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REVIEW ARTICLE

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Zebrafish as a model for studying functional pancreatic β cells development and regeneration

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Pancreatic β cells produce insulin and play a central role in glucose homeostasis. The regenerative capacity of mammalian β cells is limited, so that loss and dysfunction of β cells causes diabetes. Zebrafish have a pancreas which is functionally and morphologically conserved with human pancreas. Zebrafish have high regenerative capacity of islets. In the present review, development of zebrafish pancreas was described in comparison to that of mammals, and the regenerative process of zebrafish pancreas was also described. This review will give information on understanding islet and pancreatic β cell development and regeneration and provide possibilities of zebrafish research for developing new diabetic therapies and regenerative medicines.

KEYWORDS
pancreas, pancreatic β cell, development, regeneration

1 | INTRODUCTION

The islets of Langerhans (islets or endocrine cells) play a central role in glucose homeostasis. When the blood glucose concentration increases after a meal, pancreatic β cells secrete insulin to reduce blood glucose level. Insulin stimulates an uptake of glucose by target cells and conversion of glucose to glycogen in the liver. When the blood glucose concentration is reduced, pancreatic α cells secrete glucagon, which stimulates conversion of glucose to glycogen (Joslin, Kahn, Weir, & King, 2015). Thus, optimal control of the blood glucose level depends on delicate changes in the production and secretion of hormones from the islets. Especially, insulin is the only hormone identified that reduces the blood glucose concentration, and loss and dysfunction of β cells cause diabetes (Joslin et al., 2015).

The pancreas is an organ with two sets of functions: those pertaining to the digestive system (acinar cells and ductal cells), and those to the endocrine system (islets). Several phylogenetic analyses suggest that the digestive system and the endocrine system have evolved differently (Youson & Al-Mahrouki, 1999) and that the unified single pancreas developed as a new organ by the fusion of endocrine and digestive parts during fish evolution (Youson & Al-Mahrouki, 1999). Zebrafish (*Danio rerio*), one of the teleosts, possess the pancreas that shares its basic organization and physiology with the mammalian pancreas (Filed, Dong, Beis, & Stainier, 2003; Kamel & Ninov, 2017; Kimmel & Meyer, 2016; Kinkel & Prince, 2009; Maddison & Chen, 2017; Tiso, Moro, & Argenton, 2009). Investigations in zebrafish mutants, morphants, and transgenic lines, have revealed that zebrafish use lots of common mechanisms underlying pancreatic development, β cell differentiation, and physiology (Argenton, Zecchin, & Bortolussi, 1999; Biemar et al., 2001; Dalgin et al., 2011; Dong, Provost, Leach, & Stainier, 2008; Filed et al., 2003; Huang, Vogel, Liu, Melton, & Lin, 2001; Kimmel et al., 2015; Manfroid et al., 2012; Oliver-Krasinski & Stoffers, 2008; Parsons et al., 2009; Prince, Anderson, & Dalgin, 2016; Yee, Yusuff, & Pack, 2001). Although mammalians cannot regenerate β cells, zebrafish can regenerate β cells throughout their entire life (Curado et al., 2007; Moss et al., 2009; Pisharath, Rhee, Swanson, Leach, & Parsons, 2007), so zebrafish are an attractive model for the study of β cell regeneration. Here, the possibilities of zebrafish pancreas for the study of islet cell regeneration are reviewed by showing both the common and different mechanisms underlying pancreatic and/or islet development and physiology among zebrafish and other vertebrates.

