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REVIEW

Transcriptional control of mammalian pancreas organogenesis

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Abstract The field of pancreas development has markedly expanded over the last decade, significantly advancing our understanding of the molecular mechanisms that control pancreas organogenesis. This growth has been fueled, in part, by the need to generate new therapeutic approaches for the treatment of diabetes. The creation of sophisticated genetic tools in mice has been instrumental in this progress. Genetic manipulation involving activation or inactivation of genes within specific cell types has allowed the identification of many transcription factors (TFs) that play critical roles in the organogenesis of the pancreas. Interestingly, many of these TFs act at multiple stages of pancreatic development, and adult organ function or repair. Interaction with other TFs, extrinsic signals, and epigenetic regulation are among the mechanisms by which TFs may play context-dependent roles during pancreas organogenesis. Many of the pancreatic TFs directly regulate each other and their own expression. These combinatorial interactions generate very specific gene regulatory networks that can define the different cell lineages and types in the developing pancreas. Here, we review recent progress made in understanding the role of pancreatic TFs in mouse pancreas formation. We also summarize our current knowledge of human pancreas development and discuss developmental pancreatic TFs that have been associated with human pancreatic diseases.

Keywords Embryonic pancreas · Transcriptional regulation · Multipotent pancreatic progenitor cells · GATA factors

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Pancreas development overview

The mammalian pancreas is a mixed exocrine and endocrine organ that plays a critical role in glucose homeostasis and nutrient digestion. The exocrine compartment consists of acinar cells that produce and secrete digestive enzymes into networks of ductal cells. The endocrine portion is comprised of at least five distinct hormone-producing cell types organized into the islets of Langerhans. These include glucagon-producing α-cells, insulin-producing β-cells, somatostatin-producing δ-cells, pancreatic polypeptide-producing PP-cells, and ghrelin-producing ε-cells. The pancreas, like the other organs of the gastrointestinal tract, is derived from the endodermal germ layer during gastrulation. The morphological processes involved in the development of the pancreas have been well characterized in mice. The pancreas arises from two independent primordia (dorsal and ventral) of the foregut epithelium around 8.5–9 days of gestation (e8.5–9) in the mouse [1]. The pancreatic epithelium invades the surrounding mesenchyme and subsequently expands and branches, forming a multilayered, stratified epithelium (Fig. 1). This early pancreatic epithelium contains multipotent pancreatic progenitor cells (MPCs) with the potential to give rise to all pancreatic cell types. Around e11.5–e12.5, the dorsal and ventral buds fuse

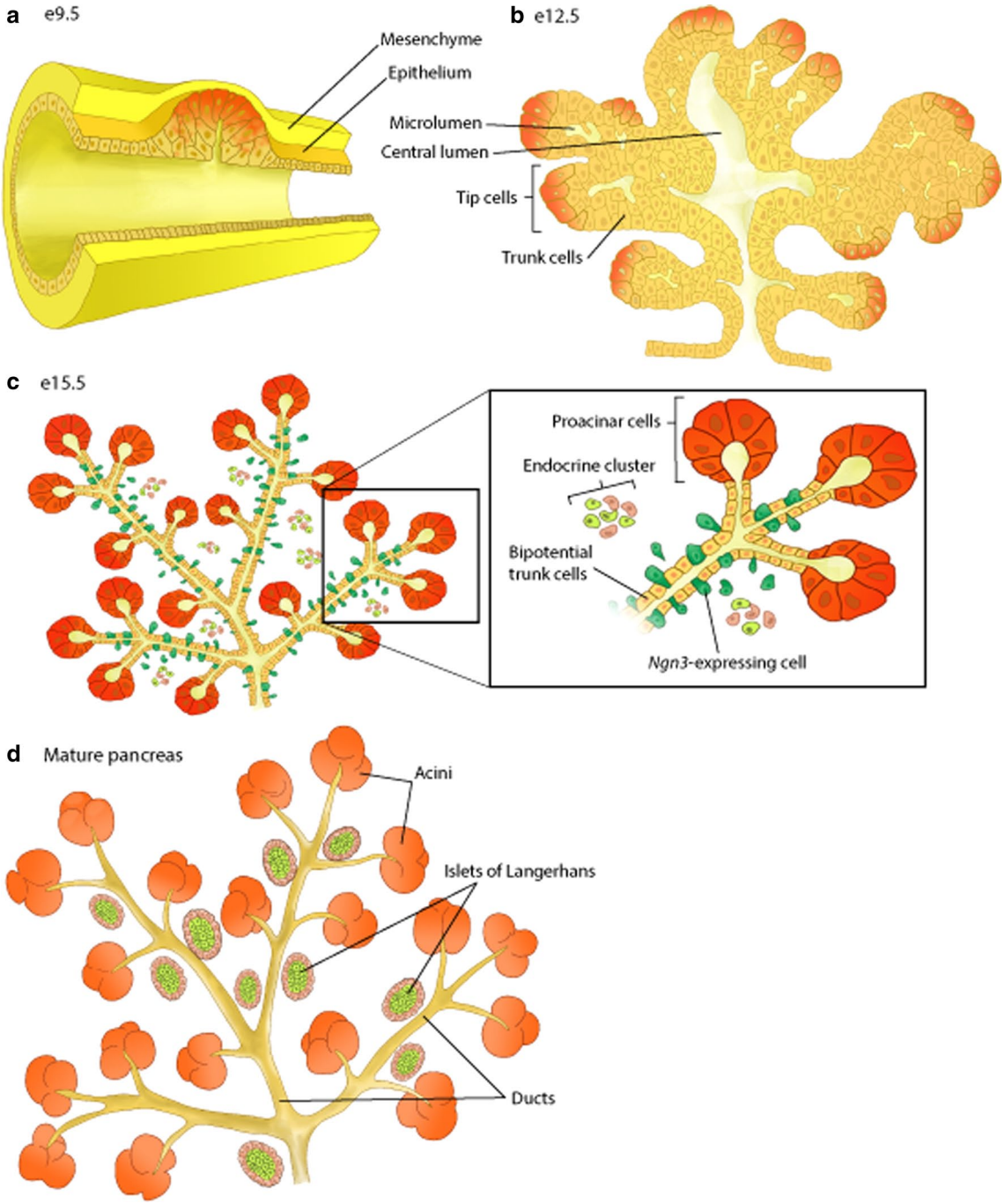

Fig. 1 Overview of pancreatic organogenesis in mice. **a** Formation of the pancreatic bud starts at approximately embryonic day (E) 9.5. **b** At E12.5, the pancreatic epithelium undergoes a marked process of expansion and branching. Segregation of tip (proacinar) and trunk (ductal/endocrine) domains begins. **c** At e14.5, cells located at the tips of the pancreatic epithelium differentiate into acinar cells. *Ngn3*-expressing endocrine precursors delaminate and migrate away from the trunk epithelium to form endocrine clusters. **d** In the mature pancreas, three cell types are found: acinar, ductal, and endocrine cells. Endocrine cells form clusters termed islets of Langerhans

when stomach and duodenum move during the rotation of the gut. At this time, a dramatic morphogenetic reorganization begins in the pancreatic epithelium that appears to be intimately connected to the formation of two distinct cellular domains. The epithelial "tips" of the branching epithelium contain MPCs while the "trunk" region harbors cells that will give rise to islet and ductal cells [2]. By e13.5, "tip" cells lose their multipotency and become acinar progenitor cells. This marks the beginning of the period known as the "secondary transition" (e13.5–e15.5),

