Molecular Medicine 8(12): 763–770, 2002

Review Articles

Extracellular Signals and Pancreatic β-cell Development: A Brief Review

Fang-Xu Jiang and Leonard C. Harrison

Autoimmunity and Transplantation Division, The Walter and Eliza Hall Institute of Medical Research, The Royal Melbourne Hospital, Parkville, Australia

Contributed by L. C. Harrison. Accepted October 9, 2002

Abstract

Cell lineage development is a finely tuned process of proliferation and differentiation, survival and apoptosis, that is regulated by numerous extracellular signals. Here we review some of the extracellular signals—including insoluble cell–cell and extracellular matrix–cell interactions, as well as soluble factors—that appear critical for pancreatic β-cell development. Knowledge of how these signals control the development of pancreatic endocrine stem/precursor cells into fully functional insulin-secreting β cells is a platform for the restoration of β-cell function and the cure therapy of type 1 diabetes.

Introduction

Type 1 (insulin-dependent) diabetes is due to insulin deficiency, following destruction of pancreatic islet β cells by autoreactive T lymphocytes. Restoration of β-cell function, the cure for type 1 diabetes, is far from a reality, partly because we understand so little about how extracellular signals (ECS) control precursor cell proliferation and differentiation into fully functional insulin-secreting β cells. Since the mid-1970s, it has been accepted that pancreas cell lineage development is independent of exogenous hormones, growth factors and innervation, and is controlled by autocrine and paracrine ECS, including extracellular matrix (ECM)–cell interactions. Thus the pancreas primordium dissected from an E11 fetal rat differentiates normally into exocrine and endocrine tissues after 9 days in culture in the absence of any exogenous factors (1). Recently, a great amount of information has appeared, witnessed by over 100 published reviews in last decade, on pancreas cell lineage development (2–4) and regulation at the cellular (5–10) and transcriptional (11–14) levels. Most transcription factor genes are activated by ECS (15). However, progress in determining the identity of ECS molecules and their mechanisms of action has been slow, because of early embryo lethality or functional redundancy after gene targeting and lack of simple and reliable in vitro assays.

Address correspondence and reprint requests to: Fang-Xu Jiang, The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Parkville 3050, Australia. Phone: 61-3-9345 2468; fax: 61-3-9347 0852; e-mail: jiang@wehi.edu.au.

There are, broadly, three types of ECS molecules: those that mediate cell–cell interactions such as Notch and cadherin receptors, insoluble ECM proteins such as laminins and collagens, and soluble factors such as growth factors, hormones, and vitamins. Here we review some ECS critical for pancreas development (Fig. 1).

Cell–Cell Interactions

Molecules that mediate cell–cell interactions are illustrated by reference to Notch and cadherin families. Notch signaling is an evolutionarily conserved mechanism for development of multicellular organisms. Four isoforms of Notch have so far been identified. The Notch receptor is a single transmembrane protein composed of an extracellular domain, a transmembrane domain, and a cytoplasmic domain. The extracellular domain recognizes cell-bound ligands of the Delta/Serrate/Lag2 (DSL) family present on neighboring cells. Activation of the Notch pathway leads to proteolysis releasing the Notch intracellular domain (NICD), summarized in Figure 1 and reviewed elsewhere (16–19). By in situ hybridization, Notch 1 mRNA was detected uniformly in both the dorsal and ventral pancreatic buds of E10.5 mouse embryos, coexpressed with Hairy and Enhancer-of-split protein-1 (HES-1), a downstream molecule in Notch signaling (20). Mice deficient for *Delta-like gene 1*or *HES-1* showed premature differentiation of islet cells and exhaustion of the precursor population (21,22), demonstrating that Notch signaling is critical for maintaining the precursor cell pool and regulating its differentiation into islet cells. However,

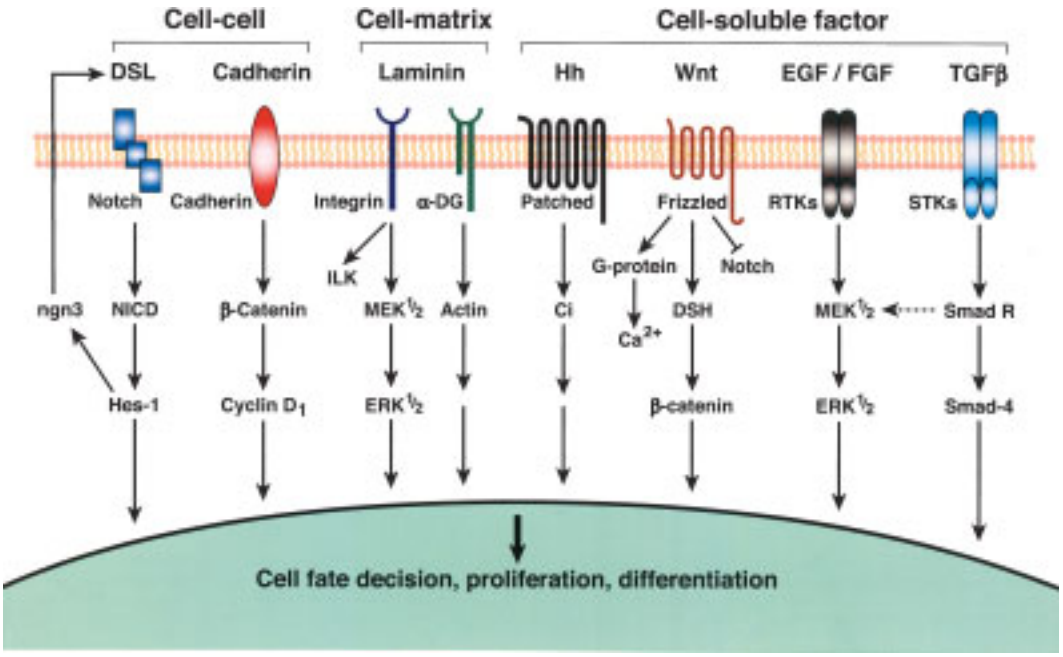

Fig. 1. Schema depicting three major classes of extracellular factors involved in pancreatic cell lineage development. See main text for details.

studies on other Notch isoforms are required to determine their physiologic roles.

