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Interplay of Transcriptional Factors In Beta Cells Development and Maturation

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Abstract

Diabetes is a group of metabolic disorders resulting from defects in insulin secretion, insulin action, or both. It is characterized by high blood sugar levels over a prolonged period of time with disturbances of carbohydrate, protein, and fat metabolism. It is a global disorder affecting about half a billion or 536.6 million people worldwide, which is said to rise to 783.2 million by 2045. To maintain normoglycemia, pancreatic β-cells release insulin in proportion to the amounts of nutrients in the blood. Through a process of postnatal development, β-cells learn to connect insulin release to food cues. The insulin secretory response in mature β-cells adjusts to alterations in nutritional status. The interaction between transcriptional programs specific to each cell type and external stimuli is necessary for both β-cell maturation and functional adaptation. In this review, we examine the growing data that suggests lineage-determining and signal-dependent transcription factors (LDTFs and SDTFs, respectively) work together to regulate β-cell activity during development and homeostasis. In-depth knowledge of β-cell SDTFs and their corresponding signals would clarify the processes involved in β-cell maturation and functional adaptation, directly affecting diabetes treatments and the production of mature β-cells from stem cells.

Keywords: Diabetes, Insulin, Sugar level, Beta cell, Transcription factors.

Introduction:

Diabetes is a group of metabolic disorders resulting from defects in insulin secretion, insulin action, or both. It is characterized by high blood sugar levels over a prolonged period of time with disturbances of carbohydrate, protein, and fat metabolism It is a global disorder affecting about half a billion or 536.6 million people worldwide, which is said to rise to 783.2 million by 2045. India is the second largest hub with 74.2 million diabetic reported cases, which will grow to 124.9 million by 2045 as per International Diabetic Federation (IDF) reports 2021.

According to research conducted throughout the globe, Type-I or Type-II diabetes are likely to develop as a result of decreased beta cell function or a considerable loss in beta cell mass. To reverse diabetes, transplantation of β cells is a promising replacement therapy, but limitations like tissue rejection and low donor availability pose a challenge to widespread application. Thus, to enhance pancreatic islet functional beta cell mass (BCM), the induction of endogenous regeneration of beta cells seems to be a potential strategy in diabetic patients. Regeneration of beta cells either by trans-differentiation of alpha or delta cells of pancreas islet or stimulation of progenitor of beta cells could be a promising curative strategy to overcome the long-term challenges of diabetes. [1]

In last decade, many of the studies used various approaches like to trigger different proliferative and trans differentiation responses, plasticity to reprogram pancreatic cells and neogenesis (differentiation of new β-cells from endocrine progenitors or stem cells) for the endogenous regeneration of pancreatic beta cell mass. Thus, regeneration of functional beta cell mass is the safe approach to diabetes therapy. In their study, Zhong and Jiang have provided comprehensive overview of the strategies for regulating β cell regeneration, and discuss the various factors that are involved in this process, such as mediators, transcription factors, signaling pathways, and potential pharmaceutical drugs. [2]

From the early pancreatic progenitor formation up to the development of functional beta cell mass, at every step, various transcription factors are involved. Transcription factors are involved in early pancreatic progenitor formation, include pancreas/ duodenum homeobox protein 1 (PDX1), forkhead box A2 (FOXA2), and sex determining region Y-box 17 (SOX17). Few transcription factors critical for endocrine lineage specification and differentiation, are Neurogenin 3 (NEUROG3) and neurogenic differentiation 1 (NEUROD1). V-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MAFA), V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (MAFB), paired box gene 6 (PAX6), and estrogen-related receptor gamma regulate late maturation of beta cells. [3]

Pancreatic development

Pancreatic development is a highly orchestrated process crucial for ensuring proper organ functionality. In mice, this process begins with the formation of dorsal and ventral pancreatic buds, both contributing to the development of endocrine and exocrine cells. These buds play a vital role in generating the first and second waves of endocrine cells, alongside exocrine cells. However, zebrafish pancreas development showcases distinct characteristics. While dorsal and ventral buds still form, their fate differs significantly. In zebrafish, the dorsal bud exclusively gives rise to first wave endocrine cells, while the ventral bud contributes to the formation of second wave endocrine cells, exocrine cells, and duct cells. Remarkably, all cells forming the mature zebrafish pancreas originate from the ventral bud. Moreover, the diversity of early endocrine cell types in zebrafish, including α-cells, β-cells, δ-cells, and ε-cells, contrasts with the predominant Gcg expression in early endocrine cells in mice. Molecular control mechanisms underlying pancreatic bud formation exhibit variances between the two species. While homeobox transcription factors like Pdx1 and Mnx1/Hb9 are crucial in both mice and zebrafish, their specific functions diverge. In mice, knockout studies reveal their necessity for early pancreas morphogenesis and β-cell maturation. Conversely, in zebrafish, morpholino-based knockdown studies demonstrate a conserved function in specifying second wave endocrine cells and β-cell maturation. This highlights the complexity of pancreatic development across species, emphasizing the importance of understanding both similarities and differences to unravel the underlying molecular mechanisms. [4]

Zebra Fish – An important model for regeneration studies

Zebrafish have been widely utilized to study human diseases and the biology of vertebrates. This is a result of the model's numerous experimental advantages. Genetically, it is very tractable due to fecundity and oviparous reproduction. With readily available chemicals and equipment, highly efficient mutagenesis and transgenesis can be accomplished with little training. Because of this, numerous resource centers offer a huge number of mutant and transgenic lines at a nominal fee. Zebrafish are an ideal model organism for research because of their rapid development of translucent embryos, which allows for the direct visualization of numerous developmental events. Because the embryo and larvae are small enough to be treated on microtiter plates and have easily observable morphological or behavioral outcomes, their compatibility with chemical screening is enhanced. Zebrafish are being utilized more and more not just in developmental biology but also in drug development and mechanistic research to represent human disorders. The ability of the zebrafish pancreas to

regenerate is astounding. We now have a better grasp of the signaling pathways and transcription factors involved in β-cell neogenesis, trans dedifferentiation, and proliferation thanks to recent discoveries on zebrafish β-cell regeneration (Fig. 1). [5,6]

