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REVIEW

Transcription factor Ptf1a in development, diseases and reprogramming

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Abstract

The transcription factor Ptf1a is a crucial helix–loop–helix (bHLH) protein selectively expressed in the pancreas, retina, spinal cord, brain, and enteric nervous system. Ptf1a is preferably assembled into a transcription trimeric complex PTF1 with an E protein and Rbpj (or Rbpjl). In pancreatic development, Ptf1a is indispensable in controlling the expansion of multipotent progenitor cells as well as the specification and maintenance of the acinar cells. In neural tissues, Ptf1a is transiently expressed in the post-mitotic cells and specifies the inhibitory neuronal cell fates, mostly mediated by downstream genes such as Tfap2a/b and Prdm13. Mutations in the coding and non-coding regulatory sequences resulting in Ptf1a gain- or loss-of-function are associated with genetic diseases such as pancreatic and cerebellar agenesis in the rodent and human. Surprisingly, Ptf1a alone is sufficient to reprogram mouse or human fibroblasts into tripotential neural stem cells. Its pleiotropic functions in many biological processes remain to be deciphered in the future.

Keywords Pancreatic development · Retinal development · Transcriptional regulation · Cell fate specification · Acinar cells · Inhibitory neurotransmitter · GABAergic · Glycinergic · Glutamatergic · Diabetes · Somatic cell reprogramming · Inheritable

Introduction

A helix–loop–helix (HLH) protein structure motif was first described by Murre and colleagues in 1989 [1]. The typical HLH motif is characterized by a loop connecting two α-helices, among which the larger helix contains the basic amino acid residues that can bind DNA in the major groove, and the smaller helix allows dimerization by folding and packing against another helix. The HLH transcription factors can form homodimers or heterodimers, and bind to a consensus DNA sequence motif called E-box, CANNTG (N: A/C/T/G) [1, 2], or an E-box-like sequence, CAYRMK (Y: C/T; R: A/G; M: A/C; K: G/T) [3].

More than 240 HLH proteins have been identified and can be categorized into seven groups based on their tissue distributions, DNA-binding specificities, and dimerization capacities [4, 5]. Notably, the class I HLH members (E proteins) are ubiquitously expressed in almost all tissues. They assemble into heterodimers or homodimers and bind to the canonical E-box only. The class I members include E12 and E47 (alternatively spliced E2A, or TCF3), E2-2 (TCF4) and HEB (TCF12) in the vertebrates. The class II members are distributed in certain tissues specifically. Most of them cannot form homodimers, but preferably form heterodimers with class I members and bind both E-box and E-box-like sequences. The majority of HLH proteins belong to class II. The class V members, including inhibitors of DNA-binding (ID), lack the basic DNA-binding region. The ID proteins are usually low in mature cells but abundant in proliferating or differentiating cells. They are capable of forming heterodimers with E proteins or class II proteins, but unable to bind DNA and hence act as negative regulators of transcription [6, 7]. Other HLH classes will not be discussed here. The variations in E-box and E-box-like sequences and diversities in homodimers and heterodimers provide many possibilities in transcriptional control. The HLH proteins represent one of the most important transcription factor

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families regulating cell proliferation, specification and fate determination in development.

The pancreas-specific transcription factor 1a, Ptf1a (alias: bHLHa29, PTF1-p48, Ptf1p48, p48 DNA-binding subunit of transcription factor PTF1), is a class II HLH member. Ptf1a was named after the transcription factor PTF1 [8], which is a transcription complex composed of three distinct subunits, p75, p64 and Ptf1-p48. The p75 subunit was later identified as the HEB protein, and could be replaced by E47 in a small fraction of the PTF1 complexes [9]. HEB or E47 forms a HLH heterodimer with Ptf1a and together bind to the E-box [9]. The p64 subunit turns out to be Rbpj (CBF1/Rbpjκ/Su(H)/Lag-1, CSL) [10] that binds the TC-box (TTTCCC) consensus sequences [11]. In mature acinar cells, Rbpj is replaced by Rbpjl in the complex [12, 13]. Simultaneous binding to the bipartite cognate sites (E-box and TC-box) is required for PTF1 transcriptional activity.

Accumulating data indicate that Ptf1a plays a critical role in controlling development and physiological function of many organs, including pancreas, brain, spinal cord, retina and others. We will give a deliberate review about the past findings and the important advances.

Genomic, RNA and protein structures of Ptf1a

The evolution history of Ptf1a gene can at least be traced back to as early as sea urchin [14]. The sea urchin orthologue of Ptf1a is expressed in a unique population of cells in the embryonic stomach. These cells co-express exocrine cell markers and resemble vertebrate pancreatic exocrine cells in genetic regulatory network [14]. Though the function and role of Ptf1a seem to be evolutionarily preserved, its genomic sequences vary highly in many species and are not much conserved when compared with the extent of conservation of ancient transcription factor genes such as the Hox family. Typical Ptf1a genomic structure is composed of two exons and one intron as shown in the mouse genome (Fig. 1a). Alignment of 60 vertebrate Ptf1a genome sequences revealed several highly conserved regions, including the 5′ promoter region, the bHLH domain region in exon 1, the exon 2 coding region, and the 3′ untranslated region (UTR) (Fig. 1a). The conserved 5′ promoter region is very close to the transcription start site (TSS) and contains transcription factor binding sites that are critical for the regulation of transcription initiation. Hundreds of potential transcription factor binding sites within 500 bp upstream of the mouse Ptf1a TSS site are predicted by PROMO (v3) with a dissimilarity margin less or equal to 15%. It must be emphasized here that the 3′ regulatory regions are extremely important for Ptf1a transcription. One 3′ conserved enhancer region, about 400 bp located at 25 kb downstream of the coding sequence, is critical for the transcription of Ptf1a in pancreatic development. Mutations in this region are associated with pancreas agenesis and diabetes [15, 16]. Another 3′ conserved enhancer region, located at 10.8 kb downstream of the coding sequence, is necessary for tissue-specific expression of Ptf1a in the dorsal neural tube [17].

The 3′ UTR region of many genes contains various sequences including microRNA response elements (MREs), AU-rich elements (AREs), and the poly(A) tail that are crucial in gene expression regulation by influencing the localization, stability, and translation efficiency of an mRNA. The 3′ UTR in the mouse Ptf1a mRNA is 335-base long and contains a binding site for RNA-binding protein Rbms3, which indirectly affects pancreas development by regulating the translation efficiency of Ptf1a protein [18]. Scanning of Ptf1a 3′ UTR sequence with TargetScan identifies many conserved miRNA targeting sites, whose biological significance is yet to be revealed.

A typical Ptf1a protein has a bHLH domain in the middle as shown in the mouse Ptf1a which is composed of 324 amino acid residues (Fig. 1b). In the mouse Ptf1a (Fig. 1b, c), there are two conserved tryptophan residues (W280 and W298 in the C1 and C2 regions, respectively) important for interaction with Rbpj/Rbpjl [10], and three serine residues (S154, S175, S262) for possible phosphorylation (predicted by GPS3.0, http://gps.biocuckoo.org) and regulation of Ptf1a activity. The lysine residue K310 (K312 in human) has been shown to be required for E3 ubiquitin ligase TRIP12 (thyroid hormone receptor-interacting protein 12) that targets Ptf1a for ubiquitination and proteasomal degradation [19]. From zebrafish, Xenopus, mouse, rat, dog, and cow to human, alignment of the seven vertebrate Ptf1a amino acid sequences indicates that the phylogenetic tree of Ptf1a is in accordance with the evolutionary history (Fig. 1d). The alignment also reveals a couple of conserved regions, including the bHLH domain and a region in the C terminus (Fig. 1c), implying that these regions are important for Ptf1a functions. Notably, the amino acid sequences of the DNA-binding domain (the basic region plus the first helix, or the larger helix) remain unchanged in the seven species. Except for the dog, all species share exactly the same sequences in the bHLH domain. Interestingly, the dog has more evolutionary changes in Ptf1a conserved domains than any other listed mammals (Fig. 1c), mostly attributing to the accelerated process with artificial selections [20]. This raises a plausible question whether the dog Ptf1a is more adaptive than other species' and hints at the future direction of Ptf1a evolution.

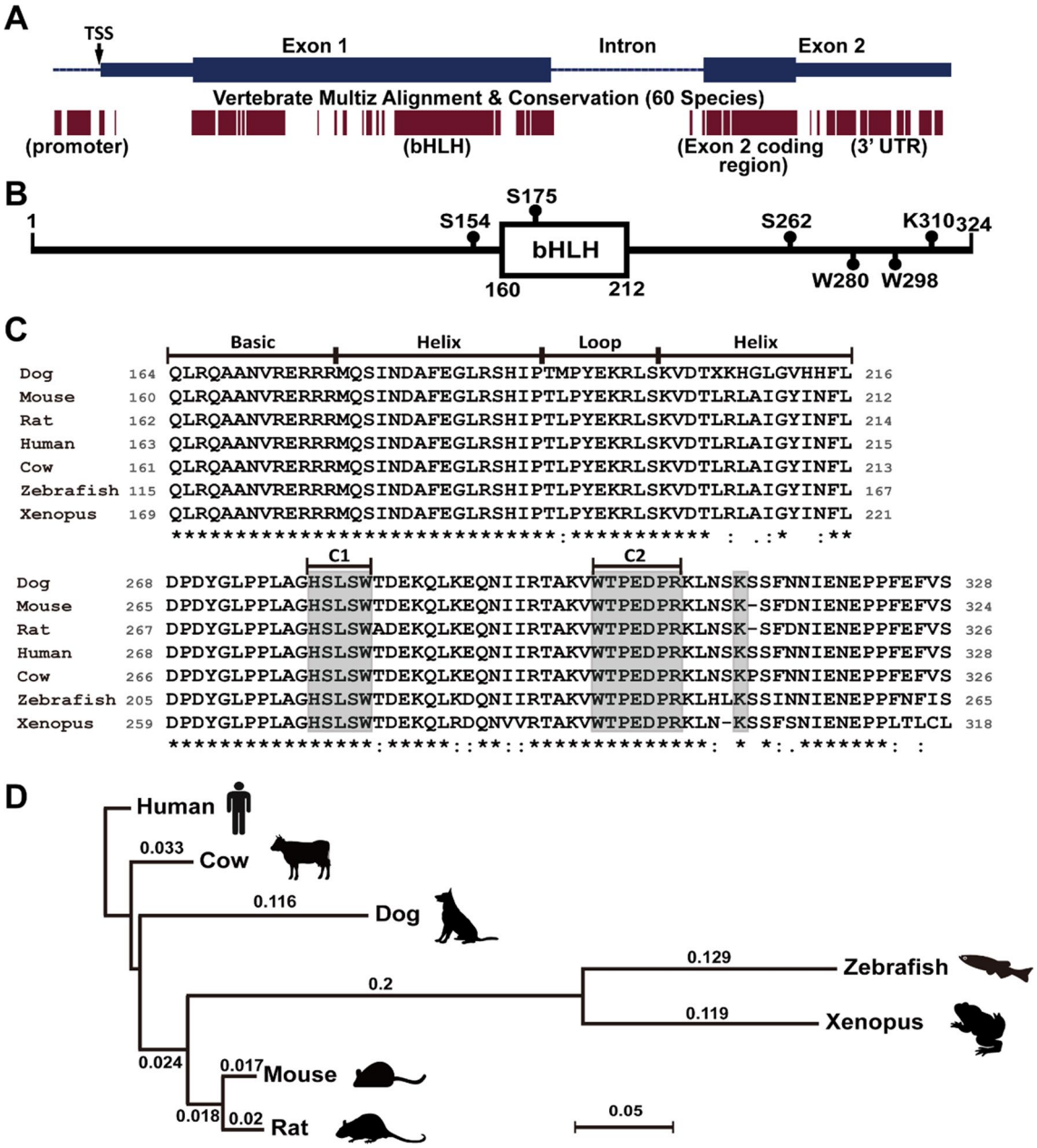


Fig. 1 Genomic structure and protein sequences of Ptf1a. **a** The upper portion of the image shows the genomic structure of the mouse *Ptf1a* gene. The blue bars represent two exons; the heightened parts are coding regions for Ptf1a. TSS, transcription start site. The lower portion of the image shows the corresponding multiz alignment and conservation of *Ptf1a* DNA sequences in 60 vertebrate species by UCSC genome browser. **b** The mouse Ptf1a protein is composed of 324 amino acid residues. The bHLH domain and several important residues are shown. **c** Multiple alignment of amino acid residues of the bHLH domains (upper image) and C termini (lower image) of Ptf1a proteins from seven vertebrate species. All sequences were downloaded from NCBI. The gray boxes in the C-terminal region (C1 and C2) outline two conserved regions important for interaction with Rbpj or Rbpjl protein, and one conserved lysine residue required for ubiquitination and proteasome degradation. **d** The phylogenetic tree for seven vertebrate Ptf1a proteins was constructed by the neighbor-joining method. The distance was calculated with Poisson correction. Gaps were distributed proportionally. The scale bar represents a distance of 0.05

Rbpj-dependent and -independent activity of Ptf1a

Literally, the major activity of a transcription factor is transcription regulation, which usually requires it to bind to a consensus site in the chromosome. As a bHLH domain member, Ptf1a assembles into a heterodimer with another HLH factor (preferably with an E protein) and binds to

the E-box or E-box-like consensus through its larger helix (Fig. 2a, b). Depending on whether Rbpj is present in the complex, the transcription activity of Ptf1a is categorized to be Rbpj-dependent or -independent.

