Developmental biology of the pancreas

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SUMMARY

The pancreas is an organ containing two distinct populations of cells, the exocrine cells that secrete enzymes into the digestive tract, and the endocrine cells that secrete hormones into the bloodstream. It arises from the endoderm as a dorsal and a ventral bud which fuse together to form the single organ. Mammals, birds, reptiles and amphibians have a pancreas with similar histology and mode of development, while in some fish, the islet cells are segregated as Brockmann bodies. Invertebrates do not have a pancreas, but comparable endocrine cells may be found in the gut or the brain.

The early pancreatic bud shows uniform expression of the homeobox gene IPF-1 (also known as IDX-1, STF-1 or PDX), which when mutated to inactivity leads to total absence of the organ. The occurrence of heterotopic pancreas in the embryo, and also the metaplasias that can be displayed by a regenerating pancreas in the adult, both suggest that only a few gene products distinguish the pancreatic cell state from that of the surrounding tissues of duodenum, gall bladder and liver.

In the developing pancreatic buds, the endocrine cells start to differentiate before the exocrine cells, and co-expression of different hormones by the same cell is often observed at early stages. Although pancreatic endocrine cells produce many gene products also characteristic of neurons, evidence from in vitro cultures and from quail-chick grafts shows that they are of endogenous and not of neural crest origin. Observational studies suggest strongly that both endocrine and exocrine cells arise from the same endodermal rudiment.

Development of the pancreas in embryonic life requires a trophic stimulus from the associated mesenchyme. In postnatal life, all cell types in the pancreas continue to grow. Destruction of acinar tissue by duct ligation or ethionine treatment is followed by rapid regeneration. Surgical removal of parts of the pancreas is followed by moderate but incomplete regeneration of both acini and islets. Poisoning with alloxan or streptozotocin can lead to permanent depletion of β cells. Although the cell kinetics of the pancreas are not understood, it seems likely that there is a continuous slow turnover of cells, fed from a stem cells population in the ducts, and that the controls on the production rate of each cell type are local rather than systemic.

Key words: pancreas, acinar cells, ducts, islet cells, β (=B) cells, amylase, insulin, glucagon, somatostatin, pancreatic polypeptide, peptide YY, amylin, insulin promoter factor-1 (IPF-1), mesenchymal factor, metaplasia, streptozotocin

ANATOMY AND HISTOLOGY OF THE PANCREAS

The pancreas is a particularly important organ from the point of view of human medicine because it suffers from two important diseases: diabetes mellitus and pancreatic cancer. Diabetes affects at least 30 million people worldwide and despite the availability of insulin remains a major problem. Pancreatic cancer causes about 6500 deaths per annum in the UK and is virtually incurable. Despite this medical importance, the developmental biology of the pancreas has attracted only a small number of workers in recent years. An index of the lack of awareness is the fact that textbooks of histology, pathology and medicine invariably devote separate chapters to the exocrine and endocrine pancreas as though they were entirely separate organs. Although the lineage of the cell types is far from understood, this makes about as much biological sense as treating neurons and glia in the brain as though they belonged to different organs.

The name pancreas derives from the Greek roots ‘pan’ meaning ‘all’ and ‘creas’ meaning ‘flesh’. In humans it consists of an organ of 70-150 grams measuring 15-25 cm in length. It is connected to the duodenum by the ampulla of Vater, where the main pancreatic duct joins with the common bile duct (Fig. 1A). In the human, the terms head, neck, body and tail are used to designate regions of the organ from proximal to distal, while in rodents the shape of the pancreas is rather less well defined. The pancreas has its embryological origin as two buds developing on the dorsal and the ventral side of the duodenum. The ventral bud arises immediately adjacent to the hepatic diverticulum, and the dorsal bud arises on the opposite side of the gut tube (Fig. 1B). As the stomach and duodenum rotate, the ventral bud and hepatopancreatic orifice move around until they come into contact and fuse with the dorsal bud. The ventral bud forms the posterior part of the head, or uncinate process, while the dorsal bud forms the remainder of the organ. The ventral duct fuses with the distal part of the dorsal duct to become the main pancreatic duct (duct of Wirsung), and the proximal part of the dorsal duct becomes a small accessory duct.
duct (duct of Santorini) usually with its own opening into the duodenum.

The pancreas consists of two quite different types of glandular tissue, the exocrine cells that secrete enzymes into the intestine, and the endocrine cells that secrete hormones into the bloodstream. The exocrine pancreas is a lobulated, branched, acinar gland. The secretory cells are grouped into acini (Figs 1C, 2A) and are pyramidal in shape with basal nuclei, regular arrays of rough endoplasmic reticulum, a prominent Golgi complex and numerous secretory (zymogen) granules, containing the digestive enzymes. There are at least 22 of these, including proteases, amylases, lipases and nucleases. Most are secreted as inactive precursors and become activated after they enter the duodenum. The lumina of the acini are small and may be terminal or intercalary. At the junction of acini and ducts are low cuboidal centroacinar cells,
rich in mitochondria, that are thought to secrete non-enzymic components of the pancreatic juice, including bicarbonate. The ducts proper are lined with columnar epithelial cells, and in the larger ducts are found small numbers of goblet and brush cells similar to those of the intestine. Secretion of the pancreatic juice is regulated by hormonal stimulation, principally by secretin, cholecystokinin and gastrin, and also by neural stimuli. The acini and smaller ducts are invested with a delicate, loose connective tissue, which becomes more extensive around the larger ducts.

