Role of transcription factors in the transdifferentiation of pancreatic islet cells

Talitha van der Meulen[1] and **Mark O Huising**[1,2]

[1]Department of Neurobiology, Physiology and Behavior, College of Biological Sciences, and [2]Department of Physiology and Membrane Biology, School of Medicine, University of California, 193 Briggs Hall, One Shields Avenue, Davis, California 95616, USA

Correspondence should be addressed to M O Huising
Email
mhuising@ucdavis.edu

Abstract

The α and β cells act in concert to maintain blood glucose. The α cells release glucagon in response to low levels of glucose to stimulate glycogenolysis in the liver. In contrast, β cells release insulin in response to elevated levels of glucose to stimulate peripheral glucose disposal. Despite these opposing roles in glucose homeostasis, α and β cells are derived from a common progenitor and share many proteins important for glucose sensing and hormone secretion. Results from recent work have underlined these similarities between the two cell types by revealing that β-to-α as well as α-to-β transdifferentiation can take place under certain experimental circumstances. These exciting findings highlight unexpected plasticity of adult islets and offer hope of novel therapeutic paths to replenish β cells in diabetes. In this review, we focus on the transcription factor networks that establish and maintain pancreatic endocrine cell identity and how they may be perturbed to facilitate transdifferentiation.

Key Words
- α cell
- β cell
- transdifferentiation
- dedifferentiation
- ARX
- PAX4
- PDX1
- NKX2-2
- FOXO1
- diabetes

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Introduction

The pancreatic islets consist of several endocrine cell types including insulin-producing β cells, glucagon-producing α cells, somatostatin-producing δ cells, pancreatic polypeptide-producing PP cells, and ghrelin-producing ε cells. The β cells are active under conditions of nutrient excess, particularly high blood glucose levels, and secrete insulin, which has peripheral actions on liver, muscle, and fat tissues that reduce blood glucose (Thorel *et al*. 2010, Lin & Accili 2011, Leto & Saltiel 2012). Insulin is functionally opposed by glucagon, which promotes liver glycogenolysis to prevent hypoglycemia during fasting or exercise (Ramnanan *et al*. 2011). Products of β cells inhibit α cell secretion (Franklin *et al*. 2005), while α-cell-derived glucagon, paradoxically, activates β cells to secrete insulin (Samols *et al*. 1965, Kawai *et al*. 1995). The δ cells are generally considered to be the brake on islet secretion as somatostatin inhibits the release of both insulin and glucagon (Strowski *et al*. 2000, Hauge-Evans *et al*. 2009), thereby preventing large swings in blood glucose levels. It was generally thought that the endocrine cell types present in the islet were distinct and terminally differentiated populations. However, recent observations indicate that, under certain conditions, pancreatic cells of one type can transdifferentiate to another. This review focuses on such interconversions between the various islet endocrine cells and does not discuss in detail the ability of other pancreatic cells, such as acinar cells, to transdifferentiate into β cells following viral transduction (Zhou *et al*. 2008) or in

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response to soluble factors (Baeyens *et al.* 2014). Two apparently distinct pathways have been described by which transdifferentiation of islet endocrine cells takes place, i.e., where a hormone-expressing islet cell converts into a hormone-expressing islet cell of a different type. One is direct transdifferentiation that proceeds via a double-hormone-positive intermediate cell (Lu *et al.* 2010, Thorel *et al.* 2010, Papizan *et al.* 2011, Yang *et al.* 2011). The other involves loss of hormone expression during a process of reversion to a precursor-like state before differentiation into a hormone-positive cell of a different type (Jonas *et al.* 1999, Talchai *et al.* 2012, Wang *et al.* 2014). Whether these different phenotypes represent mechanistically distinct transdifferentiation processes, or merely represent different gradations of a single transdifferentiation spectrum is not clear at this time and cannot always be distinguished by lineage-tracing approaches, which are best suited to evaluate the end result of the transdifferentiation process. It is clear, however, that the process of transdifferentiation of islet endocrine cells has generated considerable excitement as a potentially novel strategy for treating diabetes. While diabetes is traditionally cast as a disease of relative (type 2) or absolute (type 1) insufficiency in β cell mass, it is less appreciated that hyperglucagonemia secondary to the loss of the normal inhibitory tone on α cells from β cells contributes to the etiology of diabetes by aggravating hyperglycemia (Franklin *et al.* 2005, Unger & Cherrington 2012). Despite their opposing roles in the maintenance of a dynamic equilibrium of blood glucose levels, α and β cells are derived from a common progenitor. They continue to share expression of many transcription factors and genes for glucose uptake and glycolysis, stimulus–secretion coupling, and hormone exocytosis (Benner *et al.* 2014). This resemblance makes non-β endocrine cells the cell types that are most closely related to β cells and makes them prime candidates to target for conversion into β cells (Fig. 1).

