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Pax genes and the differentiation of hormone-producing endocrine cells in the pancreas

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Abstract

Despite the pivotal role of the pancreas in hormonally-regulated pathways in the body, e.g. glucose homeostasis, the genetic mechanisms defining it have for many years remained largely enigmatic. After years out of the spotlight, pancreas development has once again come to centre stage. To a large extent, this is due to recent advances made through the detailed analysis of transgenic mice which have been engineered to carry mutations in specific developmental control genes. This review specifically focuses on the specification of the endocrine pancreas lineage and in particular on the role of the developmental control genes *Pax4* and *Pax6* in the generation of specific endocrine cell types. The comparison of various phenotypes of different mouse mutants affecting endocrine development supports a model in which *Pax4* and *Pax6* are required for the differentiation of certain endocrine cell lineages and implies a potential for acting at different levels of endocrine development.

Keywords: Organogenesis; Pancreas; *Pax4*; *Pax6*; Developmental control genes; Differentiation; Endocrine cell lineage; Knock-out; *Small eye*; Diabetes mellitus

1. Introduction

The importance of the pancreas for glucose homeostasis and its implication in pathological states such as diabetes mellitus had been documented already by the ancient Greeks. It was not until the last 2 centuries that breakthroughs in the understanding of fundamental aspects of pancreatic cell biology and pathophysiology were achieved, for example by the identification of the cellular components of the islets of Langerhans, and the discovery by Banting and Best in 1922 that lack of insulin is the primary reason for elevated blood glucose levels in diabetic patients (Banting and Best, 1922; Banting et al., 1922). In addition, over the last 2 decades, classical embryological experiments and studies on the co-expression of pancreatic hormones led to significant progress in the understanding of pancreatic cell lineages (Pictet and Rutter, 1972; Palmiter et al., 1987; Teitelman and Lee, 1987; Teitelman et al., 1987, 1993; Evans, 1989; Ohashi et al., 1991; Gittes and Rutter, 1992; Githens, 1993). More recently, the anlage of the pancreas has received special attention by developmental biologists who have demonstrated that pancreas development requires complex combinations of transcription factors in order to control organogenesis and cell differentiation (summarized in Kim et al., 1997; Sander and German, 1997; Edlund, 1998). In particular, the Pax family of transcription factors is involved in the formation of many organs. Two of its members, *Pax4* and *Pax6*, play important roles in islet differentiation (Sosa-Pineda et al., 1997; St-Onge et al., 1997).

Pax genes encode key regulators which are involved in the embryonic development of many organs including the eyes, brain, kidney, thyroid gland, immune system, and the pancreas (reviewed in Mansouri et al., 1996a; Dahl et al., 1997). A number of murine and human genetic disorders are linked to mutations in specific *Pax* genes. For example, a mutated *Pax1* gene is responsible for the murine *undulated* (*un*) phenotype (Balling et al., 1988, 1992) and mutations in the *Pax2* gene cause the human ocular-renal syndrome (Schimmenti et al., 1997). The murine *splotch* (*sp*) allele and human Waardenburg syndrome type I are associated with *Pax3* (summarized in Epstein et al., 1993; Goulding et al., 1993), whereas the murine *small eye* (*Sey*) mutant, human aniridia, and Peter's anomaly are due to mutations in *Pax6* (Hogan et al., 1988; Hill et al., 1991; Ton et al., 1991; Jordan et al., 1992; Grindley et al., 1995; Graw, 1996)

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(reviewed in Macdonald and Wilson, 1996; Stoykova et al., 1996, 1997; Grindley et al., 1997; Götz et al., 1998).

