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Reprogramming into pancreatic endocrine cells based on developmental cues

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Introduction

Over the past decades, diabetes has become one of the most widespread metabolic disorders with epidemic dimension affecting almost 6% of the world's population. By the year 2025, the number of people affected by type 1 or 2 diabetes is projected to reach 300 million. For instance, in Asia, where a fast change in lifestyle and dietary habits is occurring, the number of diabetic patients is increasing faster than anywhere else. Herein, we will focus on Type 1 diabetes, which is characterized by a selective loss of insulin-producing beta-cells in the endocrine pancreas as a result of an autoimmune reaction. Left untreated, the induced shortage in insulin hormone and resulting hyperglycemic condition may lead to complications, such as micro- and macro-vascular damage, blindness, amputation and/or death. Current treatments involve daily injections of exogenous insulin in order to compensate the deficiency in this hormone. However, environmental factors, such as exercise, diet, or age, may cause marked variations in blood glucose levels despite insulin therapy and eventually lead to the complications discussed previously. Islet transplantation represents a substitute to insulin therapy, but the shortage in donors prevents its widespread use. Thus, other alternatives must be found in order to efficiently treat the consequences of type 1, but also type 2, diabetes, both diseases eventually resulting in loss and/or insufficient numbers of beta-cells.

In this context, the generation of pancreatic beta-cells from stem, progenitor or other cell subtypes may represent an interesting option. However, to design rational protocols allowing the *in vitro* or *in vivo* generation of beta-cells, it is imperative to gain further insight into the molecular determinants controlling the development of embryonic, but also adult beta-cells, *in vivo*.

The genetic program underlying the genesis of the endocrine pancreas

The pancreas plays a crucial role in nutritional homeostasis through synthesis and secretion of hormones and enzymes. This organ includes three tissue-types: acinar, ductal and endocrine. The exocrine pancreas consists of acinar cells secreting digestive enzymes, such as lipases, proteases and nucleases, which are emptied into the pancreatic duct forming an elaborately branched network of tubules, composed of epithelial cells. Acinar cells also produce bicarbonate ions and electrolytes, which together with exocrine enzymes, are transported through the main duct into the duodenum, where they contribute to food processing (Githens et al., 1994). Endocrine cells are organized into small clusters of cells termed islets of Langerhans. These correspond to specialized micro-organs composed of five original cell subtypes, including alpha-, beta-, delta-, epsilon-, and PP cells, which produce the hormones glucagon, insulin, somatostatin, ghrelin, and PP (Pancreatic Polypeptide), respectively. Insulin and glucagon function coordinately to control glucose homeostasis, whereas somatostatin and PP regulate the secretion of other hormones and of exocrine enzymes (Adrian et al., 1978, Roncoroni et al., 1983, Csaba and Dournaud, 2001, Wierup et al., 2002, Heller et al., 2005).

The earliest morphological signs of pancreas development are observed at approximately embryonic day 9.5 (E9.5) as dorsal and ventral protrusions of the primitive gut epithelium that will later fuse to form the definitive pancreas (reviewed in Edlund, 2002, Jorgensen et al., 2007). Concomitantly, glucagon-producing cells are the first endocrine cells detected (Herrera et al., 1991, Teitelman et al., 1993, Upchurch et al., 1994). At embryonic day 10.5 (E10.5), a few insulin-producing cells appear, often co-expressing the glucagon hormone (Teitelman et al., 1993). At E14, a peak of endocrine cell genesis leads to the generation of numerous fully differentiated insulin-expressing beta-cells and glucagon-producing alpha-cells. Endocrine cells organize themselves into small aggregates and, within the next 24 hours, the first somatostatin-producing delta-cells emerge (Pictet et al., 1972). Finally, at E18, shortly before birth, PP-

expressing PP-cells differentiate, while endocrine cells begin to form well-organized islets of Langerhans.

Until recently, the mechanisms underlying the generation of these different endocrine cell types were not well understood, but lineage studies and the generation of mice deficient for a number of pancreatic transcription factors have provided major insight into these processes. Besides, genetic manipulations, such as loss-, but also gain-of-function approaches, have permitted researchers to better define the function of such factors, including Sox9, Pdx1, Ngn3, IA1, Pax4, Arx, Nkx2.2, Nkx6.1, Nkx6.2, Pax6, and MafA.