The endocrine cells are mainly grouped into the islets of Langerhans, which are compact spheroidal clusters embedded in the exocrine tissue. There are four principal types of endocrine cell. The \( \beta \) (or B) cells secrete insulin, and also an insulin antagonist called amylin, and make up the majority of cells in the islets (Fig. 2B). The \( \alpha \) (or A or A2) cells secrete glucagon (Fig. 2C), the \( \delta \) (or D or A1) cells secrete somatostatin (SS), and the PP (or F) cells secrete pancreatic polypeptide (PP). PP-rich islets are found particularly in the posterior part of the head, derived from the ventral pancreatic bud of the embryo. A proportion of the adult islet cells make peptide YY in addition to their principal product (Al Rachedi et al., 1984), and devotees of early embryo research will be interested to learn that activin A is abundant in the A and D cells (Yasuda et al., 1993). In rodents there is quite a sharp segregation within the islets such that the \( \beta \) cells lie in the centre and the other types at the periphery, while in humans this segregation, although present, is less clearcut. The proportion of endocrine cells is a small fraction of the total, about 4% of total cells in the adult rat (Githens, 1988). Endocrine cells making glucagon, somatostatin, amylin and peptide YY are present not only in the pancreas but also elsewhere in the gut. All the types of islet cell, in addition to their specific hormones, also express a number of gene products characteristic of neuroendocrine cells, such as neuron-specific enolase, tetanus toxin receptor, A2B5 antigen (Le Douarin, 1988) and the homeodomain-LIM protein islet-1 (Thor et al., 1991).

Insulin is a dimeric SS linked protein. It is synthesised as a single chain precursor which first loses its signal peptide, then loses a segment known as the C-peptide, before becoming the mature hormone molecule. The mature insulin is stored in secretory granules and its release is controlled by the level of glucose in the perfusing blood. The effects of insulin on the target tissues are both metabolic, particularly in promoting glucose uptake, and mitogenic. It acts via a tyrosine kinase type receptor which has as a major intracellular target a \( 170 \times 10^6 \text{ M}_r \) protein called insulin receptor substrate 1 (IRS-1; Siddle, 1992). This may be an intermediate in the stimulation of phosphatidylinositol 3’ kinase and the MAP kinase pathway, but the relatively mild phenotype of the IRS-1 knockout mouse suggests the existence of parallel pathways of signal transduction (Lienhard, 1994).

There are two main types of diabetes mellitus. In type 1 or insulin-dependent diabetes, found most often in children and young people, the \( \beta \) cells are destroyed by an autoimmune reaction and severe permanent insulin deficiency results. Type 2 or non-insulin dependent diabetes, more often found in older people, is a more complex and heterogeneous range of conditions, usually involving a degree of insulin non-responsiveness in the target tissues. In some cases there may be a contribution from pancreatic pathology as the islets are often invested with a deposit of amyloid. The major component of this amyloid is a precipitated form of amylin, a peptide belonging to the calcitonin family that is made by the \( \beta \) cells (Edwards and Morley, 1992; Rink et al., 1993). Pharmacological doses of amylin can inhibit both the secretion and action of insulin and it has been suspected of playing a role in the pathogenesis of type 2 diabetes.

In addition to the glandular components, the pancreas has a rich blood supply, the arterial blood passing in each lobule first to the islets and then to the adjacent acini. There is also an extensive lymphatic drainage, and a rich sympathetic and parasympathetic nerve supply. Smooth muscle is found around the larger ducts and in the sphincter muscles of the two ampullae. The fibroblastic, lymphatic and smooth muscle components of the pancreas are presumed to arise from the abundant mesenchyme enveloping the embryonic buds.

CELLULAR HOMOLOGUES IN LOWER VERTEBRATES AND INVERTEBRATES

The description given above for rodents and human is also broadly applicable to birds, reptiles and amphibians. However the situation in other animals is more complex and has been expertly reviewed by Falkmer (1985). When assessing homologies it must be borne in mind that the identification of endocrine cells is based mainly on immunostaining with antibodies prepared against mammalian hormones, and in most cases little more is known of the hormones or the cells that make them except that they react with such antibodies.

