Diabetologia (2001) 44: 1071–1079

Diabetologia

Reviews

Factors controlling pancreatic cell differentiation and function

H. Edlund

Umeå Centre for Molecular Medicine and Dept. of Molecular Biology, University of Umea, SE-901 87 Umea, Sweden

Abstract

Diabetes affects 4 to 5 % of the population worldwide and is the most common metabolic disorder. The number of individuals diagnosed with diabetes is rapidly increasing, especially in the developed countries and the disorder frequently leads to secondary complications such as retinopathy, nephropathy, neuropathy and cardiovascular disease. Type II (non-insulin-dependent) diabetes mellitus is the most common form of diabetes, more than 90 % of diagnosed cases, and results from insulin resistance, pancreatic beta-cell dysfunction, or a combination of both. The beta-cell dysfunction seems to result in part from an inability of the beta cells to produce and secrete sufficient amounts of active insulin in response to an increased demand for insulin. Type I (insulin-dependent) diabetes mellitus is caused by an autoimmune destruction of the insulin producing beta cells, resulting in insulin deficiency. The existing therapies for both types of diabetes are unsatisfactory since they do not offer a cure and are mostly not sufficient for preventing the secondary complications associated with diabetes. Thus, there is a great need for new improved therapies. This search is, however, hampered by our currently limited knowledge of the basic processes that control the proliferation, differentiation, survival and physiology of the beta cell. Over the last 7 to 8 years our knowledge concerning the development of the pancreas has increased substantially due to the use of genetically modified mice. Nevertheless, key questions regarding the control of proliferation and differentiation of pancreatic progenitor cells into fully functional beta cells remain to be solved. [Diabetologia (2001) 44: 1071–1079]

Keywords Dorsal and ventral pancreas, notochord, lateral inhibition, notch-signalling, Type II diabetes, beta-cell dysfunction, proinsulin, PC1/3, Glut2, FGF-signalling, *Ipf1/Pdx1*.

Pancreatic islet cell transplantation is a promising approach to restore the required mass of functional beta cells in diabetic patients as a mean to achieve long-term normoglycaemia. This therapy is, however, not yet widely used because of the shortage of human islet cells and problems of viability and rejection of grafted cells. The search for new, improved therapies for diabetes could be crucially enhanced by a profound knowledge of a number of fundamental questions concerning beta-cell proliferation, differentiation, survival and physiology. One attractive approach towards a cure for diabetes involves the generation of functional beta cells from stem and/or progenitor cells. The prospect of such a scenario will require three important prerequisites, i. e. the identification of (i) pancreatic stem or progenitor cells that have the capacity to self-renew and to generate differ-

Received: 3 April 2001 and in revised form 22 May 2001

Corresponding author: Helena Edlund, Umeå Center for Molecular Medicine and Dept. of Molecular Biology, University of Umea, SE-901 87 Umea, Sweden, E-mail: Helena.Edlund@micro.umu.se

Abbreviations: bHLH, Basic-Helix-loop-Helix; e, embryonic day; dn, dominant-negative; FGF, fibroblast growth factor; FGFR, fibroblast growth receptor; HES, hairy-and-Enhancer-of-split; Hlxb9, homeobox gene Hb9; Ihh, Indian hedgehog; Ipf1, insulin promoter factor 1; ngn, neurogenin; Pdx1, pancreatic duodenal homeobox gene 1; Shh, sonic hedgehog; Glut2, glucose transporter type 2; PC, prohormone convertase

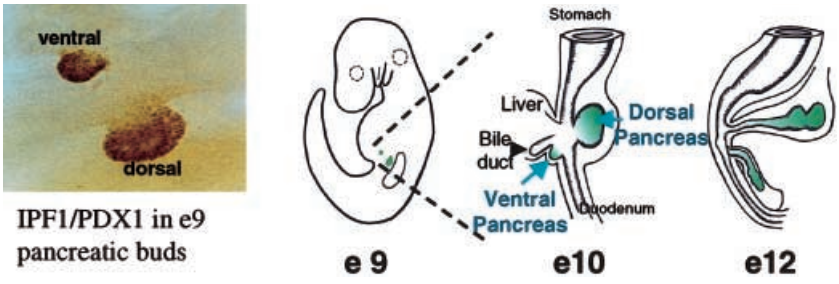

Fig. 1. Pancreatic development commences with the evagination of the gut endoderm to give rise to the dorsal and ventral pancreatic buds. IPF1/PDX1 is one of the earliest markers defining the pancreatic region of the gut endoderm during these early stages. In mice the dorsal and ventral pancreatic buds become apparent on embryonic day (e) 9. The two buds will grow, branch and eventually fuse around e13–14 to form the definitive pancreas [5]

entiated progeny, (ii) proliferative signals that can expand specified pancreatic progenitor cells, (iii) instructive signals that can induce the differentiation of these stem and/or progenitor cells into functional beta cells that secrete fully processed insulin in a pulsatile manner in response to physiological concentrations of glucose. The current interest in pancreatic stem cells is unfortunately not matched by our existing knowledge of the processes that normally operate during embryogenesis to control the proliferation, specification, commitment and differentiation of pancreatic progenitor cells. Our views on stem cells have changed dramatically during the last years and stem cells are found in many different organs of adult animals and can be manipulated to differentiate and to generate cells different from those of the organ they initially originate from [1–3]. To make use of the full potential of stem cells, the molecular mechanisms that keep cells undifferentiated and the factors that allow expansion of stem cells need to be identified. We need to understand how stem cells become specified and committed for particular fates, and the mechanism by which committed cells terminally differentiate into functional cell types. The generality of these mechanisms among different stem cell populations also needs to be elucidated. This information should provide a critical basis for efforts to recapitulate these events in the diseased organ, and/or ex vivo, with the generation of functional cell types as the ultimate goal. Several recent reviews on pancreas development have extensively discussed the role that various transcription and intercellular factors play during pancreatic development [4–9]. Hence, this review will not in detail discuss all these factors but rather a selected number of intrinsic and extrinsic factors controlling beta-cell differentiation and function.