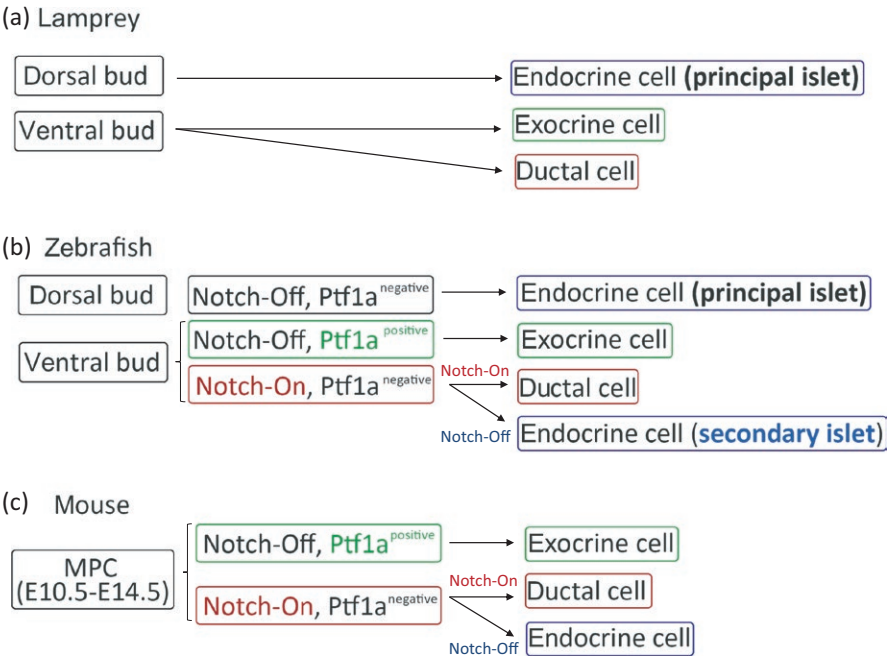

FIGURE 1 Comparison of development of pancreas lineages among vertebrates. (a) In lamprey, endocrine cells arise only in the dorsal bud, and both exocrine cell and ductal cells arise only in the ventral bud. (b) In zebrafish, dorsal bud differentiates into only endocrine cells. On the other hand, ventral bud give rise to all cell types; exocrine cells, ductal cells and endocrine cells. There are Notch-Off, Ptf1a^{positive} cells and Notch-On, Ptf1a^{negative} cells in the zebrafish ventral bud. Notch-Off, Ptf1a^{positive} cells differentiate into exocrine cells. Notch-On, Ptf1a^{negative} cells differentiate into ductal cells and endocrine cells. (c) In mouse, multipotent pancreatic progenitor cell (MPC) arise in both dorsal and ventral buds. Around E10.5, Notch-Off, Ptf1a^{positive} cells and Notch-On, Ptf1a^{negative} cells start appearing from the MPCs. Like zebrafish ventral bud, Notch-Off, Ptf1a^{positive} cells differentiate into exocrine cells, and Notch-On, Ptf1a^{negative} cells differentiate into ductal cells and endocrine cells

2 | ZEBRAFISH PANCREATIC DEVELOPMENT

In mammals, pancreatic development begins with formation of the ventral and dorsal pancreatic buds where *pancreatic and duodenal homeobox 1* (*pdx1*) is expressed (Kim & Hebrok, 2001). The ventral bud rotates dorsally and is fused with the dorsal bud (Kim & Hebrok, 2001). Thus, a final pancreas is generated (Kim & Hebrok, 2001). Similar morphogenetic events occur during pancreas development in zebrafish, as well. *pdx1*-positive cells appear from endodermal cells by 14 hr post fertilization (hpf), and become pancreatic primordia. The dorsal bud emerges from *pdx1*-positive pancreatic primordia by 24 hpf (Filed et al., 2003; Tiso et al., 2009) Around 35 hpf, *pancreas transcription factor1a* (*ptf1a*), which is a key regulator of exocrine pancreas, positive ventral bud appears within *pdx1*-positive pancreatic primordia. By 48 hpf, the ventral bud has fused with the dorsal bud (Filed et al., 2003; Lin et al., 2004; Tiso et al., 2009). In amphibians, birds, and mammals, both ventral and dorsal buds give rise to both exocrine (acinar cells and ductal cells) and endocrine (islet) cells (Filed et al., 2003; Kim & Hebrok, 2001; Matsuura et al., 2009; Pearl, Bilogan, Muhhi, Brown, & Horb, 2009). In zebrafish, dorsal bud does not produce exocrine cells, but only endocrine cells (Figure 1b), which forms a large islet, known as a principal islet. Ventral bud produces both exocrine and endocrine cell populations (Figure 1b; Filed et al., 2003; Tiso et al., 2009). Phylogenetically, islets and exocrine tissues appeared as different organs (Youson & Al-Mahrouki, 1999). In lamprey, dorsal and ventral buds do not fuse; dorsal bud differentiates into islets, and the ventral bud differentiates into the digestive tissues/organs; pancreatic acinar cells, bile ducts, liver, and gallbladder (Figure 1a; Youson & Al-Mahrouki, 1999). Teleosts have acquired the ability to differentiate islets in ventral pancreatic bud, and amphibians have acquired the ability to differentiate pancreatic exocrine compartments in dorsal bud. It is unclear how teleost ventral bud has acquired the ability of islets differentiation, because it is unclear what molecular pathways are working in the ventral bud of Lamprey. On the other hand, *ptf1a*, which is a master regulator of pancreatic exocrine cell, is expressed in only the ventral bud of zebrafish (Lin et al., 2004), but in both ventral and dorsal buds in *Xenopus* (Afelik, Chen, & Pieler, 2006; Pearl et al., 2009). Furthermore, *ptf1a* knock down results in a complete loss of acinar cells in *Xenopus* as in zebrafish ventral bud (Figure 1b; Afelik et al., 2006; Lin et al., 2004; Pearl et al., 2009). Thus, the difference of *ptf1a* expression may make a difference in the ability of differentiating pancreatic exocrine compartments in teleosts and amphibians.