Hence, Sox9, a member of the Sox gene family, is expressed in Pdx1⁺ cells of the pancreatic epithelium from E9.0 onwards. At E14.5, after the second transition its expression becomes restricted to non-differentiating cells expressing low levels of Pdx1⁺ and is absent in hormone expressing cells. Postnatally, Sox9 is localized in centroacinar cells and a small subset of ductal epithelial cells (Seymour et al., 2007). Several findings supported the notion that Sox9 labels pancreatic progenitor cells: (1) Sox9 expression appears unchanged in *Ngn3* and *Nkx6.1*-null mice, (2) in transgenic mice in which Pdx1⁺ progenitor cells are artificially maintained in their progenitor state, Sox9 expression remains abnormally sustained, and (3) Sox9 is found co-expressed with the proendocrine transcription factor Ngn3 at E15.5, but lack in cells expressing mature islet markers, such as Nkx2.2 and Isl1. Lastly, the targeted deletion of Sox9 in Pdx1⁺ progenitors was found to induce a decrease in the endocrine cell mass concomitantly to a premature differentiation into cells expressing glucagon and Isl1. Therefore, during early pancreas development, Sox9 seemingly marks a pool of pluripotent progenitor cells in which its activities appear crucial to keep such cells in a proliferative state and thereby prevent their premature differentiation (Lynn et al., 2007, Seymour et al., 2008).

Similar to Sox9, Pdx1, a homeodomain-containing transcription factor, is expressed as early as E8.5 in the dorsal and ventral endoderm, posterior to the stomach and duodenal epithelium. Subsequently, *Pdx1* expression progressively becomes restricted to the beta-cell lineage in which it controls glucose-stimulated

insulin secretion (Ohlsson et al., 1993, Stoffers et al., 1999, Guz et al., 1995). Lineage tracing experiments have demonstrated that mature pancreatic cells derive from *Pdx1*-expressing progenitor cells (Gu et al., 2003). This was further confirmed by the pancreatic agenesis detected in *Pdx1*-deficient mice (Offield et al., 1996). The conditional inactivation of this factor at different developmental stages, as well as in mature beta-cells, underscored its requirement to specify, but also maintain, the beta-cell phenotype (Ahlgren et al., 1998, Holland et al., 2005). Furthermore, Gannon et al. (2008) observed that the inactivation of *Pdx1* in late-gestational beta-cells led to a decrease in the proliferation of insulin-producing cells concomitantly with an augmentation in proliferation of glucagon-producing cells. These results suggest that *Pdx1* function is necessary for the genesis and maintenance of beta-cells, but also for late-gestational regulation of the proper endocrine cell numbers (Gannon et al., 2008). Interestingly, using a Tet-off approach, the conditional down-regulation of *Pdx1* was found to provoke an arrest of beta-cell development, but also alterations in acinar cell genesis. One likely explanation may be the absence of *Ptf1a*, a factor downstream of *Pdx1* known to play a crucial role in acinar cell development (Hale et al., 2005). During early pancreas development, *Ptf1a* is expressed throughout the pancreatic epithelium, whereas, at later stages, it is exclusively detected in acinar cells (Krapp et al., 1996). Consistently, mice with targeted ablation of *Ptf1a* do not develop any acinar cells, whereas the four endocrine cell types are present albeit misallocated in the splenic mesenchyme (Krapp et al., 1998). The combined loss of *Pdx1* and *Ptf1a* in mice leads to alterations similar to those seen in *Pdx1* single mutant mice, suggesting that *Ptf1a* acts downstream of *Pdx1* (Burlison et al., 2008). However, recent findings revealed that *Ptf1a* also interacts with the *Pdx1* promoter thereby potentiating *Pdx1* expression in early pancreatic progenitors and later in acinar cells (Wiebe et al., 2007).

Unlike *Pdx1* playing pleiotropic roles during the development of the pancreas, the bHLH transcription factor, *Ngn3* exclusively acts on the differentiation of the endocrine tissue. Accordingly, lineage tracing analyses indicated that endocrine cells are derived from cells that expressed *Ngn3* (Gu et al., 2002). Detectable

