

## 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

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### SUMMARY

The *XIHXbox 8* homeodomain protein of *Xenopus* and STF-1, its mammalian homolog, are selectively expressed by  $\beta$  cells of adult mouse pancreatic islets, where they are likely to regulate insulin expression. We sought to determine whether the expression of the homeobox protein/s during mouse embryonic development was specific to  $\beta$  cells or, alternatively, whether *XIHXbox 8*/STF-1 protein/s were initially expressed by multipotential precursors and only later became restricted to the insulin-containing cells. With two antibodies, we studied the localization of STF-1 during murine pancreatic development. In embryos, as in adults, STF-1 was expressed by most  $\beta$  cells, by subsets of the other islet cell types and by mucosal epithelial cells of the duodenum. In addition, most epithelial cells of the pancre-

atic duct and exocrine cells of the pancreas transiently contained STF-1. We conclude that in mouse, STF-1 not only labels a domain of intestinal epithelial cells but also provides a spatial and temporal marker of endodermal commitment to a pancreatic and subsequently, to an endocrine  $\beta$  cell fate. We propose a model of pancreatic cell development that suggests that exocrine and endocrine ( $\alpha$ ,  $\beta$ ,  $\delta$  and PP) cells arise from a common precursor pool of STF-1<sup>+</sup> cells and that progression towards a defined mono-specific non- $\beta$  cell type is correlated with loss of STF-1 expression.

Key words: pancreatic islets, insulin gene, ontogeny, homeoprotein, duodenum, pancreatic cell lineage, murine pancreas

### INTRODUCTION

Endocrine cells of the adult pancreas are organized into the islets of Langerhans, which are scattered throughout the exocrine tissue. There are four major islet cell types:  $\alpha$ ,  $\beta$ ,  $\delta$  and PP cells that synthesize glucagon (GLU), insulin (IN), somatostatin (SOM) and pancreatic polypeptide (PP), as their principal differentiated hormone products. In addition to the four hormones, islet cells synthesize several neuronal-specific markers such as tyrosine hydroxylase (TH) and neuronal specific enolase (Alpert et al., 1988 and references therein).

The mammalian pancreas develops by fusion of dorsal and ventral primordia that appear as evaginations of the gut. The first differentiated pancreatic cells appear in the endodermal layer of the gut in embryos of about 20 somites, corresponding to day 9.5 of development (E9.5) in mouse and E10.5 in the rat (Wessels and Evans, 1968; Pictet and Rutter, 1972; Kaufman, 1992). The two primitive glands grow independently, forming both endocrine and exocrine tissues, and finally merge at E10.5 in mouse and E11.5 in rat (Pictet and

Rutter, 1972). Although it is generally agreed that precursor cells in the pancreatic duct give rise to the endocrine and exocrine compartment of the pancreas, the origin of these two cell types from common endodermal precursors has been controversial and it was hypothesized that islet cells were neural crest derivatives (Pearse, 1977).

Previous studies suggest that all four islet cell types arise from common multipotent precursors that coexpress several hormones and neural markers when they first differentiate (Alpert et al., 1988; De Krieger et al., 1992; Lukinius et al., 1992). As these stem cells mature, their antigenic repertoire becomes restricted to a single hormone (Alpert et al., 1988). Cells coexpressing GLU and insulin C-peptide (IN C-P) first appear in primordial pancreas at E9.5. The number of IN<sup>+</sup> GLU<sup>+</sup> cells decreases during gestation and, by E14.5, a significant number of cells express only one hormone. Cells containing SOM and PP first differentiate at E14.5 and postnatal day 1 (P1) respectively. Each of these cell types also co-express IN and GLU when they first appear (Alpert et al., 1988). Co-expression of several polypeptide hormones is also

reported in individual cells of embryonic human and porcine pancreas (De Krieger et al., 1992; Lukinius et al., 1992). These findings suggested that common factors are involved in islet cell determination, whereas a distinct set of signals restrict expression of differentiated islet hormone genes in  $\alpha$ ,  $\beta$ ,  $\delta$  and PP cells.