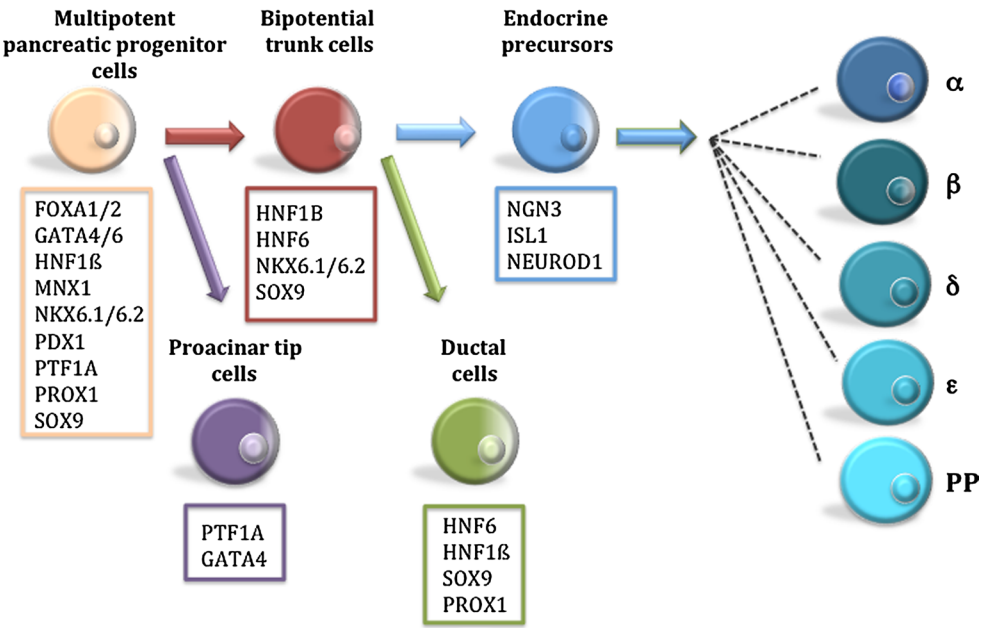

Fig. 2 Key transcription factors governing lineage decisions during pancreas development

in which extensive endocrine and exocrine cell differentiation occurs. After the secondary transition, the differentiated pancreatic cells undergo further growth and differentiation. This period is marked by the extensive proliferation of acinar cells and the organization of endocrine cells into clusters. During postnatal development, endocrine cells coalesce, forming the distinctive islet architecture. The embryonic formation of the pancreas is controlled by precisely timed signaling events, which determine the chronology of activation and repression of transcriptional networks. The signaling pathways that mediate pancreatic development have been reviewed recently in a comprehensive manner [3–8] and will only be briefly discussed throughout the subsequent sections of the present review.

Transcriptional regulation of major morphogenetic processes in pancreas development

In recent years, the number of TFs that have been implicated in pancreas formation has grown significantly. The purpose of this review is to summarize recent data regarding the TFs mediating pancreas formation. It does not aim to provide an exhaustive compilation of these TFs. Indeed, any attempt to generate a comprehensive list of TFs expressed during pancreas development would be futile, particularly given the vast array of information publicly available from gene expression profiling studies. Rather, we highlight TFs with recently discovered roles in pancreas organogenesis. For purposes of simplicity, we divide the development of the pancreas into four major stages: pancreas specification, pancreatic budding and formation of the multipotent progenitor cell (MPC) population, onset of exocrine and islet development, and endocrine and exocrine differentiation. We will review the specific functions of TFs at each of these developmental stages (Fig. 2). Emphasis will be placed on TFs involved in MPC formation due to the significant advances made in this area in recent years. We also attempt to incorporate information on how TFs are regulated by embryonic signaling pathways at the different developmental stages.

Specification of pancreas in the developing foregut endoderm

After the early patterning of the endoderm, factors secreted from the surrounding mesoderm establish the different organ domains in the primitive gut tube (see [9] for a comprehensive review). Each pancreatic bud receives distinct signals from their adjacent mesodermal tissues. Thus, signals secreted from the lateral plate mesoderm (retinoic acid) and notochord (fibroblast growth factor 2, FGF2, and activin) are essential for the development of the dorsal pancreas while FGF and bone morphogenetic protein (BMP) signaling from the cardiac mesoderm control liver/ventral pancreas fate specification (ventral pancreas being the default program). These signals activate transcription factors specific to each organ domain (for a review, see [10]).

The pancreatic domain in the primitive gut tube is defined by the overlapping expression of *Pdx1* (pancreatic and duodenal homeobox 1) and *Ptf1a* (pancreas-specific transcription factor 1a) [11, 12]. PDX1 and PTF1A are arguably the most important (and most intensely studied) TFs in pancreatic development and both perform multiples

roles at different stages of pancreas formation and in the adult organ. *Pdx1* is first expressed in the primitive gut tube at e8.5, prior to any apparent morphological changes, thus demarcating the prepancreatic endoderm. However, by e10.5, *Pdx1* is also expressed in the posterior stomach, duodenum, and bile duct [13–15]. *Pdx1* expression becomes restricted mostly to endocrine cells just before birth in the pancreas [13, 14, 16]. At these stages, *Pdx1* expression is also observed in epithelium and Brunner's glands of the duodenum and pyloric glands of the distal stomach [13, 14, 16, 17]. *Ptf1a* is first expressed in the pancreatic epithelium at e9.5. By e13.5, its expression is restricted to acinar precursor cells. In contrast to *Pdx1*, *Ptf1a* expression is exclusive to the pancreas during development [12]. Interestingly, although *Pdx1* and *Ptf1a* are expressed early in the prepancreatic endoderm and play critical roles in pancreatic development, their function is not critical for pancreas specification. Both *Pdx1* and *Ptf1a* null mice display pancreas agenesis [12, 14, 18]. However, a pancreatic rudiment is still present, indicating that activation of the pancreatic genetic program can occur in the absence of either TF. Based on the specific expression pattern of *Pdx1* and *Ptf1* in the developing foregut, it has been hypothesized that their combined expression determines the commitment toward a pancreatic fate [19]. However, a recent study has shown that an early pancreatic bud is still formed in mice with simultaneous inactivation of *Pdx1* and *Ptf1a* [20].

Considerable effort has been devoted to the elucidation of the TF mechanisms controlling *Pdx1* expression during the early stages of pancreas specification. Several conserved regulatory regions in the *Pdx1* promoter have been identified. Areas I–III direct *Pdx1* expression in the early pancreas [21, 22], areas I and II impart endocrine expression, and area III confers transient, specific ß-cell expression [23]. Earlier work identified HNF6 and FOXA2 as direct regulators of *Pdx1* expression through binding to areas I–III, but these TFs do not appear to be necessary for early pancreas formation [24, 25]. More recently, a redundant role for two members of the GATA family of TFs, GATA4 and GATA6, in activating *Pdx1* expression has been reported. Double *Gata4/Gata6* mutant mice exhibit severe pancreas agenesis and *Pdx1* expression is reduced in multipotent pancreatic progenitors [26, 27] (discussed below). Furthermore, studies with transgenic mice identified two conserved GATA sites in area III of the *Pdx1* regulatory sequence [26], a region required for *Pdx1* expression in the early pancreatic bud. However, depletion of *Gata4* and *Gata6* in the early endoderm (prior to pancreas formation) does not impair initial formation of the pancreatic bud [27]. These results indicate that GATA4 and GATA6 function are not required for initial pancreas specification. Other TFs with a role in pancreas formation, such as motor neuron and pancreas homeobox 1 (*Mnx1*), hematopoietically expressed Homeobox (*Hhex*), hepatocyte nuclear factor-1-beta (*Hnf1b*), and the SRY-box containing HMG transcription factor *Sox17*, are also expressed in the endoderm before *Pdx1*. It has not been determined whether any of these can directly regulate *Pdx1* expression, however. Strikingly, these TFs whose expression precedes *Pdx1* activation are widely expressed in the early endoderm [10]. How these TFs might activate the expression of *Pdx1* specifically in the prepancreatic endoderm remains unclear. However, it is likely that they act in a cooperative manner, perhaps with additional, undiscovered endodermal TFs. Indeed, the emerging picture in the field is that foregut organ specification is not achieved by activation of individual, organ-specific TFs, but rather through a particular combination of TFs [10]. Evidence for this hypothesis was provided in a recent study that performed a dynamic expression survey of 15 TFs important for endodermal organ formation in the developing foregut [28]. This analysis identified over a dozen specific domains of TFs expression that correspond to different organs of the gastrointestinal system. Another good example of how combinations of TFs can specify different lineages in the endoderm was presented in the recent study by Spence and colleagues [29]. These authors showed that combined expression of *Pdx1* and *Sox17*, a master regulator of endoderm formation [30], define the pancreatobiliary boundary in the ventral posterior foregut. Deletion of *Sox17* at e8.5 results in the loss of biliary structures and ectopic pancreas formation in the common duct, whereas *Sox17* overexpression suppresses pancreas formation and induces ectopic biliary tissue [29]. Additional research is required to comprehensively establish the combinatorial patterns of TFs that define the pancreatic domain in early endoderm.

