integrated events.

Importantly, the same signaling pathways described in the previous section on early development (e.g., BMPs, FGFs, etc.) are used iteratively in the specification of organ domains and the growth and differentiation of tissues. Sometimes, a single signal is involved in the development of two different organs, or one signal may mediate two different effects in the same tissue. In these instances, it is cellular *context*, the identity of the cell on which a given signal acts, that determines the signaling outcome.

GI organogenesis can be divided into several overlapping phases:

1. *specification* – a direct consequence of the patterning processes previously described, results in the commitment of cells to restricted tissue fates
2. *budding* – of liver and pancreas
3. *morphogenesis* – the formation of a three-dimensional structure that facilitates the physiological function of the tissue (e.g., hepatic sinusoids and intestinal villi)
4. *cell fate determination* – the restriction of specific lineages within the tissue (e.g., hepatocytes and cholangiocytes).

These components of organogenesis do not occur sequentially or independently, but rather, occur in parallel, in a coordinated fashion. Finally, differentiation programs are implemented within those lineages, allowing the expression of physiological function (discussed further in Section Developmental physiology).

Liver

Specification

The liver provides a good example of how a prepatterned mesenchyme can influence epithelial fate. The developing cardiac mesoderm, which gives rise to the heart, lies adjacent to the anterior endoderm fated to give rise to the liver (see Figure 1.2b). Experiments performed decades ago showed that cardiac mesoderm plays a critical role in the formation of the liver. These studies consisted of transplantation assays in which pieces of endoderm and mesenchyme were independently assembled. Such experiments demonstrated that an interaction between endoderm and cardiac mesoderm, during a critical time window, is necessary for the endoderm to activate a liver program [24]. As the cardiac mesoderm moves anteriorly, the space adjacent to the prehepatic endoderm is replaced by the septum transversum, a mesoderm derivative that later gives rise to part of the diaphragm. Other signals mediate the outgrowth of the expanding liver bud into the septum transversum mesenchyme (see also Chapter 10).

Tissue transplantation studies using molecular markers have confirmed an important role for embryonic mesenchyme in liver development. For example, ventral endoderm expresses albumin (a marker of liver specification) when it is cocultured with cardiac mesoderm. However, other studies suggest that a more complex regulatory circuit underlies the process. For example, *dorsal* endoderm expresses albumin when it is simply removed from its adjacent endoderm. This surprising result implies that the normal function of cardiac mesenchyme is permissive rather than inductive, in that it may allow the expression of a "default" liver program [47]. Such a default mechanism may also apply to the ventral pancreas, which forms from a lip of anterior endoderm that constitutes the "leading edge" of the anterior intestinal portal. This piece of endoderm exhibits "bipotential" pancreatic/hepatic properties; that is it expresses pancreatic genes if cultured on its own, but represses the pancreatic program and expresses albumin if cocultured with signals from the cardiac mesoderm [37]. Although it is enticing to interpret these experiments as an indication that intrinsic endoderm fates are reprogrammed by specific mesenchymal elements, it is more likely that the liver – like all parts of the endoderm – is specified through a combination of early signals that provide cells with an intrinsic bias as well as later permissive and inductive signals.

While it is likely that a combination of FGF and BMP signals are among the specific signals involved in hepatic specification. FGFs are both sufficient and necessary for isolated anterior endoderm to activate albumin expression [48], and BMPs expressed by the septum transversum mesenchyme appear to act in concert with these FGFs [49]. The transcription factors Foxa1 and Foxa2 are critical mediators of these signals within the adjacent endoderm, as liver specification fails to occur in mice with a targeted inactivation of both of these genes [35].

Budding

After hepatic specification by the cardiac mesoderm, a bud that will grow into the liver begins to emerge. The first morphological evidence of budding is a thickening of the adjacent endoderm into a "hepatic diverticulum," which is followed by the outgrowth of liver cells into the septum transversum mesenchyme. FGFs are also necessary for this outgrowth, although their role in budding appears to be permissive and their actions alone are not sufficient for liver bud outgrowth [50]. BMPs (specifically BMP4) are independently required for liver budding into the septum transversum, as demonstrated with the use of *Bmp4* mutant mice and the BMP antagonist noggin [49]. Furthermore, endothelial cells within the septum transversum mesenchyme are a source of growth-promoting signals, as *Flk1* mutant embryos (which are incapable of forming mature endothelial cells or blood vessels) undergo liver specification but fail to bud [50].

Many genes are required after endoderm specification for outgrowth into the septum transversum. These include three homeobox-containing transcription factors – Hex, Prox1, and Hlx – and the zinc-finger transcription factor GATA6. *Hex* is expressed during gastrulation in the first endoderm cells to pass through the primitive streak that ultimately give rise to the liver. Mice lacking *Hex* form a small hepatic diverticulum, but subsequent outgrowth and budding fails to occur [51,52]. *Hlx* and *Prox1* mutant mice also exhibit growth arrest at the bud stage, although the livers of *Prox1* mutant mice ultimately reach nearly

a third of the size of a normal liver [53,54]. *Hex* and *Prox1* are expressed in the hepatic epithelium, whereas *Hlx* is normally expressed in the septum transversum mesenchyme.

As previously noted, the GATA4 zinc finger transcription factor binds to the albumin promoter before albumin expression, suggesting a role in liver specification [33]. Another GATA family member, GATA6, also plays an important role in liver development. GATA6 regulates HNF4, an important transcriptional regulator of hepatocyte genes (described in Section Morphogenesis and cytodifferentiation), and liver bud outgrowth is retarded in mouse embryos lacking *GATA6* [55]. Further studies are needed to determine whether a regulatory relationship exists between *Hex*, *Hlx*, *Prox1*, and *GATA6*, given the similar phenotypes that mutations of these genes exhibit. Further studies are also required to determine the signaling hierarchy between soluble FGFs and BMPs and the activity of these transcription factors; for example, *Hex* expression can be induced by BMP signaling [56].

Morphogenesis and differentiation

After this migration into the septum transversum, epithelial cells intercalate with mesenchymal cells, eventually leading to the formation of the hepatic sinusoids which support embryonic hematopoiesis. These morphogenetic changes are accompanied by dramatic growth of the liver through the action of mesenchymal factors. The most important of these is hepatocyte growth factor (HGF), which signals through the c-met receptor. Mutation of either *Hgf* or c-*met* leads to marked liver cell apoptosis in some but not all analyses [57,58]. This signaling pathway also seems to modulate the response to injury in adult liver [59,60]. Mutations in several other genes, including components of the tumor necrosis factor (TNF)–nuclear factor-κB signaling pathway, lead to similar developmental apoptosis phenotypes [61–63]. Hepatocyte apoptosis in many adult liver diseases is mediated by a TNF-like "death receptor" pathway [64], suggesting that these cell death signaling mechanisms are active throughout life.

The two major parenchymal cell types of the liver – hepatocytes and bile ducts – arise from multipotent embryonic "hepatoblasts." Intrahepatic bile ducts (IHBDs) are derived from "ductal plates," precursor structures that form around branches of the portal vein. Inductive signals from the portal vein mesenchyme induce surrounding hepatoblasts to form the ductal plate, which can be recognized by the expression of distinctive cytokeratin (CK) molecules, such as CK19 (Figure 1.6). Mature intrahepatic bile ducts emerge after remodeling of the ductal plate, in conjunction with selective apoptosis of duct precursors. The "extrahepatic" bile ducts (EHBDs) and the gallbladder have

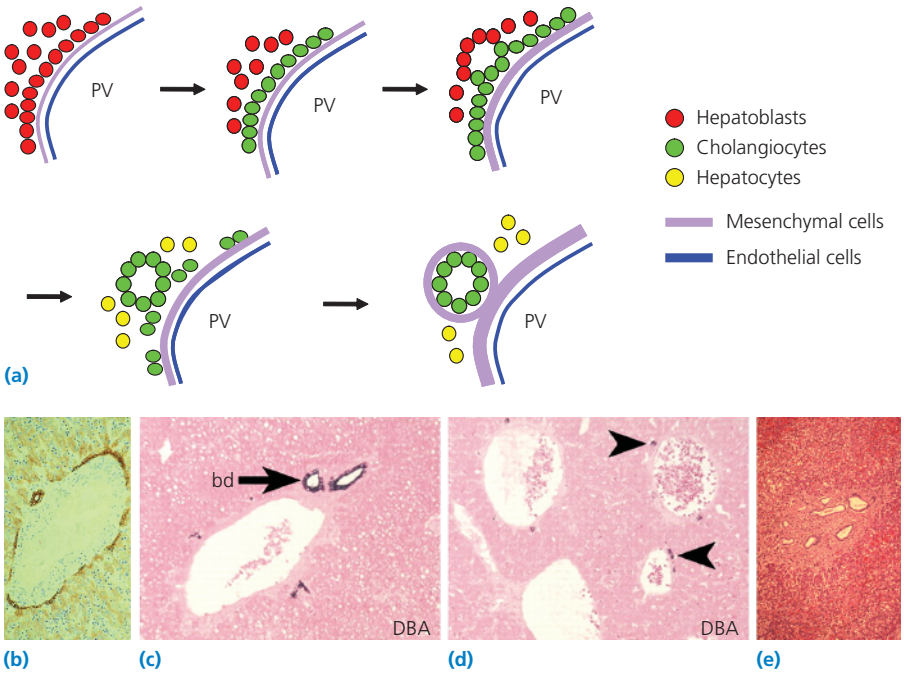


Figure 1.6 Biliary tract development. **(a, b)** Normal biliary development. **(a)** Branches of the portal vein (PV) induce embryonic hepatoblasts (red) to form the ductal plate, a ring of biliary epithelial precursor cells (green). These cells become arranged into a tube that undergoes remodeling late in embryogenesis or early in postnatal life to yield mature bile ducts. This process involves Notch and TGF-β signaling. **(b)** The ductal plate expresses cytokeratin 19, a marker of mature bile ducts. **(c, d)** Disorders of biliary development. **(c)** Normal mouse portal tract with bile duct ((bd) visualized by DBA lectin staining), hepatic artery, and portal vein branches. **(d)** Portal tract from a mouse lacking one copy of both *Jagged1* and *Notch2*, a model of human Alagille syndrome. **(e)** Periportal expansion of bile ducts in a patient with the ductal plate abnormality characteristic of congenital hepatic fibrosis. **(a)** Source: Adapted from Zong and Stanger 2011 [69]. Reproduced with permission of Elsevier. **(c, d)** Source: Adapted from McCright et al. 2002 [213]. Reproduced with permission of the Company of Biologists, Ltd.

a separate embryonic origin from the IHBDs, as these larger ductal structures arise through a process of branching from the gut tube into the liver well after budding has occurred. It remains unclear how the connection between IHBDs and EHBDs occurs.