from E8.5 onward, *Ngn3*-expression peaks around E15.5 and is maintained at lower levels in mature endocrine cells. Loss-of-function experiments have demonstrated the requirement of *Ngn3* for the specification of all enteroendocrine and endocrine lineages (Gradwohl et al., 2000, Jenny et al., 2002, Gu et al., 2003). Interestingly, the ablation of *Ngn3* in adult *Pdx1*⁺ cells was associated with reduced expression of several *Ngn3* target genes and found to result in impaired islet function, outlining the importance of sustained *Ngn3*-expression in mature endocrine cells (Wang et al., 2009). Furthermore, gain-of-function analyses established its potential in promoting endocrine cell differentiation (Schwitzgebel et al., 2000, Heremans et al., 2002). Hence, the ectopic expression of *Ngn3* in *Pdx1*-expressing cells leads to a premature onset of endocrine differentiation resulting in the near exclusive genesis of glucagon-producing cells (Apelqvist et al., 1999, Grapin-Botton et al., 2001). In the light of these results, *Ngn3* was initially considered as a proendocrine factor. However, recent data brought additional insight into the function of *Ngn3*. Indeed, Villasenor and colleagues (2008) showed that *Ngn3* expression occurs in two distinct temporal waves, correlating with the first and secondary transition as described by Pictet et al. (Pictet et al., 1972), thereby generating “early” and “late” born endocrine cells owning different developmental potentials (Villasenor et al., 2008). Interestingly, the temporal relevance of *Ngn3* expression has also been outlined recently by Johansson and colleagues (2007). Briefly, the authors crossed a *Pdx1*-controlled, tamoxifen-inducible *Ngn3* mouse line with *Ngn3*-deficient animals and specifically activated *Ngn3* in the pancreas through tamoxifen treatment at different developmental stages. It was thereby established that early *Ngn3*⁺ cells exclusively give rise to alpha-cells. Importantly, the activation of *Ngn3* at later developmental stages was found to successively promote the genesis of beta-, PP- (after E11.5) or delta- (after E14.5) cells, while the competence to give rise to alpha-cells progressively decreases.

Following *Ngn3* activation, several additional factors acting in the endocrine cell allocation have been characterized. Hence, the zinc-finger-containing transcription factor insulinoma-associated antigen 1 (IA-1) was found to

represent a direct downstream target of *Ngn3* and to act in the initial steps underlying endocrine cell differentiation. Endocrine cells are still detected in *IA-1* mutant pancreata, but the majority of these do not express any hormones (Gierl et al., 2006). Unlike *Ngn3*, the ectopic expression of *IA1* in duct cells is not sufficient to induce the endocrine specification program, although the coexpression of *Ngn3* and *IA1* was found to significantly enhance the efficiency of the *in vitro* duct-to-endocrine transdifferentiation as compared to ectopic *Ngn3*-expression only (Mellitzer et al., 2006).

Among the aforementioned factors involved in early endocrine cell differentiation, *Arx* and *Pax4* were found to play key roles in the specification of the endocrine cell subtypes, *Arx* promoting the alpha-/PP-cell fates, whereas *Pax4* induces the beta-/delta-cell lineages (Figure 1A-C). Indeed, in *Pax4*-deficient pancreata, beta-/delta-cells do not develop, while a proportional increase in alpha-cell numbers is observed (Sosa-Pineda et al., 1997). Conversely, upon the loss of *Arx*, an increase in both beta- and delta-cell populations is noticed while alpha-cells are absent (Collombat et al., 2003). It is worth noting that in both instances, the total islet cell numbers are found unchanged as compared to wild-type pancreata. Closer examination indicated that *Arx* and *Pax4* factors exhibit antagonistic roles on the specification of the endocrine subtype destinies through an inhibitory, cross-regulatory circuit that controls the transcriptional state of these two genes. The combined loss of *Pax4* and *Arx* (Figure 1D) do not result in an arrest in the differentiation of all four endocrine cell types, but rather in the depletion of beta- and alpha-cells, while the delta-cell population is increased and the PP-cell content is unaltered (Collombat et al., 2005). It was therefore concluded that *Pax4* is not required to directly specify the beta-/delta-cell destinies, but rather acts by inhibiting the alpha-cell fate through the inhibition of *Arx* expression.

In an effort to further define the transcriptional network underlying the development of the pancreas, the role of *Nkx2.2*, a homeodomain-containing factor belonging to the NK-class, was characterized. Acting early during development, *Nkx2.2* was found to play a preponderant function especially in the

specification of the beta-cell lineage. Indeed, in mature islets, *Nkx2.2* labels alpha-, beta- and PP-cells. Accordingly, mice depleted in *Nkx2.2* display a loss of beta-cells, as well as reduced alpha- and PP-cell contents, whereas the numbers of delta-cells remain unaffected. It is worth noting that the ghrelin-expressing cell population appears considerably augmented in such animals (Sussel et al., 1998, Prado et al., 2004). The analysis of *Pax4/Nkx2.2*- double-nullizygous mice revealed alterations similar to those observed in pancreata of *Nkx2.2*^{-/-} mutant mice, indicating that *Pax4* may act downstream of *Nkx2.2*. This was further supported by the lack of alteration in the expression levels of *Nkx2.2* in *Pax4*-deficient mice.

