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Expression of peptide YY in all four islet cell types in the developing mouse pancreas suggests a common peptide YY-producing progenitor

Brent H. Upchurch[1], Gregory W. Aponte[2] and Andrew B. Leiter[1,*]

[1]Division of Gastroenterology and Digestive Disease Research Center, New England Medical Center Hospitals and Tufts University School of Medicine, Boston, MA 02111, USA
[2]Department of Nutritional Sciences, College of Natural Resources, University of California at Berkeley, Berkeley, CA 94720, USA

*Author for correspondence at 750 Washington Street, Box 218, Boston, MA 02111, USA

SUMMARY

The islets of Langerhans contain four distinct endocrine cell types producing the hormones glucagon, insulin, somatostatin and pancreatic polypeptide. These cell lineages are thought to arise from a common, multipotential progenitor cell whose identity has not been well established. The pancreatic and intestinal hormone, peptide YY, has been previously identified in glucagon-producing cells in islets; however, transgenic mice expressing Simian Virus 40 large T antigen under the control of the peptide YY gene expressed the oncoprotein in β, δ and pancreatic polypeptide cells, and occasionally developed insulinomas, suggesting relationships between peptide YY-producing cells and several islet cell lineages. The four established pancreatic islet cell types were examined for coexpression of peptide YY in islets of normal and transgenic mice throughout development. Peptide YY immunoreactivity was identified in the earliest endocrine cells in the fetal pancreas and was coexpressed in each islet cell type during development. Peptide YY showed a high degree of co-localization with glucagon- and insulin-producing cells in early pancreatic development, but by adulthood, peptide YY was expressed in less than half of the α cells and was no longer expressed in β cells. Peptide YY was also coexpressed with somatostatin and pancreatic polypeptide when these cell types first appeared, but most δ and pancreatic polypeptide cells continued to express peptide YY throughout development. The use of conditions that distinguish peptide YY from the related peptides, pancreatic polypeptide and neuropeptide Y, as well as the ability of the peptide YY gene to direct expression of a reporter gene in islets of transgenic mice, establishes expression of peptide YY in the earliest pancreatic endocrine cells. Coexpression of peptide YY in all islet cell types as they first emerge suggests that the four established islet cell types may arise from a common, previously unrecognized peptide YY-producing progenitor cell.

Key words: peptide YY, islet cell lineage, transgenic mouse, pancreas

INTRODUCTION

The embryonic pancreatic duct arises from primitive gut endoderm and contains endocrine precursor cells from which the islets develop (Pictet and Rutter, 1972). The origin of the four islet cell lineages has not been established, although the glucagon-producing α cell has been identified as the first endocrine cell to appear in islet development at about day 10 of gestation, followed by cells producing insulin (day 12), somatostatin (day 15) and pancreatic polypeptide (birth) (Alpert et al., 1988; Herrera et al., 1991; Pictet and Rutter, 1972; Rall et al., 1973). One model of islet cell lineage has been proposed from studies in transgenic mice expressing Simian Virus 40 large T antigen (Tag) under the control of the rat insulin gene 5′ flanking region (Alpert et al., 1988). Glucagon was the first hormone expressed in the transgenic mice. When insulin-producing β cells subsequently appeared, a high percentage of β cells also produced glucagon, suggesting that β cells arose from the α cell lineage. In transgenic mice expressing Tag, this otherwise short-lived coexpression was prolonged and fell gradually with age. During the first few days after they appeared in developing islets, somatostatin (δ) and pancreatic polypeptide (PP) cells also transiently produced insulin. In older animals, the ability to support insulin gene expression in non-β cells was lost. The transient coexpression of insulin in PP- and somatostatin-producing cells during fetal development led to the proposal that δ and PP cells originated from the β cell lineage. In contrast, a subsequent study described coexpression of PP in islet progenitor cells and in each of the 3 remaining lineages (Herrera et al., 1991). More recently, it has been suggested that the PP-like immunoreactivity which was reported in the earliest pancreatic endocrine cell at day 9.5 post-conception (pc) was the neurotransmitter, neuropeptide Y (Teitelman et al., 1993).

Other hormones are produced by pancreatic islet cells, including the intestinal hormone, peptide YY, which has been localized to glucagon-producing α cells in many species (Ali-Rachedi et al., 1984; Böttcher et al., 1984, 1993; Lozano et al., 1991; Nilsson et al., 1991; Rombout et al., 1986). Peptide YY belongs to a multigene family, sharing 50% and 70% amino

acid identity with the islet hormone pancreatic polypeptide and the neurotransmitter neuropeptide Y, respectively (Krasinski et al., 1991; Tatemoto, 1982). The inhibitory effects of peptide YY on gastric acid secretion, pancreatic exocrine function and gastrointestinal motility are well established (Taylor, 1989). Less is known about its physiological role in islets, but others have suggested an inhibitory action on insulin and glucagon release (Böttcher et al., 1989; Greeley et al., 1988).