Initiation of the pancreatic program

Pancreatic cell types derive from endodermal cells of the upper, duodenal, region of the foregut [10, 11] and the development of the pancreas begins with the dorsal and ventral protrusion of a region of the primitive gut epithelium [5, 7] (Fig. 1). The initiation of the pancreatic program critically requires that signals specify the pancreatic region within the developing gut endoderm [5, 7, 9]. One important component of this early specification of the pancreatic program within a region of the gut endoderm involves the exclusion of the hedgehog gene family of signalling molecules, *Sonic hedgehog* (*Shh*) and *Indian hedgehog* (*Ihh*), since these factors promote an intestinal differentiation program on the expense of pancreatic development [12, 13]. At the dorsal site, factors emanating from the notochord have been suggested to ensure that the expression of the *hh* genes is prohibited [14–16]. It is, however, plausible to assume that, in addition, the endoderm is already patterned so that only the presumptive pancreatic regions respond to factors secreted from the notochord by suppressing the expression of *shh* and *ihh*. Candidate factors capable of, at least in vitro, suppressing the expression of the *hh* genes are activin-βB and fibroblast growth factor (FGF) 2 [17]. However, this is complicated by the parallel development of the ventral pancreatic primordium. At this site there is no notochord equivalent and instead the ventral pancreatic anlage develops in close proximity to the developing heart and liver (Fig. 1). In vitro cultures of early ventral foregut endoderm under different culture conditions suggest that FGFs secreted from the embryonic heart promote hepatocyte differentiation on the expense of ventral pancreatic initiation [17]. Thus in this context FGFs inhibit rather than promote a pancreatic program. A number of additional differences exist between the development of the dorsal and ventral pancreatic buds. The mesenchyme surrounding the dorsal pancreatic epithelium expresses the LIM homeodomain protein Isl1 and this mesenchyme is lost in the *Isl1* -/- mice [18]. The loss of dorsal mesenchyme in turn obstructs dorsal pancreatic development in these mutants whereas the ventral bud, which is surrounded by mesenchyme that do not normally express Isl1, still can go on and give rise to exocrine, but not endocrine cell types [18]. Another striking difference between the dorsal and

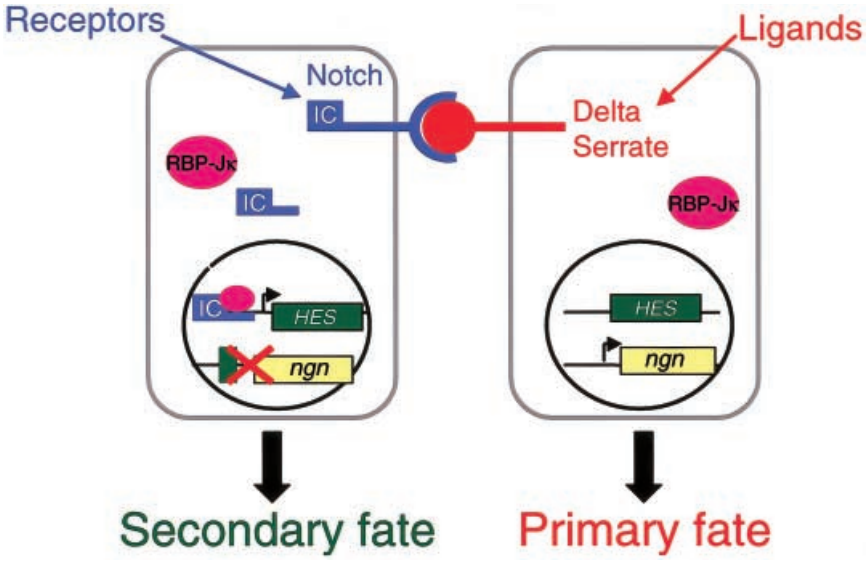

Fig. 2. Notch-signalling involves direct cell to cell signalling between adjacent cells via membrane-bound receptors, encoded by the *Notch* genes, and membrane-bound ligands, encoded by the *Delta* and *Serrate* genes. Ligand-receptor activation results in a processing of the receptor so that the intracellular (IC) part is cleaved off and activated. The IC part of Notch acts via the RPB-J$\varkappa$ transcription factor to activate expression of the *Hes* genes in the receiving cell. The *Hes* genes encode bHLH transcriptional repressors that repress the expression of primary fate genes like the *ngn* genes. In the receiving cell primary fate (*ngn*) gene expression is repressed via Notch-activation, whereas the signalling cell is free to express primary fate (*ngn)* genes and differentiate accordingly

ventral pancreatic bud is illustrated by the phenotype in mice defect in the homeobox gene *Hlxb9*, encoding Hb9 [19–21]. Hb9 is transiently expressed both at the dorsal and ventral sites at early stages and at later stages Hb9, similar to the homeodomain transcription factor IPF1/PDX1, reappears but is then restricted to the insulin-producing beta cells [20, 21]. In *Hlxb9* mutant mice dorsal pancreatic development is blocked whereas, in contrast, the ventral pancreas develops and contains both endocrine and exocrine cells [20, 21]. Thus, the requirement for *Hlxb9* in pancreatic development reveals a molecular distinction in the dorsal and ventral differentiation programs. No link has yet been found between *Hlxb9* and pancreatic disorders in humans. Nevertheless, several studies describing cases showing either a selective dorsal or ventral apancreatic phenotype have been reported in the literature but the cause underlying these abnormalities are presently not known [22, 23].