In mammals, multipotent progenitor cells (MPCs), which can differentiate into all cell types: exocrine cells, ductal cells and endocrine cells; arise in both dorsal and ventral bud during early pancreatic

embryogenesis (Figure 1c; Afelik & Jensen, 2013). At about E10.5 in mice, Notch signal starts to regulate the cell fates of the MPCs (Afelik & Jensen, 2013). In Notch-Off MPCs, *ptf1a* expression is activated and then these cells differentiate into exocrine cells (Figure 1c; Afelik & Jensen, 2013). On the other hand, Notch-On MPCs first differentiate into bipotent progenitor cells, which can differentiate into ductal cells and endocrine cells (Figure 1c; Afelik & Jensen, 2013). After becoming bipotent progenitor cells, Notch signal is reduced in some of these progenitors, which is followed by differentiation into endocrine islets (Figure 1c; Afelik & Jensen, 2013). The other Notch-On bipotent progenitor cells differentiate into ductal cells (Figure 1c; Afelik & Jensen, 2013). Ventral bud cells in zebrafish are similar to mouse MPCs in terms of cellular events and molecular pathways (Figure 1b,c). The zebrafish ventral bud contains two cell populations: Notch-Off, Ptf1a^{positive} cells that give rise to the exocrine pancreas (Figure 1b; Ghaye et al., 2015; Parsons et al., 2009; Wang, Park, Parsons, & Leach, 2015), and a Notch-On, Ptf1a^{negative} cell population that differentiates into the ductal system and endocrine cells (Figure 1b; Parsons et al., 2009; Wang, Rovira, Yusuff, & Parsons, 2011; Ghaye et al., 2015). Experiments using some Notch signal reporter lines and notch signal inhibitors revealed that the reduction of Notch signaling in the Notch-On cells triggered their proliferation and differentiation into endocrine cell types (Figure 1b; Matsuda, Parsons, & Leach, 2013; Ninov, Borius, & Stainier, 2012; Parsons et al., 2009), and that continuous Notch-On cells became ductal cells (Figure 1b, Wang et al., 2011). Endocrine cells, which originate from the ventral bud, are called secondary islets, and are distinguished from the principal islet that originates from the dorsal bud. The principal islet is single and huge, while small and multiple secondary islets exist in the pancreas. Secondary islets develop in the ventral bud even after the larval period (after 4 dpf). Secondary islets are over 10 in number by 30 dpf (Ninov et al., 2013). Zebrafish keep Notch-On cells throughout their life (Delaspre et al., 2015), although Notch-On cells disappear in mouse after birth (Afelik & Jensen, 2013). In adult zebrafish pancreas, Notch-On cells are limitedly localized in the centroacinar cells, which are a ductal cell type positioned at the center of acini (Parsons et al., 2009), and are a source of endocrine neogenesis (Ghaye et al., 2015; Matsuda et al., 2013; Ninov et al., 2012; Parsons et al., 2009). Thus, understanding the molecular and cellular mechanisms underlying secondary islet formation, including the reduction of Notch signaling and the endocrine differentiation process, can provide unique insights into their development as well as in the field of islet regeneration.