All nine *Pax* genes identified thus far have been inactivated in mice by targeted mutagenesis (Table 1). Their inactivation generally results in embryonal or neonatal death accompanied by striking developmental defects: targeted disruption of *Pax1* is associated with disturbed skeletogenesis (Wilm et al., 1998), *Pax2* knock-out mice do not develop kidneys (Torres et al., 1995) and mice deficient for the *Pax3* gene have severe defects in the developing neural tube, neural crest and skeleton (Mansouri and Gruss, 1998). *Pax5* mutant mice lack mature B-cells and show brain defects (Urbanek et al., 1994). Inactivation of the *Pax7* and *Pax8* genes leads to neural crest defects and thyroid gland dysgenesis, respectively (Mansouri et al., 1996b, 1998). *Pax9* mutant mice lack pharyngeal pouch derivatives, teeth and show craniofacial and limb defects (Peters et al., 1998).

In this review we concentrate on the development of the pancreas which consists of mainly exocrine and endocrine tissue. The exocrine tissue forms acinar glands that store and secrete digestive enzymes into the digestive tract. The endocrine cells are clustered in islets of Langerhans and secrete insulin, glucagon, somatostatin, and pancreatic polypeptide directly into the blood stream. Although a number of transcription factors have been identified to play important roles during pancreas development the molecular mechanisms that define the differentiation of endocrine cell types in the pancreas have for many years remained largely unknown.

It was only recently that the analysis of *Pax4* and *Pax6* knock-out mice revealed their important roles in the differentiation of specific endocrine cell lineages during pancreas development (Sosa-Pineda et al., 1997; St-Onge et al., 1997). Whereas mice lacking the *Pax4* gene do not generate insulin-producing β-cells and somatostatin-producing δ-cells in the pancreas, *Pax6*-deficient mice are unable to form glucagon-producing α-cells (Table 1). The focus of this review is the analysis of *Pax4* and *Pax6* gene function during embryogenesis with special emphasis on the differentiation of endocrine cells during pancreas development.

2. PAX4 and PAX6, two members of the paired domain family of transcription factors

Pax4 and *Pax6* were originally identified in a homology screen for paired box containing genes (Walther et al., 1991). Paired box genes encode nuclear transcription factors which are characterized by the presence of the paired domain, a highly conserved sequence motif of 128 amino acids which possesses DNA-binding activity (reviewed in Noll, 1993). The *Pax4* and *Pax6* genes are more closely related to each other than to other members of the *Pax* gene family. In addition to the paired domain, both proteins also have a homeodomain as well as a highly conserved octapeptide (reviewed in Dahl et al., 1997). A characteristic feature of *Pax* genes is that they are located at distinct chromosomal loci and thus act independently and not in tandem as other developmental control genes do, e.g. *Hox* genes. The murine Pax4 gene is located on chromosome 6, whereas Pax6 maps to chromosome 2 (Pilz et al., 1993; Tamura et al., 1994).

During mouse embryogenesis, *Pax6* is expressed in various cellular populations in the eyes, nose, central nervous system, and developing pancreas (Table 1) (Walther and Gruss, 1991; Walther et al., 1991; Turque et al., 1994). Analysis of the *Pax6* promoter has led to the identification of various cis-acting elements which direct expression in some of these tissues (Plaza et al., 1995; Xu and Saunders, 1997, 1998; Sharon-Friling et al., 1998; Kammandel et al., 1999; Xu et al., 1999). Although Pax6 is involved in the development of various organs, its role in the developing eye has provided the greatest level of access to elucidating its function. Homozygous *Pax6* mutants of *Drosophila* (*eyeless*, *ey*), mice (*Small eye*, *Sey*), and rats (*rSey*) fail to develop eyes. In the heterozygous state a variety of ocular abnormalities are observed in mice (*Small eye*)

Table 1
The Pax gene family and its association with mouse mutants and human syndromes