Several signaling pathways are involved in biliary specification and morphogenesis. Among those first identified were liver-enriched "hepatocyte nuclear factors" (HNFs). HNFs belong to several different transcription factor families and contribute to the expression of liver-specific genes. Inactivation of either HNF6 (a member of the onecut transcription factor family) or HNF1B (a homeodomain factor) perturbs biliary development [65,66]. The reduced HNF1B expression in the livers of *Hnf6* mutant mice suggests that HNF6 likely acts through HNF1B [65]. Alternatively, HNF6 and the onecut transcription factor OC2 act through activin/TGF-β family member(s) to regulate biliary fate decisions. The liver normally exhibits a gradient of TGF-β signaling activity, with high activity near the ductal plates and low activity in the remaining parenchyma. In livers lacking both HNF6 and OC2, this gradient is disrupted, resulting in high levels of activin/TGF-β signaling throughout the liver and the appearance of cells exhibiting features of both hepatocytes and cholangiocytes. Thus, onecut transcription factors may shape a gradient of activin/TGF-β signaling to allow localized induction of the bile ducts.

In addition, there is convincing evidence that Notch signaling is important for biliary development. Mutations in the Notch ligand Jagged 1 (*JAG1*) result in Alagille disease, a clinical syndrome that includes a paucity of intrahepatic bile ducts [67,68], and the mechanism appears to involve a failure of proper biliary differentiation [69]. The molecular pathogenesis of Alagille syndrome is discussed further in the Section Disorders of remodeling.

A cellular differentiation program is executed after the assignment of biliary or hepatocyte fate. Evidence that this program is distinct from the assignment of hepatocyte cell fate comes from the targeted inactivation of HNF4. Remarkably, this transcription factor has been reported to bind to nearly half of the actively expressed genes in the liver [70]. Among the genes whose expression "defines" the hepatocyte are albumin, apoplipoproteins A and B, and transferrin. Despite exhibiting normal morphogenesis, HNF4-deficient embryos exhibit reduced expression of all of these genes, demonstrating a role for this transcription factor in hepatocyte differentiation but not liver specification and morphogenesis [71].

In summary, FGF and BMP signals from the cardiac and septum transversum mesoderm/mesenchyme induce a portion of the ventral foregut endoderm to become the liver. Budding and parenchymal growth involves homeobox-containing transcription factors and mesenchyme-derived soluble factors, such as HGF, that mediate proliferation and suppress apoptosis. Poorly understood epithelial–mesenchymal interactions mediate the morphogenesis of the hepatic sinusoids, which support hemopoiesis during fetal life. Biliary development involves the formation of perivascular ductal plates and subsequent remodeling, a process that requires Notch and TGF-β signals and the activity of several onecut transcription factors. In hepatocytes, other transcription factors including HNF4A are required for the full manifestation of the differentiated hepatocyte program.

Pancreas

With some important exceptions, development of the pancreas follows a paradigm that is similar to that of the liver. Specifically, signals from adjacent mesoderm specify the pancreatic endoderm, FGFs mediate pancreatic growth, and a variety of signaling components (including Notch and homeobox-containing transcription factors) regulate the differentiation of the parenchymal cell types of the pancreas – its exocrine, endocrine, and duct cells.

Specification

Unlike the liver, the pancreas forms from two distinct pieces of foregut endoderm – a dorsal pancreatic domain and a ventral pancreatic domain (see Figure 1.2c) – that later fuse into a single gland. Patches of endoderm on opposite sides of the gut tube must therefore somehow be instructed to become pancreas. Transplantation studies similar to those previously described have shown that the dorsal pancreatic region of the endoderm is specified before the 13-somite stage, a period when this endoderm is in contact with the notochord [72]. At a slightly later stage, the "prepancreatic endoderm" (the patch of endoderm fated to become pancreas) is in contact with the aorta (dorsally) and the vitelline veins (ventrally). Thus, the notochord or blood vessels could be mesenchymal sources for inductive pancreatic signals, akin to the role played by the cardiac mesoderm in the developing liver.

Indeed, evidence suggests that both the notochord and the blood vessels are important for pancreatic specification and growth. Isolated dorsal endoderm fails to show evidence of pancreatic differentiation when cultured on its own, but a pancreatic program is induced on reassociation with the notochord [73]. Similarly, coculture of dorsal endoderm with aortic cells (or other endothelial cells) induces a pancreatic program, whereas removal of aortic precursor cells from the embryo causes a failure in pancreatic development [74].

The most important consequence of mesenchymal signaling appears to be the repression of Shh expression. Shh is expressed throughout the entire gut tube, with the notable exception of the prepancreatic regions (both dorsal and ventral). The notochord is directly responsible for repressing Shh in the dorsal prepancreatic endoderm, possibly through the activity of FGF2 or activin β-B [75]. Repression of Shh alone is able to reproduce the pancreatic inductive activity of notochord [75], and blocking Shh signaling with an inhibitor (cyclopamine) causes ectopic pancreas formation [76]. Furthermore, Shh repression must be maintained throughout pancreatic development, as ectopic expression of Shh after pancreatic budding inhibits further pancreas development [77]. Shh repression is therefore both

necessary and sufficient for pancreas specification. It is unclear what structure serves the function analogous to that of the notochord to repress Shh expression in the ventral prepancreatic endoderm.

Budding

The pancreatic buds form at about 3–4 weeks of embryonic development (E9.5 in the mouse), with formation of the ventral bud lagging behind that of the dorsal bud. One of the earliest and most important genes to be expressed in these nascent buds is the homeobox transcription factor *PDX1*. All mature pancreatic cell types are derived from cells that expressed *PDX1* [78], and ectopic *PDX1* expression in the intestine is sufficient to promote the early steps of pancreas formation [79]. Although pancreatic buds form in *Pdx1*-deficient embryos, further pancreas development is arrested at this stage [80,81], a phenotype that has also been observed in humans (Box 1.2) [82]. In adults, PDX1 is a major transcription factor for insulin, and its loss in adult mice causes diabetes [83]. Several other transcription factors that play roles in mature differentiated pancreas cells are also expressed in the early progenitor cells of the pancreas, including p48/PTF1A, Hes1, and Nkx6.1.

As noted, specification of the dorsal and ventral pancreas occurs by different mechanisms (notochord for dorsal, unknown for ventral). This differential regulation repeats itself later, as several genes exhibit distinct activities in dorsal versus ventral pancreatic development. One of these is *Hlxb9*, which encodes a homeobox transcription factor that is required for dorsal, but not ventral, pancreatic budding in mice [62,85]. Similarly, the homeobox transcription factor Isl1 is required in the pancreatic mesenchyme to promote dorsal, but not ventral, pancreas development [86]. Mesenchymal *Isl1* expression is maintained in *Hlxb9* mutants, suggesting that *Isl1* is not downstream of *Hlxb9*. As there are no profound functional or histological differences between the postnatal derivatives of the ventral (head and uncinate process) and dorsal (body and tail) pancreas, it is unclear why *Isl1* and *Hlxb9* mutations cause such selective phenotypes.

Once formed, the ventral pancreas rotates across the midline to meet the dorsal pancreas (Figure 1.8). The two pancreatic derivatives undergo complete functional and anatomic integration, and the ventral ductal system (duct of Wirsung) serves as the major conduit for pancreatic secretion through the major papilla. Failure of integration results in the common anatomic variant *pancreatic divisum*, which is marked by persistence of the dorsal duct of Santorini and drainage through the minor papilla.

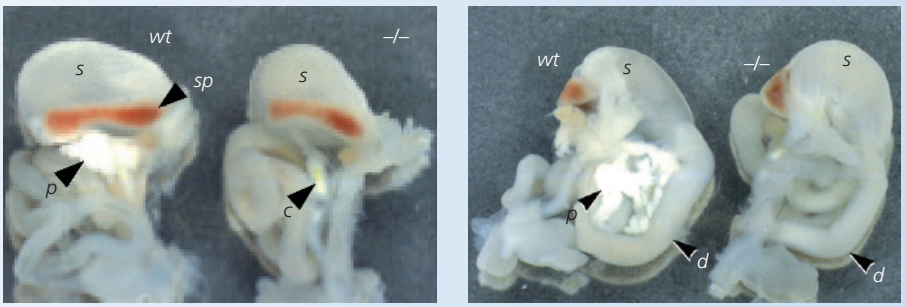
Morphogenesis and cytodifferentiation

Mutant phenotypes demonstrate that early pancreas organogenesis occurs in two steps: an early phase of pancreatic budding (which requires ISL1), and a later phase of outgrowth and branching (which requires PDX1). Wessells and Cohen [72] suggested a two-step process after observing that the substitution of heterologous mesenchyme for pancreatic mesenchyme supported later stages of development but not early budding.

For many years, investigators looked for "mesenchymal factors" that control pancreatic growth, branching, and differentiation [87]. FGF10 was discovered to be such a pancreatic mesenchymal factor. In the lung, FGF10 expression causes budding and branching of the pulmonary epithelium [88]. This growth is "stereotyped" – primary, secondary, and tertiary branch formation is spatially and temporally regulated to ensure a consistent branching pattern. Although branching in the pan-

Box 1.2 Pancreatic agenesis.

PDX1 homeodomain-containing transcription factor (also known as IDX1, STF1, and IPF1) is absolutely required for development of the pancreas, as both mice and humans lacking the gene have an arrest in pancreatic development [80,82] (Figure 1.7). Heterozygous mutations in PDX1 caused maturity-onset diabetes of youth (MODY) in a subset of patients, reflecting the protein's later role as the major transcriptional regulator of insulin gene expression [84].



(a) (b)

Figure 1.7 (a, b) Modeling pancreatic agenesis in the mouse. Images of dissected mouse stomach (s), spleen (sp), duodenum (d) and pancreas (p) from wild-type (wt) and mutant mice lacking the *Pdx1/IPF1* gene (–/–). In the absence of Pdx1/IPF1, the pancreas does not develop and is instead replaced with a cystic structure (c). Source: Offield et al. 1996 [80].

