Graded levels of Ptf1a differentially regulate endocrine and exocrine fates in the developing pancreas

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The mechanisms regulating pancreatic endocrine versus exocrine fate are not well defined. By analyzing the effects of Ptf1a partial loss of function, we uncovered novel roles for this transcription factor in determining pancreatic fates. In a newly identified hypomorphic ptf1a mutant, pancreatic cells that would normally express ptf1a and become exocrine cells, express the endocrine marker Isl1, indicating a cell fate switch. Surprisingly, a milder reduction of Ptf1a leads to an even greater increase of ectopic endocrine cells, suggesting that Ptf1a also plays a role in promoting endocrine development. We propose that low levels of Ptf1a promote endocrine fate, whereas high levels repress endocrine fate and promote exocrine fate.

Supplemental material is available at http://www.genesdev.org.

Loss of the insulin-producing β-cells from the endocrine pancreas occurs in diabetes, a disease that afflicts >125 million people worldwide. Thus, endocrine cell replacement is likely to be an integral step in the treatment of this disease (Donath and Halban 2004; Bertuzzi et al. 2006). A major challenge lies in developing practical strategies for β-cell replacement, a task that will be facilitated by a more profound understanding of the mechanisms underlying the specification and differentiation of pancreatic endocrine cells.

The pancreas is composed of digestive enzyme-producing exocrine cells, ductal cells, and hormone-producing endocrine cells. Although many genes are known to be required for the differentiation of endocrine and exocrine cells, factors regulating the endocrine versus exocrine fate decision remain elusive (Jensen 2004). The Atonal-related basic helix-loop-helix (bHLH) protein Neurogenin3, and Lim domain protein Isl1 (Isl1) are transcription factors required for the specification and differentiation of pancreatic endocrine cells (Ahlgren et al. 1997; Gradwohl et al. 2000). The bHLH transcription factor Ptf1a is required for exocrine differentiation (Krapp et al. 1998). Ptf1a lineage tracing has also revealed an earlier role for Ptf1a in allocating foregut endodermal cells to the pancreas, implicating Ptf1a in pancreas specification. This finding is consistent with the observation that Ptf1a is expressed in precursors of all pancreatic cell types, including endocrine cells (Kawaguchi et al. 2002). Importantly, it is not specifically known how Ptf1a regulates endocrine development. The PTF1 complex binds directly to the promoters of terminal exocrine differentiation genes such as Trypsin and Elastase, indicating that Ptf1a is also involved in exocrine cell differentiation (Cockell et al. 1989; Krapp et al. 1996). Furthermore, Ptf1a interacts differentially with the vertebrate Suppressor of Hairless homologs, Rbpj and Rbpf, to regulate pancreas specification and exocrine differentiation, respectively (Masui et al. 2007). Lineage tracing of cells expressing Carboxypeptidase A1, a Ptf1a target, has shown that endocrine and exocrine cells can originate from a common progenitor in the specified pancreas (Zhou et al. 2007). Therefore, it will be necessary to analyze the post-pancreas specification role of Ptf1a to determine whether it conveys other functions, such as determining the balance of endocrine versus exocrine cell fates.

Results and Discussion

To search for mutants with pancreas defects, we screened ENU mutagenized Tg(gutGFP)$^{akreas}$ zebrafish (Ober et al. 2006), which express GFP in many endodermal organs, including the pancreas. $akreas^{mut}$ is a recessive mutant that exhibits a small ventral pancreas (Fig. 1A,B). The principal islet, which consists of endocrine cells that are initially derived from the dorsal pancreas (Field et al. 2003b), is present in $akreas$ mutants, indicating that the dorsal pancreas does develop. Expression of trypsin (data not shown) and Tg(elastase:GFP)$^{akreas}$, markers of the exocrine pancreas, is lost in $akreas$ mutants (Fig. 1C,D), indicating the absence of differentiated exocrine cells.