Although the key TFs that pattern the foregut endoderm have been identified, much remains to be learned about how these TFs transduce mesodermal signals into specific gene expression programs. For example, a number of secreted factors, including RA and VEGF, are known to influence *Pdx1* expression [8, 31]. However, little is known about how these signals are actually translated into *Pdx1*-activation in the early endoderm. This is an important focus for future investigation; the knowledge generated will undoubtedly contribute to optimization of current protocols to generate insulin-producing cells from embryonic stem cells (ESC). Epigenetic mechanisms might also play an important role in pancreas fate choice during endoderm development. A recent study has found distinct histone modifications at liver and pancreas regulatory elements in the early foregut endoderm [32]. Furthermore, deletion in the foregut endoderm of the histone methyltransferase, *Ezh2*, leads to a marked expansion of the ventral pancreatic bud at the expense of liver bud development [32]. These results indicate that histone modifiers dictate the fate choice for liver or ventral pancreas induction. It would be

interesting to determine whether a similar epigenetic mechanism also controls dorsal pancreas induction.

Pancreatic budding and formation of the MPC population

Following bud formation, the pancreatic epithelium significantly expands and branches. A detailed description of the pancreatic branching process has recently been reported [33, 34]. These studies have provided a novel mechanism for pancreatic branching, which involves transient epithelial stratification and partial loss of cell polarity, de novo tubulogenesis, as well as microlumen formation and fusion. The early pancreatic bud (e9.5–e12.5) is mainly comprised of undifferentiated cells, known as multipotent pancreatic progenitor cells (MPCs). However, whether MPCs at these early stages represent a homogeneous population remains to be determined. Single cell transcript analysis and colocalization experiments with progenitor markers do, indeed, suggest MPC heterogeneity as early as e10.5 [11, 35, 36]. Clonogenic assays of MPCs, such as those recently described by Sugiyama and colleagues [37], combined with a comprehensive molecular characterization of MPCs, will help shed light on this issue. Lineage-tracing studies have demonstrated that MPCs retain the potential to give rise to all three pancreatic lineages until e12.5 [2, 12, 38–41]. However, a similar study has challenged this notion, as lineage analysis of cells expressing the TF *Sox9* (discussed below) were shown to contribute to acinar, ductal and endocrine cell compartments between e13.5 and birth [42]. Although this study suggests that MPCs might exist in the fetal pancreas beyond e12.5, experiments using conditional ablation of progenitor cells have demonstrated that the final pancreas size is determined by the number of MPCs present between e9.5 and e12.5 [43]. Thus, this period is a critical developmental window for progenitor expansion and significant efforts have been made to identify mesenchymal factors that control this process. These include, among others, Notch, FGF, and Wnt signaling pathways. Notch signaling inhibits differentiation of the MPC population whereas FGF signaling (namely the FGF10/FGFR2b ligand-receptor pair) [44] and Wnt signaling promote the expansion of the progenitor pool [45, 46].

The number of TFs shown to be critical for MPC formation has grown considerably in the last few years. These include PDX1, PTF1A, SOX9, FOXA1/2, PROX1, MNX1, ONECUT1, HNF1β, and GATA4/6. Next, we will review the roles of these TFs in the formation and maintenance of MPCs, highlighting the reported interactions.

PDX1

As described above, germ-line inactivation of *Pdx1* causes pancreas agenesis. Initial budding of the pancreas is seen in these mutants, but growth of the pancreatic epithelium is arrested around e10.5 [14, 15, 18]. These observations, along with experiments involving temporally controlled inactivation of *Pdx1* during pancreatic development [47, 48], indicate that PDX1 is necessary for epithelial growth and branching morphogenesis. How PDX1 regulates progenitor expansion remains poorly understood, but it might involve regulation of other TFs known to be involved in MPC formation and maintenance. In agreement with this notion, microarray analyses performed on e10.5 *Pdx1* mutant embryos found downregulation of several TFs expressed in MPCs, including *Nkx6.1* and *Ptf1a* [49]. In addition, a modest decrease in *Sox9* expression has been observed in *Pdx1* mutant mice [50]. However, whether PDX1 directly regulates these TFs has not yet been confirmed. It is remarkable that few direct PDX1 targets at this stage have been described. Two recent studies have identified a direct regulation of *Gata4*, *Foxa2*, and *Hnf1b* by PDX1 at early stages of pancreas formation. Experiments performed in transgenic mice have shown that PDX/HOX sites in an enhancer of the TF *Gata4* are required for enhancer function in vivo at e10.5 [51]. Chromatin immunoprecipitation (ChIP) assays using chromatin from wild-type pancreata at later stages of pancreatic development (e13.5) have demonstrated that PDX1 directly occupies sequences in both *Hnf1b* and *Foxa2* genes [52]. Interestingly, and as discussed in the earlier section about pancreas specification, many of the TFs expressed in MPCs including FOXA2, GATA4, HNF6, and PTF1A also directly regulate *Pdx1* expression in the early pancreatic bud.

PTF1A

Loss of *Ptf1a* results in pancreas agenesis although a rudimentary dorsal bud is present. Lineage-tracing experiments have shown that *Ptf1a*-deficient cells adopt an intestinal or common bile duct fate [12]. These results suggest that PTF1A is essential for the commitment and proliferation of pancreatic progenitors. It has been suggested that FGF10 signaling from the mesenchyme cells maintains *Ptf1a* expression in the dorsal pancreatic bud [53] but the underlying mechanism is unclear. PTF1A is the only bHLH TF that requires a third DNA-binding subunit for transcriptional activity called RBP-J [54]. The formation of the complex PTF1A-RBP-J is essential for proper pancreatic development. Mutations in one of the motifs of PTF1A that impairs the incorporation of RBPJ (but not the *Rbpj* homolog *Rbpjl* that appears to act with PTF1A in later stages of exocrine cell development) into the complex causes pancreatic phenotypes similar to those observed in *Ptf1a* null mice [55]. Notably, RBPJ function in the PTF1A complex is independent of its role as the effector of Notch signaling [54]. A recent study has identified a significant

number of direct targets of PTF1A in pancreatic progenitors [56]. Remarkably, several of these targets are TFs that are also expressed in the MPC population such as *Pdx1* (confirming previous results [22]), *Nkx6.1*, *Hnf6*, and *Mnx1*. It is also important to note that *Ptf1a* maintains its own expression during pancreatic development [57].