Although peptide YY and glucagon are coexpressed in the same cell type, the developmental regulation of the peptide YY gene differs from that of glucagon in rat islets (Krasinski et al., 1991). Peptide YY mRNA was detectable in fetal rat pancreas at day 15 pc, peaked in late gestation and fell to low levels in adults. In contrast, glucagon mRNA levels continued to rise after birth and remained relatively high. This divergence in developmental regulation prompted us to examine the ontogeny of peptide YY-producing cells in developing mouse pancreas and the relationship of peptide YY-producing cells to the four established islet cell lineages. Here, we show that peptide YY is expressed in the earliest pancreatic endocrine cell in fetal mouse pancreas and is coexpressed in all islet cell types during development. These findings suggest that peptide YY expression plays a central role in islet cell differentiation and support the concept of a common multipotential progenitor for all four islet cell types.

MATERIALS AND METHODS

Transgenic mice

A peptide YY-Tag fusion gene was constructed by cloning a *Bgl*II-*Xho*I fragment (–2600 to +32 bp) of the 5′ flanking region of the rat peptide YY gene into the *Bam*HI-*Xho*I sites of a Bluescript plasmid containing the coding region for SV 40 early region (*Stu*I at 5190 bp to *Bam*HI at 2533 bp). The transgene was excised with a *Bgl*II-*Kpn*I digest, and microinjected into $B_6D_2F_1 \times B_6D_2F_1$ mouse embryos (Hogan et al., 1986). Transgenic animals were identified by DNA dot blot hybridization and pedigrees were maintained on a CD1 background. Two pedigrees having similar transgene expression in pancreatic islets were identified; one pedigree was examined in detail for this developmental study.

Immunohistochemistry

Fetal tissues were obtained from timed pregnancies, using noon on the day of the vaginal plug as day 0.5 of gestation. Developmental stages were confirmed by morphological criteria (Kaufman, 1992). Tissues for immunohistochemistry were postfixed in Bouin's fixative for 4 hours, washed in PBS, stored in 70% ethanol, embedded in paraffin and sectioned at 4 μm, or embedded in plastic and sectioned at 1 μm. At least three normal, CD1 mice (Charles River Laboratories) and three transgenic mice were examined for each developmental time point.

Primary antisera for immunofluorescence included guinea pig anti-peptide YY at 1:300 dilution (G. Aponte, UC-Berkeley); rabbit anti-peptide YY at 1:300 (T. McDonald, U. Western Ontario); rabbit anti-glucagon at 1:400 (M. Appel, U. Mass.); mouse monoclonal anti-glucagon at 1:1000, guinea pig anti-insulin at 1:500, rabbit anti-PP at 1:300, and rabbit anti-neuropeptide Y at 1:100 (A. Buchan, U. British Columbia); rabbit anti-mouse proinsulin I C peptide at 1:100 and rabbit anti-rat proinsulin II C peptide at 1:100 (O. Madsen, Hagedorn Research Institute, Denmark); rabbit anti-somatostatin at 1:300 (R. Lechan, Tufts U.); and rabbit anti-Tag at 1:500 (D. Hanahan, UC-San Francisco). For immunoperoxidase labelling, primary antisera were tenfold more dilute. Primary incubations were done at 4°C overnight. Controls included non-immune primary sera, mismatched primary and secondary antisera, known positive sections, and absorption with specific and heterologous antigens. Antisera against peptide YY were absorbed with 10 μM rat PP (Sigma) to prevent cross-reactivity with this related peptide. Although the peptide YY antisera showed no immunostaining of neuropeptide Y-immunoreactive neurons in the intestine or pancreas, peptide YY antisera were absorbed with 10 μM human neuropeptide Y (Sigma) to ensure that the immunostaining was specific for peptide YY. Neuropeptide Y and PP antisera were absorbed with 10 μM porcine peptide YY (Peninsula). Antisera against somatostatin and peptide YY were also absorbed with the unrelated peptide at 10 μM. In all cases, absorption with peptides specific for the primary antisera abolished all immunostaining and absorption with heterologous antigens did not affect immunostaining.

Immunoperoxidase labelling was performed with a Vectastain ABC kit (Vector Labs) using DAB or AEC (aminoethylcarbazole) precipitation for detection. Tag detection was enhanced with subtilisin digestion (Rindi et al., 1990) and DAB-nickel chloride precipitation. Hormone co-localization was determined by double immunofluorescent labelling with FITC-, Texas red- and AMCA (aminomethlycoumarin acetic acid)-conjugated, donkey anti-guinea pig, anti-rabbit and anti-mouse IgG secondary antibodies which were immunoabsorbed for multiple labelling (Jackson Immunoresearch). Single labelled sections incubated with mismatched secondary antibodies showed no immunostaining, confirming the specificity of the secondary antisera. Immunofluorescence was observed on an Olympus BH-2 microscope fitted with appropriate barrier filters to achieve complete color separation for the different fluorophores.