In most occasions, Ptf1a is associated with Rbpj (or Rbpjl) in the PTF1 complex, namely in a Rbpj-dependent manner (Fig. 2c). Fox example, in acinar development, the PTF1 complex binds a bipartite motif containing an E box and a TC box separated by preferential spacing of one, two or three helical turns of DNA [10, 21–24]. During acinar maturation and adulthood, Rbpj is replaced by Rbpjl in the PTF1 complex to maintain exocrine expression [12]. Motif analysis of the data from ChIP-seq experiments with Ptf1a and Rbpj antibodies indicates that more than 70% Ptf1a is colocalized with Rbpj/Rbpjl in the pancreas [23]. If peak calling is less stringent and the low-affinity binding events are considered, the overlap of Ptf1a and Rbpj/Rbpjl may approach 100% in the pancreas. In neural tube development, however, ChIP-seq and motif analyses reveal that the PTF1 trimer occupies barely more than 25% of peak calling events, indicating that the majority of Ptf1a protein functions independently of PTF1 complex. Further analysis shows that Rbpj-independent Ptf1a likely activates transcription in combination with Sox, Hox, Forkhead, GATA, and homeodomain family members [23], since the Ptf1a and E-protein heterodimer alone is relatively weak in activating transcription in vitro [9, 10, 25]. There are still many unanswered questions concerning Rbpj-dependent and -independent mechanisms. For example, can both mechanisms exist in the same cell? How do they coordinate with each other in cells and tissues?

Furthermore, Ptf1a represents an important component antagonizing Notch signaling [26–28]. Upon binding with its ligands (Serrate, Delta, Jagged, etc.), Notch releases its intracellular domain (NICD) through sequential proteolysis by ADAM and γ-secretase, which then translocates into the nucleus, interacts with transcription factor Rbpj (Fig. 2d), and activates the transcription of downstream genes such as Hes1 and Hes5. Beres et al. found that the C1 and C2 regions of Ptf1a, especially the tryptophan residues in the two regions, are absolutely required for cross-interaction with Rbpj [10]. NICD interacts with Rbpj through a tryptophan-containing hydrophobic motif φWφP (φ represents a hydrophobic residue) [29]. The Ptf1a C2 motif and NICD φWφP motif are structurally similar and compete to bind exclusively to the same site on Rbpj, which means that Ptf1a and NICD antagonize each other cell autonomously in a dose-dependent manner [10]. This has been demonstrated to be a very important mechanism for regulating cell fate specification during development of many tissues.

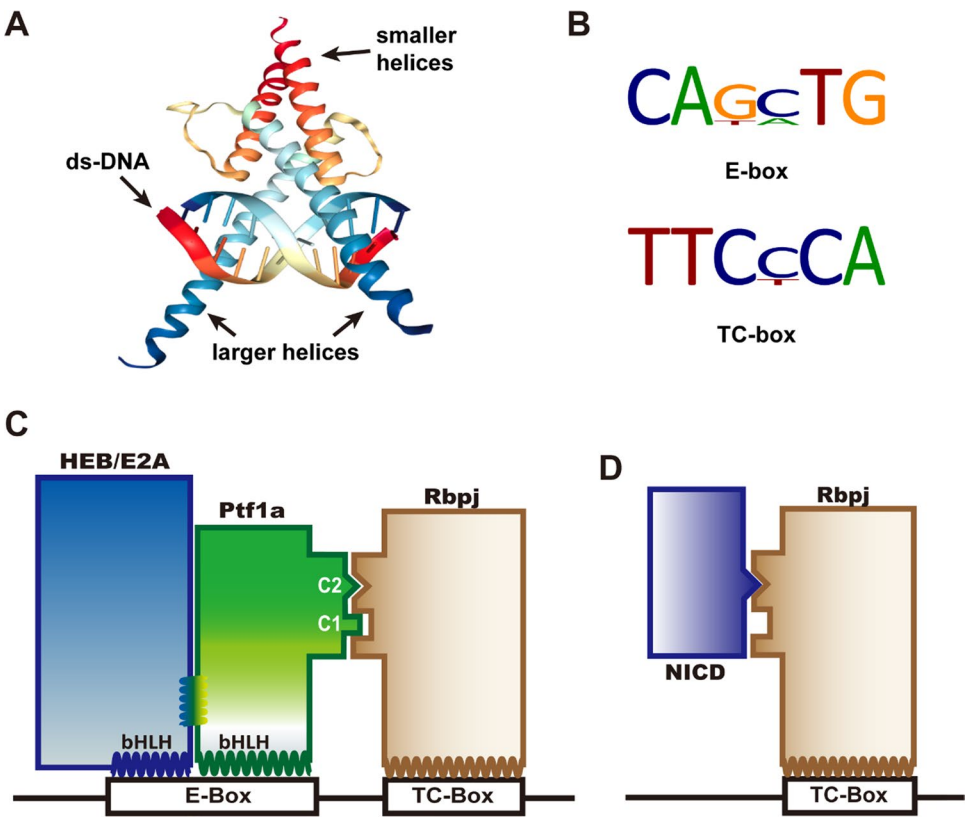


Fig. 2 DNA binding with Ptf1a and PTF1 complex. **a** The cartoon illustrates that two bHLH domains (from SCL-E47, PDB bank) bind to the major groove of DNA with their larger helices. The smaller helices fold and allow dimerization of the domains. **b** The common DNA binding consensus E-box and TC-box for the PTF1 complex. **c** Ptf1a and HEB (or E47, E12, isoforms of E2A) bind to the E-box, and Rbpj binds to the TC-box. Transcription activation requires that the PTF1 complex binds to E-box and TC-box simultaneously, which depends on Ptf1a interfacing with Rbpj via the conserved C1 and C2 regions at the C terminus. **d** The Notch intracellular domain (NICD) can compete with Ptf1a and E protein heterodimers for the binding sites on Rbpj protein

Ptf1a expression profiles

During vertebrate embryonic development, Ptf1a expression is dynamic and transient in most tissues. SAGE (serial analysis of gene expression) analysis of Ptf1a indicates that Ptf1a is expressed in the brain, retina, spinal cord, pancreas, heart, lung, liver, colon, thyroid, breast, lymph node, skin, placenta, and other major organs/tissues during development (https://cgap.nci.nih.gov/SAGE). RNA-seq data from GTEx show that Ptf1a is also expressed in the testis, ovary, stomach, small intestine, skeletal muscle, adipocyte, and other tissues (https://gtexportal.org).

In adult human tissues (data from ProteomicsDB), Ptf1a mRNA expression is much higher in pancreas, median FPKM (Fragments Per Kilobase Of Exon Per Million Fragments Mapped) value reaching 14, followed by stomach (FPKM 0.9), testis (FPKM 0.6), and cerebral cortex and prostate gland (FPKM 0.1 each). At the tissue level, Ptf1a protein abundance is highest in the ovary, median value reaching 4.52 (log10 normalized iBAQ intensity, the same below), followed by rectum (4.27), colon (4.13), and pancreas (3.35). It seems that transcription and translation of Ptf1a are not tightly coupled since the ratio of its mRNA and protein levels is not proportional among tissues. Based on the common features shared by the tissues expressing Ptf1a, an interesting question can be raised regarding whether there is a correlation between Ptf1a and cell exocrine. Notably, highest Ptf1a level is found in synovial fluid (7.18), reinforcing the idea that Ptf1a might participate in controlling synthesis and/or exocytosis of exosomes/microvesicles (including synaptic vesicles in neurons).

The expression pattern of Ptf1a, its role and associated mutant and overexpression phenotypes in mouse is listed (Table 1). Next, we will discuss these findings in detail, including the spatiotemporal expression profile of Ptf1a, its function, and its upstream and downstream signaling pathways during development of several tissues.

Ptf1a in pancreatic development and function

The pancreas has endocrine function to secrete insulin and glucagon which are antagonists and together regulate blood glucose homeostasis. The organ also serves as an exocrine unit to secrete amylase, elastase-1 and other important digestive enzymes. In origin, the pancreas develops from the endoderm at the foregut and midgut junction. Two separate pancreatic primordia, the dorsal and the ventral pancreas buds, arise from the junction and join together to form the pancreas.

Ptf1a RNA is detected at as early as 9 dpc (days post coitum) in the pancreas buds [25, 30, 31]. From 14 dpc and on, it seems that Ptf1a is expressed only in the exocrine part of pancreas [31]. In homozygous Ptf1a null mutant mice, acinar development was abolished, and some endocrine pancreatic cells were misallocated in the spleen [30]. Evidence from these early studies suggests that Ptf1a is a specific determinant for exocrine pancreatic cells only. However, lineage tracing studies with $Ptf1a^{Cre}$ or $Ptf1a^{CreERTM}$ and R26R mice revealed that most pancreatic multipotent progenitor cells (MPCs) express Ptf1a, and are capable of differentiating into acinar, ductal or endocrine cells [32, 33]. This result is confirmed in the zebrafish using a similar approach [34]. When Ptf1a activity is totally inactivated, most of the MPCs are converted into non-pancreatic cell fates, such as gut and gall bladder [32, 34]. Conversely, Ptf1a misexpression in embryonic mouse endoderm converted the entire glandular stomach, rostral duodenum and extrahepatic biliary system into pancreas [35]. These experiments demonstrate that Ptf1a is both necessary and sufficient for acquisition of pancreatic fate from foregut endoderm. Interestingly but not surprisingly, the exocrine versus endocrine cell fate depends on the dose effect of Ptf1a. Haploinsufficiency or low levels of Ptf1a will promote endocrine cell fates while repressing exocrine cell fates, and vice versa [34, 36, 37]. These findings underscore the distinct roles of Ptf1a in the specification and expansion of pancreatic progenitor cells.

How does Ptf1a participate in the expansion of early pancreatic progenitor cells? Ptf1a is not only involved in establishing the pancreatic identity in foregut cells, Ahnfelt-Rønne et al. found that it is also indispensable in the expansion of early pancreatic MPCs by controlling Dll1 expression [38]. Using *LacZ* as a reporter gene in $Dll1^{lacZ/+}$; $Ptf1a^{Cre/cre}$ mice, β-*gal* expression (viz. *Dll1* expression) was not detected in early pancreatic MPCs, indicating the requirement of *Ptf1a* in *Dll1* expression. ChIP analysis shows that Ptf1a and/or Rbpj can bind directly to the proximal promoter region of *Dll1*. Dll1 binds to the Notch receptor on the cell membrane of neighbor MPCs (Fig. 3a), leading to release of NICD, which activates Notch downstream target genes such as Hes1 that mediates MPC cell proliferation. Unexpectedly, Hes1 is also required for retaining Ptf1a protein level in the MPCs [38], owing to an unknown mechanism. It is speculated that the Hes1–Ptf1a protein–protein interaction helps to stabilize the Ptf1a protein [39]. In summary, the Ptf1a and Notch pathway constitutes a positive feed-forward loop to promote early MPC proliferation and expansion in a non-cell autonomous manner (Fig. 3a). Meanwhile, another mechanism may also participate in MPC expansion. Two groups found that Ptf1a could bind to the conserved promoter of *Pdx1* and activate its expression in early MPCs [40, 41]. This effect could be synergistically enhanced by Foxa1/2 [23, 42].

