Virchows Arch (2000) 436:527–538

REVIEW ARTICLE

Jochen Peters · Anja Jürgensen · Günter Klöppel

Ontogeny, differentiation and growth of the endocrine pancreas

Received: 23 December 1999 / Accepted: 15 February 2000

Abstract The pancreas develops from the primitive foregut endoderm, which differentiates into ductal, acinar and endocrine cells. This complex process is probably replicated in the adult pancreas when endocrine cell renewal is required, as may be the case in diabetes mellitus. This review describes what is known about the morphogenesis of the endocrine pancreas during ontogeny and the mechanisms regulating its differentiation and growth.

Key words Endocrine pancreas · Morphogenesis · Differentiation · Growth

Introduction

Interest in the development and differentiation of the endocrine pancreas has increased considerably during recent years because of the implications for our understanding of the mechanisms regulating beta cell growth in the normal and in the diabetic pancreas. The classic data on the morphogenesis of the embryonal pancreatic tissue and the timing of its development and its differentiation into endocrine and exocrine cells have been augmented by molecular biological findings and new experimental observations, which are beginning to reveal the genetic background and the factors that control the origin, neogenesis, replication and function of pancreatic beta and non-beta cells. In this review we will describe the present state of knowledge of the mechanisms that regulate the development, differentiation and growth of the endocrine pancreas, including data from both human and experimental animal studies.

J. Peters · A. Jürgensen · G. Klöppel (✉)
Department of Pathology, University of Kiel, Michaelisstrasse 11, 24105 Kiel, Germany
e-mail: gkloeppel@path.uni-kiel.de
Tel.: +49-431-5973400, Fax: +49-431-5973462

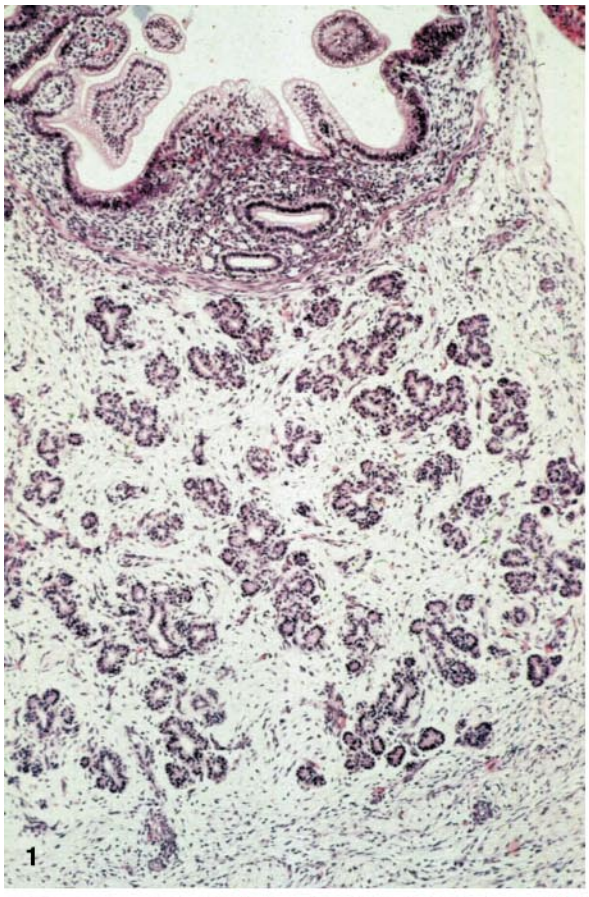
Ontogeny of the pancreas: the process of morphogenesis

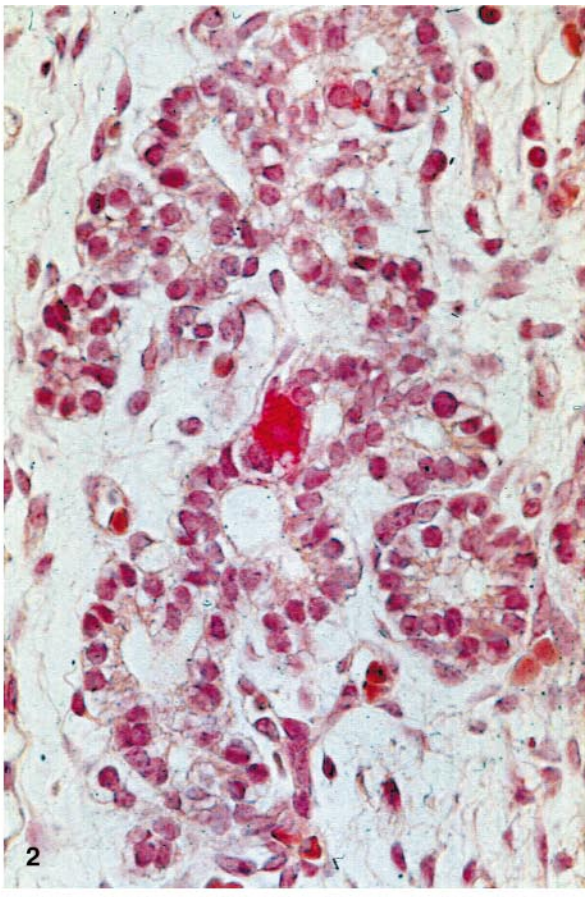
The mammalian pancreas controls nutrient resorption and glucose metabolism via the functional activity of its three major cell compartments, i.e. the ductal cells, acinar cells and endocrine cells. Although these three cell compartments differ clearly in structure and function, they all have the same origin, the primitive gut endoderm. The earlier hypothesis that pancreatic exocrine and endocrine cells are derived from different cell pools, i.e. the gut endoderm and the neural crest, respectively [63], has been refuted by a number of cell lineage experiments [3, 20, 49] and molecular studies (see below).

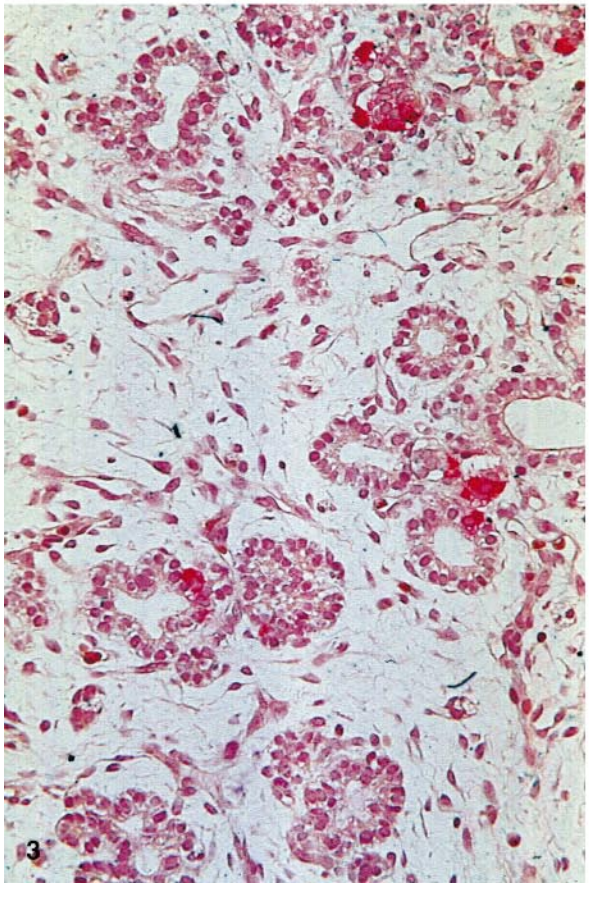
During early gestation (28 days in humans, 8 days in mice) evaginations of the embryonal foregut form the ventral and dorsal buds of the pancreas. The two buds arise opposite to each other while the gut is still surrounded by primitive mesenchyme. The ventral anlage is closely associated with the hepatic and gallbladder diverticulum and appears to start to grow somewhat later than the dorsal bud. After rotation of the stomach and the duodenum, the ventral anlage moves around and fuses with the dorsal bud. The ventral bud forms the posterior part of the pancreatic head including the uncinate process, while the dorsal bud forms the remainder of the organ. In the enlarging epithelial buds a treelike ductal system develops, which eventually gives rise to endocrine and acinar cells (Fig. 1) [75].