SOX9

Sox9, a member of the SRY/HMG box family, is expressed in the *Pdx1* domain from e9.5 [58, 59]. Lineage-tracing experiments have shown that at this stage *Sox9*-expressing cells give rise to all pancreatic cell types [58]. During the secondary transition, *Sox9* expression becomes restricted to the trunk ductal/endocrine progenitor domain. At later stages of pancreas development, *Sox9* expression is maintained in ductal cells. Conditional inactivation of *Sox9* in the *Pdx1* domain results in severe pancreatic hypoplasia [58] due to both diminished MPC proliferation and increased MPC cell death. In addition to MPC proliferation and survival, SOX9 is required to maintain MPC identity through a mechanism mediated by mesenchymal FGF signaling [50]. SOX9 regulates fibroblast growth factor receptor 2b (*Fgfr2b*) expression in the pancreatic epithelium, which is required to transduce mesenchymal FGF10 signals. In turn, FGF10 is required to maintain expression of *Sox9* and *Fgfr2b*. Thus, SOX9 and FGF10-FGFGR2b form a feed-forward loop in the MPC population that maintains pancreas identity. Disruption of this loop results in activation of the liver developmental program in the pancreatic epithelium [50]. SOX9 directly regulates the expression of other TFs expressed in MPCs, such as *Hnf1b*, *Hnf6*, and *FoxA2* suggesting a central role for SOX9 in the transcriptional network controlling MPC formation and maintenance [59].

GATA factors

The GATA transcription factor family is composed of six zinc finger TFs that play important roles in the specification and differentiation of multiple cell types [60–62]. All members of the GATA family recognize the motif WGATAR in the regulatory sequences of the target genes. In the mouse embryo, the expression pattern of *Gata4* and *Gata6* overlaps in the foregut endoderm, including the prepancreatic endoderm [61]. As embryonic development proceeds, *Gata4* and *Gata6* expression become mutually exclusive. *Gata4* is expressed in acinar cells, whereas *Gata6* expression is restricted to endocrine islets [63]. Earlier work performed in *Gata4* and *Gata6* tetraploid embryos, has suggested a role for these TFs in ventral bud formation. More recently, two studies have established a critical regulatory role for GATA4 and GATA6 in pancreas formation. While the inactivation of either *Gata4* or *Gata6* does not impair pancreas formation, inactivation of both *Gata4* and *Gata6* in the pancreatic progenitor domain leads to pancreatic agenesis, indicating a functional redundancy for these TFs during pancreas formation [26, 27]. *Gata4/Gata6* mutant embryos exhibit defects in MPC proliferation and branching morphogenesis. Furthermore, cell commitment toward endocrine and exocrine lineages is also severely impaired. As discussed in the previous section, GATA factors directly regulate *Pdx1* expression [26]. Therefore, it is possible that some of the defects observed in *Gata4/Gata6* pancreata might be due to diminished *Pdx1* expression. It would be interesting to determine whether GATA factors also directly regulate the expression of other TFs critical for the formation of the MPC population.

HNF1β

Hnf1b is expressed in the foregut endoderm prior to *Pdx1* (E8) [64]. During the secondary restriction, its expression is restricted to the epithelial trunk domain. At later stages, *Hnf1b* expression is observed only in the exocrine ducts [65, 66]. Lineage-tracing studies have shown that *Hnf1b*-expressing cells can give rise to all three pancreatic lineages until e13.5 [39]. *Hnf1b* mutants display a severely reduced dorsal pancreatic bud combined with complete absence of the ventral bud, a phenotype reminiscent of that observed in *Ptf1a* mutants. Indeed, *Ptf1a* is not expressed in the *Hnf1b* pancreatic bud rudiment, suggesting that PTF1A is downstream of HNF1β [66].

HNF6

Hnf6 is first expressed at e8.5 in the foregut-midgut region of the endoderm. It is broadly expressed within the pancreatic epithelium at early stages of pancreas formation, but becomes restricted to ductal and acinar cells prior to birth [65, 67]. Global *Hnf6* null mice exhibit pancreas hypoplasia with reduced *Pdx1* expression [24]. More recently, it was reported that inactivation of the *Hnf6* gene in *Pdx1* expression domain mice results in defects in pancreatic epithelial branching and growth [67]. HNF1β regulates *Hnf6* expression in the prepancreatic endoderm, which has led to the hypothesis that the sequential activation of *Hnf1b*, *Hnf6*, and *Pdx1* in the endoderm might control the generation of pancreatic progenitors [68].

MNX1

The Motor neuron and pancreas homeobox protein (*Mnx1*) is expressed throughout the primitive gut endoderm at e8 [69, 70]. *Mnx1* forms a transient dorsal–ventral gradient at e9.5 [28]. This *Mnx1* expression rapidly disappears

from the pancreatic epithelium by e12.5 [71]. Interestingly, *Mnx1* later becomes expressed in adult ß-cells [69, 70]. *Mnx1*-deficient embryos display agenesis of the dorsal pancreas, whereas the ventral bud is normally formed.

PROX1

The Prospero-related homeobox transcription factor1 (*Prox1*) is expressed in the prepancreatic endoderm. It is broadly expressed in the early pancreatic progenitor domain but becomes restricted to ductal and endocrine cells by e15.5 [72]. *Prox1* mutant embryos exhibit premature acinar cell differentiation and defects in pancreatic epithelial branching [73]. It has been proposed that PROX1 controls the expansion of tip progenitors in the early developing pancreas [73].

FOXA factors

The forkhead box A1 and A2 proteins (FOXA1 and FOXA2), two winged-helix TFs, are expressed in the pancreatic primordium prior to pancreas morphogenesis and persists in all pancreatic cell types throughout development and adulthood [25, 74]. FOXA1 and FOXA2 play redundant roles in early pancreas development. Individual inactivation of these TFs does not have a major impact on pancreas formation. However, conditional ablation of both *Foxa1* and *Foxa2* in the pancreatic progenitor domain leads to severe pancreatic hypoplasia [74]. These defects might be due to loss of *Pdx1* expression in MPCs. ChIP and ChIP sequencing (ChIPSeq) experiments performed in fetal pancreas have demonstrated that FOXA1 and FOXA2 are the essential transcription factors required for early *Pdx1* expression.

The studies summarized above have revealed a significant number of TFs with critical roles in MPC formation and maintenance. Many of the pancreatic TFs directly regulate each other and their own expression, suggesting that they form a specific cross-regulatory network during early pancreas formation (Fig. 3) [7, 59, 75, 76]. Future studies focused on the identification and characterization of TFs target genes and signal inputs, as well as computational approaches to integrate such knowledge, should significantly advance our understanding of how pancreatic progenitor formation is regulated.

Onset of exocrine and islet development

Between e12.5 and e15.5, the pancreatic epithelium undergoes a significant process of expansion, branching and differentiation. Concomitantly, a massive wave of endocrine and acinar cell differentiation, known as the secondary transition, begins [77]. During these stages, the epithelium

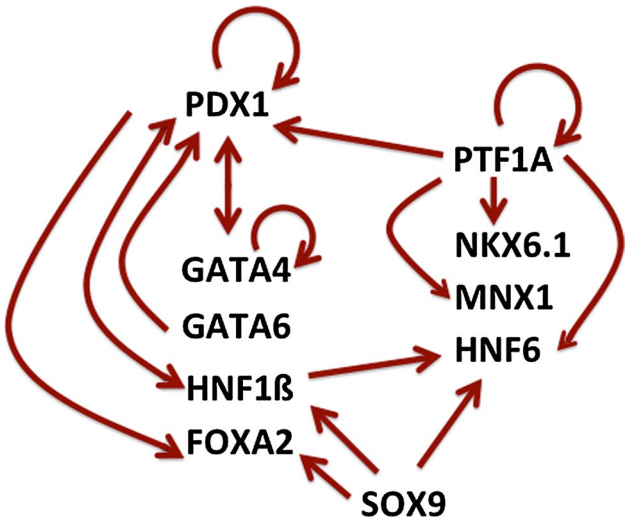

Fig. 3 Transcriptional regulatory interactions between TFs in multipotent pancreatic progenitors (MPCs). Only direct interactions are included