The $akreas$ mutation maps to chromosome 2, within a 2 cM region that contains $ptf1a$ (data not shown). Like $akreas$ mutants, $ptf1a$ mutant mice (Krapp et al. 1998) and zebrafish embryos injected with antisense $ptf1a$ morpholino oligonucleotides (MOs; Lin et al. 2004; Zecchin et al. 2004) develop pancreatic endocrine cells but no exocrine cells, prompting us to sequence the $ptf1a$ gene of $akreas$ mutants. We identified a transversion in the coding region of $ptf1a$ (Fig. 1E), causing an isoleucine 142-to-asparagine [I142N] substitution in the first helix of the highly conserved bHLH domain (Fig. 1F). This iso- leucine is present in the bHLH domain of diverse proteins in organisms from flies to humans (Krapp et al. 1996; Lin et al. 2004; Zecchin et al. 2004). The genetic linkage to $ptf1a$, expression of $ptf1a$ in the affected tissue, phenocopy of the $ptf1a$ loss of function, and a genetic lesion in a highly conserved residue of $ptf1a$ indicate that $akreas$ is a mutation in $ptf1a$.

To better understand the effect of the $akreas$ mutation on $ptf1a$ function, we examined the ability of the mutant protein to activate a modified rat Chymotrypsinogen B promoter. Both wild-type zebrafish and rat $ptf1a$, in combination with the cofactor E47, effectively activated a
mutants [Fig. 1H,I], we hypothesized the presence of a low level of Ptf1a function. To test this hypothesis, we used a ptf1a MO [ptf1aMO1], which knocks down translation of ptf1a but not Tg(ptf1a:eGFP) (Supplemental Fig. S1C; Supplemental Material), in the akreas mutant background. In -20% of ptf1aMO1 injected embryos [36 of 179] from an incross of akreas heterozygotes, we found a disorganization of retinal Tg(ptf1a:eGFP)-expressing cells similar to what is observed in the Ptf1a-null mice. Particularly, we found a reduction of Tg(ptf1a:eGFP)-expressing cells in the amacrine layer and ectopic Tg(ptf1a:eGFP) expression in scattered cells in the ganglion layer, as marked by Isl1 [Fig. 1J,K; Fischer et al. 2002] and Alcam [Weiner et al. 2004] expression. Furthermore, many of the Tg(ptf1a:eGFP)-expressing cells in the ganglion layer coexpress Isl1 and Alcam [Supplemental Fig. S1D–O], suggesting that presumptive amacrine cells are being translated toward ganglion cells, a phenotype similar to that seen in Ptf1a-null mice. We also observed that the Tg(ptf1a:eGFP) expressing horizontal cells, which in wild-type embryos coexpress Isl1, lack Isl1 expression and are severely reduced in number. In addition, we found Tg(ptf1a:eGFP) expression in the adjacent photoreceptor cells, suggesting that horizontal cells are being translated into photoreceptor cells, a phenotype not previously described. These phenotypes were not observed in uninjected akreas mutants or in ptf1aMO1-injected wild-type embryos. Therefore, a more severe ptf1a loss-of-function phenotype can be achieved by a ptf1a morpholino/mutant combination. Based on these in vivo data, as well as the transactivation and gel shift results, we conclude that the akreas mutation does not completely abolish Ptf1a function. Furthermore, the role of ptf1a in retinal cell fate decision prompted us to examine whether ptf1a might also have a similar function during pancreas development.