Table 1 Expression profile of *Ptf1a* in mouse

Organ/tissue	Developmental stages	Role of Ptf1a	Phenotypes/defects	References
Pancreas	E9–E13, in MPCs	Required for acquisition of pancreatic identity from foregut endoderm cells Required for proliferation of pancreatic MPCs	*Ptf1a* misexpression transforms endodermal organs into pancreas Inactivation of *Ptf1a* converts pancreatic MPCs into non-pancreatic cell fates. Hypomorphic *Ptf1a* leads to pancreatic aplasia or agenesis	[32, 33, 35, 38]
	E14-adult, in exocrine pancreas and acinar cells	Promotes acinar cell fates and regulates acinar cell-specific genes Maintains acinar cell identity and its physiological functions	High levels of *Ptf1a* promotes exocrine cell fates and represses endocrine cell fates *Ptf1a* inactivation may cause acinar cells to transform into tumor cells	[36, 37, 119]
Retina	E12–P3, in retinal precursors	Specifies horizontal and amacrine cell fates and promotes their differentiation	Knockout of *Ptf1a* results in losses of horizontal and amacrine cells *Ptf1a* misexpression promotes horizontal and amacrine cell fates	[59, 60, 62]
Spinal cord	E9.5–E13.5(?), in neuronal precursors	Specifies GABAergic and glycinergic inhibitory neuronal cell fates while repressing glutamatergic excitatory cell fates	In the absence of *Ptf1a*, *Ptf1a*-lineage cells switch fates to acquire an excitatory neuronal identity	[25, 83, 124]
Cerebellum	E10.5–E18.5 in neural progenitors	Specifies GABAergic cerebellar neurons including Purkinje cells, GABAergic neurons of DCN, and molecular layer interneurons while repressing glutamatergic cell fates	Misexpression of *Ptf1a* converts non-*Ptf1a*-lineage progenitors into GABAergic instead of glutamatergic neurons *Ptf1a* inactivation leads to mis-specification of *Ptf1a*-lineage progenitors into glutamatergic neurons	[93–96, 125]
Brainstem	E10.5–E13.5 in neuronal progenitors of the caudal hindbrain	Specifies GABAergic neuronal fates in development of the nucleus of the solitary tract (nTs), the spinal trigeminal nuclei (SpV) and principal trigeminal nuclei (PrV) Specifies GABAergic and glycinergic inhibitory neurons in the cochlear nucleus. Controls the cell fate specification, development and survival of glutamatergic climbing fiber (CF) neurons and their migration to the inferior olivary nucleus (ION)	*Ptf1a* inactivation results in generation of more *Lmx1b*-lineage viscerosensory and somatosensory neurons at the expense of *Pax2*-lineage GABAergic viscerosensory and somatosensory neurons, and inferior olivary neurons Development of inhibitory neurons in the cochlear nucleus is severely undermined in the absence of *Ptf1a* Loss of *Ptf1a* compromises the development and migration of CF neurons and causes some of the precursors to switch cell fates to mossy fiber neurons	[98–100]
Forebrain	E10.5–E16.5, In neuronal progenitors	*Ptf1a* does not involve cell fate specification but confers the competence to acquire sex differentiation in the developing brain	*Ptf1a*-deficient male and female mice display abnormalities in reproductive organs and sexually dimorphic behaviors	[101]

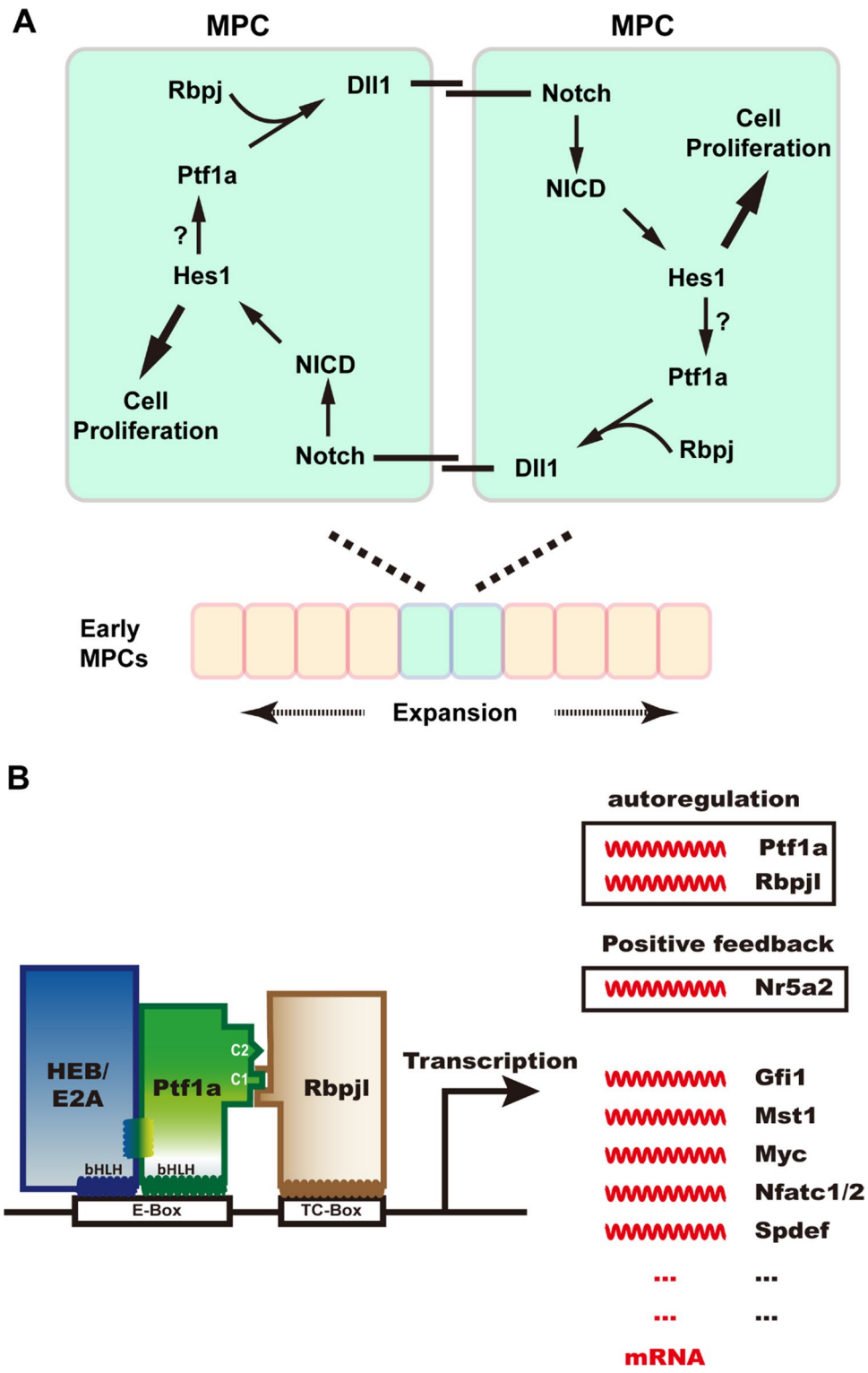


Fig. 3 Ptf1a is required for early pancreatic genesis and adult acinar cell identity. **a** Ptf1a regulates early pancreatic MPC expansion in a positive feed-forward loop (adapted from Ahnfelt-Ronne et al. [38]). Ptf1a and Rbpj activate the expression of Notch ligand Dll1, which in turn binds to Notch receptors on the neighbor MPC, and triggers the downstream pathway of Notch. The activated Notch intracellular domain (NICD) enters the nucleus and activates transcription of target genes such as *Hes1*, which promotes cell proliferation. Hes1 may also maintain the Ptf1a level by stabilizing the Ptf1a structure. This positive feed-forward mechanism guarantees the proliferation and expansion of early MPCs in pancreatic primordia. **b** Ptf1a participates not only in the fate specification of acinar cells during development, but also in the maintenance of their physiological function and identity in the adult pancreas. The PTF1 triplex is capable of autoactivation by transactivating Ptf1a and Rbpjl expression. PTF1 activates the expression of Nr5a2 which can positively regulate the expression of Ptf1a and Rbpjl in return. PTF1 also activates the expression of many transcription factors crucial for acinar cell development and function, such as *Gfi1*, *Mst1*, *Myc*, *Nfatc1/2*, and *Spdef*

Ptf1a and Pdx1 together maintain the proliferation of early MPCs before cell differentiation becomes widespread.

In later development and adulthood, Ptf1a promotes acinar differentiation and regulates acinar cell-specific gene expression (Fig. 3b) [12, 43–46], which is dependent on the PTF1 complex. PTF1 is capable of autoactivation by directly binding to the promoter regions of *Ptf1a* and *Rbpjl* and activating their transcription [12, 13, 46]. ChIP analysis with a Ptf1a antibody demonstrated that PTF1 directly regulates *Nr5a2* expression by binding to its regulatory sequences

[46]. In response, Nr5a2 also binds to the promoters of *Ptf1a* and *Rbpjl* and promotes their expression. The coordination of PTF1 and Nr5a2 guarantees the terminal differentiation and function of acinar cells [47]. Moreover, PTF1 controls the expression of many other transcription factor genes such as *Gfi1* [48], *Mst1* [49], *c-Myc* [50], *Nfatc1/2* [51], and together regulate the growth, differentiation and physiological function of acinar cells.

There are still gaps in our understanding of how *Ptf1a* expression is initiated in early pancreatic organogenesis. In *Hnf1b* (*TCF2*, *vHnf1)*-inactivated mice, expression of *Ptf1a* was not detected [52]. This can be interpreted as *Hnf1b* as a genetically upstream regulator of *Ptf1a* [53]. Alternatively, it may result from the secondary effect of failure in specifying pancreatic progenitor cell identity, since Pdx1, Mnx1, Ngn3, Isl1, Hnf6, and other critical pancreatic cell markers were all dramatically downregulated or totally disappeared in the mutant. Further evidence is needed to determine if Hnf1b is able to initiate the onset of *Ptf1a* expression. The essential mesenchymal–epithelial interactions for the proper development of early pancreas have been established since the 1960s, and the well-studied molecules mediating the process are FGFs, especially Fgf10 from the mesenchyme cells [54–56]. Loss of function study with *Fgf10* knockout mice shows that *Fgf10* signaling is essential for maintaining the proliferative capacity of pancreatic progenitor cells, mostly due to its ability to maintain normal expression levels of *Pdx1* [54] and *Ptf1a* [56]. It is likely that similar pathways from aortic endothelium or dorsal mesenchyme initiate *Ptf1a* expression in the pancreas bud [56, 57], but the exact mechanism needs to be deciphered.

The Ptf1a activity is also regulated post-translationally in acinar cells. The K (Lysine) acetyltransferase Kat2b (Pcaf, p/CAF) interacts with Ptf1a and enhances transcription activity of the PTF1 complex by promoting Ptf1a nuclear accumulation and its acetylation [44]. The catenin beta interacting protein 1 (Ctnnbip1, ICAT), however, interacts directly with Ptf1a and interferes with its acetylation modification by Kat2b, and thus inhibits its transcriptional activity [58]. The E3 ubiquitin–protein ligase, TRIP12 (thyroid hormone receptor interactor 12), interacts with Ptf1a and promotes its polyubiquitination and proteasomal degradation [19]. Inhibition of proteasomal degradation results in elevated levels of Ptf1a and its polyubiquitinated forms in acinar cells. Phosphorylation, SUMOylation or other modification of Ptf1a has not been explored so far.

Ptf1a in neural tissue development

During development, Ptf1a is selectively expressed in the central and peripheral nervous systems, including retina, spinal cord, brain, and enteric neural system. Unlike in pancreas development, Ptf1a is not involved in early neural progenitor proliferation in neural tissues. It is only expressed transiently in post-mitotic neural precursors and participates in cell fate specification and differentiation.

Retina

Retina is a delicate neural tissue responsible for light signal capture, transduction, modulation, and conduction. It is developed from a cluster of multipotent progenitors in the optic vesicle. The mature vertebrate retina has multiple structural layers and is composed of six major types of neurons (cone, rod, horizontal, amacrine, bipolar, and ganglion,) and one major type of glial cells (Müller cells). Among which, the cone and rod photoreceptors located in the outer nuclear layer capture photons and convert them into neuronal signals; the horizontal, bipolar and amacrine cells are interneurons positioned in the inner nuclear layer and modulate the signals from photoreceptors before passing on to ganglion cells; the ganglion cells further integrate the signals from interneurons and transmit them into the brain.

Ptf1a expression in the retina starts at around E12 (embryonic day 12) and disappears after P3 (postnatal day 3), coincident with the specification period of horizontal and amacrine cells [59, 60]. BrdU pulse labeling result shows that Ptf1a expression is restricted in the post-mitotic cells. In Ptf1a knockout retinae, the absence of Ptf1a results in a loss of all horizontal cells, GABAergic and glycinergic amacrine cells, and a temporary increase of ganglion cells and photoreceptors, indicating a cell fate switch during retinal development [59, 60]. Conversely, *Ptf1a* misexpression promotes the fates of horizontal cells and GABAergic and glycinergic amacrine cells at the expense of photoreceptors and ganglion cells [61, 62]. Loss- and gain-of-function results demonstrate that *Ptf1a* is necessary and sufficient for horizontal and amacrine cell fate commitment.