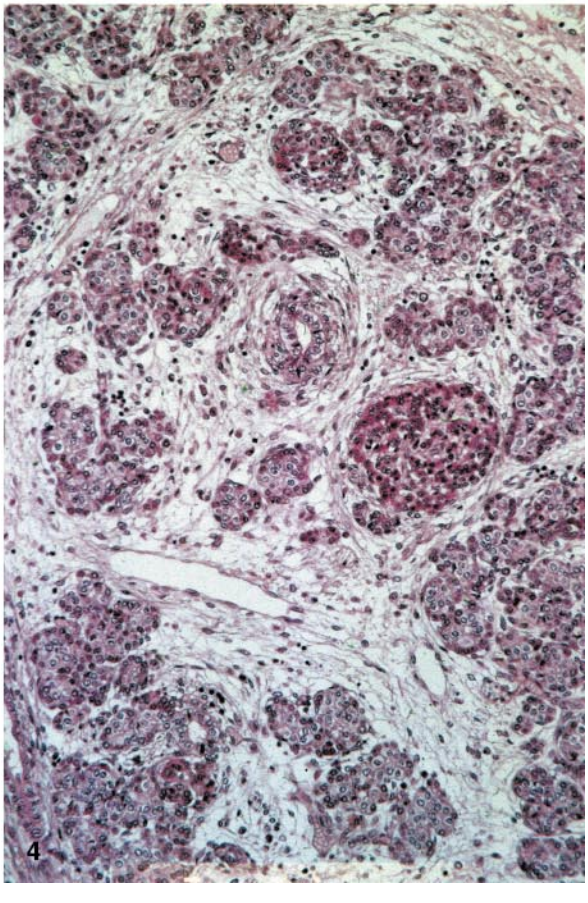
Endocrine cells in the embryonal pancreas: the hormonal markers

The first endocrine cells that can be identified by their hormone content in the human embryonal pancreas are the cells producing somatostatin and pancreatic polypeptide (PP; Fig. 2). From the 7th gestational week (gw) onward they are observed scattered among ductal cells [7]. One week later, glucagon-producing cells appear (Fig. 3), and by the beginning of gw 9 insulin cells can be detect-


1


2


3


4

ed. The C-peptide of the proinsulin molecule is detectable from gw 10 onward [57].

In mice, insulin or glucagon is the first hormonal product that can be identified in pancreatic endocrine cells. This occurs between days 9.5 and 10.5 of embryonic development [36, 87, 89], and it appears that these cells co-express both hormones over the following 2–3 days. In contrast to humans, somatostatin and PP are expressed somewhat later (day 15.5). In addition, Upchurch et al. [89] found cells staining for peptide YY, which, at least when it is first detected, colocalises with each of the four main islet hormones in the order of their appearance. Triple immunofluorescent staining revealed coexpression of peptide YY with only one of the other islet hormones, but never the production of three hormones in a single cell. The appearance and coexpression of peptide YY in the earliest pancreatic endocrine cell types suggest an origin of the different islet cells from a common peptide YY-producing progenitor cell [89]. The results of the study by Herrera et al. [36] differ from those of Upchurch et al. [89] in that Herrera et al. failed to observe cells containing both insulin and glucagon, while they did find a colocalisation of glucagon and PP.

Apart from the above hormones, a number of other hormones and bioactive amines, such as gastrin, gastrin-inhibitory polypeptide, serotonin, catecholamines and prostaglandins, have been detected in the embryonal and fetal pancreas [34]. This suggests that the embryonal/fetal pancreatic endocrine cell is able to produce more than one type of hormone. The biological significance of this ability is not yet known, but it seems to be largely lost in the adult pancreas [55].

Microdissection-based RT-PCR studies of epithelial segments isolated from the foregut of mouse embryos at different stages of development revealed that the genes for the specific pancreatic hormones and enzymes (insulin I and II, glucagon, somatostatin, PP, carboxypeptidase A1 and amylase) are transcribed in a genetically programmed chronology [28, 29]. Somatostatin mRNA was found first. It was detected in the embryonic foregut prior to the evagination of the duodenum, which precedes pancreatic morphogenesis. Interestingly, this expression was not restricted to the areas of the foregut that give rise to the pancreas. Insulin and glucagon were first expressed approximately 12 h before the evagination of the dorsal duodenal wall, from where the dorsal pancreas anlage starts to develop. Shortly after the evagination of the pancreatic buds the pancreatic polypeptide gene was expressed. Finally, carboxypeptidase and amylase mRNAs were expressed, although acinar structures had not yet been formed. It was concluded from these results that the expression of functionally related mRNAs in morphologically undifferentiated cells in the wall of the duodenal anlage may represent the recruitment/commitment of these cells to an endocrine cell lineage. Moreover, hormone expression may be viewed as a stepwise process starting with a low-level transcription (mRNA for hormones appears) in endocrine precursor cells, followed by a gradually increasing synthesis of the specific hormones, which parallels their differentiation to a mature cell type [87].

◀ **Fig. 1** Embryonal human pancreas composed of small ductal structures in the vicinity of the primitive duodenum. H&E, ×60

Fig. 2 Fetal human pancreas (9th gestational week [gw]) with a PP-positive cell within the epithelium of a ductal structure. Immunostaining for pancreatic polypeptide, ×240

Fig. 3 Fetal human pancreas (gw 10). Glucagon-positive cells associated with duct epithelium and within small endocrine cell clusters. Immunostaining for glucagon, ×120

Fig. 4 Fetal human pancreas (gw 21) with formation of definite islets. H&E, ×120

Islet development: the structural features

Islets form by aggregation of polyclonal endocrine cells [23]. This seems to occur when they start to express certain cell adhesion molecules, such as neural cell adhesion molecule (N-CAM) [47] and cadherins [22, 73].

In humans, islet formation begins at gw 12. In the first phase, i.e. gw 13–16, small aggregates of endocrine cells grow out from pancreatic ducts and become vascularised. These primordial islets of Langerhans make up approximately 4% of the total pancreatic tissue. Subsequently, from gw 17 to 20, islets lose their contact with ducts (Fig. 4) and non-beta cells form a mantle around the beta cells (‘mantle islets’). Eventually, i.e. between gw 21 and 26, non-beta cells also appear to some extent in the centre of the islets, which then display the characteristic composition of the postnatal islet [34]. In these periods of development, i.e. between gw 17 and 20 and gw 21 and 26, the proportion of islet tissue increases to 8% and then to approximately 13%, while the average size of the islets increases from 27 µm to 99 µm in diameter [35]. Apart from islets the fetal pancreas contains a large number of small beta cell clusters (<20 µm in diameter), which account for approximately 1.2% of the pancreatic tissue [67]. This proportion decreases towards the end of pregnancy, but ‘extra-insular’ beta cell units are still present in the adult pancreas, where they account for approximately 15% of all beta cells and are found to be associated with ductules [16]. The duct cell system itself makes up about one third of all cells [16], much more than was originally thought. The endocrine tissue accounts for only 1–2% of the entire pancreas [35].

Extra-insular beta cell clusters or single cells, some of them associated with ducts (Fig. 5), are also found in the rat pancreas, where they represent less than 1% of all beta cells [91, 92]. Extrapancreatic islets have also been found in the submucosal layer of the duodenum in rats [5].