Specification of pancreatic cell fate

Lateral specification mediated by the Notch signalling pathway is a classic way of specifying a particular cell fate within a field of initially equivalent cells [24–26]. Notch-signalling involves cells expressing high quantities of the ligands (Delta or Serrate) that signal to activate Notch receptors on neighbouring cells in which the activated, intracellular Notch receptor (NIC) suppress the primary cell fate, i.e. the cell fate adopted by the signalling cell (Fig. 2). In the Notch-signalling pathway NIC interacts with the DNA-binding protein RBP-J$\varkappa$ to activate expression of bHLH repressor genes, i.e. the *Hes* genes, which in turn repress expression of downstream target genes which if expressed would promote the primary cell fate [24–26].

Endocrine cells of the pancreas appear in a scattered manner in the early pancreatic anlagen (Fig. 3). Analyses of mice genetically altered at several steps in the Notch signalling pathway have shown that, similar to the generation of neurons during neurogenesis, the endocrine cells of the pancreas are specified by lateral specification mediated by the Notch signalling pathway [27, 28]. Mice deficient for the genes *Delta-like gene 1 (Dll1),* intracellular mediator *RBP-J$\varkappa$* or the bHLH repressor *Hes1*, all show accelerated differentiation of pancreatic endocrine cells paralleled by a depletion of the pool pancreatic precursor cells [27, 28]. In addition a similar phenotype was observed in mice over-expressing *ngn3* or the intracellular form of *Notch3*, which act as a repressor of Notch signalling [27]. In contrast, mice lacking a functional *ngn3* gene completely lack pancreatic endocrine cells although a grossly normal pancreas with exocrine cells still develops in these mutants [29]. Together these studies collectively demonstrate that Notch signalling controls the choice between differentiated endocrine and progenitor cell fates in the developing pancreas, and that a block in the activation of the Notch receptor, resulting in high *ngn 3* gene expression, promotes the endocrine fate (Fig. 3). In contrast, cells with active Notch-sig-

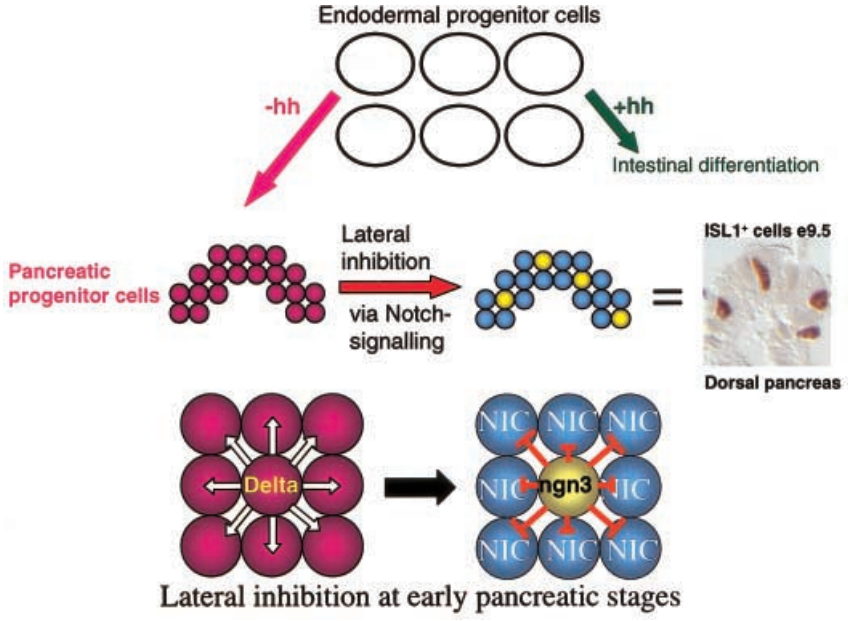

Fig. 3. The hh signalling molecules are expressed throughout the primitive gut endoderm except at the regions destined to become the pancreas. *hh* gene expression promotes intestinal differentiation but impairs pancreatic development. Among the pool of early pancreatic stem cells, differentiated endocrine cells, here illustrated by the expression of the pancreatic endocrine marker ISL1 in an e9.5 dorsal bud, appear in a scattered manner. The specification of pancreatic endocrine cells within the pool of equivalent progenitor cells is mediated via lateral inhibition through the notch-signalling pathway where ngn3 acts as the proendocrine gene

nalling adopt the exocrine fate and/or remain as undifferentiated progenitor cells which would allow the subsequent proliferation, morphogenesis and differentiation of the pancreatic epithelial cells analogous to the function of Notch-signalling during early mammalian neurogenesis [24–26].

Throughout pancreatic development the endocrine cells delineate from the epithelium upon differentiation and migrate into the adjacent mesenchyme where they cluster. This migration is likely to result in a decrease in Notch signalling (i.e. lateral inhibition) among progenitor cell and in addition allows the formation of islet structures. This would consequently permit a continued appearance of cells with a primary (endocrine) fate that can respond to later appearing inductive signals and thus generate distinct endocrine cells throughout the development of the pancreas depending on the inductive milieu. Progenitor cells that are not singled out to become endocrine cells will subsequently differentiate to become either acinar or ductal cells. Alternatively, the existence of several receptors and ligands of the Notch-pathway which are expressed in different temporal patterns during pancreatic development [27, 30] could suggest that Notch-signalling in the pancreas is complex and that depending on the type of ligand-receptor interaction different endocrine cell types could be generated. Other components that could also modulate Notch-signalling involves the *presenilin* genes *1* and *2* and *Sel-1* [31–33]. Presenilins seem to be involved in the processing of the Notch-receptor to the active, intra-cellular form [31, 32] and Sel-1 seems to antagonise Notch-signalling, presumably by participating in the degradation of Notch [33].