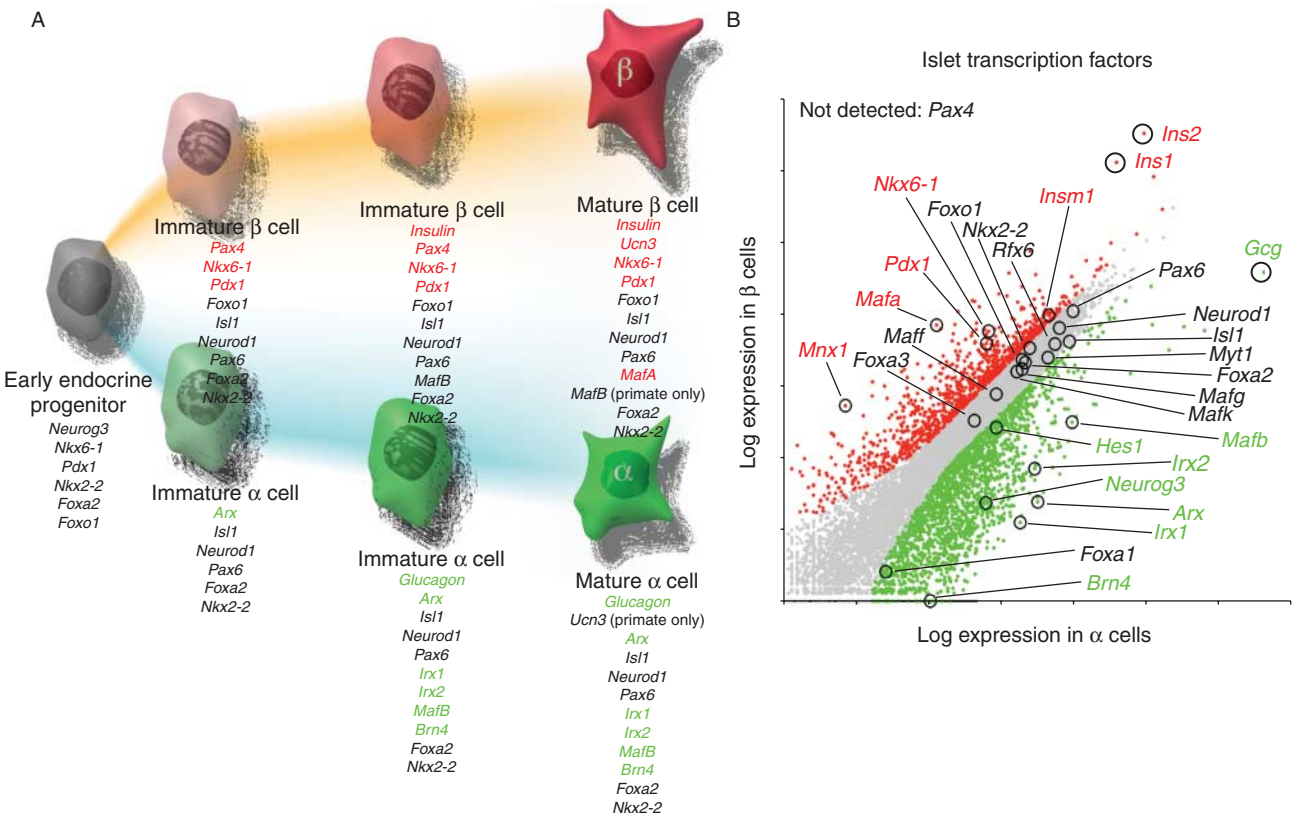

Figure 1

Development and maintenance of α and β cells from a common progenitor (A) require intricate networks of transcription factors. Transcription factors and hormones specific to β (red) and α (green) cells are indicated. Despite their opposing functions in the regulation of blood glucose levels, α and β cells continue to share the expression of many transcription factors (black). Specificity of gene expression was determined by significant ($P<10^{-7}$) enrichment in one cell type compared with the other (B). This figure was adapted from Benner C, van der Meulen T, Caceres E, Tigyi K, Donaldson CJ & Huising MO 2014 The transcriptional landscape of mouse beta cells compared to human beta cells reveals notable species differences in long non-coding RNA and protein-coding gene expression. *BMC Genomics* **15** 620, published as an open access article by Biomed Central.

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Herein, we will provide an overview of the recent literature on the differentiation and transdifferentiation of β and α cells, based largely on experimental observations of transgenic and knockout mouse models, with emphasis on the transcription factors that are implicated in transdifferentiation of β and α cells, before we address the application of transdifferentiation in managing diabetes.

Transcription factors in early pancreas formation

The pancreas is first identified by the transcription factor pancreatic and duodenal homeobox 1 (PDX1), which marks the pancreatic anlage starting at embryonic day 8.5 (E8.5) in the mouse (Ohlsson *et al*. 1993, Guz *et al*. 1995). The absolute requirement of PDX1 for pancreas development is evident from global loss of PDX1, which results in the absence of a pancreas at birth (Jonsson *et al*. 1994, Offield *et al*. 1996). A hypomorphic allele of PDX1, created by the deletion of some regulatory sequences for PDX1 expression, results in a remnant pancreas with ductal and acinar cells, but a relative lack of endocrine cell development, with α cells being least affected (Fujitani *et al*. 2006). PDX1 expression is quickly followed by expression of the basic helix–loop–helix protein pancreas-specific transcription factor 1a (PTF1A; Krapp *et al*. 1996, Hald *et al*. 2008), forkhead box O1 (FOXO1; Kitamura *et al*. 2009), NK2 homeobox 2 (NKX2-2; Sussel *et al*. 1998), and NK6 homeobox 1 and 2 (NKX6-1 (Sander *et al*. 2000) and NKX6-2 (Henseleit *et al*. 2005) respectively). PTF1A is initially expressed in progenitors comprising the early pancreatic epithelium but subsequently becomes restricted to the exocrine acinar cells and their precursors (Krapp *et al*. 1996, Kawaguchi *et al*. 2002, Hald *et al*. 2008, Pan *et al*. 2013). PTF1A expression is repressed by the NKX6 proteins and PTF1A conversely represses expression of the *NKX6* genes, thereby segregating the acinar lineage from the ductal/endocrine lineages (Schaffer *et al*. 2010). FOXO1 and PDX1 continue to be expressed in the same pancreatic cells, but either one or the other is localized to the nucleus (Kitamura *et al*. 2002, 2009). FOXO1 inhibits PDX1 function (Kitamura *et al*. 2002), yet the expression of FOXO1 is important for maintenance of normoglycemia as animals age (Talchai *et al*. 2012). The transcription factor that marks the earliest progenitor exclusive to all endocrine cells within the pancreas is the basic helix–loop–helix transcription factor neurogenin 3 (NGN3; Gu *et al*. 2002). NGN3 is required for endocrine cell formation (Gradwohl *et al*. 2000) and initiates a new set of developmental programs as expression of many transcription factors that are very important for endocrine cell lineage differentiation and maintenance depend on NGN3. These factors include the LIM homeobox protein islet 1 (Isl1), paired box 4 and 6 (PAX4 and PAX6), aristaless-related homeobox (ARX), and neuronal differentiation 1 (NEUROD), which are all lost in *Ngn3* (*Neurog3*)-deficient mice (Gradwohl *et al*. 2000, Collombat *et al*. 2003). The competency of progenitors changes with time such that $NGN3^+$ cells that arise early in development preferentially differentiate into $glucagon^+$ cells while later-born $NGN3^+$ cells give rise to $insulin^+$ or $somatostatin^+$ cells (Johansson *et al*. 2007). The number of differentiating $NGN3^+$ cells is limited by lateral inhibition via Notch signaling (Apelqvist *et al*. 1999, Murtaugh *et al*. 2003). After endocrine cell fate is specified by NGN3, PDX1 becomes restricted to β cells and a subset of δ cells by E13.5 (Ohlsson *et al*. 1993, Peshavaria *et al*. 1994) and helps maintain β cell mass (Ahlgren *et al*. 1998). NKX2-2 and NKX6-1 expression also become progressively restricted to the endocrine compartment (Sussel *et al*. 1998, Sander *et al*. 2000). NKX2-2 marks most endocrine cells with the exception of δ cells. Deletion of NKX2-2 causes major changes in the endocrine makeup of the islets, as α and PP cells are reduced in number (Sussel *et al*. 1998), while β cells are replaced by ghrelin cells (Prado *et al*. 2004). Within the islet, NKX6-1 functions downstream of NKX2-2 and is both necessary and sufficient for β cell neogenesis during the secondary transition, a time of further differentiation and major expansion of the endocrine cell mass. By E15.5, NKX6-1 is found exclusively in β cells and scattered ductal and periductal cells (Sander *et al*. 2000, Schaffer *et al*. 2013). While NKX2-2 and NKX6-1 (and in its absence NKX6-2) are required for early endocrine cell development, including α cells (Sussel *et al*. 1998, Henseleit *et al*. 2005), NKX6-1 expression is later lost from the developing α cells. In fact, continued expression of both NKX2-2 and NKX6-1 in the β cells actually represses α cell fate via repression of ARX (see also below) to maintain β cell identity (Papizan *et al*. 2011, Schaffer *et al*. 2013). These observations are indicative of a central role of the PDX1, NKX2-2, and NKX6-1 transcription factors in the early development of β cells.