RESULTS

To determine the relationship of peptide YY-producing cells to other islet lineages, we introduced a genetic marker for peptide YY-producing cells by expressing a hybrid gene containing 2.6 kb of the rat peptide YY gene 5′ flanking region linked to the protein coding sequences of Tag into the germline of transgenic mice. Occasionally, animals from two pedigrees developed solitary islet cell neoplasms expressing the oncoprotein (Fig. 1A,B). Most of the tumor cells contained insulin immunoreactivity (Fig. 1C), an unanticipated finding since peptide YY had been frequently identified only in glucagon-producing cells. Occasional foci within the tumors stained intensely for peptide YY immunoreactivity (Fig. 1D). A large subset of insulin immunoreactive tumor cells stained weakly for peptide YY by double immunofluorescence (not shown). However, glucagon, somatostatin, or PP immunoreactive cells could not be found in the tumors. The unexpected identification of peptide YY in β cell tumors suggested that peptide YY cells may be related to lineages other than the α cell, prompting us to examine the relationship of peptide YY-producing cells to other islet cell types in developing transgenic and normal mice.

Transgene expression paralleled the known developmental regulation of peptide YY (Krasinski et al., 1991). Tag was first detectable on day 12 pc in clusters of endocrine cells and co-localized with peptide YY-producing cells (Fig. 2A). Tag was detected in all islets in late gestation, but by 2 weeks after birth, fewer than half of the islets contained Tag^+ cells. In contrast, the islets of most adult transgenic animals appeared entirely normal with only about 10% of adults developing solitary insulinomas expressing the oncoprotein (Fig. 1). The infre-

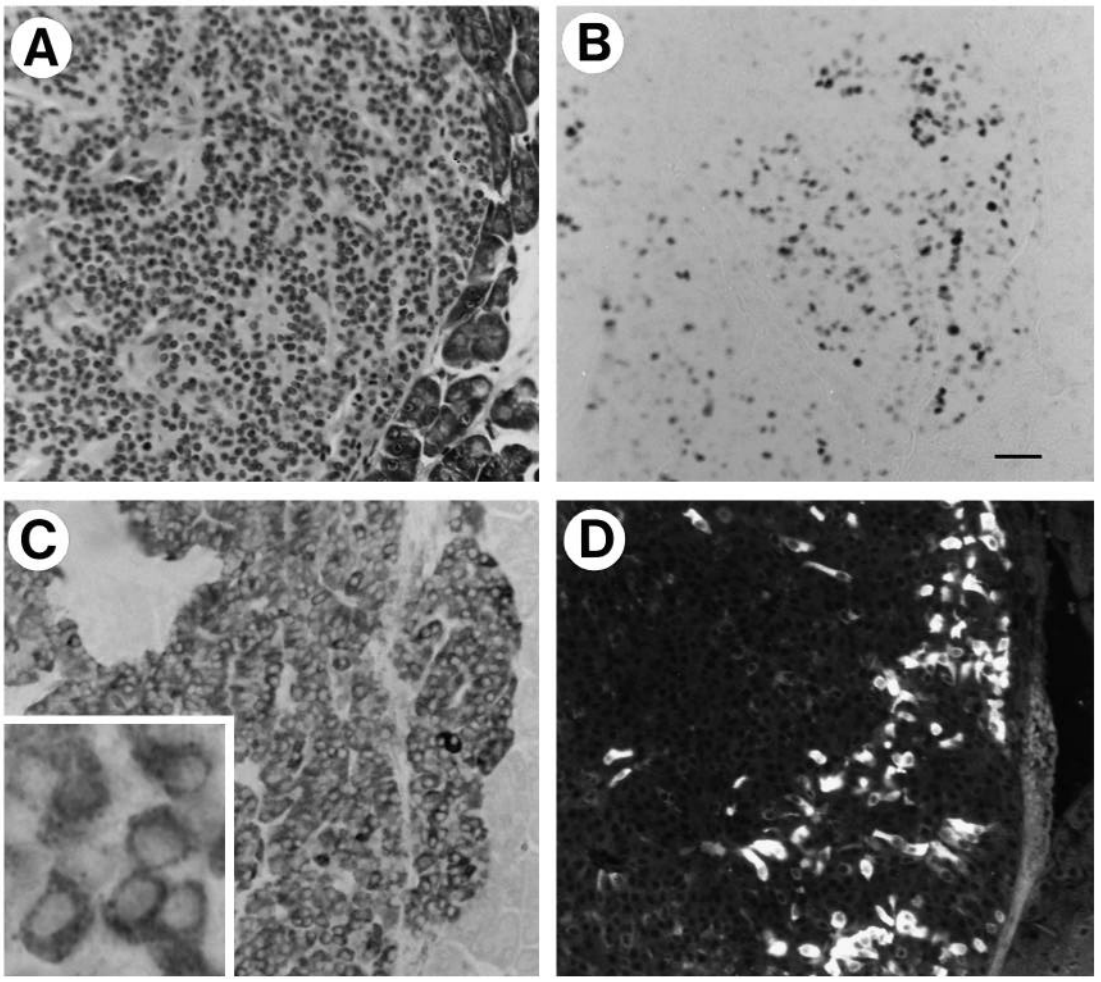

Fig. 1. Islet cell tumor in a peptide YY-T antigen adult transgenic mouse. (A) Hematoxylin- and eosin-stained section from a large, well-vascularized tumor. (B) Nuclear T antigen immunostaining. (C) Insulin immunoreactivity throughout the tumor. Inset shows cytoplasmic insulin immunostaining at higher magnification. (D) Small focus of cells strongly positive for peptide YY immunofluorescence as well as numerous weakly immunoreactive cells (lower left). Bar, 20 μm.

quent occurrence of islet tumors in the transgenic mice may be explained by the downregulation of peptide YY gene expression which normally occurs after birth, and possibly by the requirement for a second genetic event for islet cell transformation.