FGF-signalling and diabetes

Type II (non-insulin-dependent) diabetes mellitus is the most common form of diabetes, affecting about 4% of the population worldwide. Type II diabetes results from insulin resistance, beta-cell dysfunction, or a combination of both, which can lead to secondary complications such as retinopathy, nephropathy, neuropathy and cardiovascular disease [34, 35]. The beta-cell dysfunction seems to result in part from an inability of the beta cells to produce and secrete sufficient amounts of active insulin in response to an increased demand for insulin [34, 35]. Type II diabetic patients often, but not always, show a reduced number of beta cells compared to weight-matched, non-diabetic subjects, which could suggest that diabetic patients have fewer beta cells prior to onset of disease and/or fail to generate more beta cells in response to an increased demand for insulin [36–40]. The beta cells of many Type II diabetic patients have a perturbed glucose stimulation of insulin release, resulting in an impaired glucose tolerance in these patients, a condition that usually precedes the onset of the disease [34, 35, 41–43]. In fasting states the beta cell stores insulin and C-peptide within storage granules in readiness

for quick first phase release of insulin after stimulation by the uptake of glucose via the low-affinity glucose transporter type 2, Glut2. Glucose, which enters the cell, becomes phosphorylated by glucokinase and metabolism of glucose leads to an increase in the ATP:ADP ratio. The rise in the ATP:ADP ratio blocks the ATP-sensitive K^+ATP transmembrane channel, resulting in membrane depolarisation and subsequent activation of the voltage gated Ca^{2+}-channel. The following influx of Ca^{2+} then stimulates exocytic release of insulin and C-peptide into the bloodstream.

Another prominent feature of diabetic patients is an increased ratio of circulating proinsulin to insulin (P:I) compared to healthy subjects [42, 43]. Several independent studies have identified hyperproinsulinaemia as a risk factor for the progression to overt diabetes in patients showing an impaired glucose tolerance [44–51]. Thus an increased P:I ratio has been associated with a rapid conversion from a prediabetic to an overt diabetic state, irrespective of insulin resistance. Together these findings suggest that a defect in the processing of proinsulin to active insulin in the beta cells could be a direct contributing factor for the development of the disease [44–51]. Processing of proinsulin to insulin in beta cells is catalysed by the sequential actions of prohormone convertases PC1/3 and PC2, which act in concert with carboxypeptidase E (CPE). PC1/3 appears more important than PC2 in the processing of proinsulin to active insulin because inactivation of *PC2* does not result in a diabetic phenotype [52]. *PC2* plays, however, a crucial part in the processing of proglucagon and prosomatostatin in α- and δ- cells [52, 53]. In addition, a patient with a compound heterozygote defect in the *PC1/3* gene showed increased plasma proinsulin contents and perturbed glucose homeostasis [54], further emphasising the critical role of *PC1/3* in proinsulin processing.

The increased P:I ratio in Type II diabetic patients suggests that proinsulin processing to mature insulin is perturbed, implying an impaired expression or activity of prohormone convertases 1/3 and 2 in the beta cells of these patients. Until now no uniform molecular mechanism that provides an explanation for these beta-cell dysfunctions in Type II diabetic patients has been proposed.

Recent findings provide the first insights into the nature of a signalling pathway that seems to control several key aspects of beta-cell functions. Dominant-negative approaches used in transgenic mice as a way of exploring the function of FGF-signalling in pancreas links the FGF-pathway to beta-cell function [55]. FGF-signalling involves binding soluble ligands, fibroblast growth factors (FGFs), to the extra-cellular domain of high affinity membrane-bound FGF receptors (FGFRs), which belongs to the tyrosine kinase family of receptors [56, 57]. A large number of *Fgfs* are expressed in the developing mouse embryo and gene inactivation approaches to elucidate the role of FGF-signalling in the mouse have been hampered by early embryonic lethality or functional redundancy [56, 57]. An alternative genetic approach has been to attenuate FGF-signalling via expression of dominant negative (dn) forms of fibroblast growth factor receptors (FGFRs) that will bind to and block the interaction of FGFs with the normal receptor. This approach has been used successfully to antagonise FGF-signalling in a number of different tissues and organs [58–61].