Origin of endocrine cells: the stem cell compartment

It has been shown that in the developing pancreatic diverticula exocrine and endocrine cells originate from branching ‘protodifferentiated’ epithelial cells with the features of duct cells [64]. These findings strongly sug-

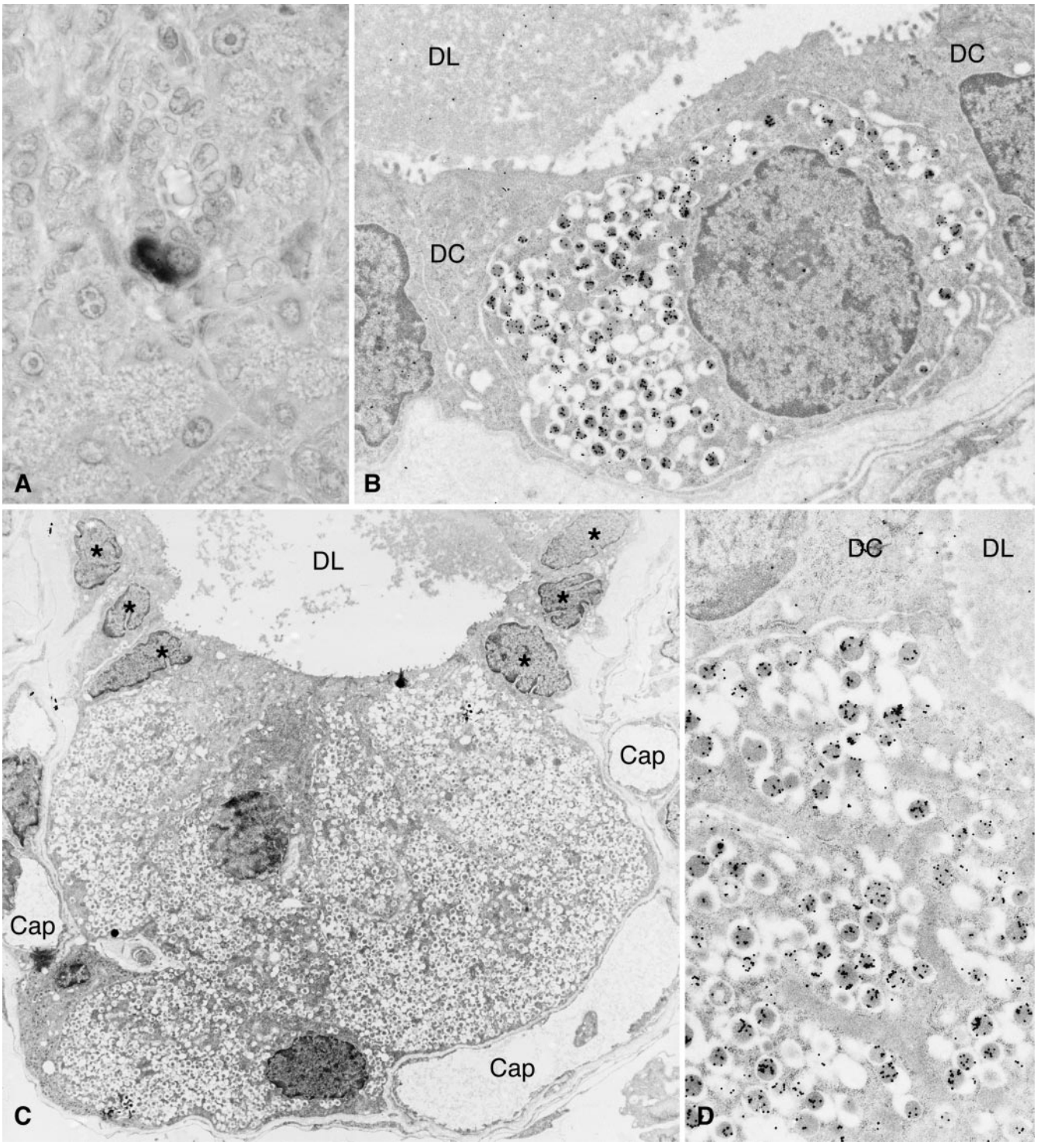
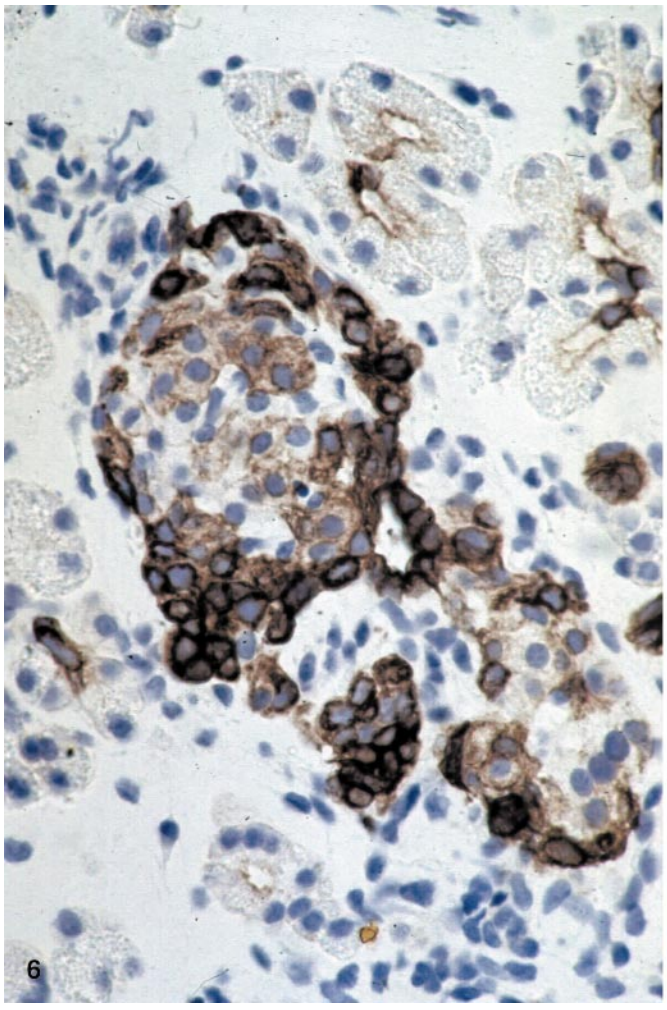


Fig. 5 Adult rat pancreas showing **A**, **B** a single beta cell and **C**, **D** a cluster of beta cells among duct epithelium (*DC* and *asterisks* in **C**). Note the open contact between the beta cell and the duct lumen (*DL*; *Cap* blood capillaries). Photographs by courtesy of Prof. J. Roth, Zürich. **A**, **B**, **D** Protein A-gold staining for insulin, **A** ×250, **B** ×3500, **C** ×1800, **D** ×8000

gest that the pancreatic duct cells harbour the stem cell compartment from which, under appropriate stimuli, acinar or islets cells may differentiate. The phenotype of the putative pancreatic stem cell has yet to be defined, however, although recent studies were able to identify proteins such as tyrosine hydroxylase [87], glucose transporter (GLUT-2) [62], cytokeratins [14], high-affinity nerve growth factor TrkA [44] and PDX-1 [43], which might serve as putative stem cell markers [13].



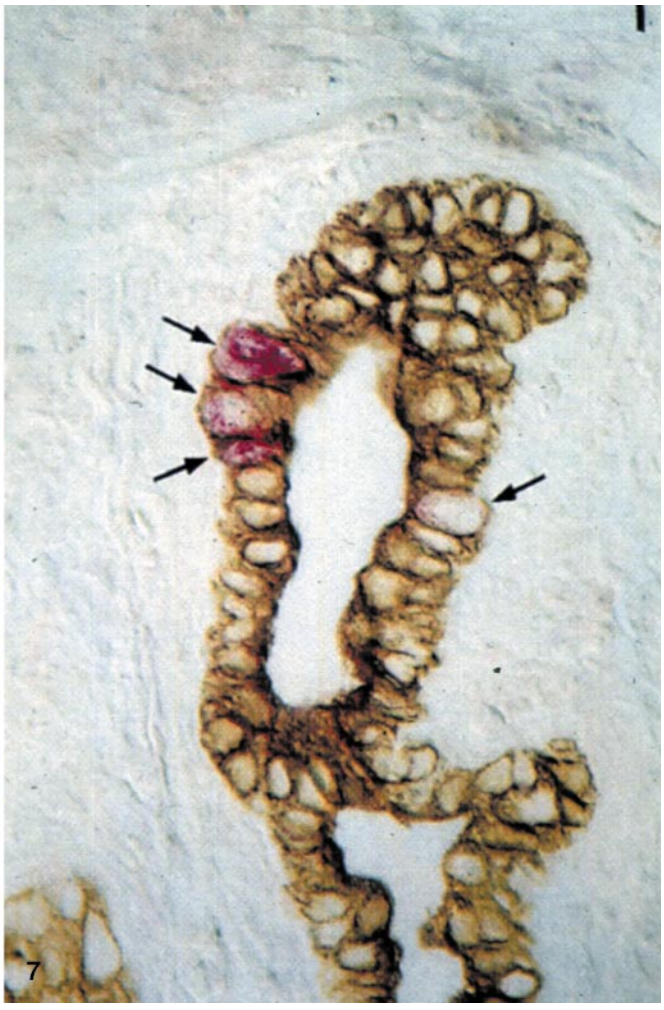


Fig. 6 Neonatal rat pancreas (day 2) showing small ductular structures surrounding islet cells. Both duct cells and islet cells are positive for CK20, although in differing intensity. Immunostaining for CK20, ×240

Fig. 7 Adult rat pancreas after duct ligation. Insulin-positive cell (*red*) arising from CK20-positive duct cells (*arrows*). Immunostaining for insulin and CK20, ×240

Tyrosine hydroxylase (TH), a neuronal marker, shows a shift of expression in the developing rat pancreas [86, 87]. TH expression was found on embryonal day 16 (e16) in pancreatic ductular cells, but not in endocrine cells. During late fetal and early postnatal life, TH immunostaining was also observed in islet cells and some ductular cells. In the adult rat pancreas, TH expression was restricted to beta cells. This expression pattern suggests that endocrine precursor cells are characterised by the expression of TH.