Cadherins comprise 80 members of a superfamily of homophilic cell–cell adhesion molecules (CAMs) (23,24). The cytoplasmic domains of cadherins bind to the submembranal proteins, β-catenins, which link to the actin cytoskeleton via α-catenin. β-catenin–mediated transcription is activated by the Wnt signaling pathway (see below). β-catenin activates genes involved in cell proliferation, such as *Cyclin D1* (25). Data from other cell systems and the pancreas demonstrate that cadherins play an important role during development. For example, N-cadherin interactions regulate cell proliferation in the sensory epithelia of the inner ear in response to changing cell density (26). Ovarian granulosa cell proliferation in vitro is significantly inhibited in the presence of monoclonal E-cadherin antibody (27). Blockage of epithelial CAM by a monoclonal antibody induces the differentiation of insulin and glucagon-positive cells in fetal human pancreatic cells (28). Though not affecting normal cell differentiation, abnormal islet architectural development was observed in N-CAM deficient mice (29) and in dominant-negative mutant E-cadherin mice (30). The expression and function of other cadherins during pancreas development remains to be investigated. Availability of more reagents for studies on cadherins could facilitate in vitro manipulation of islet cell development.

To avoid any complicating effects of cell–cell interactions, for example, via cadherins and Notch receptors, and paracrine effects from high cell density, we developed a low cell density cell culture system for pancreas precursor cells to investigate the effects of added ECS (Figure 2A) (31). A similar system was also established for multipotent adult bone marrow mesodermal progenitor cells, because cell–cell interaction at a high cell density was shown to induce their differentiation (32,33).

Cell–Matrix Interactions

The ECM is an organized network composed of numerous glycoproteins, typically including laminins, fibronectin, collagens, proteoglycans, and glycosaminoglycans. The ECM is not a static structure, but is continually produced and remodeled. ECM–cell interactions operate through receptor-mediated signaling and directly or indirectly modulate the cell response to growth factors (Fig. 1) (34–38).

The pancreas is an endoderm-derived, branching epithelial organ. At the interface between epithelial and mesenchymal tissues, there is a dense, sheet-like specialized ECM, the basement membrane (BM), composed mainly of laminins (80%) and collagen IV (39,40). Laminin is biochemically a heterotrimeric glycoprotein (Mr = 850,000) composed of disulfide bonded α (400 kDa), β (210 kDa), and γ (200 kDa) chains, whose structure and function has been well-reviewed recently (41,42). Laminin-1, the earliest described prototype and most extensively studied member of this family, plays an important role in proliferation and differentiation of many cell types during development (43). We detected laminin α1 chain, specific for laminin-1, in the BM of the developing mouse pancreas from E13.5 to E17.5 (31), but it is not present in adult pancreas (44–46). Thus laminin α_1 is expressed in the epithelial BM during early

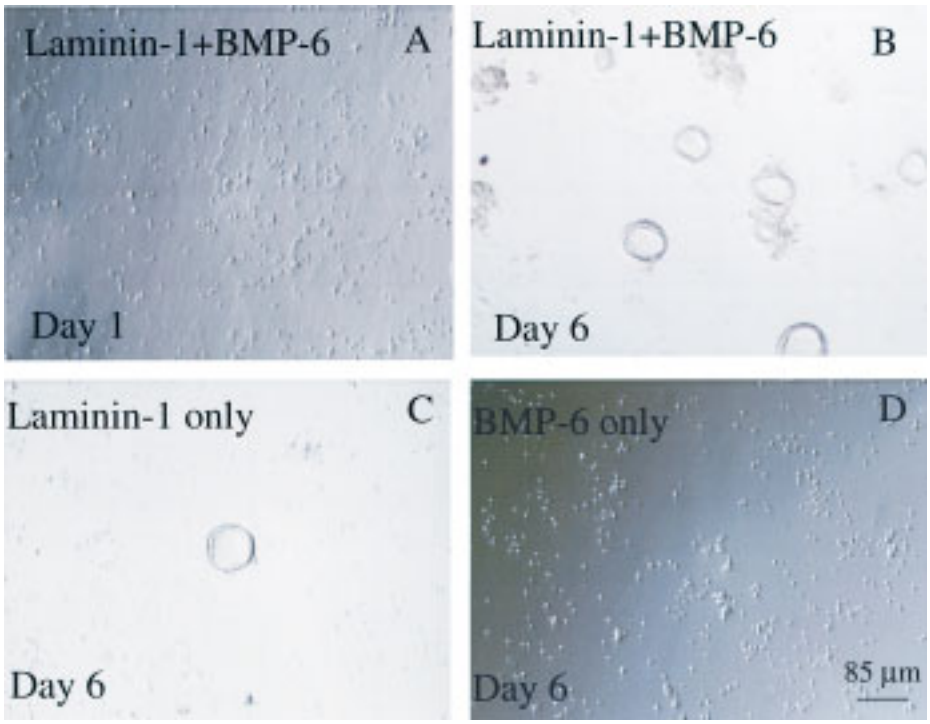

Fig. 2. Phase contrast images showing dissociated E15.5 mouse pancreas cells cultured with and without BMP-6 (10 ng/ml) and laminin-1 (160 μg/ml). (A) Day 1 in the presence of both laminin-1 and BMP-6. **(B, C, D)** Day 6: BMP-6 induced pancreatic cell colony formation in the presence of laminin-1. Without BMP-6, fewer colonies formed in the presence of laminin-1 only. BMP-6 alone failed to promote colony formation in the absence of laminin-1.

development but is subsequently down-regulated (45,47). We found that laminin-1 was required for differentiation of isolated E13.5 pancreas cells to insulin-positive β cells in vitro (31). Expression of various collagen isoforms during pancreas development has been documented (48,49). Collagen IV was shown to inhibit fetal pancreatic cell survival, whereas the non-BM ECM molecule, fibronectin, had no effect (31). Our findings demonstrate that laminin-1 and collagen IV have opposing effects on β-cell development.