Transcription factors later in β cell differentiation

The transcription factor PAX4 is induced later than NKX2-2 and NKX6-1 and acts downstream of NGN3 (Gradwohl *et al*. 2000). PAX4 is necessary for normal β and δ cell development, as α and ghrelin cell numbers are increased in its

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PDX1 expression that marks the start of insulin transcription. In the absence of MAFB, fewer α and β cells are present, although total endocrine cell mass is not changed (Artner *et al.* 2007). MAFA expression follows shortly after insulin expression (Artner *et al.* 2006, Nishimura *et al.* 2006) and persists in adult β cells (Fig. 2). MAFB expression

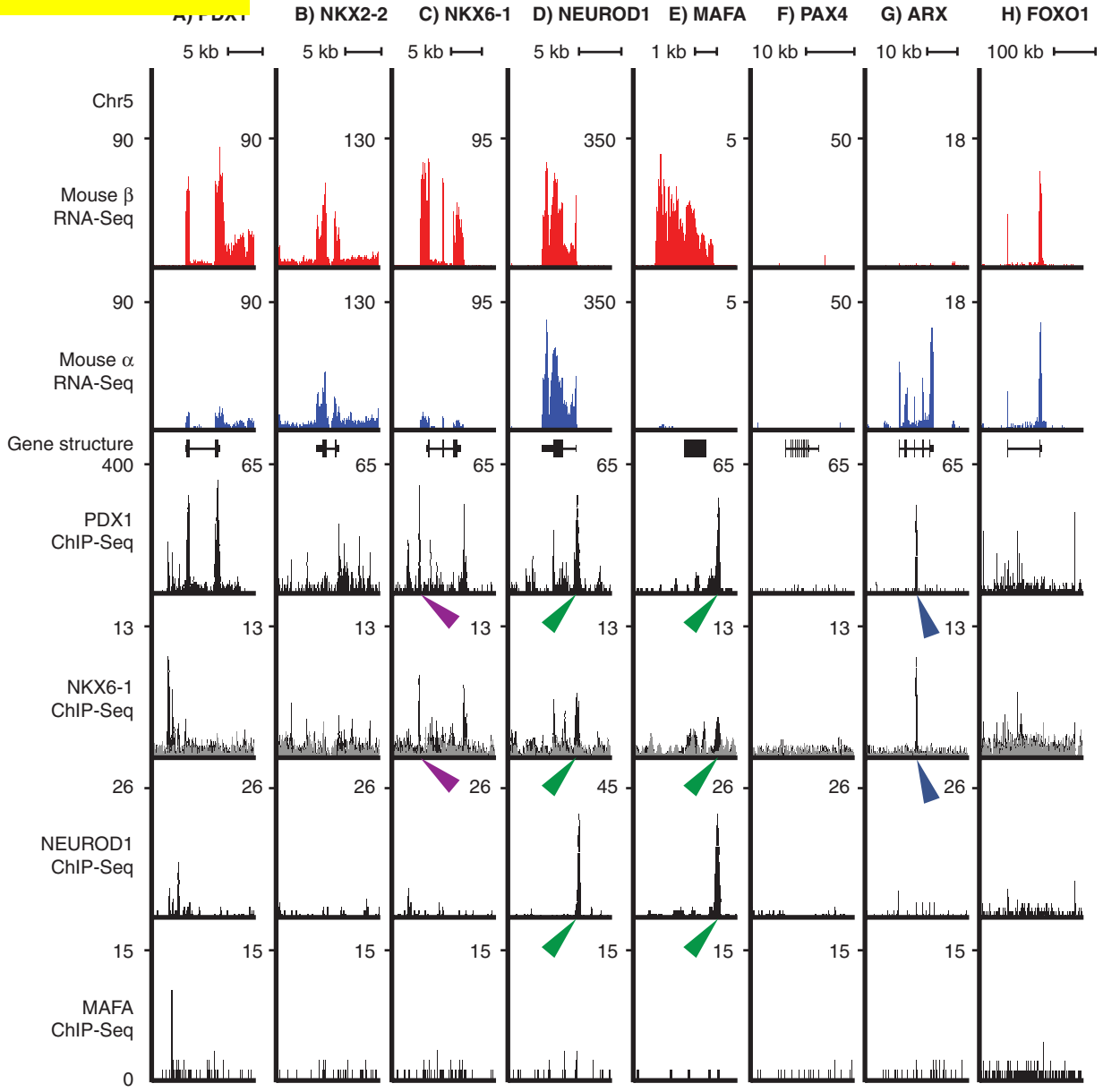

Figure 2

Illustration of the pancreatic transcription networks in α and β cells. Expression of transcription factors PDX1 (A), NKX2-2 (B), NKX6-1 (C), NEUROD1 (D), MAFA (E), PAX4 (F), ARX (G), and FOXO1 (H) in β and α cells as determined by whole transcriptome sequencing (RNA-Seq), combined with chromatin immunoprecipitation data (ChIP-Seq) for PDX1 (Khoo *et al.* 2012), NKX6-1 (Taylor *et al.* 2013), and NEUROD1 and MAFA (Tennant *et al.* 2013). Purple arrowheads indicate binding of NKX6-1 and PDX1 to the same TAAT sequence in the *NKX6-1* gene, green arrowheads indicate closely spaced but non-identical binding sites for *NEUROD1*, *NKX6-1*, and *PDX1* on the *NEUROD1* and *MAFA* genes. Blue arrowheads indicate the *PDX1* and *NKX6-1* binding sites on the *ARX* gene. NKX6-1 inhibits ARX expression via this interaction (Schaffer *et al.* 2013).