The mechanisms that govern islet-specific gene expression have been intensely investigated in recent years. Because the promoter regions required for islet expression of insulin, somatostatin and glucagon include critical AT-rich elements, it has been presumed that homeodomain-containing factors are important activators of these genes. The homeodomain is a sequence-specific DNA-binding motif present in numerous developmentally regulated transcription factors, some of which are thought to be required for the expression of lineage specific genes (Bodner et al., 1988; Ingraham et al., 1988). It was initially suggested that the homeodomain-containing factor XIHbox 8 might function in pancreatic development because its expression in *Xenopus* embryos corresponded to endodermal cells of the duodenum and developing pancreas (Wright et al., 1988). Subsequently, the putative murine and rat homologues of XIHbox 8 have been isolated and variously termed as STF-1 (Leonard et al., 1993), IPF-1 (Ohlsson et al., 1993) and IDX-1 (Miller et al., 1994). Characterization of all three mammalian proteins revealed their close similarity to XIHbox 8 in the N-terminal region and 100% identity in the homeodomain. For clarity, we will refer to this protein as STF-1. DNA-binding and transactivation assays suggest that STF-1 may be an important regulator of insulin (and somatostatin) gene expression in the islet (Ohlsson et al., 1993; Leonard et al., 1993; Peshavaria et al., 1994; Miller et al., 1994).

Immunohistochemical studies reported the localization of STF-1 to  $\beta$  cells of embryos and adults (Ohlsson et al., 1993) suggesting that in mammals, in contrast to amphibians, STF-1 was a specific  $\beta$  cell marker. We have also reported that STF-1 is primarily restricted to  $\beta$  cells (91%) of adult mice pancreata (Peshavaria et al., 1994). However, we also detected STF-1 expression in approximately 3% of  $\alpha$  and 15% of  $\delta$  cells (Peshavaria et al., 1994), indicating a less stringent distribution of the homeoprotein. In light of the differences between our observations and those reported by Ohlsson et al. (1993), we have examined STF-1 expression in pancreas during embryonic development and in gut of embryos and adults.

Here we find that STF-1 is expressed during islet development in  $\beta$  cells, in other precursor cells destined to become  $\alpha$ ,  $\delta$  and PP cells as well as in the epithelial layer of the duodenal mucosa. Moreover, we also find that exocrine cells of the pancreas and most epithelial cells of the pancreatic duct transiently express STF-1 during development. These observations suggest that STF-1 expression during development identifies an endodermal domain in the gut with two compartments, one that will generate the epithelial layer of the duodenum and a second subset that is endowed with the potential to form pancreas. This latter compartment of STF-1<sup>+</sup> precursor cells subsequently gives rise to pancreatic exocrine and endocrine cells. With further development, STF-1 expression in the pancreas becomes highly confined to  $\beta$  cells where it plays a critical role in the regulation of insulin gene transcription.

## MATERIALS AND METHODS

### Animals and tissue processing

Pregnant CD-1 mice were purchased from Charles River. The appearance of the vaginal plug was considered day 0.5 of gestation (E0.5). Pregnant females were killed by cervical dislocation, the uterus was removed and placed in 4% paraformaldehyde buffered to pH 7.4 with 0.1 M sodium phosphate buffer (PBS). The embryos were dissected in the fixative solution and were postfixed for 1 hour in the same solution. Embryos were examined at E9.0, 9.5, 11.5, 13.5, 15.5, and 16.5. Postnatal and adult CD-1 mice were perfused through the heart with the fixative solution; the pancreas was then removed and postfixed for 1 hour. The fixed tissues were infiltrated overnight in 30% sucrose, mounted in embedding matrix (Lipshaw Co., Pittsburg, Pa) and 15-20  $\mu$ m cryostat sections were collected onto gelatin-coated slides.

