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Reprogramming into pancreatic endocrine cells based on developmental cues

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Introduction

Over the past decades, diabetes became 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 person affected by type 1 or 2 diabetes is projected to reach 300 millions. 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 may lead to complications, such as micro- and macro-vascular damages, 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*.

Formation of the developing 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 duct cells. The latter 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 aggregate into small clusters of cells termed islets of Langerhans. These correspond to specialized micro-organs composed of five original cell subtypes, 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 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). Concomitantly, glucagon-producing cells are the first endocrine cells detected (Herrera et al., 1991, Teitelman et al., 1993, Upchurch et al., 1994). One day later (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. Hence, genetic manipulations, such as loss-, but also gain-of-function approaches, have permitted researchers to better define the function of the genetic regulators implicated in the development of the pancreas. The specification of the early pancreas domain was thereby found governed by a set of mesenchymal-derived factors, including members of the Fibroblast Growth Factor, Sonic HedgeHog, Retinoic Acid and Bone Morphogenetic Protein signalling pathways, and have recently been excellently reviewed (Hebrok et al., 2003, Kumar and Melton, 2003, Jensen et al., 2004, Grapin-Botton and Constam, 2007).

The genetic program underlying the genesis of the pancreas

In the following, we outline some of the most crucial key players (Pdx1, Ngn3, IA1, Pax4, Arx, Nkx2.2, Nkx6.1, Nkx6.2, Pax6, MafA) implicated in the specification of the pancreatic cell subtypes with particular emphasis on the endocrine lineages.

Pdx1, a homeodomain-containing transcription factor, is expressed as early as E8.5 in the dorsal and ventral endoderm distal to the stomach and duodenal epithelium. Subsequently, *Pdx1* expression progressively becomes restricted to the beta-cell lineage in which it controls the 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, outlined 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 and playing a crucial role in acinar cell development (Hale et al., 2005). Indeed, this factor, belonging to the bHLH transcription factor family, was initially identified as a transcriptional regulator operating in acinar cells (Cockell et al., 1989). During early pancreas development, *Ptf1a* is expressed throughout the pancreatic epithelium, whereas, at later stages, it is exclusively detected in acinar, but not

ductal, nor endocrine cells (Krapp et al., 1996). Consistently, transgenic mice lacking *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). In the light of these results, it was assumed that *Ptf1a* was exclusively required for the formation of acinar cells, whereas endocrine and duct cells derived from different progenitors. However, Kawaguchi et al. (2002) revised this hypothesis by means of lineage tracing performed *in vivo* and thereby found that almost all acinar cells, but also the majority of duct and endocrine cells, derive from *Ptf1a*-expressing progenitor cells. This suggests that both *Pdx1* and *Ptf1a* factors are required to drive pancreatic multipotent progenitors towards a pancreatic cell fate. To gain further insight into the hierarchy of *Pdx1* and *Ptf1a*, the phenotype of animals with combined loss of both factors was analyzed (Burlison et al., 2008). Such double-mutant mice displayed a similar alterations to those seen in *Pdx1* single mutant mice, suggesting that *Ptf1a* acts downstream of *Pdx1*. However, recent findings revealed that *Ptf1a* 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 for the differentiation of the endocrine tissue. Accordingly, lineages tracing analyses indicated that endocrine cells derived from cells that expressed *Ngn3* (Gu et al., 2002). Detectable from E8.5 to E15.5, its expression is subsequently reduced so that, under normal conditions, it is barely detectable in adults. 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). 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 led to a premature onset of endocrine differentiation resulting in the nearly 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 in 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 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 recently outlined 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 initially exclusively give rise to alpha-cells. Importantly, the activation of *Ngn3* at later developmental stages was found to promote the genesis of beta- and PP- (after E11.5) or delta- (after E14.5) cells, while the competence to give rise to alpha-cells was dramatically decreased.

