

Gene expression cascades in pancreatic development

Maria E. Wilson, David Scheel, Michael S. German*

Department of Medicine, UCSF Diabetes Center, Hormone Research Institute, University of California, San Francisco, 513 Parnassus Avenue, San Francisco, CA 94143-0534, USA

Accepted 29 July 2002

Abstract

The specialized endocrine and exocrine cells of the pancreas originally derive from a pool of apparently identical cells in the early gut endoderm. Serial changes in their gene expression program, controlled by a hierarchy of pancreatic transcription factors, direct this progression from multipotent progenitor cell to mature pancreatic cell. When the cells differentiate, this hierarchy of factors coalesces into a network of factors that maintain the differentiated phenotype of the cells. As we develop an understanding of the pancreatic transcription factors, we are also acquiring the tools with which we can ultimately control pancreatic cell differentiation. © 2002 Published by Elsevier Science Ireland Ltd.

Keywords: Pancreas; Organogenesis; Gene expression cascades

1. Introduction

The pancreas presents a fascinating problem for those interested in the question of how cell fate choice is established during organogenesis. Starting from a pool of apparently identical progenitor cells, the embryonic pancreas transforms into a mature organ containing three clearly distinct cell types: exocrine cells, endocrine cells and duct cells (Edlund, 2001, 2002; Sander and German, 1997; Slack, 1995). In addition, the endocrine compartment, the islets of Langerhans, further differentiates into four pancreatic endocrine cell subtypes (α , β , δ and PP) that have obvious similarities and express common genes – those involved in the regulated secretion of hormones from intracellular vesicles, for example – yet exhibit distinct control over the expression of the exclusive hormones (glucagon, insulin, somatostatin and pancreatic polypeptide, respectively) that they secrete.

It is ultimately regulation at the level of gene transcription that provides the mature pancreatic cells with their unique phenotypes. Therefore the initiation and maintenance of distinct gene expression programs are required to establish the distinct cell types in the pancreas. The development of the mature gene expression program must be a dynamic process of serial changes as cells advance from uncommitted precursor to mature, terminally differentiated cells. This evolution of the gene expression program can be observed as changes in the key players that control the process, the transcription

factors, and can be envisioned as a hierarchy or cascade of factors. Eventually, what initially transpires as serial regulation of specific transcription factors goes on to coalesce into the network of transcription factors that define and maintain each differentiated cell phenotype.

Simply cataloguing the transcription factors expressed throughout pancreas development, however, is not particularly informative without delineating how they and their downstream targets act to drive the differentiation process. Ultimately, mouse genetics is the best, although not always the most practical, method for testing the position and function of genes in the transcriptional hierarchy. This approach has yielded a wealth of information about endocrine development over the past few years, and with the advent of techniques that allow more precise engineering of gene expression in the mouse, combined with the technology to look at expression dynamics of large numbers of genes, it is likely that much more can be learned about the integration of the transcription factor cascades that determine pancreatic cell fate.

Why is this important to understand? If we are to understand the mechanisms by which pancreatic cell differentiation occurs, and ultimately control it, we need to understand the pathways through which it proceeds, identify the factors that direct cells along specific differentiation pathways, and understand the interactions among them. Alterations in the relative participation of factors in this network, caused by environment, injury or genetic variation, may precipitate events that contribute to pancreatic disease, such as the beta-cell failure seen in type 2 diabetes. So, identifying the

* Corresponding author. Tel.: +1-415-476-9262; fax: +1-415-731-3612.
E-mail address: mgerman@biochem.ucsf.edu (M.S. German).

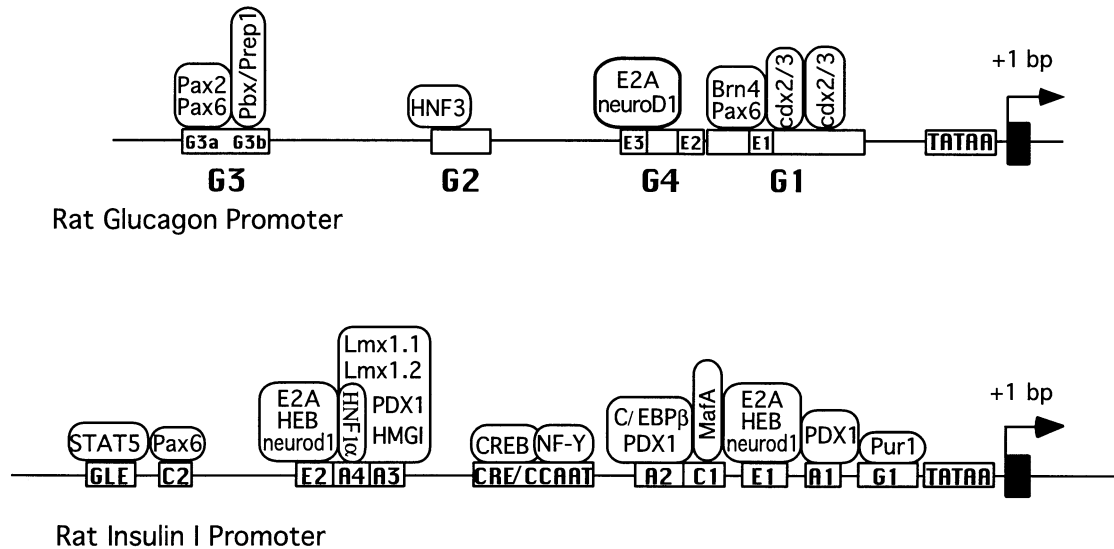


Fig. 1. Islet hormone gene promoters. The rat *insulin I* and *glucagon* gene promoters with known sequence elements and binding factors. The boxes represent characterized sequence elements. The islet transcription factors that bind to each site are circled above the promoters.

hierarchy of transcriptional regulation that controls pancreatic cell differentiation and maintains the mature cell phenotype may allow us to both understand the pathology of beta-cell failure, and give us the tools to restore beta-cell function.

2. Starting backwards: cell-type specific transcription in the mature pancreatic cells

While it is convenient to look at the differentiation process as a linear cascade of genes being induced and repressed, the reality is more complicated. The final transcription factor network in a mature cell forms from the intersection of multiple interacting pathways. One of the confounding difficulties with studies that attempt to elucidate these pathways is the fact that most of the factors involved in pancreatic development are expressed at more than one time point and play more than one role. Then in mature cells, the gene expression program becomes an interlocking network of cooperative transcriptional control that maintains the functional phenotype of the cell. As a result, insights into the gene expression program at one stage of differentiation are generally not applicable at other stages.

Nevertheless, working backwards starting from the mature pancreatic cells, the study of how the expression of particular genes is restricted to specific pancreatic cell types, using as examples the islet hormone and exocrine enzyme genes, led to the identification of a host of transcription factors from diverse families, many of which also direct the differentiation of the endocrine and exocrine cells from progenitor cells during embryonic development (Edlund, 1998; Ohneda et al., 2000a; Sander and German, 1997; Schwitzgebel, 2001). Fig. 1 shows some of the factors known to interact with the regulatory regions of the *insulin* and *glucagon* gene promoters. Developmentally important

factors originally identified due to their interactions with the *insulin* gene promoter include islet1, PDX1, and neuroD1 (also called BETA2) (Karlsson et al., 1990; Naya et al., 1995; Ohlsson et al., 1993). *Glucagon* promoter function also depends upon developmentally important factors including pax6 (Sander et al., 1997), homeobox factor cdx2/3 (Andersen et al., 1999; Hussain and Habener, 1999; Jin et al., 1997) and the forkhead family members Foxa1 and Foxa2 (previously known as HNF3 α and β) (Kaestner et al., 1999; Philippe et al., 1994; Shih et al., 1999). The observation that many of these factors are important in the expression of a broader set of cell-type specific genes, such as the role of PDX1 in the expression of the beta-cell genes *islet amyloid polypeptide* (Watada et al., 1996a), *glucose transporter 2* (Waeber et al., 1996), and *glucokinase* (Watada et al., 1996b), underlines the role of these factors in the generation of the broader gene expression program that defines the differentiated phenotype of the mature cell.

The pancreatic islet cells, with their ability to sense nutrient changes and respond with vesicular secretion of bioactive peptides, seem to have more in common with neuronal cells than with their neighboring exocrine and ductal cells. Supporting this observation, pancreatic endocrine cells express many genes originally described in neurons, such as *tyrosine hydroxylase*, *neuron-specific enolase* and *glutamic acid decarboxylase* (Bishop et al., 1982; Okada et al., 1976; Teitelman et al., 1981). It was therefore not surprising to find that pancreatic endocrine development utilizes many transcription factors originally described in neural development. Examples include the paired homeodomain family members pax4 and pax6 (Dohrmann et al., 2000; Sander et al., 1997; Sosa-Pineda et al., 1997; St-Onge et al., 1997), the NK homeodomain factors nkx2.2 and nkx6.1 (Madsen et al., 1997; Sander et al., 2000; Sussel et al.,

1998) and the pro-endocrine bHLH genes neurogenin3 and neuroD1 (Apelqvist et al., 1999; Jensen et al., 2000a; Naya et al., 1997; Schwitzgebel et al., 2000). Conversely, genes identified originally as islet transcription factors have been found to play roles in neuronal development, an example being *Isl1*, which is required for the production of motor neurons as well as playing a role in pancreas development (Ahlgren et al., 1997; Pfaff et al., 1996).

Despite the functional similarities between the endocrine pancreas and the cells of the nervous system, it has been established that pancreatic endocrine cells are derived from the embryonic endoderm, not the ectoderm from which neurons derive (Fontaine and Le Douarin, 1977; Le Douarin, 1988; Pictet et al., 1976). Many of the transcription factors involved in the formation, patterning and differentiation of endoderm and its derivatives have been identified (reviews, this issue). Some of these factors have also been shown to be important in the developing pancreas. In particular, a variety of hepatocyte nuclear factors (HNF's), identified originally as regulators of liver specific gene transcription, have been shown to be involved not only in liver organogenesis, but also in the development and function of the pancreas. Thus, the formation of the pancreatic endocrine cells can be viewed as a product of the concerted contributions of at least two groups of factors shared, respectively, with neurons and endoderm tissues including hepatocytes.

3. Pancreatic bud formation

By embryonic day 8.5 (E8.5) in the mouse, 24 h before the dorsal pancreatic bud first appears, cells in the endoderm in the region of the foregut/midgut junction are already committed to a pancreatic fate, as demonstrated by their unique ability to differentiate into all pancreatic lineages in vitro (Wessells and Cohen, 1967). This early prepancreatic endoderm selectively expresses two homeodomain transcription factors, the parahox factors PDX1 (Ahlgren et al., 1996; Guz et al., 1995; Offield et al., 1996) and HB9 (Harrison et al., 1999; Li et al., 1999). The patterning represented by the expression of these factors depends on instructive signals from adjacent tissues (see reviews in this issue and Edlund, 2002; Kim and Hebrok, 2001). Competence to respond to the instructive signals is presumably provided by factors that are expressed more broadly in the endoderm and precede PDX1 and HB9, including several transcription factors that were originally described in the adult liver, hepatic nuclear factors 1b (a POU-homeodomain factor) (Ott et al., 1991), 3a and b (forkhead factors now known as *Foxa1* and *Foxa2*) (Monaghan et al., 1993), 4a (an orphan nuclear receptor) (Duncan et al., 1994; Taraviras et al., 1994) and 6 (a cut-homeodomain factor) (Landry et al., 1997; Rausa et al., 1997), and the zinc-finger transcription factors GATA4, 5, and 6 (Arceci et al., 1993; Laverriere et al., 1994). The hierarchy of these genes in endoderm devel-

opment has been reviewed previously (Cereghini, 1996; Zaret, 1999, 1996), although the hierarchy may be somewhat different in the developing pancreas (Boj et al., 2001; Shih et al., 2001; Shih and Stoffel, 2001). Several of these endoderm factors have been directly implicated in the control of PDX1 expression (see Fig. 5).