3 | ZEBRAFISH ISLET PHYSIOLOGY AND MATURATION

Pancreatic β cells secret insulin in response to high glucose levels. In mammalian β cells under high glucose conditions, glucose moves into β cells through the glucose transporter, GLUT2. The β cells then produce more ATP, which increases the ATP/ADP ratio, through glucose metabolism. Elevation of the ATP/ADP ratio stimulates the closure of ATP-dependent potassium (K_{ATP}) channels, subsequently evoking β cell depolarization leading to activation of voltage-dependent Ca^{2+} channels that stimulate insulin secretion.

Like mammals, *glut2* is expressed in zebrafish pancreatic β cells (Marin-Juez et al., 2015). Furthermore, K_{ATP} channels and Ca^{2+} channels regulate insulin secretion according to blood glucose levels (Emfinger et al., 2017; Singh et al., 2017). These indicate that zebrafish pancreatic β cells function in the same way as other vertebrates. On the other hand, these investigations were performed using principal islets, but not secondary islets. Therefore, it is unclear how much secondary islets contributes to glucose homeostasis. Molecular mechanisms underlying secondary islet development in zebrafish is similar to that of mammalian islet development. Thus, determining whether there are functional differences between principal islets and secondary islets may become important to understand how vertebrates have acquired functional islets.

In neonatal rodents, β cells cannot respond to blood glucose levels. β cells acquire the ability to secrete insulin in response to glucose (glucose stimulated insulin secretion; GSIS) around the second week after birth (Bliss & Sharp, 1992). β cell secretory functions continue to be improved well beyond the weaning periods (Bliss & Sharp, 1992). This process is known as β cell maturation. In *Pdx1* heterozygote mice, pancreatic β cells reduced GSIS (Brissova et al., 2002). Overexpression of *musculoaponeurotic fibrosarcoma* oncogene homolog A *(Mafa)* enhanced GSIS in P2 islets (Aguayo-Mazzucato et al., 2011), and GSIS was impaired in *Mafa*-deficient mice (Zhang et al., 2005). Furthermore, *Pax6* knockdown in rat primary islets lead to disruption of GSIS (Gosmain et al., 2012). Thus, some genes have been identified to be involved in both induction and maintenance of β cell maturation. Furthermore, it is known that *Pdx1* mutant showed pancreatic agenesis (Jonsson, Carlsson, Edlund, & Edlund, 1994) and that *Pax6* mutant showed endocrine cell reduction (Sander et al., 1997). Thus, these genes are involved in early development of pancreas and/or islets as well as β cell maturation.

Vertebrate organ development often involves a two-step process. First, the formation of a functionally immature organ during embryogenesis, which is followed by differentiation into the mature form (Matsuda & Shi, 2010; Shi, 1999). The second step most often takes place during the postembryonic/postnatal period, when the plasma thyroid hormone (TH) concentration becomes high. Moreover, it has been proposed that mammalian weaning, frog metamorphosis and the zebrafish larval–juvenile transition are functionally equivalent events during postembryonic development, based on cellular and molecular events in their digestive organs, including pancreas, and elevation of plasma TH concentration during these periods (Matsuda & Shi, 2010; Shi, 1999). In rats, plasma TH levels increase dramatically around postnatal day 15 (Aguayo-Mazzucato et al., 2013) and their pancreatic β cells start GSIS around P10. This is followed by an increase in insulin secretion in response to glucose throughout suckling-weaning transition (Aguayo-Mazzucato et al., 2013). When rats are treated with TH exogenously from P1, they acquire GSIS by P7 (Aguayo-Mazzucato et al., 2013). It was also reported that TH stimulated GSIS in pancreatic β cells generated from pluripotent cells