Unlike in the pancreas, the transcription factors regulating *Ptf1a* expression are well defined in the retina (Fig. 4a). In knockout mouse models, Foxn4-null and Ptf1a-null mice display strikingly similar phenotypes in the retina, including loss of horizontal and amacrine cells, temporary increase of ganglion cells and photoreceptors, indicating that both genes engage in the same genetic pathway [59, 63–65]. *Foxn4* expression remains unchanged in *Ptf1a*-null retinae, but *Ptf1a* expression is abolished in Foxn4-null retinae, demonstrating that *Ptf1a* is a downstream target of *Foxn4* [59]. Gain-of-function studies during chick retinogenesis further verified that misexpression of Foxn4 could greatly induce the expression of Ptf1a, while misexpression of Ptf1a inhibited the expression of Foxn4 due to negative feedback inhibition [66]. *RORβ1*, an isoform of the retinoid-related orphan nuclear receptor β gene (*Rorb*, *Nr1f2*), is also an upstream regulator of *Ptf1a* gene [67]. Knockout of *RORβ1*

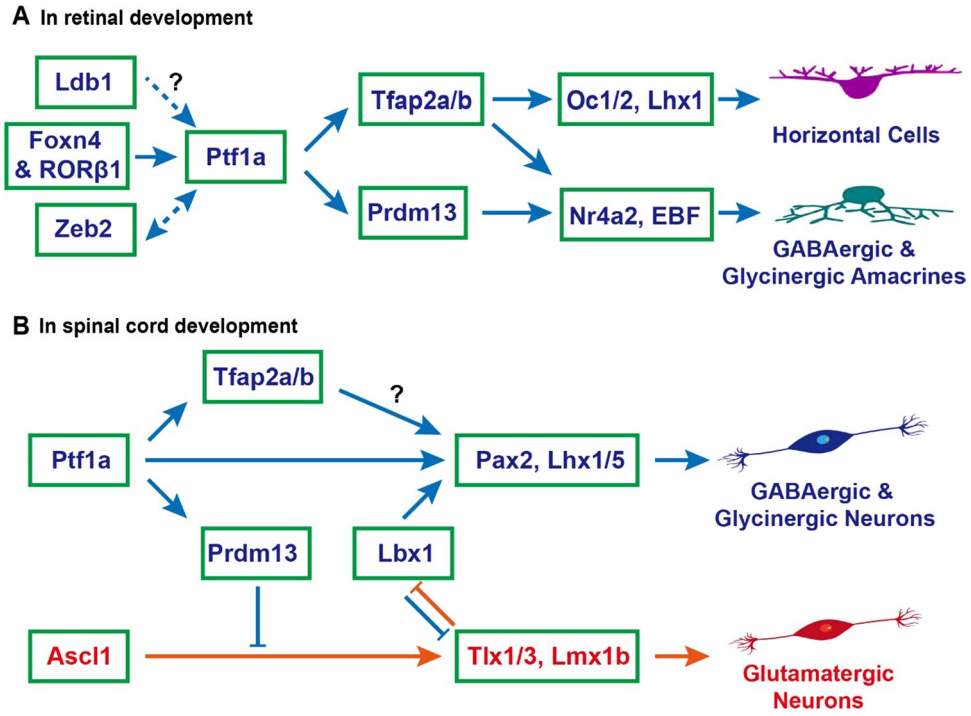


Fig. 4 Ptf1a signaling pathways to specify inhibitory neuronal fates in retinal and spinal cord development. **a** During retinal development, Foxn4 and RORβ1 jointly turn on the expression of *Ptf1a*, which in turn activates the expression of *Tfap2a/b* and *Prdm13*, determining the cell fates of horizontal and amacrine cells. Both GABAergic and glycinergic amacrine cells are inhibitory neurons. Zeb2 directly activates *Ptf1a* expression and Ptf1a may enhance *Zeb2* expression during amacrine and horizontal cell development. Ldb1 complexes may also be involved in activating *Ptf1a* expression and vice versa. **b** During spinal cord development, the upstream regulator of *Ptf1a* is unknown. Ptf1a activates expression of downstream targets *Pax2* and *Lhx1/5* directly or indirectly through *Tfap2a/b*, specifying the progenitors towards inhibitory GABAergic and glycinergic neuronal fates. Meanwhile, Ptf1a inhibits the excitatory glutamatergic neuronal fates indirectly through the downstream gene *Prdm13*. The coordinate interaction between Ptf1a and Lbx1 is unclear. In cerebellar and brainstem development, the same mechanism may be also at play as in the spinal cord

leads to almost the same phenotypes in the mutant retinae as those in Foxn4-null and Ptf1a-null retinae. Ptf1a expression is undetectable in *RORβ1*-null retinae, but Foxn4 expression is not affected, indicating that Ptf1a but not Foxn4 is a downstream target of *RORβ1*. Data from Foxn4 knockout mice implicate that *RORβ1* does not act downstream of Foxn4. Analysis of *Ptf1a* genomic regulatory sequences and electrophoretic mobility shift assay (EMSA) indicates that RORβ1 and Foxn4 may bind directly to the *Ptf1a* enhancer elements and synergistically activate Ptf1a expression [67]. However, it remains to be answered whether RORβ1 and Foxn4 form a protein complex regulating Ptf1a expression in retinal precursor cells, despite their ability to interact with each other [67]. The Zeb2 transcription factor, which is encoded by the Mowat–Wilson syndrome associated gene, has also been shown to directly bind the *Ptf1a* 3′ enhancer and therein controls the development of amacrine and horizontal cells by activating *Ptf1a* transcription [68, 69]. In the *Ptf1a*-null mouse retina, Zeb2 expression is downregulated, indicating that Ptf1a may regulate Zeb2 expression by positive feedback or other unknown mechanisms [69]. The LIM domain-binding protein Ldb1, along with its cofactors, may potentially be involved in regulating Ptf1a expression and thus participate in the differentiation of amacrine and horizontal cells in the retina [70].

Several downstream transcription factors mediating Ptf1a effects have been identified in retinal development (Fig. 4a). Using RNA-seq to compare differentially expressed genes between wild-type and Ptf1a-null retinae, a group of transcription factors, including Tfap2a, Tfap2b and Prdm13 (PR domain containing 13), were found to be significantly downregulated in the mutant retinae [62, 71]. Immunostaining with anti-Tfap2a and anti-Tfap2b antibodies showed that Tfap2a and Tfap2b proteins were undetectable in Ptf1a-null retinae [62]. Moreover, Ptf1a, Tfap2a and Tfap2b are expressed in post-mitotic cells, and have partially redundant functions in the differentiation of horizontal and amacrine cells [72]. Compound knockout of Tfap2a and Tfap2b in mouse retinae leads to similar phenotypes, i.e., loss of horizontal and amacrine cells, as seen in Ptf1a-null retinae [72]. In vivo and in vitro gain-of-function studies showed that overexpression of Ptf1a could induce the expression of

Tfap2a and *Tfap2b*, and moreover, overexpression of *Tfap2a* and *Tfap2b* phenocopied those of *Ptf1a* overexpression [62]. The loss- and gain-of-function results established *Tfap2a* and *Tfap2b* as two major downstream targets of *Ptf1a*. In comparison, Prdm13 is present only in amacrine precursors and mature amacrine cells but not in horizontal precursors or mature horizontal cells. Correspondingly, Prdm13 deficiency only affects amacrine cells [73, 74]. By analysis of spatiotemporal expression patterns and loss-of-function phenotypes, we could place *Oc1/2* [75, 76], *Lhx1* [77, 78], *EBF* [79], and *Nr4a2* [80] further downstream of *Tfap2a/b* and *Prdm13* as shown in Fig. 4a.

The question remains as to whether Ptf1a activity is also dependent on Rbpj during retinal development. There are discrepancies in published results. In *Rbpj* homozygous conditional knockout retinae obtained by the *Pax6 α-Cre* driver, the development of amacrine and horizontal cells seems unaffected [81], whereas the two cell types are missing or drastically decreased in *Ptf1a-null* retinae, implying that the Ptf1a activity does not or minimally relies on Rbpj during retinal development. However, in *Rbpj* homozygous mutants conditionally knocked out by the *Chx10-Cre* driver, the development of amacrine and horizontal cells was disrupted and both populations dropped dramatically in the mutant retinae [82], suggesting that Ptf1a activity may be partially dependent on Rbpj. The discrepancies cannot be easily reconciled with the differential expression patterns of Cre recombinase. The latter result is more persuasive since the former investigated only a subset of amacrine cells, the calbindin + and calretinin + ones, with no quantitative data to support the claim. In contrast, the latter examined using a pan-amacrine cell marker, syntaxin, with solid statistical data. In agreement with this, in gain-of-function experiments in the chick retinae, it is found that interaction with Rbpj is a prerequisite for Ptf1a to specify retinal horizontal and amacrine cell fates [66]. Ectopic expression of *Ptf1a* was sufficient to promote the fates of Prox + horizontal cells and Tfap2a + amacrine neurons. When the interacting motifs C1 and C2 or C2 alone was mutated, Ptf1a$^{\Delta C2}$ or Ptf1a$^{\Delta C1\Delta C2}$ lost the ability to specify these cell fates. In conclusion, Ptf1a activity is dependent on Rbpj during retinal development, but we still cannot rule out the importance of Rbpj-independent activity.

Spinal cord

The spinal cord is a part of the central nervous system that relays efferent motor neuron signals and afferent sensory neuron information. It is also the site controlling central pattern generators and simple neural reflex circuits. Developmentally, spinal cord is derived from neural ectoderm and neural tube.

By immunostaining, the expression of Ptf1a was detected at E10 in the neural tube and was limited to post-mitotic cells as well [83]. Specifically, Ptf1a is present in GABAergic neuronal precursors in spinal cord dorsal horns, and is required for fate determination of the GABAergic cell population [83]. The absence of Ptf1a results in a complete loss of inhibitory GABAergic neurons and a concomitant increase of excitatory glutamatergic neurons in the dorsal horn regions. Notably, many inhibitory neurons in the dorsal horn express glycinergic markers, which are also controlled by Ptf1a [84]. One explanation is that some dorsal horn GABAergic neurons co-release glycine [85, 86]. Thus, glycinergic neurons constitute a subset of GABAergic neurons, unlike their complete segregation in the retina. The imbalance of inhibitory and excitatory neuronal activity could interfere with primary sensory afferents and lead to sensory disorders, emphasizing the importance of Ptf1a in specifying inhibitory sensory neuronal fates.

As revealed by ChIP and other analyses, Ptf1a activity is mediated in both Rbpj-dependent and -independent manners in neural tube development [23]. Using gain-of-function studies, ChIP-seq, RNA-seq, and other approaches, a PR domain containing gene, *Prdm13*, was identified to be a direct downstream target of Ptf1a and Rbpj complex at the dorsal neural tube in the mouse and *Xenopus* [87, 88]. *Prdm13* and *Ptf1a* display an overlapping expression pattern in the region. Overexpression of *Ptf1a* induces upregulation of *Prdm13*, and overexpression of *Prdm13* phenocopies that of *Ptf1a*. Ptf1a protein binds to several genomic regulatory sequences of *Prdm13* in a Rbpj-dependent manner. The ability of Ptf1a to inhibit the glutamatergic neuronal fate is mostly mediated by Prdm13, which suppresses the transcription of *Tlx1*, *Tlx3* and other Ascl1 downstream genes by directly interacting with Ascl1 (Fig. 4b) [87]. The ability of Prdm13 to induce Pax2-positive GABAergic cell fates is likely an indirect effect, for example, by indirectly uplifting *Lbx1* expression, which biases toward generation of GABAergic neurons.

Are *Tfap2a* and *Tfap2b* also downstream targets of Ptf1a in spinal cord development? *Tfap2b* is expressed in the dorsal horn with a pattern highly overlapping with that of *Pax2*. Its expression is completely absent in the *Ptf1a* knockout neural tube [89], indicating that *Tfap2b* is also a downstream gene of *Ptf1a* in neural tube development (Fig. 4b). Consistent with this, RNA-seq data showed that *Tfap2b* was downregulated in E11.5 *Ptf1a-null* neural tubes [90]. As a transcription activator, Tfap2b is speculated to activate the transcription of *Lbx1* or *Pax2*, but this needs to be verified by further studies. *Tfap2a* is essential for early neural crest formation and growth. Whether it acts downstream of *Ptf1a* in later cell fate specification remains to be determined. Nevertheless, during cerebellar development in which *Ptf1a* has a similar role, it is found that both *Tfap2a* and *Tfap2b* act

downstream of *Ptf1a* and upstream of *Pax2* to determine inhibitory neuronal fates [91]. It is therefore highly possible that this is also the case during spinal cord development.

The upstream genetic regulator of *Ptf1a* is currently unknown in spinal cord development; however, it must be quite different from the ones in the retina. Though *Foxn4* is expressed in progenitors of the neural tube, it is located only in the ventral region and does not participate in the regulation of *Ptf1a* as it does in the retina [92]. Similarly, the *Rorb* expression pattern revealed by RNA in situ hybridization assay indicates that RORβ1 is not an upstream regulator of *Ptf1a* either in the neural tube [89]. Genome sequence analysis indicated that a conserved enhancer region located 10.8 kb 3′ of *Ptf1a* coding region is required for tissue-specific expression of *Pf1a* in the dorsal neural tube. In this region, a DNA-binding motif for paired homeodomain (pd-hd) protein is indispensable for activating *Ptf1a* expression [17], implying that one of the paired homeodomain proteins is an upstream regulator of *Ptf1a*. Though Pax6 could bind to the motif to activate transcription in vitro, it is unlikely the upstream regulator of *Ptf1a* since their expression patterns are undoubtedly different from each other in the spinal cord.