Like ptf1a mRNA expression, initial Tg(ptf1a:eGFP) expression in the presumptive ventral pancreas area of the endoderm occurs at ~32 hpf [hours post-fertilization] [data not shown]. At this stage, Tg(ptf1a:eGFP)-expressing cells are in a ventral endodermal domain, caudal to the liver bud. The homeodomain transcription factor Prox1 [Sosa-Pineda et al. 2000; Wang et al. 2005] is expressed in a continuous field of cells that comprises the liver bud and all Tg(ptf1a:eGFP)-expressing cells. These Prox1/Tg(ptf1a:eGFP)-coexpressing cells also express Pdx1 [data not shown], a homeodomain transcription factor involved in pancreas specification [Jonsson et al. 1994; Offield et al. 1996]. We utilized the Tg(ptf1a:eGFP) line to track cells that are expressing, or were recently expressing, ptf1a in wild-type and mutant embryos. At 38 hpf, Prox1 and the homeodomain transcription factor Nkx6.1 [Oster et al. 1998] are expressed in all Tg(ptf1a:eGFP)-expressing cells in wild type [Fig. 2A,B]. In contrast, the expression of Prox1 and Nkx6.1 in akreas mutants is markedly reduced or undetectable in Tg(ptf1a:eGFP)-expressing cells, but appears unaffected in the liver [Prox1] or dorsal pancreas [Prox1 and Nkx6.1] [Figure 2C,D]. Pdx1 expression appears to be unaffected in akreas mutants [data not shown]. These results indicate that ptf1a is required for the expression of the early pancreatic markers, Prox1 and Nkx6.1, thereby identifying novel targets of Pdt1a. However, by 46 hpf akreas mutants express Prox1 and Nkx6.1 in Tg(ptf1a:eGFP)-expressing cells of the ventral pancreatic bud [Fig. 2E–H], suggesting that ventral pancreas specification does occur.
in akreas mutants, although at a later stage. Consistent with these data, we observed that although the ventral pancreas has budded and formed a prominent outgrowth by 42 hpf in wild type, no ventral pancreatic bud has formed in the mutant siblings [Fig. 2I]. By 44 hpf, a small ventral pancreatic bud can be found in akreas mutants [Fig. 2K,L], further supporting the model that ventral pancreas specification is delayed in akreas mutants. This delay in budding and specification of the ventral pancreas in akreas mutants may be explained by the fact that the 1142N Ptf1a protein retains some function: As mutant Ptf1a proteins accumulate in akreas mutant endodermal cells, Ptf1a function becomes sufficient to allow specification and budding of the ventral pancreas. By 80 hpf, a ventral pancreatic outgrowth is evident in akreas mutants, and it can fuse with the dorsal pancreas [Fig. 2M,N]. At this stage in wild type, Nkx6.1 expression is found primarily in cells of the extrapancreatic duct (EPD) and intrapancreatic duct (IPD). In akreas mutants, whereas the IPD domain of Nkx6.1 expression appears to be absent, the EPD domain is evident. Cadherin expression at the apical/lateral membranes of the EPD cells also appears to be unaffected in akreas mutants [Fig. 2O,P], indicating that the EPD does form. Together, these findings show that the ventral pancreas is specified in akreas mutants but that exocrine cells fail to develop. Therefore, Ptf1a is required for exocrine fate in addition to its role in ventral pancreas specification.