Nkx6.1, another early marker of the pancreatic epithelium also belonging to the *Nkx* subfamily, is first detected at E9.5. Its expression is initially detectable in *Ngn3*⁺ endocrine precursor cells, but becomes restricted to mature beta-cells where it was found to be required for proper insulin secretion (Oster et al., 1998, Sander et al., 2000). Indeed, mice lacking *Nkx6.1* exhibit a dramatic reduction in mature beta-cells, while other islet cell subtypes develop normally (Sander et al., 2000). Interestingly, early insulin⁺ cells emerging during the first transition are not affected, suggesting a decisive role for *Nkx6.1* for the generation of “late-born” mature beta-cells originating from the second transition (Henseleit et al., 2005). *Nkx6.2*, a paralog to *Nkx6.1*, displays a similar expression pattern compared to *Nkx6.1*, but in contrast to the latter, it is not detected in adult beta-cells (Henseleit et al., 2005, Pedersen et al., 2005). Importantly, *Nkx6.2* mutant mice do not present any obvious phenotype, whereas mice deficient for both *Nkx6.1* and *Nkx6.2* exhibit similar alterations as compared to *Nkx6.1*-mutant mice (loss of mature beta-cells), but also a drastic reduction in glucagon⁺ cells, indicating an additional involvement of the *Nkx*-factors in alpha-cell formation (Henseleit et al., 2005). Notably, in *Nkx6.1*^{-/-} mice, misexpression of *Nkx6.2* in *Pdx1*⁺ cells was found to rescue beta-cell development, indicating that both factors possess redundant activities for beta-cell differentiation (Nelson et al., 2007).

Besides *Pax4*, another member of the *Pax* gene family, *Pax6*, plays an important role in islet cell differentiation. *Pax6* is expressed in all endocrine hormone-

producing cells throughout pancreas morphogenesis. Conventional knockout of *Pax6* in mice revealed its requirement for the development of all four endocrine cell subtypes, as well as its involvement in forming well-structured islets of Langerhans (St-Onge et al., 1997, Sander et al., 1997). Interestingly, using conditional ablation of *Pax6* in mice, an additional role of this gene in maintaining the postnatal beta-cell phenotype was outlined, as such, mice displayed a diabetic condition associated with hypoinsulinemia (Ashery-Padan et al., 2004). Numerous additional factors were characterized for their involvement in the maintenance of the beta-cell identity, including representatives of the *Maf* gene family (*MafA*, *MafB* and *c.Maf*) that were also shown to act in the terminal differentiation of beta-, but also alpha-cells. For instance, *MafA* was found to directly interact with the promoter of the *insulin* gene and to transactivate *insulin* expression (Matsuoka et al., 2003, Aramata et al., 2005, Zhao et al., 2005). Its expression is initiated at E13.5 and is restricted to insulin⁺ cells during embryogenesis as well as throughout adulthood (Matsuoka et al., 2004). Consistently, mice deficient for *MafA* develop diabetes mellitus accompanied with a significant decrease in circulating insulin levels and, in addition, abnormal islet architecture. Isolated *MafA*-deficient insulin⁺ cells are unable to secrete insulin upon glucose challenge, indicating a beta-cell autonomous defect in glucose-stimulated insulin secretion (Zhang et al., 2005). Moreover, the ectopic expression of *MafA* in the embryonic chicken endoderm, as well as in non-pancreatic cell cultures, was found to be sufficient to promote insulin secretion (Matsuoka et al., 2007, Artner et al., 2008). Altogether, this non-extensive description of the major players acting to specify the different endocrine cell subtypes outlines the complexity of the mechanisms involved. In order to exploit these findings for the *in vitro* or *in vivo* genesis of insulin-producing beta-cells, gaining more insight into the endocrine differentiation program, but also into the interplay of the different actors, will represent a challenge for the years to come.