Brain

The brain is the most important organ governing cognition, intelligence and logical thinking. Anatomically, the mammalian brain can be divided into the forebrain, midbrain and hindbrain, each of them can be further subdivided both anatomically and functionally. For example, the forebrain is composed of diencephalon (thalamus and hypothalamus) and telencephalon (cerebrum). The hindbrain contains the pons and cerebellum. At developmental stage E15.5, BAC transgenic mouse and RNA in situ hybridization data from http://Gensat.org show that Ptf1a is highly expressed in the olfactory bulb, cerebral cortex, hippocampus, and hypothalamus of the forebrain, in the pons, medulla and cerebellum of the hindbrain, as well as in the midbrain (Fig. 5a). Except in the cerebellum, brainstem and forebrain, the role of *Ptf1a* is currently unknown in these brain regions during development.

The cerebellum mainly involves in motor control, for example, movement coordination, precision and timing accuracy. In cerebellar development, the Ptf1a-positive ventricular zone (VZ) progenitors give rise to GABAergic cerebellar neurons, such as Purkinje cells, GABAergic neurons of DCN (deep cerebellar nuclei) and molecular layer interneurons [93, 94]. Ectopic expression of *Ptf1a* in Ptf1a-negative VZ progenitors or rhombic lip progenitors converts them into GABAergic instead of glutamatergic neurons [93, 95]. On the other hand, in the absence of *Ptf1a*, those Ptf1a lineage VZ progenitors were either mis-specified into glutamatergic neurons and/or migrated and contributed to the ventral brainstem, or committed to apoptosis, eventually leading to postnatal cerebellar agenesis [93, 94, 96, 97]. Similar to that in the retina and spinal cord, the *Ptf1a-Tfap2a/b* transcriptional cascade has been shown to be the force driving inhibitory neuronal differentiation in the cerebellum [91]. It remains to be determined whether there also exists the *Ptf1a–Prdm13* cascade in the cerebellum.

The brainstem is the most primitive portion of the brain. It connects the brain and spinal cord and relays sensory and motor signals. It also contains vital nuclei with diverse neuronal populations that regulate a wide range of life processes such as digestion, breath and heartbeat. For example, the nucleus of the solitary tract (nTs) relays baroreceptor, cardiac, pulmonary and other vagal afferents. The principal sensory trigeminal nuclei (PrV) and spinal trigeminal nuclei (SpV) process facial sensory information. The inferior olivary nuclei (ION) and cochlear nuclei handle motor coordination and sounds, respectively. It is found that *Ptf1a* plays a crucial role in the development of these nuclei (Table 1) [98–100]. Since these nuclei all arise from the neighboring rhombomeres, the molecular mechanism underlying Ptf1a regulation is much the same and similar to those in spinal cord and cerebellum, except in development of ION. Basically, the majority of evidence supports that the major function of *Ptf1a* is to promote GABAergic and glycinergic inhibitory neuronal cell fates while suppressing excitatory neuronal cell fates during development of these nuclei. However, in the development of ION, *Ptf1a* promotes the fates of climbing fiber (CF) neurons but suppresses mossy fiber (MF) neuronal fates [99]. This is quite unusual since CF and MF neurons are both glutamatergic excitatory neurons. It suggests that other TF(s) coexisted with Ptf1a may outweigh Ptf1a to specify excitatory neurons.

Surprisingly, unlike in other neural tissues, not all Ptf1a+ cells are post-mitotic cells; some of them are co-expressed with Ki67 in the forebrain [101]. Lineage tracing indicated that forebrain Ptf1a-lineage developed into a variety of neuronal subtypes including glutamatergic, dopaminergic, GABAergic and peptidergic neurons, in contrast to restricted inhibitory neuronal fates in the retina, spinal cord, cerebellum and cochlear nucleus [59, 83, 93, 100, 101]. It seems that forebrain Ptf1a is not involved in the excitatory versus inhibitory cell specification and fate determination, since its deletion neither alters the cell numbers nor the cell subtypes. However, *Ptf1a*-deficient mice exhibit complex abnormalities in sexually dimorphic behaviors and reproductive organs in both sexes, revealing a critical role of Ptf1a in regulating sexual differentiation of the brain (Fig. 5b). Ptf1a could alter cellular expression of sexually biased genes through cell autonomous or non-cell autonomous manners in the developing hypothalamus. One of the key downregulated genes in the *Ptf1a*-deficient

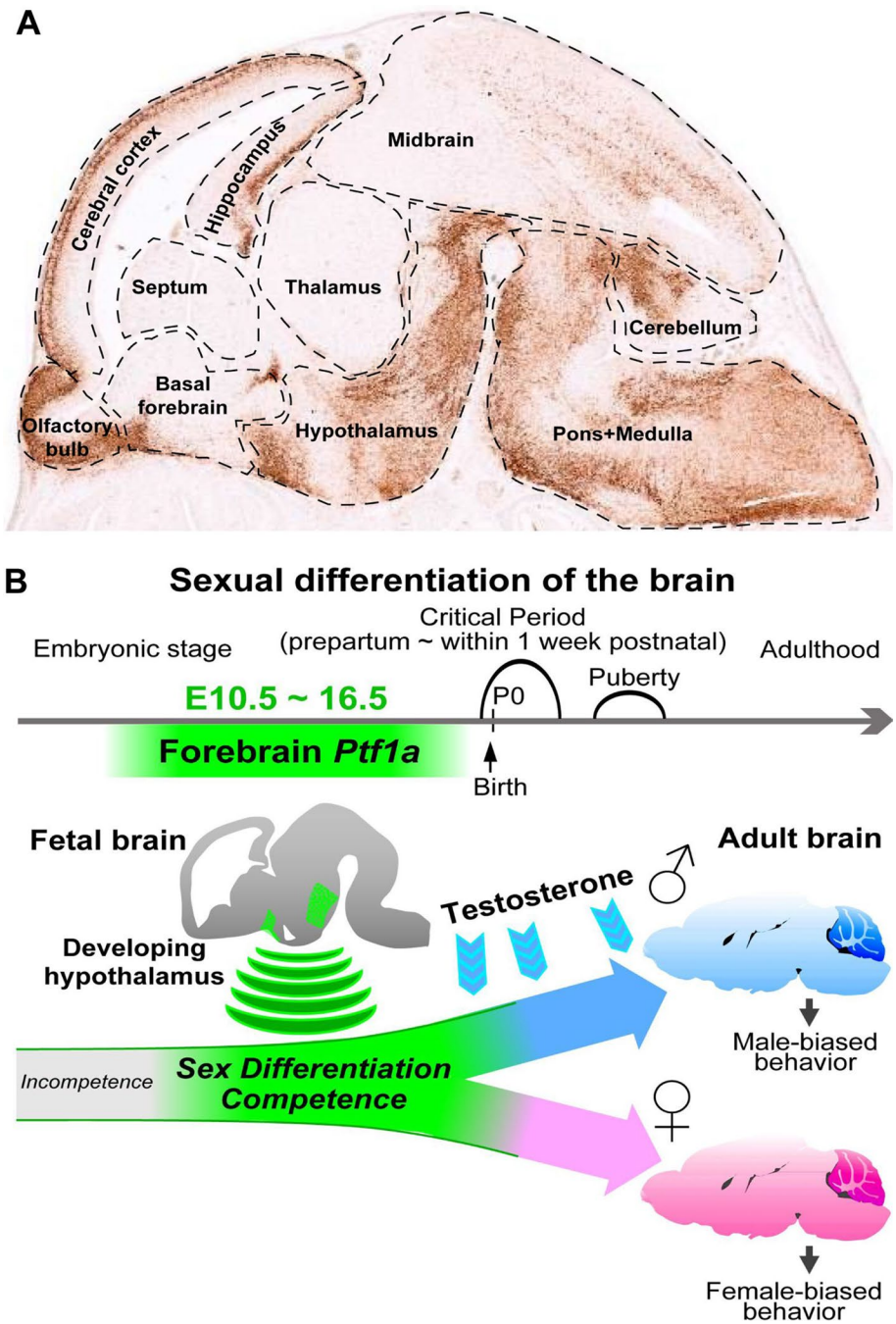


Fig. 5 Ptf1a participates in brain development and cell fate reprogramming. **a** The image shows the expression pattern of GFP/Ptf1a (brownish signal) in the E15.5 brain (sagittal section) of a BAC transgenic mouse line harboring *Ptf1a* regulatory sequences and *GFP* as a reporter gene (image adapted from http://GENSAT.org). Ptf1a is modestly or strongly expressed in many regions in the forebrain, midbrain and hindbrain. **b** Around perinatal stage is the critical period when male brain is exposed to testosterone signal and female exposed to non-testosterone signals to establish sexual differentiation of the brain. Forebrain Ptf1a confers the sex differentiation competence during brain development. Ptf1a-deficient mice display sexually dimorphic behavior abnormalities. Image adapted from Fujiyama et al. [101]

hypothalamus is *Kiss1* that is crucial in regulating brain sexual development through kisspeptin-GPR54 signaling pathway [102]. The expression of *Ptf1a* common downstream gene *Prdm13* is also dramatically downregulated. However, its connection to brain sex differentiation needs further studies. Brain masculinization or feminization does not occur by exposure or non-exposure of testosterone in *Ptf1a*-deficient males or females, respectively, suggesting that Ptf1a confers the competence to acquire sex differentiation in the developing brain.

Enteric nervous system

The enteric nervous system (ENS) is often called the second brain, reflecting its importance, complexity and independence from the CNS. The ENS is composed of interconnecting ganglia within the myenteric and submucosal plexuses of the gut wall. Its neurons and glias arise from neural crest cells that migrate from vagal and sacral levels of the neural axis. The ENS includes sensory neurons, motor neurons and interneurons, and is capable of autonomous functions. Many neurotransmitters have been identified in the ENS neurons,

such as acetylcholine, dopamine, NOS, GABA, and serotonin, most of which are identical to those found in the CNS [103]. Many researchers consider ENS as a repertoire of neurotransmitters, and diffusion, leak or uptake of the neurotransmitters into the blood stream could distribute them to the whole body. Some of the neurotransmitters can enter and/or exit the blood–brain barrier (BBB) via transporters on the brain capillary endothelial cells (BCECs), i.e., serotonin via SERT [104], GABA via GAT2/BGT-1 [105].

Some transcription factors such as Phox2b, Sox10, Ascl1, and Hand2 have been shown to be crucial in ENS development. Phox2b is essential for all autonomic ganglion formation and its deletion leads to enteric aganglionosis [106]. Sox10 is important to maintain the undifferentiated state of migrating crest cells [107]. Ascl1 promotes neurogenesis and has a role opposite to that of Sox10 [108]. Hand2 is required for late stage neurogenesis and expression of a subset of cell type-specific markers, especially vasoactive intestinal polypeptide (VIP) [109]. Genetic lineage analysis in zebrafish shows that Ptf1a is present in a subset of enteric neurons, most of which express serotonin [110], one of the major inhibitory neurotransmitters. The exact role of Ptf1a is not yet elucidated in the enteric neurons. It would be worthwhile to investigate if Ptf1a and Ascl1 are the major controllers contributing to inhibitory and excitatory ENS neurons, respectively.

Ptf1a mutations and diseases

The mutations discussed here represent naturally occurred spontaneous mutations, not genetically manipulated ones. Unlike many transcription factors with partially or fully redundant function replaceable by family members, to our knowledge, Ptf1a is very unique and indispensable. Mutations in Ptf1a coding regions and non-coding regulatory sequences often lead to genetic diseases in animals and human beings. The known disease-causing mutation sites and their associated defects are presented in Fig. 6 and Table 2.

The Danforth's short tail (Sd) is a semi-dominant mutation in the mouse, characterized by spinal defects, kidney agenesis and anorectal malformations. The Sd mutant phenotypes resemble symptoms seen in human caudal malformation syndromes, including urorectal septum malformation, caudal regression, VACTERL association, and persistent cloaca [111]. A few laboratories recently demonstrated that Sd is caused by up to tenfold overexpression of Ptf1a in the notochord, hindgut, cloaca, and mesonephros, due to an early retrotransposon insertion in the 12 kb conserved domain upstream of Ptf1a [111–113]. This raises an intriguing question of whether human caudal malformation syndromes are also caused by overexpression of Ptf1a. In addition, Sd mouse provides an excellent genetic tool for the study of Ptf1a gain-of-function effects.