Studies of the *pdx1* gene promoter have been used to look for factors that control its expression and therefore that may lie upstream of PDX1 in pancreatic development (Ben-Shushan et al., 2001; Gerrish et al., 2001, 2000; Marshak et al., 2001; Samaras et al., 2002; Sharma et al., 1997; Stoffers et al., 1999; Wu et al., 1997). Most of these studies have been performed in differentiated beta-cells, however, and their conclusions may not apply to the expression of PDX1 in the early pancreatic endoderm. *pdx1* (4.3 kb) upstream sequence can recapitulate endogenous PDX1 expression in transgenic mouse embryos (Stoffers et al.,

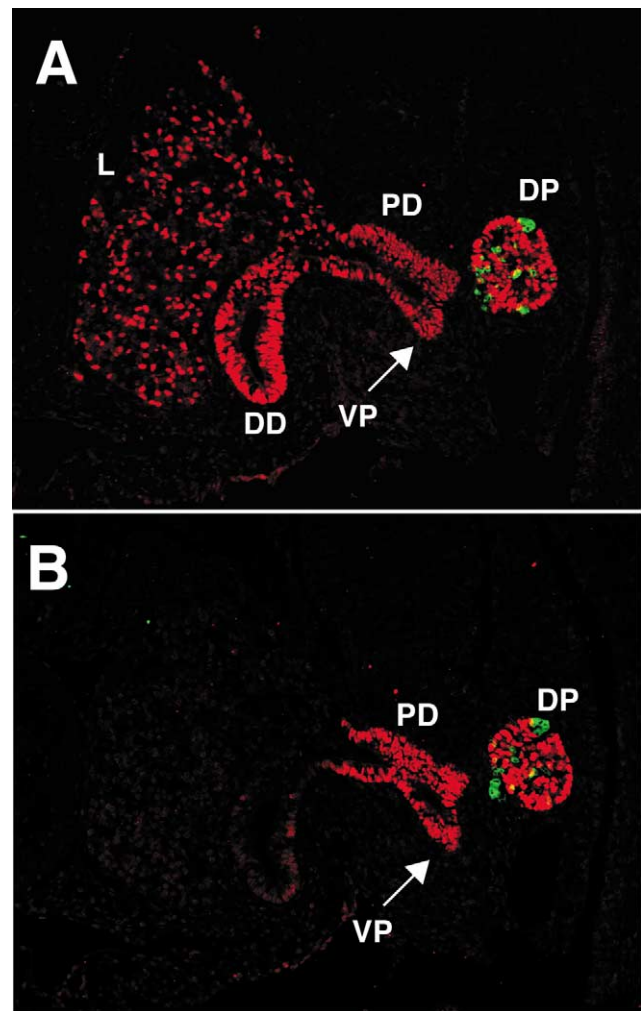


Fig. 2. Mouse pancreatic buds at E10.5. In (A), nuclei expressing *Foxa2* are stained red. In (B), nuclei expressing PDX1 are stained red. Glucagon-producing cells are shown in green in both panels. DP, dorsal pancreatic bud; VP, ventral pancreatic bud; PD, proximal duodenum; DD, distal duodenum; L, liver.

1999; Wu et al., 1997) and conserved regions have been identified that can direct expression in early pancreatic epithelium or in the mature islet (Samaras et al., 2002; Stoffers et al., 1999; Wu et al., 1997). Among the factors that bind to these important sequences are members of the HNF1 and Foxa families of transcription factors, the paired homeodomain factor pax6, and PDX1 itself (Gerrish et al., 2000; Marshak et al., 2000; Sharma et al., 1997; Wu et al., 1997) (HB9 may also bind to these same PDX1-binding sites, given its similarity to PDX1 in the DNA-binding homeodomain). HNF1a and pax6 are not expressed early enough or broadly enough to initiate the early expression of PDX1 in the embryonic gut and pancreatic buds, but the expression patterns of HNF1b, Foxa1, and Foxa2 suggest that they could play this role (see Fig. 2). Mice null for Foxa2 or HNF1b (Ang and Rossant, 1994; Coffinier et al., 1999b; Weinstein et al., 1994) die before pancreas formation, but embryoid bodies lacking Foxa2 fail to activate the *pdx1* gene, suggesting that Foxa2 may be a bona fide activator of PDX1 expression in the prepancreatic endoderm and pancreatic buds and therefore lie directly upstream of PDX1 in the hierarchy of factors involved in initiating pancreas development (Gerrish et al., 2000).

Once expressed, PDX1 plays a critical role early in pancreas formation (Hui and Perfetti, 2002; McKinnon and Docherty, 2001). As the dorsal and ventral pancreatic buds start to form, expression of PDX1 persists in the undifferentiated pancreatic epithelium, and also in the adjacent duodenum and antral stomach (Ohlsson et al., 1993; Peshavaria et al., 1994). Broad expression continues in the undifferentiated epithelial cells of the pancreatic buds until E13 when it becomes restricted, with low expression remaining in some duct and exocrine cells and enhanced expression in most beta-cells and some delta cells. Removal of PDX1 by gene targeting arrests pancreatic development after initial bud formation, resulting in an animal with no pancreas (Ahlgren et al., 1996; Jonsson et al., 1994; Offield et al., 1996). A homozygous mutation in the *IPF1* gene, which encodes human PDX1, was identified in a human patient with similar pancreatic agenesis (Stoffers et al., 1997b), and mutations in the zebra fish gene encoding PDX1 also affect pancreas development (Yee et al., 2001), reflecting an evolutionary conservation of PDX1 function.

The phenotype of mice lacking PDX1 demonstrates that PDX1 is necessary for the growth of the pancreatic buds but not for the initial induction of bud formation. Interestingly, ectopic expression of PDX1 in non-pancreatic regions of gut endoderm in chick embryos can induce evagination of the gut epithelium similar to pancreatic bud formation (Grapin-Botton et al., 2001), suggesting that PDX1 may play a sufficient, but redundant, role in pancreatic bud induction.

In contrast, HB9 is necessary for bud induction, but only for the dorsal bud. Expression of HB9 precedes PDX1 in the dorsal prepancreatic endoderm, and commences simultaneously with PDX1 in the ventral prepancreatic endoderm (Li et al., 1999). HB9 is down regulated when the pancreatic

buds form but reappears in the differentiated beta-cells. In embryonic mice homozygous for targeted mutations in the *Hlxb9* gene that encodes HB9, the dorsal prepancreatic endoderm fails to express PDX1 and formation of the dorsal pancreatic bud never initiates (Harrison et al., 1999; Li et al., 1999). This intriguing phenotype demonstrates that HB9 functions upstream of PDX1 in the dorsal, but not the ventral pancreatic bud, and adds to a body of evidence that different sets of factors control dorsal and ventral pancreatic specification.

To understand how HB9 and PDX1 drive the formation and growth of the pancreatic bud, it will be important to identify the downstream gene targets of these transcriptional regulators. PDX1 was originally identified as a transactivator of the *insulin* and *somatostatin* gene promoters (Leonard et al., 1993; Miller et al., 1994; Ohlsson et al., 1993), and has been implicated in the expression of a number of genes in differentiated beta-cells, including the glucose transporter *glut2*, glucokinase, and islet amyloid polypeptide (Chakrabarti et al., 2002; Macfarlane et al., 2000; Waeber et al., 1996; Watada et al., 1996a,b). Unfortunately, however, with the possible exception of *pax4* (Chakrabarti et al., 2002; Smith et al., 2000), no gene targets of PDX1 in the prepancreatic endoderm or the early pancreatic buds have been identified.

Transcription factors exert their effects on a particular promoter as part of a complex of DNA and non-DNA binding proteins that act in concert to stabilize and activate the RNA pol II complex on the promoter (Carey, 1998). In binding to target genes, PDX1 physically interacts with a variety of other nuclear proteins (Asahara et al., 1999; Dutta et al., 2001; Ohneda et al., 2000b; Peers et al., 1995; Qiu et al., 2002) including another homeodomain factor, Pbx1 (Asahara et al., 1999; Dutta et al., 2001; Peers et al., 1995). Pbx family members are widely expressed throughout the developing embryo as well as the pancreas, and dimerize with a variety of hox and parahox homeodomain transcription factors (Chan et al., 1994; Chang et al., 1995; van Dijk and Murre, 1994), thereby modulating both their DNA-binding specificity and function. In the absence of Pbx1, the development of both the endocrine and the exocrine pancreas is impaired in a pattern that partially replicates the defects seen in PDX1 null embryos (Kim et al., 2002). An elegant experiment by Dutta et al. demonstrated that the role of Pbx1 in the pancreas is tied to its ability to interact with PDX1, since removal of the domain of PDX1 that interacts with Pbx1 prevents a PDX1 transgene from fully rescuing pancreas development in *pdx1* $-/-$ animals (Dutta et al., 2001). Like PDX1 itself, PDX1/Pbx1 complexes are necessary for the expansion of the pancreatic buds but not for the specification of the different pancreatic cell types.

4. Endocrine and exocrine specification

Endocrine and exocrine specification in the pancreas can be viewed as a process of determining which cells out of a

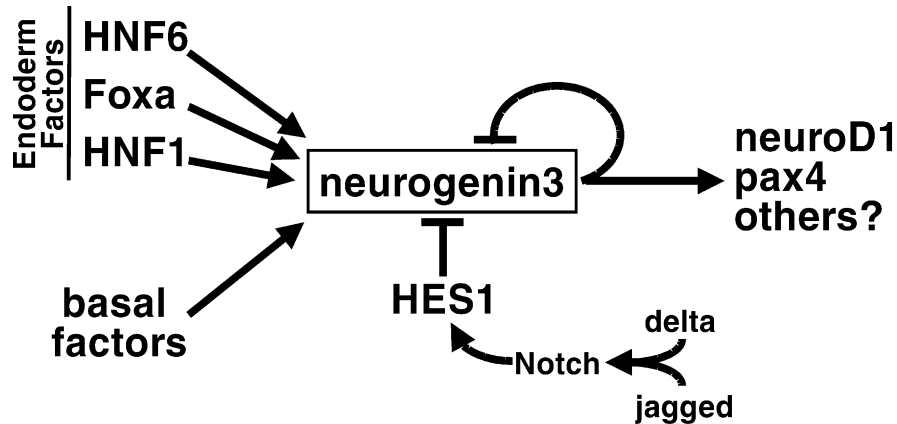


Fig. 3. Control of neurogenin3 expression. A model is shown for the positive and negative regulation of neurogenin3 expression in the pancreatic endocrine progenitor cells.

large pool of apparently uniform precursors will eventually differentiate into mature islet or acinar cells. The precursor pool is in the form of convoluted sheets of epithelial cells that make up the early pancreatic buds. Since the early pancreatic epithelium uniformly expresses PDX1 and HB9, other factors must determine the subsets of cells that will become endocrine or exocrine cells. Furthermore, the genetic studies that implicate PDX1 and HB9 in bud formation and outgrowth do not support an essential role for these factors in the specification of the different pancreatic lineages, since cells of distinct lineages can differentiate in the absence of either factor (Ahlgren et al., 1996; Harrison et al., 1999; Li et al., 1999; Offield et al., 1996).

In many ways, the specification of the endocrine cells in the developing pancreatic endoderm is reminiscent of the process by which neurons are specified in the developing neuroectoderm. In a field of initially identical cells in the neuroectoderm, the cells that first initiate the neural differentiation program inhibit neural differentiation in their neighbors through the notch signaling pathway, thereby forcing them to remain as precursors and eventually assume an alternate cell fate (Lewis, 1998). In this system, notch signaling represses the default activation of the neural differentiation program in most of the cells in the field, limiting neural differentiation to a subset of cells scattered across the neuroectoderm. In those cells that suppress neurogenesis, the target of notch signaling is a family of genes encoding inhibitory basic helix-loop-helix (bHLH) transcription factors, the hairy/enhancer-of-split (HES) genes. Once activated by notch signaling, the HES factors in turn inhibit the pro-neuronal bHLH genes that otherwise would activate the neural differentiation program (Jan and Jan, 1993; Lee, 1997a).

In an entirely analogous fashion, notch signaling determines which cells in the developing pancreas will activate the endocrine differentiation program (Apelqvist et al., 1999; Edlund, 2001; Jensen et al., 2000b). HES-1, the downstream target of notch signaling in the pancreas, inhibits the expression of the pro-endocrine bHLH transcription

factor neurogenin3 (Jensen et al., 2000b; Lee et al., 2001), a member of the larger neurogenin/neuroD family of vertebrate pro-neuronal genes (Gradwohl et al., 1996; Lee, 1997a; Ma et al., 1996; Sommer et al., 1996). Neurogenin3 is expressed only briefly in scattered cells in the pancreatic epithelium, and not in differentiated endocrine cells (Apelqvist et al., 1999; Jensen et al., 2000a; Schwitzgebel et al., 2000); but lineage tracing using cre recombinase in transgenic animals has demonstrated that these neurogenin3 expressing cells function as endocrine cell precursors (Gu et al., 2002). Animals lacking neurogenin3 fail to develop any endocrine cells (Gradwohl et al., 2000), while uniform ectopic expression of neurogenin3 throughout the pancreatic epithelium causes massive premature differentiation of the entire pancreas into endocrine cells (Apelqvist et al., 1999; Schwitzgebel et al., 2000). Together, these studies demonstrate that neurogenin3 expression, permitted by an absence of notch signaling, is both necessary and sufficient for initiating endocrine differentiation in the pancreas.

