Development 127, 2317-2322 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
DEV2561

Adult insulin- and glucagon-producing cells differentiate from two independent cell lineages

Pedro Luis Herrera

Department of Morphology, University of Geneva Medical School, 1, rue Michel-Servet, 1211 Geneva 4, Switzerland
Author for correspondence (e-mail: pedro.herrera@medecine.unige.ch)

Accepted 16 March; published on WWW 10 May 2000

SUMMARY

To analyze cell lineage in the pancreatic islets, we have irreversibly tagged all the progeny of cells through the activity of Cre recombinase. Adult glucagon (α) and insulin (β) cells are shown to derive from cells that have never transcribed insulin or glucagon, respectively. Also, the β-cell progenitors, but not α-cell progenitors, transcribe the pancreatic polypeptide (PP) gene. Finally, the homeodomain gene PDX1, which is expressed by adult β-cells, is also expressed by α-cell progenitors. Thus the islet α- and β-cell lineages appear to arise independently during ontogeny, probably from a common precursor.

Key words: Mouse, Transgenic, Cre, Pancreas, Cell lineage

INTRODUCTION

The pancreatic islets of Langerhans contain four different types of endocrine cells: insulin- (β), glucagon- (α), somatostatin- (δ) and pancreatic polypeptide- (PP) producing cells (Orci, 1982). Given the early presence of α-like cells in the pancreatic primordia (Rall et al., 1973), and the reported colocalization of glucagon and other hormones in cell subsets during development (Alpert et al., 1988; Herrera et al., 1991; Rall et al., 1973; Teitelman et al., 1993), it has been proposed that all islet cell types arise from a common precursor, probably α-like.

To determine cell lineages conclusively, we used a targeted somatic gene rearrangement catalyzed by the Cre recombinase, a technique allowing a permanent labeling of all cells arising from a given progenitor. Thus, to irreversibly tag cells that transcribe the insulin, glucagon or PP genes, or the earliest pancreatic stem cells characterized to date, which express PDX1 (pancreatic and duodenal homeobox factor 1), we employed the Cre/loxP system (Sauer, 1994, 1998). Adult islet β- and α-cells were then examined to determine whether they are derived from these 'tagged' cells.

MATERIALS AND METHODS

Preparation of hGH-encoding transgenes

InsPr-loxP-STOP-loxP-hGH

An *Eco*RI-*Msc*I 1.5 kb fragment from pBS302 (GibcoBRL, no. 10349-017) containing the loxP-STOP-loxP cassette was cloned in *Eco*RI-*Eco*RV of pBS-KS; it was then cropped with *Bam*HI(partial)-*Acc*I and inserted at *Bgl*II(partial)-*Cla*I of plasmid RIP-TGFβ-hGH (Sanvito et al., 1995) (RIP, rat insulin2 promoter), which results in a replacement of the TGFβ cDNA by the loxP-STOP-loxP sequence.

GlucPr-loxP-STOP-loxP-hGH

RIP of InsPr-loxP-STOP-loxP-hGH was excised with *Xba*I, blunted and replaced with a blunted *Xba*I-*Acc*I 2.1 kb rat glucagon promoter fragment (kind gift of Dr J. Philippe, Geneva).

PPPr-hGH

This construct was described elsewhere (Herrera et al., 1994).

Preparation of Cre-encoding transgenes

InsPr-Cre

A *Mlu*I(blunted)-*Xba*I 1 kb fragment of pBS185 (GibcoBRL, no. 10347-011) containing Cre was cloned at *Cla*I(blunted)-*Xba*I sites of a pBS-KS containing a rabbit β-globin1 intron and polyadenylation signal (plasmid pBS-βglobin-Cre). The 0.6 kb insulin promoter fragment (Hanahan, 1985), obtained from plasmid RIP-TGFβ-hGH (see above) with *Bgl*II(blunted)-*Sac*I, was inserted at sites *Not*I(blunted)-*Sac*I of pBS-βglobin-Cre.

GlucPr-Cre

A *Sac*I fragment with the glucagon promoter was inserted at *Sac*I of pBS-βglobin-Cre.

PPPr-Cre

The 0.6 kb rat PP promoter fragment contained in a *Sac*I-*Eco*RV fragment was ligated to pBS-βglobin-Cre at *Not*I(blunted)-*Sac*I.

PDX1Pr-Cre

A *Sac*I(blunted)-*Xba*I 4.5 kb fragment containing the PDX1 promoter (kind gift of Dr C. Wright, Nashville) was inserted at *Xho*I(blunted)-*Xba*I of a plasmid containing the Cre-coding region endowed with a nuclear localizing signal, upstream of a hGH minigene (kind gift of Dr T. Hennet, Zurich).