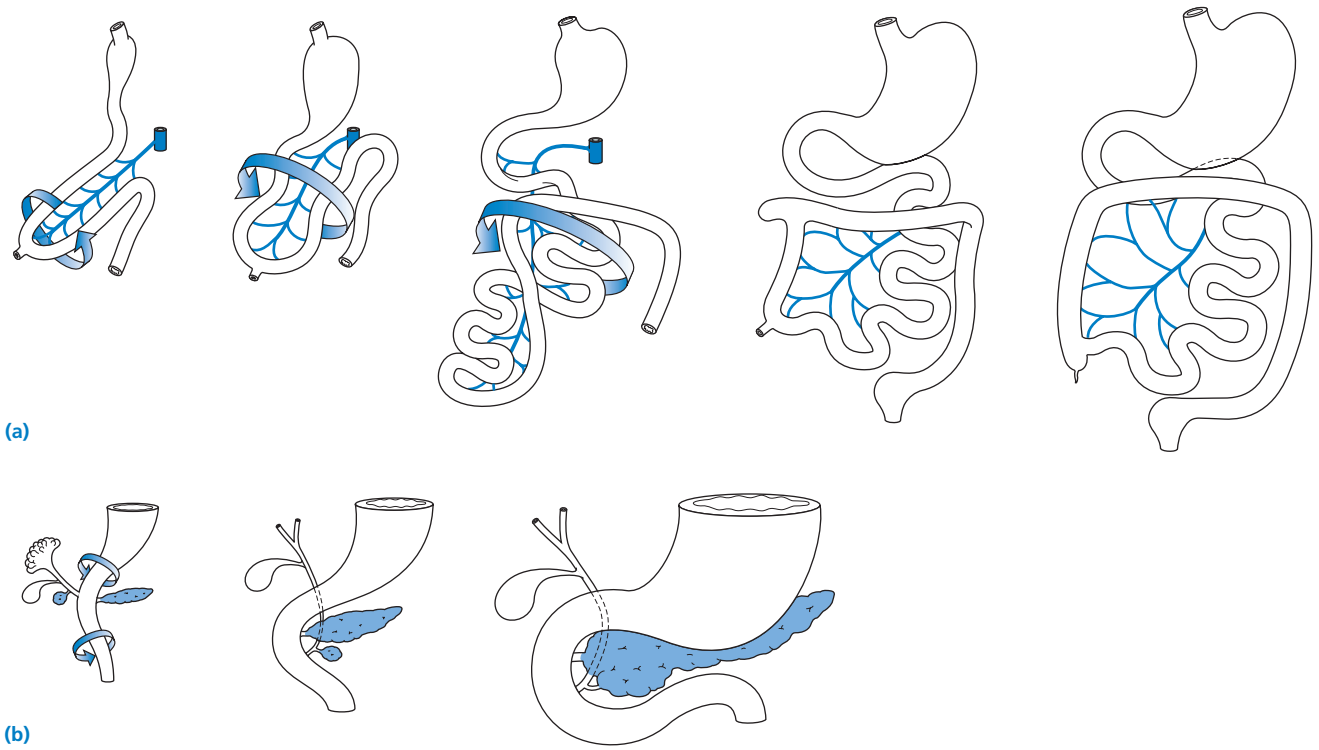


Figure 1.8 Rotation during development of the gastrointestinal tract. **(a)** Rotation of the gut. **(b)** Rotation of the pancreas.

creas does not appear to be stereotyped in the same way that it is in the lung, FGF10 has a strikingly similar function in the development of the pancreas. FGF10 is expressed in the mesenchyme and drives the proliferation of progenitor cells expressing PDX1 during branching by binding to the FGFR2b receptor on epithelial cells [89]. Consistent with this, *Fgf10* mutant mice exhibit arrested pancreas development at the bud stage [90]. An additional activity of FGF10 during pancreas growth is to keep the expanding pancreatic epithelium in an undifferentiated state. This is achieved through the activation of Notch signaling, a potent regulator of pancreatic differentiation [91–95].

The mature pancreas contains exocrine cells that make digestive enzymes, ducts that carry these enzymes to the gut, and hormone-producing endocrine cells. The exocrine pancreas is the largest compartment, comprising over 80% of the pancreatic mass. The transcription of exocrine-specific genes is dependent on the PTF1A transcriptional complex, which contains the pancreas-specific transcription factor p48. Like PDX1, p48 is expressed in the early stages of development in multipotent pancreatic progenitor cells [96], and it is the major transcription factor for the expression of exocrine-specific genes [97]. p48 is required for exocrine differentiation, as null mutant mice develop an endocrine pancreas but lack exocrine cells [98]. Another transcription factor, MIST1, is required for the assembly of the exocrine secretory machinery [99].

During the growth of the pancreatic epithelium within the bud, endocrine cells arise in waves of differentiation (glucagon-producing α cells preceding insulin-producing β cells); these cells delaminate from the epithelium and reaggregate postnatally into the islets of Langerhans (which also include somatostatin-producing δ cells and pancreatic polypeptide-producing PP cells). The development of these different endocrine lineages is complex and regulated by multiple factors. The bHLH transcription factor Neurogenin 3 (NGN3) is both necessary and sufficient for endocrine differentiation in the pancreas [79,100,101]. NGN3, and its target gene *BETA2/NEUROD*, are regulated by Notch signals [102] and the onecut transcription factor HNF6 [103]. Additional transcription factors involved in the delineation of different endocrine lineages in appropriate numbers include NKX6.1, NKX2.2, PAX4, and PAX6 [104].

Islets are not derived from the monoclonal expansion of endocrine precursor cells, but rather from the polyclonal coalescence of distinct endocrine cells or endocrine precursors [105]. The aggressive search for putative "adult stem cells" in the pancreas has yielded ambiguous results. One laboratory that used a genetic labeling method failed to show that adult stem/progenitor cells give rise to β cells, suggesting that the adult β cell mass is maintained principally by replication [106].

In summary, the repression of Shh signaling induces the formation of dorsal and ventral pancreatic buds from the endoderm. Signals provided by blood vessels, as well as mesenchymal FGF10, promote the outgrowth of multipotent pancreatic progenitor cells into a branched epithelium. Complex signals, including members of the Notch and TGF-β families, as well as numerous bHLH and homeodomain proteins, regulate the

subsequent differentiation of pancreatic endocrine, exocrine, and ductal lineages.

Gastrointestinal tract

Specification

Although the gastrointestinal tract is composed of a single continuous tube, it is partitioned into discrete domains from anterior to posterior (esophagus, stomach, small intestine, and colon) that are demarcated by sphincters (lower esophageal sphincter, pylorus, ileocecal valve, and anal sphincter). Each domain has a distinct function and a unique architecture. Similar to the liver and the pancreas, the different functional domains of the intestine are patterned after gastrulation through a repertoire of homeobox genes and epithelial–mesenchymal crosstalk.

Homeobox-containing genes are expressed in a regionalized manner in the gut epithelium and mesenchyme [13,22], and several examples of "homeotic transformations" have been observed after the dysregulated expression of homeobox genes. *Hoxa13* and *Hoxd13* are expressed in the hindgut, and ectopic expression of either of these *Hox* genes in the midgut leads to acquisition of hindgut characteristics [107,108]. Likewise, the *Hox* gene *Hoxa5* is expressed in stomach mesenchyme, and is necessary for gastric fate specification [109]. Sphincters constitute a special case of endoderm patterning, as they reside at boundaries between intestinal segments. Again, *Hox* genes are important for the process of sphincter formation. *Hoxa13/Hoxd13* mutant mice have defects in anal sphincter formation, and mice with a large deletion in the *Hoxd* cluster (*Hoxd4–d13*) lack an ileocecal valve [19,20].

In addition to the expression of homeobox genes, epithelial–mesenchymal signaling is also essential for the establishment of an intestinal pattern. In some cases, such as the murine cecum, a clear hierarchy of epithelial–mesenchymal signaling (through FGFs in cecal development) mediates organ growth [110]. In other cases, it is less clear whether transcription factors (such as homeobox-containing proteins) establish an initial pattern that is refined by further epithelial–mesenchymal signaling, or whether epithelial–mesenchymal signaling is responsible for establishing the pattern of transcription factor gene expression. An alternative possibility is that the basement membrane, an aggregate of extracellular matrix strategically placed between epithelial and mesenchymal cells, regulates crosstalk between the two tissue layers [111].

Pyloric sphincter development is a particularly instructive example of this complex process of specification. In the chicken, the pyloric sphincter forms at the junction of the gizzard (caudal stomach) and the small intestine. Two transcription factors – the homeobox factor Nkx2.5 and the HMG-box factor Sox9 – are both markers of the mesenchyme of the pyloric sphincter, and ectopic expression of either gene is sufficient to convert the gizzard into pyloric sphincter-like epithelium [112–114]. Moreover, mesenchymal BMP4 is both necessary and sufficient to induce the expression of these transcription factors [112,114,115]. This result is surprising, because BMP4 is expressed widely throughout the gut mesenchyme. The specificity of BMP4 activity to induce sphincter development likely reflects specific spatial regulation of its own expression and spatial regulation of its receptor [115,116]. Moreover, the ability of the downstream factor Nkx2.5 to induce pyloric sphincter development is spatially regulated; Nkx2.5 can induce pyloric sphincter development when it is expressed anteriorly (in the gizzard), but not posteriorly (in the duodenum) [113]. These studies provide insight into the final steps regulating the development of the pyloric sphincter but a deeper question remains: What regulates the regulators?

Complex signals that are both intrinsic and extrinsic to the developing epithelium control tissue identity. The extent to which these or similar inductive events contribute to common congenital anomalies, including intestinal stenoses and atresias, duplications, and anorectal malformations, is unknown. However, congenital anomalies of the gastrointestinal tract are commonly associated with malformations in other organ systems or chromosomal abnormalities, including trisomy 21 (Down syndrome), suggesting that the regulatory signals involved in patterning are disrupted widely.

The clinical relevance of these regulatory networks may extend beyond putative relationships to congenital errors. For example, intestinal metaplasia, a premalignant lesion in which portions of the esophagus or stomach are replaced with intestinal mucosa, may represent reactivation of developmental programs. Studies of BARX1, a homeobox-containing transcription factor that is expressed transiently in the gastric mesenchyme, provide support for this concept. BARX1 mediates gastric specification by inhibiting Wnt signaling, and mouse embryos with a targeted disruption of the gene exhibit a homeotic transformation of stomach to intestine [117]. One interpretation of this result is that intestinal differentiation represents a "default" state for gut endoderm that must be overcome (through inhibition of Wnt signaling) to allow stomach specification. Although unproved, this model may explain why intestinal metaplasia of the stomach and esophagus is common, whereas the converse, gastric metaplasia of the midgut or hindgut, is uncommon.

Morphogenesis

The lumenal gastrointestinal tract acquires it shape through rotational changes at a gross level, and through tissue remodeling at a microscopic level. Left–right (L–R) asymmetry of the intestine is generated through the same mechanisms that regulate the L–R axis of the body plan. This process involves the clockwise movement of cilia, which promotes the asymmetric distribution of inductive signals [118]. Dysregulation of cilium function leads to randomization of L–R asymmetry and clinical phenotypes including situs inversus.

The intestine undergoes tremendous growth during the initial embryonic period, and elongates about 1000-fold between the 5th and 40th weeks of human development [119]. To accommodate a large embryonic liver, the intestine exists outside the

abdominal cavity for much of its early embryonic life ("physiological herniation"). Early in development, the growing midgut and hindgut undergo a two-step rotation (Figure 1.8) totaling 270° (counterclockwise orientation viewing the embryo en face). Both growth and looping of the intestine require the action of HLX, a homeobox transcription factor that is expressed in the midgut and hindgut mesenchyme and that is also required for liver development (see Section Budding of the liver). *Hlx* mutant mouse embryos have a shortened and single-looped gut that undergoes normal differentiation [53]. Although many congenital anomalies are related to errors in these gross movements of the intestine, most notably midgut malrotation with risk of ensuing volvulus, the mechanisms underlying normal rotation are poorly understood.

Although our understanding of this dramatic intestinal growth and rotation remains mainly descriptive and phenomenological, the mechanisms controlling the cross-sectional makeup of the intestine are better understood. The stereotyped circumferential arrangement of cells according to each intestinal segment has been referred to as the *radial axis* of the gastrointestinal tract. Starting from the lumen, the radial axis goes from innermost epithelium, lamina propria, muscularis mucosae, submucosa, outer muscular layers, out to the serosa. Each intestinal segment has a unique epithelial and mesenchymal composition; for example, the stratified squamous epithelium and thin submucosa and muscular layers of the esophagus versus the columnar epithelium and thickly muscled mesenchyme of the stomach.