Gene	Expression[a]	Knock-out phenotype	Human syndrome
Pax1	Sclerotome, pharyngeal pouches, thymus	Disturbed skeletogenesis	
Pax2	Pro/mesonephros, metanephric mesenchyme, mid/hindbrain, eye, ear	No kidneys	Ocular-renal syndrome
Pax3	Dermamyotome, neural crest, CNS	Neural tube, neural crest, skeletal defects	Waardenburg syndrome
Pax4	Pancreas, neural tube	No pancreatic β- and δ-cells	
Pax5	Pro B-cells, CNS	No B-cells, brain defects	
Pax6	Eye, pancreas, CNS	Small eye, no pancreatic α-cells, brain defects	Aniridia
Pax7	Neural crest, dermamyotome, CNS	Neural crest defects	
Pax8	Neural tube, CNS	Neural crest defects, thyroid dysgenesis	
Pax9	Pharyngeal pouches, mesenchyme	No thymus, no parathyroid glands, no teeth, craniofacial and limb defects	

[a] Only prominent sites of expression are indicated. CNS, central nervous system.

and humans (aniridia and Peter's anomaly) (Hill et al., 1991; Ton et al., 1991; Jordan et al., 1992; Quiring et al., 1994; Grindley et al., 1995, 1997; Graw, 1996; Macdonald and Wilson, 1996; Stoykova et al., 1996; Xu et al., 1997). Alternatively targeted expression of *Pax6* genes from invertebrates and vertebrates induces the formation of ectopic eyes in *Drosophila*, demonstrating the ability of Pax6 to regulate complex developmental processes throughout evolution (Halder et al., 1995).

In contrast to the widespread embryonic expression of *Pax6*, the *Pax4* gene is characterized by a very restricted expression pattern with only a few *Pax4*-positive cells in the ventral spinal cord, and in the endocrine pancreas (Table 1) (Sosa-Pineda et al., 1997). *Pax4* was first identified in mice along with *Pax6* and subsequently cloned in humans and rats (Walther and Gruss, 1991; Pilz et al., 1993; Tamura et al., 1994; Bonthron et al., 1998; Inoue et al., 1998; Tao et al., 1998; Tokuyama et al., 1998). To date, there are no known naturally occurring mutations of *Pax4* in mice or in humans.

3. Expression of Pax4 and Pax6 during pancreas development

During mouse embryogenesis, the first morphological sign of pancreas formation is an epithelial evagination near the junction of foregut and midgut in an area that will become the duodenum. At embryonic day 9.5 (E9.5), this epithelial evagination forms the dorsal pancreatic bud (Wessells and Cohen, 1967; for review see Slack, 1995; Edlund, 1998). Expression of PAX6 protein can be detected already around E9.0 in a small subset of cells in the prepancreatic endoderm (Fig. 1). At E9.5, PAX6 protein is expressed in a few cells of the pancreatic bud, some of them co-expressing glucagon (Sander et al., 1997). In contrast, *Pax4* expression is first detected in E10 embryos in only a few cells of the pancreatic bud (Fig. 1) (Sosa-Pineda et al., 1997; Sosa-Pineda, pers. commun.). Approximately half a day later (E10.5), the first insulin-positive cells can be detected, as the epithelial bud enlarges to develop into a tree-like ductal system by branching and growth (Fig. 1).

Shortly after formation of the dorsal pancreatic bud, the ventral bud arises at the ventro-lateral side of the gut tube. By the time the first acini and ducts can be distinguished histologically in the dorsal bud (E14.5), the number of *Pax4* expressing cells increases significantly. Double labelling experiments reveal a small subpopulation of insulin and *Pax4* co-expressing cells, compared to a larger cell population exclusively expressing *Pax4*. The largest number of *Pax4* expressing cells is observed around E15.5, 1 day after the onset of exocrine development (Sosa-Pineda, pers. commun.). *Pax4* and *Pax6* expression has never been detected in cells of the exocrine pancreas (Sander et al., 1997; Sosa-Pineda et al., 1997; St-Onge et al., 1997). In

