Published in final edited form as:
Clin Genet. 2008 October ; 74(4): 316–324. doi:10.1111/j.1399-0004.2008.01081.x.

Promoting ectopic pancreatic fates: pancreas development and future diabetes therapies

Esther J. Pearl[1] and **Marko E. Horb**[1,2,3,4]

[1]Laboratory of Molecular Organogenesis, Institut de Recherches Cliniques de Montréal, Montreal, QC Canada

[2]Division of Experimental Medicine and Department of Anatomy and Cell Biology, McGill University

[3]Département de Médecine, Université de Montréal

[4]Montreal Diabetes Research Center, Centre de recherche du Centre Hospitalier de l'Université de Montréal

Abstract

Diabetes is a disease which could be treated more effectively with a better understanding of pancreas development. This review examines the role of master regulator genes driving crucial steps in pancreas development, from foregut specification to differentiation of the five endocrine cell types. The roles of *Pdx1*, *Ptf1a*, and *Ngn3* are particularly examined as they are both necessary and sufficient for promoting pancreatic cell fates (*Pdx1*, *Ptf1a*) and endocrine cell development (*Ngn3*). The roles of *Arx* and *Pax4* are studied as they compose part of the regulatory mechanism balancing development of different types of endocrine cells within the iselts and promote the development of α/PP and β/δ cell progenitors, respectively. The roles of the aforementioned genes, and the consequences of misexpression of them for functionality of the pancreas, are examined through recent studies in model organisms, particularly *Xenopus* and zebrafish. Recent developments in cell replacement therapy research are also covered, concentrating on stem cell research (coaxing both adult and embryonic stem cells towards a β cell fate) and transdifferentiation (generating β cells from other differentiated cell types).

Keywords

cell replacement therapy; Ngn3; Pancreas; Pdx1; Ptf1a; stem cells; transdifferentiation; *Xenopus*; zebrafish

Diabetes and the importance of basic research

Diabetes is a serious disease that could be treated more effectively with a better understanding of pancreas development. The discovery of insulin in 1921 and the first insulin treatment of a patient in 1922 has changed diabetes from an outright lethal disease

Corresponding author: Marko E. Horb, Laboratory of Molecular Organogenesis, Institut de Recherches Cliniques de Montréal, 110, avenue des Pin Ouest, Montréal, H2W1R7, Québec, Canada. marko.horb@ircm.qc.ca, telephone: 514 987 5629, fax: 514 987 5793.

into a chronic condition (1). Diabetes can be split into three main types. First, Type 1 diabetes, which includes insulin-dependent diabetes, involving autoimmune destruction of the insulin-producing β-cells, and neonatal insulin production deficiencies. Second, Type 2 diabetes, a form of the disease whereby people become resistant to insulin, often associated with obesity. Third, gestational diabetes, which develops during pregnancy and most often resolves itself after the child is born.

Insulin replacement therapy has long been the treatment for type 1 diabetes and despite the fact that this regimen has changed diabetes from a fatal to a chronic disease, complications associated with inappropriate control of glucose levels still endure. Poorly managed diabetes can lead to blindness, kidney damage, and amputations among other things. Pancreatic transplants and injections of islet cells from cadavers are new therapies, but the shortage of donor tissue and the long term effects of lifetime immunosupressive regimens means that this approach is not available nor suitable for all diabetics. In fact pancreatic transplants are mainly reserved for those in whom regular insulin treatments are not working or those with many or advanced complications. Alternative treatments need to be developed, but this requires a better understanding of the molecular pathways underlying normal and disturbed pancreas development. To this end, basic research in model organisms will lead to a better understanding of how individual pancreatic cell fates are specified and help elucidate the molecular mechanisms that break down in disrupted pancreas development. This review will examine the genetic regulation of pancreas development focusing on genes that are not only essential, but also sufficient to promote ectopic pancreatic cell fates, as these are most likely to form the basis of future diabetes therapies. Many reviews covering other aspects of pancreas development in more detail are available (2-6).

Several model organisms have been informative in pancreatic developmental studies. Mouse and chick are the traditional models, while recently *Xenopus* and zebrafish have also been utilized (7-13). The two aquatic species, *Xenopus* and zebrafish, are cheaper to work with, grow faster than mice, and have a shorter generation time allowing experiments with higher throughput. Knock down and overexpression experiments can be performed initially in *Xenopus*, for example, to confirm the involvement of genes of interest (such as those implicated by genome-wide association studies or a microarray experiment) before more expensive and time-consuming mouse knock-outs are made. In addition, *Xenopus* and zebrafish can be used for *de novo* discoveries. Indeed, one of the most important genes in pancreatic development, *Pdx1*, was first discovered in *Xenopus* (14) years before the homologue was found in mice (15-18).

The pancreas develops from the endodermal germ layer, initially as two ventral buds and a single dorsal bud. These buds arise from distinct locations and are specified by different molecular mechanisms. In *Xenopus* the two ventral pancreatic buds fuse initially with each other, and shortly thereafter with the dorsal pancreas, such that by 3 days post-fertilization the tadpole has a single pancreas (5). In mammals however, one of the ventral buds regresses and fusion with the dorsal pancreas only occurs at E12.5 in mice (reviewed in (6)) and the sixth week of fetal development in humans (reviewed in (19)). Defects in development at these early stages can lead to clinically recognisable pancreatic anomalies - inappropriate development of the ventral pancreas can lead to annular pancreas or pancreas

divisum, whereas agenesis of the dorsal pancreas can occur (reviewed in (4)). Given that defects in development can lead to disease, a more thorough understanding of pancreas development from the earliest stages is important to understanding diabetes and other pancreatic diseases.