GLUT-2 was found to be expressed in cells of the dorsal and ventral pancreatic buds of rat embryos [62]. This expression was maintained during subsequent growth and branching of the buds. Later (on day 17), cells coexpressing GLUT-2 and insulin were detected, which aggregated to form the beta cells of islets. Cells that became acinar cells lacked GLUT-2 expression.

A difference in cytokeratin (CK) 20 expression was found in pancreatic ductal and endocrine cells in the rat [14, 87, 91]. In the adult animal, CK20 labelled only the duct cells. However, during the fetal and neonatal periods and after induction of beta cell neogenesis by duct ligation in adult rats, CK20 was also expressed in endocrine cells (Fig. 6) identified by their staining for insulin or glucagon. This indicates that there are transitional forms of ductal cells to endocrine cells, which might represent a protodifferentiated cell compartment, from which the terminally differentiated endocrine cells derive [13, 91].

In the human fetal pancreas proliferation is mainly found in the duct cell compartment, followed in frequency by endocrine cells, which are synaptophysin positive but hormone negative, and, finally, insulin- or glucagon-positive cells. In addition, it was noted that all epithelial cells, including endocrine cells, express cytokeratin 19 from gw 12 to gw 16. Later this cytokeratin disappears from the endocrine cells [15].

Induction of pancreatic development: the role of the mesenchyme

Epithelial–mesenchymal interactions seem to play a major part in pancreatic development. Studies in mice and rats

even some 20–30 years ago postulated a 'mesenchymal factor' as a potential stimulus for the differentiation of pancreatic cells during embryogenesis [24, 27, 31, 51, 64, 97]. Gittes et al. [30] recently analysed the effects of the environment on the morphogenesis of the pancreas in more detail. First it was shown that in the absence of mesenchyme isolated pancreatic epithelium was unable to differentiate into exocrine or endocrine tissue. Second, grafting of pancreatic epithelium under the kidney in association with nonspecific mesenchyme resulted in the formation of mature endocrine cells, but no acinar or ductal cells. Third, when the isolated epithelium was grown in the presence of the surrounding (specific) mesenchyme and supplied with soluble mesenchymal factors, it differentiated into ductal and acinar cells. Finally, when pancreatic epithelium was cultured on Matrigel containing extracellular matrix components, it developed into ductular structures only. These results suggest that the differentiation of the exocrine component of the pancreas requires mesenchymally derived signals, which apparently differ from those necessary for the differentiation of endocrine cells.

The essential role of the mesenchyme for the development of the pancreas was further defined in 1997, when Kim et al. [46] reported that removing the notochord (representing early mesenchymal tissue) from the endoderm abrogates pancreatic development. This again suggests that signals from the mesenchyme are essential for the differentiation of primitive gut endoderm into pancreatic cells.

Induction of endocrine cell differentiation: the role of transcription factors

The investigations described in the preceding section have shown that the primitive gut endoderm has the capacity to respond to extrinsic signals (factors) from the surrounding mesenchyme. This responsiveness is probably regulated by the expression of a specific set of intrinsic factors that control the mechanisms for further cell differentiation [81]. These intrinsic factors are called transcription factors. They are proteins distinct from RNA polymerase, which are required for the initiation of transcription. They activate a promoter or a set of promoters containing a recognition target sequence such as a *cis* acting site. The transcription factor genes belong to the homeobox genes, which are known to control pattern formation during development. Distinct homeotic genes within a homeobox have a gene-regulatory function and act as transcription factors that regulate target genes in a precise spatial and temporal pattern [52, 83]. The essential role of transcription factors in pancreatic development was recently demonstrated in investigations using knockout mice lacking certain transcription factors, such as PDX-1 or ISL-1 (reviewed in [76]).

PDX-1

This homeodomain protein in mammals was found to be a transcription factor regulating the expression of insulin and somatostatin. It is also known as insulin promoter factor 1 (IPF-1) [59], somatostatin transactivating factor 1 (STF-1) [50] and islet-duodenal-homeodomain protein (IDX-1) [54]. The *pdx-1* gene represents a homologue of the *XlH*box-8 gene of *Xenopus*, which it is assumed plays a part in the molecular mechanisms controlling the regionalisation of the gut [98]. In mice, *pdx-1* expression starts at day 8.5 in the dorsal gut endoderm, preceding insulin and glucagon expression. One day later PDX-1 appears in all cells of the dorsal and ventral pancreatic buds and in the duodenal wall between them [1, 33, 58]. Subsequently, *pdx-1* expression becomes more and more restricted to the islet cells. Finally it is produced mainly in the beta cells [33, 54, 58].

Knock-out mice that were homozygous for a targeted mutation of the *pdx-1* gene selectively lacked a pancreas [43], while the rest of the gastrointestinal tract, including the common bile duct, the duodenum and the mesenchyme, showed normal development. In a patient with pancreatic agenesis the lack of a pancreas was associated with a single nucleotide deletion in the *pdx-1* gene [84].

In a second *pdx-1* knock-out mouse model [58], the defect abolished pancreatic outgrowth and the differentiation of the most proximal part of the duodenum, but failed to inhibit the differentiation of glucagon cells in the bud. In addition, this model suggested that the *pdx-1* gene acts autonomously on cells by exerting its effects within the epithelium where it is expressed, but that the growth of the pancreatic mesenchyme is independent of PDX-1 expression.

Ahlgren et al. [1] suggested an interesting model for the role of PDX-1 during early pancreatic development in mice (e8 to e15). At e8, patterned embryonic gut epithelium receives an inductive signal prior to the 15-somite stage, which leads to the onset of PDX-1 expression in the dorsal and ventral gut wall and the initiation of evagination. At this stage, no pancreatic mesenchyme is associated with the dorsal gut tissue, which instead is in close contact with the notochord. One day later (e9) high levels of PDX-1 occur in the evaginating dorsal and ventral buds and mesenchymal cells accumulate around the dorsal bud. First, glucagon-expressing cells appear. By e10, PDX-1 may have activated genes that make the pancreas buds able to grow, branch and differentiate. On this day the first insulin cells appear. Subsequently (e11), epitheliomesenchymal interaction leads to the morphogenesis and differentiation of the pancreas and PDX-1 is down-regulated. Then an increase in insulin cells, which are also PDX-1 positive, occurs.

Mice that are heterozygous for *pdx-1* develop normally, but seem to have more non-beta cells than wild-type mice, making this model an interesting one for the study of the genetic background of late-onset diabetes [26]. Once the region of the foregut has acquired the ability to develop into a pancreas due to the expression of *pdx-1*, the further differentiation of endocrine cells seems to be regulated by the *pax* genes. The expression of these genes in the pancreatic epithelium at e9.0 defines two endocrine cell populations: cells expressing both *Pax4*

and *Pax6* will develop into mature insulin-producing beta cells, while cells expressing only *Pax6* differentiate into glucagon-producing alpha cells. Deletion of *Pax4* diverts the beta cell lineage into the alpha cell lineage. Absence of *Pax6* eliminates the alpha cell lineage [82].

ISL-1

All types of islet cells in the adult pancreas express the homeoprotein ISL-1 [2]. Targeted disruption of the *Isl-1* gene has shown that it is necessary for the development of the dorsal pancreatic mesenchyme and the differentiation of islet cells.

Other transcription factors

Candidates for other potentially important transcription factors are Nkx6.1, BETA 2 and PTP-NP. Nkx6.1 was cloned from a hamster library [74] and found to be expressed in the pancreatic buds, but only in a subset of cells. Later its expression was restricted to beta cells, thus implying that it may be specific to beta cell differentiation and function [76].