Generation and analysis of mice

Transgenic mice were produced by pronuclear microinjection of B6/CBAJ-F1×B6/CBAJ-F_1 zygotes as described (Hogan et al., 1994),

using DNA solutions at 5-10 ng/μl in a 0.1 mM EDTA-containing buffer. Minimums of three different families were established per transgene. As is usual with this technique, some of the founder mice obtained were not germline; among the transmitters, for every transgene, those families in which Cre activity was either undetectable or mosaic were eliminated (see Table 1).

Mice were genotyped by performing different PCRs with genomic DNAs extracted from tail biopsies. The PCR profile was a 30 times-repeated standard cycle, either with or without DMSO (less than 5% final): 94°C, 30 seconds, 55°C, 30 seconds, 72°C 1 minute. Alternatively, a 'touch-down' program was also used: 10 initial cycles were then added with a starting annealing temperature of 65°C that was decreased by 1°C per cycle. The primers used were: InsPr-loxP-STOP-loxP-hGH and GlucPr-loxP-STOP-loxP-hGH: stop1 (5′): 5′AGG TGG CAA GTG GTA TTC CG3′; hGH1 (3′): 5′AGG ACA CAT TGT GCC AAA GG3′ (580 bp fragment). InsPr-Cre, GlucPr-Cre and PPPr-Cre: ag2 (5′): 5′CTG CTA ACC ATG TTC ATG CCT3′; cre1 (3′): 5′CCT GTT TTG CAC GTT CAC CG3′ (600 bp fragment). PDX1Pr-Cre (750 bp fragment): cre2 (5′): 5′CGG TGA ACG TGC AAA ACA GG3′; hGH1 (3′, see above). In addition, all strains of mice were screened using a 5′ primer specific for each hormone promoter and, for Cre mice, with a couple of internal oligonucleotides for Cre: cre3 (5′): 5′ATG CTT CTG TCC GTT TGC CG3′, and cre1 (3′, see above), the amplified fragment being 270 bp.

Single and double transgenic mice of the two sexes were analyzed for pancreatic α- or β-cell expression of hGH. Animals were killed either at the 7-10 days, or alternatively after weaning (i.e., when young adults). Pancreata were dissected, fixed in 2.5% glutaraldehyde and embedded in Epon 812 as described (Herrera et al., 1991, 1994; Higuchi et al., 1992). 1 μm consecutive semithin sections were fluorescence-stained with anti-hormone antibodies (insulin, glucagon and hGH) as previously reported (Herrera et al., 1994).

RESULTS AND DISCUSSION

The experimental design resulted in the generation of mice bearing two transgenes (summarized in Fig. 1). The first transgene, 'reporter', placed under the control of either the insulin2 (InsPr, β-specific) or the glucagon (GluPr, α-specific) gene promoter, contains a hGH-coding region placed downstream of a loxP-flanked transcription termination site (STOP sequence) (Sauer, 1993). The native reporter transgene cannot be expressed in β- or α-cells because of the STOP sequence; its expression requires the deletion of this sequence, which can be obtained in the presence of Cre recombinase. hGH is a good marker for immunohistochemical detection of gene expression and has been used in a number of transgenic studies (Liang et al., 1994; Lopez et al., 1995; Roth et al., 1990; Szabo et al., 1995). A second, or 'tagger' transgene, consists of the Cre recombinase gene placed under the control of a promoter active in putative progenitor cells; its expression results in the deletion of the STOP site of the reporter transgenes, thus allowing hGH synthesis. This approach has been used by others to activate the transcription of either the SV40 T antigen, the *lacZ* or the placental alkaline phosphatase genes in transgenic mice (Jacob and Baltimore, 1999; Lakso et al., 1992; Tsien et al., 1996).

We prepared mice carrying the following tagger transgenes: insulin2 gene promoter-Cre (InsPr-Cre), glucagon gene promoter-Cre (GlucPr-Cre), PP gene promoter-Cre (PPPr-Cre) and PDX1 gene promoter-Cre (PDX1Pr-Cre).

For each of these transgenes, either reporter or tagger, several families were established from no less than three independent founders and analyzed for transgene expression (see the legend of Table 1). These first screenings were necessary for selecting mouse families with stable and complete transgene expression (i.e. in about 100% of targeted cells), independently of both transgene copy number and genomic integration site.

Therefore, mouse strains with complete and stable transgene expression (Cre activity) were selected by crossings with the appropriate silenced reporter transgene-bearing strain of mice (see 'controls' in Table 1). Adult doubly transgenic mice from such mating schemes were analyzed for reporter gene expression (hGH) in islet cells by immunofluorescence on pancreatic sections (summarized in Table 1). As expected, there was no hGH expression in single transgenic mice (Figs 2A,B, 3A,B; Table 1), i.e. those bearing only either a reporter or a tagger transgene. On the contrary, and also as expected, hGH was expressed by β-cells in doubly transgenic mice bearing the β-specific reporter gene and β-expressed tagger genes (either InsPr-Cre or PDX1Pr-Cre; PDX1 is an insulin