Foregut regional specification

The developing gut is divided into three regions, foregut, midgut, and hindgut; with the pancreas arising in the posterior part of the foregut. Several growth factor families, including bone morphogenetic protein (BMP) and Wnt, play important roles in patterning the endoderm and creating these regions. Interestingly, it is the repression of Wnt and BMP signaling that is essential in establishing the foregut (20, 21). Promotion of Wnt signaling in the anterior endoderm with ectopic expression of β-catenin or wnt8 during gastrula and neurula stages inhibits normal development of the pancreas and liver (21). In contrast, inhibiting Wnt signaling in the posterior endoderm with gsk3β or Dkk1 results in ectopic development of liver and pancreas buds. Similarly, the Smad transcriptional corepressor TGIF2 is required for proper specification of the pancreas (20). In addition to BMP and Wnt, retinoic acid (RA) and sonic hedgehog (Shh) signaling play key roles in positioning the posterior foregut domain (22-27). RA signalling is not only important for regional induction of this domain, but it is also essential for dorsal pancreas development (28), perhaps by suppression of endodermal Shh (29). The absence of *Shh* expression is vital for pancreas specification in amniotes and *Xenopus* (30, 31). In zebrafish Shh is required very early in foregut development for pancreatic induction (32-34) but has inhibitory effects on development in later stages, similar to those seen in amniotes setting up the organ boundaries in the foregut (34). Once the posterior region of the foregut is specified, downstream transcription factors are activated that function to specify development of the entire pancreas, followed by the activation of lineage-specific factors in distinct regions within the pancreas.

Several genes are expressed in a broad domain encompassing the entire posterior foregut prior to initial specification of the pancreas. Two of the earliest markers of the foregut are the onecut transcription factor, hepatocyte nuclear factor 6 (HNF6), and the ParaHox transcription factor, pancreas and duodenal homeobox gene 1 (Pdx1). Expression of *HNF6* in the dorsal and ventral endoderm is evident as early as the eight somite stage in mice, prior to expression of *Pdx1* in the pancreatic and rostral duodenum region (35). In HNF6$^{-/-}$ mice, although *Pdx1* expression is delayed (resulting in pancreatic hypoplasia), endocrine cells develop, albeit abnormally (36). In *Pdx1* mutant mice a more severe pancreatic phenotype is seen; pancreatic tissue is absent (37-39). Interestingly, initial budding of the pancreatic epithelium does occur, and early glucagon and insulin cells can be detected, though they do not persist. In humans, two different cases of pancreatic agenesis have been attributed to point mutations in the human *Pdx1* homologue, *IPF1* (40, 41). Studies in zebrafish show that knockdown of *Pdx1* using morpholinos results in reduced pancreatic tissue at 2 days, although by day 5 the pancreas is normal (42). Although *Pdx1* was initially cloned in *Xenopus* the first report of a *Pdx1* knockdown in *Xenopus laevis* did not come until 2006 (13). Morpholino knockdown of *Pdx1* in *X. laevis* results in a complete absence of acinar cells, while endocrine β cells develop normally; effects on other endocrine cells were not

reported. The explanation as to why such different phenotypes are observed in *Xenopus* and zebrafish are unclear, but may be due to the fact that they were not null mutations.

Downstream of *Pdx1*, one of the earliest markers specific to the developing dorsal and ventral pancreas is the basic helix-loop-helix (bHLH) transcription factor, pancreas transcription factor 1a (Ptf1a) (43). Similar to Pdx1, loss of Ptf1a results in pancreas agenesis (43, 44). The fact that loss of either Pdx1 or Ptf1a results in agenesis of the pancreas would suggest that these two proteins may interact to specify initial pancreas development, and several facts support this. First, although *Ptf1a* is expressed slightly later than *Pdx1* (a day later in mouse development), it has recently been shown to bind to the *Pdx1* promoter suggesting a role in maintenance of *Pdx1* expression (45). Second, it was recently shown that Ptf1a and Pdx1 function interdependently to specify early pancreatic multipotent progenitor cells (46). Third, progenitors lacking *Ptf1a* expression instead become intestinal (43). Perhaps cells expressing both *Pdx1* and *Ptf1a* become pancreatic, while those expressing only *Pdx1* give rise to intestinal tissue.

What has until recently been perplexing is how Ptf1a, originally isolated as an acinar-specific transcription factor, could also be involved in specification of general pancreatic progenitors. Several recent papers provide answers to this conundrum. First, it was shown that Ptf1a binds a specific region of the *Pdx1* promoter (area III) that mediates the early pancreas-wide expression of *Pdx1* (45). Second, Ptf1a binds this region in cooperation with RBPJ, which is essential for early pancreas development (47). Third, PTF1a function in acinar cells is dependent on RBPJL (47). Thus the function of Ptf1a in early pancreas development is dependent on RBPJ, while its function in acinar cell development is dependent on RBPJL, providing a mechanism to regulate these two different functions of Ptf1a.

In contrast to the situation with loss of Pdx1, specification of endocrine cells does occur in Ptf1a mutants, albeit at a significantly reduced rate (8, 44, 46, 48). In Ptf1a mutant mice these endocrine cells are present in the small dorsal pancreatic remnant, while in humans the location of the remaining insulin-expressing cells has not been determined (49). In *Xenopus*, initial specification of β cells does not occur in tadpoles lacking Ptf1a, whereas insulin expressing cells can be detected at later stages (9, 13). The exact opposite is seen in zebrafish where only a small population of late emerging endocrine cells are Ptf1a-dependent (8). These results suggest that there are two populations of endocrine cells, a Ptf1a-dependent (ptf1a^{+}ngn3^{+} cells) and a Ptf1a-independent (present in *Ptf1a* null mice) population (44, 46). However, these Ptf1a-independent endocrine cells are insufficient to establish the normal number of endocrine cells (44, 46).

None of these studies however, address the sufficiency of Ptf1a or Pdx1 in promoting pancreatic cell fates. This is quite important because although a factor may be necessary for the development of a specific cell type, it does not follow that it will also be sufficient to promote that fate. It is also possible that a specific factor may promote different cell fates depending on the cell type in which it is expressed. Regarding Ptf1a and Pdx1, we have shown (using *Xenopus*) that both are sufficient to promote ectopic pancreatic cell fates in other organs (9, 10). The benefit of using *Xenopus* is that we can rapidly test the function of

specific pancreatic transcription factors in different contexts: either in mRNA injections into early cleavage stage embryos to target naïve endoderm prior to organogenesis, or in transgenics to overexpress in specific organs at later developmental stages. Overexpressing genes in early endoderm is similar to overexpressing the same genes in embryonic stem cells (ES cells). Our lab has shown that overexpression of an activated *Pdx1*, *Pdx1-VP16*, in the liver cells of tadpoles and *in vitro* in HepG2 cells promotes the development of both endocrine and exocrine cells (10). Similar results were obtained by other labs, most notably by the Ferber lab, using either Pdx1 or Pdx1-VP16 (50-52). Interestingly, persistent overexpression of *Pdx1* in the pancreas (in all cell types) results in acinar-ductal metaplasia (53). Therefore, the ability of *Pdx1* to promote specific pancreatic cell fates depends on the context in which it is overexpressed.