The major laminin receptors, the integrins, comprise at least 22 isoforms. They are a well-characterized family of heterodimeric transmembrane glycoprotein molecules composed of noncovalently bound α (120–180 kDa) and β (90–110 kDa) subunits, of which there are 16 and 8 isoforms, respectively. Integrins mediate both cell–matrix and cell–cell interactions in multicellular organisms (50). In addition to their ability to link cells to their extracellular microenvironment, integrins play a role in cellular signaling via association of their cytoplasmic domains with signal transduction cascades (51). All known epithelial integrin receptors for laminin are among the isoforms composed of α_6, α_3, β_1, and β_4 subunits (52). However, $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins are also detected in the developing human pancreas (53). Disruption of β_1 subunit, a partner of at least 12 integrins, causes peri-implantation lethality (54). Because the α_6 subunit shares significant (40%) identity with the α_3 subunit (55), the function of α_6 integrins can be compensated by α_3 integrins (56). This probably explains why knockout of the α_6 integrin gene failed to result in any abnormality of β-cell development in vivo (57). Although a comprehensive profile of integrin expression during pancreas development is still lacking, blocking laminin-1 binding to α_6 integrin or α_6 integrin downstream signaling pathways, such as the extracellular activated receptor kinase 1 pathway, by monoclonal antibody or specific inhibitors, respectively, promotes β-cell differentiation (Fig. 1) (57), illustrating that the role of integrins not only to physically support cells but also to transduce signals for development.

The nonintegrin receptor for laminin, α-dystroglycan (α-DG), is a glycoprotein initially identified in muscle (58) and subsequently in other tissues including pancreas (59). It is a highly glycosylated peripheral membrane protein associated with a membrane-spanning protein β-dystroglycan (β-DG), comprising the dystrophin–glycoprotein complex (DGC). In muscle, DGC is structurally organized into three distinct subcomplexes: the dystroglycans (α-DG and β-DG), the sarcoglycans (SGs, α, β, γ, δ, and ε subunits) and the cytoskeletal proteins, dystrophin (Dp), syntrophin and dystrobrevin (60,61). α-DG is associated with the F-actin cytoskeleton through dystrophin (62,63). In epithelial cells, however, neither α-, β-, γ-, nor δ-SG is expressed (59). The intracellular binding partners of β-DG have not been identified but may include utrophin and/or the shorter dystrophin isoforms

Dp71 and Dp140 (64). To study the role α-DG in laminin-1–induced ß-cell differentiation, dispersed E13.5 fetal mouse pancreatic cells were cultured for 4 days with IIH6, a mouse monoclonal anti–α-DG antibody that blocks laminin-1 binding to α-DG (62,65,66), and heparin, a known inhibitor of laminin-1 binding to α-DG. Both IIH6 and heparin significantly decreased the number of both total cells and ß cells in a dose-dependent manner, indicating that α-DG was essential for survival and differentiation of the pancreatic precursor cells in vitro (57). Further studies are required to examine the role of α-DG in islet cell development in vivo.

Soluble Factor–Cell Interactions

A plethora of soluble factors, mainly polypeptides signal via binding to cell surface receptors. In development, they include hedgehog signaling molecules, Wnt proteins and several families of growth factors.

The hedgehog family of secreted signaling molecules comprise Sonic hedgehog (Shh), Indian hedgehog (Ihh) and Desert hedgehog, which regulate growth and differentiation of many organs during development. Hedgehog signaling inhibits the proteolytic cleavage of the transcriptional factor cubitus interruptus (Ci) and has been recently reviewed (67,68). Although Shh expression is not detectable during pancreas development, $Shh^{-/-}$ and $Shh^{-/-}$ $Ihh^{+/-}$ mice had a 3-fold increase in pancreas mass and a 4-fold increase in islet cell number (69), demonstrating the importance of hedgehog signaling for pancreas development. Ectopic expression of *Shh* under the control of the homeodomain pancreas duodenum transcription factor-1 (PDX1) promoter induced myoid cell formation around pancreatic cells, but islet cell development still occurred (70).

The *Wnt* genes encode a large family of secreted glycoprotein growth factors (19 in humans) (71). The *Wnt* genes are highly conserved between vertebrates, sharing overall sequence identity. Once secreted, Wnt proteins associate with glycosaminoglycans in the ECM and are tightly linked to the cell surface. Wnt signals are transduced by binding to two distinct families of cell surface receptors: members of the Frizzled (FZD) gene family (Fig. 1) and members of low-density-lipoprotein receptor-related family, and activated FZD receptors signal through Disheveled (DSH) (71–74). Before binding to their receptors, Wnt proteins are also modulated extracellularly by various secreted proteins including Fzd-related proteins (Frzbs), Wnt-inhibitory factor-1, and Cerberus. Wnts-2b and -11 mRNAs are expressed in many human fetal organs including pancreas (75,76). A human Frzb homolog termed HFZD-1b was exclusively expressed in the human pancreas (77). Similarly, HFZD-5 was highly expressed in fetal liver and pancreas (78). Recently, Wnt6 protein was showed to induce neural crest cell differentiation (79). All these data suggest that Wnt signals play important roles during pancreas lineage development. Therefore these signals deserve closer attention with respect to pancreas development. In addition, together with the hedgehog family, Wnt signaling regulates stem cell numbers in epithelia such as those of the skin and intestine, which undergo constant renewal (80). Wnt proteins can bind to the Notch extracellular domain and the Notch signaling is inhibited through binding to the Wnt downstream molecule, DSH (19).

Growth factor signals can be transduced by receptor tyrosine kinases, for example, for the epidermal growth factor (EGF) family, fibroblast GF (FGF) family, hepatocyte GF (HGF) and nerve GF (NGF), or by serine/threonine kinases, for example, for the transforming GF [TGF]-β superfamily members such as TGF-β itself, bone morphogenetic proteins (BMPs) and activin.

Serum contains over 1000 components, including various kinds of growth factors and poorly defined growth modifying molecules. Without fetal bovine serum (FBS), for example, islet cell differentiation in chicken dorsal pancreas buds embedded in Matrigel, a purified BM component, was 5-fold more than in 1% FBS (81). Similarly, proliferation of bone marrow mesodermal progenitor cells required a serum-free environment (32,33). We chose serum-free complete medium for culture of dispersed fetal pancreas cells (31,57,82).

The EGF family consists of at least 10 members including EGF, amphiregulin (AR), betacellulin, epiregulin, heparin-binding EGF (HB-EGF), neuregulins 1–4, and transforming GF-α (TGF-α). The EGF receptor (EGFR) family members, EGFR/ErbB1/HER1, ErbB2/neu/HER2, ErbB3/ HER3, and ErbB4/HER4, are tyrosine kinases. They have some specificity for particular ligands; for example EGF, AR, and TGF-α preferentially bind EGFR/ErbB1 (83). Each EGFR family member can form a homodimer or heterodimer with another, except ErbB2, which is the critical partner for several heterodimers (84). Structures and signaling of the EGF family have been reviewed (83–87). By immunocytochemistry, ErbBs 2–4 were detected in the mouse pancreatic epithelium around E14.5 (88). Similarly, HB-EGF was observed in the rat pancreatic epithelial precursors, with a pattern similar to that of PDX1 (89). Mutant ErbB3/HER3 impaired development of the mouse pancreas (90). In vitro, EGF was shown to promote proliferation of mesenchyme-free rat pancreatic epithelium at E13 (91). All these data implicate the EGF family in pancreas development. However, in *EGFR/ErbB1* gene knockout (−/−) mice, lineage development of the pancreas was normal, except for somewhat impaired migration and delayed differentiation of the islet cells (92). This perhaps illustrates the shortcomings of gene targeting to examine the function of molecules with redundant, overlapping actions. The effect of EGF family members on pancreas development in vitro deserves further investigation.