In the adult pancreas FGFR1 and 2, along with the ligands FGF1, FGF2, FGF4, FGF5, FGF7 and FGF10 are all selectively expressed in beta cells [55]. To elucidate a role for FGF-signalling in the genesis and/or function of beta cells two transgenic mouse lines which expressed a dominant negative (dn) version of *FGFR1 c* [62], denoted FRID1, or *FGFR2 b* [62, 63], denoted FRIND2, in the developing pancreas under the control of the *Ipf1/Pdx1* promoter were generated [55]. Both FRID1 and FRIND2 mice were normal and appeared initially healthy but around 15 weeks of age the FRID1 mice developed diabetes with non-fasting blood glucose concentrations of 26.4 mmol/l ± 1.5 and fasting blood glucose concentrations of 15.8 mmol ± 1.3 [55]. In contrast the FRIND2 mice do not develop diabetes. Analyses of factors controlling key aspects of beta-cell function reveal two significant beta-cell defects in the FRID1 mice that together are likely to underlie the development of diabetes in the FRID1 mice. First, there is an impaired beta-cell expression of the glucose transporter type 2, which would result in an impaired glucose sensing. Secondly, there is a drastically reduced expression of PC1/3 in the beta cells, which most likely would impair insulin processing. Although a direct role for Glut2 in maintaining normoglycaemia in human beta cells has not been proven, Glut2 is required for maintenance of normoglycaemia in mice and hence *Glut2* null mutant mice develop early diabetes due to an impairment of glucose stimulated first, but not second, phase insulin secretion [64]. The beta-cell dysfunction of the *Glut2* mutant, and thus the early diabetes, could be rescued by expressing either Glut2 or Glut1 specifically in beta cells of those mutant mice [65]. Hence, there is direct genetic evidence on the requirement of Glut2 in maintaining glucose homeostasis in mice [64, 65]. The impaired expression of PC1/3 in beta cells of FRID1 mice appears to result in perturbed processing of proinsulin to active insulin since the beta cells of these mice were uncharacteristically loaded with proinsulin as compared to control beta cells [55]. The accumulation of proinsulin in the beta cells of FRID1 mice is strikingly similar to the increased content of proinsulin observed in beta cells of Type II diabetic patients [40]. Together these observations imply that the increased proinsu-

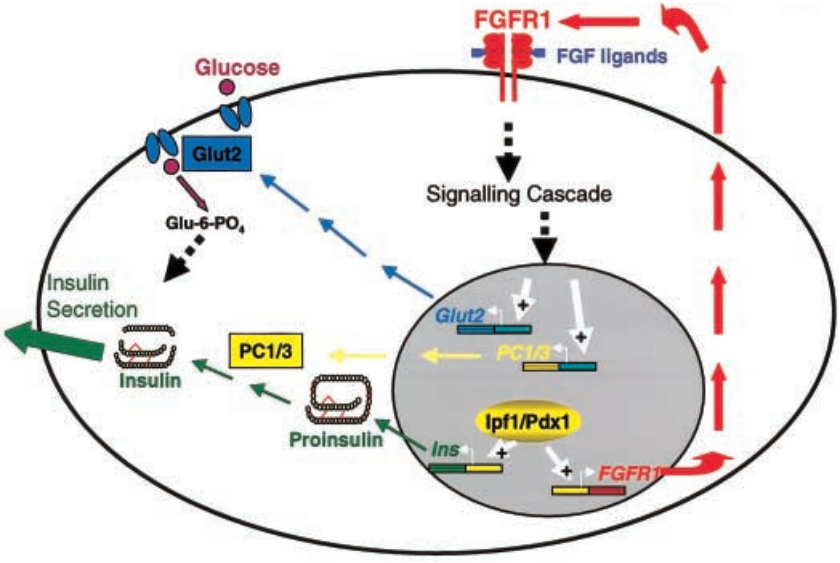

Fig. 4. In the adult mouse beta cell Ipf1/Pdx1 ensures high expression of insulin and FGFR1. Signalling via FGFR1 in the beta cell is in turn required to ensure normal expression of key components in glucose sensing (Glut2) and insulin processing machinery (PC1/3 and PC2) and thus to maintain normoglycaemia. Hence, attenuation of FGFR1-signalling in mouse β-cells leads to diabetes due to perturbation of glucose sensing and proinsulin processing. For further details please see the text

lin contents observed for many Type II diabetic patients could result from a beta-cell dysfunction involving impaired expression or activity of PC1/3.

The FRID1 mice also show a reduced number of beta cells compared with normal littermates [55]. In mice beta-cell neogenesis normally proceeds up to about 3 weeks of age in mice [66, 67] and the impaired post-natal expansion of beta-cell number observed in the FRID1 mice implies a role for FGFR1c-signalling in this process. Beta-cell hyperplasia has been observed in both humans and rodents under conditions such as pregnancy and non-diabetic obesity when there is an increased demand for insulin, showing the compensatory capacity of the beta cell [68–70]. The reduced number of beta cells in 10-week old, overt diabetic FRID1 mice as compared to control littermates indicate that FGFR1c-signalling is required for maintaining the competence of beta cells to proliferate in response to hyperglycaemia. Infants with low birth weight have been reported to have relatively fewer beta cells [71] and moreover reduced growth in early life has been linked to impaired glucose tolerance [72]. Together these observations indicate a nutritional and/or growth factor stimulus on beta cell expansion and function. Moreover an impairment of this stimulation could result in an apparent beta cell dysfunction that predisposes the affected subject to Type II diabetes. In light of the perturbed expansion of beta cells as observed in the FRID1 mice [55], signalling via FGFR1c might represent one such key stimulatory pathway for beta-cell expansion.

In humans, heterozygosity for a nonsense mutation in the *Ipf1* gene, which results in a dominant negative frameshift, has been linked to MODY 4 [73], a monogenetic form of diabetes that results from beta-cell dysfunction rather than insulin resistance. Moreover, missense mutations in the human *Ipf1* gene are implicated in predisposing subjects to Type II diabetes [74, 75]. Previous work has shown that *Ipf1/Pdx1* is required for ensuring normal amounts of insulin and Glut2 expression [76, 77]. In addition there is now genetic evidence suggesting that *Ipf1/Pd × 1* acts upstream of FGF-signalling in the beta cell, since genetic inactivation of *Ipf1/Pdx1* in beta cells, as in the *RIP1/Ipf1*$^{\Delta}$ mice [77], leads to reduced expression of FGFR1 and the ligands FGF1, FGF2, FGF4 and FGF5 [55]. Consequently, the *RIP1/Ipf1*$^{\Delta}$ mice also show reduced expression of PC1/3 paralleled by an increase in proinsulin in the beta cells of these mice [55].