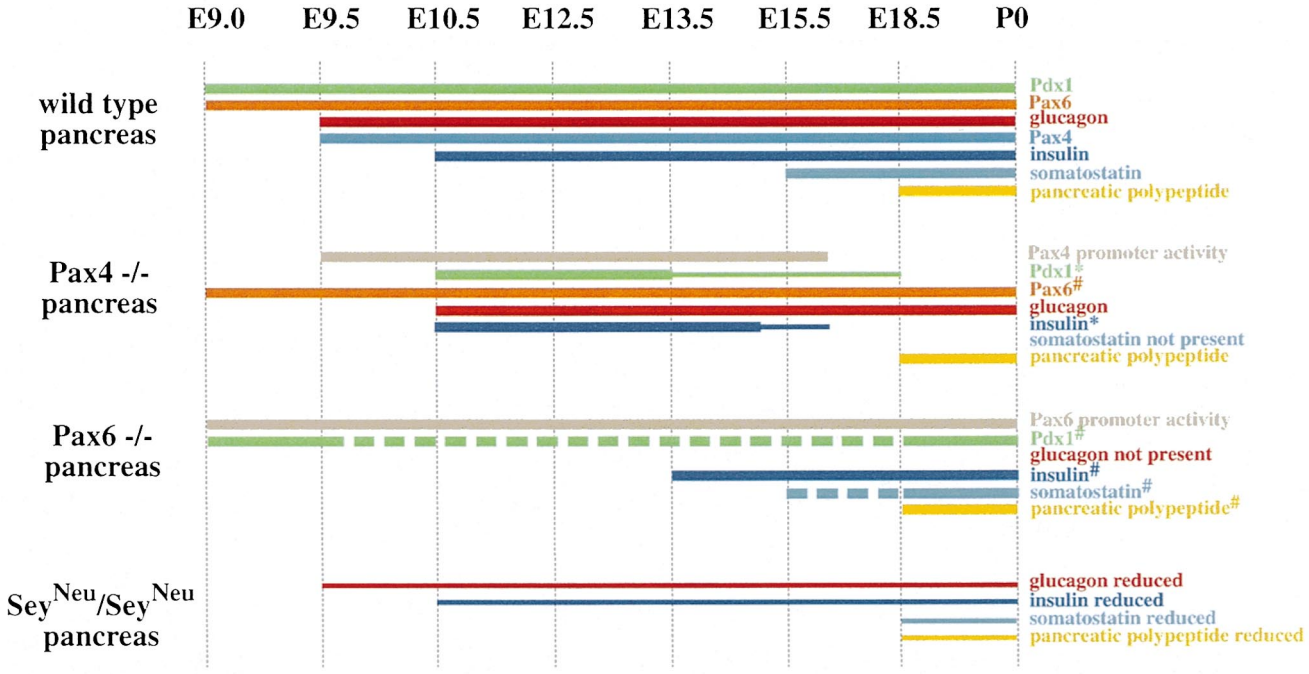

Fig. 1. Schematic summary of an expression profile of pancreas markers during wild type, *Pax4* knock-out, *Pax6* knock-out and *Sey*^{Neu} mutant pancreas development. Expression is indicated by bars; decreased expression is indicated by narrow bars; dashes indicate assumed expression although not analyzed during the corresponding stages. E, embryonic day; P, postnatal day; *, Sosa-Pineda, pers. commun.; #, St-Onge, pers. commun. Promoter activity was determined by β-galactosidase staining.

contrast to the cell type restricted expression of *Pax4* during these stages, *Pax6* expression is detected throughout all endocrine cells of the developing pancreas producing hormones. As the end of gestation approaches and all endocrine cells begin to organize into the typical islets of Langerhans, *Pax6* expression is maintained, whereas *Pax4* expression continually decreases. Finally, in the adult pancreas *Pax4* expression is no longer detectable (Sosa-Pineda, pers. commun.).

In summary, expression of *Pax4* and *Pax6* during the early stages of pancreas development is confined to cellular subsets of the endocrine lineage. *Pax6*, which can be detected throughout pancreas development, is expressed in all endocrine cells. In contrast, *Pax4* is required exclusively in cells restricted to the β- and δ-cell lineage and can only be detected during embryogenesis.