The ventral pancreas is specified in akreas mutants, thereby providing a unique opportunity to explore other potential roles of ptf1a during later stages of pancreas development. As the pancreas develops, ptf1a expression becomes more restricted to the exocrine cells. Single-cell expression profiling data have suggested that Ptf1a and Isl1 expression are mutually exclusive in the mouse pancreas [Chiang and Melton 2003]. Similarly, we found that Tg(ptf1a:eGFP) and Isl1 expression are almost completely mutually exclusive in the fish pancreas [Fig. 3A]: We found between zero and three Isl1/Tg(ptf1a:eGFP)-coexpressing cells per developing wild-type pancreas \(n > 50, 48–80\) hpf. Importantly, in all cells where Isl1 and Tg(ptf1a:eGFP) are coexpressed, Tg(ptf1a:eGFP) expression is substantially lower than in all other Tg(ptf1a:eGFP)-expressing cells [Fig. 3C,D], suggesting that these endocrine cells have arisen from cells that express, or were recently expressing, low levels of ptf1a. These double-positive cells may represent endocrine cells at early differentiation stages, since more mature endocrine cells never coexpress Tg(ptf1a:eGFP) in wild type [data not shown]. In akreas mutants, there is a significant increase in the number of Isl1/Tg(ptf1a:eGFP)-coexpressing cells \([5\text{ to } 12\text{ cells at } 80\text{ hpf}]\) [Fig. 3E–H] further suggesting that Ptf1a functions to repress Isl1 expression in the developing pancreas. Interestingly, in akreas mutants, the Tg(ptf1a:eGFP)/Isl1-coexpressing cells have relatively higher Tg(ptf1a:eGFP) intensity than the cells not coexpressing Isl1 [Fig. 3E–H]. We postulate that these high level Tg(ptf1a:eGFP)-expressing cells in akreas mutants represent cells originally fated to become exocrine. Therefore, in akreas mutants, the loss of exocrine cells and the gain of endocrine cells expressing Tg(ptf1a:eGFP) may suggest an exocrine to endocrine fate change. Furthermore, whereas Tg(ptf1a:eGFP) expression was never observed in more differentiated endocrine cells as marked by the expression of Insulin (Ins), Glucagon (Glu), and Somatostatin (Sst) in wild type, such double-positive cells were found in akreas mutants [Fig. 3I,J; data not shown]. Expression of Tg(ptf1a:eGFP) in these Isl1, Ins, Glu, and Sst-positive cells indicates that they are derived from the ventral pancreas since the dorsal pancreas does not express Tg(ptf1a:eGFP) in wild type or mutant [Lin et al. 2004; Zecchin et al. 2004; data not shown]. Altogether, these data suggest that Ptf1a is involved in regulating pancreatic endocrine and exocrine fates, in addition to its known roles in pancreas specification and exocrine differentiation.

In akreas mutants, the Isl1/Tg(ptf1a:eGFP)-coexpressing cells are mostly found in the area of the distal EPD, where Isl1-positive cells are often located in wild type. However, Tg(ptf1a:eGFP)-positive cells that are mislocalized along the rest of the EPD in akreas mutants do not express Isl1. It may be that the Tg(ptf1a:eGFP)-expressing cells in akreas mutant EPDs maintain their multipotent progenitor state, and that the cells located in the distal EPD region are instructed to become endocrine cells, as in wild type. Ptf1a levels may also be in-
is down-regulated. We hypothesize that new endocrine

Figure 3. Ptf1a represses endocrine development. [A–H, K, L] Tg(ptf1a:eGFP) [green] expression in wild-type [A, C, D, F] and akreas mutant [B, E–H, J] embryos stained for Isl1 [red] and pan-Cadherin [blue] show mutually exclusive expression of Tg(ptf1a:eGFP) and Isl1 in wild-type (except in a single cell; arrow) but not in akreas mutants at 50 hpf [A, B] and 80 hpf [C–F]. (D) Tg(ptf1a:eGFP) intensity of C showing the single cell [arrow] with relatively low Tg(ptf1a:eGFP) expression. Note that most Tg(ptf1a:eGFP)/Isl1-coexpressing cells in akreas mutants are found at the distal EPD [E–H, arrows] where the Tg(ptf1a:eGFP) intensity is relatively high [H, GFP intensity of E] [shown at higher magnification in Supplemental Fig. S3A–F]. (I, L) Tg(ptf1a:eGFP) [green] expression in 80 hpf wild-type [J] and akreas mutants [I] stained for Isl1 [red] and insulin [blue] show mutually exclusive expression of Tg(ptf1a:eGFP) and insulin in wild-type and coexpression in akreas mutants [arrows]. (K, L) At 140 hpf, Isl1 expression is no longer detectable in the principal endocrine cluster in wild-type (K), suggesting that Isl1 expression is lost from more mature endocrine cells. However, in akreas mutants [L], Isl1-positive cells, some of which coexpress Tg(ptf1a:eGFP) [arrows], are present, suggesting that endocrine neogenesis continues in akreas mutants. Asterisks mark the EPD.