Protostome-type invertebrates have nothing resembling a pancreas. PP-containing cells have been described in the brain of a flatworm, and SS-cells in the brain of an earthworm. In insects there are cells containing each of the four pancreatic hormones in the brain, and there are also SS- and PP-cells in the gut in some species. Tunicates and amphioxus, which are invertebrate chordates, have insulin, glucagon, SS and PP-cells present in the gut mucosa. SS-, PP- and glucagon cells, but not insulin cells, occur also in the brain. In the hagfish, which represents the primitive jawless vertebrates, there are some enzyme-secreting cells in the gut mucosa, and there is a separate islet-organ at the junction of the gut and bile duct. This consists almost entirely of insulin cells, with a few SS-cells. More SS-cells are found in the bile duct, and glucagon and PP-cells are found in the gut mucosa. The bony fish have a conventional exocrine pancreas but the endocrine tissue is often gathered into a separate structures called Brockmann bodies that lie adjacent in the mesentery. In some cases (e.g. the Goby) there is a single large Brockmann body, in others there may be several, or there may be additional disseminated islets. Among the cartilaginous fish, there is a distinct exocrine pancreas with islets, but in bradyodonts (e.g. ratfish) they contain only insulin, glucagon and SS cells, while PP cells are found in the gut. In selachii (sharks and rays), as in higher vertebrates, all four types are found in the islets.

If this sequence is regarded as one of evolutionary descent then we might be tempted to say that the pancreatic endocrine cells ‘migrated’ from the brain to the gut, and that they then collected themselves together in a particular gut specialisation, the exocrine pancreas. However we face the usual problem that
all of the animals that can be examined today are contemporary species, and even with molecular sequence trees it has proved very difficult to order the divergence times of the different major groups of animal in evolution. So we should not necessarily assume that the condition found in invertebrates today was that also present in the ancestor of all vertebrates 600 million years ago.

From a developmental point of view the comparative data present us with two distinct questions about mechanisms. Firstly, does the association of pancreatic endocrine cells with neuroendocrine cells in lower animals mean that pancreatic endocrine cells in mammals are of neural crest origin? This possibility has been seriously considered by some (see review by Le Douarin, 1988), although it is probably not correct (Le Douarin and Teillet, 1973; Andrew, 1976). Secondly, even if the neural crest is not involved, does the segregated nature of the endocrine organ in the hagfish, or in certain teleosts, mean that the endocrine and exocrine cells of the pancreas in mammals have distinct embryological origins? There are precedents for such a conclusion. For example, it is well known that the mammalian adrenal medulla is of neural crest origin while the adrenal cortex is of mesodermal origin. Fish have distinctly separate suprarenals, homologous to the medulla, and interrenals, homologous to the cortex. Likewise, the thyroid gland in mammals contains calcitonin secreting C-cells, while in birds these cells are found in a separate, persistent, ultimobranchial body. We shall consider the embryological evidence relating to the origin of the pancreatic endocrine cells in a later section.

There are some interesting echos in mammals of the presence of insulin cells in the brains of invertebrates. In rats and mice there are two similar but non-allelic insulin genes, and the insulin gene 2 is expressed in the brain during embryonic life (Deltour et al., 1993; Devaskar et al., 1993). A possibly associated fact is that the insulin promoter will drive transgene expression in the neural tube as well as in the pancreatic rudiments (Alpert et al., 1988; Douhet et al., 1993). Moreover, islet cells express a whole variety of typically neural markers, and in culture but not in vivo, will extend long neurofilament-containing processes (Teitelman, 1990). So, even if there is no direct embryological connection between the brain and the pancreas, there are certainly some common elements of genetic regulation. Both insulin genes are also expressed in the yolk sac of the mouse embryo, and this expression has recently been found to be imprinted, only the paternal allele being active (Giddings et al., 1994).

The insulin-like growth factors, IGF-I and IGF-II, are proteins related in sequence to insulin. They are synthesized in many tissues around the body and tend to have stronger mitogenic and weaker metabolic effects than insulin itself (Nielsen, 1992).

### MORPHOLOGICAL DEVELOPMENT IN THE EMBRYO

A detailed morphological description of early pancreatic development is given in Wessells and Cohen (1967), Pictet et al. (1972) and Pictet and Rutter (1972). Events in the rat and the mouse are similar in relation to somite number for which the timescale is approximately as shown (times in this article are corrected to the convention that the day of the appearance of the vaginal plug=0.5).

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In the early rodent embryo the endoderm is on the outside of a cup-shaped structure. The notochord is embedded in the endoderm and there is close contact between both tissues and the neural plate. The anterior intestinal portal forms at 2-3 somites, and the turning of the embryo, at about 13 somites, brings the midgut to the ventral side and helps to close it. At about this stage the notochord separates from the endoderm (Fig. 3A) and from about 13 to 20 somites they are separated by the dorsal aorta (Fig. 3B). Throughout these stages the gut epithelium consists of a single layer of columnar cells. There is a thick layer of splanchic mesoderm on either side of the gut but none on the dorsal side. During the phase 20-25 somites, mesenchyme pushes around the dorsal side of the gut and a dorsal pancreatic bulge starts to form (Fig. 3C). In vitro cultures from this stage indicate that this bulge is specified to become pancreas (Wessells and Cohen, 1967). The ventral
bulge appears at about 30 somites and the course of its develop-
ment is exactly the same as that of the dorsal bud although lagging slightly in time (Spooner et al., 1970). Although the epithelial part of the early pancreas appears to consist of a solid mass of cells (Fig. 4A), at the ultrastructural level it can be seen to consist of a highly folded epithelial sheet with apical and basal surfaces in continuity with those of the gut tube (Pictet and Rutter, 1972). It is sometimes said that the pancreas arises from the midgut. Strictly it arises from the foregut since the foregut is defined as that part of the gut cranial to the anterior intestinal portal. However, the fate map of the endoderm is poorly understood and it is not clear whether the foregut-midgut boundary separates persistent cell populations or whether it sweeps across a common cell population as the intestinal portal narrows.