Notch signaling restricts neurogenin3 to scattered cells within the pancreatic epithelium, but positive signals, permissive and inductive, must initiate neurogenin3 expression in the absence of notch signaling. Studies with the neurogenin3 promoter have identified several transcription factors that may play this role. The promoter for *neurogenin3* gene is remarkably conserved across species; and, as expected, it contains multiple-binding sites for the HES1 repressor, which potently inhibits the promoter in transient transfections (Lee et al., 2001). In addition, however, the promoter contains binding sites for several other transcriptional activators broadly expressed in the endoderm and the pancreatic buds, including HNF1, Foxa and HNF6 (Jacquemin et al., 2000; Lee et al., 2001) (see Fig. 3).

Genetic evidence in mice supports a role for HNF6 as an upstream activator of neurogenin3 expression. HNF6 is a Cut-homeodomain transcription factor originally identified as a regulator of hepatic genes including Foxa2 (Lemaigre et al., 1993, 1996; Rausa et al., 1997; Samadani and Costa, 1996). HNF6 is initially expressed throughout the pancrea-

tic buds, but after birth is excluded from the mature islets (Rausa et al., 1997). The neurogenin3 promoter contains HNF6-binding sites and can be activated by HNF6 in cultured cells (Jacquemin et al., 2000). Mice lacking HNF6 have severely reduced neurogenin3 expression in the developing pancreas, and a severely reduced number of endocrine cells with no organized islets at birth (Jacquemin et al., 2000).

Given its central role in determining the endocrine lineage in the pancreas, it will be important to understand how neurogenin3 functions on a molecular level and to identify its downstream gene targets. In other tissues where bHLH proteins play a prominent role in development, cell-type differentiation is often initiated by a cascade of bHLH proteins (Arnold and Winter, 1998; Cau et al., 1997; Lee, 1997b). Fitting with this idea of a cascade of bHLH factors, and in keeping with the concept that pancreatic endocrine cells and neurons develop in parallel pathways, neurogenin3 has been shown to activate the promoter for the gene encoding another member of the family of neural pro-neuronal bHLH factors, neuroD1 (see Fig. 3). NeuroD1 is expressed slightly later than neurogenin3 during pancreatic development; but, unlike neurogenin3, it persists in the mature islet cells, where it is the predominant member of the neuroendocrine bHLH family, and plays a role in the expression of a number of differentiated endocrine cell products including insulin (Glick et al., 2000; Naya et al., 1995; Ohneda et al., 2000b; Qiu et al., 2002). Neurogenin3 can bind to and activate the *neuroD1* gene promoter, and it can induce ectopic neuroD1 expression in *Xenopus* embryos (Huang et al., 2000). In neurogenin3 null embryos, neuroD1 expression in the pancreas is completely lost (Gradwohl et al., 2000), while neurogenin3 expression is unchanged in neuroD1 null embryos (Schwitzgebel et al., 2000).

Like neurogenin3, ectopic expression of neuroD1 induces premature endocrine differentiation in the pancreas, demonstrating that neuroD1 is also a pro-endocrine gene (Schwitzgebel et al., 2000). In the absence of neuroD1, pancreatic islet development is severely impaired; but, unlike embryos lacking neurogenin3, differentiated endocrine cells are generated, but are lost at a high rate due to accelerated apoptosis (Naya et al., 1997). The less severe phenotype of the neuroD1 null mice suggests that other genes downstream of neurogenin3, possibly other bHLH genes, may compensate for the loss of neuroD1, since neurogenin3 expression is unaffected by the loss of neuroD1 in these animals (Schwitzgebel et al., 2000).

While cells expressing neurogenin3 are destined to become endocrine cells, cells in which neurogenin3 expression is extinguished by notch signaling can become part of the exocrine pancreas. Other factors, in particular the bHLH factor p48, are then required to drive these precursor cells to the exocrine fate. P48 was originally identified as a subunit of the PTF-1 complex that drives the expression of exocrine-pancreas-specific genes such as the pancreatic enzymes (Krapp et al., 1996; Rose et al., 2001). Mice lack-

ing p48 fail to develop any exocrine pancreas; both acini and the ductal trees are absent, although islets form within the mesenchyme and migrate to the spleen (Krapp et al., 1998). No factors that may control the expression of p48 have yet been identified, and studies of the gene encoding p48 have not identified cell-type specific control regions (Knofler et al., 1996).

Taken together, these studies engender a model in which neurogenin3, downstream of HNF6 and other endoderm factors, specifies which cells in the pancreatic epithelium will differentiate into endocrine cells and initiates the differentiation program, at least in part, by activating the expression of neuroD1. At the same time, cells in which the notch signaling pathway has been activated suppress neurogenin3 and can express P48 and differentiate into exocrine cells.

5. Endocrine cell subtype determination

Once neurogenin3 has been activated in a progenitor cell in the pancreas, that cell is fated to become an endocrine cell, but it can become any one of four possible endocrine cell subtypes, and other factors control this decision. Ectopic expression of neurogenin3 in the early pancreatic bud drives cells to an endocrine fate, but the cells produced are almost exclusively alpha cells (Schwitzgebel et al., 2000). Since we know that during normal pancreatic development neurogenin3 expressing cells are progenitors for all four endocrine cell subtypes (Gu et al., 2002), then either the timing or method of ectopic neurogenin3 expression in these transgenic embryos favors the alpha-cell fate, or the alpha-cell fate is the default outcome for cells that express neurogenin3, and additional signals are required to deviate these cells to alternate fates such as beta-cells.

Part of the difficulty in understanding how endocrine cell-type fate is determined is that we do not know when it is determined. Based on the early emergence of alpha-cells and the presence of cells coexpressing insulin and glucagon in the early pancreatic buds, a previous model proposed that endocrine cell precursors initially express glucagon before differentiating into one of the other three endocrine cell subtypes (Alpert et al., 1988; Teitelman et al., 1993). In this model, endocrine cell subtype determination does not occur until after hormone genes are expressed. This model has fallen into disfavor because hormone coexpressing cells are not present during the major period of islet cell neogenesis in the mouse (E13–E16) and because transgenic lineage tracing studies do not support the idea that all islet cells express glucagon at some stage in differentiation (Herrera, 2000; Herrera et al., 1994, 1998).

Instead, subtype fate seems to be determined before hormone genes are activated. We do not know, however, whether that decision is made before, during or after neurogenin3 expression, and therefore also do not know whether neurogenin3 is necessary for expression of the factors that control endocrine subtype fate. The uniform production of

alpha cells in response to ectopic neurogenin3 expression suggests that endocrine cell subtype decisions are made independently of neurogenin3 expression. Since we know that few islet cells other than alpha cells are normally generated in the pancreatic buds prior to E13, the early bud may simply lack instructive signals that deviate progenitor cells to non-alpha-cell fates. Alternatively, the cells may lack the appropriate set of intrinsic factors involved in endocrine cell subtype decisions, and when neurogenin3 is expressed prematurely in these cells, they uniformly adopt an alpha-cell fate.

A number of transcription factors that are expressed selectively in the endocrine lineage in the developing pancreas and that could play a role in endocrine cell subtype fate decisions have been identified. To date, however, no data has convincingly demonstrated that any of these factors are either necessary or sufficient to control endocrine cell fate decisions. These factors all contain homeodomains and can be divided into early factors (*pax4*, *nkx2.2* and *nkx6.1*) that are coexpressed with neurogenin3 in endocrine progenitor cells and late factors (*pax6*, *isl1*, *brn4*, *HB9* and *PDX1*) that are found in more mature cells.

The paired-homeodomain factor *pax4* is expressed selectively in the developing pancreas and is required for the normal development of beta- and delta cells. *Pax4* expression in the pancreas crests just before the peak of endocrine cell genesis at E15 and little or no *pax4* can be detected in the mature pancreas (Dohrmann et al., 2000; Smith et al., 1999). Mice homozygous for a targeted deletion of the *pax4* gene develop diabetes at birth due to a profound deficiency of beta and delta cells, although the fates of beta and delta-cell precursors are uncertain (Sosa-Pineda et al., 1997). Despite its critical role in beta-cell and delta-cell genesis, *pax4* by itself is insufficient to drive neurogenin3-expressing precursors to a beta- or delta-cell fate (Grapin-Botton et al., 2001) (S. Smith, D. Scheel and M.S.G., unpublished observations).

Loss of the NK-homeodomain protein *nkx2.2* has an equally profound but very different effect on islet cell differentiation. *Nkx2.2* is expressed broadly in the pancreatic bud until E13 when it becomes localized to the neurogenin3-expressing progenitor cells (see Fig. 4). Unlike *pax4*, *nkx2.2* also persists in many of the mature endocrine cells including all beta-cells. Mice lacking *nkx2.2* have a complete absence of insulin-producing cells, and a reduction in alpha- and PP-cells. They develop islets consisting of alpha, delta and PP cells along with a population of unusual endocrine cells that express IAPP and *PDX1*, markers of beta cells, but lack other markers such as *Glut2* and *glucokinase*. It seems that in the absence of *nkx2.2* beta cells are specified but are unable to differentiate fully to mature, insulin-producing cells (Sussel et al., 1998).

The islets of mice null for *nkx2.2* also fail to express the distantly related NK-homeodomain factor *nkx6.1*. The pattern of *nkx6.1* expression in the pancreas is similar to the pattern of *nkx2.2* expression except that it is not expressed in non-beta islet cells (Oster et al., 1998; Sander et al., 2000). The phenotype of mice lacking *nkx6.1*,

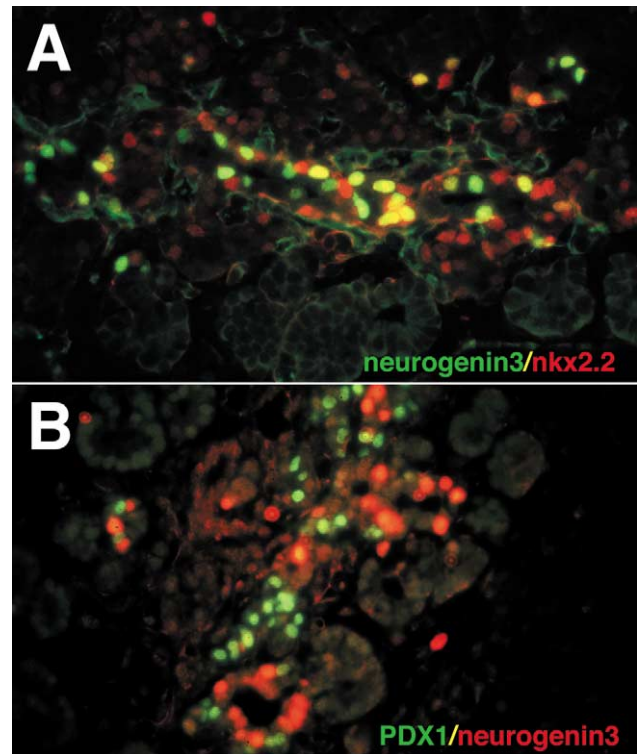


Fig. 4. Transcription factor coexpression in mouse pancreas at E15.5. In (A), nuclei containing *nkx2.2* are stained red, and nuclei containing neurogenin3 are stained green. Nuclei containing both *nkx2.2* and neurogenin3 appear yellow. In (B), nuclei containing neurogenin3 are stained red, and nuclei containing *PDX1* are stained green. No cells coexpressing *PDX1* and neurogenin3 can be detected.

however, is more reminiscent of *pax4* null animals, as they have a profound, but not complete, defect in beta-cell generation, and do not produce the aberrantly differentiated endocrine cells seen in the *nkx2.2* null pancreas (Sander et al., 2000), although they also lack the defect in delta cells seen in *pax4* null animals.