Islets were examined for transgene coexpression with peptide hormones to understand the developmental relationship between peptide YY-producing cells and other endocrine cell lineages. Although more than half of the Tag^+ cells did not appear to produce any hormone, peptide YY was expressed in about 20% of Tag^+ cells (Fig. 2B) and insulin was expressed in about 30% of Tag^+ cells (Fig. 2C) until several weeks after birth, after which the number of Tag^+ cells per islet decreased dramatically. Occasional Tag^+ cells (<10%) transiently coexpressed somatostatin when this hormone appeared in the pancreas at day 15.5 pc (Fig. 2D). When PP cells first appeared at birth, a few PP^+/Tag^+ cells (<10% of Tag^+ cells) were seen in the ventral pancreas (Fig. 2E). Since peptide YY-producing cells frequently make glucagon, Tag expression was also expected in α cells. However, $Tag^+/glucagon^+$ cells were not seen at any age (Fig. 2F).

The phenotypes of peptide YY-producing islet cells were examined in developing normal and transgenic mice. The patterns of hormone co-localization in islet cells were identical for both normal and transgenic animals, except for the prolonged appearance of a $PYY^+/insulin^+$ cell in transgenic mice as described below. The first endocrine cells to appear in the fetal pancreas at day 9.5 pc contained both peptide YY and glucagon immunoreactivity, although several peptide $YY^+/glucagon^-$ cells could be identified as early as day 10.5 (Fig. 3A,B). A subset of the α cell lineage appeared to diverge from peptide YY-producing cells by day 15.5 pc, when significant numbers of cells expressing either glucagon or peptide YY alone were seen (Fig. 3C). From late fetal development to adulthood, two distinct populations of glucagon cells were identified, $glucagon^+$/peptide YY^+ and $glucagon^+$/peptide YY^-.

Insulin-producing cells, identified with sensitive proinsulin I and II C peptide antisera, were found in the fetal pancreas as early as day 10.5 pc. At this early stage, all $insulin^+$ cells were also peptide YY^+ (Fig. 3D,E). From day 10.5 pc to birth, a few peptide YY and insulin coexpressing cells could be identified in most endocrine cell clusters by double immunofluorescence (Fig. 3F). Although seen in most endocrine cell clusters in fetal life, these peptide $YY^+/insulin^+$ cells were rarely seen after birth in normal mice. In transgenic animals, these peptide $YY^+/insulin^+$ cells were more abundant, persisting for 2-3 weeks after birth (Fig. 3G). At day 10.5 pc, all cells immunoreactive for C peptides I and II were also reactive for glucagon (not shown), but these $insulin^+/glucagon^+$ cells were never seen after day 12 pc.