As the buds grow they rapidly form new protrusions leading to a highly branched structure (Figs 4B,C, 5A-D). Acini and ducts become clearly visible as histologically differentiated structures by about 14.5d in the mouse. Low levels of the various terminal differentiation products can be detected throughout pancreatic development (the ‘protodifferentiated state’ see Pictet and Rutter, 1972, further extended to the RT-PCR level in Gittes and Rutter, 1992), but amylase becomes detectable by immunostaining from about 14.5d (Fig. 6B,C). Endocrine cells can be detected in the forming pancreas from the earliest stages (see below). By 15.5d they make up a substantial proportion of the total cells, maybe 10%. They are still largely individual and associated with the ducts (Fig. 6A) and are only found as islets towards the end of gestation, at about 18.5d in the mouse (Herrera et al., 1991). Innervation with ganglion cells and unmyelinated fibres commences about the 50 somites stage, and vascularisation from about 14d in mouse.

Fig. 4. (A) Part of the gut of an 11.5d mouse embryo from which the mesenchyme has been removed by enzyme treatment. s, stomach; dp, dorsal pancreatic bud. (B) Dorsal bud at 11.5d. (C) Dorsal pancreas at 12.5d. (D) Dorsal pancreas at 16.5d, acini and ducts are now clearly visible.

Fig. 5. Diagrams showing the progressive branching that builds up the tree-like structure of the pancreas. (A) 9.5d. (B) 10.5d. (C) 12.5d. (D) 15.5d in mouse. Figure adapted from Pictet and Rutter (1972).
EXPRESSION OF MOLECULAR MARKERS IN THE MOUSE EMBRYO

The entire early pancreatic rudiment and part of the surrounding gut tube expresses the homeobox gene IPF-1 (otherwise known as IDX-1, SF-1 or PDX: Ohlsson et al., 1993; Miller et al., 1994; Guz et al., 1995). This is a homologue of the Xenopus gene XlHbox-8, which is also expressed in the same position in the tailbud-stage Xenopus embryo (Wright et al., 1988). This gene probably has a key role in pancreatic determination because when it is removed from mice by targeted mutagenesis, the embryos completely lack a pancreas (Jonsson et al., 1994). In adult life this gene is expressed only in the β cells and is believed to function as a transcription factor for insulin. It is interesting that this gene is expressed early on in the whole pancreas and is later restricted to the islets. This is in accordance with the apparent sharing of genetic control elements between the two cell types, for example, a segment of the elastase enhancer has a quantitative effect on expression in acinar cells but on its own drives expression in islet cells (Kruse et al., 1993). Other transcription factors expressed in the early pancreas, as well as in other parts of the body, include prox-1 (Oliver et al., 1993), Tlx-1 (Raju et al., 1993) and pax6 (Turque et al., 1994) which all belong to the homeobox gene group.

The whole early rudiment also expresses the enzyme L-amino acid decarboxylase (AADC), which becomes confined to islet cells postnatally (Teitelman et al., 1987). Some endocrine cells in the early pancreas express the enzyme tyrosine hydroxylase, although this is not an invariable characteristic (Teitelman et al., 1987).

The appearance of cell type-specific products has been studied using RT-PCR, by Gittes and Rutter (1992). They found that somatostatin mRNA could be detected from 8.5d, insulin and glucagon mRNA from 9.5d, pancreatic polypeptide mRNA from 10.5d and amylase mRNA from 12.5d. Three immunostaining studies on the formation of the endocrine cells have recently been published (Herrera et al., 1991; Teitelman et al., 1993; Upchurch et al., 1994). There are a number of differences in their results which seem to arise from the differing specificities of the antibodies used and the different methods of tissue fixation and processing. However, certain common conclusions emerge. The first cells containing insulin and glucagon appear around 9.5d, and over the next 2-3 days cells are frequently found expressing both hormones together. Most or all of the endocrine cells also express a ‘PP fold’ peptide, probably peptide YY. Cells expressing somatostatin or pancreatic polypeptide appear rather later in development. Endocrine cells are initially visible as scattered individual cells, often embedded in ducts. Later they are visible in clusters, and towards the end of gestation as typical islets (Fig. 7A-C). The rate of increase of numbers of the endocrine cells could be accounted for by multiplication of the precursors present early on, but a cell kinetic study in the rat by Kaung (1994) suggested recruitment into the endocrine population both before and after birth.

Although the authors cited all make claims about the cell lineage, descriptive studies of this sort cannot prove anything for sure. This is because cells within one lineage may turn particular genes on and off at different times so there is no guarantee, for example, that the cells expressing insulin at 11.5d are precursors of mature β cells. Furthermore cells can move about, so the place where they reside at the time of detection may not be the position of origin. However, it does look as though immature endocrine cells may be characterised by expression of a PP fold peptide, probably peptide YY, and
it seems likely that the earliest endocrine cells may co-express insulin and glucagon. It is not at all clear whether new islet cells continue to be formed throughout embryonic development, but if they are, it would seem unlikely that a cell co-expressing insulin and glucagon could be an obligate precursor to the mature types since such cells are only found at the earliest times.