To date, the FGF superfamily consists of 23 members. All contain a conserved 120 amino acid core region and act extracellularly through four tyrosine kinase FGF receptors (FGFRs 1–4) with various affinities (93). The extracellular region consists of two or three immunoglobulin-like domains called loops I, II, and III, the last determining ligand specificity. In isolated chick endoderm, FGF2 can repress Shh expression and activate PDX1 and insulin expression (94), while in the mouse ventral foregut endoderm FGF2 or FGF8b induces Shh expression and leads to liver development (95). RT-PCR analysis detected FGFs-1, -7, and -10 and FGFR2b in the developing rat pancreas from E12–E18 (96). In mesenchyme-free cultures of embryonic pancreatic epithelium, FGF-1, -7, and -10 stimulated growth, morphogenesis, and differentiation of pancreatic exocrine cells (96). mRNA in situ hybridization revealed only FGF-10 before E12.5, and exogenous FGF-10 restored proliferation of the pancreatic precursor cell population in FGF-10 mutant (−/−) mice (97). Overexpression of FGF10, but not FGF8, disturbed β-cell differentiation (98). FGFR1 and FGFR4 mRNAs were detected throughout pancreas development in rats (99). Expression of dominant-negative FGFR2IIIb, specific for FGF-7 (also called keratinocyte GF), caused pancreatic aplasia (100). Similarly, replacing FGF2IIIb translational stop codons with an IRES-LacZ cassette resulted in dysgenesis of many organs including the pancreas (101). This further supports the idea that FGF7 promotes pancreas precursor cell proliferation. Furthermore withdrawal of FGF7 led to the differentiation of endocrine cells in vitro (102). FGF2IIIb signaling also induces FGF4 expression and the latter stimulates Shh expression (101). However, perturbing FGF signaling, by expressing dominant negative forms of FGFR1b and FGFR1c (dnFGFR1c) in the developing pancreas on the PDX1 promoter, did not interfere with normal lineage development, although the adult mice with a dnFGFR1c developed diabetes (103).

TGF-β superfamily members, including the TGF-β, the BMPs and the activins, bind to two types (I and II) of serine/threonine kinase receptors on the cell surface, both receptors being necessary for signal transduction (104,105). The TGF-β family consists of at least three members. Its signal transduction, regulation and function have been recently reviewed (106–108). Transgenic mice expressing a dominant negative TGF-β receptor II controlled by the mouse metallothionein 1 promoter display increased proliferation and impaired differentiation of pancreatic acinar cells (109). Transgenic mice expressing a dominant negative activin receptor controlled by the human insulin promoter have hypoplasia of pancreatic islets (110). Activin B represses endodermal expression of Shh, a prerequisite for expression of PDX1, required for pancreas development (111,112). Follistatin, an inhibitor of activins, was expressed by E12 in rat pancreas mesenchyme and its addition in vitro induced the development of exocrine tissues and repressed the differentiation of islet cells (113). TGF-β1 and activin A were detected in the developing pancreas and, as in other epithelial cells, shown to inhibit pancreatic cell proliferation in vitro (82).

For the BMPs, there are three type I receptors, activin-like kinase (ALK)-3 (also called BMPR-IA), ALK-6 (BMPR-IB), and ALK-2 (or activin receptor type IA, ActR-IA) and three type II receptors, BMP type II receptor and activin type II receptors (ActR-IIA and ActR-IIB). ALK-2, ActR-IIA, and ActR-IIB are also activin receptors (114,115). Unlike TGF-β, BMPs appear to bind cooperatively to both type I and II receptors (104). The type II receptor transphosphylates the type I receptor at a specific region of its cytoplasmic domain resulting in its activation. The activated type I receptor then recruits and phosphorylates downstream signaling molecules belonging to the Smad family, via Smad1, Smad5, and Smad8. The activated Smads then form a complex with Smad4, translocate into the nucleus and regulate the transcription of various target genes. Smads2 and 3 are phosphorylated by TGF-β type 1 and activin type 1B receptors, respectively (116,117). Smads6 and 7 are inhibitory Smads, serving as transcriptional repressors to silence the transcription of target genes.

The BMPs have been shown to be important in development of kidney tubule, lung and other organ epithelia (118,119), including the pancreas. BMP-7 was detected immunocytochemically in human fetal pancreas duct epithelium (120) and by mRNA in situ hybridization in mouse pancreas epithelium between E12.5 and E14.5 (121). In our RT-PCR analysis of mRNAs from E13.5, E15.5, and E17.5 fetal mouse pancreas, BMPs-4, -6, and -7 were detected at each age, whereas BMP-5 and BMP-2 were only detected later at E17.5 (82), consistent with other reports (120–122). In the presence of laminin-1, we found that BMPs-4, -5, and -6 promoted the development of E15.5 isolated pancreas cells into cystic epithelial colonies (Fig. 2) containing insulin-positive cells (82). At this time, one can presumably harvest a maximal number of precursor cells, because expression of neurogenin-3, a marker of islet precursors (123,124), peaks at E15.5 (125). In the absence of laminin-1, BMPs-4, -5 or, -6 failed to promote colony formation (Fig. 2), indicating that both laminin-1 and BMP signaling act in concert. BMP-6–induced colony formation was completely abolished by TGF-β1 or activin A (82), indicating that BMPs and TGFβ and activins may have opposing roles on islet development.

Other growth factors are implicated in pancreas development. Overexpression of vascular endothelial GF under the control of the PDX1 promoter, and hence hypervascularization of the pancreas, is associated with islet hyperplasia (3-fold increase in islet number and area) (126). HGF and platelet-derived GF were also shown to increase proliferation of immature human pancreas β cells in vitro (127–129). High-affinity NGF receptor $gp140^{Trk\text{-}A}$ (tyrosine receptor kinase A [TrK-A]) was detected by immunofluorescence in the

fetal rat pancreas ductal epithelium (130), implying that NGF may influence pancreas development.