The absence of *nkx6.1* expression in the islets of animals lacking *nkx2.2* suggests that *nkx2.2* lies upstream of *nkx6.1* in the beta-cell differentiation pathway (see Fig. 5). Two additional pieces of genetic evidence support this conclusion. First, pancreatic *nkx2.2* expression is unaffected in mice null for *nkx6.1*. Second, the pancreatic phenotype of mice with homozygous mutations in both the *nkx6.1* and *nkx2.2* genes is identical to the phenotype of *nkx2.2* homozygous single mutant mice, demonstrating that the loss of *nkx6.1* has no effect on beta-cell differentiation in the absence of *nkx2.2* (Sander et al., 2000). In addition, studies of the *nkx6.1* gene promoter suggest that *nkx2.2* may directly regulate *nkx6.1* expression (Watada et al., 2000).

The relationship of *pax4* to the *nkx* factors during islet cell differentiation is less clear. Based solely on timing of expression in progenitor cells, it is unlikely that *pax4* lies upstream of *nkx2.2*, although it could lie downstream of *nkx2.2* along with *nkx6.1*. Given their apparently similar roles in the beta-cell differentiation pathway, it will be inter-

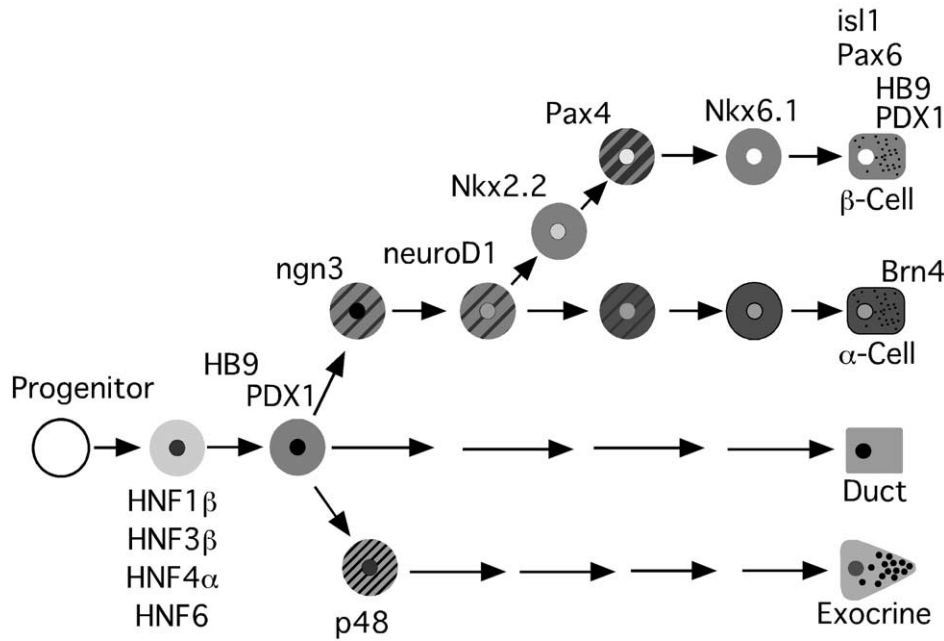


Fig. 5. A simplified model for the role of islet transcription factors in endocrine differentiation in the developing pancreas. The proposed position for each transcription factor is based on its timing of expression, timing of predominant functional role, or both. Clearly some factors function at several steps, but a single step is shown for simplicity.

esting to test the relative positions of *nkx6.1* and *pax4* in the pathway.

The *pax4* gene appears to be a direct target of neurogenin3 (see Fig. 3). Neurogenin3 can bind to and activate the *pax4* gene promoter (Smith et al., 2000), it can activate the intact gene in cultured cells (R. Gasa and M.S.G., unpublished observations), and in its absence *pax4* expression is lost in the pancreas (Gradwohl et al., 2000). In addition, studies of the *pax4* gene promoter implicate the more broadly expressed factors HNF1 and HNF4 in cooperative activation of *pax4* expression along with neurogenin3 (Smith et al., 2000).

The late factors *pax6*, *isl1*, *PDX1* and *brn4* function in the final steps of islet-cell differentiation, after neurogenin3 expression and in conjunction with hormone gene expression (see Fig. 5). As demonstrated by their roles in the expression of many of the islet hormone genes, these factors are critical to the development and maintenance of the final differentiated islet cell phenotypes. The paired-homeodomain factor *pax6* and the LIM-homeodomain factor *isl1* are expressed in all islet cells and their loss causes defects in the generation of all endocrine cell subtypes (Ahlgren et al., 1997; Sander et al., 1997; St-Onge et al., 1997). In addition, both factors activate islet hormone gene expression, with *pax6* implicated in glucagon, insulin and somatostatin expression (Sander et al., 1997), and *isl1* implicated in glucagon and somatostatin expression (Leonard et al., 1992; Vallejo et al., 1992; Wang and Drucker, 1995).

In addition to its early role in bud formation, *PDX1* also plays a role in the final differentiation of mature islet cells. After its early expression in the prepancreatic endoderm and early pancreatic buds, *PDX1* expression is down regulated.

PDX1 cannot be detected in the neurogenin3 expressing endocrine cell progenitors (Schwitzgebel et al., 2000) (Fig. 4), but its expression is reactivated in most differentiated beta-cells and some delta cells, where it plays a role in maintaining the differentiated phenotype. The expression of a number of mature beta- and delta-cell gene products is regulated by *PDX1* (Chakrabarti et al., 2002; Leonard et al., 1993; Macfarlane et al., 2000; Miller et al., 1994; Ohlsson et al., 1993; Waeber et al., 1996; Watada et al., 1996a,b), and if *PDX1* is removed from mature beta-cells by cre-recombinase-mediated deletion, the expression of many of these genes is impaired and beta-cell loss is accelerated (Ahlgren et al., 1996).

The POU-homeodomain factor *brn4* also is activated in mature islet cells, but it is restricted to non-beta-cells, predominantly alpha cells (Hussain et al., 1997; Wilson et al., 2002), where it regulates glucagon gene expression (Hussain et al., 1997). Animals null for *brn4* do not exhibit any defect in alpha-cell formation (Phippard et al., 1999). Ectopic expression of *brn4*, however, produces an interesting phenotype. When driven by the *pdx1* gene promoter, *brn4* expression gives rise to beta-cells that coexpress insulin and glucagon (Hussain et al., 2002), demonstrating the importance of the late islet differentiation factors in defining the final differentiated phenotype of the individual endocrine cell subtypes, but not their identity.

6. Summary

As mouse genetic studies have begun to outline the differ-

Table 1
Pancreas transcription factors^a

Factor	Family	Expression	Downstream pancreatic genes	Mouse mutations ^b	Human mutations	References
Neurogenin3	bHLH	Fetal pancreas (endocrine progenitor cells) and CNS	neuroD1/BETA2, pax4, nkx2.2	Diabetes, no islet cells		Apelqvist et al., 1999; Gradwohl et al., 2000; Huang et al., 2000; Jensen et al., 2000a; Schwitzgebel et al., 2000
NeuroD1/BETA2	bHLH	Islet, gut endocrine cells, CNS	Insulin	Diabetes, decreased islet cells	Het: late onset diabetes	Lee et al., 1995; Malecki et al., 1999; Mutoh et al., 1998; Naya et al., 1997, 1995; Qiu et al., 1998
P48/PTF	bHLH	Exocrine pancreas, CNS	Exocrine enzyme genes	Exocrine pancreatic agenesis, islets in spleen		Krapp et al., 1996, 1998; Obata et al., 2001
Mist1	bHLH	Exocrine pancreas, serous exocrine cells		Exocrine pancreas disorganization		Lemercier et al., 1997; Pin et al., 2000, 2001
PDX1/IPF1	Parahox homeodomain	β and δ cells, duodenum, stomach, CNS	Insulin, IAPP, glucokinase, glut2	Pancreatic agenesis	Het: MODY4 Hom: pancreatic agenesis	Ahlgren et al., 1996, 1998; Guz et al., 1995; Jonsson et al., 1994; Offield et al., 1996; Perez-Villamil et al., 1999; Schwartz et al., 2000; Stoffers et al., 1997a,b; Waeber et al., 1996; Watada et al., 1996a,b,c
HB9	Parahox homeodomain	β Cells, gut, lymphoid, CNS	glut2	Dorsal pancreatic agenesis	Het: sacral agenesis	Harrison et al., 1994, 1999; Li et al., 1999
Pax2	Paired domain	Islet, urogenital tract and CNS	Glucagon	Defects in optic nerve, CNS and urogenital tract	Het: renal-coboma syndrome	Ritz-Laser et al., 2000; Torres et al., 1996
Pax4	Paired-homeodomain	fetal pancreas and CNS	pax4 (autorepression)	Decreased β and δ cells	Het: late onset diabetes Hom: early diabetes	Shimajiri et al., 2001; Smith et al., 2000, 1999; Sosa-Pineda et al., 1997
Pax6	Paired-homeodomain	Islet, gut endocrine cells, CNS	Glucagon, insulin, somatostatin	Decrease in all islet cells, decreased glucagon	Het: Aniridia	Hill et al., 1999; Sander et al., 1997; St-Onge et al., 1997
Nkx2.2	NK-homeodomain	β , α , and PP cells and CNS	nkx6.1 insulin, glut2, GK	Diabetes, no insulin		Rudnick et al., 1994; Sander et al., 2000; Sussel et al., 1998; Watada et al., 2000
Nkx6.1	NK-homeodomain	β Cells and CNS		Decreased β cells, postnatal lethal		Jensen et al., 1996; Rudnick et al., 1994; Sander et al., 2000; Sussel et al., 1998
Cdx2/3	Caudal-homeodomain	Islet and gut	Glucagon	Het: gut tumors Hom: Embryonic lethal		Chawengsaksophak et al., 1997; German et al., 1992; Jin and Drucker, 1996; Jin et al., 1997; Laser et al., 1996
Isl1	LIM-homeodomain	Islet and CNS	Somatostatin, glucagon	No islet cells, embryonic lethal	Het: late onset diabetes	Ahlgren et al., 1997; Dong et al., 1991; Karlsson et al., 1990; Leonard et al., 1992; Shimomura et al., 2000; Thor et al., 1991; Vallejo et al., 1992
Lmx1.1	LIM-homeodomain	β Cells and CNS	Insulin	Dreher: roof plate, cerebellum defects		German et al., 1992; Millonig et al., 2000; Ohneda et al., 2000b

Table 1 (continued)

Factor	Family	Expression	Downstream pancreatic genes	Mouse mutations ^b	Human mutations	References
Brn4	Pou-homeodomain	α Cells and CNS	Glucagon		Hom: congenital neurogenic deafness	Hussain et al., 1997; Jensen et al., 2000a; Phippard et al., 1998
HNF1 α	Pou-homeodomain	Islet, liver, kidney	pax4, neurogenin3, glut2, Rat insulin I	Diabetes, impaired β cell glucose sensing	Het: MODY3	Emens et al., 1992; Lee et al., 2001; Noguchi et al., 1991; Pontoglio et al., 1998; Shih et al., 2001; Smith et al., 2000; Yamagata et al., 1996b
HNF1 β	Pou-homeodomain	Islet, pancreatic duct, liver, kidney	pax4, PDX1	Embryonic lethal	Het: MODY5	Coffinier et al., 1999a,b; Horikawa et al., 1997; Smith et al., 2000
HNF6	Cut-homeodomain	Pancreatic duct, liver	Neurogenin3	IGT, small islets		Jacquemin et al., 2000; Landry et al., 1997; Lee et al., 2001; Lemaigre et al., 1996; Rausa et al., 1997, 1998
Foxa1/HNF3a	Forkhead/Winged Helix	Islet, gut, liver	Glucagon	Hypoglycemia		Kaestner et al., 1999; Shih et al., 1999
Foxa2/HNF3 β	Forkhead/Winged Helix	Islet, pancreatic duct, gut, liver, CNS	PDX1, neurogenin3, Kir6.2, SUR1	Embryonic lethal		Duncan et al., 1998; Gerrish et al., 2000; Lee et al., 2001; Rausa et al., 1997, 1998, 1999; Sharma et al., 1997; Sund et al., 2001; Wu et al., 1997
Foxa3/HNF3 γ	Forkhead/Winged Helix	Islet, gut, liver	Glucagon	No pancreatic phenotype		Kaestner et al., 1994, 1998; Liu et al., 2002
HNF4 α	Nuclear receptor	Liver, islet, kidney	HNF1 α , glycolytic enzymes, pax4	Embryonic lethal	Het: MODY1	Duncan et al., 1994, 1998; Miquerol et al., 1994; Sladek et al., 1990; Smith et al., 2000; Yamagata et al., 1996a,b
MafA	bZip	β Cells, eye and thymus	Insulin			Olbro et al., 2002; Planque et al., 2001
c-Maf	bZip	α Cells, eye	Glucagon			Planque et al., 2001

^a Abbreviations: het, heterozygous; hom, homozygous; CNS, central nervous system; GK, glucokinase; IAPP, islet amyloid polypeptide.