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is lost postnatally from mouse (Artner *et al*. 2010) but not human (Dai *et al*. 2012, Riedel *et al*. 2012) β cells. In *Mafb*-deficient mice, insulin expression is reduced and delayed until MAFA becomes expressed, but the resulting insulin$^+$ cells lack PDX1, NKX6-1, and GLUT2 (SLC2A2) (Artner *et al*. 2007) and are therefore unlikely to represent true β cells. In MAFA-deficient pancreata, about one-third of β cells continue to express MAFB postnatally, which may partially compensate for the loss of MAFA (Artner *et al*. 2010). Nevertheless, these mice display reduced expression of several genes involved in glucose-stimulated insulin secretion and have reduced glucose tolerance (Zhang *et al*. 2005, Artner *et al*. 2010, Hang *et al*. 2014). Upon β cell specification, the transcription of insulin is initiated and maintained by MAFB (Matsuoka *et al*. 2003), PDX1 (Ohlsson *et al*. 1993), NEUROD1 (Naya *et al*. 1995), PAX6 (Sander *et al*. 1997), and MAFA (Kataoka *et al*. 2002, Olbrot *et al*. 2002, Matsuoka *et al*. 2003). Additionally, PAX4 (until expression is lost in adult, Fig. 2), NKX6-1, and PDX1 inhibit glucagon expression (Ritz-Laser *et al*. 2002, Schisler *et al*. 2005), thereby preventing the expression of glucagon by β cells.

Transcription factors that define the α cell

ARX is one of the first known transcription factors to mark the α cell lineage (Fig. 2) and is required for α cell development (reviewed in Bramswig & Kaestner (2011)). In the absence of ARX, no α cells develop and the number of β and δ cells increases without affecting the total number of endocrine cells. This indicates that precursors alter their fate from α to β and δ (Collombat *et al*. 2003). This phenotype is exactly the converse of that seen in *Pax4*-deficient mice described earlier in this review. Indeed, a competitive reciprocal interaction between ARX and PAX4 is involved in the decision of cell fate between α and β cell (Collombat *et al*. 2003, 2005). In the absence of ARX, PAX4 expression in early development takes over the ARX domain, leading to increased numbers of β/δ cells at the expense of the α cell population. Conversely, in the absence of PAX4, ARX expression in early development expands, resulting in the opposite phenotype. When both ARX and PAX4 are lost, both α and β cells are lost, while the δ cell population persists (Collombat *et al*. 2005). The same holds true for mice in which both ARX and NKX2-2 are lost (Kordowich *et al*. 2011, Mastracci *et al*. 2011). From these observations, it follows that ARX is required to generate the α cell phenotype, while NKX2-2 and PAX4 are required for the β cell phenotype and are permissive of, but not required for, δ cell formation. While ARX is necessary for the early specification of the α cell and directly maintains α cell mass (Courtney *et al*. 2013), it is not directly involved in glucagon expression (Gosmain *et al*. 2011).

Transcription factors later in α cell differentiation

The PAX4-related factor PAX6 is expressed by both α and β cells. *Pax4*-deficient mice lack α cells (St-Onge *et al*. 1997). In addition to its role in insulin transcription in the β cell, PAX6 also coordinates glucagon transcription in the α cell directly by binding to the glucagon promoter (Sander *et al*. 1997) and indirectly by inducing expression of other transcription factors such as c-Maf, MAFB, and NEUROD1, which also activate glucagon expression (Dumonteil *et al*. 1998, Artner *et al*. 2006, Gosmain *et al*. 2011). Moreover, PAX6 forms heterodimers with c-Maf on the glucagon promoter (Gosmain *et al*. 2007). A third factor that is crucial for α cell development is FOXA2. *Foxa2*-deficient pancreata show normal expression levels of ARX and PAX6, indicating that FOXA2 plays a role later in α cell development downstream of these transcription factors. Indeed, *Foxa2*-deficient pancreata display a lack of mature α cells while α cell precursors are present (Lee *et al*. 2005). Upon successful specification of the α cell lineage, pre–pro-glucagon expression is further promoted by FOXA1 (Kaestner *et al*. 1999), BRN4 (Hussain *et al*. 1997), and ISL1 (Wang & Drucker 1995, Du *et al*. 2009). Of all these transcription factors that can promote glucagon expression, expression of only two, BRN4 and MAFB, is enriched in adult mouse α cells compared with β cells, while transcripts for *Pax6, Foxa2, Foxa1*, and *Isl1* are expressed at similar levels in mouse α and β cells (Benner *et al*. 2014).

Redundant transcription factor networks

As just described, the identity of the different pancreatic lineages is coordinated by a complex network of transcription factors that first establishes and then maintains differentiated cell identity. The transcription factor networks in the endocrine pancreas in particular are self-reinforcing, which is illustrated by key transcription factors binding regulatory elements in proximity to their own genes, as well as those of other transcription factors (Pasquali *et al*. 2014). They activate lineage-appropriate genes or, equally importantly, repress expression of lineage-inappropriate genes (Collombat *et al*. 2003, Schaffer *et al*. 2010, 2013, Papizan *et al*. 2011). Multiple transcription factors bind the same promoter and the net

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effect of the individual interactions determines the transcriptional activity of a gene. This principle is illustrated for the NEUROD1, PDX1, and NKX6-1 transcription factors in Fig. 2. NEUROD1, which recognizes a different core motif from NKX6-1 and PDX1 (Seo *et al.* 2007), binds in close proximity to PDX1 and NKX6-1 on the promoters for *Neurod1* and *Mafa* (Fig. 2D and E, green arrowheads). Moreover, related transcription factors recognize highly similar consensus binding sites. For example, homeobox-containing genes such as *PDX1* (Liberzon *et al.* 2004) and NKX6-1 (Jorgensen *et al.* 1999, Taylor *et al.* 2013) recognize a core TAAT sequence, while their preference for the adjacent nucleotides is less stringent. Indeed, super-imposable peaks for PDX1 and NKX6-1 over a single TAAT sequence are evident on the *NKX6-1* gene (Fig. 2C, purple arrowheads). The β-cell-specific PDX1 and NKX6-1 bind the α-cell-specific *ARX* gene suggesting an inhibitory effect of this binding (Fig. 2, blue arrowheads). Indeed, this is known for the NKX6-1-binding site (Schaffer *et al.* 2013). The resulting redundancy within the transcriptional networks may help maintain cell identity. Conversely, severe disruptions of the network compromise cell identity and contribute to dedifferentiation and transdifferentiation.