EXPERIMENTAL STUDIES OF CELL LINEAGE

Although the descriptive studies referred to above have been used as a basis for various models, proof can only come from experimental approaches in which cell lineages can be traced through development. The early experiments involved culture of pancreatic rudiments in vitro (Wessells and Cohen, 1967). This showed that the dorsal bulge, dorsal to the forming liver, at the 20-25 somite stage was the region that formed a high proportion of acini in vitro, while segments of the gut from either side did not. This is good evidence for identifying this bulge with the later dorsal bud, although the location of the prospective pancreatic region at earlier stages is not known. Unpublished results quoted by Pictet and Rutter (1972) suggest that both exocrine and endocrine cells can arise in vitro from isolated epithelium. They also reported that both tissues could be formed from rudiments isolated before the arrival of blood vessels or nerves, showing that interactions with these later arriving tissues are not necessary for differentiation. Evidence against a neural crest origin for the endocrine cells was produced by Andrew (1976). She grafted quail neural tube into chick embryos and after differentiation of the pancreas, examined it for the presence of quail cells. She found that the endocrine cells of the pancreas did not show the quail nucleolar marker, although the sympathetic ganglia associated with the nearby parts of the gut did so. These results all suggest that endocrine cells arise from the endodermal epithelium, rather than from the neural crest, or from the mesenchyme, but leave open the possibility that there may be discrete rudiments for exocrine and endocrine tissue within the epithelium. Only one study has looked at mouse aggregation chimaeras (Deltour et al., 1991). This showed that the β cells of a single islet could be derived from both components of the chimera, suggesting that islets arise by aggregation of cells rather then by monoclonal growth from individual progenitors. This is consistent with the descriptive studies showing that the first endocrine cells arise as individuals and islets form at a relatively late stage of gestation.

The other recent lineage studies have all involved the production of transgenic mice, either stable lines or transient transgenics, in which a promoter for one of the endocrine hormones is used to drive a modifying gene or a toxin (Fig. 8). In both designs it is necessary to assume that the promoter drives the transgene with the same tissue-specificity as its own normal gene. This is not necessarily the case, and is particularly uncertain in transient transgenics in which each embryo is likely to have a different number of transgene copies and have them inserted at a different position in the genome. Unfortunately no pure reporter study has been done, but the RIP-Tag mice made by Alpert et al. (1988), in which the rat insulin promoter is used to drive the SV40 T antigen, approximate to a reporter study since the biological effects of the T antigen are modest in embryonic life. In the embryo, most of the endocrine cells express T antigen, while post-natally only the β cells do so, which supports the idea of an early endocrine progenitor in which several or all the hormones are expressed. A similar study by Upchurch et al. (1994) utilised the peptide YY-promoter. Here T antigen was expressed from 12d in cells positive for peptide YY, and cells co-expressing T antigen and one of each of the four principal hormones were found during embryonic life. This is consistent with the proposition that all endocrine precursors express peptide YY at an early stage.

The toxin experiments have used a suitable promoter to drive transcription of the diphtheria A chain. This toxin is
very potent enzyme causing adrenoribosylation of elongation factor 2, hence blockage of protein synthesis and cell death. Theoretically the effect should be cell autonomous as the toxin lacks the B chain normally required for entry into intact cells. When the elastase promoter was used, the result is mice lacking almost all exocrine pancreas and with a reduced complement of ductal tissue and islets (Palmiter et al., 1987). In a transient transgenic study, Herrera et al. (1994) used promoters for insulin, glucagon and pancreatic polypeptide to drive the toxin. The insulin and glucagon constructs depressed only the numbers of their own endocrine type, while the PP-construct depressed all types. This is rather odd, as PP itself is supposed to be expressed only in the late embryo. An associated study using human growth hormone as a reporter showed that the putative PP promoter drove expression in 12% of embryonic PP fold peptide expressing cells, but it is not clear whether these are a subset of the peptide YY cells or precocious PP cells. In general the transgenic studies to date seem bedevilled by uncertainties both about the expression of the transgene and about the cell autonomy of the ablation effects.

THE PANCREATIC STATE

Assuming that the principles of development as established by work on early embryos holds also for organ development, there must be a region within the endoderm committed to form the pancreas at some stage before the appearance of the first terminal differentiation products. This region presumably consists of a set of cells committed by the expression of a particular combination of transcription factors, this combination being the ‘epigenetic code’ for the pancreas. Since there are still uncertainties about the cell lineage, we cannot be sure whether there are just two such regions (the future dorsal and ventral buds), or whether these regions are themselves further subdivided, for example into exocrine and endocrine components.