Table 1. hGH expression in islets of transgenic mice

	Reporter transgene			
Tagger transgene	Wild-type mouse (*no reporter*)	INSPr loxP-STOP-loxP-hGH	GLUCPr loxP-STOP-loxP-hGH	PPPr-hGH (*no STOP sequence*)
Wild-type mouse (*no Cre*)	–	–	–	[PP cells: ++]
INSPr-Cre	–	[β-cells: ++]	–	N.A.
GLUCPr-Cre	–	–	[α-cells: ++]	N.A.
PPPr-Cre	–	β-cells: ++	–	N.A.
PDX1Pr-Cre	–	[β-cells: ++]	α-cells: ++	N.A

++, good staining with anti-GH antibodies; –, no staining at all; N.A., not applicable (the PPPr-hGH reporter gene is not modifiable by any tagger gene).

Brackets indicate the mice and cell types in which staining with anti-hGH is taken as positive control of transgene activity, since the promoter driving the expression of both tagger and reporter transgenes is the same, i. e. InsPr in β-cells and GluPr in α-cells (PDX1Pr is also active in β-cells). Cells using the corresponding promoter appear evenly stained for hGH in mice bearing these transgenes, allowing the conclusion that the tagger and reporter transgenes selected for the experiments work efficiently and reproducibly.

Promoter activity in the β- and α-cell progenitors can be compared using the same tagger gene, PPPr-Cre, whose promoter is not used in any mature α- or β-cell, but only in mature PP cells. This is shown in the far right column with mice bearing a PPPr reporter transgene devoid of the loxP-STOP-loxP sequence: hGH staining was detected only in PP cells but neither in α- nor β-cells (Fig. 2I,J). In mice bearing the PPPr-Cre transgene, expression of the reporter transgene occurred uniformly in β-cells but not at all in α-cells. This indicates that good PP promoter activity is present only in β-cell progenitors, demonstrating that they belong to a lineage different from that of α-cell progenitors.

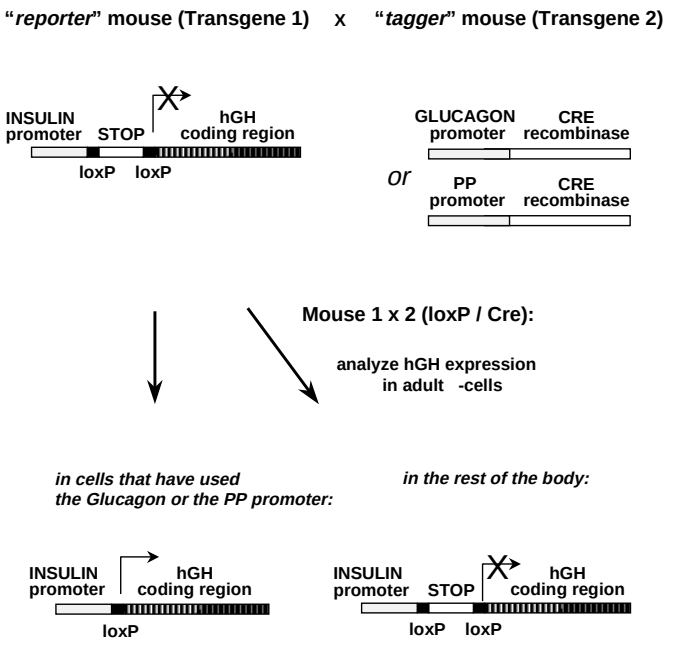

Fig. 1. General Cre/loxP-based strategy to genetically label islet endocrine cell precursors. For simplicity, only one example is illustrated: the experiment is designed to assess whether adult β-cells descend from glucagon- and/or PP-producing progenitors. The reverse experiment, planned to determine whether there is insulin, PP or PDX1 production in the α-cell lineage, was also performed (see text).

gene transcription factor and is thus expressed in adult β cells; Figs 2C,D, 4A,B; Table 1). Similarly, almost all α-cells from mice bearing both the α-expressed tagger gene (GluPr-Cre) and the α-specific reporter gene were hGH positive (Fig. 3C,D; Table 1).

As a final control, we verified that permanent expression of hGH in α- or β-cells does not alter the differentiation and function of endocrine cells in a detectable way. To this end, double transgenic mice bearing β-specific, or α-specific, tagger and reporter genes were kept alive for one year: these mice were always normoglycemic and their islets appeared histologically normal at death (not shown).

To explore whether β-cell precursors synthesize glucagon prior to or together with insulin, 15 mice bearing both the GluPr tagger gene and the β-specific reporter gene were produced: in no case was hGH detected in adult β-cells (Fig. 2E,F; Table 1), demonstrating that there is no glucagon gene expression in their precursors. For the exploration of the α lineage, five mice bearing the InsPr tagger gene and the α-specific reporter gene were obtained; no hGH presence was detected in adult α-cells (Fig. 3E,F; Table 1), demonstrating the absence of insulin gene expression in their progenitors. Thus, the glucagon and insulin coexpressing cells reported in early pancreatic buds (Larsson, 1998) are unlikely to be the progenitors of either mature α- or β-cells. Hormone coexpression patterns have been presented as evidence for the identification of common stem cells; however, cells coexpressing multiple hormones may be, at least in some instances, 'terminal' (i.e. differentiated) rather than 'stem' cells. This is in accordance with kinetic studies, which strongly suggest that most insulin cells formed between E15.5 and E18.5 must appear from proliferating hormone-negative precursors (Herrera et al., 1991; Larsson, 1998).