The phenotypes observed in the FRID1 mice; (i) reduced beta-cell number, (ii) impaired glucose sensing, a consequence of loss of Glut2 expression; (iii) perturbed proinsulin processing due to the down regulation of prohormone convertase 1/3 and 2 expression, are reflective of the beta-cell dysfunction associated with Type II diabetic patients. These observations suggest that signalling via FGFR1c could represent one factor required for beta-cell expansion during early life and in response to hyperglycaemia. Moreover these data provide evidence that FGFR1c-signalling in the beta cell is required to ensure normal expression of key components in glucose sensing (i.e. Glut 2 in mice) and insulin processing machinery (PC1/3 and PC2) and thus to maintain normoglycaemia. The analyses of the *RIP1/Ipf1*$^{\Delta}$ mice

provide genetic evidence that the Ipf1/Pdx1 transcription factor acts upstream of FGFR1-signalling in controlling key aspects of beta-cell identity (Fig. 4). The apparent conservation of *Ipf1/Pdx1* gene function from mice to humans suggests that also the downstream effects controlled by *Ipf1/Pdx1* gene activity could be conserved. Thus it is plausible that analogous to the mouse, FGF-signalling will be important for beta-cell function also in humans and that perturbation of this signalling pathway in adult human beta cells could be linked to Type II diabetes. Whether attenuation of FGF signalling contributes to diabetes in humans has yet to be determined.

Conclusion

Studies focusing on the role of transcription factors in pancreatic development and beta-cell function provide evidence for a strong conservation in the function of these genes in mice and humans [4–6]. Recent insights in the role of growth factors and inductive signals operating during the different stages of pancreas development and beta-cell function are likely to unravel a similar conserved role for these factors as well [7–9]. The understanding of how these latter types of factor control different aspects of pancreas development and function will be critical not only for our understanding of pancreatic disorders such as diabetes and cancer, but also for the prospect of establishing a system where beta cells might be selectively induced and amplified in vivo or ex vivo. In addition, the full characterisation of the different pathways by which these various extrinsic factors operate are likely to identify novel therapeutical targets for the treatment of beta-cell dysfunctions linked to diabetes.

Sources. This review is based on our own published work, other relevant literature published in the English language during 1990 to 2001, and appropriate prior contributions. The sources available to the author were integrated with sources identified through PubMed searches for "pancreas development", "hyperproinsulinaemia and diabetes" and FGF-signalling.

Acknowledgements. Work in my laboratory is supported by the Juvenile Diabetes Foundation, Swedish Research Council, and the European Union. I wish to thank Dr. Thomas Edlund, Dr. Alan Hart and members from my laboratory for their helpful discussions.

References

1. Clarke DL, Johansson CB, Wilbertz J et al. (2000) Generalized potential of adult neural stem cells. Science 288: 1660–1663
2. Brazelton TR, Rossi FM, Keshet GI, Blau HM (2000) From marrow to brain: expression of neuronal phenotypes in adult mice. Science 290: 1775–1779
3. Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR (2000) Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. Science 290: 1779–1782
4. Sander M, German MS (1997) The β cell transcription factors and the development of the pancreas. J Mol Med 75: 327–340
5. Edlund H (1998) Transcribing pancreas. Diabetes 47: 1817–1823
6. St-Ogne L, Wehr R, Gruss P (1999) Pancreas development and diabetes. Curr Opin Genet Dev 9: 295–300
7. Edlund H (1999) Pancreas: how to get there from the gut? Curr Opin Cell Biol 11: 663–668
8. Edlund H (2001) Developmental biology of the pancreas. Diabetes 50: S5–S9
9. Kim SK, Hebrok M (2001) Intercellular signals regulating pancreas development and function. Genes Dev 15: 111–127
10. Pictet RL, Rall LB, Phelps P, Rutter WJ (1976) The neural crest and the origin of the insulin-producing and other gastrointestinal hormone producing cells. Science 191: 191–192
11. Fontaine J, Le Douarin NM (1977) Analysis of endoderm formation in the avian blastoderm by the use of quail-chick chimaeras. The problem of the neurectodermal origin of the cells of the APUD series. J Embryol Exp Morphol 41: 209–222
12. Bitgood MJ, McMahon AP (1995) Hedgehog and Bmp genes are coexpressed at many diverse sites of cell-cell interaction in the mouse embryo. Dev Biol 172: 126–138
13. Apelqvist Å, Ahlgren U, Edlund H (1997) Sonic hedgehog directs specialised mesoderm differentiation in the intestine and pancreas. Curr Biol 7: 801–804
14. Kim SK, Hebrok M, Melton DA (1997) Notochord to endoderm signalling is required for pancreas development. Development 124: 4243–4252
15. Hebrok M, Kim SK, Melton DA (1998) Notochord repression of endodermal Sonic hedgehog permits pancreas development. Genes Dev 12: 1705–1713
16. Kim SK, Melton, DA (1998) Pancreas development is promoted by cyclopamine, a Hedgehog signaling inhibitor. Proc Natl Acad Sci USA 95: 13036–13041
17. Deutsch G, Jung J, Zheng M, Lora J, Zaret KS (2001) A bipotential precursor population for pancreas and liver within the embryonic endoderm. Development 128: 871–881
18. Ahlgren U, Pfaff S, Jessel TM, Edlund T, Edlund H (1997). Independent requirement for ISL1 in the formation of the pancreatic mesenchyme and islet cells. Nature 385: 257–260
19. Harrison KA, Druey KM, Deguchi Y, Tuscano JM, Kerhl JH (1994) A novel human homeobox gene distantly related to proboscipedia is expressed in lymphoid and pancreatic tissues. J Biol Chem 269: 19968–19975
20. Li H, Arber S, Jessell TM, Edlund H (1999) Selective agenesis of the dorsal pancreas in mice lacking homeobox gene *Hlxb9*. Nat Genet 23: 67–70
21. Harrison KA, Thaler J, Pfaff SL, Gu H, Kehrl JH (1999). Pancreas dorsal lobe agenesis and abnormal islets of Langerhans in *Hlxb9*-deficient mice. Nat Genet 23: 71–75
22. Theodor F (1909) Angeborene aplasie der gallenwege verbunden mit lebercirrose, durch operation behandelt. Archio für Kinderheilkunde 49: 538
23. Wilding R, Schnedl WJ, Reisinger EC et al. (1993) Agenesis of the dorsal pancreas in a woman with diabetes mellitus and in both of her sons. Gastroenterology 104: 1182–1186