Obviously, it would be good to know the identity of the genes involved in the epigenetic code for the pancreas. One important component is likely to be the IPF-1 gene referred to above, since targeted ablation results in the total absence of the pancreas. This gene is, however, expressed in a wider domain than the future pancreatic buds themselves, so cannot itself represent the entire pancreatic code. One way of filling in the remainder is to look at new expression patterns as they are published, and see which transcription factors have boundaries in the appropriate places. Some of these have been mentioned above (prox-1, Tlx-1, pax-6), but there are others such as the HNF-3 (forkhead domain) family whose expression patterns form a nested set in the endoderm suggesting that they may act to pattern the endoderm in an analogous way to the patterning of the ecto- and mesoderm by the HOX genes (Monaghan et al., 1993).

The other way of investigating the pancreatic state is to ask whether pancreas can be turned into other tissues, or vice versa, since such transformations are likely to result from the change of activity of one or a few genes and would therefore indicate which other tissues had similar epigenetic codes. There are two types of transformation of this sort. Firstly there is pancreatic heterotopia, which occurs during human embryonic development in about 2% of people examined post-mortem and is most common in the stomach, the duodenum, the jejunum, the ileum, the vitellointestinal duct and the gall bladder (Branch and Gross, 1935; de Castro Barbosa et al., 1946). Such heterotopic patches usually have ducts opening into the gut lumen, and the presence of a duct strongly suggests in situ differentiation rather than migration from elsewhere. Heterotopic pancreas does not occur elsewhere in the body, so it seems reasonable to suppose that the stomach, intestine and bile duct have epigenetic codes closer to pancreas than does any other tissue. This is quite plausible embryologically as these are all tissues derived from nearby parts of the endodermal epithelium.

It is also possible to bring about one transformation experimentally, which is the metaplasia of pancreas to liver. If hamsters are fed on a diet containing ethionine instead of methionine, this causes severe damage to the exocrine pancreas. Restoration of methionine provokes tissue regeneration and if the animals are simultaneously treated with a mutagen, then numerous foci of hepatocytes develop within the pancreas (Scarpelli and Rao, 1981). A similar effect can be achieved in rats by means of prolonged copper deprivation. This causes substantial ablation of acinar tissue while leaving ducts and islets unchanged. Restoration of copper provokes regeneration leading to substantial hepatic development (Fig. 9; Rao et al., 1990). Early signs of hepatic differentiation occur in both periductal and interstitial cells Rao et al., 1989). In these experiments there is also some intestinal metaplasia.

GROWTH CONTROL OF THE EMBRYONIC AND POSTNATAL PANCREAS

The evidence reviewed above suggests that the mesenchyme does not contribute cells to either the exocrine or the endocrine pancreas. It does, however, have an important role in the control of growth and differentiation, the embryonic pancreas presenting us with a classic example of permissive induction. Golosow and Grobstein (1962) found that an 11.5d mouse pan-

Fig. 8. Diagram to show the logic of transgenic reporter and transgenic ablation studies. In this model, X, Y and Z are precursor cell types and A,B,C are terminal cell types. The Y to B transition needs a permissive inductive signal from C. The tables show which of the terminal types would be labelled or ablated in a transgenic strain in which the reporter or toxin is driven off an X,Y or Z specific promoter.
creatinic bud would develop very well in vitro, but that the isolated epithelium would not grow or differentiate in the absence of mesenchyme. They showed that salivary mesenchyme was even more effective than pancreatic mesenchyme and that the effect could be exerted across a Millipore filter, suggesting that the mesenchyme secreted a soluble growth factor needed by the epithelium. In some circumstances the mesenchyme could be replaced by a chick embryo extract, the necessary factor being trypsin-sensitive (Rutter et al., 1964). Wessells and Cohen (1967) further refined this picture by showing that there was a requirement for pancreatic mesenchyme at the earliest stage (6-10 somites), for heterologous mesenchyme later on (15 somites) and eventually just for embryo extract (30 somites). A partial purification of the mitogen from chick embryos was achieved by Ronzio and Rutter (1973), but the identity of the ‘mesenchymal factor’ has remained elusive to this day. It may in fact be a mixture rather than a single substance. Recent studies by Sanvito et al. (1994) show no effect on pancreatic bud cultures of bFGF, rhHGF, IGF-II, PDGF, or NGF. EGF tended to promote ductal development, and TGFβ-1 promoted endocrine development, but as the cultures are quite long term these may be selective effects on the cell populations rather than direct controlling effects on the developmental pathways.

Growth control in the postnatal pancreas has been a subject of research since the nineteenth century, and a remarkable number of earlier studies are mentioned by Hughes (1956). A brief and more up to date summary is provided by Goss (1978). The pancreas grows with the animal although it becomes somewhat reduced as a proportion of total body weight. During growth the number of islets increases, according to Hughes (1956) from 664 in the newborn rat to 4673 in the adult. However the proportion of islet cells falls, in the rat reaching a plateau of about 4% of total cells (2% total area) by 3 months (McEvoy, 1981). Studies of mitotic index, or of labelling by tritiated thymidine, bromodeoxyuridine (BrdU) or antibody to proliferating cell nuclear antigen (PCNA), all show low but measurable levels of cell division in the acini, islets and ducts (Wenzel et al., 1972; Githens, 1988; Muller et al., 1990; Elsässer et al., 1994). Because it is not possible to calculate the cell production rate from labelling studies alone we do not know whether this rate of division is just enough to provide for the growth of the organ, or whether there is some overproduction accompanied by some cell turnover.