### Source of antibodies and purified peptides

The following antisera were used to stain cryostat sections: guinea pig antibodies to bovine insulin and rat C-peptide were purchased from Linco Research Inc (Eureka, MO); rabbit antisera to human glucagon was purchased from Calbiochem (San Diego, CA); rabbit antisera to human PP and somatostatin were supplied by Peninsula Labs (Belmont CA); rabbit antiserum to human  $\alpha$  amylase was purchased from Accurate Chem. Sci. Corp. (Westbury, N.Y.) Biotinylated goat anti-rabbit IgG, goat anti-guinea pig IgG and avidin-labelled peroxidase were purchased from Vector Laboratories (Burlingame, CA). Antiserum to the N-terminal domain of XIHbox 8 was raised against the first seventy five amino acids of XIHbox 8 as a GST/XIHbox 8 fusion protein (Wright et al., 1988; Peshavaria et al., 1994). STF-1 antiserum was raised in rabbits using a synthetic STF-1 peptide extending from amino acids 196-214 (Leonard et al., 1993).

### Characterization of homeoprotein antibodies

Anti-XIHbox 8 serum was purified with a GSE-*E. coli* extract depletion matrix and then affinity purified on a column containing immobilized N-terminal fusion protein. The affinity purified antibody reacts with N-terminal fusion protein on western blots of bacterial proteins. Preincubation of the antibody with the fusion protein blocks all staining in western blots (results not shown). Western blot analysis of XIHbox 8 and STF-1 antisera revealed that both interact with a  $47 \times 10^{-3}$  Mr protein on HIT and  $\beta$  TC cells (Peshavaria et al., 1994). Occasionally, immunoblots of  $\beta$  TC cells also revealed a  $39 \times 10^{-3}$  band, which could be a degradation product; this band was never observed in HIT or  $\alpha$  cell extracts.