24. Lewis J (1996) Neurogenic genes and vertebrate neurogenesis. Curr Opin Neurobiol 6: 3–10
25. Beatus P, Lendahl U (1998) Notch and neurogenesis. J Neurosci Res 54: 125–136
26. Bray S (1998) Notch signalling in Drosophila: three ways to use a pathway. Semin Cell Dev Biol 9: 591–597
27. Apelqvist Å, Li H, Sommer L et al. (1999) Notch-signalling controls pancreatic cell differentiation. Nature 400: 877–881
28. Jensen J, Pedersen EE, Galante P et al. (2000) Control of endodermal endocrine development by Hes-1. Nat Genet 24: 36–44
29. 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 U S A 97: 1607–1611
30. Lammert E, Brown J, Melton DA (2000) Notch gene expression during pancreatic organogenesis. Mech Dev 94: 199–203
31. Donoviel DB, Hadjantonakis AK, Ikeda M et al. (1999) Mice lacking both presenilin genes exhibit early embryonic patterning defects. Genes Dev 13: 2801–2810
32. Hardy J, Israël A (1999) In search of γ-secretase. Nature 398: 466–467
33. Donoviel DB, Donoviel MS, Fan E, Hadjantonakis A, Bernstein A (1998) Cloning and characterization of Sel-1 l, a murine homolog of the C. elegans sel-1 gene. Mech Dev 78: 203–207
34. Kahn BB (1998) Type 2 diabetes: when insulin secretion fails to compensate for insulin resistance. Cell 92: 593–596
35. Taylor SI (1999) Deconstructing type 2 diabetes. Cell 97: 9–12
36. Gepts W, Lecompte PM (1981) The pancreatic islets in diabetes. Am J Med 70: 105–115
37. Clark A, Wells CA, Buley ID et al. (1988) Islet amyloid, increased A-cells, reduced B-cells and exocrine fibrosis: quantitative changes in the pancreas in type 2 diabetes. Diabetes Res 9: 151–159
38. Kloppel G, Lohr M, Habich K, Oberholzer M, Heitz PU (1985) Islet pathology and the pathogenesis of type 1 and type 2 diabetes mellitus revisited. Surv Synth Pathol Res 4: 110–125
39. Westermark P, Wilander E (1978) The influence of amyloid deposits on the islet volume in maturity onset diabetes mellitus. Diabetologia 15: 417–421
40. Guiot Y, Sempoux C, Moulin P, Rahier J (2001) No decrease of the beta-cell mass in type 2 diabetic patients. Diabetes 50 [Suppl 1]: S188
41. Kahn BB, Rossetti L (1998) Type 2 diabetes–who is conducting the orchestra? Nat Genet 20: 223–225
42. Porte D Jr, Kahn SE (1989) Hyperproinsulinemia and amyloid in NIDDM. Clues to etiology of islet beta-cell dysfunction? Diabetes 38: 1333–1336
43. Hales CN (1994) The pathogenesis of NIDDM. Diabetologia 37 [Suppl 2] S162–S168
44. Mykkanen L, Haffner SM, Kuusisto J et al. (1995) Serum proinsulin levels are disproportionately increased in elderly prediabetic subjects. Diabetologia 38: 1176–1182
45. Mykkanen L, Zaccaro D, Hales CN, Festa A, Haffner SM (1999) The relation of proinsulin and insulin to insulin sensitivity and acute insulin response in subjects with newly diagnosed type II diabetes: the Insulin Resistance Atherosclerosis Study. Diabetologia 42: 1060–1066
46. Kahn SE, Leonetti DL, Prigeon RL et al. (1995) Proinsulin as a marker for the development of NIDDM in Japanese-American men. Diabetes 44: 173–179
47. Kahn SE, Leonetti DL, Prigeon RL et al. (1995) Relationship of proinsulin and insulin with noninsulin-dependent diabetes mellitus and coronary heart disease in Japanese-American men: impact of obesity–clinical research center study. J Clin Endocrinol Metab 80: 1399–1406
48. Larsson H, Ahren B (1999) Relative hyperproinsulinemia as a sign of islet dysfunction in women with impaired glucose tolerance. J Clin Endocrinol Metab 84: 2068–2074
49. Nijpels G, Popp-Snijders C, Kostense PJ, Bouter LM, Heine RJ (1996) Fasting proinsulin and 2-h post-load glucose levels predict the conversion to NIDDM in subjects with impaired glucose tolerance: the Hoorn Study. Diabetologia 39: 113–118
50. Rachman J, Levy JC, Barrow BA, Manley SE, Turner RC (1997) Relative hyperproinsulinemia of NIDDM persists despite the reduction of hyperglycemia with insulin or sulfonylurea therapy. Diabetes 46: 1557–1562
51. Haffner SM, Gonzalez C, Mykkanen L, Stern M (1997) Total immunoreactive proinsulin, immunoreactive insulin and specific insulin in relation to conversion to NIDDM: the Mexico City Diabetes Study. Diabetologia 40: 830–837
52. Furuta M, Carroll R, Martin S et al. (1998) Incomplete processing of proinsulin to insulin accompanied by elevation of Des-31,32 proinsulin intermediates in islets of mice lacking active PC2. J Biol Chem 273: 3431–3437
53. Furuta M, Yano H, Zhou A et al. (1997) Defective prohormone processing and altered pancreatic islet morphology in mice lacking active SPC2. Proc Natl Acad Sci U S A 94: 6646–6651
54. Jackson RS, Creemers JW, Ohagi S et al. (1997) Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. Nat Genet 16: 303–306
55. Hart A, Baeza N, Apelqvist A, Edlund H (2000) Attenuation of FGF-signalling in mouse β-cells leads to diabetes. Nature 408: 864–868
56. Kato S, Sekine K (1999) FGF-FGFR signaling in vertebrate organogenesis. Cell Mol Biol 45: 631–638
57. Szebenyi G, Fallon JF (1999) Fibroblast growth factors as multifunctional signaling factors. Int Rev Cytol 185: 45–106
58. Itoh N, Mima T, Mikawa T (1996) Loss of fibroblast growth factor receptors is necessary for terminal differentiation of embryonic limb muscle. Development 122: 291–300
59. Campochiaro PA, Chang M, Ohsato M et al. (1996) Retinal degeneration in transgenic mice with photoreceptor-specific expression of a dominant-negative fibroblast growth factor receptor. J Neurosci 16: 1679–1688
60. Jackson D, Bresnick J, Rosewell I et al. (1997) Fibroblast growth factor receptor signalling has a role in lobuloalveolar development of the mammary gland. J Cell Sci 110: 1261–1268
61. Ye W, Shimamura K, Rubenstein JL, Hynes MA, Rosenthal A (1998) FGF and Shh signals control dopaminergic and serotonergic cell fate in the anterior neural plate. Cell 93: 755–766
62. Cheon HG, LaRochelle WJ, Bottaro DP, Burgess WH, Aaronson SA (1994) High-affinity binding sites for related fibroblast growth factor ligands reside within different receptor immunoglobulin-like domains. Proc Natl Acad Sci U S A 91: 989–993
63. Celli G, LaRochelle WJ, Mackem S, Sharp R, Merlino G (1998) Soluble dominant-negative receptor uncovers essential roles for fibroblast growth factors in multi-organ induction and patterning. Embo J 17: 1642–1655
64. Guillam MT, Hummler E, Schaerer E et al. (1997) Early diabetes and abnormal postnatal pancreatic islet development in mice lacking Glut-2. Nat Genet 17: 327–330