Transdifferentiation of β to α cells

Forcing β-to-α transdifferentiation by over-expression of ARX

One of the first pieces of evidence that indicated that β cells can be transdifferentiated into α cells resulted from the forced mis-expression of ARX within the pancreas. Transgenic mice were generated that expressed ARX as well as β-galactosidase from the human β-actin promoter (CAG), but only upon Cre-recombinase-mediated recombination. When ARX was expressed in all pancreas cells (by PDX1-Cre (Gu *et al.* 2002)) or all endocrine cells (by PAX6-Cre (Ashery-Padan *et al.* 2000)), pancreata showed massive reductions in β and δ cell numbers and increased α and PP cell numbers, predictably resulting in hyperglycemia (Collombat *et al.* 2007). The total number of endocrine cells was not altered upon over-expression in the entire pancreas, indicating that ARX is not able to divert pancreatic non-endocrine progenitor cells to an α cell fate, but instead acts on endocrine progenitors and/or their offspring. Persistent ARX expression in all β cells (using rat Ins2-Cre (Herrera 2000)) also resulted in the transdifferentiation of β cells toward α and PP cells (Collombat *et al.* 2007). The numbers of δ cells were unchanged. No double-hormone-positive cells were reported, indicating that β cells first down-regulated insulin before expressing glucagon (Collombat *et al.* 2007). Taken together, these results indicate that ARX expression not only directs endocrine progenitors toward the α and PP cell fate early in development, but also is able, later in development, to overcome an established β cell fate in favor of an α cell fate.

The importance of PDX1 for β cell identity

In addition to the importance of PDX1 for early pancreas specification, several lines of evidence indicate that PDX1 is also important for subsequent β cell generation and maintenance of β cell identity. Forced expression of PDX1 in all $NGN3^+$ cells and their offspring via NGN3-Cre resulted in a reduction in the embryonic α cell population with a concomitant increase in the β cell population (Yang *et al.* 2011). Deletion of PDX1 slightly later in development, upon insulin expression using Cre recombinase under the control of the rat insulin 1 promoter (RIP1), resulted in the opposite phenotype: reduced β and increased α cell numbers, with many double-hormone-positive cells as well as overt diabetes by 3–5 months of age (Ahlgren *et al.* 1998). Cre-mediated recombination in this mouse line was inefficient and only became prominent by 3–5 weeks of age. Similar experiments using a more efficient rat insulin 2 promoter-driven Cre recombinase (RIP-Cre; Postic *et al.* 1999, Gannon *et al.* 2000) revealed earlier recombination, but essentially the same phenotype, except in an accelerated fashion and without double-hormone-positive cells. Results of lineage tracing of the recombined β cells using RIP-Cre indicated that α cells exhibited an increased proliferation rate, while β cells decreased proliferation, with no detectable β-to-α transdifferentiation (Gannon *et al.* 2008). However, a more recent study using tamoxifen-inducible RIP-CreER (Dor *et al.* 2004) to delete *Pdx1* in the β cells of young adult (30-day-old) mice reaches a different conclusion (Gao *et al.* 2014). Herein, β cell-specific *Pdx1* ablation also resulted in diabetes and increased numbers of α cells at the expense of β cells, but herein the mechanism is transdifferentiation of β to α cells (Gao *et al.* 2014). It is possible that the difference in outcome between these studies is attributable to the timing of the onset of *PDX1* ablation, which is commensurate with the onset of insulin expression in immature β cells during embryonic development using Rip1-Cre (Gannon *et al.* 2008), but was started at 30 days post parturition using RIP-CreER (Gao *et al.* 2014) and thus did not occur until most β cells have matured (van der Meulen *et al.* 2012). Moreover, these two stages

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differ substantially in the way new pancreatic cells can be generated. During embryonic development and up to the first 3 weeks of postnatal life, islet cells continue to be generated through neogenesis from ductal precursors (Kopp *et al.* 2011). In contrast, self-replication is the main mechanism by which β cell mass is maintained in the adult (Dor *et al.* 2004). This is indicative of an age-dependent difference in plasticity between the embryonic pancreas and the pancreas of young adult mice. *Pdx1* ablation mediated by RIP-Cre and RIP1-Cre takes place during late embryonic development and can activate compensatory changes in endocrine cell ratios via both neogenesis and proliferation. In contrast, the window for neogenesis has largely closed upon tamoxifen-dependent *Pdx1* ablation in RIP-CreER mice at p30 and leads to more pronounced β-to-α transdifferentiation undiluted by neogenesis. The tamoxifen-inducible RIP-CreER line offers an opportunity to directly compare the effects of *PDX1* ablation at different onsets on the ensuing putative α-to-β cell transdifferentiation.

Maintaining β cell identity through NKX2-2 expression

Results of a recent study have highlighted the importance of NKX2-2, in particular its tinman domain, for the maintenance of β cell identity (Papizan *et al.* 2011). The tinman domain is located outside the homeobox domain and is not directly involved in DNA binding, but instead recruits Groucho corepressors (Muhr *et al.* 2001). Deletion of the tinman domain of NKX2-2 (NKX2-2$^{TNmut/TNmut}$) phenocopies some aspects of total NKX2-2 deficiency, including reduced β cell mass, but the phenotype diverges in several important respects from that of global *Nkx2-2* null mice. First, until E12.5, α and β cell development appears normal (Papizan *et al.* 2011), while *Nkx2-2*-deficient mice have reduced α cell numbers and lack β cells altogether (Sussel *et al.* 1998). Secondly, in contrast to the lower α cell numbers in *Nkx2-2*-deficient mice, NKX2-2$^{TNmut/TNmut}$ pancreata show increased α cell numbers from E18.5 onward as well as an increase in the number of cells co-expressing glucagon and insulin. Yet, NKX2-2$^{TNmut/TNmut}$ mutants have no overt metabolic phenotype until 3.5 weeks of age when progressive hyperglycemia develops, in accordance with the increase in α cell numbers paired with a decrease in β cells (Papizan *et al.* 2011). Results of lineage tracing of the existing β cells using rat Ins2-Cre (Herrera 2000) indicate that at least a few of the increased population of α cells in the NKX2-2$^{TNmut/TNmut}$ mutant mice derive from transdifferentiated β cells, as more than 2% of α cells carried a β cell lineage mark (Papizan *et al.* 2011). Irreversibly labeling β cells at 3 weeks of age using a PDX1-CreER (Gu *et al.* 2002) reveals that mature β cells continue to transdifferentiate before any metabolic phenotype is evident (Papizan *et al.* 2011). While these results are very exciting, they should be interpreted with the understanding that the constitutive lack of the tinman domain of NKX2-2 could have affected β cell development in ways that were not immediately obvious from histological analyses, for example, by inhibiting final differentiation steps, potentially making it easier for these β cells to transdifferentiate. Of note is the fact that deletion of *Arx* from β cells in NKX2-2$^{TNmut/TNmut}$ mice prevented β-to-α transdifferentiation (Papizan *et al.* 2011), as this highlights once more the requirement of ARX for differentiation along the α cell lineage. Mechanistically, NKX2-2 achieves inhibition of the *Arx* promoter by forming a complex with both the *de novo* methyltransferase 3a (Dnmt3a), which methylates CG dinucleotides to repress transcription, and histone deacetylase 1 (Hdac1; Papizan *et al.* 2011). This NKX2-2/Dnmt3a/Hdac1 complex occupies the *Arx* promoter and inhibits ARX transcription (Papizan *et al.* 2011). The β-cell-specific deletion of *Dnmt3a* (Papizan *et al.* 2011), or of the related *Dnmt1* (Dhawan *et al.* 2011), predictably resulted in the de-repression of ARX in β cells followed by β-to-α transdifferentiation. As NKX2-2 and the other known components of the ARX repression complex are also expressed in adult ARX^{+} α cells, there are probably additional β-cell-specific factors that aid in ARX repression in the β cell. In summary, NKX2-2 is required to facilitate endocrine differentiation early in embryonic development. Later in β cell development, NKX2-2 takes on the role of maintaining β cell identity and in this capacity prevents transdifferentiation to the α cell lineage by epigenetically modifying the *Arx* promoter.