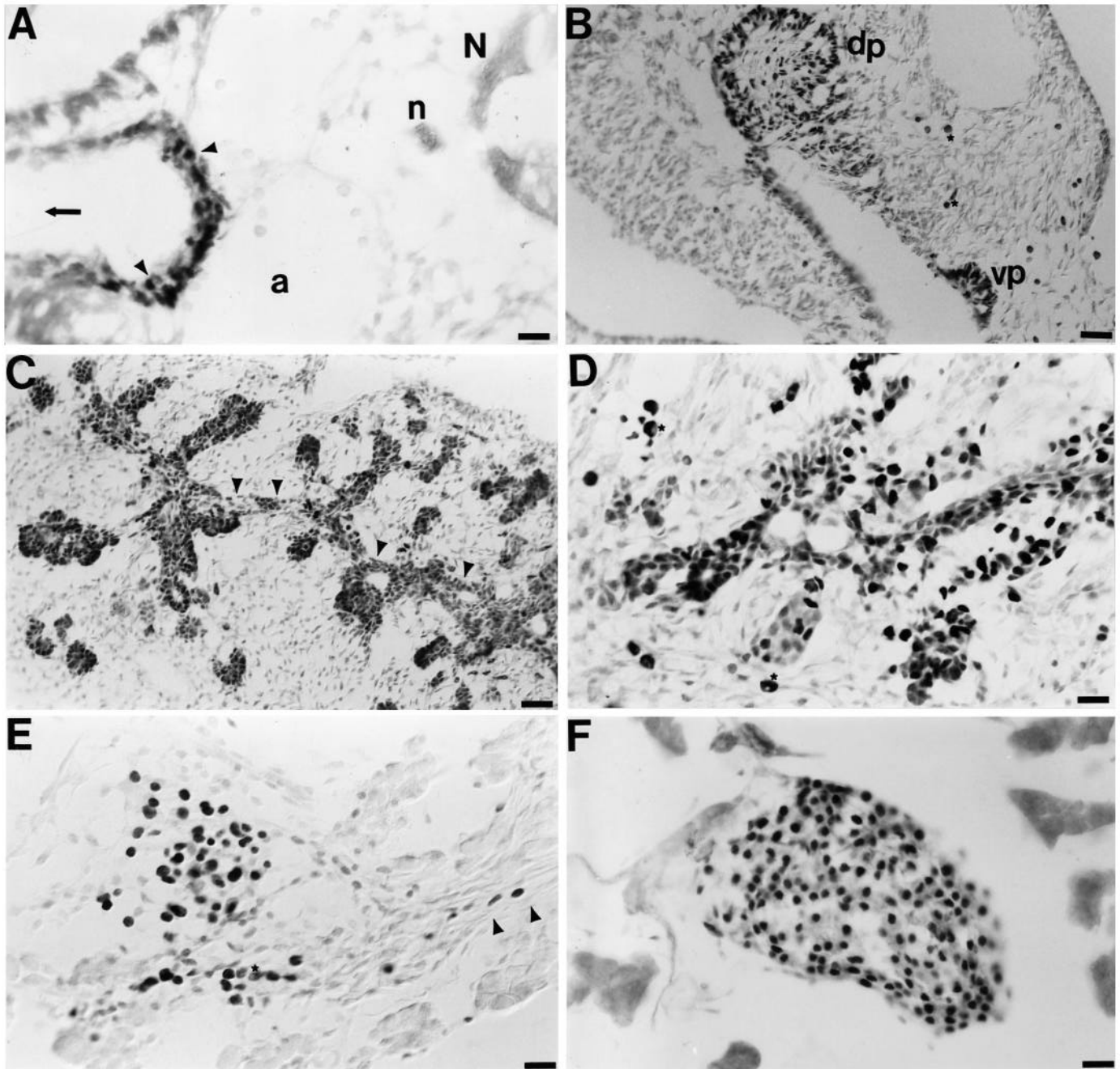
### Immunolabeling of cryostat sections using peroxidase techniques

Sections on slides were transferred to Tris-saline solution (TS; 0.9% NaCl in 0.1 M Tris, pH 7.4) and were immunostained using the avidin-biotin-HRP method. In brief, the sections were incubated sequentially in: (a) 0.3% Triton X-100 in a 1% solution of goat serum in TS for 15 minutes; (b) a 1:30 dilution of goat serum (Gibco) in TS for 30 minutes; (c) an empirically derived optimal dilution of control serum or primary antibody raised in species 'X' containing 1% goat serum in TS for 18 hours; (d) a 1:50 dilution of anti-(species x) biotinylated IgG solution in 1% goat serum in TS for 30 minutes and (e) a 1:100 dilution of peroxidase-avidin complex for 30 minutes. Following these incubations, the bound peroxidase was visualized by reaction for 6 minutes in a solution containing 22 mg of 3,3'-diaminobenzidine (DAB) and 10  $\mu$ l of 30% H<sub>2</sub>O<sub>2</sub> in 100 ml of 0.1 M TS. All incubations were carried out at room temperature. After the DAB step, sections were dehydrated and mounted with Permount. Antibodies were used at the following dilutions: guinea pig anti-bovine insulin, 1:400; guinea pig anti-rat insulin C-peptide, 1:300;

rabbit anti-human glucagon, 1:12,000; rabbit anti-human somatostatin, 1:8,000; rabbit anti-human pancreatic polypeptide, 1:20,000; rabbit anti-human amylase, 1:1000; Rabbit anti-XIHbox 8 and rabbit anti-STF-1, 1:250.