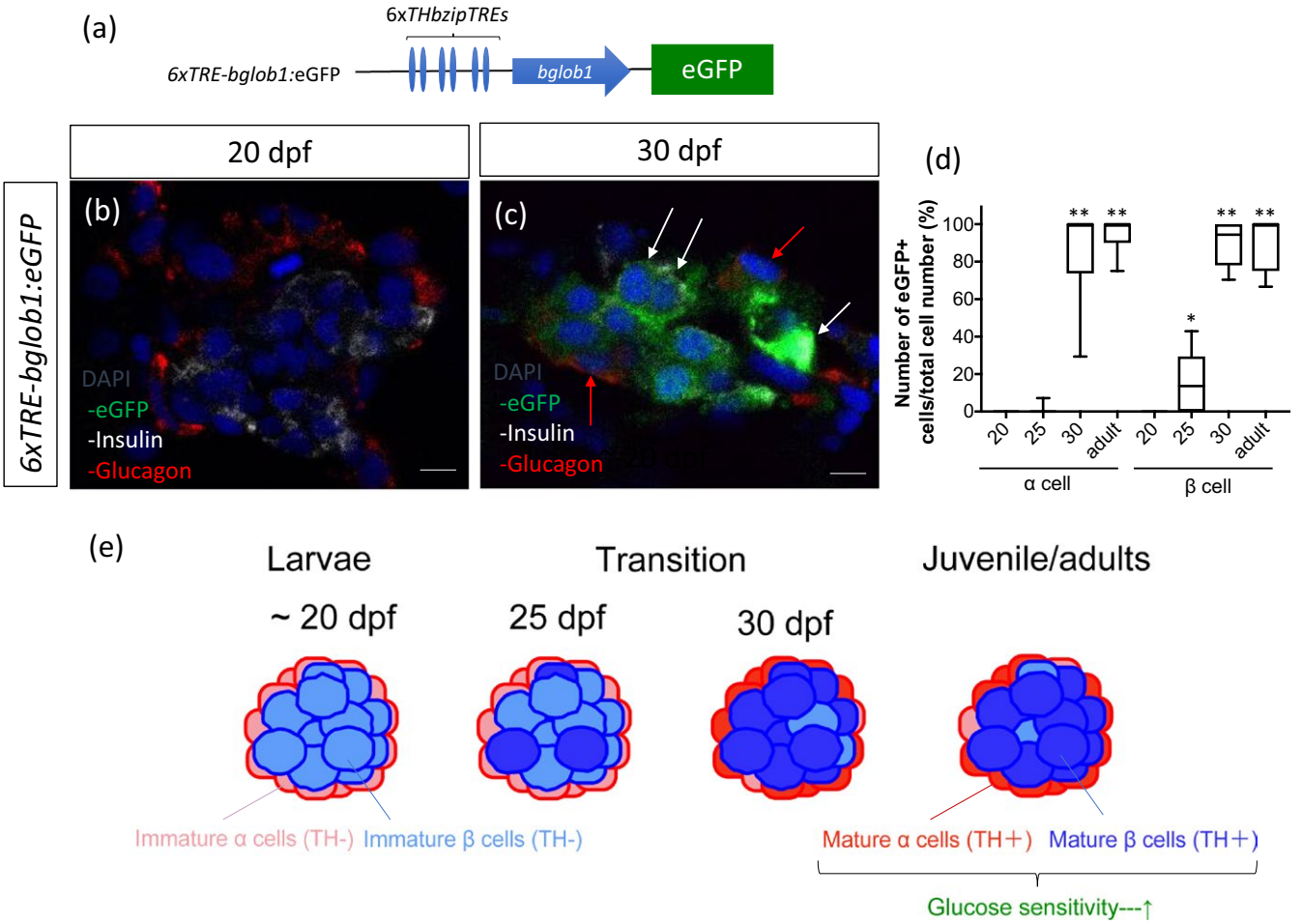

FIGURE 2 Model of islet maturation. (a) Schematic representation of the TH reporter constructs (*6xTRE-bglob1:eGFP*). 6 TREs, which TH receptor (THR)biding sites, of *Xenopus THbzip* gene are inserted into the upstream of *bglob1* minimal promoter. eGFP is expressed when TH-bound-THRs bind to TREs. (b–d) eGFP expression cannot be detected in the pancreatic islets at 20 dpf. eGFP expression in β cells begins at 25 dpf. eGFP was expressed in both α and β cells at later stages than 30 dpf. (e) Functional analysis indicates that TH simulates α and β cells maturation through enhancing glucose sensitivity. Scale bars, 5 μm. ((A–D) ©2017 by the American Diabetes Association ®Diabetes 2017 Oct; 66(10): 2623–2635. Reprinted with permission from the American Diabetes Association ®)

in vitro (Pagliuca et al., 2014; Rezania et al., 2014). Thus, it is clear that TH can stimulate pancreatic β cell function.