Maintaining β cell identity through FOXO1 expression

Another transcription factor that is integral for maintaining β cell identity is FOXO1. While *FOXO1* mRNA is similarly expressed in both α and β cells (Benner *et al.* 2014), results of immunofluorescence analysis indicate that FOXO1 protein is more restricted to β cells (Kitamura *et al.* 2009). In β cells, FOXO1 is normally found in the cytoplasm, but mild hyperglycemia causes nuclear translocation of FOXO1 (Talchai *et al.* 2012), possibly contributing to the maintenance of MAFA and NEUROD1 expression and preservation of β cell function (Kitamura *et al.* 2005). Indeed, ablation of *Foxo1* from β cells via rat *Ins2*-Cre (Herrera 2000) increases susceptibility of β cells to metabolic stress

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(Kobayashi *et al.* 2012, Talchai *et al.* 2012). In younger mice on a *db/db* background, that is associated with severe obesity, β-cell-specific deletion of *Foxo1* further impaired glucose tolerance (Kobayashi *et al.* 2012). Older multiparous females and aging males that lack β cell FOXO1 expression demonstrate increased α cell mass, reduced β cell mass, and reduced glucose tolerance accompanied by concomitant increases in plasma glucagon and decreases in plasma insulin levels (Talchai *et al.* 2012). Increased α cell mass in these mice was not due to increased proliferation (Talchai *et al.* 2012). Instead, during metabolic stress, *Foxo1*-deficient β cells lose MAFA, PDX1, and insulin expression (Talchai *et al.* 2012), degranulate (Kobayashi *et al.* 2012, Talchai *et al.* 2012), and dedifferentiate into progenitor-like cells (Talchai *et al.* 2012). These dedifferentiated β cells start to express the endocrine progenitor marker NGN3 (Talchai *et al.* 2012), possibly owing to reduced expression of the Notch effector and NGN3 repressor HES1 in the absence of FOXO1 (Kitamura *et al.* 2007), resulting in de-repression of NGN3 (Lee *et al.* 2001). The increase in NGN3 expression was confirmed using *db/db* and GIRKO mice (Talchai *et al.* 2012); the latter mouse model lacks insulin receptors in muscle and fat and has a high incidence of diabetes (Lin *et al.* 2011). Surprisingly, deletion of *Foxo1* in the *db/db* model decreases NGN3 again (Kobayashi *et al.* 2012). In addition to NGN3, dedifferentiated β cells express the pluripotency markers octamer-binding protein 4 (OCT4 (POU5F1)), avian myelocytomatosis viral oncogene homolog (c-MYC), and the homeobox transcription factor nanog (Talchai *et al.* 2012). Forced expression of c-MYC alone in β cells is already sufficient to induce dedifferentiation in β cells, as indicated by the loss of insulin, PDX1, and SLC2A2 (Cheung *et al.* 2010). Taken together, the expression of NGN3, POU5F1, c-MYC, and NANOG indicate that the progenitor state is a regulated state rather than a regression to a degenerative state (Talchai *et al.* 2012) and raises the question as to whether dedifferentiated β cells can be re-differentiated into mature glucose-responsive β cells. Indeed, the results of lineage tracing of β cells indicated that, after dedifferentiation, they can subsequently re-differentiate into α, δ, and PP cells (Talchai *et al.* 2012). The authors could not demonstrate re-differentiation of dedifferentiated β cells owing to the limitations of the conventional lineage-tracing approaches. However, it is reasonable to expect that dedifferentiated β cells will re-differentiate into β cells under these conditions as well. Thus, the results of these studies indicate that β cells lacking FOXO1 under mild metabolic stress associated with aging dedifferentiate into progenitor-like cells capable of re-differentiating into any endocrine cell type of the islet, including α cells.

Human β-to-α transdifferentiation without genetic manipulation of transcription factor expression

The effects of the specific genetic manipulations described earlier in this review indicate the importance of transcription factors in the transdifferentiation process. Human β cells show unexpected plasticity as they too have the capacity to convert into α cells, and apparently do so without genetic perturbation of transcription factor networks. This was demonstrated by irreversibly labeling more than 80% of β cells by transducing dissociated human islets with lentiviral vectors containing a rat *Ins2*-Cre and a GFP reporter (Russ *et al.* 2009), with less than 1% of α cells being labeled (Spijker *et al.* 2013). Following labeling, the dissociated cells were re-associated into islet-like clusters. Over the course of 2 weeks, the percentage of β cells in these clusters declined and that of α cells increased. Low levels of both apoptosis and proliferation indicated that these processes are not major contributors to the changing cluster composition. Instead, up to 15% of α cells showed a β cell lineage mark 2 weeks following transduction (Spijker *et al.* 2013). Generalized inhibition of ARX in the cultures reduced the percentage of α cells carrying a β cell lineage label, while at the same time increasing the number of remaining β cells with a lineage label. This was interpreted as a reduction in β-to-α transdifferentiation and, given the central role of ARX in the α cell, could also be due in part to a loss of α cells (Spijker *et al.* 2013). No cells that co-expressed insulin and glucagon were found. Instead, β cells degranulated before expressing glucagon and did so despite the continued presence of PDX1 and NKX6-1 (Spijker *et al.* 2013), β-cell-specific transcription factors that inhibit glucagon expression (Schisler *et al.* 2005). The α cell identity persisted for at least 2 weeks after transplantation into mice (Spijker *et al.* 2013). These results indicate that human β cells, similar to those in mouse, have the capacity to transdifferentiate into α cells, at least under experimental conditions *in vitro* and dependent on ARX. The question as to whether such plasticity of endocrine cell types exists in the human islet *in situ* remains yet to be answered.