The expression of the bHLH (basic helix–loop–helix) protein BETA2 is restricted to brain and islet cells, but little is known about its occurrence during the course of development [56]. Some of these factors, i.e. the bHLH proteins, may act as a molecular clock during development [78].

Recently, PTP-NP, a new member of the tyrosine phosphatase receptor family, was suggested as a factor controlling the development of pancreatic endocrine cells [21]. The transcription of PTP-NP is observed as early as e8.5 (mouse) in the endodermal layer of the dorsal region of the gut that gives rise to the pancreas. One day later and thereafter, as the pancreatic rudiment becomes morphologically distinguishable, cells containing PTP-NP appeared to be restricted to the pancreatic rudiment, thus making it a more specific marker than PDX-1, which is also expressed in the adjacent duodenum in early stages of development [60]). In the fetal and in the adult pancreas PTP-NP expression is specific to all four endocrine lineages and is not found in exocrine cells.

Induction of embryonal endocrine cell growth and functional differentiation: the growth factors

The soluble factors that have been found to exert an influence on both cell proliferation and cell differentiation are usually referred to as growth factors [69]. On the one hand, they act as mitogens, initiating cell growth, cell division and cell differentiation; on the other hand, they influence cell function. They act in a paracrine or an autocrine manner. Among the growth factors that may have a role in the endocrine pancreas are insulin-like growth factors (IGF) I and II, transforming growth factors (TGF) alpha and beta, hepatocyte growth factor/scatter factor (HGF/c-met), nerve growth factor (NGF), betacellulin (BTC), vascular endothelial growth factor (VEGF) and gastrin. Neogeneration of pancreatic endocrine tissue was also observed in transgenic animals that express interferon gamma (INF-γ) [32], interleukin 6 (IL-6) [18] or tumour necrosis factor (TNF) alpha [37].

Insulin-like growth factor

IGF-I and IGF-II exert a number of diverse effects, including mitogenic effects (together with growth hormone), insulin-like action, stimulation of chemotaxis and induction of cell differentiation. IGF-I binds to the type-I IGF receptor, a tyrosine kinase that shares considerable sequence identity with the insulin receptor [88]. A number of different IGF-binding proteins (IGF-BP 1–6) act as transporters in the serum and also have other biological effects. IGFBPs have been shown to both stimulate and inhibit IGF-I action. IGF-I and IGF-II are expressed in the rat pancreas from e20 at the latest, the latter being predominant in fetal life and the former during fetal development [39]. In addition, the expression of IGF-BPs 1, 2, 3 and 4 shows distinct developmental patterns. IGF-BP-3 and 4 are predominant in the fetal and neonatal periods, while increased expression of IGFBP-1 and 2 occurs 2–3 weeks after birth. The ontogeny of *IGFBP* gene expression in the pancreas may be related to changes in the nutritional input.

Transforming growth factor alpha

TGFα belongs to a family of peptides that play an important part in the regulation of cell growth and differentiation in both developing and mature mammals. Its effects are mediated by binding to the epidermal growth factor (EGF) receptor [40]. After pancreatic duct ligation, elevated TGFα protein expression is found in the cells of the tubular complexes and ducts, as remodelling of the ligated pancreas parenchyma occurs. Because its expression coincides with increased duct cell proliferation and gastrin expression as well as with the formation of new beta cells [92, 94], it has been suggested that it might be one of the factors involved in islet cell neogenesis [94]. The observed neogeneration of islet tissue in TGFα/gastrin double-transgene mice further supports this assumption [95].

Transforming growth factor beta

The general role of the TGFβ superfamily in embryogenesis was investigated by Hogan et al. [38]. In the human pancreas, immunohistochemistry and in situ hybridisation techniques revealed the presence of TGFβ_1, -β_2 and -β_3 in islet, acinar and duct cells. TGFβ type II receptor-negative transgenic mice have aberrant ductular cells and

ductules [12]. This indicates that TGFβ has an inhibitory effect on the growth of ductular cells and ductules. $TGF\beta_1$ induces the regression of the acinar compartment of the developing pancreas and promotes differentiation of the endocrine tissue [77].

Hepatocyte growth factor/scatter factor

HGF, a glycoprotein, exerts morphogenetic effects on several tissues, including digestive epithelium [45]. Its receptor is encoded by the *c-met* proto-oncogene. In the partial pancreatectomy model of endocrine cell regeneration ([9]; see below) the expression of HGF mRNA was found to be increased after 24 h, with a peak at days 2–3 [11]. This expression pattern coincided with proliferation, branching and regeneration of the ductules, suggesting that HGF might have a role in the regenerative process after partial pancreatectomy, possibly as both an autocrine and paracrine mitogen for the ductal epithelium.

Nerve growth factor

The high-affinity NGF receptor Trk-A, which is required for NGF signal transduction, is expressed in various beta cell lines and in normal rat islet cells both in primary culture and in vivo in the fetal and in the adult pancreas [44, 79]. By double immunofluorescence analysis using anti-Trk-A plus anti-insulin or anti-glucagon, Trk-A expression was found on e16 in pancreatic ductular cells, but not in endocrine cells. During late fetal and early postnatal life, Trk-A immunostaining was also observed in islet cells and adjacent ductular cells. In the adult rat pancreas, Trk-A expression is restricted to beta islet cells. This expression pattern suggests that endocrine precursor cells might be targets for the Trk-A ligand NGF.

Betacellulin

BTC was isolated from the medium of beta-TC-3 insulinoma cells and is considered to belong to the EGF family [80]. It stimulates growth in fibroblasts and vascular smooth muscle cells and converts pancreatic AR42J-B20 cells into insulin-producing cells [53]. It is expressed in human pancreatic islets and is therefore thought to be involved in the differentiation of pancreatic beta cells [42].

Vascular endothelial growth factor

In the rat pancreas, the angiogenic factor VEGF was found to be expressed in normal islet cells, while its receptor Flk-1 was demonstrated on the apical membrane of ducts. When rat duct cells in primary culture were treated with VEGF, the proliferation index of the cells more than doubled, but no endocrine differentiation was induced [68]. These observations suggest that VEGF may stimulate the growth of pancreatic ductal cells, but has no effect on islet neogenesis.

Gastrin

Gastrin appears to have a dual function as a hormone and as a trophic factor. As a trophic factor gastrin exerts a potent effect on the enterochromaffin-like cell, and possibly on other gastric and intestinal cells as well [85]. It has been suggested that gastrin and the homologous cholecystokinin are responsible for increased ^{3}H-thymidine labelling of pancreatic ductal and acinar cells in rats fed raw soya flour [57]. In the mammalian pancreas, and particularly in the rat pancreas, gastrin is expressed at both the mRNA and the protein level [4, 17, 48] during late fetal gestation when cytodifferentiation into ductal, acinar and endocrine cells occurs [64], but disappears rapidly from the pancreas in the postnatal period. These findings suggest that pancreatic gastrin may play a part in the growth and development of the endocrine pancreas.

Postnatal endocrine cell growth and regeneration: the experimental models

Whenever the postnatal growth of the endocrine pancreas is considered the discussion focuses on the beta cells, because they are the most frequent and functionally most important cells of the pancreatic islets. The capacity of postnatal beta cells to grow or regenerate via replication (mitotic division of pre-existing, differentiated beta cells) or neogenesis (differentiation from an undifferentiated precursor or stem cell) seems to be low. However, several experimental models have shown that under certain conditions and in certain stages of development, the growth of beta cells can be reactivated. The most important of these models are duct ligation in adult rats [41, 92], partial (90%) pancreatectomy in rats [10], cellophane wrapping of the pancreatic head in adult hamsters [70, 72], streptozotocin-induced beta cell depletion in newborn rats [8, 10, 19, 92, 96] and beta cell destruction by selective alloxan perfusion in adult mice [90].

Duct ligation model

It has long been known that duct ligation may trigger islet neogenesis. Only recently, however, was this process studied in detail [92]. It was shown in the ligated rat pancreas that the beta cell population nearly doubles within the first week after duct ligation. Moreover, CK20-positive ductal cells were identified that coexpressed insulin or the beta cell marker GLUT 2 (Fig. 7). These results suggested neogenesis of beta cells from duct cells rather than replication from pre-existing beta cells.