In summary, understanding and manipulating pancreas lineage development will require investigation of many ECS and their complex interactions. Together with genetic analysis, low cell density, serum-free culture of pancreatic precursor cells facilitates the identification of ECS molecules required for pancreas development. An outcome of these studies will be the generation of insulin-secreting β cells in vitro for cell replacement therapy in type 1 diabetes.

Acknowledgments

This study was supported by a Juvenile Diabetes Research Foundation Fellowship and a Diabetes Australia Research Grant (2001) (F.-X.J.) and by the National Health and Medical Research Council of Australia (L.C.H.). We thank Catherine O'Shea for secretarial assistance.

References

1. Rutter WJ, Pictet RL, Harding JD, et al. (1978) An analysis of pancreatic development: role of mesenchymal factor and other extracellular factors. *Symp. Soc. Dev. Biol.* **35:** 205–227.
2. Slack JM. (1995) Developmental biology of the pancreas. *Development* **121:** 1569–1580.
3. Bock P, Abdel-Moneim M, Egerbacher M. (1997) Development of pancreas. *Microsc. Res. Tech.* **37:** 374–383.
4. Larsson LI. (1998) On the development of the islets of Langerhans. *Microsc. Res. Tech.* **43:** 284–291.
5. Hill DJ, Duvillie B. (2000) Pancreatic development and adult diabetes. *Pediatr. Res.* **48:** 269–274.
6. Scharfmann R. (2000) Control of early development of the pancreas in rodents and humans: implications of signals from the mesenchyme. *Diabetologia* **43:** 1083–1092.
7. Soria B. (2001) In-vitro differentiation of pancreatic beta-cells. *Differentiation* **68:** 205–219.
8. Docherty K. (2001) Growth and development of the islets of Langerhans: implications for the treatment of diabetes mellitus. *Curr. Opin. Pharmacol.* **1:** 641–650.
9. Edlund H. (2002) Organogenesis: pancreatic organogenesis developmental mechanisms and implications for therapy. *Nat. Rev. Genet.* **3:** 524–532.
10. Yamaoka T, Itakura M. (1999) Development of pancreatic islets (review). *Int. J. Mol. Med.* **3:** 247–261.
11. Sander M, German MS. (1997) The beta cell transcription factors and development of the pancreas. *J. Mol. Med.* **75:** 327–340.
12. Edlund H. (1998) Transcribing pancreas. *Diabetes* **47:** 1817–1823.
13. McKinnon CM, Docherty K. (2001) Pancreatic duodenal homeobox-1, PDX-1, a major regulator of beta cell identity and function. *Diabetologia* **44:** 1203–1214.
14. 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.
15. Brivanlou AH, Darnell JE, Jr. (2002) Signal transduction and the control of gene expression. *Science* **295:** 813–818.
16. Artavanis-Tsakonas S, Rand MD, Lake RJ. (1999) Notch signaling: cell fate control and signal integration in development. *Science* **284:** 770–776.
17. Mumm JS, Kopan R. (2000) Notch signaling: from the outside in. *Dev. Biol.* **228:** 151–165.
18. Fortini ME. (2001) Notch and presenilin: a proteolytic mechanism emerges. *Curr. Opin. Cell Biol.* **13:** 627–634.
19. Baron M, Aslam H, Flasza M, et al. (2002) Multiple levels of notch signal regulation (review). *Mol. Membr. Biol.* **19:** 27–38.
20. Lammert E, Brown J, Melton DA. (2000) Notch gene expression during pancreatic organogenesis. *Mech. Dev.* **94:** 199–203.
21. Apelqvist A, Li H, Sommer L, et al. (1999) Notch signalling controls pancreatic cell differentiation. *Nature* **400:** 877–881.
22. Jensen J, Pedersen EE, Galante P, et al. (2000) Control of endodermal endocrine development by Hes-1. *Nat. Genet.* **24:** 36–44.
23. Takeichi M. (1991) Cadherin cell adhesion receptors as a morphogenetic regulator. *Science* **251:** 1451–1455.
24. Fukata M, Kaibuchi K. (2001) Rho-family GTPases in cadherin-mediated cell-cell adhesion. *Nat. Rev. Mol. Cell Biol.* **2:** 887–897.
25. Shtutman M, Zhurinsky J, Simcha I, et al. (1999) The cyclin D1 gene is a target of the beta-catenin/LEF-1 pathway. *Proc. Natl. Acad. Sci. U.S.A.* **96:** 5522–5527.
26. Warchol ME. (2002) Cell density and N-cadherin interactions regulate cell proliferation in the sensory epithelia of the inner ear. *J. Neurosci.* **22:** 2607–2616.
27. Kirkup KM, Mallin AM, Bagnell CA. (2000) Inhibition of pig granulosa cell adhesion and growth in vitro by immunoneutralization of epithelial cadherin. *J. Reprod. Fertil.* **120:** 275–381.
28. Cirulli V, Crisa L, Beattie GM, et al. (1998) KSA antigen Ep-CAM mediates cell-cell adhesion of pancreatic epithelial cells: morphoregulatory roles in pancreatic islet development. *J. Cell. Biol.* **140:** 1519–1534.
29. Esni F, Taljedal IB, Perl AK, et al. (1999) Neural cell adhesion molecule (N-CAM) is required for cell type segregation and normal ultrastructure in pancreatic islets. *J. Cell Biol.* **144:** 325–337.
30. Dahl U, Sjodin A, Semb H. (1996) Cadherins regulate aggregation of pancreatic beta-cells in vivo. *Development* **122:** 2895–2902.
31. Jiang FX, Cram DS, DeAizpurua HJ, Harrison LC. (1999) Laminin-1 promotes differentiation of fetal mouse pancreatic beta-cells. *Diabetes* **48:** 722–730.
32. Reyes M, Lund T, Lenvik T, et al. (2001) Purification and ex vivo expansion of postnatal human marrow mesodermal progenitor cells. *Blood* **98:** 2615–2625.
33. Jiang Y, Jahagirdar BN, Reinhardt RL, et al. (2002) Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* **418:** 41–49.
34. Juliano R. (1996) Cooperation between soluble factors and integrin-mediated cell anchorage in the control of cell growth and differentiation. *Bioessays* **18:** 911–917.
35. Giancotti FG. (1997) Integrin signaling: specificity and control of cell survival and cell cycle progression. *Curr. Opin. Cell Biol.* **9:** 691–700.
36. Taipale J, Keski-Oja J. (1997) Growth factors in the extracellular matrix. *Faseb J.* **11:** 51–59.
37. Streuli C. (1999) Extracellular matrix remodelling and cellular differentiation. *Curr. Opin. Cell Biol.* **11:** 634–640.
38. Miranti CK, Brugge JS. (2002) Sensing the environment: a historical perspective on integrin signal transduction. *Nat. Cell Biol.* **4:** E83–E90.
39. Timpl R, Brown JC. (1996) Supramolecular assembly of basement membranes. *Bioessays* **18:** 123–132.
40. Pujuguet P, Simian M, Liaw J, et al. (2000) Nidogen-1 regulates laminin-1-dependent mammary-specific gene expression. *J Cell Sci* **113:** 849–858.
41. Colognato H, Yurchenco PD. (2000) Form and function: the laminin family of heterotrimers. *Dev. Dyn.* **218:** 213–234.
42. Tunggal P, Smyth N, Paulsson M, Ott MC. (2000) Laminins: structure and genetic regulation. *Microsc. Res. Tech.* **51:** 214–227.
43. Ekblom P. (1996) Receptors for laminins during epithelial morphogenesis. *Curr. Opin. Cell Biol.* **8:** 700–706.
44. Falk M, Ferletta M, Forsberg E, Ekblom P. (1999) Restricted distribution of laminin alpha1 chain in normal adult mouse tissues. *Matrix Biol.* **18:** 557–568.
45. Virtanen I, Gullberg D, Rissanen J, et al. (2000) Laminin alpha1-chain shows a restricted distribution in epithelial