volved in regulating this endocrine fate decision. As indicated above, in wild type, the Tg(ptf1a:eGFP)/Isl1-coexpressing cells have relatively low Tg(ptf1a:eGFP) intensity, whereas the Tg(ptf1a:eGFP)/Isl1-coexpressing cells in akreas mutants have relatively higher Tg(ptf1a:eGFP) intensity. These data suggest that in the specified pancreas, cells with relatively low levels of wild-type Ptf1a, or high levels of mutant Ptf1a, preferentially differentiate into endocrine cells, whereas cells that do not express wild-type Ptf1a or have low levels of mutant Ptf1a remain in a progenitor state. Furthermore, we also observed that in contrast to wild type, akreas mutants continue to express Isl1 even as late as 140 hpf [Fig. 3K, L]. After 100 hpf, little to no Isl1 expression can be detected in the wild-type pancreatic endocrine cluster, suggesting that as endocrine cells differentiate, Isl1 is down-regulated. We hypothesize that new endocrine
cells continue to appear in akreas mutants as more Tg(ptf1a:eGFP)-positive cells accumulate sufficient levels of mutant Ptf1a protein.

If a certain level of Ptf1a function is involved in promoting endocrine fate, a level of Ptf1a higher than that present in akreas mutants should lead to an increase in the number of Tg(ptf1a:eGFP)/Isl1-coexpressing cells along the EPD. To test this hypothesis, we knocked down Ptf1a expression to various levels by injecting wild-type embryos with different quantities of ptf1aMO1. Wild-type embryos injected with 4 ng or more of ptf1aMO1 exhibit a pancreas phenotype similar to akreas mutants. Specifically, we found between five and 12 Tg(ptf1a:eGFP)/Isl1-coexpressing cells, typically localized in, or distal to, the distal EPD [n > 20] [data not shown]. Importantly, a milder knockdown of Ptf1a expression via injecting 2 ng of ptf1aMO1 led to an extensive coexpression of Tg(ptf1a:eGFP) and Isl1 along the entire EPD [15–30 cells, n > 20] [Fig. 4A–I]. These results are consistent with the hypothesis that Tg(ptf1a:eGFP)-expressing cells in the EPD of akreas mutants do not adopt an endocrine fate because of insufficient levels of Ptf1a function.

Furthermore, in embryos injected with 2 ng of ptf1aMO1, as with akreas mutants, all Tg(ptf1a:eGFP)-positive cells distal to the EPD coexpress Isl1, indicating a lack of exocrine cells. This phenotype is opposite to what is observed in wild type, or embryos injected with 0.5 ng of ptf1aMO1, where most Tg(ptf1a:eGFP)-positive cells distal to the EPD do not coexpress Isl1 [n > 20] [Fig. 4K–L]. These cells that do not coexpress Isl1 always have the highest intensity of Tg(ptf1a:eGFP) relative to all other pancreatic cells and are also Nkx6.1-negative [data not shown], indicating that they are neither endocrine nor duct, but more likely exocrine. These data [schematized in Supplemental Fig. S4] suggest that high levels of Ptf1a function are required for the exocrine fate.

By examining Tg(ptf1a:eGFP) expression in embryos injected with a morpholino [ptf1aMO2] designed to knock down both Ptf1a and Tg(ptf1a:eGFP) expression [Supplemental Fig. S1C], we can evaluate the relative level of Ptf1a knockdown in individual cells. Thus, cells with relatively high Tg(ptf1a:eGFP) intensity have, or recently had, relatively high Ptf1a expression, whereas cells with relatively low Tg(ptf1a:eGFP) intensity have, or recently had, relatively low Ptf1a expression. In embryos injected with ptf1aMO2, we found that pancreatic cells with very high Tg(ptf1a:eGFP) intensity never coexpressed Isl1, whereas cells with relatively low Tg(ptf1a:eGFP) intensity have, or recently had, relatively low Ptf1a expression. In embryos injected with ptf1aMO1, all Tg(ptf1a:eGFP)-positive cells distal to the EPD coexpress Isl1 [n > 20] (Fig. 4A–I). These results further support the model that a lower level of Ptf1a can function, in a cell-autonomous manner, to promote endocrine fate, whereas high levels repress it.