Mechanisms of beta-cell replenishment

Among the several approaches envisaged to treat diabetes-related beta-cell insufficiency, the *in vivo* regeneration of insulin-producing beta-cells appears of great potential. The fact that the adult beta-cell mass is not static, but fluctuates in response to changing physiological conditions, such as pregnancy and insulin-resistance, underscores the activity of adaptive mechanisms (Lingohr et al., 2002, Bonner-Weir, 2000). Furthermore, several approaches, including surgical ablation of pancreatic tissue as well as partial duct ligation (PDL), were found to initiate endogenous regenerative programs acting to replace missing cells. These findings raise the question of the cellular origin of neogenerated cells. Using a pulse-chase labeling of beta-cells, Dor et al. established that, throughout adult life, or following partial pancreatectomy, new beta-cells arise from pre-existing beta-cells rather than from any other cell subtype (Dor et al., 2004). Similar results were obtained after the diphtheria-toxin-mediated ablation of the beta-cell mass: treated mice recovered from hyperglycemia through the genesis of new beta-cells that mainly originated from surviving beta-cells (Nir et al., 2007). Using sequential administration of CldU and IdU, Teta and coworkers were able to label cells that had undergone more than one round of cell division and therefore identify potential stem- or progenitor-cells. Focusing on pancreatic beta-cells, they observed that repeatedly dividing progenitors did not contribute to the adult beta-cell mass neither under physiological conditions nor following 50% pancreatectomy nor during pregnancy (Teta et al., 2007). However, several other studies suggested alternative origins for beta-cells during pancreas regeneration, thereby encouraging the pancreatic stem cell controversy.

One of the first hints for the contribution of non beta-cells to the beta-cells mass was provided by Wang and colleagues (Wang et al., 1995). Provoking a regenerative response in rats through partial duct ligation combined with BrdU pulse-chase labeling, the results obtained indicated that proliferation could not fully account for the drastic beta-cell hyperplasia. It was therefore concluded that multipotent precursors and/or stem cells may be implicated in these regeneration

events. Ductal cells were thought to be at the origin of such neogenerated cells due to the detection of metaplastic cells expressing both ductal and beta cell marker and the increased proliferative activity noted around ducts. Interestingly, in 2008, a pioneering study from Heimberg's laboratory conclusively established the involvement of duct-lining precursor cells in pancreatic islet regeneration following PDL. Besides the mechanism of self-duplication of pre-existing beta-cells, the authors also identified cells re-expressing the proendocrine gene *Ngn3* in the ductal lining. Such isolated cells were found capable to give rise to all four endocrine cell subtypes (Xu et al., 2008). Along the same vein, Inada et al. were able to trace the lineage of newly formed beta-cells following PDL in adult mice. They thereby determined that duct cells could indeed give rise to islets and acinar cells. Remarkably, in non-injured neonates, the naturally occurring postnatal increase in islet cell mass was also partially attributed to duct cell conversion (Inada et al., 2008).

Altogether, the findings that beta-cells can be regenerated by replication of pre-existing beta-cells, but also through the conversion of exocrine cells opens new perspectives for the treatment of diabetes. However, new questions arise, especially concerning the nature of the extrinsic and/or intrinsic signals implicated into such conversion. Specifically, whether PDL induces the reprogramming of duct-lining cells or outlines the presence of precursor cells will have to be determined. Deciphering the detailed mechanisms underlying beta-cell neogenesis from pancreatic cells and finding ways to chemically induce such conversion will be one of the greatest challenges facing the future of diabetes research.

Reprogramming into pancreatic cells

In the last years, several studies outlined an unsuspected plasticity of mature pancreatic cells (Tosh and Slack, 2002, Eberhard and Tosh, 2008). Such findings opened new avenues for the treatment of type 1 diabetes since it was established that particular pancreatic cell types may be forced to adopt an alternate phenotype through the misexpression of selected transcription factors. The analysis of the genetic determinants underlying the genesis of the different cell subtypes and the determination of mechanisms by which a particular fate is specified at the expense of another during the development is therefore crucial, as such knowledge may be used to induce specific cells to adopt another identity. This research could (1) be applied to the programming of stem or progenitor cells into endocrine cells *in vitro*, and (2) lay the basis towards the ultimate goal of reprogramming abundant and easily accessible patient-specific human cells, such as fibroblasts, blood cells or adipocytes, into becoming insulin-producing beta-cells.

Numerous protocols aiming at differentiating ES-cells have been reviewed previously (Nir and Dor, 2005, D'Amour et al., 2006, Oliver-Krasinski and Stoffers, 2008). In the following, we will therefore focus on the *in vivo* reprogramming of pancreatic and non-pancreatic cells into endocrine cells, with emphasis on the beta-cell end-point (Figure 2).