Our previous results suggested that the PP gene promoter is used either in β-cell progenitors or in cells that are necessary (through a paracrine effect on unidentified progenitors) for β-cells to differentiate, since mice with a diphtheria toxin (DT-A) transgene driven by this promoter fail to develop β-cells (Herrera et al., 1994). Islet β-cells do not produce PP; this is also shown here in adult mice bearing only a PPPr-hGH reporter gene (without a STOP sequence), which produce hGH only in their PP cells and not in their β-cells (Fig. 2I,J; Table 1). Therefore, in order to determine whether: (1) β-cells derive from PP-expressing progenitors, and (2) β- and α-cell progenitors are also distinct by a different use of the PP gene promoter, double transgenic mice bearing both a PPPr tagger gene (i.e. PPPr-Cre) and either the β- or α-specific reporter genes were produced. We thus explored 35 juvenile and adult double transgenic mice bearing both the PPPr tagger gene and the β-specific reporter gene. In all cases, all islet β-cells contained hGH (Fig. 2G,H; Table 1). On the contrary, 6 double transgenic mice bearing both the same PPPr tagger and the α-specific reporter gene did not show any expression of hGH in their islet α-cells (Fig. 3G,H; Table 1). These contrasting findings are of special interest since they deal with a tagger gene (PPPr-Cre) whose promoter appears to be used in neither mature α- nor β-cells, in contrast to the glucagon or insulin promoters.

It could be argued that, in doubly transgenic mice bearing the GluPr tagger and β-specific reporter transgenes, the failure to activate the β-specific reporter gene, and thus to express hGH in adult β-cells, does not completely rule out the use of the glucagon promoter in their progenitors, and vice versa, since a transient and minimal expression of Cre may be insufficient to remove the STOP segment of the reporter gene. This pitfall requires three considerations. First, the threshold of the effect of Cre expression, at the cellular level, is '*all-or-none*': if partial deletion of the STOP sequence was achieved, then a patchy or mosaic phenotype would be expected. This was not the case. Second, we must recall the results obtained with the DT-A-encoding toxigenes (Herrera et al., 1994) that only one molecule of the toxin is required to kill a cell (Yamaizumi et al., 1978). Hence, a transient and minimal use of the glucagon promoter in cells in GluPr-DTA mice should be sufficient to kill those cells, yet in such mice β-cell differentiation occurs normally (Herrera et al., 1994). Similarly, an InsPr-DTA toxigene does not alter normal α-cell differentiation (Herrera et al., 1994), thus proving the exquisite specificity of these two promoters. Third, the results obtained with the PPPr-Cre tagger transgene, and with the PPPr-DTA toxigene (Herrera et al., 1994), demonstrate in a fully reciprocal and controlled way that β- and α-cell progenitors differ in their use of the PP promoter, and independently confirm that adult α- and β-cells belong to distinct cell lineages.

It is noticeable that detection of PP immunoreactivity occurs only late in ontogeny, from embryonic day 16 (E16) in the mouse (Jackerott et al., 1996, and unpublished observations of the author; reviewed by Larsson, 1998); however, PP mRNA is detected in pancreatic buds by RT-PCR, a much more sensitive technique, from E10-10.5 (Gittes and Rutter, 1992;