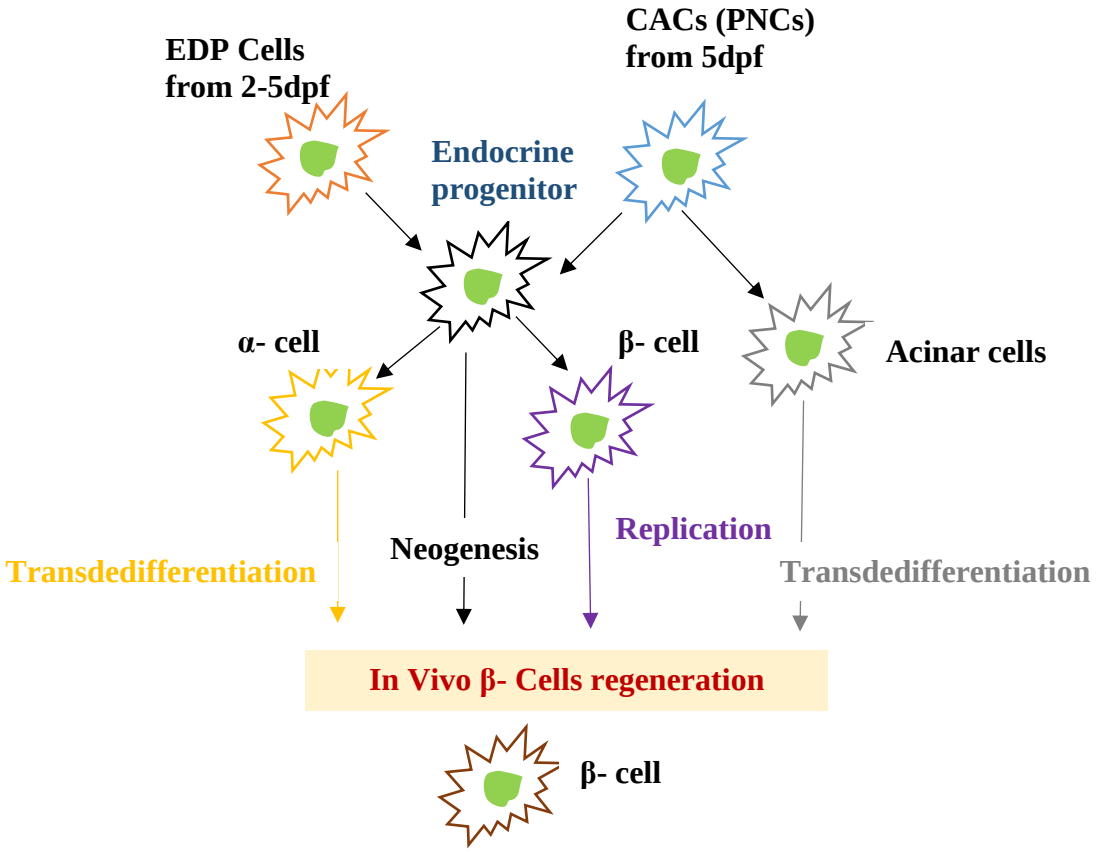

Fig.1 In vivo generation of β cells in Zebrafish using cell sources

Significance of transcriptional factors (TFs) in beta cell regeneration/development

Transcription factors (TFs) play a critical role in the development and regeneration of beta cells within the pancreas, essential for maintaining metabolic balance in vertebrates. The pancreatic islets, known as the islets of Langerhans, house various cell subtypes, each responsible for producing specific hormones crucial for metabolic regulation. During embryonic development, pancreatic progenitor cells emerge from the dorsal and ventral pancreatic buds, regulated by a cascade of TFs. Initially, TFs such as PDX1 and PTF1a specify pancreatic progenitors. Following this, HLH factors including NGN3 and NEUROD induce the formation of endocrine precursors. Finally, homeodomain factors such as NKX2.2, NKX6.1, PAX6, PAX4, and ARX guide the differentiation into distinct endocrine cell subtypes.[7]

Two groups of TFs are vital for pancreas development. The first group, including Pdx1, Ptf1a, and Hlxb9, directs the specification of the pancreatic anlage, essential for both endocrine and exocrine lineage development. Pdx1, ubiquitous in the duodenum and pancreatic anlage, is crucial for beta- and exocrine cell formation. Similarly, Ptf1a, initially recognized for its role in exocrine differentiation, also steers pancreatic precursors, including endocrine lineages, away from a duodenal fate. Hlxb9, expressed throughout the pancreatic anlage, contributes to this process.[8]

The second set of TFs determines specific cell types within the pancreas from partially differentiated precursor cells. NeuroD, Pax6, Pax4, and Isl1 are pivotal members guiding endocrine lineage differentiation. For instance, Nkx2.2 transitions from broad expression in pancreatic buds to confined expression in endocrine lineage cells during mouse development. Many of these TFs are also active in zebrafish pancreas development, including pdx1, nkx2.2a, pax6b, and islet1, contributing significantly to pancreas development. Understanding the intricate regulatory roles of TFs in pancreas development is crucial for unravelling mechanisms underlying

beta cell regeneration and dysfunction, holding promise for therapeutic interventions targeting metabolic disorders. [9]

Expression of TFs in mammals and zebrafish

Understanding TF expression patterns and functions is pivotal for deciphering the complex mechanisms underlying development and physiology in different species. Mammals boast a rich repertoire of TFs, intricately orchestrating cellular functions. In parallel, Zebrafish, renowned for their transparency and genetic tractability, offer a unique model to study vertebrate development. While TF conservation exists between mammals and zebrafish, evolutionary divergence has also led to distinctive expression profiles and functional adaptations. This review aims to delve into TF expression in both mammals and zebrafish, uncovering shared regulatory networks alongside species-specific nuances. Table no. 1 illustrates the genes expressed prominently in Zebrafish.