Shh–BMP crosstalk appears to be important for determining the composition of the radial axis in each intestinal segment. This conclusion is based on several lines of evidence. First, Shh is expressed throughout the gut epithelium (except for the pancreas, as discussed in the Section Specification of the pancreas) and is a potent activator of mesenchymal BMP expression so that the two signaling pathways regulate each other. Second, ectopic BMP expression affects the degree of muscularity of the mesenchyme along the anterior–posterior axis [110], suggesting that it regulates mesenchymal morphology. Third, Shh signaling is necessary for normal crypt–villus structure [120], and Shh regulates mesenchymal fate according to the distance from the epithelium [121]. These results are consistent with a model in which a concentration gradient of Shh (expressed by the innermost epithelium) organizes the mesenchymal rings of the gut, possibly through the activity of BMPs. According to this model, mesenchymal cells closest to the epithelium are induced to adopt a lamina propria or submucosal fate, whereas only those cells furthest from the epithelium adopt a muscle fate [121].

The intestinal lumen forms after 7–8 weeks of gestation (human) and arises through the processes of canalization and morphogenesis. The failure of canalization is thought to account for some cases of duodenal atresia, a partial or complete obstruction of the duodenum that occurs with a frequency of 1 in 5000 to 1 in 10 000 births, while morphogenesis involves polarization of the epithelium and transformation of a stratified epithelium to a columnar epithelium. As villi emerge from the stratified epithelium, they acquire a distinctive crypt–villus architecture, a process that is dependent on the cytoskeleton. One of these cytoskeletal elements is the "bridge" protein ezrin, which links membrane proteins to the actin cytoskeleton. Ezrin-deficient mice exhibit normal intestinal differentiation and polarity but abnormal villi, including nascent villus structures that are unable to break away from each other [122].

Cell proliferation and kinetics

The adult small intestinal epithelium has a rapid and regular turnover, with the average lifespan of intestinal enterocytes measured in days [123]. To support this constant need for new cells, the intestine recapitulates the embryonic processes of differentiation from stem cells throughout life (Figure 1.9). Stem cells are specialized cells that can generate multiple differentiated cell types ("multipotentiality") and also produce more stem cells ("self-renewal"). Intestinal stem cells reside near or at the bottom of the crypts and are characterized by their relatively low rate of cell division and long life [124].

Progenitor cells with a more limited potential and shorter half-life coexist with stem cells in the crypts [125]. A subset of stem cell-derived progenitor cells, known as the *transient amplifying population*, undergoes rapid cell division within a region of the crypt–villus axis known as the *proliferative zone* (see Figure 1.9). Mesenchymal factors, including the winged helix transcription factor Fkh6 [126], and several intercellular signaling pathways regulate cell division in this zone. Stem cell regulation in the GI tract is discussed in detail in Chapter 2.

Differentiation

The differentiated cells of the intestine (see also Chapter 5) can be divided into *absorptive* and *secretory* cells on the basis of cellular function. The precise identity and relative abundance of absorptive and secretory cells varies along the anterior–posterior axis. The major secretory cells of the stomach (and their secretory products) are parietal cells (acid), chief cells (digestive enzymes), and endocrine G cells (gastrin). By contrast, the major secretory cells of the small intestine are goblet cells (mucous), Paneth cells (antimicrobial peptides), and enteroendocrine cells (myriad hormones). Nevertheless, the genetic mechanisms that regulate the development of these different cells are shared between different segments of the gastrointestinal tract.

As in the pancreas, Notch signaling plays a critical role in the regulation of intestinal cell fate by mediating the expression of several downstream bHLH proteins – NGN3, BETA2/NEUROD, HES1, and MATH1. The general conclusion from studies of mice with mutations of these proteins is that differentiation of the secretory cell lineage is triggered by repression of Notch signaling and HES1, with the resulting derepression of MATH1 [127,128]. Additional signals control the selection of different intestinal secretory cell lineages (endocrine, goblet, and Paneth). One of these signals, NGN3, is absolutely required

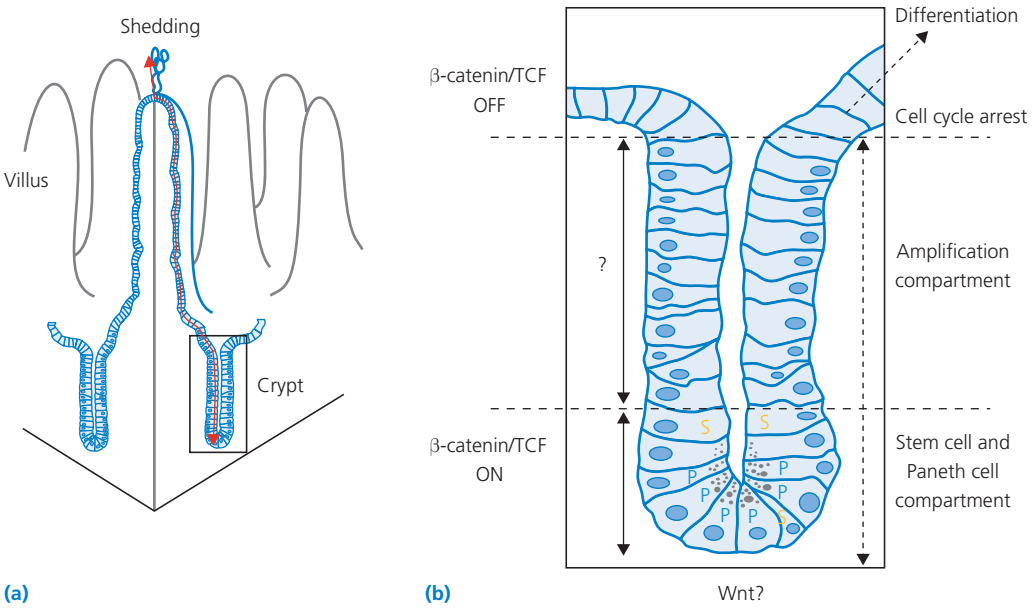


Figure 1.9 Small intestinal maturation. **(a)** Maturation of the crypt–villus axis. Stem cells near the base of the crypt give rise to a transient amplifying multipotent population of cells that reside in the middle and upper portions of the crypt. These cells give rise to mature enterocytes, enteroendocrine cells, and goblet cells, which migrate up the villus and are eventually shed into the intestinal lumen. Paneth cells are also derived from the transient amplifying population, but these cells migrate down to the crypt base where they intermingle with stem cells. **(b)** Small intestinal crypt. Schematic showing the crypt is divided into compartments. At the base are stem cells (S), which are thought to reside slightly above the crypt base, and Paneth cells (P). Canonical Wnt signaling is active in these cells. Above this compartment is the transient amplifying population, containing actively dividing cells. Cells withdraw from the cell cycle as they reach the crypt–villus interface and adopt an absorptive or secretory fate. Source: Adapted from Sancho et al. 2003 [228]. Reproduced with permission of Elsevier.

for endocrine cells to form in the intestine, but not in the stomach; by contrast, the formation of goblet and Paneth cells is normal in *Ngn3*-deficient mice [129,130]. Other complex signals and lineage relationships underlie the development of the 10 or so different types of enteroendocrine cells [131].

There is additional evidence that Notch signals are coordinated with Wnt signals to regulate the balance between proliferation and differentiation. Either embryonic activation of Notch or inhibition of Wnt results in the loss of secretory cell lineages [132–134]. Furthermore, Wnts have a special role in ensuring proper compartmentalization of the crypt–villus axis by regulating another family of cell–cell signaling molecules known as ephrins [135].

Several studies suggest that major regulators of embryonic differentiation – Notch and Wnt – are also involved in adult intestinal homeostasis. These are discussed in Chapter 2. Of note, activation of Wnt signaling through the loss of the *APC* gene and the subsequent activation of β-catenin is known to be a key step in colorectal carcinogenesis (see Chapters 31 and 78), suggesting that intestinal stem cells or transient amplifying cells are most sensitive to the inactivation of *APC* and represent a likely target for malignant transformation.

In summary, the formation of sphincters during midgestation divides the gut tube into segments – esophagus, stomach, small intestine, and large intestine – that prefigure their distinct morphologies and functions. The mechanisms underlying sphincter formation and gut tube patterning are incompletely understood but involve significant epithelial–mesenchymal crosstalk. Similar crosstalk is involved in the morphogenesis of the different intestinal segments. Subsequently, intestinal development proceeds by differentiation from stem cells, a process that depends on Wnt and Notch signaling, among other pathways. Such signals control proliferation and differentiation in the adult intestine and are dysregulated during carcinogenesis.

Conclusions

After gastrulation, the gut tube is exposed to regional signals from adjacent nonendodermal cells. In prespecified organ domains, the epithelium responds by growing into adjacent mesoderm-derived mesenchyme, resulting in the budding of lung, liver, and pancreas. In the remaining gut epithelium, reciprocal signaling with mesenchyme results in the formation of sphincters or regionally distinct submucosal layers. Complex morphogenetic changes and differentiation events occur in each of these developing organs, giving rise to organized functional tissues. Tissue-specific gene expression begins, setting the stage for further refinement of regulated expression and function. In the adult intestine, differentiated cell types are generated throughout life from stem cells that reside within the crypts, an ongoing process that recapitulates many developmental events. In the liver and the pancreas, by contrast, the replication of

existing cell types appears to be the major mechanism for tissue maintenance.

Developmental physiology

The development of complex anatomical structures with distinct differentiated cell lineages would be purposeless if it did not facilitate function. The functions of the gastrointestinal organs include assimilation of nutrients, detoxification and elimination of waste, maintenance of blood glucose, and synthesis of plasma proteins. In addition, the gastrointestinal tract subserves secondary roles in water and electrolyte balance and immunological defense. The physiology of the intestine, pancreas, and liver is considered in Chapter 12.

The connection between form and function is reflected in an economy of mechanism. Many genes exhibit dual function and are involved in both development and physiological regulation. As previously described, FOXA2 and GATA4 bind to the albumin promoter as part of a program of endoderm commitment, and are thus involved in both patterning as well as the functional expression of a liver-specific gene. There are several other instances of such developmental "parsimony"; for example, the *Pdx1* and *p48* genes play important roles in pancreatic development and are also the major transcriptional regulators of insulin and of several exocrine genes, respectively [81,97]. Similarly, the *CDX2* homeobox gene product plays an important role in endoderm patterning [13,15,16,136] and also functions as a major transcription factor for the expression of brush border enzymes and intestinal carbonic anhydrase [137–139]. Such economy is not surprising because the use of a limited set of genetic tools reduces the need for additional layers of complexity during specification and differentiation. Fortuitously, this arrangement facilitates the study of developmental physiology, as identification of the genes that regulate development provides a list of candidate regulators of function, and vice versa.