It is possible to alter the growth of the pancreas, or of its individual cellular constituents, by various types of experimental intervention. We shall consider first the methods for augmenting growth and then the types of regeneration seen following selective or generalised ablation.

It has been known since the 1940s that pancreatic hypertrophy can be produced by feeding animals on a diet rich in soy beans. This is due to the existence of a trypsin inhibitor in the food, which neutralises the trypsin produced by the pancreas and thereby lifts the normal inhibition of cholecystokinin (CCK) secretion by trypsin. As mentioned above, CCK stimulates secretion of pancreatic juice, but it also has a direct trophic effect on the acinar cells, as does its analogue cerulein (Elsässer et al., 1994). The growth stimulation produced by CCK or cerulein is rapid and substantial. A different sort of effect on the acini is produced in transgenic mice overexpressing transforming growth factor α (TGFα) on an elastase or a metallothionein promoter (Sandgren et al., 1990; Jhappan et al., 1990). This also increases the size of the pancreas, but mainly by fibrosis, there being a considerable pseudoductular metaplasia of the acini.

As far as the islets are concerned, there is some hyperplasia during pregnancy in diabetic mothers. This has been ascribed to the high levels of glucose in the foetal circulation, but it has been difficult to reproduce the effect in animals and so it is not known whether it represents a direct or indirect effect of glucose (Hellerström and Swenne, 1985). Measures that provoke a continuous inflammation seem to lead to some new islet formation (‘nesidioblastosis’) by budding from ductules. One way of provoking this is to wrap the head of a hamster pancreas with cellophane tape such that there is a stasis of secretions in the smaller ducts (Rosenberg et al., 1983). This leads to a local inflammation and fibrosis. The ducts show hyperplasia and goblet cell metaplasia and there is some budding of islets from hyperplastic ductules. A rather similar result seems to be produced in transgenic mice in which interferon γ is driven off an insulin promoter (Gu and Sarvetnik, 1993; Gu et al., 1994). This provokes an autoimmune attack on the β cells and leads to continuous inflammation and tissue destruction, with consequent goblet cell and hepatic metaplasia. In this case the origin of the new β cells from the ducts is supported by the kinetics of labelling with BrdU. As in

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**Fig. 9.** Metaplasia of pancreas into liver. A, residual acini; I, islet; D, duct, arrows show clusters of hepatocytes. From Rao et al. (1989), photograph kindly provided by Dr Rao.
embryonic life, a proportion of newly formed endocrine cells coexpress more than one hormone.

The most traditional method for destroying the pancreas is the ligation of the main duct. This prevents the outflow of the pancreatic secretions and although most of the enzymes are in the form of inactive precursors, enough activity is presumably released to lead to a rapid destruction of the acinar tissue, and a slower destruction of ducts and islets (Hughes, 1956; Goss, 1978). Prolonged ligation leads to a permanent and irreversible fibrosis. A temporary ligation represents a rough and ready way of ablating most of the acinar cells while sparing most of the ducts and islets. A similar result can be achieved by administering the protein synthesis inhibitor ethionine in combination with a protein free diet (Fitzgerald et al., 1966). Once the obnoxious stimulus has been removed, any of these treatments is followed by a rapid regeneration of the acinar tissue, with complete restitution after a few weeks.

This sort of result shows that the acinar tissue of the adult has the capacity for rapid regenerative growth. However this is not so apparent following surgical removal of parts of the pancreas. It is possible to remove up to 90% of the rat pancreas without serious effects on the animal’s growth rate. There is some regeneration, defined as growth of the remnant in excess of that shown by the same region of the pancreas in sham-operated animals, but the total organ size is never restored to that of the controls (Friedmann and Marble, 1941; Lehv and Fitzgerald, 1968; Sidorova, 1978). There is an elevation of mitotic index both in the acinar and the islet cells (Brockenbrough et al., 1988), but this dies away after the first two weeks whereas the slight growth advantage of the remnant persists for about 6 months. Once again we see that the growth rate cannot be deduced from the labelling index alone. There is some sprouting of ducts at the cut surface of the organ and probably some new formation of both acini and islets from them. Islet sprouting of ducts at the cut surface of the organ and probably be deduced from the labelling index alone. There is some sprouting of ducts at the cut surface of the organ and probably some new formation of both acini and islets from them. Islet sprouting of ducts at the cut surface of the organ and probably some new formation of both acini and islets from them.

Animals can survive the removal of 90% of the pancreas, but removal of 100% necessitates insulin therapy. The appetite for such studies seems to have waned since the available references are very old, but they all maintain that the residual duct remnant can regenerate substantially and that the cut surface can produce clumps of pancreatic tissue including both acini and islets (Fisher, 1924; Grauer, 1926; Shaw and Latimer, 1926).