The association of *PTF1A* with human diseases was first reported in 2004. Two families from Pakistan and Northern Europe with permanent diabetes mellitus were diagnosed as pancreatic and cerebellar agenesis [97]. Both families were found to have truncated mutations in *PTF1A*. In family 1, affected individuals had homozygous nonsense mutation p.R296X in PTF1A, leading to loss of the C2 motif that is required for interaction with Rbpj (Figs. 1c, 2c). In family 2, an insertion mutation c.705insG caused frameshift (p.P236fsX270) and premature truncation of PTF1A protein at codon 270, resulting in a loss of both C1 and C2 motifs. More mutations in PTF1A have since been identified [114–116]. It appears that the severity of the symptoms

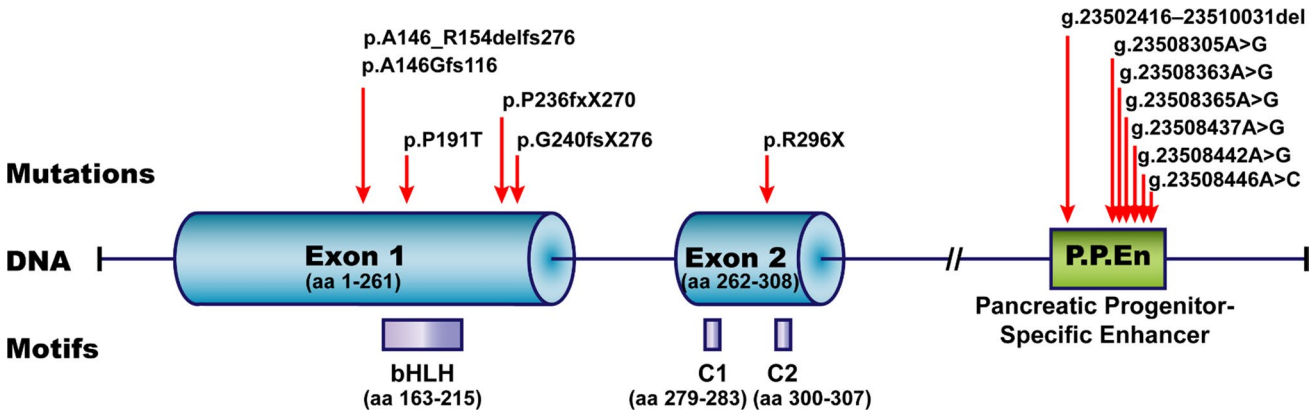


Fig. 6 Known disease-causing mutations in human *PTF1A*. The human genomic (DNA) structure of *PTF1A* gene is illustrated (non-proportionally). Exons 1 and 2 with the corresponding numbers of coded amino acid residues, and the 3′ downstream pancreatic progenitor-specific enhancer are highlighted. The disease-causing mutations at protein level (p.xxx) or genomic level (g.xxx) are listed on top of the DNA structure. Red arrows point to the mutation sites. The critical peptide motifs, bHLH, C1 and C2, and their positions in PTF1A protein, are placed below the corresponding DNA structure

Table 2 Ptf1a spontaneous mutations and diseases

Species	Mutation position	Mutation type	Hetero- or homozygous	Diseases/defects	References
Mouse	A retrotransposon insertion at 12 kb upstream of *Ptf1a*	Insertional mutation causing *Ptf1a* gain-of-function	Heterozygous	Danforth's short tail, characterized by spinal defects, kidney agenesis and anorectal malformations	[111–113, 126]
Human	p.R296X	Nonsense mutation causing PTF1A truncation	Homozygous	Diabetes mellitus, cerebellar agenesis, and more	[97]
	p.P236fsX270	Insertional mutation causing PTF1A frameshift and truncation	Homozygous	Diabetes mellitus, cerebellar agenesis, and more	[97]
	p.G240fsX276	Frameshift causing PTF1A truncation	Homozygous	Diabetes mellitus, cerebellar agenesis, craniofacial defects, irregular breathing and more	[114]
	p.A146_R154delfsX115	Deletional mutation causing PTF1A frameshift and truncation	Homozygous	Diabetes mellitus, cerebellar agenesis, craniofacial defects, irregular breathing, optic atrophy and more	[115]
	p.P191T	Missense mutation causing hypomorphic PTF1A	Homozygous	Diabetes mellitus	[116]
	A conserved regulatory sequence at 25 kb downstream of *PTF1A*	Point mutations or deletions in the distal pancreatic-specific enhancer	Homozygous, or compound heterozygous	Pancreatic agenesis	[15, 16]
	p.A146GfsX116 and g.23508442A > G	A frameshift and truncation mutation in *PTF1A* coding region in the chromosome. A point mutation at distal enhancer in the sister chromosome	Compound heterozygous	Pancreatic agenesis	[117]
	Unknown	Unknown mutation(s) leading to hypomorphic PTF1A in acinar cells	Unknown, possibly homozygous or compound heterozygous	Pancreatic cancer	[118–120]

is associated with the severity of the mutations. Some hypomorphic *PTF1A* mutations may only cause isolated pancreatic aplasia but without any cerebellar phenotypes. For example, the p.P191T missense mutation in the bHLH domain affects the dimerization of PTF1A with E proteins, resulting in isolated pancreatic aplasia or neonatal diabetes mellitus, but the patients show no obvious neurodevelopmental defects [116]. In addition to symptoms caused by pancreatic and cerebellar hypoplasia, optic atrophy was recorded in a patient with *PTF1A* deletion and frameshift [115]. No associated symptoms in the spinal cord and urorectal system have been reported in human patients.

The contribution of non-coding variants to human diseases remains a challenge to gauge. However, mutations in the *PTF1A* non-coding enhancer region are found to cause human diseases. Analysis of whole genome sequencing data from patient families with isolated pancreatic agenesis uncovered different recessive mutations in a 400-bp enhancer region located at 25 kb downstream of *PTF1A* [15, 16]. This enhancer is required to target *PTF1A* in human embryonic pancreatic progenitor cells, and mutations in the enhancer leads to its inactivation and PTF1A deprivation in these cells. One rare case of neonatal diabetes mellitus and pancreatic agenesis was also reported with compound heterozygous mutation of *PTF1A* [117]. The patient had a deletional and frameshift mutation in the *PTF1A* coding region on one chromosome, and a point mutation in the 400-bp enhancer region on another chromosome. Given the fact that there are other enhancers for *PTF1A* specific expression in the spinal cord, it will be challenging to discover the association of these enhancer mutations with some sensorineural diseases, if there is any.

Additionally, downregulation of PTF1A is associated with pancreatic intraepithelial neoplasia (PanIN), the first stage of pancreatic ductal adenocarcinoma (PDAC) [118–120]. PTF1A is required to maintain the identity of acinar cells, and downregulation of PTF1A in these cells cause them to transdifferentiate into ductal cells. The underlying molecular mechanism of *PTF1a* downregulation and development of PanIN/PDAC remains poorly understood. However, it is tempting to propose that small molecules or drugs targeting *PTF1A* upregulation may provide an entry point for the treatment of these diseases.

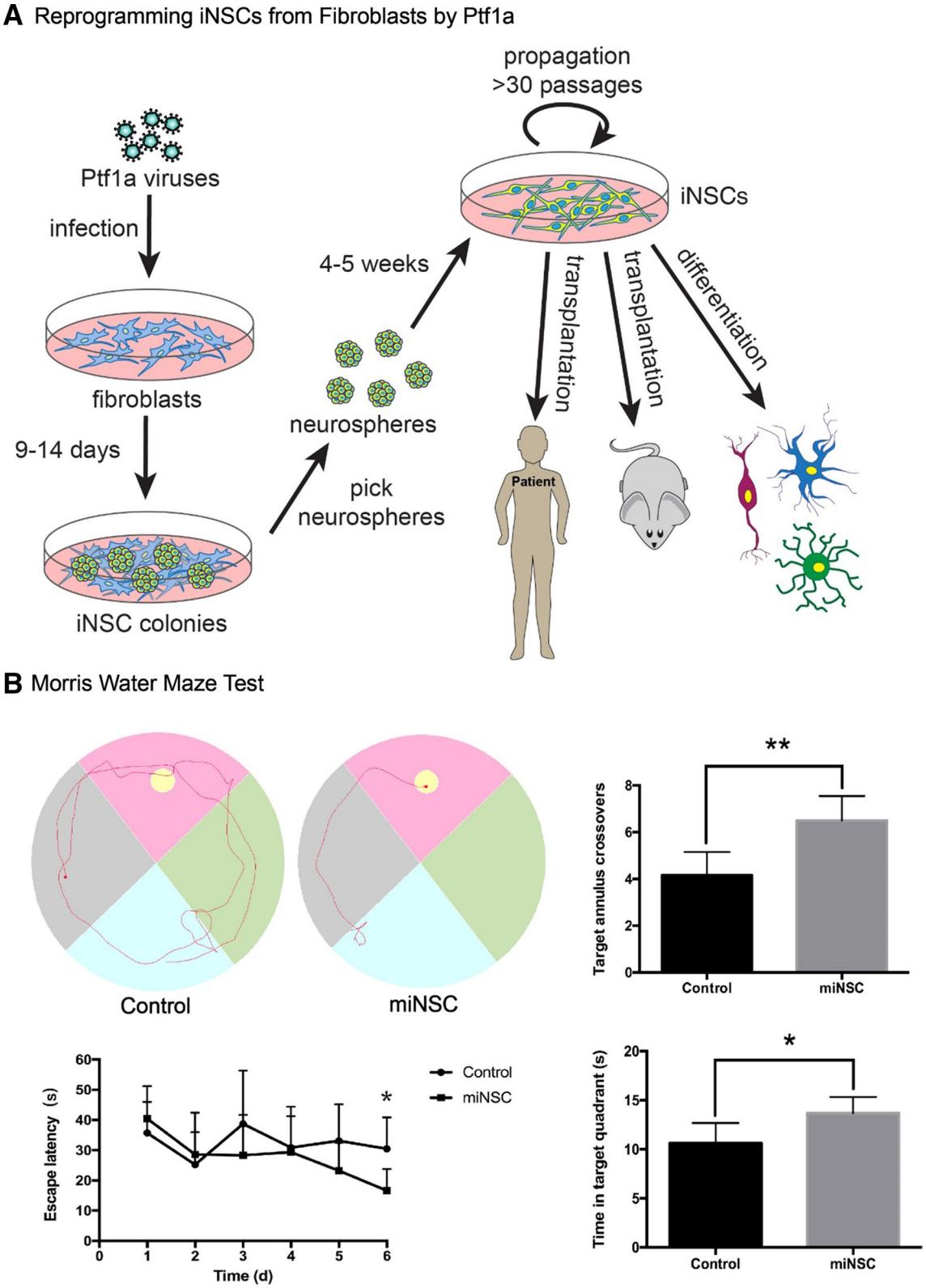
Ptf1a in cell fate reprogramming and transdifferentiation

Given the importance of *Ptft1a* in the establishment of pancreatic identity, researchers wondered if *Ptf1a* could be used in the pursuit of cell fate reprogramming. Indeed, transient ectopic expression of *Ptf1a* in the mouse embryonic stem cells (ESCs) triggered the cells to launch pancreatic programs and express *Pdx1* [121]. The differentiated cells further formed cells of all three pancreatic lineages, the acinar, endocrine and duct cells.

Reprogramming somatic cells and transdifferentiating them into other cell types is also a common practice in stem cell research. We had hypothesized that ectopic expression of *Ptf1a* in fibroblasts would convert them into pancreatic lineages or inhibitory neurons. When we overexpressed *Ptf1a* in human or mouse fibroblasts, surprisingly, these somatic cells were reprogrammed into neurospheres containing neural stem cells (NSCs) instead of pancreatic lineages [122]. The induced NSCs (iNSCs) were capable of differentiating into various types of neurons, astrocytes and oligodendrocytes in differentiation culture media (Fig. 7a). The underlying reprogramming mechanism is also dependent on the Ptf1a–Rbpj interaction, since mutation of the interaction site on Ptf1a (W298A), or knockdown of *Rbpj*, would abolish or drastically decrease the reprogramming activity.

The Ptf1a-reprogrammed iNSCs also have the tripotence to differentiate into neurons, astrocytes and oligodendrocytes when transplanted in vivo. After injected into the mouse hippocampus, Ptf1a-reprogrammed mouse or human iNSCs survived and successfully integrated into the local tissue [122]. The integrated cells differentiated into many astrocytes, some oligodendrocytes, and some inhibitory and excitatory neurons that established synaptic connections with neighbor endogenous neurons. These results have demonstrated that Ptf1a-induced iNSCs have the differentiation tripotence both *in intro* and in vivo. Moreover, in the Aβ1-40 injected or *APP/PS1* Alzheimer's disease (AD) mouse models, implanted iNSCs significantly improved spatial learning and memory and cognitive dysfunction of the AD mouse models as evaluated by behavioral tests [122] (Fig. 7b). Despite the promising results in animal models, the question remains whether Ptf1a-induced iNSCs have any therapeutic potential in treating neurodegenerative diseases in preclinical and clinical settings.