#### Double label

Sections were first incubated with antisera to STF-1 or XIHbox 8 and the bound antibody was visualized by DAB (brown precipitate), followed by incubation with antiserum to a hormone, which was visu-



**Fig. 1.** Gradual restriction of STF-1 expression to pancreatic islets during development. Photomicrographs illustrating immunohistochemical localization of STF-1. (A) Cross section of E8.5 embryo. Note distribution of labelled nuclei (arrowheads) in the dorsal wall of the gut. Bar, 15  $\mu$ m. N, neural tube; n, notochord; a, dorsal aorta; arrow indicates the communication of the foregut diverticulum with the yolk sac. (B) Cross section of an E9.5 embryo illustrates the presence of labelled nuclei in the primordia of the dorsal pancreas (dp) and ventral (vp) pancreatic primordia, bar, 50  $\mu$ m. (C) E13.5 embryo. A large number of immunoreactive cells are distributed throughout the pancreatic duct (indicated with arrowheads) and in small clusters; bar, 50  $\mu$ m. (D) High magnification microphotograph of E13.5 pancreas illustrates the presence of darkly and lightly stained nuclei; bar, 15  $\mu$ m. (E) E17.5 pancreas. Stained cells are located in newly formed islets. Some cells expressing the homeoprotein are seen in the pancreatic duct. Note that exocrine tissue is devoid of immunoreactive cells; bar, 15  $\mu$ m. (F) In adults, the homeoprotein is expressed exclusively by islet cells; bar, 15  $\mu$ m. Darkly stained cells indicated with asterisks in B and D are nucleated red blood cells that contain peroxidase. These cells are also seen in controls in which incubation with the first antibody has been omitted.

alized with the blue reaction product of the Vector SG substrate (Vector Labs).

#### Analysis of double label staining

Slides were examined with a Nikon Microphot SA microscope equipped with Nomarski optics and using a 10× ocular and a 100× oil immersion objective. Depth of focus was calculated according to manufacture specifications and Klein and Furtak (1986) as follows:

$$D = n \times \lambda / 2(NA)^2 + n/7 \times NA \times M$$

where  $n$  = refractive index on object side,  $\lambda$  = wavelength of light (nm) and  $M$  = total magnification

$$D = 1.515 \times 550 / 2(1.25)^2 + 1.515 / 7 \times 1.25 \times 1250^* \\ = 0.35807$$

\*Due to 1.25 differential interference contrast (DIC) magnification factor in microscope.

With a depth of focus of approximately 0.4  $\mu\text{m}$  only objects located at  $\pm 0.4 \mu\text{m}$  are within the same plane of focus. Therefore, structures from different cells can be easily distinguished since they are at different plane of focus and cannot be focused simultaneously for photography. The small depth of focus used allowed us to distinguish clearly the presence (or absence) of staining in the nucleus and cytoplasm of individual cells.