As all pancreatic cells derive from *Pdx1*-expressing progenitor cells (Gu et al., 2003), this factor was the focus of several studies aiming at converting various cell types into pancreatic cells (Ber et al., 2003, Taniguchi et al., 2003, Shternhall-Ron et al., 2007). Hence, following partial pancreatectomy or PDL, both characterized by regeneration of pancreatic cells, *Pdx1* was found ectopically expressed in mature ductal structures. This suggests that *Pdx1* may play a crucial role in regenerative processes and direct the conversion of different pancreatic cell subtypes. To conclusively determine its function, a cre-mediated conditional misexpression approach was used to ectopically express *Pdx1* in acinar cells. Thus, persistent *Pdx1*-expression in acinar cells was found to induce

their conversion into cells exhibiting a ductal identity (Miyatsuka et al., 2006). Since both the liver and pancreas arise from adjacent regions of the embryonic endoderm, it was thought that these closely-related tissues may be interconverted. The transient expression of the *Pdx1* homolog *Xlhbox8* in *Xenopus* liver cells, as well as in cultured hepatocytes, was thereby found to induce the reprogramming of these cells into pancreatic exocrine and endocrine cell types (Horb et al., 2003). Similar results were obtained following adenoviral-mediated misexpression of *Pdx1* in the mouse liver (Ferber et al., 2000, Ber et al., 2003, Shternhall-Ron et al., 2007). This transient misexpression of *Pdx1* was found sufficient to prevent streptozotocin-induced hyperglycemia in mice for several months. The concomitant adenoviral application of two factors, *Pdx1* and *Ngn3* or *NeuroD* in the liver of mice, was likewise associated with the conversion of hepatic cells into insulin-producing cells, and to a significant amelioration of glucose-tolerance (Kaneto et al., 2005). Interestingly, a study from Yechoor et al. revealed that the endocrine specification factor *Ngn3* alone has likewise the competence to induce a pancreatic differentiation program when ectopically expressed in the liver (Yechoor et al., 2009). The adenoviral application of *Ngn3* combined with the islet growth factor betacellulin into the liver of streptozotocin-diabetic mice led to the emergence of all four major islet cell types including insulin-expressing cells possessing glucose responsive properties. Lineage tracing experiments revealed both parenchymal hepatocytes and multipotential hepatic progenitor cells to be the origin. Interestingly, parenchymal hepatocytes gave rise to insulin⁺ cells only transiently, thereby retaining their hepatic properties. In contrast, neogenerated islet-like cells originating from the progenitor population displayed the full set of endocrine transcription factors acting downstream of *Ngn3* during pancreas development and maintained their islet characteristics even 6 months post-*Ngn3* injection. This study impressively demonstrated how a single transcription factor induces a lineage-switch towards a pancreatic endocrine fate when ectopically expressed in endodermal derived hepatic progenitor cells.

Focusing on the directed generation of endocrine cells, several recent studies concentrated on the characterization of additional transcription factors known for their involvement in the specification of the different islet cell lineages during pancreas morphogenesis. Hence, using loss- and gain-of-function approaches, the crucial and opposed roles of Pax4 and Arx in specifying the fates of the different endocrine subtypes were outlined. While Pax4 promotes the beta/delta-cell lineages, Arx drives progenitor cells towards the alpha/PP-cell fate (Collombat et al., 2003). Aiming to determine whether these factors may not only act during pancreas morphogenesis, but also in adult animals, the expression of Arx was triggered in adult beta-cells using an inducible Cre/Lox system combined with lineage tracing. It was thereby demonstrated that the ectopic expression of Arx in adult beta-cells forces these to adopt alternative alpha- or PP-cell identities (Collombat et al., 2007). This discovery was of fundamental importance in the context of beta-cell-based therapy as it implied that the opposite conversion might be achieved, that is, to generate beta-cells from other endocrine cells misexpressing the Arx-counterpart, Pax4. To verify this hypothesis, the ectopic expression of Pax4 was induced, notably, in glucagon-producing cells using the Cre-loxP system (Collombat et al., 2009). Lineage tracing experiments indicated a conversion of these glucagon⁺ cells into cells exhibiting most features of true beta-cells. The resulting glucagon deficiency was found to induce a compensatory and continuous glucagon⁺ cell neogenesis requiring the re-expression of the proendocrine gene *Ngn3*. However, such cells failed to correct the hypoglucagonemia as they subsequently acquired a beta-cell phenotype upon Pax4 misexpression. Notably, this cycle of neogenesis/conversion was found sufficient to restore a functional beta-cell mass in animals with chemically-induced type 1 diabetes. Altogether, this study revealed that a single factor is sufficient to promote both a beta-cell fate during pancreas morphogenesis, but also a beta-cell phenotype in differentiated pancreatic cells. Along the same line, using the extensive findings from diverse loss-of-function analyses, Zhou et al. (2008) highlighted nine transcription factors displaying alterations in beta-cell function or number when mutated. The adenoviral infection of combinations of