There are several advantages for Ptf1a to reprogram iNSCs compared with other approaches [122]. (1) As a non-tumorigenic factor, Ptf1a is much safer than Sox2 or Zfp521 or other factors that are tumor-prone. (2) Ptf1a is more efficient in reprogramming iNSCs. Ptf1a could convert 0.5% fibroblasts into neurospheres. In contrast, Sox2 alone could only reach less than 0.1% [123]. The efficiency is even lower with multiple transcription factors. (3) Ptf1a-induced iNSCs possess stronger self-renewal ability and could be passaged more than 40 generations without any aging signs, while iNSCs derived from a few other approaches have only limited passaging ability. (4) Ptf1a-induced iNSCs are more efficient in directed differentiation. They are capable of generating 83.3% of neurons, 87.3% of astrocytes or 26.6% of oligodendrocytes in respective differentiation media. In comparison, Sox2-induced iNSCs could only generate 67%



of neurons and 25% of astrocytes [123]. (5) Ptf1a-induced iNSCs are closer to endogenous NSCs. RNA-seq data demonstrated that Ptf1a-induced iNSCs have a higher correlation coefficient with SCR029 cells, a well-characterized mouse cortical NSCs, than those of NS5 cells (mouse ES cell-derived NSCs) and ciNSCs (NSCs chemically induced from

◂**Fig. 7** Ptf1a alone is sufficient to reprogram human and mouse fibroblasts into induced neural stem cells (iNSCs). **a** After infected with lentiviruses expressing *Ptf1a*, human or mouse fibroblasts were transformed into neurospheres. Individual neurosphere can be further expanded and form typical single layered iNSCs in culture dishes. The iNSCs have the tripotence to differentiate into various types of neurons, astrocytes and oligodendrocytes both in vitro and in vivo. The iNSCs constitute an ideal cell source for preclinical transplantation experiments and clinical studies. **b** To determine the therapeutic effect of the Ptf1a-derived iNSCs, they were transplanted into the hippocampus of the Alzheimer disease mouse models. After training for 5 days, the iNSCs-transplanted mice spent much less time (bottom left) to find the target platform in the Morris water maze test. An example of travel pathways for a control mouse and an iNSC-transplanted mouse (top left) was given. Target annulus crossovers revealed that iNSC-transplanted mice displayed a preference for the target platform location (top right). Moreover, they also spent more time in the target quadrant (bottom right). These results demonstrate that iNSC transplantation improves the memory and cognitive functions of the AD mouse models

MEFs). Therefore, Ptf1a-induced iNSCs may provide a better cell source for disease cell modeling, drug screening and cell replacement therapies.

Conclusions and perspectives

Although many advances have been made toward understanding various aspects of the important transcription factor Ptf1a in the past 30 years, beyond its role in the development of pancreas, spinal cord, retina, cerebellum, brainstem, and forebrain, many uncharted territories still remain.

Ptf1a is highly expressed in the stomach, colon and rectum along the digestive tract. In these organs, Ptf1a is possibly expressed specifically in neurons in the local ganglion plexus, controlling muscle motility and secretion of digestive enzymes, as indicated in the ENS [110]. However, it cannot be ruled out that Ptf1a may participate in the specification of other cell types beyond neuroglial cells, either cell autonomously or non-cell autonomously, as demonstrated by the anorectal developmental defects in Sd mice [111–113]. Sd mice also display kidney defects, indicating that Ptf1a affects kidney development as well. Ptf1a is also highly expressed in the auxiliary genital organs such as ovary, testis and prostate gland. Ptf1a in these organs might have a similar role as in the pancreas, or an alternative role as in the intestine. Further studies are necessary to distinguish these possibilities using the Ptf1a knockout and Sd mice.

As aforementioned, the role of Ptf1a is unexplored in the cerebral cortex, hippocampus, and other important brain regions. Does Ptf1a also determine the inhibitory versus excitatory neuronal fates in these regions? Does Ptf1a control those neurons involved in memory, learning, feelings, or space localization? Does it have any potential to promote regeneration of neurons damaged in Alzheimer's, Parkinson's or other neurodegenerative diseases? It would be enticing and thrilling to address these questions.

In brief, here we have discussed the past findings about and insights into the crucial functions of the transcription factor Ptf1a gained since its identification. In particular, we have enumerated its genomic and protein structures, expression profiles, upstream and downstream regulators, mutations and diseases, somatic cell reprogramming activities, and so on. We have also introduced some unsolved issues about Ptf1a that may inspire colleagues to explore uncharted territories and achieve many more exciting findings in the future.

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References

1. Murre C et al (1989) Interactions between heterologous helix–loop–helix proteins generate complexes that bind specifically to a common DNA sequence. Cell 58(3):537–544
2. Chaudhary J, Skinner MK (1999) Basic helix–loop–helix proteins can act at the E-box within the serum response element of the c-fos promoter to influence hormone-induced promoter activation in Sertoli cells. Mol Endocrinol 13(5):774–786
3. Grove CA et al (2009) A multiparameter network reveals extensive divergence between *C. elegans* bHLH transcription factors. Cell 138(2):314–327
4. Murre C et al (1994) Structure and function of helix–loop–helix proteins. Biochim Biophys Acta 1218(2):129–135
5. Massari ME, Murre C (2000) Helix–loop–helix proteins: regulators of transcription in eucaryotic organisms. Mol Cell Biol 20(2):429–440
6. Benezra R et al (1990) The protein Id: a negative regulator of helix–loop–helix DNA binding proteins. Cell 61(1):49–59
7. Wang LH, Baker NE (2015) E proteins and ID proteins: helix–loop–helix partners in development and disease. Dev Cell 35(3):269–280
8. Cockell M et al (1989) Identification of a cell-specific DNA-binding activity that interacts with a transcriptional activator of genes expressed in the acinar pancreas. Mol Cell Biol 9(6):2464–2476
9. Rose SD et al (2001) The role of PTF1-P48 in pancreatic acinar gene expression. J Biol Chem 276(47):44018–44026
10. Beres TM et al (2006) PTF1 is an organ-specific and Notch-independent basic helix–loop–helix complex containing the mammalian Suppressor of Hairless (RBP-J) or its paralogue, RBP-L. Mol Cell Biol 26(1):117–130

11. Rose SD, MacDonald RJ (1997) Evolutionary silencing of the human elastase I gene (ELA1). Hum Mol Genet 6(6):897–903
12. Masui T et al (2010) Replacement of Rbpj with Rbpjl in the PTF1 complex controls the final maturation of pancreatic acinar cells. Gastroenterology 139(1):270–280
13. Masui T et al (2007) Early pancreatic development requires the vertebrate Suppressor of Hairless (RBPJ) in the PTF1 bHLH complex. Genes Dev 21(20):2629–2643
14. Perillo M et al (2016) A pancreatic exocrine-like cell regulatory circuit operating in the upper stomach of the sea urchin Strongylocentrotus purpuratus larva. BMC Evol Biol 16(1):117
15. Weedon MN et al (2014) Recessive mutations in a distal PTF1A enhancer cause isolated pancreatic agenesis. Nat Genet 46(1):61–64
16. Gonc EN et al (2015) Variable phenotype of diabetes mellitus in siblings with a homozygous PTF1A enhancer mutation. Horm Res Paediatr 84(3):206–211
17. Mona B et al (2016) Regulating the dorsal neural tube expression of Ptf1a through a distal 3′ enhancer. Dev Biol 418(1):216–225
18. Lu CK et al (2012) Rbms3, an RNA-binding protein, mediates the expression of Ptf1a by binding to its 3′UTR during mouse pancreas development. DNA Cell Biol 31(7):1245–1251
19. Hanoun N et al (2014) The E3 ubiquitin ligase thyroid hormone receptor-interacting protein 12 targets pancreas transcription factor 1a for proteasomal degradation. J Biol Chem 289(51):35593–35604
20. Driscoll CA, Macdonald DW, O'Brien SJ (2009) From wild animals to domestic pets, an evolutionary view of domestication. Proc Natl Acad Sci USA 106(Suppl 1):9971–9978
21. Masui T et al (2008) Transcriptional autoregulation controls pancreatic Ptf1a expression during development and adulthood. Mol Cell Biol 28(17):5458–5468
22. Meredith DM et al (2009) Multiple transcriptional mechanisms control Ptf1a levels during neural development including autoregulation by the PTF1-J complex. J Neurosci 29(36):11139–11148
23. Meredith DM et al (2013) Program specificity for Ptf1a in pancreas versus neural tube development correlates with distinct collaborating cofactors and chromatin accessibility. Mol Cell Biol 33(16):3166–3179
24. Thompson N et al (2012) RNA profiling and chromatin immunoprecipitation-sequencing reveal that PTF1a stabilizes pancreas progenitor identity via the control of MNX1/HLXB9 and a network of other transcription factors. Mol Cell Biol 32(6):1189–1199
25. Obata J et al (2001) p48 subunit of mouse PTF1 binds to RBP-Jkappa/CBF-1, the intracellular mediator of Notch signalling, and is expressed in the neural tube of early stage embryos. Genes Cells 6(4):345–360
26. Petcherski AG, Kimble J (2000) LAG-3 is a putative transcriptional activator in the *C. elegans* Notch pathway. Nature 405(6784):364–368
27. Kovall RA (2008) More complicated than it looks: assembly of Notch pathway transcription complexes. Oncogene 27(38):5099–5109
28. Kopan R, Ilagan MX (2009) The canonical Notch signaling pathway: unfolding the activation mechanism. Cell 137(2):216–233
29. Kovall RA, Hendrickson WA (2004) Crystal structure of the nuclear effector of Notch signaling, CSL, bound to DNA. EMBO J 23(17):3441–3451
30. Krapp A et al (1998) The bHLH protein PTF1-p48 is essential for the formation of the exocrine and the correct spatial organization of the endocrine pancreas. Genes Dev 12(23):3752–3763
31. Krapp A et al (1996) The p48 DNA-binding subunit of transcription factor PTF1 is a new exocrine pancreas-specific basic helix–loop–helix protein. EMBO J 15(16):4317–4329
32. Kawaguchi Y et al (2002) The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. Nat Genet 32(1):128–134
33. Pan FC et al (2013) Spatiotemporal patterns of multipotentiality in Ptf1a-expressing cells during pancreas organogenesis and injury-induced facultative restoration. Development 140(4):751–764
34. Kim DY et al (2015) Functional regulation of FoxO1 in neural stem cell differentiation. Cell Death Differ 22(12):2034–2045
35. Willet SG et al (2014) Dominant and context-specific control of endodermal organ allocation by Ptf1a. Development 141(22):4385–4394
36. Dong PD et al (2008) Graded levels of Ptf1a differentially regulate endocrine and exocrine fates in the developing pancreas. Genes Dev 22(11):1445–1450
37. Hesselson D, Anderson RM, Stainier DY (2011) Suppression of Ptf1a activity induces acinar-to-endocrine conversion. Curr Biol 21(8):712–717
38. Ahnfelt-Ronne J et al (2012) Ptf1a-mediated control of Dll1 reveals an alternative to the lateral inhibition mechanism. Development 139(1):33–45
39. Ghosh B, Leach SD (2006) Interactions between hairy/enhancer of split-related proteins and the pancreatic transcription factor Ptf1-p48 modulate function of the PTF1 transcriptional complex. Biochem J 393(Pt 3):679–685
40. Wiebe PO et al (2007) Ptf1a binds to and activates area III, a highly conserved region of the Pdx1 promoter that mediates early pancreas-wide Pdx1 expression. Mol Cell Biol 27(11):4093–4104
41. Miyatsuka T et al (2007) Ptf1a and RBP-J cooperate in activating Pdx1 gene expression through binding to Area III. Biochem Biophys Res Commun 362(4):905–909
42. Gao N et al (2008) Dynamic regulation of Pdx1 enhancers by Foxa1 and Foxa2 is essential for pancreas development. Genes Dev 22(24):3435–3448
43. Holmstrom SR et al (2011) LRH-1 and PTF1-L coregulate an exocrine pancreas-specific transcriptional network for digestive function. Genes Dev 25(16):1674–1679
44. Rodolosse A et al (2009) p/CAF modulates the activity of the transcription factor p48/Ptf1a involved in pancreatic acinar differentiation. Biochem J 418(2):463–473
45. Jiang Z et al (2008) Exdpf is a key regulator of exocrine pancreas development controlled by retinoic acid and ptf1a in zebrafish. PLoS Biol 6(11):e293
46. Hoang CQ et al (2016) Transcriptional maintenance of pancreatic acinar identity, differentiation, and homeostasis by PTF1A. Mol Cell Biol 36(24):3033–3047
47. Hale MA et al (2014) The nuclear hormone receptor family member NR5A2 controls aspects of multipotent progenitor cell formation and acinar differentiation during pancreatic organogenesis. Development 141(16):3123–3133
48. Qu X et al (2015) Growth factor independence-1 (Gfi1) is required for pancreatic acinar unit formation and centroacinar cell differentiation. Cell Mol Gastroenterol Hepatol 1(2):233–247.e1
49. Gao T et al (2013) Hippo signaling regulates differentiation and maintenance in the exocrine pancreas. Gastroenterology 144(7):1543–1553.e9
50. Bonal C et al (2009) Pancreatic inactivation of c-Myc decreases acinar mass and transdifferentiates acinar cells into adipocytes in mice. Gastroenterology 136(1):309–319.e9
51. Chen NM et al (2015) NFATc1 links EGFR signaling to induction of Sox9 transcription and acinar-ductal transdifferentiation in the pancreas. Gastroenterology 148(5):pp. 1024–1034 e9
52. Haumaitre C et al (2005) Lack of TCF2/vHNF1 in mice leads to pancreas agenesis. Proc Natl Acad Sci USA 102(5):1490–1495