Initial results suggesting that Ptf1a may promote ectopic pancreatic cell fates in the stomach and duodenum came from results in Hes1 mutant mice. In these mice the patches of differentiated ectopic pancreatic tissue that develop in the stomach, duodenum and common bile duct re-express *Ptf1a* (54). Although suggestive, the data did not show that Ptf1a directly promotes ectopic pancreas formation. We used *Xenopus laevis* to directly test the ability of Ptf1a and an activated form, Ptf1a-VP16, to promote ectopic pancreatic cell fates. We found that each was sufficient to promote ectopic pancreatic cell fates in the posterior foregut, but found differences in their activities (9). While overexpression of *Ptf1a* was sufficient to promote both endocrine and exocrine cell fates in the stomach and duodenum (both in early endoderm and in transgenics), *Ptf1a-VP16* was only able to promote acinar fates. We also found differences in the activity of *Ptf1a-VP16* depending on when it was overexpressed. In early endoderm, we found that Ptf1a-VP16 was able to convert most posterior foregut derivatives into acinar cells, including prospective liver, stomach, duodenum and pancreas, whereas in transgenics (where expression was much later) Ptf1a-VP16 was only able to convert liver cells to an acinar cell fate (9). The ability of Ptf1a to promote both endocrine and acinar cell fates only in posterior foregut derivatives suggests that it requires other coactivators that are localized to the foregut, such as Pdx1 (13).

Regulation of ductal, endocrine and acinar cell fates

Once a general pancreatic fate has been determined, individual ductal, acinar and endocrine cell fates need to be established. Although all three lineages arise from a common progenitor pool, specification of these cells within the pancreas occurs in a temporal manner, such that ductal cell fates are determined prior to the commitment of endocrine and acinar cells (55, 56). By E12.5 a subset of $Pdx1^+$ cells have been specified as ductal cells; after this stage $Pdx1^+$ cells will only give rise to endocrine and acinar cells (55, 56). Pancreatic progenitor cells that express *Pdx1* also express *Sox9*, a homeobox gene shown to be essential for maintenance of the pancreatic progenitor cells (57). Conditional inactivation of *Sox9* in the pancreas leads to pancreatic hypoplasia, reminiscent of that seen in Notch signaling pathway mutants. In agreement with this, expression of the downsteam Notch effector, *Hes-1*, is severely reduced in Sox9 mutants (57). Furthermore, Sox9 and several other pancreatic progenitor transcription factors, including FoxA2, Tcf2 and HNF6, interact to directly regulate each other's expression (58). Subsequently, these factors activate the expression of the endocrine progenitor marker, *neurogenin3* (*Ngn3*), to specifiy the endocrine lineage.

The bHLH transcription factor Ngn3 is the earliest marker of endocrine progenitor cells, both in the embryo and the adult (55, 59). Expression of *Ngn3* is transient in the progenitor cell population, and it is not expressed in differentiated cells (60); this allows tight regulation ensuring a balance between islet cell differentiation and progenitor cell proliferation. Ngn3 is essential for endocrine cell development, as demonstrated in Ngn3 knockout mice where no endocrine cells develop (60). One of the first genes Ngn3 activates is the zinc finger transcription factor *insulinoma associated protein 1* (*IA1*) (61). Much like *Ngn3*, *IA1* is only expressed in the progenitor cell population, and is required for the differentiation of all endocrine cells (62). The localized expression of both of these factors in endocrine progenitors coupled with the fact that they are indispensable for endocrine cell development would suggest that they ought to be sufficient for promoting endocrine cell fates. Indeed, overexpression of *Ngn3* in the pancreas is sufficient to promote differentiation of all endocrine cells (see Fig. 1), but this effect is context dependent (63). When expressed in early embryonic pancreas, *Ngn3* promotes differentiation of only the alpha cell population, but when expressed at progressively later stages it is also able to promote differentiation of three other endocrine lineages (64). In contrast to *Ngn3*, overexpression of *IA1* does not lead to any obvious changes in pancreas development (Horb ME, unpublished data). Thus, although Ngn3 is both necessary and sufficient for endocrine cell development, its ability to promote endocrine cell fates is context dependent.

Downstream of the Ngn3 pan-endocrine progenitor population there appear to be separate α/PP and β/δ lineages that are specified by the opposing actions of two transcription factors, aristaless related homeobox gene (Arx) and paired box 4 (Pax4), respectively (Fig. 1). In Arx mutants the α cell population does not develop, and there is an increase in the numbers of β and δ cells (65). Interestingly, overexpression of *Arx* is sufficient to promote the differentiation of both α and PP cell populations at the expense of β and δ cells, even though only the α cell population is affected in Arx mutants (66). The exact opposite is seen with Pax4. In Pax4 mutant mice the β and δ cell lineages do not develop, and there is an increase in the α cell lineage (67). Interestingly, the phenotype of the Arx/Pax4 double mutant mice is not simply additive of each single mutant – in these mice the α and β cell populations are absent as expected but there are excessive numbers of δ and PP cells (68). These results would seem to indicate that there is a relationship between the δ and PP cell populations. This is supported by the recent results obtained in Rfx3 mutant mice; in these mice the α and β cell lineages do not develop, and while the δ cell lineage is normal there is a large increase in the PP cell population (69). These results suggest a relationship whereby the δ and PP cells arise from the same progenitor population and the β and δ cells arise from a common progenitor.

In contrast to the four endocrine lineages described above, the factors responsible for promoting development of the fifth endocrine cell lineage, ε cells which produce ghrelin, have yet to be identified (70, 71). Several reports however have shown that loss of Nkx2.2, Pax4 or Pax6 leads to an increase in the ghrelin cell population (72, 73). Although several other transcription factors, such as Pax6, are required for proper development of the different endocrine lineages, it is unclear whether they are able to promote the development of ectopic pancreatic tissue. It is also important to remember that there are further

transcription factors responsible for the final maturation of the different cell types. As we do not have the space to discuss these factors in detail here the reader is recommended to several excellent recent reviews (2, 3, 5-7, 74, 75).