Maturation of the gastrointestinal tract

After parturition, the gastrointestinal tract faces two challenges. Immediately after birth, the individual must convert from a diet that is predominantly parenteral (provided by the maternal circulation), to one that is completely enteral (consisting of colostrum and breast milk). Later, at weaning, the gastrointestinal tract must be able to assimilate nutrients from a vast array of solid foods. These adjustments occur in a hormonal milieu that is increasingly under the control of the infant. Therefore, unlike structural development, which follows a set of preprogrammed genetic events, functional development is likely to be considerably more dependent on environmental forces [111].

Gastrointestinal "maturation" refers to the progressive attainment of features of adult gastrointestinal physiology during development. Given the imprecise nature of such a definition, several surrogate markers are used to understand how the physiology of the gastrointestinal tract changes over time. These include biochemical measurements of intestinal enzyme and hormone activities, morphological grading, mutant analysis, and measurements of permeability, motility, and immune performance. It has been suggested that the human gastrointestinal tract is structurally and functionally mature at the time of parturition, whereas the rodent gastrointestinal tract is altricial, or immature, at birth. However, given the comprehensive nature of maturation, and the fact that the neonatal diet of all mammals is similar (i.e., milk), the implications of such a distinction are unclear.

Carbohydrate digestion and absorption

A focal point in the study of intestinal maturation has been the characterization of the major brush border enzymes that digest carbohydrates. Lactase–phlorizin hydrolase (LPH), cleaves lactose, the major dietary carbohydrate of breast milk. In rats, LPH is expressed at high levels early in embryogenesis, whereas peak expression in human embryos occurs during the third trimester. LPH expression declines with age in both species. The observation that LPH expression across species is highest after birth and subsequently lower likely reflects the critical requirement for lactase during nursing [140].

Sucrase–isomaltase (SI) is another well-studied brush border enzyme. In contrast to LPH, the expression of SI is discordant between humans and other mammals. In rats and pigs, SI expression is undetectable before a dramatic burst of expression in the postnatal period, corresponding to the time just before weaning when the major carbohydrate source shifts from milk to starch. By contrast, SI expression in humans begins in the first trimester and reaches its peak level just before birth [140]. The earlier expression of SI during human ontogeny is not understood, and it is unclear whether differences in enzyme expression levels reflect differences in overall functional maturation between species.

Protein digestion and absorption

The embryo has a limited capacity to digest proteins, a result of the late expression of digestive zymogens, the low-level expression of the activating enzyme enterokinase, and the insensitivity of embryonic pancreatic exocrine cells to the action of secretagogues (see the Section Hormonal control of gastrointestinal development). Furthermore, gastric pH is neutral until birth, dropping rapidly from 6.0 to 2.2 in the first day of life [141]. Instead, other systems handle the limited protein load delivered to the intestine pre- and perinatally. Brush border and microvillar peptidases and dipeptidases, which complete peptide digestion, are present in the fetal small intestine at levels of activity comparable to levels in the adult small intestine. High levels of amino acid transporters in the newborn permit the uptake of free amino acids.

Macromolecular transport also plays an important role in the digestion of proteins and lipids in the fetus and the neonate [142–144]. In experimental animals, the small intestinal epithelium is

most permeable to amino acids and peptides in the immediate postnatal period. Macromolecular tracers infused into the amniotic fluid or the intestinal lumen late in gestation are absorbed into the enterocytes of humans, monkeys, guinea pigs, and rats, reflecting a high rate of pinocytosis [144]. This process is extremely active in the first 2 weeks postnatally and decreases at weaning. This mechanism accounts for the absorption of intact maternal immunoglobulins and other proteins from milk.

In parallel with pinocytosis, enterocytes exhibit high levels of lysosomal proteases, such as cathepsins and other peptidases, during the first 2 weeks postnatally. These intracellular enzymes provide a mechanism for protein digestion before the appearance of the pancreatic proteolytic enzymes. Intact proteins also are absorbed in premature and term human infants during the first few months of life. Macromolecules may continue to cross the healthy adult small intestine, but the quantity is low compared to those in the newborn. The relative permeability of the intestine during the first months of life may play an important role in conferring tolerance or sensitivity to dietary proteins during the development of immune function.

Lipid digestion and absorption

Fats and unhydrolyzed triglycerides are present in the stools of human neonates at a rate that is higher than that of adults, a phenomenon that correlates with the low activity of pancreatic lipase and the low intralumenal concentrations of bile acids. Although pancreatic lipase levels rise significantly during the third trimester, lipase activity at week 32 of gestation is only 50% of term levels, which are themselves only 10% of adult levels. Fat digestion in human neonates is aided by "preduodenal" lipases (lingual and gastric lipases) and maternal milk lipase. Lingual lipase rises to adult levels by 2 years of age [145]. Gastric lipase appears as early as 10–13 weeks into gestation and reaches adult levels by 16 weeks [146]. Gastric lipase appears to be a major determinant of lipolytic activity in gastric aspirates of premature infants. As with peptides, the newborn intestine exhibits increased permeability to both triglycerides and cholesterol [147].

The synthesis of bile acids from cholesterol and their conjugation with taurine and glycine can be demonstrated in organ culture in vitro with human liver tissue obtained from fetuses after 15 weeks of gestation. Biliary secretion is observed as early as the 22nd week of gestation. Bile acid reabsorption occurs in the neonate by passive diffusion throughout the small intestine, but active sodium-dependent ileal transport of bile acids does not occur until weaning [148]. As a result, the bile acid pool is reduced in neonates; this is of particular concern in premature infants, in whom 10%–20% of ingested fat may not be absorbed.

Dietary control of gastrointestinal development

The expression of SI, LPH, and other brush border enzymes appears to be under autonomous control, because their normal expression pattern does not change significantly with delayed or early weaning or early introduction of dietary sucrose [149]. Indeed, transplanted human fetal intestine is able to undergo normal cytodifferentiation in an immunodeficient "nude" mouse host [150]. However, other aspects of gastrointestinal development, particularly growth, are regulated by diet. Exposure of the gut lumen to nutrients begins in utero with the swallowing of amniotic fluid, which contains amino acids and carbohydrates, and which the embryo uses to meet some of its nutritional requirements.

The importance of the lumenal environment is supported by studies in which the timing, the composition, or the route of delivery of nutrition is varied. Ligation of the embryonic sheep esophagus causes reversible and specific inhibition of growth of the gastrointestinal tract [151]. Although normally absent in human amniotic fluid, galactose can nevertheless be absorbed by the embryonic jejunum. Intraamniotic infusion (and therefore increased enteral delivery) of galactose induces an increase in the mucosal transport of galactose by the rabbit intestine, as well as an overall increase in mucosal weight, suggesting that the fetal intestine is competent to respond to small changes in enteral carbohydrate composition [152]. Consistent with this, intestinal growth in the first day of life depends on the composition of milk [153]. Importantly, it is not simply the metabolic consequences of feeding that provide a signal; the intestinal mucosa itself must be exposed to these nutritional components [154]. A requirement for lumenal stimulation has long been appreciated in the "adaptation" observed after massive intestinal resection – a compensatory increase in intestinal surface area that depends on enteral feeding [155]. It is possible that this adaptation reflects a reemergence of a developmental program that regulates intestinal size and surface area. Indeed, microarray analysis of gene transcription during development and adaptation supports this hypothesis [156].

Hormonal control of gastrointestinal development

A possible regulatory role for corticosteroids and thyroid hormone in intestinal development has been extensively explored because of the dramatic increase in the level of both hormones observed in rats immediately before the spike in SI activity and coinciding with a reduction in LPH activity. Direct effects on the activity of several disaccharidases have been documented after the administration of exogenous hormones. Notably, prenatal administration of cortisone reduces the incidence of necrotizing enterocolitis in a rat model, presumably by accelerating the maturation of the mucosal barrier [157]. Conversely, intestinal maturation is slowed by treatments that reduce levels of circulating corticosteroids. Similar effects are seen with enhancement or inhibition of thyroid hormone expression, although some of these effects may be mediated through corticosteroids [140]. However, mice lacking corticotropin-releasing hormone or thyrotropin releasing hormone do not exhibit an overt gastrointestinal phenotype [158,159]. The regulation of gastrointestinal maturation by other hormones and circulating growth factors has also been

investigated through similar approaches. In particular, cholecystokinin, gastrin, insulin, and members of the insulin-like growth factor (IGF), epidermal growth factor (EGF), and TGF families have been the focus of numerous studies.

These analyses have yielded evidence for the involvement of hormones and systemic growth factors in gastrointestinal development. However, distinguishing between primary and secondary effects is challenging, and for the most part, the precise functions of these molecules in development remain to be delineated. It is worth noting mice with a targeted inactivation of the gastrin gene exhibit a deficiency of acid-producing parietal cells [160,161], suggesting a role in cellular differentiation rather than maturation per se.

Despite lingering uncertainty over the precise role of hormones in intestinal maturation, it is clear that the responsiveness of some gastrointestinal tissues to hormones changes over the course of fetal and postnatal life. The responsiveness of the exocrine pancreas is an example of such regulation. Pancreatic digestive and lipolytic enzymes are detected in the early bud stage, and high levels of protein are detected in the acinar cells before term. Despite the abundance of these proteins, embryonic acini are insensitive to secretagogues until after birth [162]. Similarly, sensitivity to the acid-secretory action of gastrin develops during the first week of life; poor expression of the gastrin receptor in the immediate postnatal period renders newborns relatively insensitive to gastrin [163]. Finally, as a source of insulin, the pancreas is the major regulator of glucose homeostasis, and the intestine contains numerous peptides that regulate motility, ion transport, feeding, and satiety [164].

Development of the enteric nervous system

The enteric nervous system (ENS) regulates many aspects of gastrointestinal physiology, including peristalsis and smooth muscle activity, sphincter tone, glandular output, microcirculation, and possibly, inflammation [165]. Through these activities, the ENS controls the response to feeding by coordinating intestinal transit, secretion, and continence. The cells that give rise to the ENS migrate from the neural crest during the first trimester, induced by complex and poorly understood signals. Similar to other neural crest derivatives, the ENS is part of the peripheral nervous system, constituting its largest subdivision. Although the ENS receives input from the vagus nerve, it possesses marked independence from the central nervous system, exhibiting function even after complete dissociation from all brain and spinal inputs. On the basis of its size and autonomy, the ENS has been referred to as the "second brain" [166]. Although many disorders may be related to ENS dysfunction, only Hirschsprung disease is clearly attributable to developmental errors in ENS formation (see Section Disorders of specification and formation).

Fate mapping in the chick has shown that enteric neurons are largely derived from rostral (vagal) and caudal (sacral) precursors that migrate from the neural tube and intermingle to populate the entire gut tube [167]. Although some details remain controversial, studies in mice have confirmed the general picture of neural crest migration mapped out by studies in the chick. The ENS is composed of two types of ganglionated plexuses: the Auerbach (myenteric) plexus, which is located in the outer muscular layer and regulates gastrointestinal tract motility and function of extralumenal organs, and the Meissner (submucosal) plexus, which regulates enteral secretory activity [166]. Enteric neurons can be further subclassified according to the neurotransmitters (e.g., vasoactive intestinal polypeptide and serotonin) and enzymes (e.g., tyrosine hydroxylase and choline acetyltransferase) they express. Although details regarding the migration and terminal differentiation of neural crest precursors are still emerging, neuronal subtypes appear to arise in overlapping developmental waves [168]. The functional roles of these neuronal subtypes and specific neuropeptides in gastrointestinal physiology are described elsewhere in this textbook (see Chapters 13 and 15).