53. Zaret KS (2008) Genetic programming of liver and pancreas progenitors: lessons for stem-cell differentiation. Nat Rev Genet 9(5):329–340
54. Bhushan A et al (2001) Fgf10 is essential for maintaining the proliferative capacity of epithelial progenitor cells during early pancreatic organogenesis. Development 128(24):5109–5117
55. Ye F, Duvillie B, Scharfmann R (2005) Fibroblast growth factors 7 and 10 are expressed in the human embryonic pancreatic mesenchyme and promote the proliferation of embryonic pancreatic epithelial cells. Diabetologia 48(2):277–281
56. Jacquemin P et al (2006) An endothelial-mesenchymal relay pathway regulates early phases of pancreas development. Dev Biol 290(1):189–199
57. Yoshitomi H, Zaret KS (2004) Endothelial cell interactions initiate dorsal pancreas development by selectively inducing the transcription factor Ptf1a. Development 131(4):807–817
58. Campos ML et al (2013) ICAT is a novel Ptf1a interactor that regulates pancreatic acinar differentiation and displays altered expression in tumours. Biochem J 451(3):395–405
59. Fujitani Y et al (2006) Ptf1a determines horizontal and amacrine cell fates during mouse retinal development. Development 133(22):4439–4450
60. Nakhai H et al (2007) Ptf1a is essential for the differentiation of GABAergic and glycinergic amacrine cells and horizontal cells in the mouse retina. Development 134(6):1151–1160
61. Dullin JP et al (2007) Ptf1a triggers GABAergic neuronal cell fates in the retina. BMC Dev Biol 7:110
62. Jin K et al (2015) Tfap2a and 2b act downstream of Ptf1a to promote amacrine cell differentiation during retinogenesis. Mol Brain 8:28
63. Li S et al (2004) Foxn4 controls the genesis of amacrine and horizontal cells by retinal progenitors. Neuron 43(6):795–807
64. Luo H et al (2012) Forkhead box N4 (Foxn4) activates Dll4-Notch signaling to suppress photoreceptor cell fates of early retinal progenitors. Proc Natl Acad Sci USA 109(9):E553–E562
65. Xiang M, Li S (2013) Foxn4: a multi-faceted transcriptional regulator of cell fates in vertebrate development. Sci China Life Sci 56(11):985–993
66. Lelievre EC et al (2011) Ptf1a/Rbpj complex inhibits ganglion cell fate and drives the specification of all horizontal cell subtypes in the chick retina. Dev Biol 358(2):296–308
67. Liu H et al (2013) An isoform of retinoid-related orphan receptor beta directs differentiation of retinal amacrine and horizontal interneurons. Nat Commun 4:1813
68. Menuchin-Lasowski Y et al (2016) Sip1 regulates the generation of the inner nuclear layer retinal cell lineages in mammals. Development 143(15):2829–2841
69. Wei W et al (2018) Requirement of the Mowat-Wilson syndrome gene Zeb2 in the differentiation and maintenance of non-photoreceptor cell types during retinal development. Mol Neurobiol. https://doi.org/10.1007/s12035-018-1186-6
70. Xiao D, Jin K, Xiang M (2018) Necessity and sufficiency of Ldb1 in the generation, differentiation and maintenance of non-photoreceptor cell types during retinal development. Front Mol Neurosci 11:271
71. Jin K (2017) Transitional progenitors during vertebrate retinogenesis. Mol Neurobiol 54(5):3565–3576
72. Bassett EA et al (2012) Overlapping expression patterns and redundant roles for AP-2 transcription factors in the developing mammalian retina. Dev Dyn 241(4):814–829
73. Watanabe S et al (2015) Prdm13 regulates subtype specification of retinal amacrine interneurons and modulates visual sensitivity. J Neurosci 35(20):8004–8020
74. Goodson NB et al (2018) Prdm13 is required for Ebf3+ amacrine cell formation in the retina. Dev Biol 434(1):149–163
75. Sapkota D et al (2014) Onecut1 and Onecut2 redundantly regulate early retinal cell fates during development. Proc Natl Acad Sci USA 111(39):E4086–E4095
76. Wu F et al (2013) Onecut1 is essential for horizontal cell genesis and retinal integrity. J Neurosci 33(32):13053–13065, 13065a
77. Poche RA et al (2007) Lim1 is essential for the correct laminar positioning of retinal horizontal cells. J Neurosci 27(51):14099–14107
78. Margeta MA (2008) Transcription factor Lim1 specifies horizontal cell laminar position in the retina. J Neurosci 28(15):3835–3836
79. Jin K et al (2010) Early B-cell factors are required for specifying multiple retinal cell types and subtypes from postmitotic precursors. J Neurosci 30(36):11902–11916
80. Jiang H, Xiang M (2009) Subtype specification of GABAergic amacrine cells by the orphan nuclear receptor Nr4a2/Nurr1. J Neurosci 29(33):10449–10459
81. Riesenberg AN et al (2009) Rbpj cell autonomous regulation of retinal ganglion cell and cone photoreceptor fates in the mouse retina. J Neurosci 29(41):12865–12877
82. Zheng MH et al (2009) The transcription factor RBP-J is essential for retinal cell differentiation and lamination. Mol Brain 2:38
83. Glasgow SM et al (2005) Ptf1a determines GABAergic over glutamatergic neuronal cell fate in the spinal cord dorsal horn. Development 132(24):5461–5469
84. Huang M et al (2008) Ptf1a, Lbx1 and Pax2 coordinate glycinergic and peptidergic transmitter phenotypes in dorsal spinal inhibitory neurons. Dev Biol 322(2):394–405
85. Todd AJ (1996) GABA and glycine in synaptic glomeruli of the rat spinal dorsal horn. Eur J Neurosci 8(12):2492–2498
86. Todd AJ, Sullivan AC (1990) Light microscope study of the coexistence of GABA-like and glycine-like immunoreactivities in the spinal cord of the rat. J Comp Neurol 296(3):496–505
87. Wapinski OL et al (2013) Hierarchical mechanisms for direct reprogramming of fibroblasts to neurons. Cell 155(3):621–635
88. Hanotel J et al (2014) The Prdm13 histone methyltransferase encoding gene is a Ptf1a–Rbpj downstream target that suppresses glutamatergic and promotes GABAergic neuronal fate in the dorsal neural tube. Dev Biol 386(2):340–357
89. Wildner H et al (2013) Genome-wide expression analysis of Ptf1a- and Ascl1-deficient mice reveals new markers for distinct dorsal horn interneuron populations contributing to nociceptive reflex plasticity. J Neurosci 33(17):7299–7307
90. Borromeo MD et al (2014) A transcription factor network specifying inhibitory versus excitatory neurons in the dorsal spinal cord. Development 141(14):2803–2812
91. Zainolabidin N et al (2017) Distinct Activities of Tfap2A and Tfap2B in the Specification of GABAergic Interneurons in the Developing Cerebellum. Front Mol Neurosci 10(281):281
92. Li S et al (2005) Foxn4 acts synergistically with Mash1 to specify subtype identity of V2 interneurons in the spinal cord. Proc Natl Acad Sci USA 102(30):10688–10693
93. Hoshino M et al (2005) Ptf1a, a bHLH transcriptional gene, defines GABAergic neuronal fates in cerebellum. Neuron 47(2):201–213
94. Pascual M et al (2007) Cerebellar GABAergic progenitors adopt an external granule cell-like phenotype in the absence of Ptf1a transcription factor expression. Proc Natl Acad Sci USA 104(12):5193–5198
95. Yamada M et al (2014) Specification of spatial identities of cerebellar neuron progenitors by ptf1a and atoh1 for proper production of GABAergic and glutamatergic neurons. J Neurosci 34(14):4786–4800
96. Millen KJ et al (2014) Transformation of the cerebellum into more ventral brainstem fates causes cerebellar

agenesis in the absence of Ptf1a function. Proc Natl Acad Sci USA 111(17):E1777–E1786
97. Sellick GS et al (2004) Mutations in PTF1A cause pancreatic and cerebellar agenesis. Nat Genet 36(12):1301–1305
98. Iskusnykh IY, Steshina EY, Chizhikov VV (2016) Loss of Ptf1a leads to a widespread cell-fate misspecification in the brainstem, affecting the development of somatosensory and viscerosensory nuclei. J Neurosci 36(9):2691–2710
99. Yamada M et al (2007) Origin of climbing fiber neurons and their developmental dependence on Ptf1a. J Neurosci 27(41):10924–10934
100. Fujiyama T et al (2009) Inhibitory and excitatory subtypes of cochlear nucleus neurons are defined by distinct bHLH transcription factors, Ptf1a and Atoh1. Development 136(12):2049–2058
101. Fujiyama T et al (2018) Forebrain Ptf1a is required for sexual differentiation of the brain. Cell Rep 24(1):79–94
102. Nakamura N et al (2016) Neonatal kisspeptin is steroid-independently required for defeminisation and peripubertal kisspeptin-induced testosterone is required for masculinisation of the brain: a behavioural study using Kiss1 knockout rats. J Neuroendocrinol 28. https://doi.org/10.1111/jne.12409
103. Sasselli V, Pachnis V, Burns AJ (2012) The enteric nervous system. Dev Biol 366(1):64–73
104. Wakayama K et al (2002) Localization of norepinephrine and serotonin transporter in mouse brain capillary endothelial cells. Neurosci Res 44(2):173–180
105. Takanaga H et al (2001) GAT2/BGT-1 as a system responsible for the transport of γ-aminobutyric acid at the mouse blood–brain barrier. J Cereb Blood Flow Metab 21(10):1232–1239
106. Pattyn A et al (1999) The homeobox gene Phox2b is essential for the development of autonomic neural crest derivatives. Nature 399(6734):366–370
107. Watanabe Y et al (2013) Sox10 and Itgb1 interaction in enteric neural crest cell migration. Dev Biol 379(1):92–106
108. Memic F et al (2016) Ascl1 is required for the development of specific neuronal subtypes in the enteric nervous system. J Neurosci 36(15):4339–4350
109. Lei J, Howard MJ (2011) Targeted deletion of Hand2 in enteric neural precursor cells affects its functions in neurogenesis, neurotransmitter specification and gangliogenesis, causing functional aganglionosis. Development 138(21):4789–4800
110. Uribe RA, Gu T, Bronner ME (2016) A novel subset of enteric neurons revealed by ptf1a:GFP in the developing zebrafish enteric nervous system. Genesis 54(3):123–128
111. Vlangos CN et al (2013) Next-generation sequencing identifies the Danforth's short tail mouse mutation as a retrotransposon insertion affecting Ptf1a expression. PLoS Genet 9(2):e1003205
112. Semba K et al (2013) Ectopic expression of Ptf1a induces spinal defects, urogenital defects, and anorectal malformations in Danforth's short tail mice. PLoS Genet 9(2):e1003204
113. Lugani F et al (2013) A retrotransposon insertion in the 5′ regulatory domain of Ptf1a results in ectopic gene expression and multiple congenital defects in Danforth's short tail mouse. PLoS Genet 9(2):e1003206
114. Tutak E et al (2009) A Turkish newborn infant with cerebellar agenesis/neonatal diabetes mellitus and PTF1A mutation. Genet Couns 20(2):147–152
115. Al-Shammari M et al (2011) A novel PTF1A mutation in a patient with severe pancreatic and cerebellar involvement. Clin Genet 80(2):196–198
116. Houghton JA et al (2016) Isolated pancreatic aplasia due to a hypomorphic PTF1A mutation. Diabetes 65(9):2810–2815
117. Gabbay M et al (2017) Pancreatic agenesis due to compound heterozygosity for a novel enhancer and truncating mutation in the PTF1A gene. J Clin Res Pediatr Endocrinol 9(3):274–277
118. Krah NM et al (2015) The acinar differentiation determinant PTF1A inhibits initiation of pancreatic ductal adenocarcinoma. Elife 4:e07125
119. Aichler M et al (2012) Origin of pancreatic ductal adenocarcinoma from atypical flat lesions: a comparative study in transgenic mice and human tissues. J Pathol 226(5):723–734
120. Naqvi AAT, Hasan GM, Hassan MI (2018) Investigating the role of transcription factors of pancreas development in pancreatic cancer. Pancreatology 18(2):184–190
121. Nair GG, Vincent RK, Odorico JS (2014) Ectopic Ptf1a expression in murine ESCs potentiates endocrine differentiation and models pancreas development in vitro. Stem Cells 32(5):1195–1207
122. Xiao D et al (2018) Direct reprogramming of fibroblasts into neural stem cells by single non-neural progenitor transcription factor Ptf1a. Nat Commun 9(1):2865
123. Ring KL et al (2012) Direct reprogramming of mouse and human fibroblasts into multipotent neural stem cells with a single factor. Cell Stem Cell 11(1):100–109
124. Zhang J et al (2017) A role for dystonia-associated genes in spinal GABAergic interneuron circuitry. Cell Rep 21(3):666–678
125. Seto Y et al (2014) Temporal identity transition from Purkinje cell progenitors to GABAergic interneuron progenitors in the cerebellum. Nat Commun 5:3337
126. Hamilton BA (2013) Retrotransposon activates ectopic Ptf1 expression: a short tail. PLoS Genet 9(2):e1003331