Future therapies based on current research

Current research into diabetes treatment is focused on generating replacement cells that can mimic the exquisite glucose-responsiveness of the normal pancreatic β cell (76). Two main sources of cells that can be used to produce ectopic β cells for cell replacement therapy are stem cells and differentiated cells (Fig. 2). In the first instance both embryonic (ES) and adult stem cells are possible sources. Recent studies using ES cells have demonstrated that these cells can be directed towards a pancreatic lineage, although these studies are still in their infancy (77-79). It is interesting to note that much of the information required to guide the differentiation of ES cells into a pancreatic lineage came from earlier developmental biology studies (80). Although adult stem cells do not possess the same pluripotency of ES cells, they are multipotent, and several recent reports have demonstrated their potential usefulness for diabetes therapy. Bone marrow derived stem cells have been examined most frequently, and can be used to either directly generate islet cells or to initiate endogenous regeneration of the pancreas (81-86). Other adult stem cell populations that may be used include umbilical cord, intestinal, liver, adipose and spleen (87-90). Although their existence remains controversial, pancreatic stem cells may also provide a rich source of cells (87, 91, 92). The pluripotent nature of ES cells would seem to favor their use, but questions surrounding their tumorigenic potential need to be answered (92). Adult stem cells provide less of an ethical dilemma, although it is unclear how competent they are to produce (or induce) fully functional β cells.

An alternative strategy is to generate pancreatic tissue from differentiated cells, known as transdifferentiation. (reviewed in (93, 94)). Transdifferentiation of liver tissue is the most promising since liver and pancreatic cells arise from common progenitors in the posterior foregut coupled with the fact that the liver has the ability to regenerate (9, 13, 50, 95, 96). The ability to alter a small population of a patient's own liver cells to become insulin-producing pancreatic cells would eliminate the problems of tissue shortage and rejection associated with islet cell or whole pancreas transplants. Transdifferentiation amongst different cell types in the pancreas is also possible as both ductal and acinar cells have been successfully transdifferentiated into β-cells (97, 98). In order for this to become a legitimate therapy a better understanding of the molecular control of transdifferentiation is required. *Xenopus laevis* is an excellent model organism in which to study the transdifferentiation process as liver-to-pancreas transdifferentiation been shown to occur in *Xenopus* (9, 10, 13). Furthermore, gene overexpression and knock down studies are easily, cheaply and quickly performed in *Xenopus* compared to other model organisms such as the mouse, enabling faster elucidation of the molecular networks regulating the process. Understanding the regulation of the transdifferentiation process in a model organism is the first step towards understanding it in humans and eventually being able to control the process in human therapies.

Acknowledgments

Work in the lab is supported in part by grants from the NIH (DK077197), the Canadian Cancer Society (016243) and the JDRF. M.E.H. is a Junior 2 Research Scholar of the Fonds de la recherche en santé du Québec (FRSQ).

References

1. Banting FG, Best CH, Collip JB, et al. Pancreatic extracts in the treatment of diabetes mellitus: preliminary report. Canadian Mediacal Association Journal. 1922; 12:141–146.
2. Jensen J. Gene regulatory factors in pancreatic development. Dev Dyn. 2004; 229:176–200. [PubMed: 14699589]
3. Murtaugh LC. Pancreas and beta-cell development: from the actual to the possible. Development. 2007; 134:427–438. [PubMed: 17185316]
4. Cano DA, Hebrok M, Zenker M. Pancreatic development and disease. Gastroenterology. 2007; 132:745–762. [PubMed: 17258745]
5. Kelly OG, Melton DA. Development of the pancreas in Xenopus laevis. Dev Dyn. 2000; 218:615–627. [PubMed: 10906780]
6. Jorgensen MC, Ahnfelt-Ronne J, Hald J, et al. An illustrated review of early pancreas development in the mouse. Endocr Rev. 2007; 28:685–705. [PubMed: 17881611]
7. Ober EA, Field HA, Stainier DY. From endoderm formation to liver and pancreas development in zebrafish. MechDev. 2003; 120:5–18.
8. Lin JW, Biankin AV, Horb ME, et al. Differential requirement for ptf1a in endocrine and exocrine lineages of developing zebrafish pancreas. Dev Biol. 2004; 274:491–503. [PubMed: 15570689]
9. Jarikji ZH, Vanamala S, Beck CW, et al. Differential ability of Ptf1a and Ptf1a-VP16 to convert stomach, duodenum and liver to pancreas. Dev Biol. 2007; 304:786–799. [PubMed: 17320068]
10. Horb ME, Shen CN, Tosh D, et al. Experimental conversion of liver to pancreas. Curr Biol. 2003; 13:105–115. [PubMed: 12546783]
11. Field HA, Dong PD, Beis D, et al. Formation of the digestive system in zebrafish. II. Pancreas morphogenesis. Dev Biol. 2003; 261:197–208. [PubMed: 12941629]
12. Blitz IL, Andelfinger G, Horb ME. Germ layers to organs: using Xenopus to study “later” development. Seminars in cell & developmental biology. 2006; 17:133–145. [PubMed: 16337415]
13. Afelik S, Chen Y, Pieler T. Combined ectopic expression of Pdx1 and Ptf1a/p48 results in the stable conversion of posterior endoderm into endocrine and exocrine pancreatic tissue. Genes Dev. 2006; 20:1441–1446. [PubMed: 16751182]
14. Wright CV, Schnegelsberg P, De Robertis EM. XlHbox 8: a novel Xenopus homeo protein restricted to a narrow band of endoderm. Development. 1989; 105:787–794. [PubMed: 2574662]
15. Leonard J, Peers B, Johnson T, et al. Characterization of somatostatin transactivating factor-1, a novel homeobox factor that stimulates somatostatin expression in pancreatic islet cells. Mol Endocrinol. 1993; 7:1275–1283. [PubMed: 7505393]
16. Ohlsson H, Karlsson K, Edlund T. IPF1, a homeodomain-containing transactivator of the insulin gene. EMBO J. 1993; 12:4251–4259. [PubMed: 7901001]
17. Miller CP, McGehee RE Jr, Habener JF. IDX-1: a new homeodomain transcription factor expressed in rat pancreatic islets and duodenum that transactivates the somatostatin gene. EMBO J. 1994; 13:1145–1156. [PubMed: 7907546]
18. Peshavaria M, Gamer L, Henderson E, et al. XIHbox 8, an endoderm-specific Xenopus homeodomain protein, is closely related to a mammalian insulin gene transcription factor. Mol Endocrinol. 1994; 8:806–816. [PubMed: 7935494]
19. Polak M, Bouchareb-Banaei L, Scharfmann R, et al. Early pattern of differentiation in the human pancreas. Diabetes. 2000; 49:225–232. [PubMed: 10868939]
20. Spagnoli FM, Brivanlou AH. The Gata5 target, TGIF2, defines the pancreatic region by modulating BMP signals within the endoderm. Development. 2008; 135:451–461. [PubMed: 18094028]

