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# The molecular and morphogenetic basis of pancreas organogenesis

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ABSTRACT

The pancreas is an essential endoderm-derived organ that ensures nutrient metabolism via its endocrine and exocrine functions. Here we review the essential processes governing the embryonic and early post-natal development of the pancreas discussing both the mechanisms and molecules controlling progenitor specification, expansion and differentiation. We elaborate on how these processes are orchestrated in space and coordinated with morphogenesis. We draw mainly from experiments conducted in the mouse model but also from investigations in other model organisms, complementing a recent comprehensive review of human pancreas development (Jennings et al., 2015) [1]. The understanding of pancreas development in model organisms provides a framework to interpret how human mutations lead to neonatal diabetes and may contribute to other forms of diabetes and to guide the production of desired pancreatic cell types from pluripotent stem cells for therapeutic purposes.



**Contents**



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## 1. Introduction

The pancreas is a compound exocrine and endocrine gland located retroperitoneally in the abdominal cavity. The concerted functions of the exocrine and endocrine compartment are essential in systemic nutrient metabolism, as they facilitate digestion of nutrients and the subsequent regulation of blood glucose homeostasis, respectively. The exocrine pancreas consists of acinar cells organized into acini at the terminal ends of an elaborate network of ductal cells. The acinar cells secrete proenzymes catalyzing the breakdown of carbohydrates, proteins and lipids following their proteolytic activation after secretion to the duodenum. Proenzymes secreted by the acinar cells are transported via a highly branched network of hydrogen bicarbonate-producing ductal cells converging into sequentially larger ducts, eventually mediating the secretion of the enzyme-rich pancreatic fluid through the main pancreatic duct of Wirsung and the accessory duct of Santorini into the duodenum. The acinar cells make up the bulk of the pancreas, constituting as much as 90% of the organ. Interspersed between the acinar tissue, the endocrine compartment of the pancreas is organized into the highly vascularized and innervated discrete islets of Langerhans, comprising about 1–2% of the pancreas. The islets of Langerhans are composed of five different endocrine cellular subtypes producing different peptide-based hormones. These hormones regulate nutrient metabolism through systemic processes such as blood glucose homeostasis, coordination of digestion and appetite [2].

The systemic nature of the processes regulated by the pancreas is reflected by the considerable morbidity and mortality associated with debilitating pancreatic diseases such as diabetes mellitus, pancreatitis and pancreatic cancer. Driven by the desire to understand the underlying pathology and develop therapeutic strategies for these diseases, the mechanisms governing pancreas development have been extensively studied. Recent advances in generating insulin-producing cells from stem cell sources have spurred the optimism of a cell-based therapy for diabetes [3–5]. Since such differentiation protocols rely on an informed approach from developmental biology, increasing the knowledge of mechanisms governing cell fate decisions during embryonic and perinatal pancreas organogenesis might prove essential in the identification of control parameters for *in vitro* cell differentiation or reinstatement of differentiation- and proliferative control in pancreatic cancer. Here we review the essential processes governing embryonic and perinatal development of the pancreas with special emphasis on the mechanisms controlling cell fate allocation during murine pancreas organogenesis. We draw mainly from experiments conducted in the mouse model but also from investigations in other model organisms, complementing a recent comprehensive review of human pancreas development [1].

## 2. Endodermal patterning and induction of the pancreatic primordia

During mouse embryogenesis, the definitive pancreatic anlage are first evident at approximately embryonic day (E)8.5 by detection of pancreatic and duodenal homeobox 1 (PDX1)-expressing dorsal and ventral domains in the posterior foregut endoderm [6]. Prior to the specification of the pancreatic anlage, the primitive gut tube has undergone multiple patterning events leading to progressive spatial refinement of broad and then more discrete regions specified to give rise to the various endoderm-derived organs.

Antero-posterior patterning of endoderm starts at gastrulation as presumptive endodermal progenitors migrate through the primitive streak. In the mouse and chick, cells migrating through the primitive streak at different time points contribute to different parts of the gut and express different gene signatures in the newly formed endoderm [7–10]. Hence, at the end of mouse gastrulation, the definitive endodermal sheet consists of anterior cells expressing hematopoietically expressed homeobox (HEX), Sex-determining region Y-box 2 (SOX2) and forkhead box protein a2 (FOXA2), whereas the posterior half expresses caudal type homeobox (CDX) genes (reviewed by Zorn & Wells) [11]. Immediately after gastrulation, the naïve endoderm remains plastic and competent to respond to patterning cues from mesenchymal sources [6,12]. Reinforcement of the initial gastrulation-associated anterior-posterior patterning is achieved by mesenchymal wingless-related integration site (WNT)-, fibroblast growth factor (FGF)-, bone morphogeneic protein (BMP)- and retinoic acid (RA)-signaling, which all have a posteriorizing effect on the endoderm [13–17]. The higher posterior levels of signaling through these pathways are reinforced by anterior expression of signaling inhibitors [18,19]. Endodermal patterning occurs concomitantly with morphogenesis of the endodermal sheet into a primitive gut tube, which is essential for proper regionalization and induction of organ primordia [8,9,20,21]. Gut tube formation is accompanied by extensive cell movements along the longitudinal axis, especially ventrally and continuous signaling over this period enables the coordination of the antero-posterior identity of dorsal and ventral cells [22]. Patterning of the primitive gut tube generates a permissive environment for the onset of multiple genes, including *Pdx1* expression at the boundary between the *Sox2*-expressing anterior endoderm and the *Cdx2*-expressing posterior endoderm, facilitating subsequent induction of dorsal and ventral pancreatic anlage [23]. However, how specific signals result in the local induction of organ-specific transcription factors remains to be elucidated. Moreover, even though the BMP and Sonic Hedgehog (SHH) pathways pattern other germ layers along the dorso-ventral axis, no global dorsal and ventral organ inducer is known in endoderm. The concerted function of Pdx1 and Sox9 was recently demonstrated to be essential for stabilizing pancreatic identity in the primitive gut tube, in part by repressing *Cdx2* expression [24]. Despite the largely common set of transcription factors induced, the dorsal and ventral pancreatic anlage display some noticeable differences in the requirements for inducing signals.

### 2.1. Induction of the ventral pancreas

The ventral posterior foregut endoderm gives rise to the liver, the ventral pancreas as well as the extrahepatic biliary system consisting of the gall bladder, the cystic duct and the common bile duct. Multiple lines of evidence suggest that these ventral derivatives originate from a common progenitor population or bipotent single cells in Zebrafish [21,25–27]. Following specification of this common progenitor population, FGF-signaling emanating from the cardiac mesoderm and septum transversum mesenchyme leads to the induction of the hepatic lineage and segregation of a population of pancreas and extrahepatobilliary progenitors [25,28]. Mesenchymal BMP-signaling is also required to specify the hepatic lineage [26,29–32], and recently prostaglandin E2 from the lateral endoderm was demonstrated to promote a liver fate at the expense of the pancreas lineage in the zebrafish, putatively through interaction with the BMP pathway [33]. Morphogenesis of the endodermal sheet into the primitive gut tube is speculated to be essential for the proper specification of the liver and pancreatic anlage [34]. Upon lateral folding of the endodermal sheet, *Pdx1* expression is maintained in the lip of the endoderm advancing beyond the liver-inducing mesenchymal tissues, whereas the ventral endodermal domain remaining in prolonged contact with these FGF- and BMP-sources is instructed towards the hepatic lineage instead of the default pancreatic fate [25,35] (Fig. 1a,a'). Subsequent maintenance of *Pdx1* expression and evagination of the ventral pancreatic bud