Transdifferentiation of α to β cells

Forcing α-to-β transdifferentiation by over-expression of PAX4

As β cells transdifferentiate into α cells as a result of forced ARX expression and with the knowledge that ARX and PAX4 play opposing roles in the differentiation of α and β cells during embryonic development, it follows that the

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over-expression of PAX4 might promote a β cell identity. PAX4 is normally expressed only in the subset of endocrine cells that will become β and δ cells (Sosa-Pineda *et al*. 1997) and is undetectable in adult mouse islets (Smith *et al*. 1999, Benner *et al*. 2014). Cre-recombinase-mediated over-expression of PAX4 in the PDX1 domain (all pancreas cell types), the PAX6 domain (all endocrine cells), or the glucagon domain (α cells) invariably reduced α cell numbers (Collombat *et al*. 2009). Despite decreased α cell numbers, blood glucose levels remained normal or were slightly repressed at birth, although glucose clearance was improved in line with reduced glucagon tone (Collombat *et al*. 2009). Results of lineage tracing in mice with PAX4 over-expression in the α cell revealed that α cells were lost through their transdifferentiation into β cells. An unforeseen effect was the development of progressive hyperglycemia, despite increased β cell mass and reduced α cell numbers (Collombat *et al*. 2009). This paradoxical observation probably relates to the continued over-expression of PAX4 by β cells, which is normally absent from adult mouse β cells (Fig. 2; Smith *et al*. 1999, Benner *et al*. 2014). Over-expression of PAX4 in β cells induces β cell proliferation via expression of the oncogene *c-myc*, but impairs glucose-stimulated insulin secretion (Brun *et al*. 2004), consistent with C-Myc repressing the protein levels of insulin, SLC2A2, and PDX1 (Cheung *et al*. 2010). The combined effects of PAX4 over-expression and c-MYC induction appear to cause partial dedifferentiation and thus explain the phenomenon of β cell failure despite increased β cell mass in PAX4-over-expressing β cells.

Deletion of ARX from α cells results in loss of α cell phenotype

Transdifferentiation from α to β can also be induced independently of PAX4 over-expression in β cells by deleting *Arx* from α cells via glucagon-Cre-mediated recombination (Herrera 2000) of a floxed *Arx* allele (Fulp *et al*. 2008). This results in α-to-β transdifferentiation (Courtney *et al*. 2013, Wilcox *et al*. 2013). One group reported continued α cell neogenesis from a proposed ductal progenitor and enlargement of islets over time (Courtney *et al*. 2013), whilst the other observed no differences in total hormone-positive-cell numbers, possibly due to the younger ages of mice examined as well as a much lower observed efficiency of Cre recombination (Wilcox *et al*. 2013). In contrast to the over-expression of PAX4 in α cells, which precipitates hyperglycemia, presumably attributable to continuation of forced PAX4 expression upon α-to-β cell trans-differentiation, deletion of *Arx* in α cells improves glucose tolerance (Courtney *et al*. 2013). Combined deletion of *Pax4* and *Arx* from the α cell produced a phenotype similar to that resulting from *Arx* deletion alone. This indicates that while PAX4 and ARX transcriptionally repress each other (Collombat *et al*. 2003) and global deletion of both *Arx* and *Pax4* results in a lack of both α and β cells (Collombat *et al*. 2005), α cells can transdifferentiate into β cells upon loss of *Arx* in the absence of *Pax4* (Courtney *et al*. 2013). These results, combined with the above-mentioned roles for ARX, indicate that ARX is a key driver of α cell differentiation and maintenance as well as transdifferentiation of β cells-to-α cells.

Maintaining α cell identity through expression of MEN1 tumor suppressor

An interesting example of possible α-to-β cell transdifferentiation in the human pancreas is found in patients with MEN syndrome, caused by mutations in the *MEN1* tumor suppressor gene. MEN1 is important for normal development of all islet cell lineages (Fontaniere *et al*. 2008). MEN syndrome patients present with tumors of various endocrine origins (Lemos & Thakker 2008). The development of MEN1 tumors is likely to be a multistep process in which progressive loss of the two alleles of the *MEN1* gene precipitates hyperplasia and atypia (with abnormally shaped cells), while the development of full-blown adenomas may require additional somatic mutations (Crabtree *et al*. 2003). Glucagonomas, while abundant in microadenomas of the pancreas, become increasingly rare as *MEN1*-deficient tumors grow and insulinomas and mixed islet tumors prevail (Anlauf *et al*. 2006, Perren *et al*. 2007). The deletion of *MEN1* from the α cell using glucagon-Cre produced a phenotype that resembles that of patients with MEN syndrome. An α-cell-specific deletion resulted in glucagonomas, hyperglucagonemia, and hyperglycemia as expected. Interestingly, *MEN1*-deficient insulinomas subsequently developed and became the dominant effectors of blood glucose over time (Lu *et al*. 2010). Such insulinomas developed via a glucagon–insulin double-positive intermediate and the location of the insulin granules within the cell resembled those of glucagon in α cells. Indeed, the results of lineage-tracing experiments were indicative of an α cell origin of these insulinoma cells (Lu *et al*. 2010). Glucagon–insulin double-positive cells appeared before islet tumors arose, indicating the possibility of a direct MEN1 effect on α cell maintenance, rather than other somatic mutations that are acquired over time. These results not only indicate that α cells in a *MEN1*-deficient tumor model transdifferentiate

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into β cells, but also indicate that a similar process of transdifferentiation may take place in human MEN syndrome patients.