4. The pancreas of Pax6 knock-out and Small eye (Sey^{Neu}) mice

Sey and Sey^{Neu} mice carry semi-dominant *Pax6* alleles which are due to point mutations in the *Pax6* locus resulting in truncated PAX6 proteins (Hill et al., 1991; Sander et al., 1997). In *Sey* mutants the protein is truncated directly after the paired domain while in Sey^{Neu} mice the protein is truncated after the homeodomain, thus leaving both protein domains intact. To generate complete knock-out mice, the *Pax6* start codon along with the entire paired box was replaced with the β-galactosidase gene resulting in mutant mice in which no protein is detectable (St-Onge et al., 1997). In the homozygous state, Sey^{Neu} and *Pax6* knock-out mutants lack eyes, show severe brain defects, and die shortly after birth. However, differences in the pancreatic defects are observed in the mutant animals. Whereas *Pax6* knock-out mice do not form glucagon-producing α-cells throughout all developmental stages, Sey^{Neu} mice express normal levels of glucagon during the early stages of pancreas development (Fig. 1). A decrease in the number of glucagon-positive cells becomes evident around E10.5, and at E12.5 glucagon- and also insulin-positive cells are reduced significantly in Sey^{Neu} mice (Fig. 1). Although glucagon-producing α-cells are most strongly affected in Sey^{Neu} mice, all four endocrine cell types are decreased significantly in number by E18.5 (Fig. 1). In contrast, *Pax6* knock-out mice only lack cells of the α-cell lineage (Fig. 1). Furthermore, islet morphology is disrupted in *Pax6* knock-out mice. The remaining insulin-, somatostatin-, and pancreatic polypeptide-producing cells found in *Pax6* knock-out mice do not form proper spherical islet structures, but organize in elongated, stream-like clusters. δ- and γ-cells (together with α-cells) which normally form a rim around a β-cell core, mix instead with the insulin-producing cells in the *Pax6* mutant pancreas. Insulin expression is detectable from E13.5 onwards, and expression of *Pdx1* (coding for a transcription factor with a major involvement in islet development) appears to be normal (Fig. 1) (St-Onge, pers. commun.). The exocrine tissue on the other hand seems unaffected, synthesizing α-amylase. In Sey^{Neu} mutant mice, islet morphology is also altered although in a more subtle manner. The initial aggregation of endocrine cells seems unaffected and the number of developing islets appears normal. However, Sey^{Neu}/Sey^{Neu} islets do not form properly with a β-cell core and a mantle of α-, δ- and PP-cells but the cell populations appear mixed within a given islet. Furthermore, hormone production is markedly reduced in Sey^{Neu} mutants, pointing to a decrease in PAX6-regulated glucagon and insulin gene transcription. In support of this observation, biochemical studies involving the PAX6 protein demonstrate its binding to regulatory sequence elements of the glucagon, insulin and somatostatin genes, and transactivation of these genes has been confirmed in vitro (Sander et al., 1997).

Thus, although the phenotypes of Sey^{Neu} and *Pax6* knock-out largely overlap, there are some important differences. First, *Pax6* knock-out mice do not form α-cells whereas in Sey^{Neu} mutant mice the number of α-cells is reduced. Secondly, in Sey^{Neu} mutant mice all endocrine cell types seem to be affected whereas in *Pax6* knock-out mice only α-cells are missing.

5. The pancreas of Pax4 knock-out mice

Homozygous *Pax4*-deficient new-borns are indistinguishable from their litter mates, but die 3–5 days after birth. The pancreas of *Pax4* mutant mice is normal in size and weight but mature insulin- and somatostatin-producing cells are absent (Sosa-Pineda et al., 1997). Nevertheless, insulin-producing cells can be detected during the early stages of pancreas development (E10.5), but the expression levels of insulin and *Pdx1* are drastically decreased by E15.5 (Fig. 1). At this stage the pancreas of *Pax4* mutant mice contains cells expressing β-cell markers such as amylin, Islet1, Nkx6.1, Nkx2.2 and Pdx1. However, no mature β-cell marker, like *Nkx6.1* or *Pdx1*, can be detected, confirming the absence of mature β-cells in new-born $Pax4^{-/-}$ mice (Sosa-Pineda et al., 1997; Sosa-Pineda, pers. commun.).