Thus, we found that pancreatic cells with partial Ptf1a function do not assume an exocrine fate, but maintain their pancreatic progenitor fate, and, with sufficient levels of Ptf1a, adopt an endocrine fate. Therefore, in addition to its role in pancreas specification and exocrine cell differentiation, ptf1a also appears to function in the endocrine versus exocrine fate decision: at low levels promoting endocrine fates, and at high levels repressing endocrine and promoting exocrine fates. We propose that modulation of Ptf1a levels is involved in the endocrine versus exocrine fate decision during pancreas development. It was recently shown that the shift in role of Ptf1a
Ptf1a regulates pancreatic cell fates

Figure 4. Low levels of Ptf1a expression promote, whereas high levels repress, endocrine fate. (A–L) Tg(pft1a:eGFP) expression (green) in control wild type (A–C) and ptf1aMO1 injected wild type (D–I) stained for Isl1 (red) and pan-Cadherin (blue). (A) Three-dimensional rendering of a 56 hpf control. (B, C) Z-focal plane of A at 2× magnification revealing mostly mutually exclusive expression of Tg(pft1a:eGFP) and Isl1 in control with the exception of a single cell (arrows, red and blue channels in C). (D) Three-dimensional rendering of a 2 ng ptf1aMO1 injected wild type at 56 hpf. (E, F) Magnification (2×) of D shows ectopic Isl1-expressing cells all along the EPD (arrows, red and blue channels of E shown in F). (G, H) Two different Z-focal planes of E revealing extensive coexpression of Tg(pft1a:eGFP) and Isl1 along the entire EPD (arrows, red and blue channels of H shown in I). (I) Three-dimensional rendering of a wild-type embryo injected with 0.5 ng of ptf1aMO1 at 76 hpf. (J) Z-focal plane of J showing a cluster of high level Tg(pft1a:eGFP)-expressing cells that do not express Isl1 (arrowheads). (K) Tg(pft1a:eGFP) intensity of K showing relatively high GFP expression in a cluster of cells that do not express Isl1 (arrowheads). (L) Tg(pft1a:eGFP) intensity of L showing relatively low levels of Tg(pft1a:eGFP) expression (arrowheads) do not express Isl1. (P) Three-dimensional rendering of ptf1aMO2 [which knocks down both Ptf1a and Tg(pft1a:eGFP) expression] injected wild type at 76 hpf (M, green channel shown in N). (O–R) Z-focal plane through M showing that cells with higher levels of Tg(pft1a:eGFP) expression (arrowheads) do not express Isl1. (P) Shows red and blue and (Q) shows green channels of O. Cells with relatively low levels of Tg(pft1a:eGFP) expression (arrows) coexpress Isl1 (shown at higher magnification in Supplemental Fig. S3G–I). (R) Tg(pft1a:eGFP) intensity of O.

from pancreas specification to exocrine differentiation involves a change of cofactors from Rbpj to Rbpj [Masui et al. 2007]. Because high levels of Ptf1a appear to be critical in promoting exocrine and repressing endocrine fates, this shift in cofactors may require an increase in Ptf1a levels to form the Ptf1a/Rbpj complex.

The role of Notch signaling in repressing differentiation has been linked to the maintenance of epithelial stem/progenitors cells [Blanpain et al. 2007]. In the developing pancreas, inactivation of Notch signaling leads to the excessive differentiation of endocrine cells [Apelqvist et al. 1999; Jensen et al. 2000]. Intriguingly, it was also reported that Notch signaling represses exocrine differentiation, and that targets of Notch signaling directly inhibit Ptf1a activity [Eski et al. 2004]. Therefore, Notch signaling may be regulating pancreatic progenitor differentiation through interactions with Ptf1a.