Somatostatin-producing δ cells were the next endocrine cell

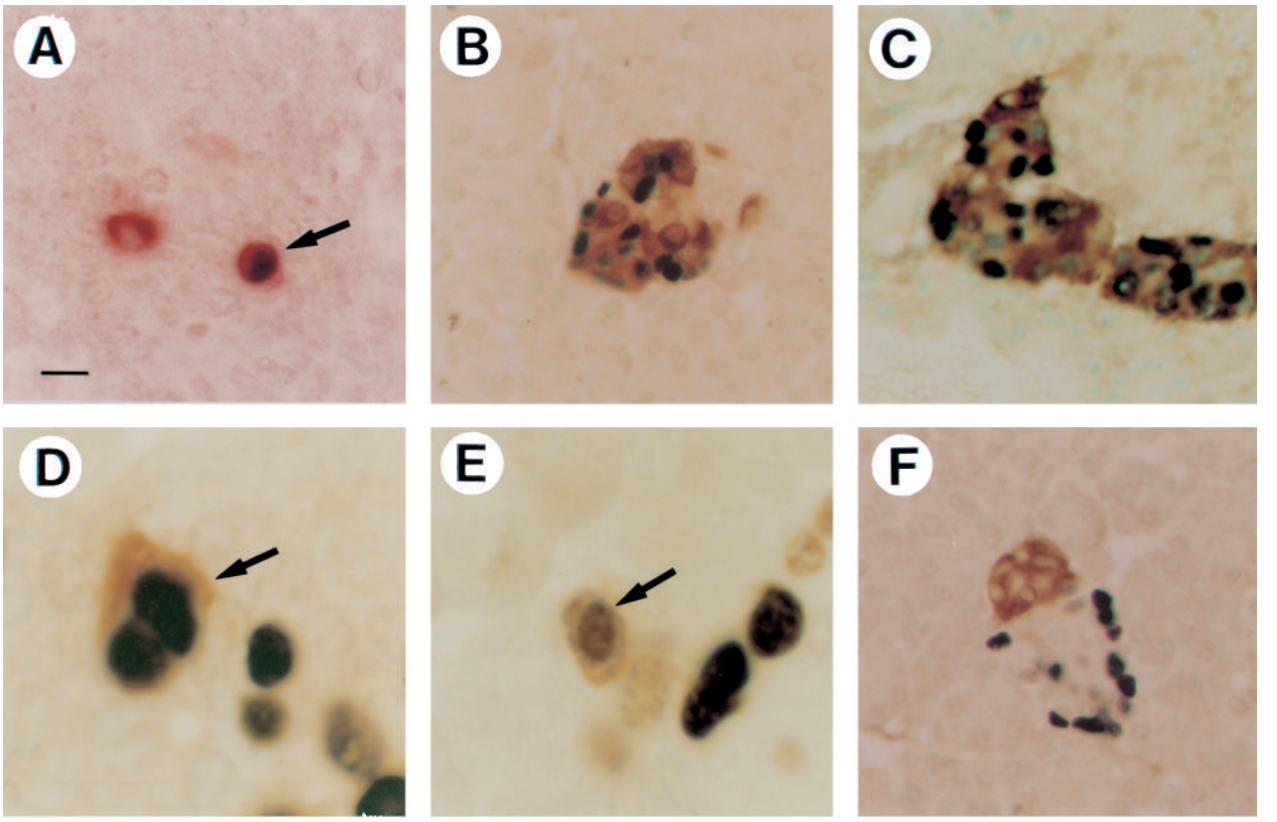

Fig. 2. Coexpression of T antigen with islet hormones in pancreata of transgenic mice. (A) Double immunoperoxidase staining showing black, nuclear Tag immunostaining which co-localized with red (AEC), cytoplasmic staining for peptide YY at day 12 pc (arrow). (B) Co-localization of Tag with brown (DAB), cytoplasmic staining for peptide YY in 14 day old mouse. (C) Co-localization of Tag with insulin in 14 day old mouse. (D) Tag co-localization with somatostatin at day 15 pc (arrow). (E) Co-localization of Tag with PP in ventral pancreas at birth (arrow). (F) Black, nuclear Tag immunostaining which does not co-localize with brown, cytoplasmic staining for glucagon. Bar, 5 μm (A), 10 μm (B,C,F), 2 μm (D,E).

type to appear in the fetal pancreas. From their first appearance at day 15.5 pc through adulthood, all δ cells in the dorsal pancreas and most δ cells in the ventral pancreas coexpressed peptide YY (Fig. 4A,B). Triple immunofluorescent staining of fetal and adult islets in the dorsal pancreas for peptide YY, glucagon and somatostatin demonstrated that peptide YY-producing cells coexpressed either glucagon or somatostatin, but not both (not shown). Insulin expression in δ cells was not seen at any stage of development.

PP cells, the last endocrine cell type to appear in the developing islets, were examined for coexpression of peptide YY using single-labelled, 1 μm, serial sections and antisera preabsorbed with an excess of the heterologous antigen to eliminate potential problems with cross-reactivity due to the homology between these related peptides. Two days after birth, shortly after PP first appeared, and in adult mice, most PP cells coexpressed peptide YY (Fig. 4C,D). A fraction of the peptide YY^+/PP^+ cells in the ventral pancreas also expressed glucagon (not shown). Peptide YY-producing cells were equally distributed between the dorsal and ventral pancreas, in contrast to the predominant localization of PP cells in the ventral pancreas. We were unable to detect PP immunoreactivity until birth, suggesting that little peptide accumulates until late in gestation.

Islets from fetal and adult mice were also examined for neuropeptide Y immunoreactivity using antisera preabsorbed with an excess of peptide YY (not shown). Without peptide YY preabsorption, the neuropeptide Y antiserum readily detected both pancreatic neurons and islet cells. However, preabsorption of the neuropeptide Y antiserum with peptide YY abolished immunostaining of islet cells, but did not diminish immunostaining of pancreatic neurons. Conversely, peptide YY immunostaining of islet cells was unaffected by preabsorption with excess neuropeptide Y.

DISCUSSION

This study reports two novel observations concerning the ontogeny of pancreatic islet cells: first, the appearance of peptide YY expressing cells when pancreatic endocrine cells first emerge, and second, the coexpression of peptide YY in all of the four lineages in the developing pancreas. These findings indicate a previously unappreciated developmental relationship between peptide YY-containing cells and the α, β, δ and PP lineages, suggesting that other models for islet differentiation need to be revised (Fig. 5). Peptide YY^+/glucagon$^+$ cells were seen beginning at day 9.5 pc. Insulin C peptide immunoreactive cells were seen one day later and coexpressed both peptide YY and glucagon at that stage. Using sensitive insulin C peptide antisera, others have convincingly demon-