In contrast to the neural crest-derived cells of the enteric plexuses, interstitial cells of Cajal (ICCs), which serve as the "pacemakers" of the intestine, arise from intestinal mesenchyme [169,170]. The development of these cells requires the function of another receptor tyrosine kinase, c-Kit. Mice with reduced or absent c-Kit function exhibit abnormal slow-wave activity in the small intestine and develop paralytic ileus [171,172]. It has been discovered that those mesenchymal tumors known as gastrointestinal stromal tumors (GISTs) have activating mutations in *KIT* that confer constitutive kinase activity in the absence of ligand [173,174]. Ultrastructural similarities between ICCs and GISTS, and other shared features, have led investigators to propose that GISTs arise from ICCs [175] or from a common ICC–smooth muscle precursor cell [176].

A central role for the c-Ret tyrosine kinase pathway in the development of most enteric neurons has been demonstrated through targeted inactivation of pathway components (see the discussion of Hirschsprung disease). Furthermore, important neural crest subpopulations have been recognized from the more limited phenotypes that result from the targeted mutation of other genes. For example, the basic helix-loop-helix protein MASH1 is required for the development of a subset of enteric neuronal precursors with noradrenergic features, and endothelin B signaling is required to prevent the differentiation of neuronal precursors that will enervate the distal colon [177]. The significance of these different subtypes is unclear, and the mechanisms by which they achieve regulatory integration require further study.

The ENS begins to function early in embryonic development, but its maturation continues well into postnatal life. Fetal swallowing is first detectable during the first trimester [178], and by term, the fetus swallows about 450 mL amniotic fluid (half of the total amniotic volume) per day [179]. A spectrum of neuropeptides is detectable between weeks 11 and 18 of human development [180]. Peak numbers of both neurons and ganglion cells are achieved during the second trimester and decrease during the third trimester [181]. Although the

structural elements of the esophagus and stomach are largely developed by midgestation, gastroesophageal motility does not fully mature until after birth. Lower esophageal sphincter pressure increases dramatically during the last trimester and again postnatally [182], achieving adult levels by 3–6 weeks of age. Despite this, free gastroesophageal reflux is common postnatally and persists in up to 10% of infants for the first year [183].

Mucosal immune system

The gastrointestinal tract, particularly the small intestine, contains a highly complex mixture of immune cell populations. The gut-associated lymphoid tissue (GALT) encompasses organized aggregates dominated by lymphocytes (Peyer patches) and a diffuse heterogeneous population of lymphocytes, monocytes, or macrophages, and other cells, such as eosinophils and mast cells in the lamina propria. Intraepithelial lymphocytes are also scattered throughout the surface epithelium. Structures resembling Peyer patches are evident as early as 11 weeks of human gestation; by 14 weeks, CD4+ and CD8+ lymphocytes can be detected. By the end of the second trimester, Peyer patches histologically resemble the adult structure, indicating that antigen exposure or bacterial colonization are not necessary for their development; however, germinal centers do not form until after birth. Mice carrying a null mutation for TNF-α do not develop Peyer patches or lymph nodes, and splenic organization is markedly abnormal; if the 55-kDa receptor for TNF-α is disrupted, lymph nodes and splenic tissue develop normally, but Peyer patches are still absent, suggesting that the 55-kDa receptor provides specificity for Peyer patch development. Other targeted mutations that result in the absence of Peyer patch development in mice include knockout of the inhibitory helix-loop-helix transcription factor Id2, lympohotoxins, and the lymphotoxin-β receptor. Mice lacking Peyer patches do not develop oral tolerance. Targeted disruption of the homeodomain-containing the transcription factor gene *Nkx2.3* in mice results in significant defects in intestinal development and also smaller Peyer patches and loss of expression of the mucosal cell adhesion molecule 1 (MadCam1), which is normally responsible for B-cell and T-cell homing to peripheral lymphoid organs. Full maturation of the immune system, and specifically Peyer patch formation, is dependent on postnatal bacterial colonization.

Lamina propria lymphocytes are first detected after 11 weeks of gestation. Macrophages are present at 12 weeks, but increase greatly in number after birth. Recruitment and maturation of mucosal lymphocytes depend on retinoic acid, presumably produced by intestinal epithelial populations. During fetal life, lymphocytes consist of increasing numbers of scattered T cells and B cells. In contrast to αβ T cells, γδ T cells, which make up 5%–15% of small intestinal and 40% of colonic intraepithelial lymphocytes, can develop extrathymically as well as in the thymus. The γδ T cells undergo clonal expansion soon after birth but with further maturation they become clonally restricted and unique in each individual. Targeted deletion of γδ T cells in mice results in a lack of mucosal B cells that produce immunoglobulin A (IgA) but has no effect on αβ T-cell development, which is thought to occur within the thymus. IgA- and IgM-producing plasma cells are not found in the lamina propria until after birth and antigenic exposure. Intraepithelial lymphocytes appear at 11 and 12 weeks of gestation. Fetal lamina propria lymphocytes are mostly CD4+ as in the adult lamina propria, and fetal intraepithelial lymphocytes are often CD4− CD8−; CD8+ cells become more predominant after birth.

As noted, exposure to the lumenal flora is necessary for maturation of the mucosal immune compartment. In rats, suckling and germ-free animals have fewer intestinal lymphocytes than adults, and weaning – associated with intestinal maturation and increasing bacterial colonization – is also characterized by marked development of the mucosal immune system. Cyclosporine (cyclosporin), an inhibitor of T-lymphocyte activation, retards normal lymphocyte development in the small intestine. Natural killer activity of intraepithelial and lamina propria lymphocytes is absent before birth, rising dramatically after weaning.

Conclusions

The genes and signals that give rise to the primitive structures of the gastrointestinal tract become progressively invested with functionality during embryogenesis and postnatal life. Some features (e.g., synthesis of pancreatic hormones, neuropeptides, and certain digestive enzymes) are largely under autonomous control, whereas other features (e.g., intestinal growth and development of mucosal immunity) are highly dependent on interactions with the environment.

Disorders of development

The sections above have described the basic events and mechanisms that allow the normal development of the gastrointestinal tract, the pancreas, and the liver. While dysgenesis may result from disturbances of any one of these steps, errors in gastrulation or endoderm formation do not present clinically because the global importance of these early steps for further development render them lethal during embryonic development. The range of observable clinical phenotypes is therefore confined to those that are compatible with advanced embryonic development. It should be emphasized that developmental disorders involving the gastrointestinal tract are most commonly observed as part of multigenic disorders. Of these, the most common is Down syndrome (trisomy 21 syndrome), which is associated with duodenal atresia, tracheoesophageal fistula, Hirschsprung disease, and imperforate anus. In the following sections, disorders have been selected to illustrate key events in organ formation and organogenesis along with their (known) molecular underpinnings.

Disorders of specification and formation

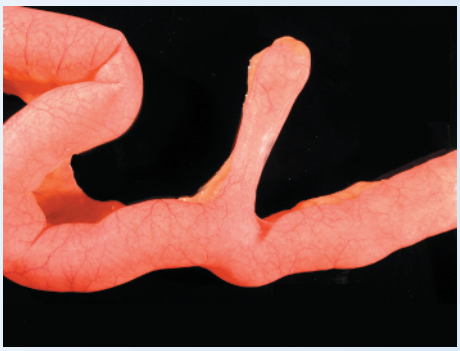
Congenital gastrointestinal malformations may occur in the setting of Down syndrome or other syndromes, or they may

Box 1.3 Meckel syndrome.

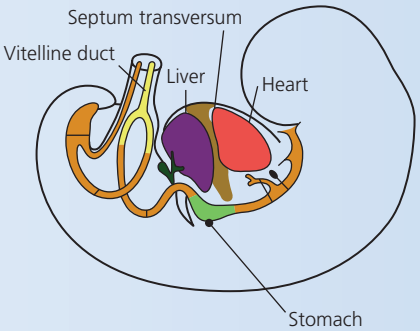
Meckel diverticulum is the most common congenital malformation of the gastrointestinal tract, occurring with a frequency of 2% of births [205]. The disorder reflects a persistence of the vitelline duct – the embryonic structure connecting the gut to the yolk sac (Figure 1.10). Meckel diverticula are generally located near the terminal ileum, and in about 50% of patients the diverticulum contains ectopic tissue, most commonly gastric or pancreatic, but occasionally also colonic, duodenal, jejunal, hepatic, and endometrial [206]. Secretion of gastric acid (and in some cases pancreatic bicarbonate) causes ulceration of adjacent small intestinal mucosa; the disorder commonly presents as unexplained gastrointestinal hemorrhage in a child or young adult (see Chapters 5 and 69). Note that heterotopia is distinct from metaplasia, which represents an acquired replacement of one tissue type with another over time.

What mechanism might account for the defective patterning leading to heterotopia?

Bossard and Zaret [207] observed that 3% of mouse embryos exhibit an albumin-expressing ectopic bud at the site of the vitelline duct, near the terminal ileum, which led them to propose that Meckel diverticula result from the loss of normal mesenchymal inhibitory signals at the site of the vitelline duct. According to this attractive model, heterotopic tissue forms not as a result of ectopic cells "left behind" by the nonregressed vitelline structure, but because a signal required for patterning and specification was disrupted by the error in regression (see Section Heterotopias in disorders of development).



(a)



(b)

Figure 1.10 **(a)** Gross specimen showing a Meckel diverticulum in the embryo. Source: Courtesy of Beth Furth, University of Pennsylvania School of Medicine. **(b)** Embryonic vitelline duct.

occur as isolated findings. For example, anorectal malformations are common birth defects that may be found in isolation or as part of a syndrome, such as the VACTERL syndrome (vertebral, anal, cardiac, tracheal, esophageal, renal, and limb abnormalities). Clinical features of anorectal malformations are discussed in Chapters 6 and 81.

The etiology for most congenital malformations is unknown. Certain anomalies result from lesions in a single gene (e.g., see Box 1.2), whereas others may be associated with a disruption of a signaling pathway (e.g., see Box 1.3: Meckel syndrome and the discussion of heterotopias). Another class of congenital syndromes may reflect a common final pathophysiological pathway that can be disrupted by any of a number of events. Hirschsprung disease is an instructive example of this last class.

Hirschsprung disease

As already noted, neural crest cells migrate from the neural tube during midgestation to give rise to the ganglion cells of the ENS. Absence of these cells (aganglionosis) in the colon results in Hirschsprung disease, a male-predominant disorder that most commonly presents in the perinatal period. Absent peristalsis in the affected segment of colon causes constipation (or failure to pass meconium), distal obstruction, and megacolon. Hirschsprung disease always affects the rectum; more proximal segments are affected in a few patients, and, rarely, the small bowel (see Chapters 6 and 81 for a detailed clinical discussion). Although Hirschsprung disease can be inherited in an autosomal or recessive fashion, most cases exhibit non-Mendelian inheritance with a genetic component. Hirschsprung disease is commonly associated with Down syndrome.