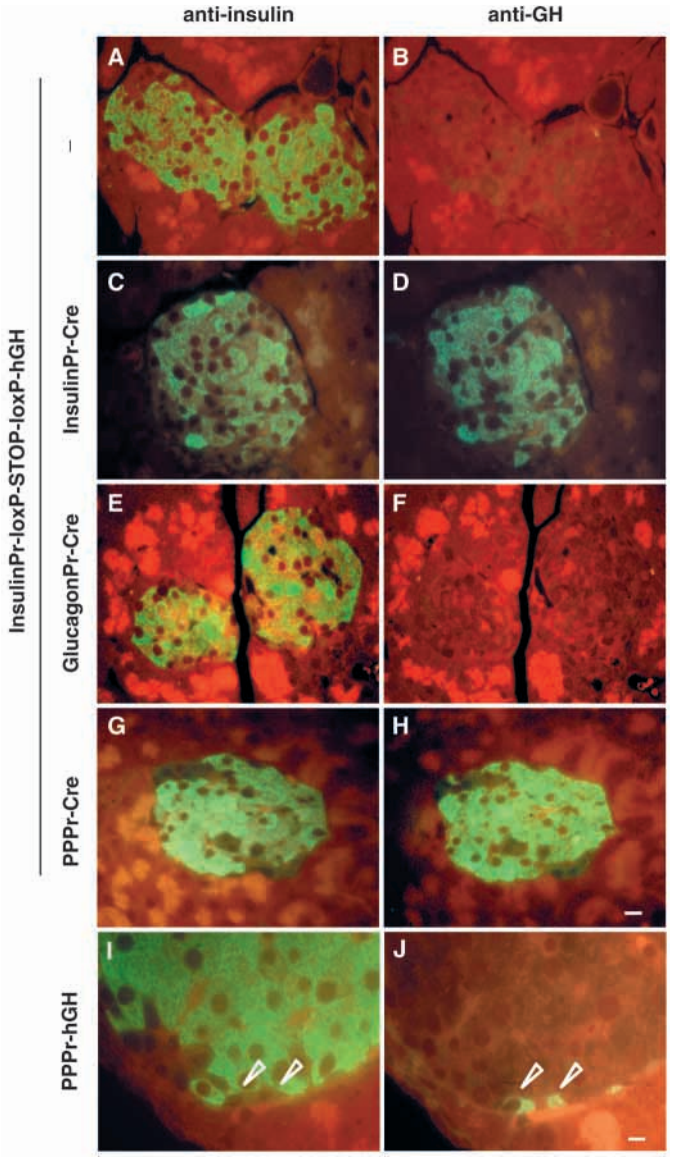

Fig. 2. The β-cell lineage. 1 μm-thick consecutive semi-thin sections of pancreata from InsPr-loxP-STOP-loxP-hGH mice, either single (A,B) or double transgenic (C-H), and PPPr-hGH mice (I,J). The sections were stained by indirect immunofluorescence with anti-insulin (A,C,E,G,I) or anti-hGH (B,D,F,H,J) antibodies. (A,B) The native InsPr-loxP-STOP-loxP-hGH transgene is inactive, so that there is no hGH production in β-cells (B). (C,D) An InsPr-Cre transgene, as expected, activates hGH expression, whereas (E,F) a GlucPr-Cre transgene fails to label adult β-cells. On the contrary, in PPPr-Cre transgenics, there is hGH staining (G,H) even though the PP promoter is inactive in adult β-cells, as demonstrated in PPPr-hGH transgenic mice (I,J; arrowheads show two PP cells, which lack insulin and contain GH). Bar, 20 μm (A-H); 13 μm (I,J).

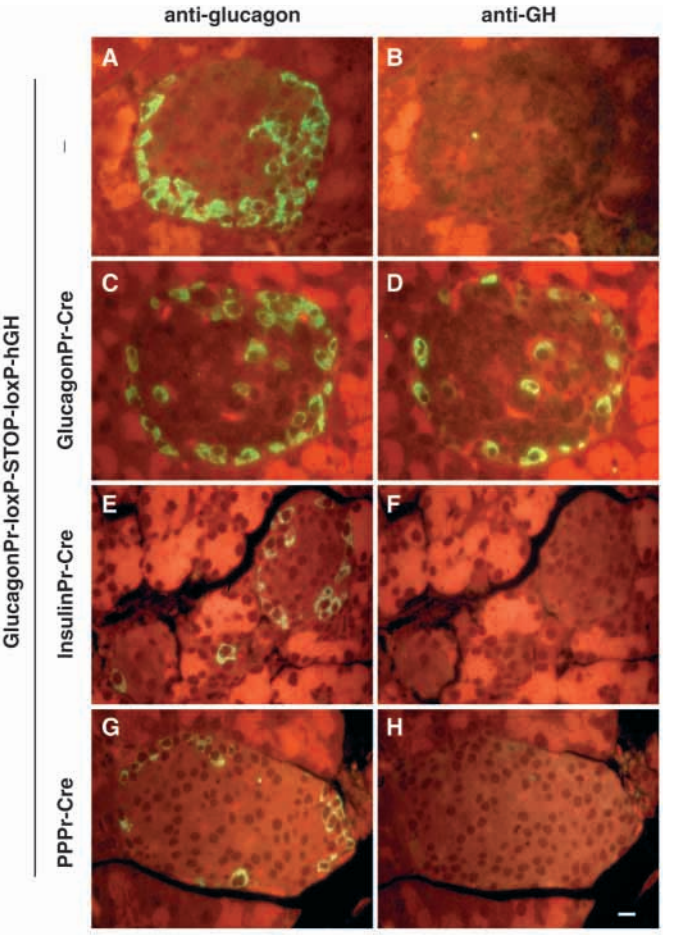

Fig. 3. The α-cell lineage. 1 μm-thick consecutive semi-thin sections of pancreata from GlucPr-loxP-STOP-loxP-hGH mice, either single (A,B) or double transgenic (C-H). The sections were stained with either anti-glucagon (A,C,E,G) or anti-hGH (B,D,F,H) antibodies. (A,B) The native GlucPr-loxP-STOP-loxP-hGH transgene is inactive, thus there is no hGH production in adult α-cells (B). (C,D) A GlucPr-Cre transgene, as expected, activates hGH expression, whereas (E,F) an InsPr-Cre transgene fails to label α-cells. (G,H) Similarly, PPPr-Cre transgenics have no hGH production in α-cells. Bar, 20 μm.