Down-regulation of Ptf1a in exocrine cell culture was shown to disrupt the exocrine differentiated state [Krap et al. 1996]. In addition, it was shown that adult exocrine cells can transdifferentiate into endocrine cells [Minami et al. 2005]. Based on our findings, we postulate that a down-regulation of Ptf1a may regulate this exocrine to endocrine switch in the mature pancreas. According to this model, down-regulation of Ptf1a may represent a new approach toward coaxing adult exocrine cells to transdifferentiate into endocrine cells.

Materials and methods

Zebrafish strains and immunohistochemistry

Fish were raised and maintained under standard laboratory conditions. We used the following mutant and transgenic lines: aktaes (aktn1), Tg(gutGFPfluorescd) [Field et al. 2003a], Tg(elastase:GFP), Tg(lapb:duRed) [Wan et al. 2006], and Tg(pft1a:eGFP) [Godinho et al. 2005]. Immunohistochemistry was performed as described previously [Dong et al. 2007; see the Supplemental Material].

Ptf1a functional assay

Information detailing the generation of constructs, the luciferase assay, and the EMSA is provided in the Supplemental Material.

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References

Ahlgren, U., Pfaff, S.L., Jessell, T.M., Edlund, T., and Edlund, H. 1997. Independent requirement for ISL1 in formation of pancreatic mesenchyme and islet cells. Nature 385: 257–260.

Apelqvist, A., Li, H., Sommer, L., Beatus, P., Anderson, D.J., Honjo, T., Hrabe de Angelis, M., Lendahl, U., and Edlund, H. 1999. Notch signalling controls pancreatic cell differentiation. Nature 400: 877–881.

Bertuzzi, F., Marzorati, S., and Secchi, A. 2006. Islet cell transplantation. Curr. Mol. Med. 6: 369–374.

Blanpain, C., Horsley, V., and Fuchs, E. 2007. Epithelial stem cells: Turning over new leaves. Cell 129: 445–458.

Chiang, M.K. and Melton, D.A. 2003. Single-cell transcript analysis of pancreatic cell differentiation. Dev. Cell 4: 383–393.

Cockrell, M., Stevenson, B.J., Strubin, M., Hagenbuchle, O., and Wellauer, P.K. 1989. Identification of a cell-specific DNA-binding activity which interacts with a transcriptional activator of genes expressed in the acinar pancreas. Mol. Cell. Biol. 9: 2464–2476.

Donath, M.Y. and Halban, P.A. 2004. Decreased β-cell mass in diabetes: Significance, mechanisms and therapeutic implications. Diabetologia 47: 581–589.

Dong, P.D.S., Munson, C.A., Norton, W., Cossier, C., Pan, X., Gong, Z., Neumann, C.J., and Stainier, D.Y.R. 2007. Fgf10 regulates hepatopancreatic ductal system patterning and differentiation. Nat. Genet. 39: 397–402.
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Esni, F., Ghosh, B., Biankin, A.V., Lin, J.W., Albert, M.A., Yu, X., Mac-Donald, R.J., Cavin, C.I., Real, F.X., Pack, M.A., et al. 2004. Notch inhibits Ptf1 function and acinar cell differentiation in developing mouse and zebrafish pancreas. Development 131: 4213–4224.

Field, H.A., Ober, E.A., Roesser, T., and Stainier, D.Y.R. 2003a. Formation of the digestive system in zebrafish. I. Liver morphogenesis. Dev. Biol. 253: 279–290.

Field, H.A., Dong, P.D.S., Beis, D., and Stainier, D.Y.R. 2003b. Formation of the digestive system in zebrafish. II. Pancreas morphogenesis. Dev. Biol. 261: 197–208.

Fischer, A.J., Diercks, B.D., and Reh, T.A. 2002. Exogenous growth factors induce the production of ganglion cells at the retinal margin. Development 129: 2283–2291.

Fujitani, Y., Fujitani, S., Luo, H., Qiu, F., Burlison, J., Long, Q., Kawagu-chi, Y., Edlund, H., MacDonald, R.J., Furukawa, T., et al. 2006. Ptf1a determines horizontal and amacrine cell fates during mouse retinal development. Development 133: 4439–4450.