basement membranes of fetal and adult human tissues. *Exp. Cell Res.* **257:** 298–309.
46. Jiang FX, Naselli G, Harrison LC. (2002) Distinct distribution of laminin and its integrin receptors in the pancreas. *J. Histochem. Cytochem.* **50:** in press.
47. Miner JH, Patton BL, Lentz SI, et al. (1997) The laminin alpha chains: expression, developmental transitions, and chromosomal locations of alpha1-5, identification of heterotrimeric laminins 8-11, and cloning of a novel alpha3 isoform. *J. Cell Biol.* **137:** 685–701.
48. Hisaoka M, Haratake J, Hashimoto H. (1993) Pancreatic morphogenesis and extracellular matrix organization during rat development. *Differentiation* **53:** 163–172.
49. Meyer T, Czub S, Chodnewska I, et al. (1997) Expression pattern of extracellular matrix proteins in the pancreas of various domestic pig breeds, the Goettingen Minipig and the Wild Boar. *Ann. Transplant.* **2:** 17–26.
50. Newham P, Humphries MJ. (1996) Integrin adhesion receptors: structure, function and implications for biomedicine. *Mol. Med. Today* **2:** 304–313.
51. Hannigan GE, Dedhar S. (1997) Protein kinase mediators of integrin signal transduction. *J. Mol. Med.* **75:** 35–44.
52. Belkin AM, Stepp MA. (2000) Integrins as receptors for laminins. *Microsc. Res. Tech.* **51:** 280–301.
53. Cirulli V, Beattie GM, Klier G, et al. (2000) Expression and function of alpha(v)beta(3) and alpha(v)beta(5) integrins in the developing pancreas: roles in the adhesion and migration of putative endocrine progenitor cells. *J. Cell Biol.* **150:** 1445–1460.
54. Fassler R, Meyer M. (1995) Consequences of lack of beta 1 integrin gene expression in mice. *Genes Dev.* **9:** 1896–1908.
55. Hogervorst F, Kuikman I, van Kessel AG, Sonnenberg A. (1991) Molecular cloning of the human alpha 6 integrin subunit. Alternative splicing of alpha 6 mRNA and chromosomal localization of the alpha 6 and beta 4 genes. *Eur. J. Biochem.* **199:** 425–433.
56. De Arcangelis A, Mark M, Kreidberg J, et al. (1999) Synergistic activities of alpha3 and alpha6 integrins are required during apical ectodermal ridge formation and organogenesis in the mouse. *Development* **126:** 3957–3968.
57. Jiang FX, Georges-Labouesse E, Harrison LC. (2001) Regulation of laminin 1-induced pancreatic beta-cell differentiation by alpha6 integrin and alpha-dystroglycan. *Mol. Med.* **7:** 107–114.
58. Ibraghimov-Beskrovnaya O, Ervasti JM, Leveille CJ, et al. (1992) Primary structure of dystrophin-associated glycoproteins linking dystrophin to the extracellular matrix. *Nature* **355:** 696–702.
59. Durbeej M, Henry MD, Ferletta M, et al. (1998) Distribution of dystroglycan in normal adult mouse tissues. *J. Histochem. Cytochem.* **46:** 449–457.
60. Suzuki A, Yoshida M, Hayashi K, et al. (1994) Molecular organization at the glycoprotein-complex-binding site of dystrophin. Three dystrophin-associated proteins bind directly to the carboxy-terminal portion of dystrophin. *Eur. J. Biochem.* **220:** 283–292.
61. Chan YM, Bonnemann CG, Lidov HGW, Kunkel LM. (1998) Molecular organization of sarcoglycan complex in mouse myotubes in culture. *J. Cell Biol.* **143:** 2033–2044.
62. Ervasti JM, Campbell KP. (1993) A role for the dystrophin-glycoprotein complex as a transmembrane linker between laminin and actin. *J. Cell Biol.* **122:** 809–823.
63. Rybakova IN, Ervasti JM. (1997) Dystrophin-glycoprotein complex is monomeric and stabilizes actin filaments in vitro through a lateral association. *J. Biol. Chem.* **272:** 28771–28778.
64. Durbeej M, Ekblom P. (1997) Dystroglycan and laminins: glycoconjugates involved in branching epithelial morphogenesis. *Exp. Lung Res.* **23:** 109–118.
65. Durbeej M, Larsson E, Ibraghimov-Beskrovnaya O, et al. (1995) Non-muscle alpha-dystroglycan is involved in epithelial development. *J. Cell Biol.* **130:** 79–91.