65. Thorens B, Guillam MT, Beermann F, Burcelin R, Jaquet M (2000) Transgenic Reexpression of GLUT1 or GLUT2 in Pancreatic{beta} Cells Rescues GLUT20 Null Mice from Early Death and Restores Normal Glucose-Stimulated Insulin Secretion. J Biol Chem 275: 23751–23758
66. Deltour L, Leduque P, Paldi A et al. (1991) Polyclonal origin of pancreatic islets in aggregation mouse chimaeras. Development 112: 1115–1121
67. Githens S (1988) The pancreatic duct cell: proliferative capabilities, specific characteristics, metaplasia, isolation, and culture. J Pediatr Gastroenterol Nutr 7: 486–506
68. Hellerström C, Anderson A, Swenne I, Welsh N, Sjöholm A (1988) Replication of B cells in the normal and diabetic pancreas. In: Lefebvre P, Pipeleers D (eds) The Pathology of the Endocrine Pancreas in Diabetes. Springer-Verlag, Heidelberg, pp141–170
69. Marynissen G, Aerts L, Van Assche FA (1983) The endocrine pancreas during pregnancy and lactation in the rat. J Dev Physiol 5: 373–381
70. Parsons JA, Brelje TC, Sorenson RL (1992) Adaptation of islets of Langerhans to pregnancy: increased islet cell proliferation and insulin secretion correlates with the onset of placental lactogen secretion. Endocrinology 130: 1459–1466
71. Van Assche FA, Aerts L (1979) The fetal endocrine pancreas. Contrib Gynecol Obstet 5: 44–57
72. Hales CN, Barker DJ, Clark PM et al. (1991) Fetal and infant growth and impaired glucose tolerance at age 64. BMJ 303: 1019–1022
73. Stoffers DA, Ferrer J, Clarke WL, Habener JF (1997) Early-onset type-II diabetes mellitus (MODY4) linked to IPF1. Nat Genet 17: 138–139
74. Macfarlane WM, Frayling TM, Ellard S et al. (1999) Missense mutations in the insulin promoter factor-1 gene predispose to type 2 diabetes. J Clin Invest 104: R33–R39
75. Hani EH, Stoffers DA, Chevre JC et al. (1999) Defective mutations in the insulin promoter factor-1 (IPF-1) gene in late-onset type 2 diabetes mellitus. J Clin Invest 104: R41–R48
76. Ohlsson H, Karlsson K, Edlund T (1993) IPF1, a homeodomain-containing transactivator of the insulin gene. EMBO J 12: 4251–4259
77. Ahlgren U, Jonsson J, Jonsson L, Simu K, Edlund H (1998) β-Cell-specific inactivation of the mouse Ipf1/Pdx1 gene results in loss of the beta-cell phenotype and maturity onset diabetes. Genes Dev 12: 1763–1768