some of these factors into the exocrine pancreas of adult mice resulted in the conversion of acinar cells into insulin-secreting cells. The combination of factors with the highest inductive efficiency consisted of Pdx1, Ngn3 and MafA. Such insulin-secreting cells were morphologically indistinguishable from endogenous beta-cells and expressed all known beta-cell molecular markers. Furthermore, streptozotocin-treated mice recovered from diabetes after the injection of the three factors. Notably, this reprogramming of acinar cells did not involve any dedifferentiation or proliferating steps, suggesting a process of direct transdifferentiation (Zhou et al., 2008). Interestingly, Pdx1 and MafA were required only transiently as they induced their endogenous homologues. *Ngn3* expression was found down-regulated one month post-infection, thereby mimicking its transient expression pattern as normally seen during embryonic development.

This study clearly outlined the stable and exclusive conversion of one cell type into another. Interestingly, using the same factors, it was not possible to force the acquisition of the beta-cell identity using muscle and fibroblast cells as starting material. The close relation and epigenetic similarities of acinar- and beta-cells might be a prerequisite for a successful conversion. This raises the question of whether reprogramming of abundant and easily accessible patient-specific human cells, such as fibroblasts, blood cells or adipocytes, into insulin-producing beta-cells might be achieved. It is likely that such cells would have to be dedifferentiated to some extent prior to force them to acquire an alternative identity. Toward this goal, the recently reported reprogramming of human skin cells into induced pluripotent stem cells (iPS) suggests the possibility of generating patient-specific pluripotent stem lines that could ultimately be used for further differentiation into insulin-producing beta-cells (Takahashi and Yamanaka, 2006).

Conclusions and future aspects

An active research aiming at characterizing the mechanisms underlying normal and pathological pancreas development uncovered numerous key factors and allowed the elucidation of some of their mutual interactions. This knowledge was/is used as a roadmap for the directed *in vivo* and *in vitro* generation of functional beta-cells and raises hopes for a suitable beta-cell replacement-therapy in order to improve type 1, but also type 2, diabetes treatment. However, despite these promising progresses, we need to gain further insight into the complex transcriptional network and interplay governing the differential development of the four endocrine cell subtypes. It is also obvious that additional factors expressed in progenitors, as well as mature beta-cells, have to be identified in order to design rational protocols faithfully mimicking natural islet cell genesis. Comprehensive Chip-assay and *in situ* hybridization screens already widened our view of the participating genes (Zhou et al., 2007). A closer examination of their function and mutual relationship will doubtless contribute to our understanding of the orchestrated interplay underlying pancreas development and improve the protocols aiming at generating fully differentiated and stable cells exhibiting a beta-cell phenotype from ES cells, iPS cells or other cell types.