Receptor tyrosine kinase RET

Heterozygous mutations in *RET*, a transmembrane tyrosine kinase (chromosome 10q11.2), represent the most common genetic alteration resulting in Hirschsprung disease. The gene for RET is expressed in ENS precursors, whereas those for its ligands (which include GDNF and neurturin [NRTN]), are expressed in the mesenchyme of the developing gut. On binding to one of its cognate ligands, RET normally activates a membrane complex that includes a glycosylphosphatidylinositol-anchored signaling component (GFRA1–4) [177]. Mutations in the *RET* gene cause disease by reducing kinase function, which interferes with the proper differentiation, survival, or migration of these cells. Such mutations are present in up to 50% of patients with familial disease. A small percentage of patients with sporadic disease have inactivating RET mutations, and

polymorphisms in the gene may also play a role [177,184,185]. Mutations in the RET ligand GDNF have also been found in patients with Hirschsprung disease [186,187], and mutations in the NRTN ligand may contribute to disease severity [188]. Polymorphisms in the homeobox transcription factor PHOX2B, a putative regulator of RET [189], are also associated with Hirschsprung disease [190]. Consistent with a specific role for RET in neural crest cell biology, a high frequency of activating mutations occur in patients with multiple endocrine neoplasia type 2, who develop a spectrum of neural crest-derived tumors [191].

Sox10

Mutations in genes with no apparent link to RET signaling are also associated with Hirschsprung disease. One of the first models of Hirschsprung disease was Dominant megacolon (Dom), a naturally occurring mouse mutant that exhibited pigmentary defects and aganglionosis [192]. Mutations in the SRY-related transcription factor SOX10 are responsible for the Dom phenotype [193,194]. In contrast to most mouse models of Hirschsprung disease, haploinsufficiency of SOX10 is sufficient to cause colonic aganglionosis in *Sox10*$^{+/-}$ mice. *SOX10* mutations are also found in patients with Waardenburg–Shah syndrome, who exhibit Hirschsprung disease, pigmentary defects, and deafness. Thus, like RET, SOX10 also likely has a general role in the development of neural crest derivatives.

Endothelins

Mutations in endothelin 3 (*EDN3*) and its receptor (*EDNRB*) have also been found in patients with isolated Hirschsprung disease or the Waardenburg–Shah syndrome. Similar to *RET*, *EDNRB* is expressed in neural crest cells before and during migration, whereas its ligand is expressed by the gut mesenchyme; mutations in these genes account for about 10% of Hirschsprung disease cases [195]. In addition, a mutation in an endothelin-processing enzyme (ECE1) has been found in a patient with Hirschsprung disease [196].

The final common pathology in Hirschsprung disease is aganglionosis; hence, the disorder may reflect defects in the specification, migration, or survival of enteric neurons. Much work remains to be done to understand precisely how the identified genes function in normal ENS development and how mutations in these genes result in a Hirschprung disease phenotype. Given that most patients with Hirschsprung disease lack identifiable mutations, polygenic contributions are likely to be important. Alternatively, "errors" in migration, without a genetic contribution, may play a role in some cases.

Disorders of differentiation and patterning

Clinical phenotypes caused by the developmental failure to form a particular cell type are rarely observed. It is likely that many mutations affecting critical regulatory pathways (e.g., Notch signaling) are incompatible with life. Alternatively, redundancy or plasticity may lead to adaptive compensatory changes that permit normal or nearly normal differentiation in a mutant background.

Instead, disorders that affect patterning leading to *misplacement* of differentiated tissues occur with some frequency. These conditions may be the result of an acquired (metaplasia) or congenital (heterotopia) tissue placement. Metaplasia is often the harbinger of malignant transformation, as mentioned in the Section on Specification of the gastrointestinal tract and discussed in greater detail in Chapter 31. While the possible mechanism of heterotopia is discussed in the following section, note that the mechanism of metaplasia is entirely unknown. In particular, it is not clear whether the premalignant intestinal epithelium that replaces the normal squamous mucosa of the esophagus is a consequence of *transdifferentiation* between the two cell types or the growth and replacement of squamous cells by a quiescent stem/progenitor cell that exists within the esophagus.

Heterotopias

The presence of ectopic cell types (heterotopia) is observed in several tissue types, although in some cases the displacement is the result of faulty migration. Ectopic placement of gastric, pancreatic, and liver tissues have all been described and may occur in the setting of congenital gastrointestinal duplications. Of the simple heterotopias, two types occur with relative frequency: inlet patches and pancreatic heterotopias.

Inlet patches consist of a segment of gastric mucosa within the cervical esophagus and occur with a frequency of up to 4.5% in autopsy studies. Inlet patches contain true gastric mucosa and most exhibit oxyntic histology. Most cases are asymptomatic, although some may be complicated by infection with *Helicobacter pylori*, inflammation, bleeding, and malignant transformation [197]. Inlet patches are sometimes associated with intestinal metaplasia and pancreatic heterotopia.

Pancreatic heterotopias, also known as pancreatic rests, consist of ectopic pancreatic tissue, most often located within the proximal gastrointestinal tract. Autopsy studies estimate their frequency to range from 0.5% to 14%, although the true prevalence is probably on the lower end of the scale [198]. As with inlet patches, most pancreatic heterotopias are asymptomatic.

Both of these conditions are believed to be congenital, but the causes are unknown. One study shed light on a possible mechanism: the segmental absence of a developmental signal. As discussed in the Section Organogenesis of the pancreas, a key signal during the specification of the pancreas is the *repression* of Shh expression in the endoderm. Consistent with hedgehog repression being sufficient to specify pancreatic development, the exposure of mouse embryos to the drug cyclopamine, an inhibitor of Shh signaling, results in ectopic pancreas formation with an anatomic distribution that mimics that of human pancreatic heterotopia (stomach > duodenum > small intestine; Figure 1.11; see also Box 1.4 for a discussion of the related subject of annular pancreas). Thus, the failure of a patch of endoderm to receive a hedgehog signal could result in the specification of an

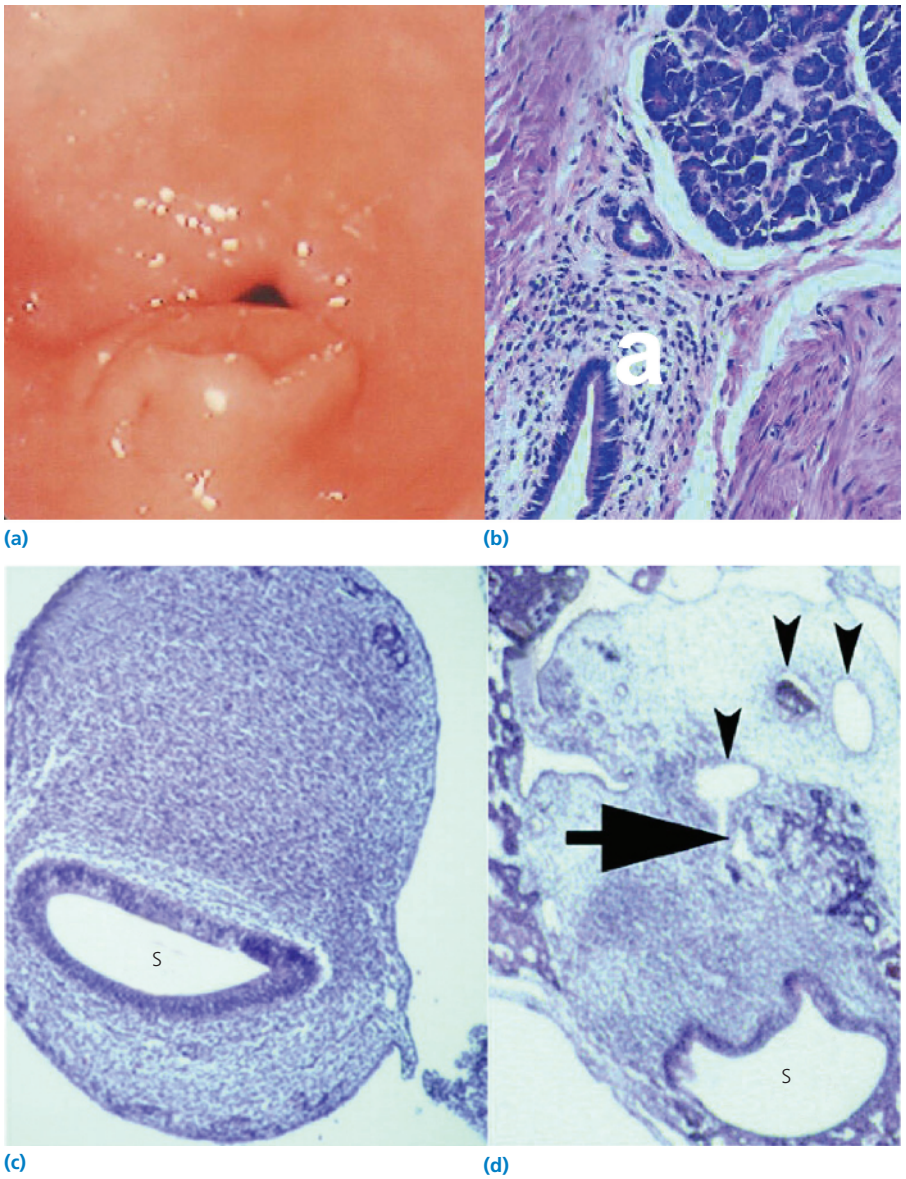


Figure 1.11 Pancreatic heterotopias (rests) in human and mouse. **(a, b)** Pancreatic heterotopia in human. **(a)** Endoscopy reveals dimpling of gastric epithelium. **(b)** Histology reveals pancreatic acini (a) adjacent to gastric mucosa. **(c, d)** Mouse model of pancreatic heterotopia. **(c)** Normal mouse stomach (S). **(d)** Mouse treated with cyclopamine exhibits pancreatic differentiation within the stomach (arrow). Arrowheads show vascular structures. **(a)** Source: Courtesy of William R. Brugge, MD, Massachusetts General Hospital. **(c, d)** Source: Kim and Melton 1998 [76]. Reproduced with permission, Copyright (1998) National Academy of Sciences U.S.A.

ectopic patch of pancreatic tissue. Similarly, the cervical esophagus could be particularly susceptible to the absence of a normally inhibitory signal during development, giving rise to an inlet patch. This presumptive mechanism could account for the development of ectopic tissues in other organs as well (see also the discussion of Meckel syndrome in Box 1.3).

Disorders of remodeling

Much is known about remodeling – the molding of patterned tissue through growth and development – in certain tissues, especially the developing central nervous system. By contrast, little is known about remodeling during gastrointestinal development. How are the vascular supplies of the intestine, pancreas, and liver tailored to physiological need? What mediates the integration of the ventral and dorsal pancreatic ductal systems (the failure of which causes pancreatic divisum)? How are the different endocrine cells in the pancreas guided to coalesce into the islets of Langerhans? Because the pathophysiology of some developmental disorders (e.g., Hirschsprung disease) may have a component of defective remodeling, the following discussion focuses on biliary tract remodeling as an example.