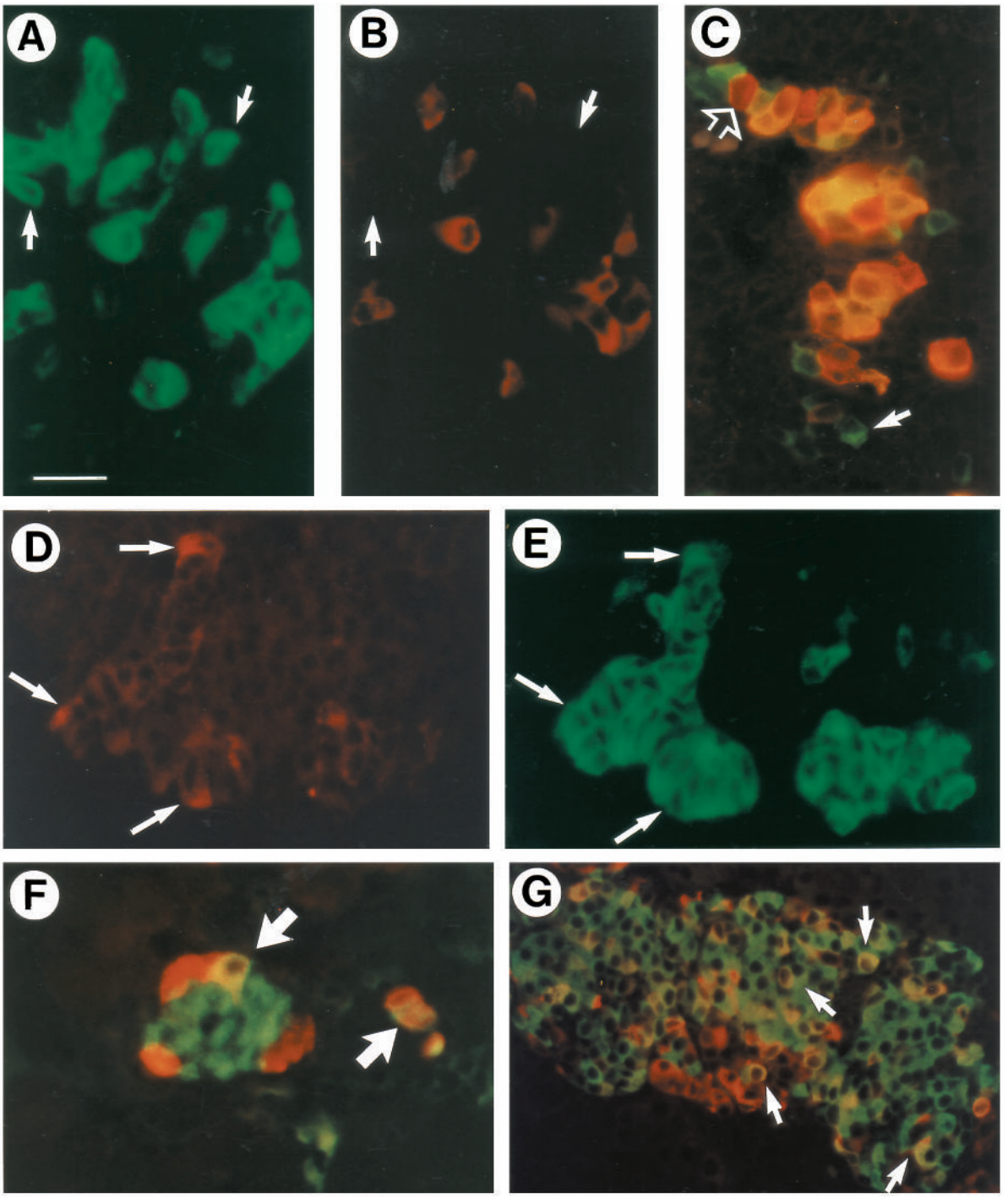

Fig. 3. Coexpression of peptide YY with glucagon and insulin cells in developing pancreas. (A-C) Double immunofluorescent staining for peptide YY and glucagon, localized with FITC and Texas red-conjugated secondary antibodies, respectively, in normal mouse pancreata. (A,B) Peptide YY immunoreactivity (A, FITC) in pancreatic endocrine cells at day 10.5 of gestation which co-localized with glucagon immunoreactivity (B, Texas red) in the same section, showing complete co-localization of glucagon with peptide YY immunoreactive cells. Several peptide YY^+/$glucagon^-$ cells are demonstrated at this early stage (A, arrows). (C) Multiple exposure photomicrograph of double immunofluorescent staining for peptide YY and glucagon at day 15 pc, showing separate populations of both peptide YY^+/$glucagon^-$ cells (green, closed arrow), $glucagon^+$/peptide YY^- cells (red, open arrow), as well as $glucagon^+$/peptide YY^+ cells (yellow). (D-G) Double immunofluorescent staining for peptide YY (FITC) and insulin (Texas red) in normal and transgenic mice. (D,E) Proinsulin II C peptide immunostaining (Texas red) in a day 10.5 pc, non-transgenic, CD1 mouse pancreas (D) which co-localized completely with peptide YY (E, FITC) on the same section. Three $proinsulin^+$/peptide YY^+ cells are indicated by arrows. (F) $Insulin^+$/peptide YY^+ cells (yellow, arrows) at day 17 of gestation in a non-transgenic CD1 mouse. (G) Numerous $insulin^+$/peptide YY^+ cells (yellow, arrows) in a hyperplastic islet from a 14 day old transgenic mouse. Bar, 10 μm.