Table1. Expression and function of genes in developing pancreas of zebrafish

Transcription Factor	Onset	Domain Expression	Mammalian disease association
Sox4b	11.5 hpf	Pancreas primordium	α,β- Cells reduced
mnx1 **(was *hlxb9, hb9*)**	14 hpf	Endocrine pancreas	Pancreatic dorsal lobe agenesis, abnormal islets
pdx1	14 hpf	Pancreas primordium later endocrine specific	Pancreatic agenesis, Pancreatic hypoplasia
nkx2.2a	14 hpf	Endocrine pancreas, pancreatic duct	Hyperglycemia
isl1 (islet1)	15 hpf	Endocrine pancreas	Islets agenesis
pax6b **(was *pax6.2*)**	15 hpf	Pancreas primordium	Reduced hormone production, Hypomorphic Islet
neurod **(was *neuroD*)**	15 hpf	Endocrine pancreas β-Cells	Non- insulin dependent Diabetes mellitus
hhex	18 hpf	Pancreas primordium	Non- insulin dependent Diabetes mellitus
hlxb9la **(was *mnr2a*)**	24 hpf	Exocrine Pancreas	-
hnf1b **(was *vhnf1,tcf2*)**	26 hpf	General Pancreas	MODY5
ptf1a **(*p48*)**	32 hpf	Exocrine, later endocrine pancreas	Pancreatic agenesis, permanent neonatal diabetes mellitus

Sox4 (SRY- BOX Transcription Factor 4)

In zebrafish, **Sox4**, a member of the SRY-like HMG-box (SOX) family, is essential for proper differentiation of endocrine cells. Sox4b's involvement in differentiation is supported by its increased expression in mind bomb mutant embryos, which exhibit accelerated pancreatic cell development. Knockdown of sox4b leads to a notable decrease in glucagon expression, while insulin, somatostatin, and trypsin levels remain relatively unaffected. This disruption in α cell differentiation is linked to reduced expression of the homeobox gene arx specifically within the pancreas. Sox4b serves as an early and temporary marker in the pancreatic epithelium, primarily guiding α cell differentiation, the final step in endocrine cell differentiation. However, while its expression suggests a role in pancreas development, its precise function remains unclear. Notably, in sox4b morphant embryos, endocrine cells fail to cluster into compact islets, indicating potential involvement in cell migration and islet formation. Additionally, sox4b may contribute to the differentiation of other types of pancreatic hormone cells, possibly in conjunction with other Sox genes like sox11 and sox12. Despite the overlap in expression patterns between Sox4 and Sox11 in mouse pancreas development, zebrafish orthologs of Sox11 (sox11a and sox11b) and sox12 do not show expression in the developing pancreas. [10,11]

Mnx-class homeobox transcription factor Hb9 (Homeobox gene 9)

In mice, the (**Motor Neuron and Pancreas Homeobox) Mnx-class homeobox transcription factor Hb9** (Homeobox gene 9) plays a crucial role in pancreas development, being necessary for both the initial formation of the pancreas and the differentiation of insulin-producing h-cells. However, in zebrafish, knockdown studies using antisense morpholinos reveal that while hb9 is essential for h-cell differentiation, it is not required for early pancreas morphogenesis, contrasting with observations in mice. Additionally, another related gene, mnr2a, is implicated in late morphogenesis of the exocrine pancreas in zebrafish. This suggests a tissue-specific expression pattern for mnx genes in the zebrafish pancreas and highlights a novel role for an mnr2-related gene.

In humans, the homeodomain protein HB9 is crucial for various stages of pancreas development, with its loss of function associated with Currarino syndrome. While only one hb9 gene is known in mammals, both hb9 and mnr2 genes have been identified in chickens, indicating different subfamilies within the Mnx Proteins. Expression patterns of hb9 in zebrafish suggest its involvement in early gut progenitors, potentially marking progenitors of h-cells similar to mice. [12]

Pancreas/ duodenum homeobox protein 1 (PDX1)

Pdx1, also known as insulin promoter factor 1, is a homeodomain transcription factor. Pdx1 expression is observed around gestational week 4 in humans. Along with early embryonic development of the pancreas, Pdx1 is also required for the subsequent differentiation of pancreatic lineages. In mature beta cells, depletion and reduction of Pdx1 induce glucose intolerance. The maturity-onset diabetes of the young 4 (MODY4), is also caused due to monogenic defects (heterozygous) in the Pdx1 gene. In human T1DM patients, Pdx1 autoantibodies are detected, suggesting Pdx1 could be an autoantigen for T1DM, whereas, in human type 2 diabetes mellitus (T2DM), Pdx1 expression levels of islet beta cells are compromised. Pdx1 is expressed in the entire duodenum, including the pancreatic anlage, and loss of Pdx1 function leads to the formation of a rudimentary pancreas devoid of beta- and exocrine cells [13]

Cdx4 transcription factor

The **Cdx4 transcription factor** plays a crucial role in determining the normal posterior boundary of the pancreatic field. Zebrafish mutants lacking cdx4/kgg not only exhibit a pancreas located abnormally far towards the posterior, but also show an excess of b-cells. This suggests that while cdx4 is expressed across all three germ layers during early development, its mesodermal expression is not essential for pancreas localization; rather, it is the endodermal expression of Cdx4 that is crucial. There is a cooperative interaction between mesodermal retinoic acid (RA) and endodermal Cdx4, where RA positively influences mesodermal development while Cdx4 negatively regulates endodermal development, collectively establishing the pre-pancreatic region within the endoderm. [14]

Fibroblast growth factor (FGF) signaling is pivotal in various aspects of pancreatic development. Wells and Melton demonstrated that different concentrations of FGF4 influenced the fate of cultured E7.5 mouse endoderm, with higher concentrations favoring intestinal fates and lower concentrations favoring pancreas/duodenal fates. Further studies by Wells and colleagues revealed that local application of FGF to chick embryos shifted the expression domain of CdxB (the zebrafish Cdx4 homolog) anteriorly during early somite stages, resembling the effect of Cdx4 deficiency observed in zebrafish, where the Pdx1 progenitor marker domain expanded posteriorly. This suggests that the regulation of Cdx4 by FGF4 could be a fundamental mechanism in regionalizing endoderm, likely conserved across vertebrates. Additionally, FGFs appear to have multiple sequential roles in pancreas development, including the later role of chick FGF2 in repressing sonic hedgehog (Shh) expression in the posterior foregut, as reported by Hebrok et al. Furthermore, Manfroid et al. reported that zebrafish fgf24 expressed in the endoderm plays a later role in patterning the adjacent pancreatic lateral plate mesoderm (LPM), with fgf24 and fgf10 from the LPM signaling back to the endoderm to aid in specifying the exocrine pancreas. [15]

Isl1(Insulin gene enhancer protein 1)