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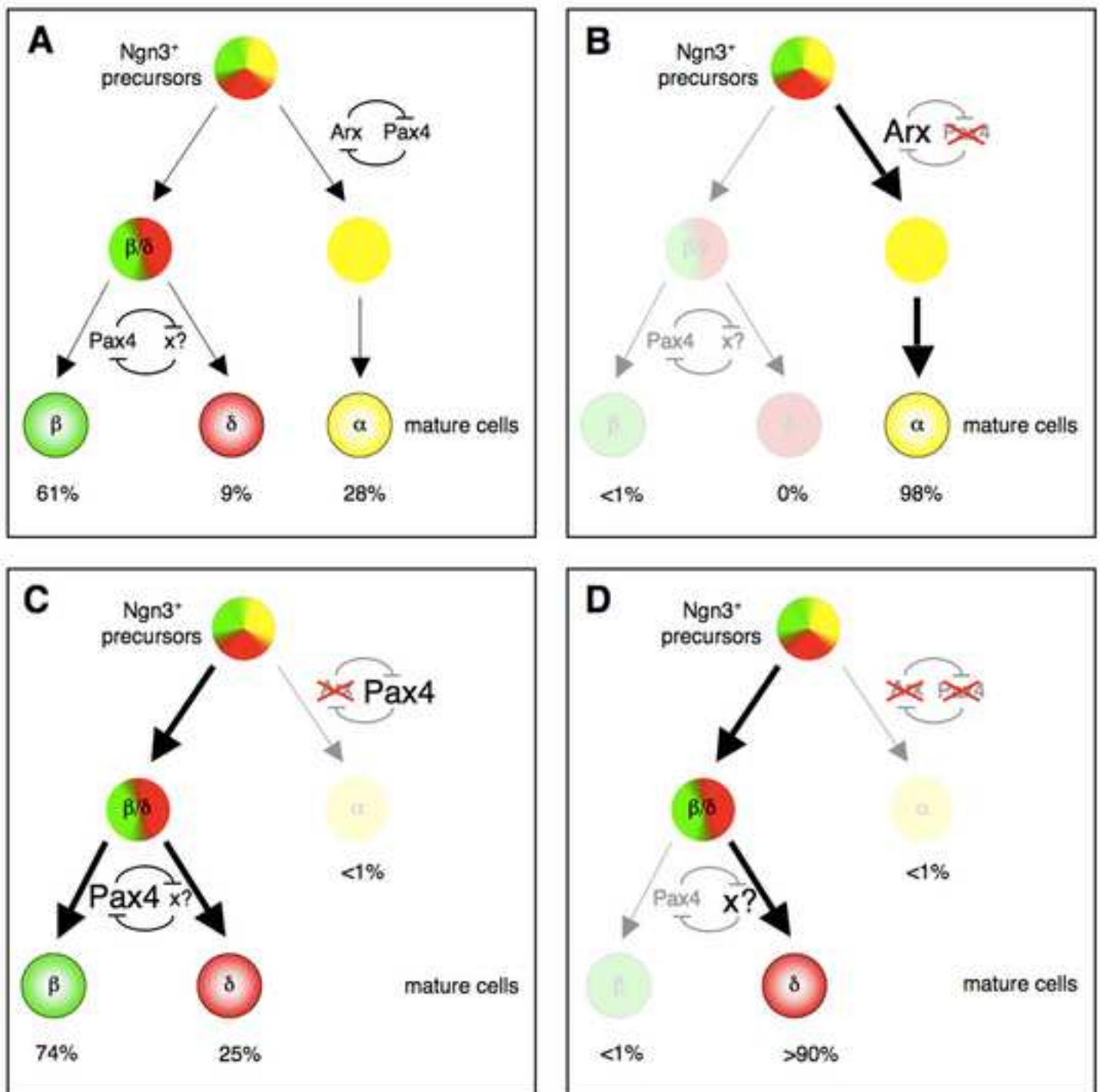
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Figure legends:

Figure 1: Schematic model of endocrine subtype specification during pancreatic development. **A:** An endocrine progenitor cell (E), with the potential of becoming either an α -cell or a second progenitor with β - and δ -cell potentials, expresses both *Arx* and *Pax4*. Mutual inhibitory interactions between *Arx* and *Pax4* lead to a “competition” between the two transcription factors resulting in subtype allocation. If *Arx* prevails (possibly stimulated by extrinsic factors), *Pax4* expression is extinguished and an α -cell fate is chosen. If *Pax4* predominates and *Arx* expression is extinguished, the resulting progenitor cell (β/δ) is posed to undergo a second round of competitive fate allocation. In this second event a hypothetical “factor X” is envisioned to promote the δ -cell fate and to display a mutual inhibitory interaction with *Pax4* similar to the interaction between *Arx* and *Pax4* in the first round of fate allocation. **B, C,** and **D:** Fate changes in the case of *Pax4*- (**B**), *Arx*- (**C**), or combined *Arx*-/*Pax4*- (**D**) deficiency. For the purpose of simplification, PP-cells are not figured.

Figure 2: Inter-conversions of hepatic and pancreatic cell types *in vivo* with focus on a beta-cell end-point. Forced conversions of hepatic and pancreatic cell types *in vivo* through transgene- or virus mediated misexpression of the indicated pancreatic developmental key regulators. Dashed arrows indicate conversions detectable after partial duct ligation (PDL) or during normal development, but whose underlying transcription factors remain hitherto unknown. For the purpose of clarity, delta- and PP-cells are not figured.

Kordowich *et al.*, 2009 - Figure 1

Kordowich et al., 2009 - Figure 2