Furthermore, in new-born $Pax4^{-/-}$ mice the number of glucagon-producing cells is significantly increased compared to heterozygous litter mates and they appear to be clustered atypically in the core of an islet-like structure (Sosa-Pineda et al., 1997). This relative increase in glucagon expressing cells suggests that endocrine progenitors instead of differentiating into β-/δ-cell precursors take on an α-cell fate. The apparent phenotype in new-borns suggests that insulin-producing β- and somatostatin-producing δ-cells have been replaced by glucagon-producing α-cells, supporting the hypothesis that β- and δ-cells are derived from a common lineage (Apert et al., 1988; Teitelman et al., 1993; Guz et al., 1995). The detailed analysis of the progression of the *Pax4* knock-out phenotype reveals

that the mutants are able to form immature β-cells which subsequently fail to differentiate into mature β-cells, but alternatively acquire α-cell characteristics.

Finally, mice lacking both *Pax4* and *Pax6* fail to develop mature endocrine cells entirely, indicating that both *Pax4* and *Pax6* are required for endocrine development in the pancreas (St-Onge et al., 1997).

6. The role of PAX4, PAX6 and other transcription factors in pancreas development

In addition to PAX4 and PAX6, a number of transcription factors, ISL1, PDX1, BETA2/NEUROD, NKX6.1, and NKX2.2, have been implicated in pancreas development and the differentiation of endocrine cells. Although it is still difficult to assign specific functions to some of these factors, the emerging picture suggests that there are three levels to endocrine development. The first step involves the generation of endocrine progenitors which are able to differentiate into all four endocrine cell types, α-, β-, δ-, and PP-cells, within the pancreatic epithelium. In a second step these pluripotent endocrine progenitors become restricted to either the β-/δ- or α-/PP-cell fate. In a third and final step, precursor cells which are already committed to the β- or δ-cell fate develop in either β- or δ-cells. The differention pathway of the α- and PP-cell lineage is less clear. It is not understood if α- and PP-cells develop from a common precursor or separately develop directly from pluripotent endocrine progenitors (Fig. 2).

Pluripotent endocrine progenitor cells are thought to arise from a duodenal portion of the foregut which is located just posterior to the developing stomach. Little is known about the factors involved in the formation of endocrine progenitor cells, with the exception of ISL1, a LIM homeodomain protein. *Isl1*-deficient mice completely lack all endocrine islet cells, indicating a function for *Isl1* in the generation of endocrine progenitor cells (Ahlgren et al., 1997). However, *Isl1* is also required for exocrine cell differentiation in the dorsal bud. *Isl1*-deficient mice lack the dorsal bud mesenchyme and fail to develop exocrine tissue in the dorsal bud. Therefore, *Isl1* is not only specifically required for endocrine, but also exocrine development (Pfaff et al., 1996; Ahlgren et al., 1997). Consequently, early differentiation of the embryonic gut epithelium towards the endo- or exocrine lineages requires additional factors acting downstream of *Isl1*, which would subsequently specify the endocrine and exocrine lineage (Fig. 2).