^b All mouse phenotypes are for homozygous mutant animals unless stated otherwise.

ent roles of the pancreatic transcription factors, this information along with knowledge of their expression patterns and insights into their molecular function enables us to start placing the factors in a loose hierarchy (Fig. 5). While clearly this model reduces complicated relationships to an overly simplistic outline, it provides a starting point for thinking about how the gene expression program evolves as cells move from multipotent endodermal progenitors to mature pancreatic cells. The model also provides testable hypotheses regarding the relative positions of individual transcription factors within the different lineages. A number of important questions remain regarding the relative positions of known factors in this scheme, the identity of unknown factors, and how each of the factors controls the expression of the genes downstream of it.

One of the key unanswered questions is how and when are the cell fate decisions made that determine which of the specific endocrine cell subtypes the endocrine cell precursors will become? Is this decision made after neurogenin3 expression, and therefore downstream of neurogenin3, or is the decision made independently of neurogenin3? Are individual endocrine cell subtype fates controlled by single factors analogous to the role of neurogenin3 in specifying endocrine cells, or are these decisions orchestrated by an interplay of several factors? Careful analysis of the roles of the known islet differentiation factors and possibly the identification of new players will help answer these questions.

In addition, many of the transcription factors discussed in this review have been found to be mutated in monogenic forms of diabetes (Table 1), and indeed transcription factors found in Fig. 5 account for five of the six genes known to cause Maturity Onset Diabetes of the Young (MODY) (Horikawa et al., 1997; Malecki et al., 1999; Shih and Stoffel, 2001; Stoffers et al., 1997a; Yamagata et al., 1996a,b). In addition, mutations in the coding sequence of *isll* and *pax4* have been implicated in families with later onset diabetes (Malecki et al., 1999; Shimomura et al., 2000). Analysis of these mutations has given insight into the process by which beta-cell dysfunction may lead to the development of diabetes.

Finally, one additional important goal of understanding how cell fate decisions are controlled and organs develop is to apply that understanding to the engineering of cells and tissues for patients. The insights into the mechanisms that control the generation of pancreatic cells during development may eventually be applicable to the development of strategies for tissue regeneration for patients and for replacing the beta cells in patients with diabetes.

Acknowledgements

We would like to thank the many members of the German laboratory who contributed to the work discussed here. The work from our laboratory was supported by NIH grants DK21344, DK41822, DK553401, and DK61245 and grants

from the Nora Eccles Treadwell Foundation, the American Diabetes Association, and the Juvenile Diabetes Research Foundation.

References

- Ahlgren, U., Jonsson, J., Edlund, H., 1996. The morphogenesis of the pancreatic mesenchyme is uncoupled from that of the pancreatic epithelium in IPF1/PDX1-deficient mice. *Development* 122, 1409–1416.
- Ahlgren, U., Pfaff, S.L., Jessell, T.M., Edlund, T., Edlund, H., 1997. Independent requirement for ISL1 in formation of pancreatic mesenchyme and islet cells. *Nature* 385, 257–260.
- Ahlgren, U., Jonsson, J., Jonsson, L., Simu, K., Edlund, H., 1998. Beta-cell-specific inactivation of the mouse *Ipfl/Pdx1* gene results in loss of the beta-cell phenotype and maturity onset diabetes. *Genes Dev.* 12, 1763–1768.
- Alpert, S., Hanahan, D., Teitelman, G., 1988. Hybrid insulin genes reveal a developmental lineage for pancreatic endocrine cells and imply a relationship with neurons. *Cell* 53, 295–308.
- Andersen, F.G., Heller, R.S., Petersen, H.V., Jensen, J., Madsen, O.D., Serup, P., 1999. Pax6 and Cdx2/3 form a functional complex on the rat glucagon gene promoter G1-element. *FEBS Lett.* 445, 306–310.
- Ang, S.L., Rossant, J., 1994. HNF-3 beta is essential for node and notochord formation in mouse development. *Cell* 78, 561–574.
- Apelqvist, A., Li, H., Sommer, L., Beatus, P., Anderson, D.J., Honjo, T., Hrabe De Angelis, M., Lendahl, U., Edlund, H., 1999. Notch signalling controls pancreatic cell differentiation. *Nature* 400, 877–881.
- Arceci, R.J., King, A.A., Simon, M.C., Orkin, S.H., Wilson, D.B., 1993. Mouse GATA-4: a retinoic acid-inducible GATA-binding transcription factor expressed in endodermally derived tissues and heart. *Mol. Cell. Biol.* 13, 2235–2246.
- Arnold, H.H., Winter, B., 1998. Muscle differentiation: more complexity to the network of myogenic regulators. *Curr. Opin. Genet. Dev.* 8, 539–544.
- Asahara, H., Dutta, S., Kao, H.Y., Evans, R.M., Montminy, M., 1999. Pbx-Hox heterodimers recruit coactivator-corepressor complexes in an isoform-specific manner. *Mol. Cell. Biol.* 19, 8219–8225.
- Ben-Shushan, E., Marshak, S., Shoshkes, M., Cerasi, E., Melloul, D., 2001. A pancreatic beta-cell-specific enhancer in the human PDX-1 gene is regulated by hepatocyte nuclear factor 3beta (HNF-3beta), HNF-1alpha, and SPs transcription factors. *J. Biol. Chem.* 276, 17533–17540.
- Bishop, A.E., Polak, J.M., Facer, P., Ferri, G.L., Marangos, P.J., Pearce, A.G., 1982. Neuron specific enolase: a common marker for the endocrine cells and innervation of the gut and pancreas. *Gastroenterology* 83, 902–915.
- Boj, S.F., Parrizas, M., Maestro, M.A., Ferrer, J., 2001. A transcription factor regulatory circuit in differentiated pancreatic cells. *Proc. Natl. Acad. Sci. USA* 98, 14481–14486.
- Carey, M., 1998. The enhanceosome and transcriptional synergy. *Cell* 92, 5–8.
- Cau, E., Gradwohl, G., Fode, C., Guillemot, F., 1997. Mash1 activates a cascade of bHLH regulators in olfactory neuron progenitors. *Development* 124, 1611–1621.
- Cereghini, S., 1996. Liver-enriched transcription factors and hepatocyte differentiation. *FASEB J.* 10, 267–282.
- Chakrabarti, S.K., James, J.C., Mirmira, R.G., 2002. Quantitative assessment of gene targeting in vitro and in vivo by the pancreatic transcription factor, Pdx1. Importance of chromatin structure in directing promoter binding. *J. Biol. Chem.* 277, 13286–13293.
- Chan, S.K., Jaffe, L., Capovilla, M., Botas, J., Mann, R.S., 1994. The DNA binding specificity of Ultrabithorax is modulated by cooperative interactions with extradenticle, another homeoprotein. *Cell* 78, 603–615.
- Chang, C.P., Shen, W.F., Rozenfeld, S., Lawrence, H.J., Largman, C., Cleary, M.L., 1995. Pbx proteins display hexapeptide-dependent coop-