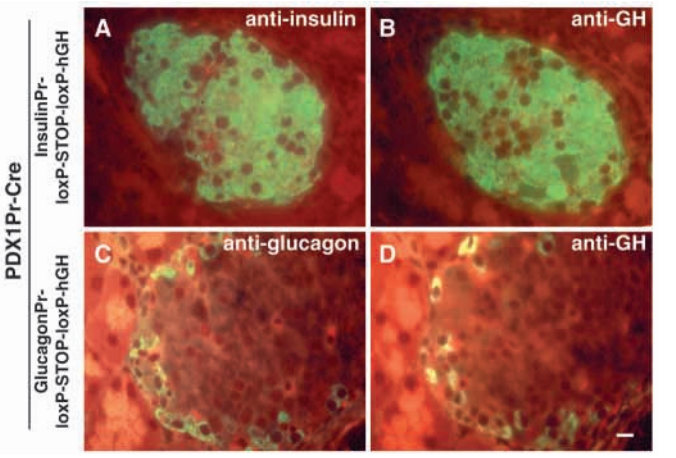

Fig. 4. The α-cell lineage: α-cells derive from PDX1-expressing precursors. 1 μm consecutive semithin sections of pancreata from double transgenic PDX1Pr-Cre mice, also bearing either the InsPr-loxP-STOP-loxP-hGH (A,B) or the GlucPr-loxP-STOP-loxP-hGH (C,D) reporter transgene. The sections were stained by indirect immunofluorescence with anti-insulin (A), anti-glucagon (C) or anti-hGH (B,D) antibodies. (A,B) Positive control for a PDX1 promoter-dependent Cre activity; (C,D) α-cells (C) are also hGH positive (D). Bar, 20 μm.

Herrera et al., 1991), at a time when the first insulin transcripts are also detected (Herrera et al., 1991; Szabo et al., 1995)

It has been proposed, on the basis of the Glut2 gene fetal expression pattern (Pang et al., 1994), that there might be two separate β-cell lineages. Since all β-cells were hGH positive in the double transgenic mice using the PP-tagger and β-specific reporter genes, the two putative β-cell lineages would be characterized by their common use of the PP promoter.

Expression of PDX1 is the earliest and most specific 'endoderm marker' of the developing pancreas. The great majority of mature α-cells do not express this gene and, using a 4.5 kb PDX1 promoter/enhancer with a *lacZ* reporter sequence as a transgene, only 3-5% of all α-cells from adult transgenic mice were found to have *lacZ* activity (C. Wright and M. Gannon, Vanderbilt University, Nashville, personal communication). It has been suggested that mature α-cells derive from early epithelial precursors in which the PDX1 gene is not expressed either (Edlund, 1998), since early differentiation of glucagon-containing cells has been observed in pancreatic primordia of PDX1$^{-/-}$ mice (Offield et al., 1996). Nevertheless, colocalization of PDX1 and glucagon early in development has been detected (Guz et al., 1995). Five young adult mice bearing both a 4.5 kb PDX1 promoter-driven tagger transgene and the α-specific reporter transgene were studied; virtually all adult α-cells expressed hGH (Fig. 4C,D; Table 1). Taken together, these observations suggest that the progenitors of adult α-cells express PDX1 and that embryonic glucagon-containing cells differ in nature from mature islet α-cells. Although it is not possible to explore with this technique whether the progenitors of β-cells also express the PDX1 gene, since it is required by adult β-cells (Fig. 4A,B; Table 1), it is reasonable to presume that all differentiated pancreatic cells arise from common stem cells expressing this gene (Fig. 5).

As a final consideration, the ontogenic relationship described here between different islet endocrine cells, in particular between PP- and insulin-secreting cells, may also be viewed from an evolutionary perspective. In the course of evolution, the endocrine pancreas has taken up, the task of participating in the control of homeostasis of metabolic parameters, in particular with respect to nutrients. In early Protostomians, this might have been achieved by subsets of nerve cells and mediated by the secretion of neuropeptides, for instance from the PP-fold family (Falkmer et al., 1985). In developed forms of Protostomians, like Insects, the four 'classical' islet hormones are present in brain nerve cells (Falkmer et al., 1985); these may be the evolutionary descendants of the putative earlier PP-family neuropeptide-secreting cells. According to this view, expression of the PP gene family would be a hallmark of the brain insulin cell precursors during evolution, as it appears to be for islet β-cells in the pancreas during ontogeny.