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 be a direct downstream target of *Ngn3* and to act in the initial steps underlying the endocrine cell differentiation. Endocrine cells were still detected in *IA-1* mutant pancreata, but the majority of these did 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 duct-to-endocrine-transdifferentiation 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 lacked (Collombat et al., 2003). It worth noticing 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 a depletion of beta- and alpha-cells, while the delta-cell population is increased and the PP-cell content 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 worth noticing 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. Throughout pancreas development, its expression is initially detectable in *Ngn3*⁺ endocrine precursor cells, but becomes restricted to mature beta-cells where it was found 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 display 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, the misexpression of *Nkx6.2* in *Pdx1*-producing cells of *Nkx6.1*^{-/-} mice 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). 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-cells as well as alpha-cells. For instance, *MafA* was found to directly interact with the promoter of the *insulin* gene and 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, an 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.

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 as some of them 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 are 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 bases towards the ultimate goal of reprogramming abundant and easily accessible patient-specific human cells, such as fibroblasts, blood cells or adipocytes, into insulin-producing beta-cells.

Numerous protocols aiming at differentiating ES-cells have been previously elegantly reviewed (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 Pancreatic Duct Ligation (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). Along the same vein, as both liver and pancreas arise from adjacent regions of the embryonic endoderm, it was supposed 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 to a conversion of hepatic cells into insulin-producing cells, and a significantly amelioration of glucose-tolerance (Kaneto et al., 2005).

Focusing on the directed generation of endocrine cells, several recent studies concentrated on the characterization of additional transcription factors acting downstream of *Pdx1* and 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 is of fundamental importance in the context of beta-cell-based therapy as it implies that the opposite conversion might be achieved, that is, to generate beta-cells from other endocrine cells. It is therefore suspected that the conditional

misexpression of the *Arx* counterpart, *Pax4*, in different pancreatic cell populations may lead to their dedifferentiation/redifferentiation or transdifferentiation into insulin- and/or somatostatin-producing beta-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 was found composed 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 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 found only required 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 muscles 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 the 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 be ultimately be used

for further differentiation into insulin-producing beta-cells (Takahashi and Yamanaka, 2006).

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Mechanisms of beta-cell replenishment *in vivo*

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, outlines the activity of adaptive mechanisms (Lingohr et al., 2002, Bonner-Weir, 2000). Furthermore, several approaches, including surgical ablation of pancreatic tissue as well as pancreatic duct ligation, 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: several weeks after discontinued toxin application, treated mice recovered from hyperglycaemia through the genesis of new beta-cells that mainly originated from surviving beta-cells (Nir et al., 2007). Teta and coworkers, using an elegant approach, provided more insight into the mitotic behavior of beta-cells under normal, as well as regenerative conditions. Through the sequential administration of CldU and IdU, they were able to label cells that had undergone more than one round of cell division and therefore identify potential stem- or progenitor-cells. Relating to pancreatic beta-cells, they observed that repeatedly dividing progenitors did not contribute to adult beta-cell mass under physiological conditions. Even during regeneration following 50% pancreatectomy as well as during pregnancy, beta-cells were found to arise from preexisting beta-cells (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 has been brought by Wang and colleagues (Wang et al., 1995). Employing

pancreatic duct ligation (PDL) to provoke a regenerative response in rats, they noticed a significant islet cell hyperplasia, these being mostly composed of cells exhibiting beta-cell features. BrdU pulse-chase labeling experiments indicated that beta-cell proliferation could not fully account for the drastic beta-cell hyperplasia observed in the ligated portion of the pancreas. It was therefore concluded that multipotent precursors and/or stem cells may be implicated in these regeneration events. The concomitant augmentation in the numbers of beta-cells or duct cells led to the assumption that newly formed beta-cells may have a ductal origin. In 2008, a pioneering study from Heimberg's laboratory conclusively established the involvement of duct-lining precursor cells in pancreatic islet regeneration following PDL. Similar to the observations made next to partial pancreatectomy, newly formed beta-cells were found to originate from self-duplication of pre-existing beta-cells. However, the authors also identified cells re-expressing the proendocrine gene *Ngn3* in the ductal lining of adult pancreas that underwent PDL. Such cells were subsequently FACS sorted and were found capable to give rise to all four endocrine cell subtypes (Xu et al., 2008). Interestingly, through the generation of transgenic mice expressing an inducible form of the cre-recombinase under the control of a ductal promoter, 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. This conversion was accompanied by several rounds of replication followed by dedifferentiation and redifferentiation. Remarkably, in non-injured neonates, the naturally occurring postnatal increase in islet cell mass was also partially attributed duct cell conversion (Inada et al., 2008). Altogether, the finding 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 future greatest challenges of diabetes research.