66. Brown SC, Fassati A, Popplewell L, et al. (1999) Dystrophic phenotype induced in vitro by antibody blockade of muscle alpha-dystroglycan-laminin interaction. *J. Cell Sci.* **112:** 209–216.
67. Ingham PW, McMahon AP. (2001) Hedgehog signaling in animal development: paradigms and principles. *Genes Dev.* **15:** 3059–3087.
68. Nybakken K, Perrimon N. (2002) Hedgehog signal transduction: recent findings. *Curr. Opin. Genet. Dev.* **12:** 503.
69. Hebrok M, Kim SK, St Jacques B, et al. (2000) Regulation of pancreas development by hedgehog signaling. *Development* **127:** 4905–4913.
70. Apelqvist A, Ahlgren U, Edlund H. (1997) Sonic hedgehog directs specialised mesoderm differentiation in the intestine and pancreas [published erratum appears in Curr Biol 1997 Dec 1;7(12):R809]. *Curr. Biol.* **7:** 801–804.
71. Miller JR. (2002) The Wnts. *Genome Biol.* **3:** REVIEWS3001
72. Kuhl M, Sheldahl LC, Park M, Miller JR, Moon RT. (2000) The Wnt/Ca2+ pathway: a new vertebrate Wnt signaling pathway takes shape. *Trends Genet.* **16:** 279–283.
73. McEwen DG, Peifer M. (2001) Wnt signaling: the naked truth? *Curr. Biol.* **11:** R524–R526.
74. Huelsken J, Birchmeier W. (2001) New aspects of Wnt signaling pathways in higher vertebrates. *Curr. Opin. Genet. Dev.* **11:** 547–553.
75. Kirikoshi H, Sekihara H, Katoh M. (2001) Molecular cloning and characterization of WNT14B, a novel member of the WNT gene family. *Int. J. Oncol.* **19:** 947–952.
76. Lin Y, Liu A, Zhang S, et al. (2001) Induction of ureter branching as a response to Wnt-2b signaling during early kidney organogenesis. *Dev. Dyn.* **222:** 26–39.
77. Hu E, Zhu Y, Fredrickson T, et al. (1998) Tissue restricted expression of two human Frzbs in preadipocytes and pancreas. *Biochem. Biophys. Res. Commun.* **247:** 287–293.
78. Saitoh T, Katoh M. (2001) Molecular cloning and characterization of human WNT5B on chromosome 12p13.3 region. *Int. J. Oncol.* **19:** 347–351.
79. Garcia-Castro MI, Marcelle C, Bronner-Fraser M. (2002) Ectodermal Wnt function as a neural crest inducer. *Science* **297:** 848–851.
80. Taipale J, Beachy PA. (2001) The Hedgehog and Wnt signalling pathways in cancer. *Nature* **411:** 349–354.
81. Rawdon BB, Andrew A. (1997) Development of embryonic chick insulin cells in culture: beneficial effects of serum-free medium, raised nutrients, and biomatrix. *In Vitro Cell Dev. Biol. Anim.* **33:** 774–782.
82. Jiang FX, Stanley EG, Gonez LJ, Harrison LC. (2002) Bone morphogenetic proteins promote development of fetal pancreas epithelial colonies containing insulin-positive cells. *J. Cell Sci.* **115:** 753–760.
83. Hynes NE, Horsch K, Olayioye MA, Badache A. (2001) The ErbB receptor tyrosine family as signal integrators. *Endocr. Relat. Cancer* **8:** 151–159.
84. Prenzel N, Fischer OM, Streit S, et al. (2001) The epidermal growth factor receptor family as a central element for cellular signal transduction and diversification. *Endocr. Relat. Cancer* **8:** 11–31.
85. Groenen LC, Nice EC, Burgess AW. (1994) Structure-function relationships for the EGF/TGF-alpha family of mitogens. *Growth Factors* **11:** 235–257.
86. Dunbar AJ, Goddard C. (2000) Structure-function and biological role of betacellulin. *Int. J. Biochem. Cell Biol.* **32:** 805–815.
87. Iwamoto R, Mekada E. (2000) Heparin-binding EGF-like growth factor: a juxtacrine growth factor. *Cytokine Growth Factor Rev.* **11:** 335–344.
88. Kritzik MR, Krahl T, Good A, et al. (2000) Expression of ErbB receptors during pancreatic islet development and regrowth. *J. Endocrinol.* **165:** 67–77.
89. Kaneto H, Miyagawa J, Kajimoto Y, et al. (1997) Expression of heparin-binding epidermal growth factor-like growth factor during pancreas development. A potential role of PDX-1 in transcriptional activation. *J. Biol. Chem.* **272:** 29137–29143.