Near-total β cell ablation replenishes β cells through an α or δ cell source

Significant loss of β cell mass is accompanied by β cell regeneration, achieved largely through self-duplication of existing β cells in adult mice (Dor *et al.* 2004). Depletion of approximately 90% of β cells has been shown to result in hyperglycemia and to induce a β cell proliferative response (Cano *et al.* 2008). However, results of recent studies have indicated that more acute (>90%) β cell loss is able to invoke alternative mechanisms in efforts to replenish the β cell deficit. Even more extreme depletion was achieved in a genetic mouse model in which β cells express the diphtheria toxin receptor under the control of the rat *Ins2* promoter (Thorel *et al.* 2010). Administration of diphtheria toxin resulted in near-total β cell ablation (99.6%; in 2-month-old mice). This extreme loss of β cells triggered α-to-β cell transdifferentiation and was, over time, able to regenerate sufficient β cell mass to restore normoglycemia (Thorel *et al.* 2010). Results of subsequent work by the same group indicate that near-complete β cell ablation in the juvenile (2-week-old) pancreas triggers a rapid transdifferentiation response from δ cells (Chera *et al.* 2014). Transdifferentiated β cells of α cell descent expressed insulin and the β cell transcription factors PDX1 and NKX6-1 and proceed through a transient co-positive stage, followed by the loss of glucagon (Thorel *et al.* 2010). In contrast, transdifferentiated β cells of δ-cell descent proceed through a hormone-negative stage before acquisition of insulin expression (Chera *et al.* 2014). When only 50% of β cells were depleted, no α-to-β cell transdifferentiation was observed (Thorel *et al.* 2010). The authors therefore suggested that transdifferentiation is induced under circumstances of extreme β cell loss, where β cell self-replication can no longer restore significant β cell mass, with the source of transdifferentiation dependent on the postnatal age of the animal. This age-dependence may stem from differences in α versus δ cell development. The α cells are among the first endocrine cells to differentiate, while the β and δ lineages separate later (Habener *et al.* 2005, Ben-Othman *et al.* 2013). Thus, δ and β cells may be more closely related, as exemplified by the continued expression of PDX1 in both cell types (Peshavaria *et al.* 1994). Perhaps, the β and δ cell lineages are still diverging in early postnatal life, enabling the observed rapid transdifferentiation of δ-to-β cells upon near-total β cell depletion (Chera *et al.* 2014). As δ cells mature, they may lose the ability to readily transdifferentiate into β cells, leaving the slower process of α-to-β cell conversion to emerge as the main source of new β cells by transdifferentiation.

Partial α-to-β transdifferentiation in human islets by modulation of epigenetic marks

In accordance with the importance of epigenetic modifications for maintenance of β cell fate, there is evidence from studies of cultured human islets indicating that the same holds true for α cell fate. The epigenome of the α cells appears to make them more amenable to transdifferentiation (Bramswig *et al.* 2013). This conclusion is based on the interesting observation that many more genes in the α cell fraction were associated with both activating (H3K4me3) and inhibiting (H3K27me3) histone modifications when compared with β and exocrine cells. Resolution of dual marks into a single mark is a common feature during the course of normal differentiation (Mikkelsen *et al.* 2007), and the overrepresentation of dual marks in the α cell was interpreted as indicating that the α cell might be poised to change fate (Bramswig *et al.* 2013). However, the increased prevalence of bivalently labeled genes in the α cell fraction could have been embellished by contaminating β cells. This possibility is difficult to exclude when applying cell-surface markers to purify populations for FACS analysis. Moreover, the study was based on *ex vivo* conditions that may be far more predisposed to induce transdifferentiation as an artifact of culture. Nevertheless, treatment of human islets with the histone methyltransferase inhibitor Adox resulted in mis-expression of PDX1 in α cells and the induction of glucagon–insulin co-positive cells (Bramswig *et al.* 2013). Although ectopic expression of α cell transcription factors in β cells under these conditions was not addressed, these observations once more highlight the surprising plasticity that exists among endocrine cells of the mouse and human pancreas.

Concluding remarks

Diabetes is characterized by an absolute or relative deficiency of β cells. Thus, increasing β cell mass will be instrumental in restoring normoglycemia in patients. Stimulating human β cell proliferation has proven challenging, even when results from rodent models seemed promising (Kulkarni *et al.* 2012, Bernal-Mizrachi *et al.* 2014). Moreover, particularly in later stages of T1D, the dearth of residual β cells in combination with their notoriously slow proliferation rate makes the restoration

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of β cell mass through the stimulation of β cell proliferation a tall order. Direct transplantation of β cells in the form of islets or whole pancreas has proven more successful in managing diabetes but is hindered by: i) a lack of donor material and ii) the requirement for life-long immunosuppression (Shapiro 2012). Efforts to generate mature, functional β cells from induced pluripotent or embryonic stem cells are in full swing (Kelly *et al*. 2011, Pagliuca *et al*. 2014, Rezania *et al*. 2014), but will have many safety hurdles to overcome. Alternative sources of new β cells, including ductal, acinar, and, most recently, non-β endocrine cells, provide alternative strategies for treating diabetes (Lysy *et al*. 2013). Together these efforts have revealed a remarkable degree of plasticity among the different endocrine cells of the islet and have firmly established that transdifferentiation takes place under certain experimental circumstances in mouse models.

The dynamic landscape of identity switches that is now coming to light offers hope for novel therapeutic strategies with which to treat diabetes. The α-to-β cell transdifferentiation, in particular, is not only an avenue for the (partial) restoration of β cell mass, but it also simultaneously reduces α cell mass and thus potentially restores the balance between insulin and glucagon, which is perturbed in diabetes (Unger & Orci 2010, Unger & Cherrington 2012). While non-islet cells far outnumber islet cells as a potential source for new β cells, non-β islet cells may be more amenable to transdifferentiation into β cells than duct or acinar cells, because they share a more recent progenitor in development. Moreover, non-β endocrine cells functionally resemble β cells in many ways, which is reflected in a high degree of overlap in expression between many of the genes that are required for glucose sensing, stimulus–secretion coupling, and exocytosis (Benner *et al*. 2014).

In order to capitalize on the potential for transdifferentiation in the islet, important questions remain unanswered that will undoubtedly be addressed in future. Firstly, it remains to be demonstrated that individual transdifferentiated β cells have fully completed their transdifferentiation and are therefore functionally equivalent to endogenous β cells. Secondly, it is imperative to identify molecules that can channel transdifferentiation toward the desired outcome of increased β cell mass by promoting α- or δ-to-β cell transdifferentiation, while preventing the transdifferentiation or dedifferentiation of β cells. Finding such molecules and ways to deliver them to the pancreas is essential to replace strategies that relied on genetic manipulation to establish proof-of-principle for transdifferentiation in the islet. Whether these molecules will be as efficient as transcription factors at inducing transdifferentiation is a separate question and is related to the question of phenotype stability for these molecules. While the possibility of depleting the islet of α and/or δ cells is a potential concern, the identification of druggable targets that can induce islet cell transdifferentiation remains the more immediate challenge, before we worry about the consequences of succeeding at this task. We are encouraged by these new opportunities despite the fact that such key questions regarding transdifferentiation in the islet remain unanswered as yet.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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