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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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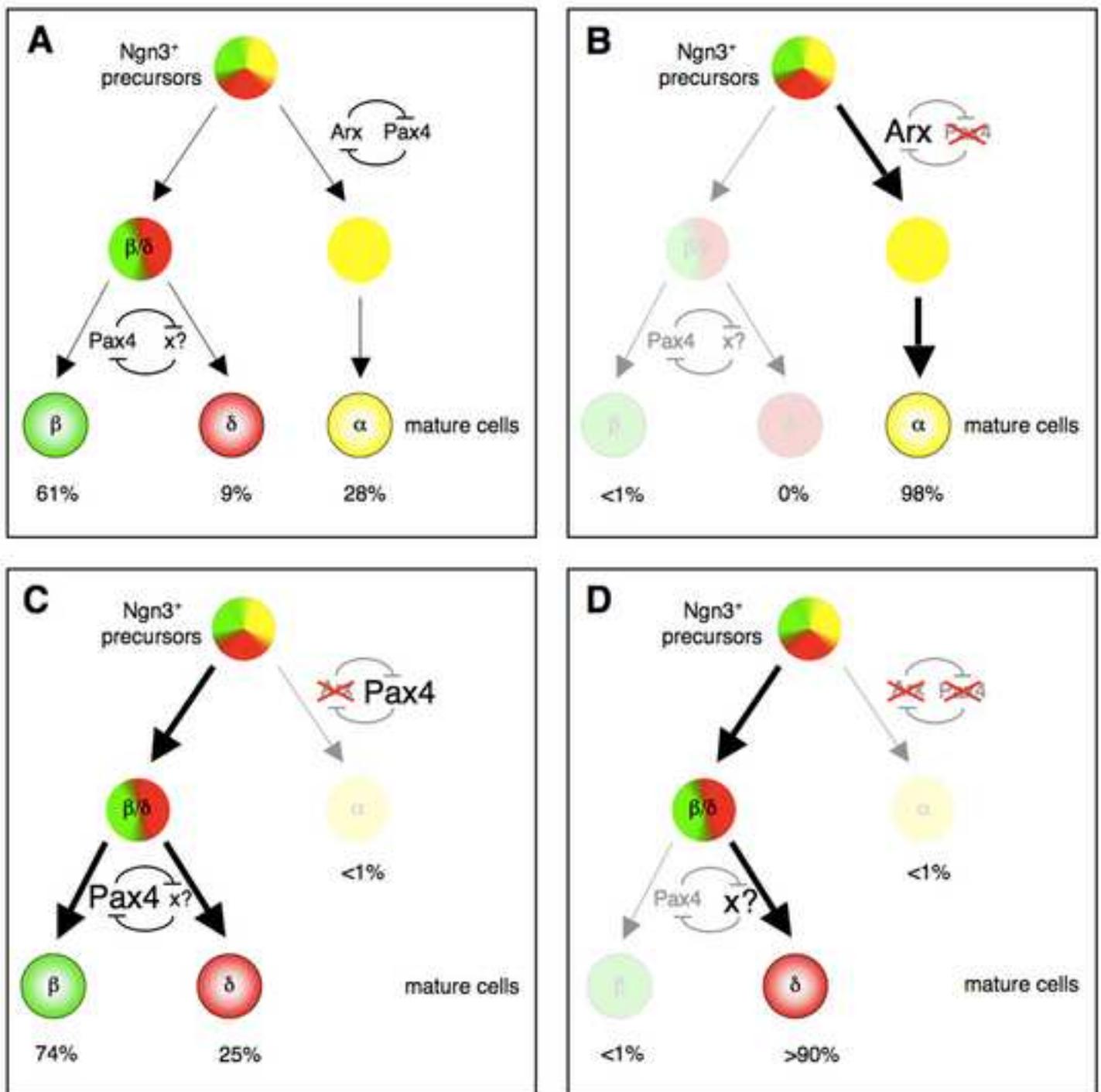
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Figure legends:

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 post-pancreatic duct ligation 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