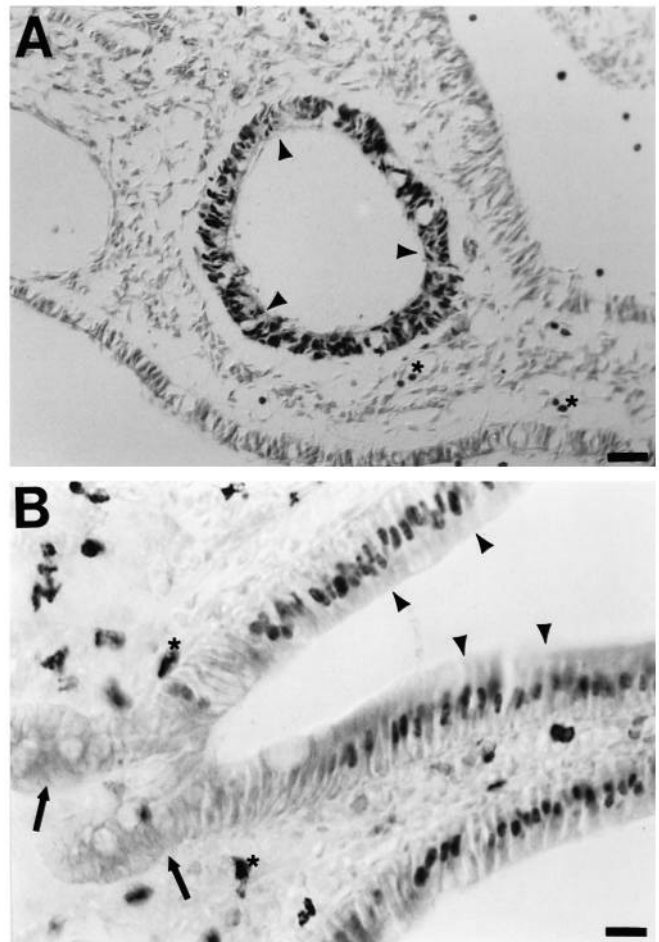
## RESULTS

These immunohistochemical studies were conducted with two different polyclonal antisera that recognized the STF-1 protein, one raised to the N-terminal region of XIH box 8 (Peshavaria et al., 1994) and the other to the C-terminal region of the rat STF-1 (Leonard et al., 1993). The same results were obtained with each. In all stages examined, STF-1 immunoreactivity was localized to the nucleus. The distribution of STF-1 was examined throughout development beginning at day 8 (E8; 5 to 7 somites). Cells containing STF-1 were first seen at E8.5 (Fig. 1A), which corresponded to embryos with 11-13 somites (Kaufman, 1992). At E8.5, STF-1<sup>+</sup> cells were present in the endodermal layer of the dorsal region of the gut (Fig. 1A) but not in the laterally located endodermal cells. At this stage the midgut is still wide open into the yolk sac and lacks a ventral wall. In 13- to 20-somite embryos (E9) when the wide communication between the midgut and yolk sac narrows and the gut has become tubular, cells containing STF-1 were seen in a transverse band of endoderm along the dorsal, lateral and ventral regions of the presumptive duodenum (Fig. 2A) and in cells forming the dorsal pancreatic primordium. The appearance of STF-1 immunoreactivity at E8.5 preceded that of GLU or IN C-P, which were first seen at E9.5 (20 somites; Teitelman et al., 1993). At E9.5, STF-1<sup>+</sup> cells were also seen in the ventral pancreatic primordia (Fig. 1B) and at E11.5, groups of cells adjacent to the pancreatic duct were immunostained. Two days later, at E13.5, the great majority of cells of the epithelium of the pancreatic duct contained STF-1 immunoreactivity (Fig. 1C,D). In addition, the pancreatic primordia contained numerous clusters of STF-1<sup>+</sup> cells, some of which had darkly stained nuclei while other cell nuclei contained less immunoreactive product (Fig. 1D). At E16.5, when endocrine cells began to aggregate to form pancreatic islets, STF-1<sup>+</sup> cells were located in the newly formed islets, surrounded mainly by unstained exocrine tissue. The number of immunostained pancreatic duct cells had also significantly decreased. One day later, at E17.5, STF-1<sup>+</sup> cells in the pancreas were seen exclusively within islets (Fig. 1E) and were nearly absent from the pancreatic duct.

As shown previously, STF-1 protein in adult pancreas is restricted to islet cells (Fig. 1F), with no expression detected in exocrine cells (Peshavaria et al., 1994).

In contrast to the non- $\beta$  cells of the pancreas, expression of STF-1 in the duodenal mucosa persisted throughout life. At E9.5 STF-1 was expressed by most cells of the epithelium of the mucosal layer but not by cells of the adjacent connective tissue sheath. In duodenum of older embryos and adults, almost all cells forming the simple columnar epithelium that line the villi were STF-1<sup>+</sup> whereas the crypt cells did not contain the homeoprotein. Cells of the other layers of the mucosa as well as cells of the submucosa, muscularis and adventitial layers of the wall of the duodenum also lacked STF-1 expression (Fig. 2B,C). Cells of the gut wall in other regions of the digestive tract never contained STF-1 immunoreactivity (not shown).