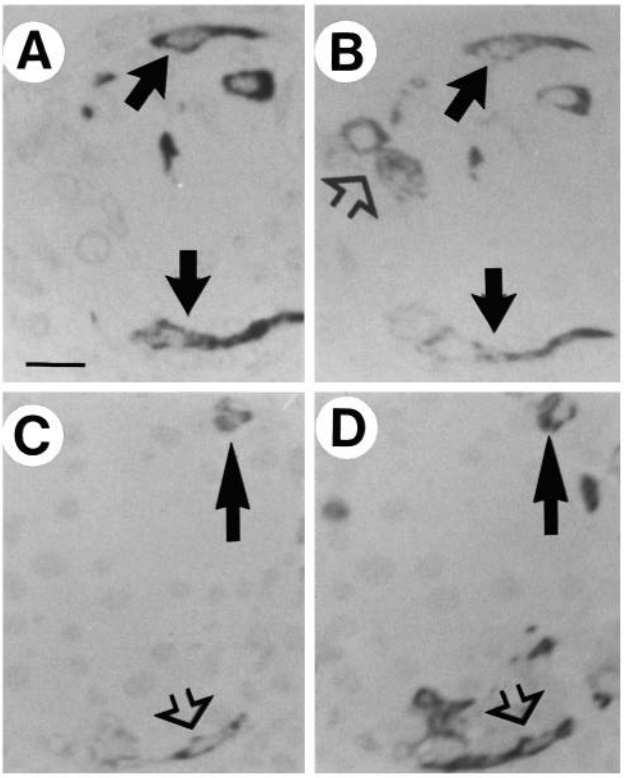

Fig. 4. Peptide YY expression in somatostatin and pancreatic polypeptide cells in normal, 2 day old CD1 mouse pancreas. (A,B) Co-localization of somatostatin (A) and peptide YY (B) immunoreactivity in adjacent 1 μm, Epon-embedded sections. All somatostatin cells co-localized with peptide YY-producing cells (closed arrows). Two peptide YY^+/somatostatin$^-$ cells are shown (open arrow). (C,D) Co-localization of PP (C) with peptide YY cells (D) in adjacent 1 μm thick, Epon-embedded sections from ventral pancreas (2 PP cells shown with open and closed arrows). Bar, 10 μm.

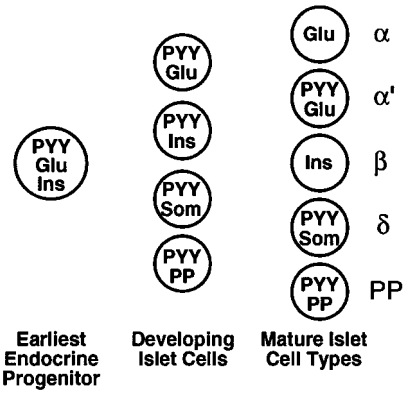

Fig. 5. Proposed model of the origin and differentiation of the four established islet lineages from a peptide YY-producing progenitor. The earliest endocrine cells appear in the fetal pancreas at day 9.5 and are peptide YY^+/glucagon$^+$/insulin$^+$. During development, peptide YY is co-expressed in each of the four islet cell types when they first appear (intermediate cell types). In mature islet cells, peptide YY expression is restricted to somatostatin and PP cells and a subpopulation of glucagon cells (α′).

strated insulin expression in fetal mouse pancreas one day earlier, at day 9.5 pc (Deltour et al., 1993; Teitelman et al., 1993) which co-localized with glucagon immunoreactivity (Teitelman et al., 1993). Thus, it seems likely that the earliest pancreatic endocrine cell type, which appears at day 9.5 pc, is a peptide YY^+/glucagon$^+$/insulin$^+$ cell.

Hormone gene expression appears to be selectively repressed as the islet cell lineages differentiate from this multipotential progenitor cell. Peptide YY gene expression appears to be repressed in most β cells by day 12.5 pc, but a few peptide YY^+/insulin$^+$ cells are present until birth. Glucagon gene expression is repressed in β cells early in development, with no glucagon$^+$/insulin$^+$ cells detectable after day 12 pc. Beginning about day 15.5 pc, peptide YY gene expression is repressed in a subset of glucagon-producing cells, and, by adulthood, less than half of the α cells coexpress peptide YY. Subsets of peptide YY-producing cells also become somatostatin and PP cells but, in these lineages, peptide YY gene expression is not repressed. The marked postnatal reduction in peptide YY gene expression in islets (Krasinski et al., 1991) may reflect restriction of peptide YY expression in α and β cells during islet cell differentiation.