To understand exactly when and where TH functions in the pancreas, my colleagues and I generated a TH-responsive reporter zebrafish line (*TRE:eGFP*) to visualize endogenous TH target tissues (Matsuda, Mullapudi, Zhang, Hesselson, & Stainier, 2017). In this transgenic reporter line, TH target cells express eGFP (Figure 2a). The *TRE*:eGFP signal could not be detected in pancreatic islets at 20 dpf (Figure 2b, d and e). *TRE*:eGFP signal was first detected in β cells at 25 dpf and in α and β cells at 30 dpf (Figure 2c–e). For functional analysis of TH signaling, conditional TH knock down experiments were performed using *tg:venus-nfsb* line (McMenamin et al., 2014), in which the thyroid is ablated when treated with metronidazole. Thyroid ablation results in loss of endogenous TH (McMenamin et al., 2014). This transgenic line can be used to perform conditional TH knock down during the larval to juvenile transition. After thyroid ablation, *insulin* expression was downregulated and *glucagon* expression was upregulated, followed by elevation of blood glucose levels during the larval to juvenile transition. Reduction of insulin secretion and elevation of glucagon secretion and resultant elevation of blood glucose level continued in adult fish. TH treatment of thyroid-ablated adult fish elevated insulin secretion and reduced glucagon secretion, and reduced blood glucose level. These results suggest that TH is important to generate α and β cells and to maintain their maturation (Figure 2e). In addition, we found that glucose sensitivity was improved in both α cells and β cells after T3 treatment (Matsuda et al., 2017). Thus, TH may regulate on α cells and β cells maturation by enhancing glucose sensitivity (Figure 2e; Matsuda et al., 2017).

4 | ZEBRAFISH PANCREATIC β CELL REGENERATION

Zebrafish can regenerate functional pancreatic β cells throughout their life (Curado et al., 2007; Moss et al., 2009; Pisharath et al., 2007), while mammals cannot regenerate β cells. Anderson and their

colleagues screened small molecules to identify stimulators of β cell regeneration in zebrafish larvae. They found that adenosine signaling promoted β cell regeneration in larval zebrafish (Andersson et al., 2012). Based on this, they treated hyperglycemic mice, in which numbers of β cell were reduced, with the adenosine agonist, NECA, and succeeded in inducing β cell proliferation and partial recovery of blood glucose level.

Notch-On cell contributes to β cell regeneration as well as β cell neogenesis in zebrafish (Delaspre et al., 2015). During β cell regeneration, Notch-On cells proliferate and differentiate into β cells (Delaspre et al., 2015). In addition, α cell is, also, another source of β cell regeneration (Ye, Robertson, Hesselson, Stainier, & Anderson, 2015). Ye and colleagues performed each pancreatic α cell, β cell and δ cell lineage tracing experiment during β cell regeneration using *glucagon*:Cre, *insulin*:Cre, *somatostatin*:Cre, zebrafish transgenic line. Their results showed that only α cells, but not surviving β and δ cells, contributed to β cell regeneration in zebrafish (Ye et al., 2015) and that α cell transdifferentiate into β cell during β cell regeneration in zebrafish (Ye et al., 2015). Thus, two different systems, "Notch-On islet stem cell" and "α-cell transdifferentiation", have been identified for β cell regeneration in zebrafish. Currently, critical methods for β cell regeneration in mammalian systems have not yet been identified. Researchers have been attempting to regenerate β cells by pancreatic stem cells and by inducing α cell transdifferentiation to β cell (Zhou & Melton, 2018). Studies in zebrafish will help to provide new regenerative methods for β cells.

5 | CONCLUSION

I have reviewed the common and unique phenomena between zebrafish and other vertebrate pancreases. I have emphasized that zebrafish are an attractive and unique model for investigation of pancreas development and functional pancreatic β cell regeneration. In the future, transcriptome and proteome studies at several different times and under different conditions will help to understand these phenomena. Additionally, chemical screening using zebrafish larvae will become a powerful tool to identify new molecules that stimulate islet maturation and functional regeneration. Discovering simple methods to generate conditional mutants in zebrafish is of major importance for zebrafish research, including the field of regenerative biology.

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