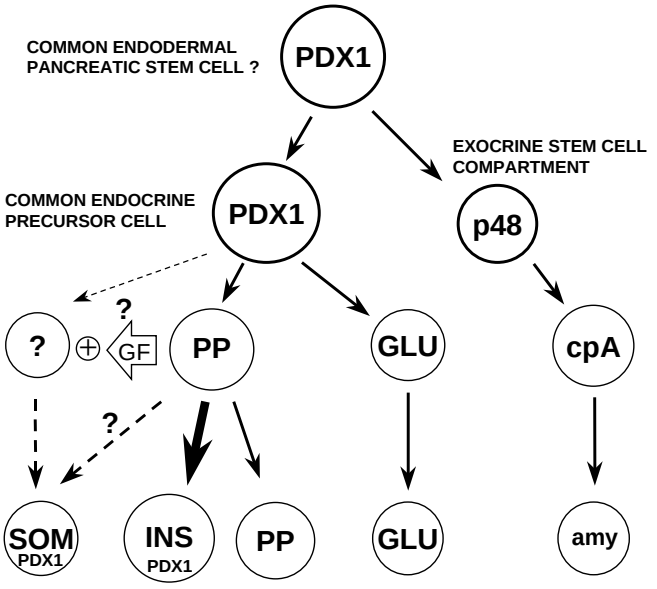

Fig. 5. Proposed pancreatic cell lineages, as deduced from current results and literature data (Larsson, 1998; Krapp et al., 1998; Gradwohl et al., 2000). p48, one of the two subunits of the exocrine pancreas-specific transcription factor PTF1, is the earliest exocrine marker known to date (Krapp et al., 1998). That somatostatin-producing cells could descend from PP-expressing precursors is deduced from the cell ablation experiments in transgenic mice (Herrera et al., 1994). cpA, carboxypeptidase A; amy, amylase; GLU, glucagon; SOM, somatostatin; INS, insulin; GF, unknown growth or differentiation factor.

I am most grateful to Mr Jordi Ritz, Mrs Gissela Flores, Mr Nicolas Steiner, Ms Caroline Raveraud, Ms Eliane Dubois, Mr Gregory Meichtry, Mrs Danielle Ben-Nashr, Dr Anthony Nichols, Dr Beate Ritz-Laser and Dr María Vázquez for their help; to Professor Jean-Dominique Vassalli for fruitful discussions and criticism, and Professor Pierre Vassalli for carefully reading the manuscript. This work was possible thanks to the financial support provided to the author by the Juvenile Diabetes Foundation International (Research Grant) and the Fonds National Suisse pour la Recherche Scientifique.

REFERENCES

Alpert, S., Hanahan, D. and Teitelman, G. (1988). Hybrid insulin genes reveal a developmental lineage for pancreatic endocrine cells and imply a relationship with neurons. *Cell* **53**, 295-308.

Edlund, H. (1998). Transcribing pancreas. *Diabetes* **47**, 1817-1823.

Falkmer, S., Dafgard, E., el-Salhy, M., Engstrom, W., Grimelius, L. and Zetterberg, A. (1985). Phylogenetical aspects on islet hormone families: a minireview with particular reference to insulin as a growth factor and to the phylogeny of PYY and NPY immunoreactive cells and nerves in the endocrine and exocrine pancreas. *Peptides* **6 Supplement 3**, 315-320.

Gittes, G. K. and Rutter, W. J. (1992). Onset of cell-specific gene expression in the developing mouse pancreas. *Proc. Natl Acad. Sci. USA* **89**, 1128-1132.

Gradwohl, G., Dierich, A., LeMeur, M. and Guillemot, F. (2000). Neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. *Proc. Natl Acad. Sci. USA* **97**, 1607-1611.

Guz, Y., Montminy, M. R., Stein, R., Leonard, J., Gamer, L. W., Wright, C. V. and Teitelman, G. (1995). Expression of murine STF-1, a putative insulin gene transcription factor, in beta cells of pancreas, duodenal epithelium and pancreatic exocrine and endocrine progenitors during ontogeny. *Development* **121**, 11-18.

Hanahan, D. (1985). Heritable formation of pancreatic beta-cell tumours in transgenic mice expressing recombinant insulin/simian virus 40 oncogenes. *Nature* **315**, 115-122.

Herrera, P. L., Huarte, J., Sanvito, F., Meda, P., Orci, L. and Vassalli, J. D. (1991). Embryogenesis of the murine endocrine pancreas; early expression of pancreatic polypeptide gene. *Development* **113**, 1257-1265.

Herrera, P. L., Huarte, J., Zufferey, R., Nichols, A., Mermillod, B., Philippe, J., Muniesa, P., Sanvito, F., Orci, L. and Vassalli, J. D. (1994). Ablation of islet endocrine cells by targeted expression of hormone-promoter-driven toxigenes. *Proc. Natl Acad. Sci. USA* **91**, 12999-13003.