Our identification of islet cells expressing multiple hormones contrasts with recently proposed models of islet cell differentiation (Alpert et al., 1988; Teitelman et al., 1993), although the sequence of individual hormone appearance agrees with previous reports (Alpert et al., 1988; Herrera et al., 1991; Pictet and Rutter, 1972; Rall et al., 1973; Teitelman et al., 1993). We could detect glucagon$^+$/insulin$^+$ cells at the earliest stage of islet development, but these cells were not seen at any subsequent stage of murine islet development. In addition, coexpression of insulin in somatostatin or PP cells was not seen when these cell types first appeared in development. The coexpression of insulin in other cell types observed by Alpert et al. (1988) probably resulted from examination of relatively thick, 15 μm sections, which included more than one cell.

The observations reported here are potentially supported by the recent descriptions of PP-like immunoreactivity (Herrera et al., 1991) and neuropeptide Y-like immunoreactivity (Teitelman et al., 1993) in early islet differentiation. The reported PP-like immunoreactivity may actually have been peptide YY, since appropriate conditions to eliminate known cross-reactivity of most PP antisera with peptide YY were not included (Ali-Rachedi et al., 1984; Fiocca et al., 1987; Leduque et al., 1983; Sundler et al., 1984). Although PP transcripts can be amplified from the fetal pancreas (Gittes and Rutter, 1992; Herrera et al., 1991), in most cases PP immunoreactivity cannot be detected before birth in rodents (Ali-Rachedi et al., 1984; Alpert et al., 1988; Sundler et al., 1977). Using conditions that discriminate between these two hormones, others have demonstrated peptide YY, but not PP immunoreactivity at day 14 in fetal mouse pancreas (Ali-Rachedi et al., 1984).

Using carefully controlled absorption experiments, a more recent study argued that the PP-like immunoreactivity reported by Herrera et al. (1991) in islet progenitor cells was actually neuropeptide Y, rather than PP (Teitelman et al., 1993). However, neither study (Herrera et al., 1991; Teitelman et al., 1993) examined the developing endocrine pancreas for peptide YY immunoreactivity or absorbed the PP and neuropeptide Y

antisera with peptide YY. Using specific antisera with the appropriate absorption controls that can unequivocally distinguish peptide YY from both pancreatic polypeptide and neuropeptide Y, we have demonstrated that the PP-like and neuropeptide Y-like immunoreactivity previously reported in islet progenitor cells is actually peptide YY. Our observations indicate that PP does not appear until birth, and that little or no neuropeptide Y exists in developing islet cells.

In addition, the concept that peptide YY is expressed early in islet development is supported by the ability of the peptide YY gene to direct expression of a reporter gene to at least three islet lineages in transgenic mice. Thus, we can confirm our histological findings demonstrating expression of peptide YY, rather than PP or neuropeptide Y, in developing islet cells with genetic evidence which is independent of the ability to identify peptide YY immunoreactivity. Transgene expression was readily detected in many peptide YY-producing cells and showed a pattern of developmental regulation in islets similar to that reported for the rat peptide YY gene (Krasinski et al., 1991).

The expression of the transgene in other islet cell types does not reflect inappropriate expression since the endogenous peptide YY gene is expressed in all islet cell types during development. Many β cells, as well as some δ and PP cells, expressed the transgene during development, but over 50% of the cells expressing the transgene did not produce any hormone, suggesting that expression of the viral oncogene at detectable levels may interfere with terminal endocrine cell differentiation. The absence of glucagon expression in cells expressing Tag may have resulted from blocked differentiation, a phenomenon frequently observed in some cell types of transgenic mice expressing this viral oncoprotein (Bradl et al., 1991; Lee et al., 1992; Mahon et al., 1987). In a study using a glucagon-Tag transgene, islet cells expressing Tag only occasionally stained for glucagon immunoreactivity (Lee et al., 1992), suggesting that the ability of α cells to express glucagon may be particularly sensitive to the effects of Tag expression. Alternatively, additional regulatory elements in the peptide YY gene may be necessary for transgene expression in α cells. Nevertheless, Tag coexpression with insulin-, somatostatin-, and PP-producing cells correctly predicted the coexpression of peptide YY in β, δ, and PP cells of non-transgenic mice. The prolongation of the normally transient peptide YY^+/$insulin^+$ phenotype, which was seen frequently in transgenic mice, probably reflects the effect of Tag expression in delaying terminal differentiation (Bradl et al., 1991; Mahon et al., 1987). The predilection for islet cell tumors to appear as insulinomas may reflect increased susceptibility of β cells to transformation early in development when these cells coexpress peptide YY.

The use of conditions that distinguish peptide YY from related peptides as well as expression of a peptide YY gene-directed transgene in early islet cell types indicate that the peptide YY immunoreactivity in these cells is indeed recognizing peptide YY, and not the highly homologous peptides, PP or neuropeptide Y. The appearance of peptide YY in the earliest pancreatic endocrine cell type, and its coexpression in glucagon, insulin, somatostatin and PP cells when they first appear in development, suggests that all four islet lineages may arise from a common, previously unrecognized peptide YY-producing progenitor cell. Unequivocal evidence in support of this model will eventually require targeted ablation of the peptide YY cell population in transgenic mice.

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