Isl1(Insulin gene enhancer protein 1), a LIM homeobox transcription factor, exhibits conserved expression patterns in vertebrate pancreas development. In zebrafish, as in mice, Isl1 transiently expresses in mesenchyme adjacent to pancreatic endoderm and continuously in all endocrine cells. Mutant zebrafish lacking Isl1 display reduced glucagon expression in early forming cells and fewer somatostatin-expressing cells, while later-born cells show reduced somatostatin expression but normal insulin and glucagon levels. Additionally, Isl1 mutants exhibit a smaller exocrine pancreas. In mice, Isl1 is essential for early pancreas morphogenesis and endocrine differentiation, with roles in insulin regulation and pancreatic mesenchyme induction demonstrated in both species. Molecular studies in mice reveal Isl1's direct regulation of genes like MafA and Arx involved in β- and α-cell differentiation, as well as its influence on islet-cell proliferation through c-Myc and Cyclin D1 regulation. Moreover, the co-regulator Ldb1 is crucial for Isl1-dependent expression of key genes regulating insulin synthesis and release. [16]

Paired box protein 6 (PAX6)

PAX6, also known as aniridia type II (AN2) protein, is a transcription factor essential for the normal development and maturation of the central nervous system (eyes and brain) and pancreas (St-Onge et al.; Turque et al.). The targeted mutation of the PAX6 gene in mice has been shown to result in the absence of glucagon-producing cells, underscoring the pivotal role of this gene in the morphogenesis of pancreatic islets and the differentiation of alpha cells (Sander et al., 1997; St-Onge et al., 1997). Recent investigations into zebrafish genetics have revealed the presence of two homologous genes to PAX6 within their genome (Nornes et al., 1998). Specifically, the expression of pax6.2 has been observed in the developing zebrafish pancreas starting at the 12-somite stage, forming a distinct layer above the yolk sac. This expression persists throughout somitogenesis, remaining visibly detectable at 24 hours post-fertilization (hpf). Conversely, there is no evidence of pax6.1 expression in the pancreatic primordia at any developmental stage. Insights from gene targeting studies involving pax4 and pax6 in mice have suggested a close interrelationship between beta and delta cells, challenging previous notions of independent lineage differentiation (Sosa-Pineda et al., 1997; St-Onge et al., 1997; Herrera et al., 1998). The selective expression of pax6.2, but not pax6.1, in the zebrafish pancreas exemplifies the divergence in gene expression domains and functions following gene duplication in the evolutionary lineage leading to teleosts. [17]

Neurod

The differentiation of alpha and beta cells in zebrafish is intricately regulated by Neurod, a transcription factor whose levels dictate cell fate determination. This process necessitates varying levels of Neurod, with higher concentrations required for the differentiation of glucagon-positive alpha cells compared to insulin-positive beta cells. Neurod deficiency leads to diminished endocrine hormone expression in the primary islet, prompting premature production of endocrine precursors from the Islet Progenitor Domain (IPD). However, these precursors fail to undergo complete differentiation, resulting in an inability to regulate glucose levels effectively in larval zebrafish. Notably, Neurod plays a critical role in the differentiation of endocrine cells from both dorsal and ventral buds. While glucagon-positive alpha cells exhibit heightened sensitivity to reduced Neurod function, insulin-positive beta cells are comparatively less affected. The interplay between Neurod and other factors such as Mnx1 and Ascl1b further influences cell fate determination, with Neurod collaborating with Mnx1 for beta cell differentiation and complementing the role of Ascl1b in zebrafish endocrine cell development. Overall, Neurod's differential activation levels are pivotal in determining the fate choice between alpha and beta cells, with its deficiency primarily hindering the differentiation of endocrine precursors and subsequent hormone production in zebrafish specimens. [18]
Notch-responsive cells (NRCs) in the duct are a shared source of endocrine precursor cells in mice and zebrafish. Studies of Dalgin et al. show that Neurod expression levels influence cell fate decisions following Notch signaling inhibition in these precursors. Notch-mediated suppression of cellular differentiation involves Hes1 activation, which inhibits bHLH proteins like neurog3 and neurod, both implicated in pancreatic development. Notably, inhibiting Notch signaling increases neurod expression, supported by observations in zebrafish mind bomb mutants. Considering the HES1 binding sites in the human NEUROG3 gene, investigating direct regulation of zebrafish neurod by Hes1 in the IPD is crucial. Additionally, Sussel et al. propose that Nkx2.2 and NeuroD1 jointly regulate various endocrine cell types in mice, emphasizing the intricate role of transcription factors in endocrine cell differentiation. [19]

Nkx2.2

Nkx2.2 emerges as a putative downstream target of Ngn3, exhibiting dynamic expression patterns across species. In mice, Nkx2.2 initially presents in most pancreatic bud cells but transitions to being confined to the endocrine lineage as development progresses. Conversely, zebrafish nkx2.2a expression initiates early in the pancreatic primordium, followed by detection in pancreatic islets, aligning with the onset of pdx1 expression. Subsequently, the appearance of group-2 genes like pax6b and islet1 occurs later in development. Through genetic manipulation and targeting of nkx2.2a, it's unveiled that its function and regulation during endocrine pancreas development are conserved between teleosts and mammals. Remarkably, nkx2.2a in zebrafish also extends its expression to precursor and differentiated cells of pancreatic ducts, playing a crucial role in the proper development of this segment of the exocrine pancreas, thus highlighting the evolutionary conservation and multifaceted roles of nkx2.2a in pancreatic development across vertebrate.

PTF1a, (pancreas associated transcription factor 1a)

Zebrafish **ptf1a,** expressed in cells of the anterior pancreatic bud at 30 hpf, exhibits conserved functionality in exocrine marker expression, as evidenced by trypsin deficiency in ptf1a morphants, while pancreatic islet development remains unaffected. Notably, ptf1a's involvement in late-differentiating endocrine cells from the anterior pancreatic bud suggests its significance in pancreas organogenesis. Additionally, parallels between zebrafish and mammalian ptf1a expression patterns underscore its role in both pancreatic and neural tissues. The initiation of pancreatic ptf1a expression at 32 hpf, alongside its association with ventral gut epithelium cells, suggests a pivotal role in pancreatic bud specification. These findings corroborate a morphogenetic model of zebrafish pancreas development, proposing ptf1a as a marker for anteroventral pancreatic bud formation and implicating its involvement in intestinal to pancreatic cell conversion, thereby emphasizing its crucial function in vertebrate pancreatic development. [12,19]

A novel gene, **exocrine differentiation and proliferation factor (exdpf**), identified in zebrafish, exhibits high conservation in mice and humans. It serves as a crucial determinant of exocrine cell fate and regulates cell proliferation. Expression analysis shows exdpf's significant presence in developing exocrine cells of the zebrafish pancreas. Knockdown experiments using antisense morpholino reveal a loss or reduction of exocrine markers, while exdpf overexpression leads to enlarged exocrine pancreas size and decreased endocrine cell count. This suggests exdpf misexpression can alter endocrine precursor fate. Additionally, exdpf morphants exhibit lineage-specific cell cycle arrest in exocrine cells, with increased expression of cell cycle inhibitor genes p21Cip and p27Kip. [11,12,19]