90. Erickson SL, O'Shea KS, Ghaboosi N, et al. (1997) ErbB3 is required for normal cerebellar and cardiac development: a comparison with ErbB2-and heregulin-deficient mice. *Development* **124:** 4999–5011.
91. Cras-Meneur C, Elghazi L, Czernichow P, Scharfmann R. (2001) Epidermal growth factor increases undifferentiated pancreatic embryonic cells in vitro: a balance between proliferation and differentiation. *Diabetes* **50:** 1571–1579.
92. Miettinen PJ, Huotari M, Koivisto T, et al. (2000) Impaired migration and delayed differentiation of pancreatic islet cells in mice lacking EGF-receptors. *Development* **127:** 2617–2627.
93. Boilly B, Vercoutter-Edouart AS, Hondermarck H, et al. (2000) FGF signals for cell proliferation and migration through different pathways. *Cytokine Growth Factor Rev.* **11:** 295–302.
94. Hebrok M, Kim SK, Melton DA. (1998) Notochord repression of endodermal Sonic hedgehog permits pancreas development. *Genes Dev.* **12:** 1705–1713.
95. Deutsch G, Jung J, Zheng M, et al. (2001) A bipotential precursor population for pancreas and liver within the embryonic endoderm. *Development* **128:** 871–881.
96. Miralles F, Czernichow P, Ozaki K, Itoh N, Scharfmann R. (1999) Signaling through fibroblast growth factor receptor 2b plays a key role in the development of the exocrine pancreas. *Proc. Natl. Acad. Sci. U.S.A.* **96:** 6267–6272.
97. Bhushan A, Itoh N, Kato S, et al. (2001) Fgf10 is essential for maintaining the proliferative capacity of epithelial progenitor cells during early pancreatic organogenesis. *Development* **128:** 5109–5117.
98. Yamaoka T, Yoshino K, Yamada T, et al. (2002) Transgenic expression of FGF8 and FGF10 induces transdifferentiation of pancreatic islet cells into hepatocytes and exocrine cells. *Biochem. Biophys. Res. Commun.* **292:** 138–143.
99. Le Bras S, Miralles F, Basmaciogullari A, et al. (1998) Fibroblast growth factor 2 promotes pancreatic epithelial cell proliferation via functional fibroblast growth factor receptors during embryonic life. *Diabetes* **47:** 1236–1242.
100. Celli G, LaRochelle WJ, Mackem S, et al. (1998) Soluble dominant-negative receptor uncovers essential roles for fibroblast growth factors in multi-organ induction and patterning. *Embo J.* **17:** 1642–1655.
101. Revest JM, Spencer-Dene B, Kerr K, et al. (2001) Fibroblast growth factor receptor 2-IIIb acts upstream of Shh and Fgf4 and is required for limb bud maintenance but not for the induction of Fgf8, Fgf10, Msx1, or Bmp4. *Dev. Biol.* **231:** 47–62.
102. Elghazi L, Cras-Meneur C, Czernichow P, Scharfmann R. (2002) Role for FGFR2IIIb-mediated signals in controlling pancreatic endocrine progenitor cell proliferation. *Proc. Natl. Acad. Sci. U.S.A.* **99:** 3884–3889.
103. Hart AW, Baeza N, Apelqvist A, Edlund H. (2000) Attenuation of FGF signalling in mouse beta-cells leads to diabetes. *Nature* **408:** 864–868.
104. Hogan BL. (1996) Bone morphogenetic proteins: multifunctional regulators of vertebrate development. *Genes Dev.* **10:** 1580–1594.
105. Kawabata M, Imamura T, Miyazono K. (1998) Signal transduction by bone morphogenetic proteins. *Cytokine Growth Factor Rev.* **9:** 49–61.
106. Massague J, Chen YG. (2000) Controlling TGF-beta signaling. *Genes Dev.* **14:** 627–644.
107. Miyazono K. (2000) Positive and negative regulation of TGF-beta signaling. *J. Cell Sci.* **113:** 1101–1109.
108. Liu X, Sun Y, Weinberg RA, Lodish HF. (2001) Ski/Sno and TGF-beta signaling. *Cytokine Growth Factor Rev.* **12:** 1–8.
109. Bottinger EP, Jakubczak JL, Roberts IS, et al. (1997) Expression of a dominant-negative mutant TGF-beta type II receptor in transgenic mice reveals essential roles for TGF-beta in regulation of growth and differentiation in the exocrine pancreas. *Embo J.* **16:** 2621–2633.
110. Yamaoka T, Idehara C, Yano M, et al. (1998) Hypoplasia of pancreatic islets in transgenic mice expressing activin receptor mutants. *J. Clin. Invest.* **102:** 294–301.
111. Jonsson J, Carlsson L, Edlund T, Edlund H. (1994) Insulin-promoter-factor 1 is required for pancreas development in mice. *Nature* **371:** 606–609.
112. Offield MF, Jetton TL, Labosky PA, et al. (1996) PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. *Development* **122:** 983–995.
113. Miralles F, Czernichow P, Scharfmann R. (1998) Follistatin regulates the relative proportions of endocrine versus exocrine tissue during pancreatic development. *Development* **125:** 1017–1024.
114. ten Dijke P, Yamashita H, Sampath TK, et al. (1994) Identification of type I receptors for osteogenic protein-1 and bone morphogenetic protein-4. *J. Biol. Chem.* **269:** 16985–16988.
115. Kim SK, Hebrok M, Li E, et al. (2000) Activin receptor patterning of foregut organogenesis. *Genes Dev.* **14:** 1866–1871.
116. Macias-Silva M, Abdollah S, Hoodless PA, et al. (1996) MADR2 is a substrate of the TGF-beta receptor and its phosphorylation is required for nuclear accumulation and signaling. *Cell* **87:** 1215–1224.
117. Zhang Y, Feng X, We R, Derynck R. (1996) Receptor-associated Mad homologues synergize as effectors of the TGF-beta response. *Nature* **383:** 168–172.
118. Hogan BL. (1996) Bone morphogenetic proteins in development. *Curr. Opin. Genet. Dev.* **6:** 432–438.
119. Weaver M, Yingling JM, Dunn NR, et al. (1999) Bmp signaling regulates proximal-distal differentiation of endoderm in mouse lung development. *Development* **126:** 4005–4015.
120. Vukicevic S, Latin V, Chen P, et al. (1994) Localization of osteogenic protein-1 (bone morphogenetic protein-7) during human embryonic development: high affinity binding to basement membranes. *Biochem. Biophys. Res. Commun.* **198:** 693–700.
121. Lyons KM, Hogan BL, Robertson EJ. (1995) Colocalization of BMP 7 and BMP 2 RNAs suggests that these factors cooperatively mediate tissue interactions during murine development. *Mech. Dev.* **50:** 71–83.
122. Crisera CA, Maldonado TS, Kadison AS, et al. (2000) Transforming growth factor-beta 1 in the developing mouse pancreas: a potential regulator of exocrine differentiation [in process citation]. *Differentiation* **65:** 255–259.
123. 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.
124. Jensen J, Heller RS, Funder-Nielsen T, et al. (2000) 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.
125. Schwitzgebel VM, Scheel DW, Conners JR, et al. (2000) Expression of neurogenin3 reveals an islet cell precursor population in the pancreas. *Development* **127:** 3533–3542.
126. Lammert E, Cleaver O, Melton D. (2001) Induction of pancreatic differentiation by signals from blood vessels. *Science* **294:** 564–567.
127. Hayek A, Beattie GM, Cirulli V, et al. (1995) Growth factor/matrix-induced proliferation of human adult beta-cells. *Diabetes* **44:** 1458–1460.
128. Beattie GM, Rubin JS, Mally MI, et al. (1996) Regulation of proliferation and differentiation of human fetal pancreatic islet cells by extracellular matrix, hepatocyte growth factor, and cell–cell contact. *Diabetes* **45:** 1223–1228.
129. Beattie GM, Itkin-Ansari P, Cirulli V, et al. (1999) Sustained proliferation of PDX-1 + cells derived from human islets. *Diabetes* **48:** 1013–1019.
130. Kanaka-Gantenbein C, Tazi A, Czernichow P, Scharfmann R. (1995) In vivo presence of the high affinity nerve growth factor receptor Trk- A in the rat pancreas: differential localization during pancreatic development. *Endocrinology* **136:** 761–769.