In another experiment rat pancreatic tissue was examined 24 h after duct ligation. Intermediate cells, i.e. cells that showed both insulin and amylase granules, were de-

tected. This finding, which was confirmed at the mRNA level, was interpreted as an indication of neoformation of beta cells through transdifferentiation of acinar cells [6].

Cellophane wrapping

Rosenberg et al. [70, 71] developed a model of partial pancreatic duct obstruction in hamsters that provides further evidence of a continuous development from duct to islet cells. Fourteen days after the head of the pancreas had been wrapped in cellophane, cells migrated from the epithelium of small intralobular ductules and began to form new islet structures. After 8 weeks there was a 2.5-fold increase in islet cell mass. With in situ hybridisation for insulin and glucagon mRNA three patterns of endocrine differentiation were observed: (a) mature cells expressing glucagon, somatostatin, or insulin, (b) foci of new islet formation expressing only one islet hormone, and (c) individual cells in the ductular epithelium expressing glucagon or insulin [69]. Moreover, a protein called ilotropin was identified, which was expressed during islet neogenesis and thought to be capable of initiating duct cell proliferation [65, 66].

Partial pancreatectomy

The 90% (partial) pancreatectomy rat model is another model for studying the mechanism of pancreatic growth and regeneration [9]. Bonner-Weir et al. [10] reported that after 90% pancreatectomy in young rats there was regeneration of both acinar and islet tissue. By 8 days after surgery the remaining 10% of the pancreas had regenerated to 27% of the normal pancreas weight and 45% of the islet cell mass. This led to the formation of new acini and islets that were indistinguishable from the pre-existing ones. The increase in beta cell mass has been shown to be due in part to neogenesis from proliferating ducts.

By applying immunohistochemistry, RNA analysis and in situ hybridisation, Bonner-Weir also examined changes in the expression of different growth factors. IGF-I, TGFβ and HGF all showed increased expression at different times during the regenerative process, thus indicating that they might be potential factors in pancreatic cell neogenesis. The reg/PSP (regenerating gene or pancreatic stone protein), however, showed changes unrelated to islet growth. Bonner-Weir's group [11] hypothesises that there are factors that influence each pathway of beta cell renewal, with some factors influencing proliferation of the ductal cells and others stimulating the differentiation of ductal cells into various pancreatic cell types.

Neonatal streptozotocin model

Beta cell growth after injury has been studied in rats treated with streptozotocin during the neonatal period [8, 25, 93, 96]. Given on the first day of life, streptozotocin induces subtotal beta cell damage with concomitant hyperglycaemia, which is followed by rapid beta cell regeneration restoring normoglycaemia. Although beta cell regeneration leads to an increase in cell number of almost 50% of normal by 6 weeks of age, beta cell function is diminished, resulting in the gradual reappearance of diabetes. Wang et al. [93] showed that the extent of beta cell regeneration depends on the timing of beta cell damage and that this potential declines rapidly during the first days of life. In the adult rat the regenerative capacity after streptozotocin treatment is therefore only minimal. The critical period in postnatal life which determines the regenerative capacity of the beta cell population seems to be the day of birth, indicating that the mechanism of beta cell growth is generated by progenitor cells. Later on the regeneration seems to be mainly due to the replication of pre-existing beta cells and to a lesser extent to precursor cells located in the pancreatic ducts.

Alloxan perfusion model

Waguri et al. [90] established a model of transient diabetes for 48 weeks in mice by perfusing the body and tail of the pancreas with alloxan after clamping the superior mesenteric artery. In the alloxan-perfused compartment all beta cells were destroyed within 5 days. In this model both processes, proliferation and differentiation, took place in the same pancreas. In the beta-cell-depleted pancreas neogenesis of beta cells from extra-islet precursors located within the ductular epithelium was mainly observed, while in the non-alloxan-perfused part of the pancreas beta cell regeneration was based mainly on proliferation from pre-existing beta cells located in islets.

Conclusions and perspectives

In recent years the combined use of different techniques, such as immunocytochemistry, molecular genetics, microdissection and in vitro culturing, and the availability of transgenic mice have considerably furthered our understanding of the processes and factors that regulate the development, differentiation and growth of the pancreas as a whole and the endocrine pancreas in particular. It has been clarified that the primitive gut endoderm gives rise to progenitor cells for both exocrine and endocrine cells. The differentiation of these cells, as evidenced by gene transcription for islet hormones and enzymes, is already initiated in the gut epithelium prior to the formation of the pancreatic diverticula. Morphogenesis and further differentiation of the pancreas, which finally results in a complex tissue structure combining duct cells, acinar cells and islet cells, requires the presence of notochord and mesenchyme and is controlled – inter alia – by transcription factors such as PDX-1, Pax4 and Pax6. Growth of the fetal endocrine pancreas results from replication of pre-existing, differentiated endocrine cells, particularly beta cells, and by neogenesis from undiffer-

entiated precursor or stem cells. After birth both mechanisms continue to maintain beta cell growth, albeit at a low level. In the adult pancreas there is evidence that, under certain conditions, duct cells can be stimulated to differentiate into islet cells. However, it remains to be elucidated whether all duct cells retain a stem cell potential and which among the transcription and growth factors are required to induce islet cell neogenesis. The understanding of these processes would give us more insight into beta cell growth and regeneration, which probably contribute to a greater or lesser degree to the pathogenesis of all types of diabetes mellitus and thus also have implications for the potential treatment of this disease group. As for islet cell transplantation, a still experimental treatment of insulin-dependent diabetes mellitus, it might become possible to augment islet cell mass after transplantation by providing appropriate growth factors and an adequate microenvironment.

Acknowledgements The authors thank Mrs. K. Dege for editing the manuscript. They are also grateful to Professors Philipp Heitz and Jürgen Roth (Department of Pathology, University of Zürich, Switzerland) for their critical comments on the manuscript. Figure 5 was contributed by Jürgen Roth.