is separated into tip and trunk domains that generate acinar progenitor cells, and ductal/endocrine progenitor cells, respectively [2, 39, 42]. These domains are also defined by the specific expression of TFs. *Ptf1a* is expressed exclusively in the tip cells whereas *Sox9*, *Hnf1b*, and *Nkx6*.1 are expressed in the trunk domain [2, 36, 39, 42, 78]. Recent work has begun to shed light on the molecular mechanisms that establish this lineage allocation. PTF1A and the NKX6 factors (NKX6.1 and NKX6.2) mutually antagonize each other to specify an exocrine or ductal/endocrine fate [36]. The interaction might be mediated by direct transcriptional repression of *Ptf1a* by Nkx6 proteins. Notch signaling pathway might mediate this process. Activation of Notch signaling in MPCs promotes a ductal/endocrine progenitor fate [36, 79, 80]. Conversely, Notch signaling attenuation in the early pancreatic epithelium results in an increase in proacinar tip cells [80, 81] and diminished expression of trunk-specific TFs such as *Nkx6.1* and *Sox9* [80]. Interestingly, *Nkx6.1* appears to be a direct target of Notch signaling, suggesting that this pathway might act initially on NKX6.1 during tip-trunk patterning [80]. An exciting recent discovery is the link between epithelial tubulogenesis and cell specification. Inactivation of the Rho-GTPase *Cdc42* in pancreatic progenitors disrupted the developing epithelial structure that led to a perturbed tip-trunk compartmentalization and an increase in acinar cell differentiation at the expense of endocrine fate [33]. It is not yet clear how defects in tubulogenesis affect pancreatic cell fate. However, it has been suggested that it might be related to the fact that tip and trunk domains are exposed to different microenvironments during pancreatic development, with tip cells in close proximity to extracellular matrix components.

Two relevant recent studies have reported that endothelial cells are preferentially positioned close to the trunk cells of the epithelium [82, 83]. In agreement with this spatial organization of endothelial cells in the pancreatic epithelium, endothelial cells have been shown to limit acinar differentiation, perhaps by repressing the expression of *Ptf1a* [82, 83]. During the secondary transition, scattered individual cells within the trunk transiently express the master regulator of endocrine formation, neurogenin 3, (*Ngn3*) and differentiate into the endocrine lineage. Endocrine progenitor cells delaminate from the ductal epithelium and form clusters, where they continue to differentiate. Little is known about the mechanisms governing endocrine delamination, migration, and aggregation. Epithelial-to-mesenchymal transition (EMT) might be involved in endocrine delamination [84, 85], although conclusive evidence remains elusive. More recently, *Grg3* (Groucho-related gene 3), a member of the Groucho/TLE family of co-repressors, has been implicated in endocrine delamination [86]. GRG3 appears to promote delamination of endocrine cells by suppressing E-cadherin gene expression. Interestingly, NGN3 is required for *Grg3* expression suggesting a possible interaction between NGN3 and GRG3. These observations are consistent with recent research describing a role for NGN3 in endocrine delamination [87–89]. E-cadherin function might also be important for endocrine migration. Current evidence suggests that RAC1, a member of the Rho family of small GTPases, acts as a key regulator of endocrine cell migration by modulating E-cadherin-mediated cell–cell adhesion [90]. Clearly, this is an area of research that merits further investigation.

Endocrine cell specification begins with the induction of the bHLH (basic helix loop helix) TF NGN3. *Ngn3* expression is first observed at e9.5. Its expression peaks during the secondary transition and markedly decreases during later stages of pancreatic development [91]. NGN3 is essential for endocrine cell formation. *Ngn3* null mice lack all endocrine cell types, but display normal exocrine development [92]. Furthermore, overexpression of *Ngn3* under the *Pdx1* promoter results in premature differentiation of MPCs and increases the formation of endocrine cells [93, 94]. Consistent with these results, lineage-tracing studies have demonstrated that all pancreatic endocrine cells derive from *Ngn3*-positive progenitor cells [38]. In addition, achieving high NGN3 levels has been shown to be critical for allocation of endocrine cell fate [95]. NGN3 activation is directly regulated by a complex cross-regulatory TF network that includes FOXA2, GLIS3, HNF1β, HNF6, and PDX1 and SOX9 [52, 59, 76, 96–98]. Although the key TFs that regulate *Ngn3* expression in pancreatic progenitors have been identified in recent years, the mechanistic details of how this TF network activates *Ngn3* expression in specific cells within the trunk domain have not been fully elucidated. The Notch pathway appears to play a key role in this process by a classical mechanism of lateral inhibition [93, 99]. Endocrine progenitors induce the production of Notch ligands. Notch signaling activates the target gene, *Hes1*, in neighboring cells, which directly downregulates *Ngn3* expression [100]. Consequently, these cells are maintained as undifferentiated progenitors. This mechanism helps to ensure the proper balance between proliferation and differentiation in ductal/endocrine trunk progenitors. However, recent studies have challenged this lateral inhibition model, suggesting further complexities in Notch-mediated regulation of endocrine differentiation [101–104]. It has been proposed that *Ngn3* activation is governed by a gradient of Notch activity in the pancreatic epithelium. SOX9 appears to play a key role in this process [101]. Epigenetic mechanisms might also play a key role in the regulation of endocrine progenitor selection: histone modifications, such as acetylation, alter endocrine cell fate allocation in embryonic pancreas [105]. Another particularly exciting discovery is that planar cell polarity controls endocrine differentiation from pancreatic progenitor cells [106]. These studies have provided new insight into the intrinsic mechanisms that govern endocrine commitment. We can expect to see more studies focused on the interaction between these cell-intrinsic mechanisms and the endocrine-specific transcriptional network in the next few years. In particular, elucidation of the mechanism that allows TFs to work in concert with chromatin modifiers to direct endocrine differentiation is an important area of investigation.

Ngn3-expressing precursors can give rise to the five different endocrine cell lineages. *Ngn3*-expressing cells are postmitotic and lineage-tracing studies have determined that they are allocated to a single endocrine cell lineage. Therefore, *Ngn3*-expressing cells are unipotent endocrine precursors [107]. At present, it is not understood how *Ngn3*-expressing precursors are instructed to specific islet cell fates. However, elegant studies by Johansson et al. [94] have shown that the timing of *Ngn3* activation in precursors cells can determine the endocrine cell type generated. For example, *Ngn3* expression induced during early pancreas development promotes the formation of α-cells. The induction of *Ngn3* expression from e11.5 onward favored the generation of β- and PP-cells. From e14.5 onward, *Ngn3*-expressing cells become competent to generate δ-cells, whereas the competence to produce α-cells markedly diminishes.

NGN3 induces the endocrine developmental program by directly activating the expression of TFs necessary for endocrine differentiation, including *Neurod1*, *Pax4*, *Insm1*, *Rfx6*, *Nkx2.2*, and *Myt1* [76, 108–115]. It is likely that the list of TFs directly regulated by NGN3 and presumably with a potential role in endocrine formation will increase in the future. Microarray analysis of *Ngn3* mutant mice,

as well as gene expression profiling studies performed in *Ngn3*-expressing cells, have already yielded a long list of TFs that might potentially be regulated by NGN3 [115–119].

Endocrine and exocrine differentiation

Endocrine cells

A remarkable number of TFs that are required for different aspects of endocrine development, including cell differentiation, survival, maturation and endocrine subtype allocation development have been identified (see [7, 120] for comprehensive reviews). Here, we highlight TFs with recently discovered roles in endocrine formation.