21. McLin VA, Rankin SA, Zorn AM. Repression of Wnt/beta-catenin signaling in the anterior endoderm is essential for liver and pancreas development. Development. 2007; 134:2207–2217. [PubMed: 17507400]
22. Stafford D, Hornbruch A, Mueller PR, et al. A conserved role for retinoid signaling in vertebrate pancreas development. Dev Genes Evol. 2004; 214:432–441. [PubMed: 15322880]
23. Moriya N, Komazaki S, Takahashi S, et al. In vitro pancreas formation from Xenopus ectoderm treated with activin and retinoic acid. Dev Growth Differ. 2000; 42:593–602. [PubMed: 11142681]
24. Molotkov A, Molotkova N, Duester G. Retinoic acid generated by Raldh2 in mesoderm is required for mouse dorsal endodermal pancreas development. Dev Dyn. 2005; 232:950–957. [PubMed: 15739227]
25. Martin M, Gallego-Llamas J, Ribes V, et al. Dorsal pancreas agenesis in retinoic acid-deficient Raldh2 mutant mice. Dev Biol. 2005
26. van den Brink GR. Hedgehog signaling in development and homeostasis of the gastrointestinal tract. Physiol Rev. 2007; 87:1343–1375. [PubMed: 17928586]
27. Hebrok M. Hedgehog signaling in pancreas development. MechDev. 2003; 120:45–57.
28. Pan FC, Chen Y, Bayha E, et al. Retinoic acid-mediated patterning of the pre-pancreatic endoderm in Xenopus operates via direct and indirect mechanisms. Mechanisms of development. 2007; 124:518–531. [PubMed: 17643968]
29. Chen Y, Pan FC, Brandes N, et al. Retinoic acid signaling is essential for pancreas development and promotes endocrine at the expense of exocrine cell differentiation in Xenopus. Dev Biol. 2004; 271:144–160. [PubMed: 15196957]
30. Zhang J, Rosenthal A, de Sauvage FJ, et al. Downregulation of Hedgehog signaling is required for organogenesis of the small intestine in Xenopus. Dev Biol. 2001; 229:188–202. [PubMed: 11133163]
31. Hebrok M, Kim SK, Melton DA. Notochord repression of endodermal Sonic hedgehog permits pancreas development. Genes Dev. 1998; 12:1705–1713. [PubMed: 9620856]
32. Roy S, Qiao T, Wolff C, et al. Hedgehog signaling pathway is essential for pancreas specification in the zebrafish embryo. Curr Biol. 2001; 11:1358–1363. [PubMed: 11553330]
33. diIorio PJ, Moss JB, Sbrogna JL, et al. Sonic hedgehog is required early in pancreatic islet development. Dev Biol. 2002; 244:75–84. [PubMed: 11900460]
34. Chung WS, Stainier DY. Intra-endodermal interactions are required for pancreatic beta cell induction. Dev Cell. 2008; 14:582–593. [PubMed: 18410733]
35. Jacquemin P, Lemaigre FP, Rousseau GG. The Onecut transcription factor HNF-6 (OC-1) is required for timely specification of the pancreas and acts upstream of Pdx-1 in the specification cascade. Dev Biol. 2003; 258:105–116. [PubMed: 12781686]
36. Jacquemin P, Durviaux SM, Jensen J, et al. Transcription factor hepatocyte nuclear factor 6 regulates pancreatic endocrine cell differentiation and controls expression of the proendocrine gene ngn3. Mol Cell Biol. 2000; 20:4445–4454. [PubMed: 10825208]
37. Offield MF, Jetton TL, Labosky PA, et al. PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. Development. 1996; 122:983–995. [PubMed: 8631275]
38. Jonsson J, Carlsson L, Edlund T, et al. Insulin-promoter-factor 1 is required for pancreas development in mice. Nature. 1994; 371:606–609. [PubMed: 7935793]
39. Ahlgren U, Jonsson J, Edlund H. The morphogenesis of the pancreatic mesenchyme is uncoupled from that of the pancreatic epithelium in IPF1/PDX1-deficient mice. Development. 1996; 122:1409–1416. [PubMed: 8625829]
40. Stoffers DA, Zinkin NT, Stanojevic V, et al. Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. NatGenet. 1997; 15:106–110.
41. Schwitzgebel VM, Mamin A, Brun T, et al. Agenesis of human pancreas due to decreased half-life of insulin promoter factor 1. J ClinEndocrinol Metab. 2003; 88:4398–4406.
42. Yee NS, Yusuff S, Pack M. Zebrafish pdx1 morphant displays defects in pancreas development and digestive organ chirality, and potentially identifies a multipotent pancreas progenitor cell. Genesis. 2001; 30:137–140. [PubMed: 11477692]