References

1. 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
2. Ahlgren U, Pfaff SL, Jessell TM, Edlund T, Edlund H (1997) Independent requirement for ISL1 in formation of pancreatic mesenchyme and islet cells. Nature 385:257–260
3. Andrew A (1976) An experimental investigation into the possible neural crest origin of pancreatic APUD (islet) cells. J Embryol Exp Morphol 35:577–593
4. Bardram L, Hilsted L, Rehfeld JF (1990) Progastrin expression in mammalian pancreas. Proc Natl Acad Sci USA 87:298–302
5. Bendayan M, Park IS (1997) Extrapancreatic islets of Langerhans: ontogenesis and alterations in diabetic condition. J Endocrinol 153:73–80
6. Bertelli E, Bendayan M (1997) Intermediate endocrine-acinar pancreatic cells in duct ligation conditions. Am J Physiol 273 [Cell Physiol 42]:C1641–C1649
7. Bocian-Sobkowska J, Zabel M, Wozniak W, Surdyk-Zasada J (1997) Prenatal development of the human pancreatic islets. Immunocytochemical identification of insulin-, glucagon-, somatostatin- and pancreatic polypeptide-containing cells. Folia Histochem Cytobiol 35:151–154
8. Bonner-Weir S, Trent DF, Honey RN, Weir GC (1981) Responses of neonatal rat islets to streptozotocin: limited B-cell regeneration and hyperglycemia. Diabetes 30:64–69
9. Bonner-Weir S, Trent DF, Weir GC (1983) Partial pancreatectomy in the rat and subsequent defect in glucose-induced insulin release. J Clin Invest 71:1544–1553
10. Bonner-Weir S, Baxter LA, Schuppin GT, Smith FE (1993) A second pathway for regeneration of adult exocrine and endocrine pancreas. A possible recapitulation of embryonic development. Diabetes 42:1715–1720
11. Bonner-Weir S, Stubbs M, Reitz P, Taneja M, Smith FE (1997) Partial pancreatectomy as a model of pancreatic regeneration. In: Sarvetnick N (ed) Pancreatic growth and regeneration. Karger Landes Systems, Basel, pp 138–153
12. Bottinger EP, Jakubczak JL, Roberts IS, Mumy M, Hemmati P, Bagnall K, Merlino G, Wakefield LM (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
13. Bouwens L, Klöppel G (1996) Islet cell neogenesis in the pancreas. Virchows Arch 427:553–560
14. Bouwens L, Wang RN, De Blay E, Pipeleers DG, Klöppel G (1994) Cytokeratins as markers of ductal cell differentiation and islet neogenesis in the neonatal rat pancreas. Diabetes 43:1279–1283
15. Bouwens L, Lu WG, De Krijger R (1997) Proliferation and differentiation in the human fetal endocrine pancreas. Diabetologia 40:398–404
16. Bouwens L, Pipeleers DG (1998) Extra-insular beta cells associated with ductules are frequent in adult human pancreas. Diabetologia 41:629–633
17. Brand SJ, Fuller PJ (1989) Differential gastrin gene expression in rat gastrointestinal tract and pancreas during neonatal development. J Biol Chem 263:5341–5347
18. Campbell IL, Hobbs MV, Dockter J, Oldstone MBA, Allison J (1994) Islet inflammation and hyperplasia induced by the pancreatic islet-specific overexpression of interleukin-6 in transgenic mice. Am J Pathol 145:157–166
19. Cantenys D, Portha B, Dutrillaux MC, Hollande E, Rozé C, Picon L (1981) Histogenesis of the endocrine pancreas in newborn rats after destruction by streptozotocin. Virchows Arch [B] 35:109–122
20. Cheng H, Leblond CP (1974) Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. V. Unitarian theory of the origin of the four epithelial cell types. Am J Anat 141:537–561
21. Chiang MK, Flanagan JG (1996) PTP-NP, a new member of the receptor protein tyrosine phosphatase family, implicated in development of nervous system and pancreatic endocrine cells. Development 122:2239–2250
22. Dahl U, Sjodin A, Semb H (1996) Cadherins regulate aggregation of pancreatic beta-cells in vivo. Development 122:2895–2902
23. Deltour L, Leduque P, Paldi A, Ripoche MA, Dubois P, Jami J (1991) Polyclonal origin of pancreatic islets in aggregation mouse chimaeras. Development 112:1115–1121
24. Dudek RW, Lawrence IE Jr, Hill RS, Johnson RC (1991) Induction of islet cytodifferentiation by fetal mesenchyme in adult pancreatic ductal epithelium. Diabetes 40:1041–1048
25. Dutrillaux MC, Portha B, Roze C, Hollande E (1982) Ultrastructural study of pancreatic B cell regeneration in newborn rats after destruction by streptozotocin. Virchows Arch [B] 39:173–185
26. Dutta S, Bonner-Weir S, Montminy M, Wright C (1998) Regulatory factor linked to late-onset diabetes? Nature 392:560
27. Filosa S, Pictet R, Rutter W (1975) Positive control of cyclic AMP on mesenchymal factor controlled DNA synthesis in embryonic pancreas. Nature 257:702–705
28. Gittes GK (1994) Studies of early events in pancreatic organogenesis. Ann N Y Acad Sci 733:68–74
29. Gittes GK, Rutter WJ (1992) Onset of cell-specific gene expression in the developing mouse pancreas. Proc Natl Acad Sci USA 89:1128–1132
30. Gittes GK, Galante PE, Hanahan D, Rutter WJ, Debase HT (1996) Lineage-specific morphogenesis in the developing pancreas: role of mesenchymal factors. Development 122: 439–447
31. Golosow N, Grobstein C (1962) Epithelio-mesenchymal interactions in pancreatic morphogenesis. Dev Biol 4:242–255
32. Gu D, Sarvetnick N (1993) Epithelial cell proliferation and islet neogenesis in IFN-γ transgenic mice. Development 118:33–46
33. Guz Y, Montminy MR, Stein R, Leonard J, Gamer LW, Wright CVE, Teitelman G (1995) Expression of murine STF-1, a putative insulin gene transcription factor, in β cells of pancreas, duodenal epithelium and pancreatic exocrine and endocrine progenitors during ontogeny. Development 121:11–18

34. Hahn Dorsche H von, Reiher H, Hahn HJ (1988) Phases in the early development of the human islet organ. Anat Anz 166:69–76
35. Hahn Dorsche H von, Falt K, Titlbach M, Reiher H, Hahn HJ, Falkmer S (1989) Immunohistochemical, morphometric, and ultrastructural investigations of the early development of insulin, somatostatin, glucagon, and PP cells in foetal human pancreas. Diabetes Res 12:51–56
36. Herrera PL, Huarte J, Sanvito F, Meda P, Orci L, Vassalli JD (1991) Embryogenesis of the murine endocrine pancreas: early expression of pancreatic polypeptide gene. Development 113:1257–1265
37. Higuchi Y, Herrera P, Muniesa P, Huarte J, Belin D, Ohashi P, Aichele P, Orci L, Vassalli JD, Vassalli P (1992) Expression of a tumor necrosis factor alpha transgene in murine pancreatic beta cells results in severe and permanent insulitis without evolution towards diabetes. J Exp Med 176:1719–1731
38. Hogan BLM, Blessing M, Winnier GE, Suzuki N, Jones CM (1994) Growth factors in development: the role of TGF-β related polypeptide signalling molecules in embryogenesis. Development [Suppl]:53–60
39. Hogg J, Hill DJ, Han VK (1994) The ontogeny of insulin-like growth factor (IGF) and IGF-binding protein gene expression in the rat pancreas. J Mol Endocrinol 13:49–58
40. Hormi K, Lehy T (1994) Developmental expression of transforming growth factor-alpha and epidermal growth factor receptor proteins in the human pancreas and digestive tract. Cell Tissue Res 278:439–450
41. Hultquist GT, Jönsson LE (1965) Ligation of the pancreatic duct in rats. Acta Soc Med Upsal 70:82–88
42. Ishiyama N, Kanzaki M, Seno M, Yamada H, Kobayashi I, Kojima I (1998) Studies on the betacellulin receptor in pancreatic AR42J cells. Diabetologia 41:623–628
43. Jonsson J, Carlsson L, Edlund T, Edlund H (1994) Insulin-promoter-factor 1 is required for pancreas development in mice. Nature 371:606–609
44. Kanaka-Gantenbein C, Dicou E, Czernichow P, Scharfmann R (1995) Presence of nerve growth factor and its receptors in an in vitro model of islet cell development: implication in normal islet morphogenesis. Endocrinology 136:3154–3162
45. Kermorgant S, Walker F, Hormi K, Dessirier V, Lewin MJ, Lehy T (1997) Developmental expression and functionality of hepatocyte growth factor and c-Met in human fetal digestive tissues. Gastroenterology 112:1635–1647
46. Kim SK, Hebrok M, Melton DA (1997) Notochord to endoderm signaling is required for pancreas development. Development 124:4243–4252
47. Lackie PM, Zuber C, Roth J (1994) Polysialic acid of the neural cell adhesion molecule (N-CAM) is widely expressed during organogenesis in mesodermal and endodermal derivatives. Differentiation 57:119–131
48. Larsson LI, Rehfeld JF, Håkanson R, Sundler F (1976) Pancreatic gastrin in foetal and neonatal rats. Nature 262:609–610
49. Le Douarin NM (1988) On the origin of pancreatic endocrine cells. Cell 53:169–171
50. Leonard J, Peers B, Johnson T, Ferreri K, Lee S, Montminy MR (1993) Characterization of somatostatin transactivating factor1, a novel homeobox factor that stimulates somatostatin expression in pancreatic islet cells. Mol Endocrinol 7: 1275–1283
51. Levine S, Pictet R, Rutter WJ (1973) Control of cell proliferation and cytodifferentiation by a factor reacting with the cell surface. Nat New Biol 246:49–52
52. Manak JR, Scott MP (1994) A class act: conservation of homeodomain protein functions. Development [Suppl]:61–77
53. Mashima H, Ohnishi H, Wakabayashi K, Mine T, Miyagawa J, Hanafusa T, Seno M, Yamada H, Kojima I (1996) Betacellulin and activin A coordinately convert amylase-secreting pancreatic AR42J cells into insulin-secreting cells. J Clin Invest 97: 1647–1654
54. Miller CP, McGehee RE Jr, Habener JF (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
55. Myrsen-Axcrona U, Ekblad E, Sundler F (1997) Developmental expression of NPY, PYY and PP in the rat pancreas and their coexistence with islet hormones. Regul Pept 68: 165–175
56. Naya FJ, Stellrecht CM, Tsai MJ (1995) Tissue-specific regulation of the insulin gene by a novel basic helix-loop-helix transcription factor. Genes Dev 9:1009–1019
57. Oates PS, Morgan RGH (1982) Pancreatic growth and cell turnover in the rat fed raw soya flour. Am J Pathol 108: 217–224
58. Offield MF, Jetton TL, Labosky PA, Ray M, Stein RW, Magnuson MA, Hogan BL, Wright CV (1996) PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. Development 122:983–995
59. Ohlsson H, Thor S, Edlund T (1991) Novel insulin promoter- and enhancer-binding proteins that discriminate between pancreatic alpha- and beta-cells. Mol Endocrinol 5:897–904
60. Ohlsson H, Karlsson K, Edlund T (1993) IPF1, a homeodomain-containing transactivator of the insulin gene. EMBO J 12: 4251–4259
61. O'Rahilly R (1983) The timing and sequence of events in the development of the human endocrine system during the embryonic period proper. Anat Embryol (Berl) 166:439–451
62. Pang K, Mukonoweshuro C, Wong GC (1994) Beta cells arise from glucose transporter type 2 (Glut2)-expressing epithelial cells of the developing rat pancreas. Proc Natl Acad Sci USA 91:9559–9563
63. Pearse AG (1969) The cytochemistry and ultrastructure of polypeptide hormone-producing cells of the APUD series and the embryologic, physiologic and pathologic implications of the concept. J Histochem Cytochem 17:303–313
64. Pictet R, Rutter WJ (1972) Development of the embryonic endocrine pancreas. In: Geiger SR (ed) Handbook of physiology, sect 7: Endocrinology. Waverley Press, Baltimore, pp 25–66
65. Pittenger GL, Vinik AI, Rosenberg L (1992) The partial isolation and characterization of ilotropin, a novel islet-specific growth factor. Adv Exp Med Biol 321:123–130
66. Rafaeloff R, Pittenger GL, Barlow SW, Qin XF, Yan B, Rosenberg L, Duguid WP, Vinik AI (1997) Cloning and sequencing of the pancreatic islet neogenesis associated protein (INGAP) gene and its expression in islet neogenesis in hamsters. J Clin Invest 99:2100–2109
67. Rahier J, Goebbels RM, Henquin JC (1983) Cellular composition of the human diabetic pancreas. Diabetologia 24:366–371
68. Rooman I, Schuit F, Bouwens L (1997) Effect of vascular endothelial growth factor on growth and differentiation of pancreatic ductal epithelium. Lab Invest 76:225–232
69. Rosenberg L (1995) In vivo cell transformation: neogenesis of beta cells from pancreatic ductal cells. Cell Transplant 4: 371–383
70. Rosenberg L, Brown RA, Duguid WP (1983) A new approach to the induction of duct epithelial hyperplasia and nesidioblastosis by cellophane wrapping of the hamster pancreas. J Surg Res 35:63–72
71. Rosenberg L, Duguid WP, Vinik AI (1989) The effect of cellophane wrapping of the pancreas in the Syrian golden hamster: autoradiographic observations. Pancreas 4:31–37
72. Rosenberg L, Rafaeloff R, Clas D, Kakugawa Y, Pittenger G, Vinik AI, Duguid WP (1996) Induction of islet cell differentiation and new islet formation in the hamster – further support for a ductular origin. Pancreas 13:38–46
73. Rouiller DG, Cirulli V, Halban PA (1991) Uvomorulin mediates calcium-dependent aggregation of islet cells, whereas calcium-independent cell adhesion molecules distinguish between islet cell types. Dev Biol 148:233–242
74. Rudnick A, Ling TY, Odagiri H, Rutter WJ, German MS (1994) Pancreatic beta cells express a diverse set of homeobox genes. Proc Natl Acad Sci USA 91:12203–12207
75. Russu IG, Vaida A (1959) Neue Befunde zur Entwicklung der Bauchspeicheldrüse. Acta Anat 38:114–125