Among the NGN3 regulated TFs, NEUROD1 and INSM1 appear to occupy relevant positions in the hierarchy of endocrine development. Mutant embryos deficient in these TFs exhibit reduced numbers of all endocrine cell types (although not to the degree observed in *Ngn3* mutant mice) [121, 122], consistent with defects in early endocrine formation. Similar defects in islet formation have recently been described in mice lacking Regulatory X-box binding 6 (*Rfx6*) and the LIM homeodomain protein Islet1 (*Isl1*) [114, 115, 123]. Although RFX6 and ISL1 appear to act downstream of NGN3 [92, 115], direct regulation of these TFs by NGN3 has not been reported. *Rfx6* is broadly expressed in the gut endoderm including the nascent pancreatic bud at e9.0 [114, 115]. From e10.5, *Rfx6* expression in the pancreas is found only in endocrine progenitor cells. *Rfx6* expression is maintained in all adult islet cell types [114, 115]. *Rfx6* mutant mice display a dramatic reduction of all endocrine cell types with the exception of PP-cells [114]. This is not related to loss of endocrine progenitors, because *Ngn3* remains normally expressed. Indeed, endocrine cells are present in the *Rfx6* pancreata, but they do not appear to express hormone genes or other mature markers. These results indicate that *Rfx6* is required for the differentiation of endocrine cell types (with the exception of PP-cells). ISL1 is required for the differentiation, proliferation and survival of endocrine cells after the secondary transition [123]. A second level of transcriptional control during endocrine formation involves the specification of individual endocrine cell types. A number of TFs whose inactivation causes differential loss of endocrine cell types have been described, including *Pax4*, *Arx* and NKX-homeodomain factors. Three NKX genes are expressed during pancreas development, *Nkx2.2*, *Nkx6.1* and *Nkx6.2*. These TFs are broadly expressed in the MPC compartment during early pancreatic development. However, the role of the NKX factors in MPCs formation and maintenance is, at present unclear, since they are dispensable for early pancreas development [124–126]. *Nkx6.1* and its paralog *Nkx6.2* show some degree of functional redundancy during endocrine formation. *Nkx6.1/Nkx6.2* double mutants exhibit reduced numbers of α- and β-cells. However, only β-cells are affected in *Nkx6.1* mutant mice, while *Nkx6.2* mice do not show any apparent pancreatic defects [126]. *Nkx2.2* null mice display a total loss of β-cells and decreased numbers of α- and PP-cells [124]. Interestingly, a significant increase in ghrelin-producing ε-cells is observed indicating a switch in lineage specification [127]. NKX2.2 genetically interacts with other TFs to regulate the specification of endocrine cell lineages. Simultaneous inactivation of *NeuroD1* and *Nkx2.2* restores α- and PP-cell, but not β-cell formation and decreases the number of ε-cells [128]. NKX2.2 differentially regulates *NeuroD1* in progenitor cells to specify different cell lineages. Thus, activation of *NeuroD1* by NKX2.2 is necessary for β-cell formation, while NKX2.2 must repress *NeuroD1* to allow proper allocation of the α-cell lineage [129]. NKX2.2 also interacts with aristaless-related homeobox x-linked (*Arx*) in the regulation of the PP-cell lineage [130]. *Pax4* mutant mice display a phenotype similar to *Nkx2.2* mice (reduced β- and δ-cell numbers and increased α- and ε-cells), although it is unclear whether a genetic interaction between these TFs exists [127]. Recent studies have provided insight into the mechanisms regulating the fate choice between the α- and β-cell lineage (see [131] for an detailed review on this subject). The opposing activities of PAX4, an important β-cell differentiation TF, and ARX, a key factor in α-cell specification, appear to play a key role in this process. Both *Pax4* and *Arx* are initially expressed in Ngn3-expressing progenitor cells [132, 133]. During endocrine differentiation, *Pax4* and *Arx* expression becomes restricted to β- and α-cell lineages, respectively. *Pax4* inactivation results in the loss of β- and δ-cells and a corresponding increase in α-cell number [133] while deficiency in *Arx* leads to a complete loss of α-cells, concomitant with an increase in β- and δ-cells [132]. Ectopic expression of *Pax4* and *Arx* in endocrine progenitor cells induces β- and α-cell formation, respectively [134, 135]. Thus, *Pax4* promotes β- and δ-cell fate, while *Arx* promotes the α-cell fate at the expense of β- and δ-cell fate. The antagonistic interaction between these TFs might be mediated by reciprocal transcriptional repression [134, 135]. A similar antagonistic relationship between NKX6.1 and ARX function might also participate in α- versus β-cell lineage commitment [136]. Misexpression of *Nxk6.1* in endocrine progenitor cells promotes β-cell formation at the expense of α-cell lineage [136] while ectopic expression of *Arx* results in the opposite alteration, as discussed previously. Conversely, inactivation of *Nkx6.1* in endocrine precursors switches β-cells to different endocrine lineages. The underlying molecular mechanism by which *Nkx6.1* regulates α- and β-cell fate allocation might involve the direct transcriptional repression of the *Arx* gene

through competition with the *Arx* activator ISL1, as suggested by in vitro studies [136]. PDX1 might also act as a regulator of α- and β-cell lineage commitment. Enforced *Pdx1* expression in endocrine progenitors induces a slight increase in β-cell formation, which is accompanied by a decrease in α-cell numbers [98]. These results are consistent with the expansion of α-cell number observed in mice with specific ablation of *Pdx1* in embryonic β-cells [137]. However, it is important to note that in this study, lineage-tracing experiments indicated that α-cells did not arise from β-cells. Once endocrine cell fate has been established, additional TFs are required for terminal cell differentiation and maintenance of cell function. A significant number of TFs involved in this process have been described, including FOXA2, GLIS3, ISL1, MAFA, MAFB, NEUROD1, PAX6, RFX3 and PDX1 (see [7, 120] for detailed reviews). Here it is important to note that maintenance of endocrine cell identity requires not only the activation of genes specific for that particular cell lineage, but also the repression of genes. This is best illustrated in a recent study that inactivated *Nkx2.2* specifically in β-cells [138]. Loss of *Nxx2.2* causes a β-to-α cell transdifferentiation due to derepression of the α-cell regulator *Arx*, proving that the repressor activities of NKX2.2 are required for maintaining β-cell identity.

The studies summarized above have revealed significant developmental plasticity among the different endocrine cell types as a consequence of forced expression of lineage-specific TFs. Recent studies have suggested that epigenetic mechanisms such as DNA methylation might play a role in the regulation of cell plasticity in endocrine cells [138, 139]. These results might be particularly relevant in light of reports characterizing α-to-β cell reprogramming in adult stages [140] and the suggestion that reprogramming strategies might lead to novel sources of β-cells for diabetes therapies.

Acinar cells

PTF1A is the master regulator of acinar cell differentiation [141, 142]. As discussed above, PTF1A forms a complex with the subunit RBPJ during early pancreas development. PTF1A initiates acinar differentiation by a mechanism that involves the replacement of the subunit RBPJ by RBPJL [142]. The PTF1A-RBP-JL complex appears to directly activate the expression of acinar-specific genes, such as secretory proteins, as suggested by Chip-Seq and gene profiling experiments [142]. Interestingly, PDX1 also seems to be required for exocrine differentiation. *Pdx1* inactivation from e13.5 results in the formation of immature acini [48]. These results indicate that while PDX1 is not required for acinar cell specification, it is needed to complete the final steps of acinar differentiation. Recent studies have suggested that GATA factors might also play a role in exocrine formation. As discussed above, *Gata6* is coexpressed with *Gata4* in the early pancreatic epithelium. At later stages, *Gata4* expression is observed only in acinar cells, whereas *Gata6* expression is restricted to endocrine cells [26, 143]. Inactivation of either *Gata4* or *Gata6* does not have a major impact on acinar cell formation. A minor defect in acinar architecture is observed in *Gata4* neonatal mice, but these phenotypes are resolved postnatally [27]. However, mice lacking both alleles of *Gata4*, but retaining one *Gata6* allele display severe acinar cell loss. The architecture of remaining acini was disorganized, but cell differentiation appears unaffected [26, 27]. Interestingly, mice lacking both alleles of *Gata6*, but retaining one *Gata4* allele, do not exhibit any apparent exocrine defects. In other words, one allele of *Gata4*, but not *Gata6*, was sufficient for normal acinar formation. These results indicate that GATA6 is more critical than GATA4 in supporting exocrine pancreas development. These results appear to be at odds with the lack of expression of *Gata6* in the exocrine lineage during late pancreas development. A possible explanation is that the exocrine phenotypes in *Gata4* homozygous, *Gata6* heterozygous mice are due to defects in acinar cell lineage allocation during early pancreas development. However, in support of a role for GATA6 in acinar cell development, a recent study has described that GATA6 is required for maintenance of cell identity in adult acinar cells [144]. Inactivation of *Gata4* and *Gata6*, individually as well as in combination, within the acinar lineage is required to conclusively establish whether GATA4 or GATA6 are necessary for exocrine formation. *Mist1* and *Nr5a2* are two TFs that are specifically expressed during acinar differentiation. Although these TFs are dispensable for the embryonic development of the acinar lineage, they are critical for postnatal maturation and adult homeostasis of acinar cells [145, 146]. Importantly, NR5A2 cooperates with the PTF1A-RBJL complex in activating acinar-specific gene expression in adult stages [147]. Several other TFs highly expressed in the acinar lineage have been reported, but their roles in acinar formation have not been determined (see [148] for a comprehensive list of these TFs).