43. Kawaguchi Y, Cooper B, Gannon M, et al. The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. NatGenet. 2002; 32:128–134.
44. Krapp A, Knofler M, Ledermann B, et al. The bHLH protein PTF1-p48 is essential for the formation of the exocrine and the correct spatial organization of the endocrine pancreas. Genes Dev. 1998; 12:3752–3763. [PubMed: 9851981]
45. Wiebe PO, Kormish JD, Roper VT, et al. Ptf1a binds to and activates area III, a highly conserved region of the Pdx1 promoter that mediates early pancreas-wide Pdx1 expression. Molecular and cellular biology. 2007; 27:4093–4104. [PubMed: 17403901]
46. Burlison JS, Long Q, Fujitani Y, et al. Pdx-1 and Ptf1a concurrently determine fate specification of pancreatic multipotent progenitor cells. Dev Biol. 2008; 316:74–86. [PubMed: 18294628]
47. Masui T, Long Q, Beres TM, et al. Early pancreatic development requires the vertebrate Suppressor of Hairless (RBPJ) in the PTF1 bHLH complex. Genes Dev. 2007; 21:2629–2643. [PubMed: 17938243]
48. Zecchin E, Mavropoulos A, Devos N, et al. Evolutionary conserved role of ptf1a in the specification of exocrine pancreatic fates. Dev Biol. 2004; 268:174–184. [PubMed: 15031114]
49. Sellick GS, Barker KT, Stolte-Dijkstra I, et al. Mutations in PTF1A cause pancreatic and cerebellar agenesis. NatGenet. 2004; 36:1301–1305.
50. Fodor A, Harel C, Fodor L, et al. Adult rat liver cells transdifferentiated with lentiviral IPF1 vectors reverse diabetes in mice: an ex vivo gene therapy approach. Diabetologia. 2007; 50:121–130. [PubMed: 17131142]
51. Meivar-Levy I, Sapir T, Gefen-Halevi S, et al. Pancreatic and duodenal homeobox gene 1 induces hepatic dedifferentiation by suppressing the expression of CCAAT/enhancer-binding protein beta. Hepatology. 2007; 46:898–905. [PubMed: 17705277]
52. Shternhall-Ron K, Quintana FJ, Perl S, et al. Ectopic PDX-1 expression in liver ameliorates type 1 diabetes. J Autoimmun. 2007; 28:134–142. [PubMed: 17383157]
53. Miyatsuka T, Kaneto H, Shiraiwa T, et al. Persistent expression of PDX-1 in the pancreas causes acinar-to-ductal metaplasia through Stat3 activation. Genes Dev. 2006; 20:1435–1440. [PubMed: 16751181]
54. Fukuda A, Kawaguchi Y, Furuyama K, et al. Ectopic pancreas formation in Hes1 - knockout mice reveals plasticity of endodermal progenitors of the gut, bile duct, and pancreas. J ClinInvest. 2006; 116:1484–1493.
55. Gu G, Dubauskaite J, Melton DA. Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. Development. 2002; 129:2447–2457. [PubMed: 11973276]
56. Esni F, Ghosh B, Biankin AV, et al. Notch inhibits Ptf1 function and acinar cell differentiation in developing mouse and zebrafish pancreas. Development. 2004; 131:4213–4224. [PubMed: 15280211]
57. Seymour PA, Freude KK, Tran MN, et al. SOX9 is required for maintenance of the pancreatic progenitor cell pool. Proc Natl Acad Sci U S A. 2007; 104:1865–1870. [PubMed: 17267606]
58. Lynn FC, Smith SB, Wilson ME, et al. Sox9 coordinates a transcriptional network in pancreatic progenitor cells. Proc Natl Acad Sci U S A. 2007; 104:10500–10505. [PubMed: 17563382]
59. Schwitzgebel VM, Scheel DW, Conners JR, et al. Expression of neurogenin3 reveals an islet cell precursor population in the pancreas. Development. 2000; 127:3533–3542. [PubMed: 10903178]
60. Gradwohl G, Dierich A, LeMeur M, et al. neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. Proc Natl Acad Sci U S A. 2000; 97:1607–1611. [PubMed: 10677506]
61. Mellitzer G, Bonne S, Luco RF, et al. IA1 is NGN3-dependent and essential for differentiation of the endocrine pancreas. EMBO J. 2006; 25:1344–1352. [PubMed: 16511571]
62. Gierl MS, Karoulias N, Wende H, et al. The zinc-finger factor Insm1 (IA-1) is essential for the development of pancreatic beta cells and intestinal endocrine cells. Genes Dev. 2006; 20:2465–2478. [PubMed: 16951258]
63. Apelqvist A, Li H, Sommer L, et al. Notch signalling controls pancreatic cell differentiation. Nature. 1999; 400:877–881. [PubMed: 10476967]

64. Johansson KA, Dursun U, Jordan N, et al. Temporal control of neurogenin3 activity in pancreas progenitors reveals competence windows for the generation of different endocrine cell types. Dev Cell. 2007; 12:457–465. [PubMed: 17336910]

65. Collombat P, Mansouri A, Hecksher-Sorensen J, et al. Opposing actions of Arx and Pax4 in endocrine pancreas development. Genes Dev. 2003; 17:2591–2603. [PubMed: 14561778]

66. Collombat P, Hecksher-Sorensen J, Krull J, et al. Embryonic endocrine pancreas and mature beta cells acquire alpha and PP cell phenotypes upon Arx misexpression. The Journal of clinical investigation. 2007; 117:961–970. [PubMed: 17404619]

67. Sosa-Pineda B, Chowdhury K, Torres M, et al. The Pax4 gene is essential for differentiation of insulin-producing beta cells in the mammalian pancreas. Nature. 1997; 386:399–402. [PubMed: 9121556]

68. Collombat P, Hecksher-Sorensen J, Broccoli V, et al. The simultaneous loss of Arx and Pax4 genes promotes a somatostatin-producing cell fate specification at the expense of the alpha- and beta-cell lineages in the mouse endocrine pancreas. Development. 2005; 132:2969–2980. [PubMed: 15930104]

69. Ait-Lounis A, Baas D, Barras E, et al. Novel function of the ciliogenic transcription factor RFX3 in development of the endocrine pancreas. Diabetes. 2007; 56:950–959. [PubMed: 17229940]

70. Wierup N, Svensson H, Mulder H, et al. The ghrelin cell: a novel developmentally regulated islet cell in the human pancreas. Regul Pept. 2002; 107:63–69. [PubMed: 12137967]

71. Prado CL, Pugh-Bernard AE, Elghazi L, et al. Ghrelin cells replace insulin-producing beta cells in two mouse models of pancreas development. Proc Natl Acad Sci U S A. 2004; 101:2924–2929. [PubMed: 14970313]

72. Wang Q, Elghazi L, Martin S, et al. Ghrelin is a novel target of Pax4 in endocrine progenitors of the pancreas and duodenum. Dev Dyn. 2008; 237:51–61. [PubMed: 18058910]

73. Heller RS, Jenny M, Collombat P, et al. Genetic determinants of pancreatic epsilon-cell development. Dev Biol. 2005; 286:217–224. [PubMed: 16122727]

74. Collombat P, Hecksher-Sorensen J, Serup P, et al. Specifying pancreatic endocrine cell fates. Mechanisms of development. 2006; 123:501–512. [PubMed: 16822656]

75. Wilson ME, Scheel D, German MS. Gene expression cascades in pancreatic development. Mechanisms of development. 2003; 120:65–80. [PubMed: 12490297]

76. Jun HS. Regeneration of pancreatic beta cells. Front Biosci. 2008; 13:6170–6182. [PubMed: 18508651]

77. Phillips BW, Hentze H, Rust WL, et al. Directed differentiation of human embryonic stem cells into the pancreatic endocrine lineage. Stem Cells Dev. 2007; 16:561–578. [PubMed: 17784830]