- erative DNA binding with a subset of Hox proteins. *Genes Dev.* 9, 663–674.
- Chawengsaksophak, K., James, R., Hammond, V.E., Kontgen, F., Beck, F., 1997. Homeosis and intestinal tumours in Cdx2 mutant mice. *Nature* 386, 84–87.
- Coffinier, C., Barra, J., Babinet, C., Yaniv, M., 1999a. Expression of the vHNF1/HNF1beta homeoprotein gene during mouse organogenesis. *Mech. Dev.* 89, 211–213.
- Coffinier, C., Thepot, D., Babinet, C., Yaniv, M., Barra, J., 1999b. Essential role for the homeoprotein vHNF1/HNF1beta in visceral endoderm differentiation. *Development* 126, 4785–4794.
- Van Dijk, M.A., Murre, C., 1994. Extracellular DNA binding specificity of homeotic selector gene products. *Cell* 78, 617–624.
- Dohrmann, C., Gruss, P., Lemaire, L., 2000. Pax genes and the differentiation of hormone-producing endocrine cells in the pancreas. *Mech. Dev.* 92, 47–54.
- Dong, J., Asa, S.L., Drucker, D.J., 1991. Islet cell and extrapancreatic expression of the LIM domain homeobox gene *isl-1*. *Mol. Endocrinol.* 5, 1633–1641.
- Duncan, S.A., Manova, K., Chen, W.S., Hoodless, P., Weinstein, D.C., Bachvarova, R.F., Darnell Jr, J.E., 1994. Expression of transcription factor HNF-4 in the extraembryonic endoderm, gut, and nephrogenic tissue of the developing mouse embryo: HNF-4 is a marker for primary endoderm in the implanting blastocyst. *Proc. Natl. Acad. Sci. USA* 91, 7598–7602.
- Duncan, S.A., Navas, M.A., Dufort, D., Rossant, J., Stoffel, M., 1998. Regulation of a transcription factor network required for differentiation and metabolism. *Science* 281, 692–695.
- Dutta, S., Gannon, M., Peers, B., Wright, C., Bonner-Weir, S., Montminy, M., 2001. PDX:PBX complexes are required for normal proliferation of pancreatic cells during development. *Proc. Natl. Acad. Sci. USA* 98, 1065–1070.
- Edlund, H., 1998. Transcribing pancreas. *Diabetes* 47, 1817–1823.
- Edlund, H., 2001. Developmental biology of the pancreas. *Diabetes* 50 (Suppl. 1), S5–S9.
- Edlund, H., 2002. Organogenesis: pancreatic organogenesis developmental mechanisms and implications for therapy. *Nat. Rev. Genet.* 3, 524–532.
- Emens, L.A., Landers, D.W., Moss, L.G., 1992. Hepatocyte nuclear factor 1 α is expressed in a hamster insulinoma line and transactivates the rat insulin I gene. *Proc. Natl. Acad. Sci. USA* 89, 7300–7304.
- Fontaine, J., Le Douarin, N.M., 1977. Analysis of endoderm formation in the avian blastoderm by the use of quail-chick chimaeras. The problem of the neuroectodermal origin of the cells of the APUD series. *J. Embryol. Exp. Morphol.* 41, 209–222.
- German, M.S., Wang, J., Chadwick, R.B., Rutter, W.J., 1992. Synergistic activation of the insulin gene by a LIM-homeodomain protein and a basic helix-loop-helix protein: building a functional insulin minienhancer complex. *Genes Dev.* 6, 2165–2176.
- Gerrish, K., Gannon, M., Shih, D., Henderson, E., Stoffel, M., Wright, C.V., Stein, R., 2000. Pancreatic beta cell-specific transcription of the *pdx-1* gene. The role of conserved upstream control regions and their hepatic nuclear factor 3beta sites. *J. Biol. Chem.* 275, 3485–3492.
- Gerrish, K., Cissell, M.A., Stein, R., 2001. The role of hepatic nuclear factor 1 alpha and PDX-1 in transcriptional regulation of the *pdx-1* gene. *J. Biol. Chem.* 276, 47775–47784.
- Glick, E., Leshkowitz, D., Walker, M.D., 2000. Transcription factor BETA2 acts cooperatively with E2a and PDX1 to activate the insulin gene promoter. *J. Biol. Chem.* 275, 2199–2204.
- Gradwohl, G., Fode, C., Guillemot, F., 1996. Restricted expression of a novel murine atonal-related bHLH protein in undifferentiated neural precursors. *Dev. Biol.* 180, 227–241.
- Gradwohl, G., Dierich, A., Lemeur, M., Guillemot, F., 2000. Neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. *Proc. Natl. Acad. Sci. USA* 97, 1607–1611.
- Grapin-Botton, A., Majithia, A., Melton, D., 2001. Key events of pancreas formation are triggered in gut endoderm by ectopic expression of pancreatic regulatory genes. *Genes Dev.* 15, 444–454.
- Gu, G., Dubauskaite, J., Melton, D.A., 2002. Direct evidence for the pancreatic lineage: NGN3 + cells are islet progenitors and are distinct from duct progenitors. *Development* 129, 2447–2457.
- Guz, Y., Montminy, M.R., Stein, R., Leonard, J., Gamer, L.W., Wright, C.V., Teitelman, G., 1995. Expression of murine STF-1, a putative insulin gene transcription factor, in beta cells of pancreas, duodenal epithelium and pancreatic exocrine and endocrine progenitors during ontogeny. *Development*.
- Harrison, K.A., Druey, K.M., Deguchi, Y., Tuscano, J.M., Kehrl, J.H., 1994. A novel human homeobox gene distantly related to proboscipedia is expressed in lymphoid and pancreatic tissues. *J. Biol. Chem.* 269, 19968–19975.
- Harrison, K.A., Thaler, J., Pfaff, S.L., Gu, H., Kehrl, J.H., 1999. Pancreas dorsal lobe agenesis and abnormal islets of Langerhans in Hlx9-deficient mice. *Nat. Genet.* 23, 71–75.
- Herrera, P.L., 2000. Adult insulin- and glucagon-producing cells differentiate from two independent cell lineages. *Development* 127, 2317–2322.
- Herrera, P.L., Huarte, J., Zufferey, R., Nichols, A., Mermillod, B., Philippe, J., Muniesa, P., Sanvito, F., Orci, L., Vassalli, J.D., 1994. Ablation of islet endocrine cells by targeted expression of hormone-promoter-driven toxigenes. *Proc. Natl. Acad. Sci. USA* 91, 12999–13003.
- Herrera, P.L., Orci, L., Vassalli, J.D., 1998. Two transgenic approaches to define the cell lineages in endocrine pancreas development. *Mol. Cell. Endocrinol.* 140, 45–50.
- Hill, M.E., Asa, S.L., Drucker, D.J., 1999. Essential requirement for Pax6 in control of enteroendocrine proglucagon gene transcription. *Mol. Endocrinol.* 13, 1474–1486.
- Horikawa, Y., Iwasaki, N., Hara, M., Furuta, H., Hinokio, Y., Cockburn, B.N., Lindner, T., Yamagata, K., Ogata, M., Tomonaga, O., Kuroki, H., Kasahara, T., Iwamoto, Y., Bell, G.I., 1997. Mutation in hepatocyte nuclear factor-1 beta gene (TCF2) associated with MODY [letter]. *Nat. Genet.* 17, 384–385.
- Huang, H.P., Liu, M., El-Hodiri, H.M., Chu, K., Jamrich, M., Tsai, M.J., 2000. Regulation of the pancreatic islet-specific gene BETA2 (neuroD) by neurogenin 3. *Mol. Cell. Biol.* 20, 3292–3307.
- Hui, H., Perfetti, R., 2002. Pancreas Duodenum Homeobox-1 regulates pancreas development during embryogenesis and islet cell function in adulthood. *Eur. J. Endocrinol.* 146, 129–141.
- Hussain, M.A., Habener, J.F., 1999. Glucagon gene transcription activation mediated by synergistic interactions of pax-6 and cdx-2 with the p300 co-activator. *J. Biol. Chem.* 274, 28950–28957.
- Hussain, M.A., Lee, J., Miller, C.P., Habener, J.F., 1997. POU domain transcription factor brain 4 confers pancreatic alpha-cell-specific expression of the proglucagon gene through interaction with a novel proximal promoter G1 element. *Mol. Cell. Biol.* 17, 7186–7194.
- Hussain, M.A., Miller, C.P., Habener, J.F., 2002. Brn-4 transcription factor expression targeted to the early developing mouse pancreas induces ectopic glucagon gene expression in insulin-producing beta cells. *J. Biol. Chem.* 277, 16028–16032.
- Jacquemin, P., Durviaux, S.M., Jensen, J., Godfraind, C., Gradwohl, G., Guillemot, F., Madsen, O.D., Carmeliet, P., Dewerchin, M., Collen, D., Rousseau, G.G., Lemaigre, F.P., 2000. Transcription factor hepatocyte nuclear factor 6 regulates pancreatic endocrine cell differentiation and controls expression of the proendocrine gene *ngn3*. *Mol. Cell. Biol.* 20, 4445–4454.
- Jan, Y.N., Jan, L.Y., 1993. HLH proteins, fly neurogenesis, and vertebrate myogenesis. *Cell* 75, 827–830.
- Jensen, J., Serup, P., Karlsen, C., Nielsen, T.F., Madsen, O.D., 1996. mRNA profiling of rat islet tumors reveals *nkx 6.1* as a beta-cell-specific homeodomain transcription factor. *J. Biol. Chem.* 271, 18749–18758.
- Jensen, J., Heller, R.S., Funder-Nielsen, T., Pedersen, E.E., Lindsell, C., Weinmaster, G., Madsen, O.D., Serup, P., 2000a. Independent development of pancreatic alpha- and beta-cells from neurogenin3-expressing precursors: a role for the notch pathway in repression of premature differentiation. *Diabetes* 49, 163–176.
- Jensen, J., Pedersen, E.E., Galante, P., Hald, J., Heller, R.S., Ishibashi, M.,

- Kageyama, R., Guillemot, F., Serup, P., Madsen, O.D., 2000b. Control of endodermal endocrine development by Hes-1. *Nat. Genet.* 24, 36–44.
- Jin, T., Drucker, D.J., 1996. Activation of proglucagon gene transcription through a novel promoter element by the caudal-related homeodomain protein *cdx-2/3*. *Mol. Cell. Biol.* 16, 19–28.
- Jin, T., Trinh, D.K., Wang, F., Drucker, D.J., 1997. The caudal homeobox protein *cdx-2/3* activates endogenous proglucagon gene expression in InR1-G9 islet cells. *Mol. Endocrinol.* 11, 203–209.
- Jonsson, J., Carlsson, L., Edlund, T., Edlund, H., 1994. Insulin-promoter-factor 1 is required for pancreas development in mice. *Nature* 371, 606–609.
- Kaestner, K.H., Hiemisch, H., Luckow, B., Schutz, G., 1994. The HNF-3 gene family of transcription factors in mice: gene structure, cDNA sequence, and mRNA distribution. *Genomics* 20, 377–385.
- Kaestner, K.H., Hiemisch, H., Schutz, G., 1998. Targeted disruption of the gene encoding hepatocyte nuclear factor 3 γ results in reduced transcription of hepatocyte-specific genes. *Mol. Cell. Biol.* 18, 4245–4251.
- Kaestner, K.H., Katz, J., Liu, Y., Drucker, D.J., Schutz, G., 1999. Inactivation of the winged helix transcription factor HNF3 α affects glucose homeostasis and islet glucagon gene expression in vivo. *Genes Dev.* 13, 495–504.
- Karlsson, O., Thor, S., Norberg, T., Ohlsson, H., Edlund, T., 1990. Insulin gene enhancer binding protein Isl-1 is a member of a novel class of proteins containing both a homeo- and a Cys-His domain. *Nature* 344, 879–882.
- Kim, S.K., Hebrok, M., 2001. Intercellular signals regulating pancreas development and function. *Genes Dev.* 15, 111–127.
- Kim, S.K., Selleri, L., Lee, J.S., Zhang, A.Y., Gu, X., Jacobs, Y., Cleary, M.L., 2002. Pbx1 inactivation disrupts pancreas development and in *Ipfl*-deficient mice promotes diabetes mellitus. *Nat. Genet.* 30, 430–435.
- Knofler, M., Krapp, A., Hagenbuchle, O., Wellauer, P.K., 1996. Constitutive expression of the gene for the cell-specific P48 DNA-binding subunit of pancreas transcription factor 1 in cultured cells is under control of binding sites for transcription factors Sp1 and α Cbf. *J. Biol. Chem.* 271, 21993–22002.
- Krapp, A., Knofler, M., Frutiger, S., Hughes, G.J., Hagenbuchle, O., Wellauer, P.K., 1996. The p48 DNA-binding subunit of transcription factor PTF1 is a new exocrine pancreas-specific basic helix-loop-helix protein. *EMBO J.* 15, 4317–4329.
- Krapp, A., Knofler, M., Ledermann, B., Burki, K., Berney, C., Zoerkler, N., Hagenbuchle, O., Wellauer, P.K., 1998. The bHLH protein PTF1-p48 is essential for the formation of the exocrine and the correct spatial organization of the endocrine pancreas. *Genes Dev.* 12, 3752–3763.
- Landry, C., Clotman, F., Hioki, T., Oda, H., Picard, J.J., Lemaigre, F.P., Rousseau, G.G., 1997. HNF-6 is expressed in endoderm derivatives and nervous system of the mouse embryo and participates to the cross-regulatory network of liver-enriched transcription factors. *Dev. Biol.* 192, 247–257.
- Laser, B., Meda, P., Constant, I., Philippe, J., 1996. The caudal-related homeodomain protein *Cdx-2/3* regulates glucagon gene expression in islet cells. *J. Biol. Chem.* 271, 28984–28994.
- Laverriere, A.C., Macneill, C., Mueller, C., Poelmann, R.E., Burch, J.B., Evans, T., 1994. GATA-4/5/6, a subfamily of three transcription factors transcribed in developing heart and gut. *J. Biol. Chem.* 269, 23177–23184.
- Le Douarin, N.M., 1988. On the origin of pancreatic endocrine cells. *Cell* 53, 169–171.
- Lee, J.E., 1997a. Basic helix-loop-helix genes in neural development. *Curr. Opin. Neurobiol.* 7, 13–20.
- Lee, J.E., 1997b. NeuroD and neurogenesis. *Dev. Neurosci.* 19, 27–32.
- Lee, J.E., Hollenberg, S.M., Snider, L., Turner, D.L., Lipnick, N., Weintraub, H., 1995. Conversion of *Xenopus* ectoderm into neurons by NeuroD, a basic helix-loop-helix protein. *Science* 268, 836–844.
- Lee, J.C., Smith, S.B., Watada, H., Lin, J., Scheel, D., Wang, J., Mirmira, R.G., German, M.S., 2001. Regulation of the pancreatic pro-endocrine gene neurogenin3. *Diabetes* 50, 928–936.
- Lemaigre, F.P., Durviaux, S.M., Rousseau, G.G., 1993. Liver-specific factor binding to the liver promoter of a 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase gene. *J. Biol. Chem.* 268, 19896–19905.
- Lemaigre, F.P., Durviaux, S.M., Truong, O., Lannoy, V.J., Hsuan, J.J., Rousseau, G.G., 1996. Hepatocyte nuclear factor 6, a transcription factor that contains a novel type of homeodomain and a single cut domain. *Proc. Natl. Acad. Sci. USA* 93, 9460–9464.
- Lemerrier, C., To, R.Q., Swanson, B.J., Lyons, G.E., Konieczny, S.F., 1997. Mist1: a novel basic helix-loop-helix transcription factor exhibits a developmentally regulated expression pattern. *Dev. Biol.* 182, 101–113.
- Leonard, J., Serup, P., Gonzalez, G., Edlund, T., Montminy, M., 1992. The LIM family transcription factor Isl-1 requires cAMP response element binding protein to promote somatostatin expression in pancreatic islet cells. *Proc. Natl. Acad. Sci. USA* 89, 6247–6251.
- Leonard, J., Peers, B., Johnson, T., Ferreri, K., Lee, S., Montminy, M.R., 1993. Characterization of somatostatin transactivating factor-1, a novel homeobox factor that stimulates somatostatin expression in pancreatic islet cells. *Mol. Endocrinol.* 7, 1275–1283.
- Lewis, J., 1998. Notch signalling and the control of cell fate choices in vertebrates. *Semin. Cell. Dev. Biol.* 9, 583–589.
- Li, H., Arber, S., Jessell, T.M., Edlund, H., 1999. Selective agenesis of the dorsal pancreas in mice lacking homeobox gene *Hlxb9*. *Nat. Genet.* 23, 67–70.
- Liu, Y., Shen, W., Brubaker, P.L., Kaestner, K.H., Drucker, D.J., 2002. Foxa3 (HNF-3 Gamma) binds to and activates the rat proglucagon gene promoter but is not essential for proglucagon gene expression. *Biochem. J.* 366, 633–641.
- Ma, Q., Kintner, C., Anderson, D.J., 1996. Identification of neurogenin, a vertebrate neuronal determination gene. *Cell* 87, 43–52.
- Macfarlane, W.M., Campbell, S.C., Elrick, L.J., Oates, V., Bermano, G., Lindley, K.J., Aynsley-Green, A., Dunne, M.J., James, R.F., Docherty, K., 2000. Glucose regulates islet amyloid polypeptide gene transcription in a PDX1- and calcium-dependent manner. *J. Biol. Chem.* 275, 15330–15335.
- Madsen, O.D., Jensen, J., Petersen, H.V., Pedersen, E.E., Oster, A., Andersen, F.G., Jorgensen, M.C., Jensen, P.B., Larsson, L.I., Serup, P., 1997. Transcription factors contributing to the pancreatic beta-cell phenotype. *Horm. Metab. Res.* 29, 265–270.
- Malecki, M.T., Jhala, U.S., Antonellis, A., Fields, L., Doria, A., Orban, T., Saad, M., Warram, J.H., Montminy, M., Krolewski, A.S., 1999. Mutations in *NEUROD1* are associated with the development of type 2 diabetes mellitus. *Nat. Genet.* 23, 323–328.
- Marshak, S., Benshushan, E., Shoshkes, M., Havin, L., Cerasi, E., Melloul, D., 2000. Functional conservation of regulatory elements in the *pdx-1* gene: PDX-1 and hepatocyte nuclear factor 3 β transcription factors mediate beta-cell-specific expression. *Mol. Cell. Biol.* 20, 7583–7590.
- Marshak, S., Ben-Shushan, E., Shoshkes, M., Havin, L., Cerasi, E., Melloul, D., 2001. Regulatory elements involved in human Pdx-1 gene expression. *Diabetes* 50 (Suppl. 1), S37–S38.
- Mckinnon, C.M., Docherty, K., 2001. Pancreatic duodenal homeobox-1, PDX-1, a major regulator of beta cell identity and function. *Diabetologia* 44, 1203–1214.
- Miller, C.P., McGehee Jr, R.E., Habener, J.F., 1994. IDX-1: a new homeodomain transcription factor expressed in rat pancreatic islets and duodenum that transactivates the somatostatin gene. *EMBO J.* 13, 1145–1156.
- Millonig, J.H., Millen, K.J., Hatten, M.E., 2000. The mouse *Dreher* gene *Lmx1a* controls formation of the roof plate in the vertebrate CNS [see comments]. *Nature* 403, 764–769.
- Miquerol, L., Lopez, S., Cartier, N., Tulliez, M., Raymondjean, M., Kahn, A., 1994. Expression of the L-type pyruvate kinase gene and the hepatocyte nuclear factor 4 transcription factor in exocrine and endocrine pancreas. *J. Biol. Chem.* 269, 8944–8951.
- Monaghan, A.P., Kaestner, K.H., Grau, E., Schutz, G., 1993. Postimplanta-