76. Sander M, German MS (1997) The beta cell transcription factors and development of the pancreas. J Mol Med 75:327–340
77. Sanvito F, Herrera PL, Huarte J, Nichols A, Montesano R, Orci L, Vassalli JD (1994) TGF-beta 1 influences the relative development of the exocrine and endocrine pancreas in vitro. Development 120:3451–3462
78. Sassone-Corsi P (1998) Molecular clocks: mastering time by gene regulation. Nature 392:871–874
79. Scharfmann R, Tazi A, Polak M, Kanaka C, Czernichow P (1993) Expression of functional nerve growth factor receptors in pancreatic beta-cell lines and fetal rat islets in primary culture. Diabetes 42:1829–1836
80. Shing Y, Christofori G, Hanahan D, Ono Y, Sasada R, Igarashi K, Folkman J (1993) Betacellulin: a mitogen from pancreatic beta cell tumors. Science 259:1604–1607
81. Sonnenberg E, Meyer D, Weidner KM, Birchmeier C (1993) Scatter factor/hepatocyte growth factor and its receptor, the c-met tyrosine kinase, can mediate a signal exchange between mesenchyme and epithelia during mouse development. J Cell Biol 123:223–235
82. St-Onge L, Sosa-Pineda B, Chowdhury K, Mansouri A, Gruss P (1997) *Pax6* is required for differentiation of glucagon-producing α-cells in mouse pancreas. Nature 387:406–409
83. Sternberg PW, Felix MA (1997) Evolution of cell lineage. Curr Opin Genet Dev 7:543–550
84. Stoffers DA, Zinkin NT, Stanojevic V, Clarke WL, Habener JF (1997) Pancreatic agenesis attributable to a single nucleotide deletion in the human *IPF1* gene coding sequence. Nat Genet 15:106–110
85. Teitelman G (1990) Insulin cells of the pancreas extend neurites but do not arise from the neuroectoderm. Dev Biol 142:368–379
86. Teitelman G, Lee GK, Alpert S (1987) Cell lineage analysis of pancreatic exocrine and endocrine cells. Cell Tissue Res 250:435–439
87. Teitelman G, Alpert S, Polak JM, 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
88. Ullrich A, Schlessinger J (1990) Signal transduction by receptors with tyrosine kinase activity. Cell 61:203–212
89. Upchurch BH, Aponte GW, Leiter AB (1994) Expression of peptide YY in all four islet cell types in the developing mouse pancreas suggests a common peptide YY-producing progenitor. Development 120:245–252
90. Waguri M, Yamamoto K, Miyagawa JI, Tochino Y, Yamamori K, Kajimoto Y, Nakajima H, Watada H, Yoshiuchi I, Itoh N, Imagawa A, Namba M, Kuwajima M, Yamasaki Y, Hanafusa T, Matsuzawa Y (1997) Demonstration of two different processes of beta-cell regeneration in a new diabetic mouse model induced by selective perfusion of alloxan. Diabetes 46: 1281–1290
91. Wang RN, Bouwens L, Klöppel G (1994) Beta-cell proliferation in normal and streptozotocin-treated newborn rats: site, dynamics and capacity. Diabetologia 37:1088–1096
92. Wang RN, Klöppel G, Bouwens L (1995) Duct-to-islet-cell differentiation and islet growth in the pancreas of duct-ligated adult rats. Diabetologia 38:1405–1411
93. Wang RN, Bouwens L, Klöppel G (1996) Beta-cell growth in adolescent and adult rats treated with streptozotocin during the neonatal period. Diabetologia 39:548–557
94. Wang RN, Rehfeld JF, Nielsen FC, Klöppel G (1997) Expression of gastrin and transforming growth factor-alpha during duct to islet cell differentiation in the pancreas of duct-ligated adult rats. Diabetologia 40:887–893
95. Wang TC, Bonner-Weir S, Oates PS, Chulak M, Simon B, Merlino GT, Schmidt EV, Brand SJ (1993) Pancreatic gastrin stimulates islet differentiation of transforming growth factor alpha-induced ductular precursor cells. J Clin Invest 92: 1349–1356
96. Weir GC, Clore ET, Zmachinski CJ, Bonner-Weir S (1981) Islet secretion in a new experimental model for non-insulin-dependent diabetes. Diabetes 30:590–595
97. Wessells NK, Cohen JH (1967) Early pancreas organogenesis: morphogenesis, tissue interactions, and mass effects. Dev Biol 15:237–270
98. Wright CV, Schnegelsberg P, De Robertis EM (1989) XlHbox 8: a novel *Xenopus* homeo protein restricted to a narrow band of endoderm. Development 105:787–794