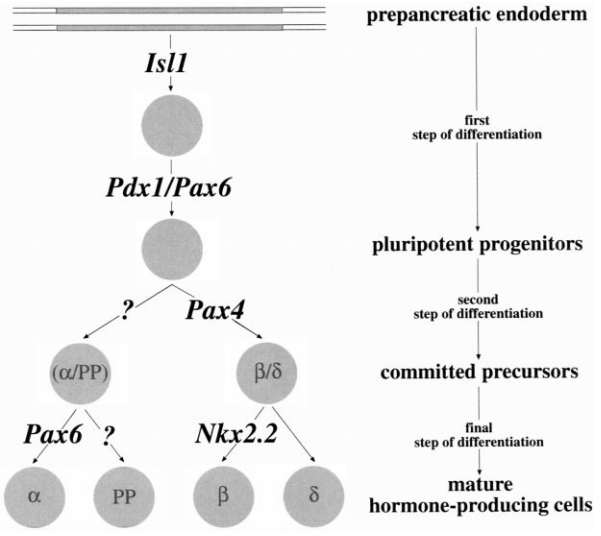

Fig. 2. Model for endocrine cell differentiation based on functional analysis of developmental control genes. α, glucagon-producing cell; β, insulin-producing cell; δ, somatostatin-producing cell; PP, pancreatic polypeptide-producing cell.

The area of the foregut which is thought to give rise to the pluripotent endocrine progenitor can be identified with the help of molecular markers before morphological signs of pancreas development become apparent. The pancreatic epithelium is marked by a lack of sonic hedgehog expression (a secreted factor influencing polarity of the developing gut) (Ahlgren et al., 1997), which is detected in the endoderm just anterior and posterior to pancreatic epithelium, and the restricted expression of *Pdx1* (Ohlsson et al., 1993; Jonsson et al., 1994; Offield et al., 1996). Although *Pdx1* expression specifies this early pancreatic epithelium, it is not required for the early differention of insulin- and glucagon-producing cells. It is more likely that *Pdx1* is involved in the proliferation of endocrine progenitor cells or, alternatively, in the proliferation of already differentiated precursors expressing insulin and glucagon (Fig. 2) (Jonsson et al., 1994; Ahlgren et al., 1998).

Once endocrine progenitors are established, *Pax* genes play a crucial role in the differentiation of specific endocrine cell lineages and even specific endocrine cell types. Loss of *Pax4* function results in a complete lack of the β- and δ-cell lineage (Sosa-Pineda et al., 1997). This suggests that after the establishment of a pluripotent endocrine progenitor which has the potential to develop into all four endocrine cell types, those cells that express *Pax4* adopt a more restricted cell fate, i.e. only insulin- and somatostatin-producing cells (Fig. 2). In fact, in the absence of *Pax4* function endocrine progenitors are unable to fully differentiate into β- or δ- cells and instead convert to an α-cell fate.

In contrast, *Pax6* is specifically involved in the differentiation of α-cells (St-Onge et al., 1997). Whether α-cells directly differentiate from a pluripotent endocrine progenitor or a precursor that is already restricted to the α- and PP-cell fate is presently unclear (Fig. 2). In either case, additional factors are required for the differentiation of γ-cells and possibly for the differentiation of an α-/PP-precursor.

There is further evidence that *Pax6* function is not just required for α-cell differentiation but that it is also involved in the proliferation of all endocrine cells (Fig. 2). Although the *Pax6*-deficient pancreas completely lacks glucagon-producing cells, no increase in cell number of other cell types has been observed. Moreover, in Sey^{Neu} mutants all

Pax6 expressing endocrine cell types (α-, β-, γ-, and PP-cells) are reduced in number indicating their inability to proliferate properly (Sander et al., 1997). A role for Pax6 in the general proliferation of endocrine cells is also supported by the phenotype of double-mutant *Pax4*/*Pax6* mice which seem to lack all endocrine cells (St-Onge et al., 1997). Since Pax4-deficient mice only lack β- and δ-cells and *Pax6*-deficient mice only lack α-cells, it is not clear why PP-cells are missing as well.

The cell type-restricted basic helix-loop-helix (bHLH) transcription factor BETA2/NEUROD is likely to be involved in the proliferation rather than the differentiation of endocrine cells. *Beta2*/*NeuroD* is expressed in all endocrine cells of the pancreatic epithelium and targeted gene disruption results in mice suffering severe diabetes and dying shortly after birth (Lee et al., 1995; Naya et al., 1997). Although the proliferation of insulin-producing cells seems to be most strongly affected, all endocrine cell types are significantly reduced in number, suggesting a role for *BETA2*/*NEUROD* not only in the differentiation of β-cells but in the general proliferation of endocrine cells.