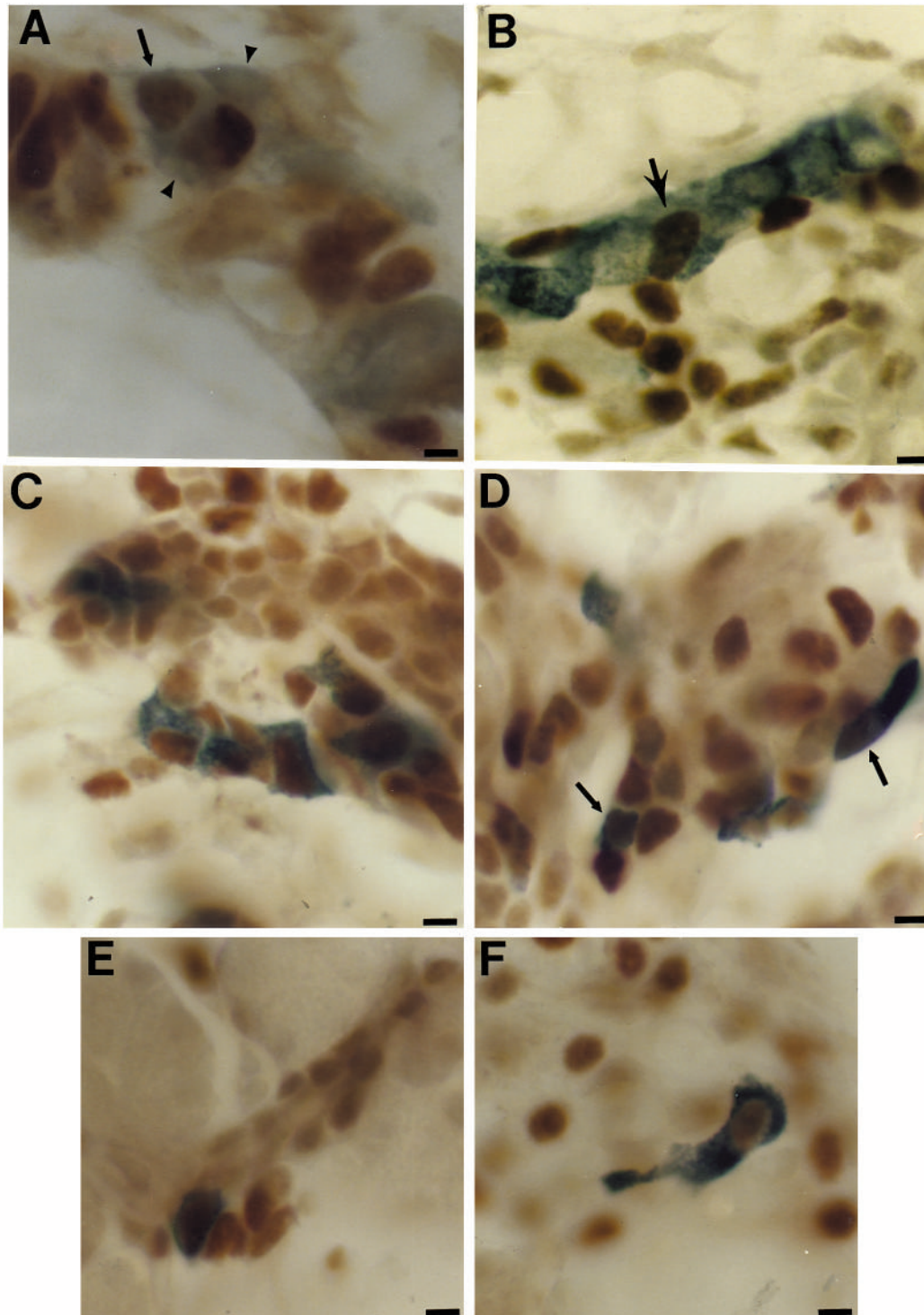
To determine the identity of the islet cells expressing STF-1, we performed double immunohistochemical visualization of the homeoprotein with each of the four pancreatic hormones. At E9.5, a time when glucagon and insulin first appeared, some STF-