Box 1.4 Annular pancreas.

The pancreas forms from two buds – a ventral bud and a dorsal bud – that only later fuse into a single integrated gland during the rotation of the abdominal viscera (at which time the ventral portion rotates *behind* the duodenum to meet the dorsal portion). Dysregulation of this process is thought to result in annular pancreas, a condition in which the duodenum is encircled by pancreatic tissue (Figure 1.12). Annular pancreas was first described in 1818 by Tiedemann [199] and is the most common congenital anomaly of the pancreas to present in childhood, although nearly half of cases are first recognized in adults [200], in whom the condition presents with early satiety, nausea, and vomiting [201,202]. In pediatric patients, the disorder is associated with other congenital anomalies, and it is more common in patients with Down syndrome.

The etiology of annular pancreas is not understood, although several theories have been proposed, including hypertrophy or failure of atrophy of the left ventral pancreatic bud, fusion of heterotopic pancreatic rests, and malrotation [200]. Others have suggested that annular pancreas is not a primary malformation at all, but instead is a secondary consequence of duodenal obstruction from other causes. A mouse model of annular pancreas was serendipitously discovered while looking at the role of hedgehog signaling in pancreas development. Inactivation of Indian hedgehog (Ihh) and rarely Sonic hedgehog (Shh) results in a high frequency of an annular pancreas that encircles the duodenum [203]. This observation provides an experimental framework for determining whether rare cases of familial annular pancreas [204], or the more common annular pancreas associated with Down syndrome, are caused by disruptions in hedgehog signaling.

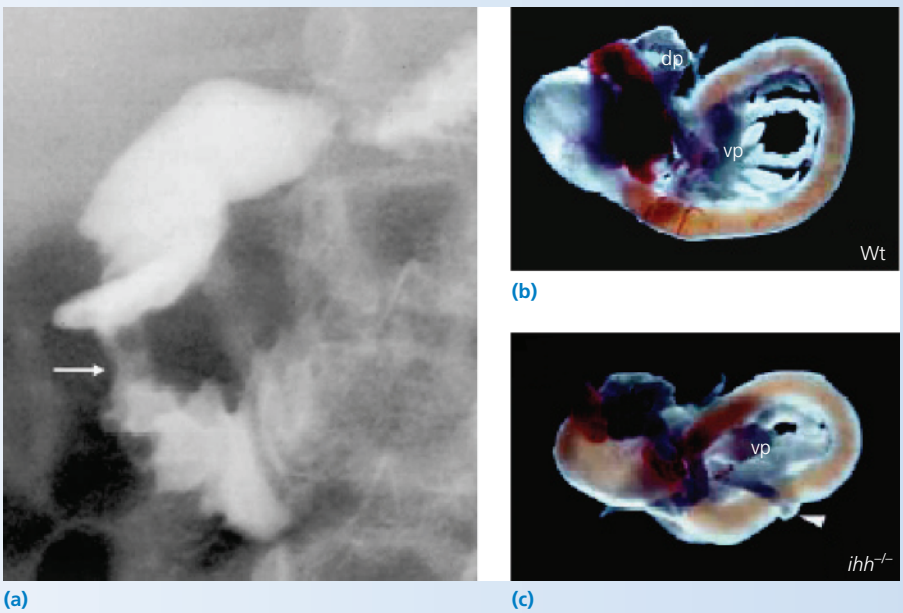


Figure 1.12 **(a)** Upper GI radiograph showing narrowing of the duodenum in the area of a pancreatic annulus. **(b)** Foregut structures from a wild-type (Wt) mouse have been dissected out, revealing strands of ventral pancreatic tissue (vp) within the duodenal loop. **(c)** In mutant animals lacking Indian hedgehog ($Ihh^{-/-}$), a piece of pancreatic tissue encircles the proximal duodenum (arrowhead). Source: Hebrok et al. 2000 [203].

Abnormal biliary development

As discussed in the Section Morphogenesis and differentiation in the liver, the ductal plate – a ring of specialized cells surrounding branches of the portal vein – gives rise to the intrahepatic bile ducts. Ductal plate remodeling appears to occur in two steps: formation of discrete tubules within the ductal plate followed by elimination of remaining cells through apoptosis, attrition, or differentiation.

Developmental or neonatal biliary disorders fall into two categories: ductal plate malformations and bile duct paucity. Ductal plate malformations refer to a collection of overlapping disorders that are characterized by faulty remodeling of the intrahepatic bile ducts, resulting in the persistence of the embryonic ductal plate configuration (see Figure 1.5; [208]). Congenital hepatic fibrosis is an autosomal recessive disease with variable histological and clinical features in which the portal tracts and bile ducts exhibit fibrosis and a ductal plate configuration. The histopathology of congenital hepatic fibrosis is seen in association with both autosomal recessive and autosomal dominant polycystic kidney disease. A related disorder, Caroli syndrome, is characterized by the ductal dilation of Caroli disease (type IVA/type V choledochal cysts; see Chapters 9 and 91) with the superimposed fibrosis of congenital hepatic fibrosis, suggesting an overlapping pathophysiology [208]. While the shared histopathological characteristics observed in these and similar abnormalities (e.g., von Meyenburg complexes) are intriguing, the etiology of these disorders remains completely unknown.

Insight into one potential mechanism for biliary malformation comes from studies of patients with a paucity of intrahepatic bile ducts, also known as Alagille syndrome. Although bile duct paucity is the sine qua non of Alagille syndrome, patients may also have several extrahepatic manifestations, including abnormalities of the great vessels, skeletal and ocular malformations, as well as characteristic facies (see Chapters 9 and 91). Two studies have shown mutations in the Notch ligand JAG1 are responsible for Alagille syndrome and strengthened the link between this developmental signaling pathway and the disease [67,68]. Consistent with this notion, many Alagille syndrome patients lacking JAG1 mutations have mutations in the NOTCH2 receptor instead [209].

An understanding of the molecular mechanisms underlying this disorder has come from mouse studies. JAG1 is expressed by portal veins and hepatic arteries [210–212], and mice with compound heterozygous *Jagged*/*Notch* mutations exhibit a paucity of intrahepatic bile ducts [213]. Furthermore, embryos deficient in the Notch target Hes1 develop ductal plates with normal appearance at the appropriate developmental time, but these ductal plates fail to form the tubular structures that precede normal duct development [214]. Finally, mutations in the Notch effector RBP-J lead to bile duct paucity, while activation of Notch signaling leads to bile duct excess [215]. Taken together, these studies suggest that Alagille syndrome results from the faulty specification of bile ducts in the absence of Notch signaling.

Biliary atresia, by contrast, is characterized principally by the loss of the extrahepatic rather than intrahepatic bile ducts. Biliary atresia is a heterogeneous disorder that presents with two major clinical patterns – a prenatal form that presents almost immediately after birth and is associated with other congenital anomalies, and a perinatal form that presents in the first few weeks of life. Although the etiology of both forms is poorly understood, defective morphogenesis of the bile ducts may play a role in the prenatal form of the disease [216]. Congenital anomalies affecting body symmetry, such as cardiac anomalies, intestinal malrotation, and abdominal situs inversus, often accompany the prenatal form of biliary atresia [217]. Mice with a mutation of the *inversin* gene exhibit abdominal situs inversus and a defective extrahepatic biliary tree [218,219]. Furthermore, missense mutations in *JAG1* have been observed in patients with severe refractory biliary atresia, suggesting that this Notch ligand contributes to disease progression [220]. Consistent with a connection between intra- and extrahepatic ductal pathology, some patients with biliary atresia exhibit the histological characteristics of ductal plate malformation observed with congenital hepatic fibrosis [208].

Disorders of growth control

Several rare disorders that affect the growth of specific parts of the body highlight another developmental phenomenon: genomic *imprinting*. In mammals, which contain sets of paired chromosomes, the maternally inherited chromosome differs from the paternally inherited chromosome both in terms of primary sequence (polymorphisms) and in additional epigenetic (noninherited) differences. Epigenetic differences are conferred by DNA methylation, a process that occurs early in embryonic development and results in the differential expression of genes from maternal and paternal alleles. Imprinting is enormously important in normal development, and improper allele-specific methylation is a major cause of defective embryos and newborns after nuclear transplantation (cloning). Several human disorders that exhibit growth abnormalities and an increased cancer susceptibility are linked to abnormalities in genomic imprinting, as exemplified by Beckwith–Wiedemann syndrome.

Beckwith–Wiedemann syndrome

This disorder is characterized by variable growth defects, including generalized overgrowth (pre- and postnatal) as well as macroglossia, visceromegaly, and hemihypertrophy (enlargement of one half of the body). Patients with Beckwith–Wiedemann syndrome have an increased frequency of several tumors, including Wilm tumor, hepatoblastoma, and pancreatoblastoma. In the last decade, it has become clear that Beckwith–Wiedemann syndrome is linked to chromosome 11p15, a region containing several imprinted genes.

Two genes in this imprinted region are thought to play a causative role in Beckwith–Wiedemann syndrome: *CDKN1C* (a negative regulator of cell proliferation that acts by inhibiting cyclin-dependent kinase) and *IGF2* (a major regulator of fetal growth). Classical mutations of either of these genes affect growth. For example, mutations in *CDKN1C* have been described in patients with Beckwith–Wiedemann syndrome [221], and the overexpression of *Igf2* in mice is sufficient to cause an overgrowth syndrome [222]. However, the more common mechanism of gene activation (*IGF2*) or inactivation (*CDKN1C*) is related to abnormalities in methylation-dependent imprinting.

Under conditions of normal imprinting, *CDKN1C* is expressed from the maternal allele and *IGF2* is expressed from the paternal allele. Two different patterns of abnormal imprinting are associated with the development of Beckwith–Wiedemann syndrome. In most cases, abnormal methylation results in the loss of *CDKN1C* expression from both alleles, whereas in a few cases, abnormal methylation results in *IGF2* expression from both alleles [223]. Notably, the converse pattern of dysregulated methylation (resulting in loss of *IGF2* expression from both alleles) is associated with Silver–Russell syndrome, a congenital disorder characterized by growth retardation and asymmetry [224–226]. Although the mechanism by which dysregulation of *CDKN1C* or *IGF2* results in isolated growth phenotypes is not known, it is likely that alterations in cell proliferation underlie both the abnormal growth and the tumor propensity in patients with Beckwith–Wiedemann syndrome.

Conclusions

Despite a detailed conceptual framework for understanding the events that govern normal patterning, organogenesis, and

physiological adaptation of the gastrointestinal tract, the pathogenesis of congenital disorders of the gastrointestinal tract is poorly understood, reflecting the numerous questions about gastrointestinal development that remain unanswered. To date, most insights have come from human (reverse) genetics and serendipitous similarities between animal and human phenotypes. Specific challenges to further advances include the association of many developmental disorders with complex genetic syndromes and the separation in time between a developmental lesion and its phenotypic manifestations.

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