Nkx6.1, a member of the NK homeobox family, is widely expressed in the pancreatic epithelium during the early stages of pancreas development, whereas in new-borns *Nkx6.1* expression is restricted to β-cells. Knock-out data reveal a severe reduction in insulin-producing cells, but normal numbers of other islet cell types, indicating an important role for *Nkx6.1* in β-cell differentiation (Sander et al., 1998).

NKX2.2 also belongs to the NK2 homeodomain family of transcription factors. Similar to *Nkx6.1*, early expression of *Nkx2.2* can be detected in all endocrine cell types. As development proceeds, *Nkx2.2* becomes restricted to β-cells, most α-cells and PP-cells but is excluded from mature somatostatin-producing δ-cells. *Nkx2.2* inactivation is associated with arrested β-cell differentiation resulting in complete absence of insulin-producing cells (Sussel et al., 1998). Additionally, a severe reduction in glucagon-producing cells and a slight reduction in pancreatic polypeptide-producing cells is observed, whereas somatostatin-producing cells remain unaffected. Late markers, including insulin, glucose transporter 2 and glucokinase, indicative of β-cell maturity are not expressed in the mutant islet, whereas early markers such as *Pdx1* and *Pax6* are present. These results indicate that *Nkx2.2*-deficient mice can initiate β-cell development but these cells are unable to terminally differentiate into functional hormone-producing cells (Fig. 2).

7. Conclusions

The generation and analysis of mouse mutants has led to the identification of various transcription factors that play important roles in pancreas development. Some of these factors, ISL1, PDX1, and BETA2/NEUROD, affect the development of all endocrine cell types, whereas other factors seem to specifically affect only certain lineages. Whereas *Pax4* is involved in the differentiation of β- and δ-cells, *Pax6* and *Nkx2.2* specifically affect the development of α- and β-cells, respectively. These differences in phenotype suggest that there are at least three levels to endocrine differentiation (Fig. 2). In the first step endocrine progenitors are generated which have the capacity to differentiate into all four endocrine cell types. Although *Isl1* is involved in the generation of endocrine progenitors it also affects exocrine development. Therefore, it is likely that there are additional, yet to be identified, factors that specify the generation of pluripotent endocrine progenitors. In a second step, these pluripotent progenitors subsequently commit to either the β-/δ- or the α-/PP-cell lineage. The best evidence for such a binary decision that results in precursor cells already committed to the β-/δ-lineage is the lack of β- and δ-cells in Pax4 mutant mice. However, it remains to be determined if there are precursor cells which, in a similar manner, are committed to the α-/PP-lineage. In the final step, precursor cells which are committed to the β-/δ- lineage differentiate into either β- or δ-cells. Factors that are required for this final differentiation step from the β-/δ-precursor to the mature β-cell are NKX2.2 and possibly NKX6.1, whereas PAX6 is required for the final differentiation of α-cells. Factors which are specifically required for the final differentiation of δ- and PP-cells have yet to be identified.

Although the studies outlined here have accumulated significant knowledge, the real power of these observations lies in their potential application to diseases involving pancreatic dysfunction. In particular, it appears that the highly influential role of the *Pax* genes in the developing embryo encompasses the process of proliferation and differentiation of the endocrine pancreas. This potential of *Pax* genes to trigger proliferation and differentiation is enormously valuable with regard to the regenerative capacity of the adult pancreas. Current thinking suggests the exciting idea that developmental control genes contribute to a genetic network where genes are interconnected by regulatory feedback loops. Once an entity of the network is active it results in triggering the mature phenotype. Novel therapeutics that exploit these loops are becoming reality for diseases such as diabetes. Among different approaches, there are innovative attempts to regenerate insulin-producing β-cells using *Pax*, *Pdx1* and other developmental control genes from pancreatic or embryonal stem cells. The successful regeneration of cellular populations represents an ideal opportunity towards novel therapeutics for a growing patient population.

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