- tion expression patterns indicate a role for the mouse forkhead/HNF-3 alpha, beta and gamma genes in determination of the definitive endoderm, chordamesoderm and neuroectoderm. *Development* 119, 567–578.
- Mutoh, H., Naya, F.J., Tsai, M.J., Leiter, A.B., 1998. The basic helix-loop-helix protein BETA2 interacts with p300 to coordinate differentiation of secretin-expressing enteroendocrine cells. *Genes Dev.* 12, 820–830.
- Naya, F.J., Stellrecht, C.M., Tsai, M.J., 1995. Tissue-specific regulation of the insulin gene by a novel basic helix-loop-helix transcription factor. *Genes Dev.* 9, 1009–1019.
- Naya, F.J., Huang, H.P., Qiu, Y., Mutoh, H., Demayo, F.J., Leiter, A.B., Tsai, M.J., 1997. Diabetes, defective pancreatic morphogenesis, and abnormal enteroendocrine differentiation in BETA2/neuroD-deficient mice. *Genes Dev.* 11, 2323–2334.
- Noguchi, T., Yamada, K., Yamagata, K., Takenaka, M., Nakajima, H., Imai, E., Wang, Z., Tanaka, T., 1991. Expression of liver type pyruvate kinase in insulinoma cells: involvement of LF-B1 (HNF1). *Biochem. Biophys. Res. Commun.* 181, 259–264.
- Obata, J., Yano, M., Mimura, H., Goto, T., Nakayama, R., Mibu, Y., Oka, C., Kawaichi, M., 2001. p48 subunit of mouse PTF1 binds to RBP-Jkappa/CBF-1, the intracellular mediator of Notch signalling, and is expressed in the neural tube of early stage embryos. *Genes Cells* 6, 345–360.
- Offield, M.F., Jetton, T.L., Labosky, P.A., Ray, M., Stein, R.W., Magnuson, M.A., Hogan, B.L., Wright, C.V., 1996. PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. *Development* 122, 983–995.
- Ohlsson, H., Karlsson, K., Edlund, T., 1993. IPF1, a homeodomain-containing transactivator of the insulin gene. *EMBO J.* 12, 4251–4259.
- Ohneda, K., Ee, H., German, M., 2000a. Regulation of insulin gene transcription. *Semin. Cell. Dev. Biol.* 11, 227–233.
- Ohneda, K., Mirmira, R.G., Wang, J., Johnson, J.D., German, M.S., 2000b. The homeodomain of PDX-1 mediates multiple protein–protein interactions in the formation of a transcriptional activation complex on the insulin promoter. *Mol. Cell. Biol.* 20, 900–911.
- Okada, Y., Taniguchi, H., Shimada, C., 1976. High concentration of GABA and high glutamate decarboxylase activity in rat pancreatic islets and human insulinoma. *Science* 194, 620–622.
- Olbrot, M., Rud, J., Moss, L.G., Sharma, A., 2002. Identification of beta-cell-specific insulin gene transcription factor RIPE3b1 as mammalian MafA. *Proc. Natl. Acad. Sci. USA* 99, 6737–6742.
- Oster, A., Jensen, J., Serup, P., Galante, P., Madsen, O.D., Larsson, L.I., 1998. Rat endocrine pancreatic development in relation to two homeobox gene products (Pdx-1 and Nkx 6.1). *J. Histochem. Cytochem.* 46, 707–715.
- Ott, M.O., Rey-Campos, J., Cereghini, S., Yaniv, M., 1991. vHNF1 is expressed in epithelial cells of distinct embryonic origin during development and precedes HNF1 expression. *Mech. Dev.* 36, 47–58.
- Peers, B., Sharma, S., Johnson, T., Kamps, M., Montminy, M., 1995. The Pancreatic islet factor STF-1 binds cooperatively with Pbx to a regulatory element in the somatostatin promoter: importance of the FPWMK motif and of the homeodomain. *Mol. Cell. Biol.* 15, 7091–7097.
- Perez-Villamil, B., Schwartz, P.T., Vallejo, M., 1999. The pancreatic homeodomain transcription factor IDX1/IPF1 is expressed in neural cells during brain development. *Endocrinology* 140, 3857–3860.
- Peshavaria, M., Gamer, L., Henderson, E., Teitelman, G., Wright, C.V.E., Stein, R., 1994. XIHbox 8, an endoderm-specific *Xenopus* homeodomain protein, is closely related to a mammalian insulin gene transcription factor. *Mol. Endocrinol.* 8, 806–816.
- Pfaff, S.L., Mendelsohn, M., Stewart, C.L., Edlund, T., Jessell, T.M., 1996. Requirement for LIM homeobox gene *Isl1* in motor neuron generation reveals a motor neuron-dependent step in interneuron differentiation. *Cell* 84, 309–320.
- Philippe, J., Morel, C., Prezioso, V.R., 1994. Glucagon gene expression is negatively regulated by hepatocyte nuclear factor 3 beta. *Mol. Cell. Biol.* 14, 3514–3523.
- Phippard, D., Heydemann, A., Lechner, M., Lu, L., Lee, D., Kyin, T., Crenshaw 3rd, E.B., 1998. Changes in the subcellular localization of the Brn4 gene product precede mesenchymal remodeling of the otic capsule. *Hear. Res.* 120, 77–85.
- Phippard, D., Lu, L., Lee, D., Saunders, J.C., Crenshaw 3rd, E.B., 1999. Targeted mutagenesis of the POU-domain gene *Brn4/Pou3f4* causes developmental defects in the inner ear. *J. Neurosci.* 19, 5980–5989.
- Pictet, R.L., Rall, L.B., Phelps, P., Rutter, W.J., 1976. The neural crest and the origin of the insulin-producing and other gastrointestinal hormone-producing cells. *Science* 191, 191–192.
- Pin, C.L., Bonvissuto, A.C., Konieczny, S.F., 2000. *Mist1* expression is a common link among serous exocrine cells exhibiting regulated exocytosis. *Anat. Rec.* 259, 157–167.
- Pin, C.L., Rukstalis, J.M., Johnson, C., Konieczny, S.F., 2001. The bHLH transcription factor *Mist1* is required to maintain exocrine pancreas cell organization and acinar cell identity. *J. Cell. Biol.* 155, 519–530.
- Planque, N., Leconte, L., Coquelle, F.M., Benkhelifa, S., Martin, P., Felder-Schmittbuhl, M.P., Saule, S., 2001. Interaction of Maf transcription factors with Pax-6 results in synergistic activation of the glucagon promoter. *J. Biol. Chem.* 276, 35751–35760.
- Pontoglio, M., Sreenan, S., Roe, M., Pugh, W., Ostrega, D., Doyen, A., Pick, A.J., Baldwin, A., Velho, G., Froguel, P., Levisetti, M., Bonner-Weir, S., Bell, G.I., Yaniv, M., Polonsky, K.S., 1998. Defective insulin secretion in hepatocyte nuclear factor 1alpha-deficient mice. *J. Clin. Invest.* 101, 2215–2222.
- Qiu, Y., Sharma, A., Stein, R., 1998. p300 mediates transcriptional stimulation by the basic helix-loop-helix activators of the insulin gene. *Mol. Cell. Biol.* 18, 2957–2964.
- Qiu, Y., Guo, M., Huang, S., Stein, R., 2002. Insulin gene transcription is mediated by interactions between the p300 coactivator and PDX-1, BETA2, and E47. *Mol. Cell. Biol.* 22, 412–420.
- Rausa, F., Samadani, U., Ye, H., Lim, L., Fletcher, C.F., Jenkins, N.A., Copeland, N.G., Costa, R.H., 1997. The cut-homeodomain transcriptional activator HNF-6 is coexpressed with its target gene HNF-3 beta in the developing murine liver and pancreas. *Dev. Biol.* 192, 228–246.
- Rausa, F.M., Ye, H., Lim, L., Duncan, S.A., Costa, R.H., 1998. In situ hybridization with 33P-labeled RNA probes for determination of cellular expression patterns of liver transcription factors in mouse embryos [published erratum appears in *Methods* 1998 Nov 16(3), 359–60]. *Methods* 16, 29–41.
- Rausa, F.M., Galarneau, L., Belanger, L., Costa, R.H., 1999. The nuclear receptor fetoprotein transcription factor is coexpressed with its target gene HNF-3beta in the developing murine liver, intestine and pancreas. *Mech. Dev.* 89, 185–188.
- Ritz-Laser, B., Estreicher, A., Gauthier, B., Philippe, J., 2000. The paired homeodomain transcription factor Pax-2 is expressed in the endocrine pancreas and transactivates the glucagon gene promoter. *J. Biol. Chem.* 275, 32708–32715.
- Rose, S.D., Swift, G.H., Peyton, M.J., Hammer, R.E., Macdonald, R.J., 2001. The role of PTF1-P48 in pancreatic acinar gene expression. *J. Biol. Chem.* 276, 44018–44026.
- Rudnick, A., Ling, T.Y., Odagiri, H., Rutter, W.J., German, M.S., 1994. Pancreatic beta cells express a diverse set of homeobox genes. *Proc. Natl. Acad. Sci. USA* 91, 12203–12207.
- Samadani, U., Costa, R.H., 1996. The transcriptional activator hepatocyte nuclear factor 6 regulates liver gene expression. *Mol. Cell. Biol.* 16, 6273–6284.
- Samaras, S.E., Cissell, M.A., Gerrish, K., Wright, C.V., Gannon, M., Stein, R., 2002. Conserved sequences in a tissue-specific regulatory region of the *pdx-1* gene mediate transcription in pancreatic beta cells: role for hepatocyte nuclear factor 3beta and Pax6. *Mol. Cell. Biol.* 22, 4702–4713.
- Sander, M., German, M.S., 1997. The beta cell transcription factors and development of the pancreas. *J. Mol. Med.* 75, 327–340.
- Sander, M., Neubuser, A., Kalamaras, J., Ee, H.C., Martin, G.R., German, M.S., 1997. Genetic analysis reveals that PAX6 is required for normal transcription of pancreatic hormone genes and islet development. *Genes Dev.* 11, 1662–1673.