78. D'Amour KA, Bang AG, Eliazer S, et al. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. Nat Biotechnol. 2006; 24:1392–1401. [PubMed: 17053790]

79. Madsen OD. Stem cells and diabetes treatment. APMIS. 2005; 113:858–875. [PubMed: 16480455]

80. Madsen OD, Serup P. Towards cell therapy for diabetes. Nat Biotechnol. 2006; 24:1481–1483. [PubMed: 17160042]

81. Lu P, Liu F, Yan L, et al. Stem cells therapy for type 1 diabetes. Diabetes Res Clin Pract. 2007; 78:1–7. [PubMed: 17349714]

82. Karnieli O, Izhar-Prato Y, Bulvik S, et al. Generation of insulin-producing cells from human bone marrow mesenchymal stem cells by genetic manipulation. Stem Cells. 2007; 25:2837–2844. [PubMed: 17615265]

83. Hess D, Li L, Martin M, et al. Bone marrow-derived stem cells initiate pancreatic regeneration. Nat Biotechnol. 2003; 21:763–770. [PubMed: 12819790]

84. Gao X, Song L, Shen K, et al. Transplantation of bone marrow derived cells promotes pancreatic islet repair in diabetic mice. Biochemical and biophysical research communications. 2008; 371:132–137. [PubMed: 18420028]

85. Ai C, Todorov I, Slovak ML, et al. Human marrow-derived mesodermal progenitor cells generate insulin-secreting islet-like clusters in vivo. Stem Cells Dev. 2007; 16:757–770. [PubMed: 17999597]

86. Lee RH, Seo MJ, Reger RL, et al. Multipotent stromal cells from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic NOD/scid mice. Proc Natl Acad Sci U S A. 2006; 103:17438–17443. [PubMed: 17088535]

87. Lee DD, Grossman E, Chong AS. Cellular therapies for type 1 diabetes. Horm Metab Res. 2008; 40:147–154. [PubMed: 18283633]

88. Gao F, Wu DQ, Hu YH, et al. In vitro cultivation of islet-like cell clusters from human umbilical cord blood-derived mesenchymal stem cells. Transl Res. 2008; 151:293–302. [PubMed: 18514140]

89. Robertson SA, Rowan-Hull AM, Johnson PR. The spleen--a potential source of new islets for transplantation? J Pediatr Surg. 2008; 43:274–278. [PubMed: 18280273]

90. Bajada S, Mazakova I, Richardson JB, et al. Updates on stem cells and their applications in regenerative medicine. J Tissue Eng Regen Med. 2008; 2:169–183. [PubMed: 18493906]

91. Gangaram-Panday ST, Faas MM, de Vos P. Towards stem-cell therapy in the endocrine pancreas. Trends Mol Med. 2007; 13:164–173. [PubMed: 17307397]

92. Limbert C, Path G, Jakob F, et al. Beta-cell replacement and regeneration: Strategies of cell-based therapy for type 1 diabetes mellitus. Diabetes Res Clin Pract. 2008; 79:389–399. [PubMed: 17854943]

93. Slack JM, Tosh D. Transdifferentiation and metaplasia--switching cell types. Curr Opin Genet Dev. 2001; 11:581–586. [PubMed: 11532402]

94. Tosh D, Slack JM. How cells change their phenotype. NatRev Mol Cell Biol. 2002; 3:187–194.

95. Tang DQ, Cao LZ, Chou W, et al. Role of Pax4 in Pdx1-VP16-mediated liver-to-endocrine pancreas transdifferentiation. Lab Invest. 2006

96. Tang DQ, Lu S, Sun YP, et al. Reprogramming liver-stem WB cells into functional insulin-producing cells by persistent expression of Pdx1- and Pdx1-VP16 mediated by lentiviral vectors. Lab Invest. 2006; 86:83–93. [PubMed: 16294197]

97. Bonner-Weir S, Inada A, Yatoh S, et al. Transdifferentiation of pancreatic ductal cells to endocrine beta-cells. Biochem Soc Trans. 2008; 36:353–356. [PubMed: 18481956]

98. Minami K, Seino S. Pancreatic acinar-to-beta cell transdifferentiation in vitro. Front Biosci. 2008; 13:5824–5837. [PubMed: 18508625]