In both humans and zebrafish, mutations in **vhnf1** affect the kidney and pancreas, as observed in patients with MODY5. Additionally, one MODY5 patient exhibited delayed motor development. Studies by Sun Hopkin et al. indicate that vhnf1 acts upstream of pdx1 in regulating pancreatic development, suggesting the involvement of other components in this pathway in pancreatic diseases. Furthermore, vhnf1 operates within the same genetic network as pax2 and wt1 to regulate pronephros patterning. vHnf1 is implicated in regulating the expression of genes critical for adult organ functions, such as the insulin gene in the pancreas and the albumin gene in the liver. This suggests that MODY5 and GCKD may stem from vHnf1 dysfunctions unrelated to its role in organogenesis.[25]

GHRL, present in the pancreas along with its receptor GHS-R, serves as a marker for endocrine pancreatic development in zebrafish. Knockdown of the crucial transcription factor nkx2.2a leads to its utilization in this context. GHRL upregulates glucagon (GCG) while downregulating insulin (INS) synthesis in the zebrafish brain, akin to mammalian studies. This imbalance favors glycogen degradation facilitated by GP, while compromised INS signaling occurs downstream. FGF2 inhibits Shh expression in chick explants, a crucial step in amniote pancreas development. Conversely, in zebrafish, Hh signaling from the notochord is essential for pancreas development, with inhibition resulting in pancreas absence. This apparent species-specific difference reflects a disparity in developmental timing, with notochord-derived Shh playing an early positive role, while endoderm-derived Shh acts later, negatively regulating pancreas development. In summary, both FGFs and Hh signaling likely play sequential roles in pancreas development, with potential implications for adult endocrine pancreas cell function. [20]

Targeted studies have elucidated the critical roles of several transcription factors during various stages of endocrine pancreas development. These include Pdx-1, Isl-1, pax4, pax6, beta2/neuroD, nkx2.2, hb9, and hnf6. Additionally, numerous signaling molecules such as Sonic hedgehog (Shh), TGF-bs, FGFs, and the Notch/Delta system contribute to pancreatic endocrine development. Furthermore, genes essential for β-cell development (e.g., Nkx6.1, Mafa) and function (e.g., Ins1/2, Slc2a2, Glp1r, Chga) are regulated by various transcription factors, including GLIS3, alongside other islet-enriched transcription factors. Interactions between GLIS3 and key transcription factors like PDX1, NEUROD1, and MAFA underscore its significant impact on β-cell generation and function. These findings collectively shed light on the intricate regulatory mechanisms governing β-cell development and function, with implications for understanding and treating related disorders. [21]

Pancreatic Development in Zebrafish, Mouse and Lamprey

Pancreatic and duodenal homeobox 1 (pdx1) is expressed in the ventral and dorsal pancreatic buds, which arise at the start of pancreatic development in mammals. Dorsally rotating, the ventral bud merged with the dorsal bud. As a result, an ultimate pancreas is produced. Zebrafish undergo similar morphogenetic events when developing their pancreas. By 14 hours after fertilization (hpf), pdx1-positive cells emerge from endodermal cells and develop into pancreatic primordia. By 24 hours post fertilization, the dorsal bud separates from pdx1-positive pancreatic primordia.

In the lamprey, the ventral bud divides into the digestive tissues/organs, including the liver, gallbladder, bile ducts, and pancreatic acinar cells, while the dorsal bud differentiates into islets. Amphibians have learned to distinguish pancreatic exocrine compartments in the dorsal bud, while teleosts have learned to distinguish islets in the ventral pancreatic bud. Since it is unknown what molecular pathways are active in the Lamprey ventral bud, it is unclear how the teleost ventral bud acquired the capacity for islet differentiation.

Notch-On cells are retained by zebrafish for the whole of their lives, whereas in mice, these cells vanish after birth. Notch-On cells are a source of endocrine neogenesis in the adult zebrafish pancreas. They are restricted to the centroacinar cells, a type of ductal cell located at the center of acini. Thus, unique insights into their development as well as in the field of islet regeneration can be gained by comprehending the molecular and cellular mechanisms underpinning secondary islet formation, including the reduction of Notch signaling and the endocrine differentiation process. The comparison of pancreas lineages are shown in Fig.2. [22]

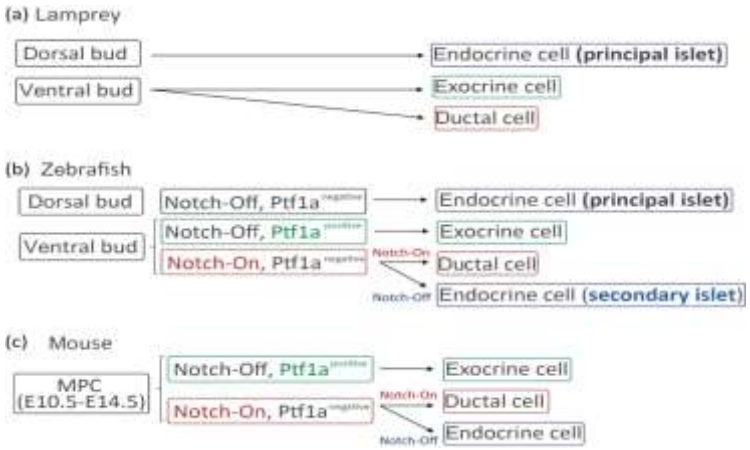

Fig.2 Comparison of development of pancreatic lineages in vertebrates

Physiology and Maturation of Islets in Vertebrates

Insulin is secreted by pancreatic β cells in response to elevated glucose levels. Under high glucose circumstances, glucose enters mammalian β cells via the glucose transporter GLUT2. Through glucose metabolism, the β cells then produce more ATP, increasing the ATP/ADP ratio. An increase in the ratio of ATP to ADP causes potassium (KATP) channels that are dependent on ATP to close, which in turn causes depolarization of the β cell and the activation of voltage-dependent Ca2+ channels that in turn cause the release of insulin.