Higuchi, Y., Herrera, P., Muniesa, P., Huarte, J., Belin, D., Ohashi, P., Aichele, P., Orci, L., Vassalli, J. D. and 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.

Hogan, B., Beddington, R., Costantini, F. and Lacy, E. (1994). *Manipulating the Mouse Embryo. A Laboratory Manual.* Cold Spring Harbor Laboratoy Press.

Jackerott, M., Oster, A. and Larsson, L. I. (1996). PYY in developing murine islet cells: comparisons to development of islet hormones, NPY, and BrdU incorporation. *J Histochem Cytochem* **44**, 809-817.

Jacob, J. and Baltimore, D. (1999). Modelling T-cell memory by genetic marking of memory T cells in vivo [see comments]. *Nature* **399**, 593-597.

Krapp, A., Knofler, M., Ledermann, B., Burki, K., Berney, C., Zoerkler, N., Hagenbuchle, O. and Wellauer, P. (1998). The bHLH protein PTF1-p48 is essential for the formation of the exocrine and to correct the spatial organization of the endocrine pancreas. *Genes Dev.* **12**, 3752-3763.

Lakso, M., Sauer, B., Mosinger, B., Jr., Lee, E. J., Manning, R. W., Yu, S. H., Mulder, K. L. and Westphal, H. (1992). Targeted oncogene activation by site-specific recombination in transgenic mice. *Proc. Natl Acad. Sci. USA* **89**, 6232-6236.

Larsson, L. I. (1998). On the development of the islets of Langerhans. *Microsc. Res. Tech.* **43**, 284-291.

Liang, Y., Jetton, T. L., Zimmerman, E. C., Najafi, H., Berner, D. K., Matschinsky, F. M. and Magnuson, M. A. (1994). Effects of glucose on insulin secretion, glucokinase activity, and transgene expression in transgenic mouse islets containing an upstream glucokinase promoter-human growth hormone fusion gene. *Diabetes* **43**, 1138-1145.

Lopez, M. J., Upchurch, B. H., Rindi, G. and Leiter, A. B. (1995). Studies in transgenic mice reveal potential relationships between secretin-producing cells and other endocrine cell types. *J. Biol. Chem.* **270**, 885-891.

Offield, M. F., Jetton, T. L., Labosky, P. A., Ray, M., Stein, R. W., Magnuson, M. A., Hogan, B. L. and Wright, C. V. (1996). PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. *Development* **122**, 983-995.

Orci, L. (1982). Macro- and micro-domains in the endocrine pancreas. *Diabetes* **31**, 538-565.

Pang, K., Mukonoweshuro, C. and Wong, G. G. (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.

Rall, L. B., Pictet, R. L., Williams, R. H. and Rutter, W. J. (1973). Early differentiation of glucagon-producing cells in embryonic pancreas: a possible developmental role for glucagon. *Proc. Natl Acad. Sci. USA* **70**, 3478-3482.

Roth, K. A., Hertz, J. M. and Gordon, J. I. (1990). Mapping enteroendocrine cell populations in transgenic mice reveals an unexpected degree of complexity in cellular differentiation within the gastrointestinal tract. *J. Cell Biol.* **110**, 1791-1801.

Sanvito, F., Nichols, A., Herrera, P. L., Huarte, J., Wohlwend, A., Vassalli, J. D. and Orci, L. (1995). TGF-beta 1 overexpression in murine pancreas induces chronic pancreatitis and, together with TNF-alpha, triggers insulin-dependent diabetes. *Biochem. Biophys. Res. Commun.* **217**, 1279-1286.

Sauer, B. (1993). Manipulation of transgenes by site-specific recombination: use of Cre recombinase. *Methods Enzymol.* **225**, 890-900.

Sauer, B. (1994). Site-specific recombination: developments and applications. *Curr Opin. Biotechnol.* **5**, 521-527.

Sauer, B. (1998). Inducible gene targeting in mice using the Cre/lox system. *Methods* **14**, 381-392.

Szabo, M., Butz, M. R., Banerjee, S. A., Chikaraishi, D. M. and Frohman, L. A. (1995). Autofeedback suppression of growth hormone (GH) secretion in transgenic mice expressing a human GH reporter targeted by tyrosine hydroxylase 5′-flanking sequences to the hypothalamus. *Endocrinology* **136**, 4044-4048.

Teitelman, G., Alpert, S., Polak, J. M., Martinez, A. and 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.

Tsien, J. Z., Chen, D. F., Gerber, D., Tom, C., Mercer, E. H., Anderson, D. J., Mayford, M., Kandel, E. R. and Tonegawa, S. (1996). Subregion- and cell type-restricted gene knockout in mouse brain [see comments]. *Cell* **87**, 1317-1326.

Yamaizumi, M., Mekada, E., Uchida, T. and Okada, Y. (1978). One molecule of diphtheria toxin fragment A introduced into a cell can kill the cell. *Cell* **15**, 245-250.