- Sander, M., Sussel, L., Conners, J., Scheel, D., Kalamaras, J., Dela Cruz, F., Schwitzgebel, V., Hayes-Jordan, A., German, M., 2000. Homeobox gene *Nkx6.1* lies downstream of *Nkx2.2* in the major pathway of beta-cell formation in the pancreas. *Development* 127, 5533–5540.
- Schwartz, P.T., Perez-Villamil, B., Rivera, A., Moratalla, R., Vallejo, M., 2000. Pancreatic homeodomain transcription factor *IDX1/IPF1* expressed in developing brain regulates somatostatin gene transcription in embryonic neural cells. *J. Biol. Chem.* 275, 19106–19114.
- Schwitzgebel, V.M., 2001. Programming of the pancreas. *Mol. Cell. Endocrinol.* 185, 99–108.
- Schwitzgebel, V.M., Scheel, D.W., Conners, J.R., Kalamaras, J.R., Lee, J.E., Anderson, D.J., Sussel, L., Johnson, J.D., German, M.S., 2000. Expression of *neurogenin3* reveals an islet cell precursor population in the pancreas. *Development* 127, 3533–3542.
- Sharma, S., Jhala, U.S., Johnson, T., Ferreri, K., Leonard, J., Montminy, M., 1997. Hormonal regulation of an islet-specific enhancer in the pancreatic homeobox gene *STF-1*. *Mol. Cell. Biol.* 17, 2598–2604.
- Shih, D.Q., Stoffel, M., 2001. Dissecting the transcriptional network of pancreatic islets during development and differentiation. *Proc. Natl. Acad. Sci. USA* 98, 14189–14191.
- Shih, D.Q., Navas, M.A., Kuwajima, S., Duncan, S.A., Stoffel, M., 1999. Impaired glucose homeostasis and neonatal mortality in hepatocyte nuclear factor 3alpha-deficient mice. *Proc. Natl. Acad. Sci. USA* 96, 10152–10157.
- Shih, D.Q., Screenan, S., Munoz, K.N., Philipson, L., Pontoglio, M., Yaniv, M., Polonsky, K.S., Stoffel, M., 2001. Loss of *HNF-1alpha* function in mice leads to abnormal expression of genes involved in pancreatic islet development and metabolism. *Diabetes* 50, 2472–2480.
- Shimajiri, Y., Sanke, T., Furuta, H., Hanabusa, T., Nakagawa, T., Fujitani, Y., Kajimoto, Y., Takasu, N., Nanjo, K., 2001. A missense mutation of *Pax4* gene (*R121W*) is associated with type 2 diabetes in Japanese. *Diabetes* 50, 2864–2869.
- Shimomura, H., Sanke, T., Hanabusa, T., Tsunoda, K., Furuta, H., Nanjo, K., 2000. Nonsense mutation of *islet-1* gene (*Q310X*) found in a type 2 diabetic patient with a strong family history. *Diabetes* 49, 1597–1600.
- Slack, J.M., 1995. Developmental biology of the pancreas. *Development* 121, 1569–1580.
- Sladek, F.M., Zhong, W.M., Lai, E., Darnell Jr, J.E., 1990. Liver-enriched transcription factor *HNF-4* is a novel member of the steroid hormone receptor superfamily. *Genes Dev.* 4, 2353–2365.
- Smith, S., Watada, H., Scheel, D., Mrejen, C., German, M., 2000. Auto-regulation and maturity onset diabetes of the young transcription factors control the human *PAX4* promoter. *J. Biol. Chem.* 275, 36910–36919.
- Smith, S.B., Ee, H.C., Conners, J.R., German, M.S., 1999. Paired-homeodomain transcription factor *PAX4* acts as a transcriptional repressor in early pancreatic development. *Mol. Cell. Biol.* 19, 8272–8280.
- Sommer, L., Ma, Q., Anderson, D.J., 1996. Neurogenins, a novel family of atonal-related bHLH transcription factors, are putative mammalian neuronal determination genes that reveal progenitor cell heterogeneity in the developing CNS and PNS. *Mol. Cell. Neurosci.* 8, 221–241.
- Sosa-Pineda, B., Chowdhury, K., Torres, M., Oliver, G., Gruss, P., 1997. The *Pax4* gene is essential for differentiation of insulin-producing beta cells in the mammalian pancreas. *Nature* 386, 399–402.
- St-Onge, L., Sosa-Pineda, B., Chowdhury, K., Mansouri, A., Gruss, P., 1997. *Pax6* is required for differentiation of glucagon-producing alpha-cells in mouse pancreas. *Nature* 387, 406–409.
- Stoffers, D.A., Ferrer, J., Clarke, W.L., Habener, J.F., 1997a. Early-onset type-II diabetes mellitus (*MODY4*) linked to *IPF1* [letter]. *Nat. Genet.* 17, 138–139.
- Stoffers, D.A., Zinkin, N.T., Stanojevic, V., Clarke, W.L., Habener, J.F., 1997b. Pancreatic agenesis attributable to a single nucleotide deletion in the human *IPF1* gene coding sequence. *Nat. Genet.* 15, 106–110.
- Stoffers, D.A., Heller, R.S., Miller, C.P., Habener, J.F., 1999. Developmental expression of the homeodomain protein *IDX-1* in mice transgenic for an *IDX-1* promoter/*lacZ* transcriptional reporter. *Endocrinology* 140, 5374–5381.
- Sund, N.J., Vatamaniuk, M.Z., Casey, M., Ang, S.L., Magnuson, M.A., Stoffers, D.A., Matschinsky, F.M., Kaestner, K.H., 2001. Tissue-specific deletion of *Foxa2* in pancreatic beta cells results in hyperinsulinemic hypoglycemia. *Genes Dev.* 15, 1706–1715.
- Sussel, L., Kalamaras, J., Hartigan-O'Connor, D.J., Meneses, J.J., Pedersen, R.A., Rubenstein, J.L., German, M.S., 1998. Mice lacking the homeodomain transcription factor *Nkx2.2* have diabetes due to arrested differentiation of pancreatic beta cells. *Development* 125, 2213–2221.
- Taraviras, S., Monaghan, A.P., Schutz, G., Kelsey, G., 1994. Characterization of the mouse *HNF-4* gene and its expression during mouse embryogenesis. *Mech. Dev.* 48, 67–79.
- Teitelman, G., Joh, T.H., Reis, D.J., 1981. Transformation of catecholaminergic precursors into glucagon (A) cells in mouse embryonic pancreas. *Proc. Natl. Acad. Sci. USA* 78, 5225–5229.
- Teitelman, G., Alpert, S., Polak, J.M., Martinez, A., Hanahan, D., 1993. Precursor cells of mouse endocrine pancreas coexpress insulin, glucagon and the neuronal proteins tyrosine hydroxylase and neuropeptide Y, but not pancreatic polypeptide. *Development* 118, 1031–1039.
- Thor, S., Ericson, J., Brannstrom, T., Edlund, T., 1991. The homeodomain LIM protein *isl-1* is expressed in subsets of neurons and endocrine cells in the adult rat. *Neuron* 7, 1–9.
- Torres, M., Gómez-Pardo, E., Gruss, P., 1996. *Pax2* contributes to inner ear patterning and optic nerve trajectory. *Development* 122, 3381–3391.
- Vallejo, M., PENCHUK, L., Habener, J.F., 1992. Somatostatin gene upstream enhancer element activated by a protein complex consisting of CREB, *Isl-1*-like, and alpha-CBF-like transcription factors. *J. Biol. Chem.* 267, 12876–12884.
- Waeber, G., Thompson, N., Nicod, P., Bonny, C., 1996. Transcriptional activation of the *GLUT2* gene by the *IPF-1/STF-1/IDX-1* homeobox factor. *Mol. Endocrinol.* 10, 1327–1334.
- Wang, M., Drucker, D.J., 1995. The LIM domain homeobox gene *isl-1* is a positive regulator of islet cell-specific proglucagon gene transcription. *J. Biol. Chem.* 270, 12646–12652.
- Watada, H., Kajimoto, Y., Kaneto, H., Matsuoka, T., Fujitani, Y., Miyazaki, J., Yamasaki, Y., 1996a. Involvement of the homeodomain-containing transcription factor *PDX-1* in islet amyloid polypeptide gene transcription. *Biochem. Biophys. Res. Commun.* 229, 746–751.
- Watada, H., Kajimoto, Y., Miyagawa, J., Hanafusa, T., Hamaguchi, K., Matsuoka, T., Yamamoto, K., Matsuzawa, Y., Kawamori, R., Yamasaki, Y., 1996b. *PDX-1* induces insulin and glucokinase gene expressions in *AlphaTC1* clone 6 cells in the presence of betacellulin. *Diabetes* 45, 1826–1831.
- Watada, H., Kajimoto, Y., Umayahara, Y., Matsuoka, T., Kaneto, H., Fujitani, Y., Kamada, T., Kawamori, R., Yamasaki, Y., 1996c. The human glucokinase gene beta-cell-type promoter: an essential role of insulin promoter factor 1/*PDX-1* in its activation in *HIT-T15* cells. *Diabetes* 45, 1478–1488.
- Watada, H., Mirmira, R.G., Leung, J., German, M.S., 2000. Transcriptional and translational regulation of beta-cell differentiation factor *Nkx6.1*. *J. Biol. Chem.* 275, 34224–34230.
- Weinstein, D.C., Ruiz I Altaba, A., Chen, W.S., Hoodless, P., Prezioso, V.R., Jessell, T.M., Darnell Jr, J.E., 1994. The winged-helix transcription factor *HNF-3 beta* is required for notochord development in the mouse embryo. *Cell* 78, 575–588.
- Wessells, N.K., Cohen, J.H., 1967. Early pancreas organogenesis: morphogenesis, tissue interactions and mass effects. *Dev. Biol.* 15, 237–270.
- Wilson, M.E., Kalamaras, J.A., German, M.S., 2002. Expression pattern of *IAPP* and prohormone convertase 1/3 reveals a distinctive set of endocrine cells in the embryonic pancreas. *Mech. Dev.* 115, 171–176.
- Wu, K.L., Gannon, M., Peshavaria, M., Offield, M.F., Henderson, E., Ray, M., Marks, A., Gamer, L.W., Wright, C.V., Stein, R., 1997. Hepatocyte nuclear factor 3beta is involved in pancreatic beta-cell-specific transcription of the *pdx-1* gene. *Mol. Cell. Biol.* 17, 6002–6013.
- Yamagata, K., Furuta, H., Oda, N., Kaisaki, P.J., Menzel, S., Cox, N.J., Fajans, S.S., Signorini, S., Stoffel, M., Bell, G.I., 1996a. Mutations in the hepatocyte nuclear factor-4alpha gene in maturity-onset diabetes of the young (*MODY1*). *Nature* 384, 458–460.

- Yamagata, K., Oda, N., Kaisaki, P., Menzel, S., Furuta, H., Vaxillaire, S., Southam, L., Cox, R., Lathrop, G., Boriraj, V., Chen, X., Cox, N., Oda, Y., Yano, H., Le Beau, M., Yamada, S., Nishigori, H., Takada, J., Fajans, S., Hattersley, A., Iwasaki, N., Hansen, T., Pedersen, O., Polonsky, K., Turner, R., Velho, G., Chevre, J., Froguel, P., Bell, G., 1996b. Mutations in the hepatocyte nuclear factor-1alpha gene in maturity-onset diabetes of the young (MODY3). *Nature* 384, 455–458.
- Yee, N.S., Yusuff, S., Pack, M., 2001. Zebrafish *pdx1* morphant displays defects in pancreas development and digestive organ chirality, and potentially identifies a multipotent pancreas progenitor cell. *Genesis* 30, 137–140.
- Zaret, K.S., 1996. Molecular genetics of early liver development. *Annu. Rev. Physiol.* 58, 231–251.
- Zaret, K., 1999. Developmental competence of the gut endoderm: genetic potentiation by GATA and HNF3/fork head proteins. *Dev. Biol.* 209, 1–10.