Ductal cells

The molecular mechanisms involved in ductal specification and development are poorly understood. This is somewhat surprising considering that these cells have received extensive attention due to their potential involvement in adult pancreatic regeneration, as well as pathologies like pancreatitis and cancer [149]. An increasing number of TFs that are expressed in the ductal lineage during late pancreas formation and adult stages have been described in recent years, including SOX9, HES1, HNf1β, HNF6, and PROX1. Some of these TFs might play a role in ductal development.

Inactivation of *Hnf6* or *Sox9* results in ductal cyst formation [67, 101, 150]. The underlying mechanism might be (partly) related to the lack of primary cilia in *Hnf6*- and *Sox9*-deficient ductal cells [67, 101, 150]. Primary cilia have been demonstrated to be necessary for proper pancreatic duct organization [151, 152]. More recently, a role for PROX1 in ductal development has also been reported [73]. Mutant mice deficient in *Prox1* display increased ductal proliferation and altered duct morphogenesis. Interestingly, *Hnf6* mutants exhibit decreased *Prox1* expression. The interactions between HNF6, HNf1β, and PROX1 suggest that they might form a transcriptional network involved in ductal development. It is important to note that in all of these genetic studies, TFs have been inactivated during early pancreas formation, and thus, it remains entirely possible that some of the ductal phenotypes are consequence of early effects on pancreatic development rather than defects in ductal differentiation. Conditional inactivation of these factors in the ductal lineage should determine their precise role in pancreatic duct formation. Recently, a microarray analysis of pancreatic ductal cells at both later stages of embryonic development and neonatal stages has been published [153]. The authors report an increase in expression of several TFs, including *Prox1*, *Hnf1B* and *Hhex* in neonatal ducts. Detailed analysis of this microarray data might help to elucidate the regulatory mechanisms involved in ductal differentiation.

Human pancreas development

Our current knowledge of human pancreatic development is mainly derived from histological studies. These studies are limited by the difficulty in obtaining high-quality fetal human tissues, especially at early stages of pancreas development. Despite these challenges, the information obtained from these studies indicates that the morphogenesis events of pancreas development are largely conserved between human and mouse. Furthermore, all the key regulators of pancreas formation are expressed in human fetal pancreatic development [154–159]. In this section. we briefly review the expression pattern of key developmental TFs in human pancreas development highlighting key differences between mouse and human. In addition, we will discuss genetic studies that have identified mutations in genes encoding TFs that have been linked to human pancreatic disorders.

During embryonic development, the human pancreas forms from a dorsal and a ventral evagination of the primitive gut epithelium. The dorsal pancreatic bud is first evident on day 26 post-conception (dpc), while the ventral pancreas buds over the next few days. The pancreatic buds fuse by 56 dpc [154]. Thus, pancreas formation in human is initiated at an equivalent stage to that in mouse. Human endocrine differentiation occurs during the first trimester of gestation and β-cells can be detected as early as 8 weeks post-conception (wpc). Scattered α- and δ-cells are observed a week later [154–158]. Thus, it appears that the beginning of endocrine differentiation is slightly delayed in human compared to mouse. This might be related to the apparent lack of the first wave of endocrine cell formation that is observed in mouse [159]. Interestingly, despite this delayed endocrine differentiation, clustering of endocrine cells into islet-like structures occurs comparatively earlier in human embryos. Endocrine cell clusters are observed around 11–14 wpc [154, 157, 158] in human islet-like structures that are formed just prior to birth in mouse. Another significant disparity between human and mouse endocrine development is that a single, prolonged period of endocrine cell formation appears to exist in humans [157, 159, 160]. Protoacinar structures emerge by 14 wpc [157, 159]. Further pancreatic expansion and growth occur during the rest of the gestation period. Adult human and mouse islets are markedly different, both in terms of islet architecture and cell composition [161–163]. Thus, human islets contain fewer β-cells and more α- and δ-cells than mouse islets [161]. In mouse, β-cells are located at the core of islets while non-β-cells are found in the periphery. In contrast, β-cells are intermingled with α and δ cells in adult human islets. The unique cellular arrangement of adult human islets may have important effects on islet function [162]. Interestingly, human fetal islet architecture resembles that found in adult mouse islets with non-β-cells found in the periphery of islets [158]. It has been suggested that after 21 wpc, human fetal islets undergo a reorganization process that results in the establishment of the characteristic cell organization of adult human islets with intermingled β- and α-cells [158].

Several studies have analyzed the expression of key TFs in developing human fetal pancreas [154–159, 164]. These studies have revealed that TFs expression patterns are generally similar in human and mouse pancreas development (see [159] for a detailed description). This has led to hypothesize that the same key regulatory TFs might govern human and mouse pancreas formation. However, some notable differences exist. For example, *PDX1* expression appears slightly later in human pancreatic development, when dorsal foregut is separated from the notochord and aorta [159]. Another significant difference is that *NKX2.2* is not expressed in early human pancreatic progenitors prior to endocrine formation [159]. Also, a single phase of *NGN3* expression is observed in human embryonic pancreata [159, 160]. However, *NGN3* expression in the developing human pancreas is prolonged [157, 160], consistent with the described extensive phase of endocrine cell differentiation. Interestingly, differences between endocrine TFs expression in mouse and human have also been found

Table 1 Transcription factors linked to human pancreatic disorders

Gene	Name	Pancreatic abnormalities
ARX	Aristaless-related homeobox	Hemizygous male: partial loss of endocrine and exocrine cells [185]
FOXP3	Forkhead box P 3	Hemizygous male: permanent diabetes [188]
GATA6	Gata binding protein 6	Heterozygous: neonatal diabetes, pancreatic agenesis [186]. Adult-onset diabetes [187]
GLIS3	Glis family zinc finger 3	Homozygous: neonatal diabetes [178]
HNF1A	Hepatocyte nuclear factor *1α*	Heterozygous: MODY3 [182]
HNF1B	Hepatocyte nuclear factor *1β*	Heterozygous: MODY5 [181]
HNF4A	Hepatocyte nuclear factor *4α*	Heterozygous: MODY1 [180]
MNX1	Motor neuron and pancreas homeobox 1	Homozygous: permanent neonatal diabetes [189]
NEUROD1	Neurogenin differentiation factor 1	Heterozygous: MODY6 [190]. Homozygous: neonatal diabetes [177]
NGN3	Neurogenin 3	Homozygous (hypomorphic mutation): diabetes [191, 192]. Biallelic mutations: permanent neonatal diabetes [179]
PAX4	Paired box 4	Heterozygous: MODY9 [193]
PDX1	Pancreatic and duodenal homeobox 1	Homozygous: congenital pancreatic agenesis [175]. Heterozygous: MODY4 [194]
PTF1A	Pancreatic-specific transcription factor 1A	Homozygous: pancreatic agenesis [176]
RFX6	Regulatory factor X6	Homozygous: neonatal diabetes and pancreatic hypoplasia [114]
SOX9	Sex determine region Y (SRY)-box 9	Heterozygous: pancreatic abnormalities [195]

in adult stages. *MAFB* expression is observed in human β-cells, whereas it is absent in mouse β-cells [165, 166].