The last method of cell ablation in the pancreas that we shall consider is the specific destruction of β cells by treatment with alloxan or streptozotocin. These substances have been widely used in order to produce animal models of diabetes (Chang and Diani, 1985). Both produce a rapid necrosis of β cells, and since streptozotocin has somewhat fewer side effects it is more often used today than alloxan. It is a glucose-conjugated nitrosourea, which presumably gains access to the β cells by the mechanism that senses the blood glucose level. Its action can be prevented by previous treatment with 3-deoxyglucose or nicotinamide. If given in a sufficiently large amount, a single dose will destroy most of the β cells and induce a permanent severe diabetes. Despite some reports of increases of labelling index among the surviving β cells, there seems to be no significant recovery in β cell numbers (Brosky and Logothetopoulos, 1969; Hamming and Reynolds, 1977). A lower dose will kill fewer cells and provoke a less severe diabetes, but this is also permanent (Steiner et al., 1970; Bonner-Weir et al., 1981).

So, acinar cell destruction is followed by rapid regeneration, surgical ablation by limited regeneration and β cell ablation by almost no regeneration at all. On the face of it, it is surprising that the different cellular components of the pancreas show such very different behaviour. The existing data do not make it possible to propose a definitive model for postnatal pancreatic growth control, but certain points are fairly clear. First, it seems that all the cell types have some division potential. Secondly, it seems that there is no systemic means of sensing the total size of the organ. In the liver there is such a mechanism and it leads to rapid regeneration of a remnant back to its original size within a matter of days. In the case of the pancreas the relative health of animals suffering loss of large parts of the organ suggests that the normal size represents a considerable functional excess capacity for both exocrine and endocrine functions. However, thirdly, there do exist pathological stimuli that can increase growth of acini, ducts or islets, so there must exist local signals that are potentially capable of promoting growth in the postnatal animal. Descriptive evidence can never be conclusive in relation to the cell lineage, but it can be suggestive, and does seem likely that the ducts represent or contain the stem cells for the whole organ (Githens, 1988). Acini and islets budding from hyperplastic ducts have been described in many circumstances, including duct ligation, surgical ablation, cellophane wrapping, ethionine treatment and the interferon γ transgenics. A ductal origin for the other cell types would also be consistent with the likely state of affairs in the embryo. The failure of regeneration following treatment with streptozotocin or alloxan may suggest that these drugs target the potential stem or transit cells as well as the differentiated β cells. It is not possible to know whether the pancreas is simply an ‘expanding compartment’, as proposed by Leblond (1964), or whether there is a slow renewal of cells fed from a stem cell population. Our understanding of other tissue renewal systems shows that there is no particular need for the stem population to have a higher cell division rate than the rest. Cell numbers and proportions are often regulated by changing the size of transit compartments rather than by speeding up division of the stem cells (Wright and Allison, 1984). We do not know whether there actually are stem cells, or transit compartments, or whether the division potential of the differentiated cells is limited, or how much cell turnover there is. In short we know very little about growth control in the pancreas despite the considerable period of time over which the existing data have been collected.

CONCLUSIONS

The data reviewed above make it pretty certain that the
exocrine and the endocrine components of the pancreas are both derived from the endoderm, and that the pancreas is thereby a single integrated organ from a biological point of view. Beyond this we know little for sure. It is not yet possible to exclude a separate endocrine rudiment within the endoderm, although this may seem unlikely. It is not clear whether there is at any stage an identifiable population of endocrine precursor cells with common properties. If there is such a population at an early embryonic stage it is not known whether it alone produces all the endocrine cells or whether there is continuous recruitment from ductal or other undifferentiated epithelium.

Furthermore we do not know whether there is any obligatory sequence of intermediate types leading to each of the terminal endocrine cell types.

In the area of growth control it is remarkable that a substance as well known as the pancreatic mesenchymal factor should so long have eluded identification. This may of course be because it is not a single substance but a complex mixture, or may just be because the great juggernaut of scientific fashion deserted it before modern growth factor purification technology had become available. The various phenomena of postnatal growth and regeneration are very interesting but also perplexing. The acinar tissue has a high regenerative potential following generalised damage but a much lower one following surgical removal. Islets seem to regenerate better following surgical ablation than following treatment with streptozotocin or alloxan. There is considerable descriptive evidence for the formation of both acini and islets from ductal tissue. A better understanding of these processes must hold out some promise for better therapy for type 1 diabetes, either by provoking β cell regeneration or by being able to culture and graft β cells between individuals.

In recent years, centre stage in developmental biology has been held by the mechanisms of patterning in the early embryo. Massive progress has been achieved, both with Drosophila and with vertebrates. In this period the study of organ development has become something of a poor relation. However, since the important embryological work of Grobstein, Wessells and Rutter in the 1960s, there has been an enormous increase in technical facilities. Many new antibodies and probes are now available for cell identification. Tissue culture media are much improved and many pure growth factors and matrix components are available. Last but not least, there is in the mouse an explosion in the potential availability of tissues and cells genetically modified by transgenesis or targeted mutagenesis. Like the other problems of organ development, our study of the pancreas should benefit greatly from these innovations and we can expect to see rapid progress over the next few years.

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