Zebrafish pancreatic β cells express glut2, just like mammals do. Additionally, insulin production is regulated by KATP and Ca2+ channels in response to blood glucose levels. These suggest that the pancreatic β cells of zebrafish function similarly to those of other vertebrates. Nevertheless, primary islets—rather than subsidiary islets—were used in these studies. As a result, the extent to which secondary islets support glucose homeostasis is unknown. The molecular pathways that underlie the creation of secondary islets in zebrafish bear similarities to those in mammals. In order to comprehend how vertebrates have gained functional islets, it may be necessary to ascertain whether major and secondary islets differ functionally.[23]

The development of vertebrate organs frequently occurs in two stages. First, during embryogenesis, an organ forms that is functionally immature; this is followed by differentiation into the mature. The second stage often occurs when the concentration of thyroid hormone (TH) in plasma rises, during the postembryonic/postnatal

phase. Furthermore, based on cellular and molecular events in their digestive organs, including the pancreas, and elevation of plasma TH concentration during these periods, it has been proposed that mammalian weaning, frog metamorphosis, and the zebrafish larval–juvenile transition are functionally equivalent events during postembryonic development. Rats' pancreatic β cells begin GSIS about P10, and their plasma TH levels rise sharply around postnatal day 15. Following this, during the nursing-to-weaning transition, there is an increase in insulin release in response to glucose. Rats given TH exogenously starting at P1 develop GSIS by. It was also found that TH promoted GSIS in pancreatic β cells produced from pluripotent cells in vitro. It is evident from this that TH can activate the function of pancreatic β cells. [24]

Regeneration of Pancreatic Cells

Mammals are unable to regenerate β cells in the pancreas, whereas zebrafish may do so for the entirety of their lives. In order to find stimulators of β cell regeneration in zebrafish larvae, Anderson and colleagues screened a limited library of compounds. In larval zebrafish, they discovered that β cell regeneration was stimulated by adenosine signaling. Based on this, they administered NECA, an adenosine agonist, to hyperglycemic mice, which had decreased β cell numbers. This allowed them to induce β cell proliferation and partially restore blood glucose levels.

In zebrafish, notch-on cells support both β cell neogenesis and regeneration. Notch-On cells multiply and develop into β cells during β cell regeneration. Furthermore, Ye et al. (2015) reported that α cells serve as an additional source of β cell regeneration. Glucagon: Cre, insulin: Cre, somatostatin: Cre, and zebrafish transgenic lines were used by Ye and colleagues to conduct each pancreatic α cell, β cell, and δ cell lineage tracing investigation during β cell regeneration.

According to Ye et al. (2015), their findings demonstrated that α cells were the only ones that contributed to β cell regeneration in zebrafish, with β and δ cells not surviving. Additionally, they showed that α cells transdifferentiate into β cells during β cell regeneration in zebrafish. Thus, for β cell regeneration in zebrafish, two distinct systems - "Notch-On islet stem cell" and "α-cell transdifferentiating"—have been found. [25,26]
As of right now, crucial techniques for β cell regeneration in mammalian systems are unknown. By using pancreatic stem cells and promoting α cell trans differentiation to β cell, researchers have been trying to regenerate β cells. Research on zebrafish will contribute to the development of novel β cell regenerating techniques.

Discussion

In this review, the gene expression patterns of the primary pancreatic cell types in Zebrafish, identifying the unique gene expression profiles of acinar and endocrine cells, including the alpha, beta, and delta subtypes was studied. Through this analysis, numerous novel markers specific to each cell type, whose functions within the pancreas are not yet understood were also discovered. [21]

By comparing the gene expression profiles of endocrine and exocrine cells in zebrafish, humans, and mice, we established a set of evolutionarily conserved genes and pathways, indicating their importance across vertebrate species. Notably, over half of the transcription factors identified in these conserved profiles are known to regulate the differentiation of pancreatic cells, such as neuroD, isl1, pax6, insm1, ptf1a, and rbpjl. Among these, Myt1 is part of the conserved signature for endocrine cells, and our findings demonstrate that disrupting the myt1b gene in zebrafish results in reduced alpha cell mass in embryos at 2 days post-fertilization (dpf). Furthermore, it was found that cdx4, which is specifically expressed in delta cells, is essential for their differentiation. [27, 28]

Fish pancreas cells frequently use a number of signaling channels that control hormone release and cellular homeostasis, according to the endocrine signature shared by the three vertebrate species animals. For instance, the RNA binding protein Elavl4/Hud is part of the evolutionarily conserved signature. It includes the ionotropic glutamate receptor Gria2, which has been demonstrated to control the production and secretion of hormones in rodents. Likewise, the glucose response system that controls the release of glucagon and insulin in mammals appears to may be employed in zebrafish as well, since some elements of this system are present in the conserved endocrine genes, including the voltage-dependent Ca++ channel cacna2d2 and the ATP-sensitive K+ channels abcc8 and kcnj11. [29,30]

The extremely high expression of glucokinase (gck) and the glucose transporter glut2 (slc2a2) in zebrafish beta-endocrine cells supports the usage of this pathway in fish. Furthermore, a few GPCR receptors which have been shown to regulate the function of pancreatic islet cells in mice, including the somatostatin receptor Sstr3, CasR, and Celsr3 optimized endocrine profile. The identification of in human alpha and beta cells gene expressions are described in Fig3. [31,32]

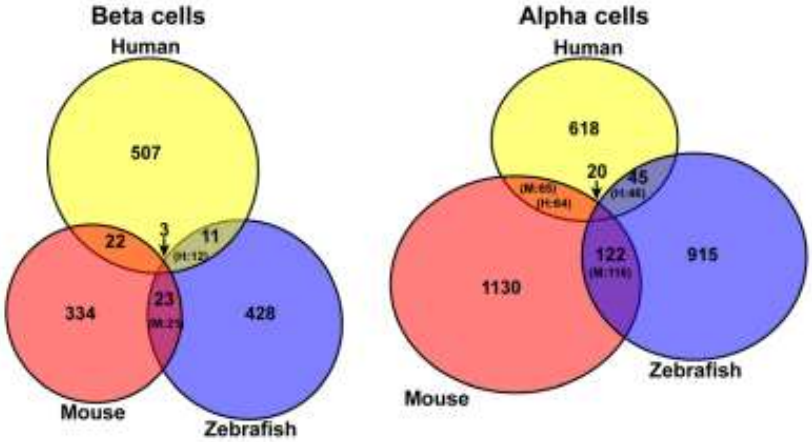

Fig.3 Alpha and Beta cells conserved gene expression identification

Future research should pay particular attention to the numerous unique potential regulatory genes that are identified by the conserved endocrine and exocrine pancreatic signatures. For example, the signaling proteins gpr158, rgs7, and rgs17, or transcription factors lmo1 and npas4a, which are all exclusively expressed in pancreatic endocrine cells in zebrafish, mice, and humans, most likely have a significant function in pancreatic cells. [33]

Conclusion

The conclusion highlights the intricate interplay among transcription factors crucial for the development and maturation of beta cells. Through our investigation, we have elucidated the collaborative efforts of various transcription factors in orchestrating the complex processes involved in beta cell development and maturation. Our findings underscore the importance of understanding the regulatory networks governing beta cell biology to uncover potential therapeutic targets for diabetes and related disorders.