Although informative, these histological studies only provide a limited snapshot of the specific developmental stage and therefore cannot indicate whether the transcriptional regulatory mechanisms governing pancreas development are conserved between human and mouse. Thus, there is an urgent need for longitudinal and functional studies on human pancreas development (for an excellent discussion on this issue, see [167]). Ex vivo culture of human fetal pancreas (mainly late fetal stages) have been used for decades to dynamically investigate human pancreatic differentiation. More recently, the Scharfmann's group has developed models of xenografts of early human fetal pancreas under the kidney and the epimysium of the thigh muscle [160, 168]. These fetal xenografts develop properly into a functional endocrine pancreas allowing detailed examination of human pancreas development. These studies have validated the expression pattern of key TFs, such as *PDX1*, *NKX2.2*, and *NGN3*, during human pancreas development obtained from earlier immunohistochemical analysis [160]. Protocols have been established to efficiently generate endocrine cells from human embryonic stem cells (hESCs) and induced pluripotent stem cells (hPSCs) as a potential source of β-cells for replacement therapies in type 1 diabetes. The most efficient protocols utilize signals known to regulate embryonic pancreas formation in vivo (reviewed in [6, 169, 170]). In addition to their potential therapeutic applications, these stem-cell-based strategies offer a valuable in vitro model for the study of human pancreatic development. Thus, expression of major developmental TFs during the differentiation process has revealed an expression pattern consistent with embryonic pancreas formation in mouse [171–173]. Of note, cells simultaneously expressing *PDX1*, *SOX9*, *PTF1A*, *GATA4*, and *NKX6.1* have been observed during intermediate stages that might correspond to mouse MPCs. Genetic tools including gene targeting and virus-mediated gene transfer have recently begun to be applied to human fetal grafts and hESCs [160, 174]. Widespread application of genetic methodologies in these ex vivo and in vitro models might provide additional, powerful experimental lines of investigation, including lineage-tracing and gain-/loss-of-function studies to investigate the role of TFs in human pancreatic development.

Genetic studies linking human pancreatic disorders to mutations in developmentally relevant transcription factors provide further support to the notion that regulatory developmental programs are conserved between human and mouse (Table 1). Thus, homozygous mutations in *PDX1 and PTF1A* lead to pancreatic agenesis in human [175, 176]. Homozygous mutations in *GLIS3*, *NEUROD1*, and *RFX6* have been associated with permanent neonatal diabetes [114, 177, 178]. Compound heterozygous mutations in *NGN3* have also been linked to permanent neonatal diabetes [179]. These results are consistent with the reported function of these TFs in mouse. However, human genetic studies have also revealed apparent differences in the roles of several developmental TFs between mouse and human. Perhaps the best-known examples are some of the TFs linked to Maturity-onset diabetes of the young (MODY). MODY is a form of monogenic diabetes caused by mutations in an autosomal dominant gene. Heterozygous loss-of-function mutations in the TFs hepatocyte nuclear factors *HNF1A* (MODY3), *HNF1B* (MODY5), and *HNF4A*

(MODY1), cause diabetes in human but not in mouse (although homozygosity does) [180–182]. Why haploinsufficiency of these TFS cause diabetes in human but not in mouse is not completely understood but it has been suggested that the extended period of endocrine cell differentiation in human might make this process more sensitive to changes in gene dosage [183]. Differential functions for ARX in human and mouse pancreas development have been suggested based on the analysis of pancreatic tissue of patients with mutations in *ARX* linked to X-linked lissencephaly with abnormal genitalia (XLAG) [184]. Defects in pancreatic α-cell formation were observed in these patients, in agreement with the described role of ARX in mouse pancreas development. However, acinar abnormalities were also observed in the pancreata of XLAG patients suggesting that ARX contributes not only to endocrine development, but also to exocrine development of the human pancreas [185]. The regulatory function of GATA factors appears to constitute a major disparity between human and mouse transcriptional developmental program. Hattersley and colleagues [186] have recently shown that mutations in *GATA6* are the most common cause of human pancreas agenesis, accounting for approximately 50 % of the cases. An exome sequence analyses of a cohort of patients with neonatal diabetes associated with pancreatic agenesis or severe pancreatic hypoplasia has revealed that 15 of 27 patients exhibit loss of function mutations in *GATA6*. Interestingly, these de novo mutations were found in heterozygotes, highlighting the important role of GATA6 in human pancreas development. More recently, a study of a larger cohort of patients by the same group has confirmed the high prevalence of pancreatic agenesis associated with mutations in *GATA6* [187]. Remarkably, four patients with heterozygous *GATA6* mutations developed diabetes without exocrine sufficiency at adult stages. A more extensive study of families carrying mutations in *GATA6* is necessary to establish the prevalence of mutations in *GATA6* as a cause of monogenic diabetes in adults. This human data is in stark contrast to the results obtained in *Gata4* and *Gata6* mutant mice. As discussed earlier, single inactivation of *Gata4* or *Gata6* in mouse does not result in any major pancreatic abnormalities. Indeed, three or four *Gata* alleles need to be eliminated to cause a severe pancreatic phenotype [26, 27]. The discrepancy in phenotypes is not completely understood but it is important to note that *Gata6* is expressed in other mesodermal and endodermal tissues during mouse embryonic development. If that is also the case in human embryonic development, mutations in *GATA6* might affect these mesodermal and endodermal tissues potentially interfering with pancreas development. Alternatively, differences in the pancreatic genetic program between species might account for this discrepancy. The spatiotemporal pattern of *GATA6* expression during human pancreas development has not been reported. Thus, it remains to be determined whether *GATA4* and *GATA6* expression overlap and are functionally redundant in early human pancreatic formation. To this end, a detailed report of *GATA4* expression in human pancreas development was recently published [159]. Interestingly, *GATA4* is strongly expressed during early human pancreas budding, consistent with the observed expression in mouse. However, *GATA4* expression appears to rapidly decrease in the early human pancreatic progenitor domain (defined by simultaneous expression of *PDX1*, *PTF1A*, *SOX9* and *NKX6.1*) [159], thus making this stage potentially more sensitive to reductions in *GATA6* gene dosage.

In summary, human genetic studies have revealed significant distinctions between human and mouse pancreas development. This highlights the need for experimental studies in human tissue to fully elucidate the roles of TFs in pancreas formation. The generation of PSCs form patients harboring mutations in genes encoding TFs, coupled with in vitro production of functional β-cells, will facilitate studies aimed at understanding the regulatory mechanisms that govern human pancreas development.

Concluding remarks

Genetic studies in mice have led to the discovery of a vast number of TFs that participate in pancreas organogenesis. Functional genomics assays have made it possible to determine gene expression profiles and to identify transcription factor-binding sites on a genome-wide scale. These studies have revealed the complexity of the transcriptional interactions and networks underlying pancreatic differentiation. Although our understanding of the transcription factors and the genetic regulatory networks they form in pancreatic development has greatly increased over the last decade, some key gaps in our knowledge need to be filled before a comprehensive model of transcriptional pathways during pancreas formation can be built. Functional integration of TFs with the extrinsic signaling pathways that regulate the multiple steps of pancreatic organogenesis, in particular, is poorly understood. Similarly, more information is needed about how pancreatic lineage-specific transcription factors interact with the epigenetic machinery to drive pancreatic cell differentiation. Also, more studies on human tissue are required since differences between mouse and human pancreas development might exist. We can expect that the identification of new TFs with roles in pancreas formation, together with the advent of more sensitive functional genomics tools and the emerging field of epigenetics, will lead to a greater understanding of the transcriptional networks that operate in pancreas organogenesis over the next few years.

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