Godinho, L., Mummm, J.S., Williams, P.D., Schroeter, E.H., Koerber, A., Park, S.W., Leach, S.D., and Wong, R.O.L. 2005. Targeting of amacrine cell neurites to appropriate synaptic laminae in the developing zebrafish retina. Development 132: 5069–5079.

Gradwohl, G., Dierich, A., LeMeur, M., and Guillemot, F. 2000. Neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. Proc. Natl. Acad. Sci. 97: 1607–1611.

Jensen, J. 2004. Gene regulatory factors in pancreatic development. Dev. Dyn. 229: 176–200.

Jensen, J., Pedersen, E.E., Galante, P., Hald, J., Heller, R.S., Ishibashi, M., Kageyama, R., Guillemot, F., Serup, P., and Madsen, O.D. 2000. Control of endodermal endocrine development by Hes-1. Nat. Genet. 24: 36–44.

Jonsson, J., Carlsson, L., Edlund, T., and Edlund, H. 1994. Insulin-promoter-factor1 is required for pancreas development in mice. Nature 371: 606–609.

Kawaguchi, Y., Cooper, B., Cannon, M., Ray, M., MacDonald, R.J., and Wright, C.V.E. 2002. The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. Nat. Genet. 32: 128–134.

Krapp, A., Knofler, M., Frutigerl, S., Hughes, G.J., Hagenbuchle, O., and Wellauer, P.K. 1996. The p48 DNA-binding subunit of transcription factor PTF1 is a new exocrine pancreas-specific basic helix-loop-helix protein. EMBO J. 15: 4317–4329.

Krapp, A., Knofler, M., Ledermann, B., Burki, K., Berney, C., Zoerkler, N., Hagenbuchle, O., and Wellauer, P.K. 1998. The HLH protein PTF1-p48 is essential for the formation of the exocrine and the correct spatial organization of the endocrine pancreas. Genes & Dev. 12: 3753–3763.

Lin, J.W., Riankin, A.V., Horb, M.E., Ghosh, B., Prasad, N.B., Yee, N.S., Pack, M.A., and Leach, S.D. 2004. Differential requirement for ptf1a in endocrine and exocrine lineages of developing zebrafish pancreas. Dev. Biol. 274: 491–503.

Masui, T., Long, Q., Beres, T.M., Magnuson, M.A., and MacDonald, R.J. 2007. Early pancreatic development requires the vertebrate Suppressor of Hairless (RBPJ) in the PTF1 bHLH complex. Genes & Dev. 21: 2629–2643.

Minami, K., Okuno, M., Miyawaki, K., Okumachi, A., Ishizaki, K., Oyama, K., Kawaguchi, M., Ishizuka, N., Iwanaga, T., and Seino, S. 2005. Lineage tracing and characterization of insulin-secreting cells generated from adult pancreatic acinar cells. Proc. Natl. Acad. Sci. 102: 15116–15121.

Nakahai, H., Sel, S., Favor, J., Mendoza-Torres, L., Paulsen, F., Duncker, G.I.W., and Schmid, R.M. 2007. Ptf1a is essential for the differentiation of GABAergic and glycinergic amacrine cells and horizontal cells in the mouse retina. Development 134: 1151–1160.

Ober, E.A., Verkade, H., Field, H.A., and Stainier, D.Y.R. 2006. Mesodermal Wnt2b signalling positively regulates livers specification. Nature 442: 688–691.

Offield, M.F., Jetton, T.L., Labosky, P.A., Ray, M., Stein, R.W., Magnuson, M.A., Hogan, B.L., and Wright, C.V. 1996. PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. Development 122: 983–995.

Oster, A., Jensen, J., Serup, P., Galante, P., Madsen, O.D., and Larsson, L.I. 1998. Rat endocrine pancreatic development in relation to two homeobox gene products (Pdx-1 and Nkx6.1). J. Immunohistochem.
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