In vivo β-cell regeneration holds promise as a potential cure for both types of diabetes, yet substantial work remains before this strategy can be clinically realized. Ongoing drug screening endeavors in zebrafish are expected to play a crucial role in advancing this goal. Besides screening larger compound libraries, future zebrafish research should also prioritize assessing the toxicity and specificity of identified compounds. The ultimate aim should be translation to humans. For any compound identified to promote β-cell regeneration in zebrafish, its effectiveness and specificity should be evaluated in rodent models, as demonstrated in previous studies. Additionally, aside from those promoting neogenesis, compounds should also undergo testing on human islets whenever feasible. Given the likelihood of multiple pathways influencing β-cell proliferation or trans differentiation in humans, combined treatment with multiple compounds may yield productive outcomes. Positive results from human islet studies should pave the way for clinical trials and eventual drug approval.

In summary, it sheds light on the significance of transcriptional regulation in beta cell development and maturation, offering valuable insights into the molecular mechanisms underlying these processes. This knowledge not only enhances our understanding of beta cell biology but also holds promise for the development of innovative strategies aimed at improving beta cell function and treating diabetes. Further research in this field is warranted to fully unravel the intricate network of transcriptional factors and their roles in beta cell biology, paving the way for novel therapeutic interventions.

The commonalities and distinctions between the pancreases of zebrafish and other vertebrates, emphasizing the attractiveness and uniqueness of zebrafish as a model for investigating pancreas development and the regeneration of functional pancreatic β cells has been analysed. In forthcoming research, examining the transcriptome and proteome at various time points and under diverse conditions will be instrumental in elucidating these phenomena further. Moreover, leveraging chemical screening techniques with zebrafish

larvae holds significant promise in identifying novel molecules that promote islet maturation and functional regeneration. The development of straightforward methods for generating conditional mutants in zebrafish is of paramount importance for advancing zebrafish research, particularly in the realm of regenerative biology.

References

1. International Diabetes Federation. IDF Diabetes Atlas, 7 edn. International Diabetes Federation: Brussels, Belgium, 2015
2. The Bastidas-ponce, A., Scheibner, K., Lickert, H., & Bakhti, M. (2017). Cellular and molecular mechanisms coordinating pancreas development, 1, 2873–2888.
3. Derrickson BH, Tortora GJ. Tortora's Principles of anatomy & physiology. 2017.
4. Chawla, P., Delgadillo Silva, L. F., & Ninov, N. (2020). Insights on β-cell regeneration from the zebrafish shoal: From generation of cells to functional integration. *Current Opinion in Physiology*, *14*, 27–34. https://doi.org/10.1016/j.cophys.2019.11.009
5. Zhu, Y., Liu, Q., Zhou, Z., & Ikeda, Y. (2017). PDX1, Neurogenin-3, and MAFA: Critical transcription regulators for beta cell development and regeneration. *Stem Cell Research & Therapy*, *8*(1), 240. https://doi.org/10.1186/s13287-017-0694-z
6. Jiang, Z., Song, J., Qi, F., Xiao, A., An, X., Liu, N., Zhu, Z., Zhang, B., & Lin, S. (2008). Exdpf Is a Key Regulator of Exocrine Pancreas Development Controlled by Retinoic Acid and ptf1a in Zebrafish. *PLoS Biology*, *6*(11), e293. https://doi.org/10.1371/journal.pbio.0060293
7. Kim, H. J., Sumanas, S., Palencia-Desai, S., Dong, Y., Chen, J.-N., & Lin, S. (2006). Genetic Analysis of Early Endocrine Pancreas Formation in Zebrafish. *Molecular Endocrinology*, *20*(1), 194–203. https://doi.org/10.1210/me.2005-0189
8. Kinkel, M. D., & Prince, V. E. (2009). On the diabetic menu: Zebrafish as a model for pancreas development and function. *BioEssays*, *31*(2), 139–152. https://doi.org/10.1002/bies.200800123
9. Jörgens, K., Hillebrands, J.-L., Hammes, H.-P., & Kroll, J. (2012). Zebrafish: A Model for Understanding Diabetic Complications. *Experimental and Clinical Endocrinology & Diabetes*, *120*(04), 186–187. https://doi.org/10.1055/s-0032-1304565
10. Musson, M. C., Jepeal, L. I., Mabray, P. D., Zhdanova, I. V., Cardoso, W. V., & Wolfe, M. M. (2009). Expression of glucose-dependent insulinotropic polypeptide in the zebrafish. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *297*(6), R1803–R1812. https://doi.org/10.1152/ajpregu.00288.2009
11. Tarifeño-Saldivia, E., Lavergne, A., Bernard, A., Padamata, K., Bergemann, D., Voz, M. L., Manfroid, I., & Peers, B. (2017). Transcriptome analysis of pancreatic cells across distant species highlights novel important regulator genes. *BMC Biology*, *15*(1), 21. https://doi.org/10.1186/s12915-017-0362-x
12. Pauls, S., Zecchin, E., Tiso, N., Bortolussi, M., & Argenton, F. (2007). Function and regulation of zebrafish nkx2.2a during development of pancreatic islet and ducts. *Developmental Biology*, *304*(2), 875–890. https://doi.org/10.1016/j.ydbio.2007.01.024
13. Conrad, E., Dai, C., Spaeth, J., Guo, M., Cyphert, H. A., Scoville, D., Carroll, J., Yu, W.-M., Goodrich, L. V., Harlan, D. M., Grove, K. L., Roberts, C. T., Powers, A. C., Gu, G., & Stein, R. (2016). The MAFB transcription factor impacts islet α-cell function in rodents and represents a unique signature of primate islet β-cells. *American Journal of Physiology-Endocrinology and Metabolism*, *310*(1), E91–E102. https://doi.org/10.1152/ajpendo.00285.2015
14. Wen, J. H., Chen, Y. Y., Song, S. J., Ding, J., Gao, Y., Hu, Q. K., Feng, R. P., Liu, Y. Z., Ren, G. C., Zhang, C. Y., Hong, T. P., Gao, X., & Li, L. S. (2009). Paired box 6 (PAX6) regulates glucose metabolism via proinsulin processing mediated by prohormone convertase 1/3 (PC1/3). *Diabetologia*, *52*(3), 504–513. https://doi.org/10.1007/s00125-008-1210-x
15. Cruz, S. A., Tseng, Y.-C., Kaiya, H., & Hwang, P. P. (2010). Ghrelin affects carbohydrate-glycogen metabolism via insulin inhibition and glucagon stimulation in the zebrafish (Danio rerio) brain. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *156*(2), 190–200. https://doi.org/10.1016/j.cbpa.2010.01.019
16. Scoville, D. W., Kang, H. S., & Jetten, A. M. (2020). Transcription factor GLIS3: Critical roles in thyroid hormone biosynthesis, hypothyroidism, pancreatic beta cells and diabetes. *Pharmacology & Therapeutics*, *215*, 107632. https://doi.org/10.1016/j.pharmthera.2020.107632
17. Elo, B., Villano, C. M., Govorko, D., & White, L. A. (2007). Larval zebrafish as a model for glucose metabolism: Expression of phosphoenolpyruvate carboxykinase as a marker for exposure to anti-diabetic compounds. *Journal of Molecular Endocrinology*, *38*(4), 433–440. https://doi.org/10.1677/JME-06-0037