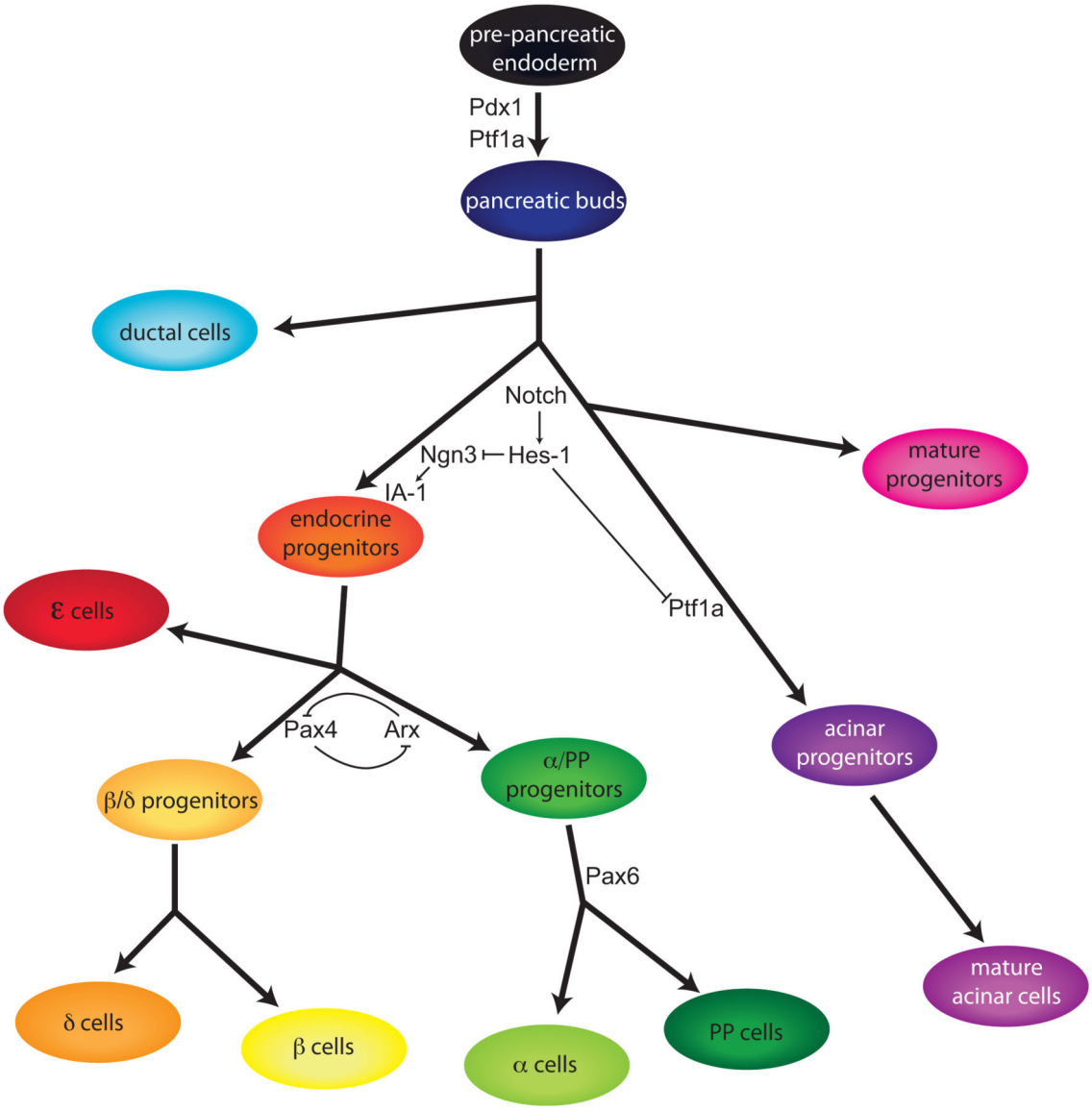

Figure 1. From pre-pancreatic endoderm to pancreas

A schematic diagram outlining the major developmental steps involved in creating different pancreatic cell types. Important progenitors are inlcuded along with genes important in key steps in pancreas development.

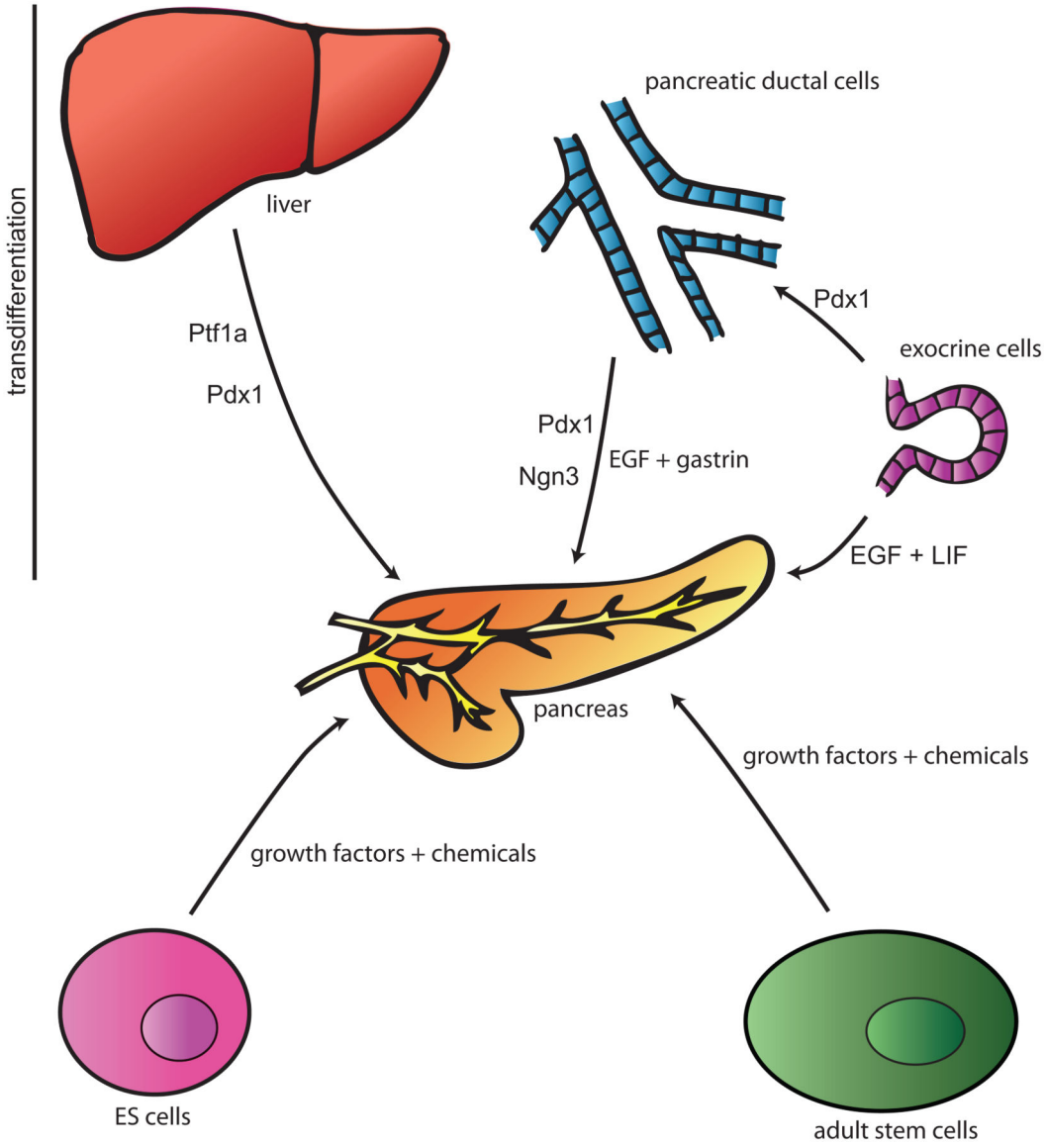

Figure 2. Creating a new pancreas, the future of diabetes therapy

Many future diabetes therapies revolve around the idea of creating new pancreas cells. There are two major ways to do this. Transdifferentiation involves changing a differentiated cell type into β cells by turning on pancreatic transcription factors in non-β cells. The other method is guiding stem cells (either from adult or embryonic tissue) to become pancreatic β cells utilizing combinations of growth factors and chemicals.