18. Aguayo-Mazzucato C, Bonner-Weir S. Pancreatic β Cell Regeneration as a Possible Therapy for Diabetes. Cell Metabolism. 2018; 27:57–67. doi: 10.1016/j.cmet.2017.08.007.
19. Beer RL, Parsons MJ, Rovira M. Centroacinar cells: At the center of pancreas regeneration. Developmental Biol. 2016; 413:8–15. doi: 10.1016/j.ydbio.2016.02.027.
20. Benthuysen JR, Carrano AC, Sander M. Advances in β cell replacement and regeneration strategies for treating diabetes. J Clin Invest. 2016; 126:3651–3660. doi: 10.1172/JCI87439
21. Afelik, S., & Jensen, J. Notch signaling in the pancreas: Patterning and cell fate specification. *Wiley Interdisciplinary Reviews. Developmental Biology*, 2013; 2:531–544. https://doi.org/10.1002/wdev.99
22. Aguayo-Mazzucato, C., Koh, A., El Khattabi, I., Li, W. C., Toschi, E., Jermendy, A.,Bonner-Weir, S. Mafa expression enhances glucose-responsive insulin secretion in neonatal rat beta cells. *Diabetologia*, 2011;54:583–593. https://doi.org/10.1007/s00125-010-2026-z
23. Andersson, O., Adams, B. A., Yoo, D., Ellis, G. C., Gut, P., Anderson, R. M. Stainier, D. Y. Adenosine signaling promotes regeneration of pancreatic beta cells in vivo. *Cell Metabolism*, 2012; 15:885–894. https://doi.org/10.1016/j.cmet.2012.04.018
24. Biemar, F., Argenton, F., Schmidtke, R., Epperlein, S., Peers, B., & Driever, W. (2001). Pancreas development in zebrafish: Early dispersed appearance of endocrine hormone expressing cells and their convergence to form the definitive islet. *Developmental Biology*, **230**, 189–203. https://doi.org/10.1006/dbio.2000.0103
25. Sun, Z., & Hopkins, N. (2001). *Vhnf1* , the MODY5 and familial GCKD-associated gene, regulates regional specification of the zebrafish gut, pronephros, and hindbrain. *Genes &* Wallace, K. N., & Pack, M. (2003). Unique and conserved aspects of gut development in zebrafish. *Developmental Biology*, *255*(1), 12–29. https://doi.org/10.1016/S0012-1606(02)00034-9
26. *Development*, *15*(23), 3217–3229. https://doi.org/10.1101/gad946701
27. Wendik, B., Maier, E., & Meyer, D. (2004). Zebrafish mnx genes in endocrine and exocrine pancreas formation. *Developmental Biology*, *268*(2), 372–383. https://doi.org/10.1016/j.ydbio.2003.12.026
28. Wilfinger, A., Arkhipova, V., & Meyer, D. (2013). Cell type and tissue specific function of islet genes in zebrafish pancreas development. *Developmental Biology*, *378*(1), 25–37. https://doi.org/10.1016/j.ydbio.2013.03.009
29. Zecchin, E., Mavropoulos, A., Devos, N., Filippi, A., Tiso, N., Meyer, D., Peers, B., Bortolussi, M., & Argenton, F. (2004). Evolutionary conserved role of ptf1a in the specification of exocrine pancreatic fates. *Developmental Biology*, *268*(1), 174–184. https://doi.org/10.1016/j.ydbio.2003.12.016
30. Mavropoulos, A., Devos, N., Biemar, F., Zecchin, E., Argenton, F., Edlund, H., Motte, P., Martial, J. A., & Peers, B. (2005). Sox4b is a key player of pancreatic α cell differentiation in zebrafish. *Developmental Biology*, *285*(1), 211–223. https://doi.org/10.1016/j.ydbio.2005.06.024
31. Dalgin, G., & Prince, V. E. (2015). Differential levels of Neurod establish zebrafish endocrine pancreas cell fates. *Developmental Biology*, *402*(1), 81–97. https://doi.org/10.1016/j.ydbio.2015.03.007
32. Jiang, Z., Song, J., Qi, F., Xiao, A., An, X., Liu, N., Zhu, Z., Zhang, B., & Lin, S. (2008). Exdpf Is a Key Regulator of Exocrine Pancreas Development Controlled by Retinoic Acid and ptf1a in Zebrafish. *PLoS Biology*, *6*(11), e293. https://doi.org/10.1371/journal.pbio.0060293
33. Scoville, D. W., Kang, H. S., & Jetten, A. M. (2020). Transcription factor GLIS3: Critical roles in thyroid hormone biosynthesis, hypothyroidism, pancreatic beta cells and diabetes. *Pharmacology & Therapeutics*, *215*, 107632. https://doi.org/10.1016/j.pharmthera.2020.107632