**Fig. 2.** Expression of STF-1 in the duodenum. (A) Photomicrographs of a cross section of the duodenum at E9.5 illustrates that most cells of the mucosa (arrowheads) express the homeoprotein; bar, 50  $\mu\text{m}$ . (C) Photomicrograph of an adult duodenum illustrates the presence of STF-1<sup>+</sup> cells in the epithelium that lines the villi (arrowheads). In contrast, nuclei of cells in the crypt (arrows) lack STF-1 immunoreactivity. Dark cells, indicated with asterisks, are red blood cells. Bar, 20  $\mu\text{m}$ .

$1^+$  cells coexpressed IN C-P or GLU (Fig. 3). While most  $IN^+$  cells expressed the homeoprotein, few  $GLU^+$  cells at E9.5 and at later stages contained the antigen (Fig. 3A,B and Table 1). During

development, the number of  $IN^+$  cells increased and almost all of them expressed STF-1 (Fig. 3C). At E14.5 and at P1, when  $\delta$  and PP cells first differentiate, STF-1 was detected in approximately



**Fig. 3.** Coexpression of STF-1 with hormones in the embryonic pancreas. These photomicrographs illustrate the immunohistochemical localization of a hormone (visualized with a blue reaction product) and STF-1 (visualized with a brown reaction product) in the same tissue section of embryonic pancreas. (A,B) Coexpression of STF-1 and glucagon at E9.5 (A), and E13.5 (B). A shows glucagon cells expressing the homeoprotein (arrow), alpha cells with unlabeled nuclei (arrowheads) and cells in which only the nucleus is stained. Similar cells are also visualized in B. Bar, 4  $\mu$ m. (C) Coexpression of insulin and STF-1 at E14.5. Bar, 4  $\mu$ m. (D) Cells indicated with arrows contain somatostatin and STF-1. Bar, 4  $\mu$ m. (E,F) Coexpression of STF-1 and PP at P1 (E) and in the adult (F). Also note that nuclei of exocrine cells at P1 are unstained. Bar, 4  $\mu$ m.

40% of SOM<sup>+</sup> and PP<sup>+</sup> cells (Fig. 3D,E and Table 1). We found that most  $\beta$  cells of adults contained STF-1 whereas this protein was only present in a fraction of the  $\alpha$  and  $\delta$  cells (Table 1). In addition we determined that some PP cells (8.6%) of adult islets contain STF-1 immunoreactivity (Fig. 3F).

To ascertain whether STF-1 was expressed in precursors of the exocrine pancreas, we co-localized amylase, an exocrine cell marker, and STF-1 in embryonic and adult pancreas respectively. While previous studies indicated that amylase immunoreactivity was first detected at E14.5 (Teitelman et al., 1987), we now detect cells expressing amylase at E13.5. The appearance of amy<sup>+</sup> cells at an earlier stage is probably due to the improved sensitivity of the avidin-biotin system. Examination of E13.5 pancreas revealed that 41.3% of amylase<sup>+</sup> cells contained STF-1 (Fig. 4A,B and Table 1). Coexpression of amylase and the homeoproteins decreased dramatically at E17.5 (Fig. 4C and Table 1) and, in adults, no amylase<sup>+</sup> cells expressed STF-1 (Fig. 4D and Table 1).

## DISCUSSION

The studies reported here sought to determine whether STF-1 expression was restricted to the pancreatic primordia, and, if so, whether a specific cell type of the pancreas contained the homeoprotein or, alternatively, whether the homeodomain protein was initially expressed by multipotential precursors and only later became restricted to a single cell type during islet cell maturation.

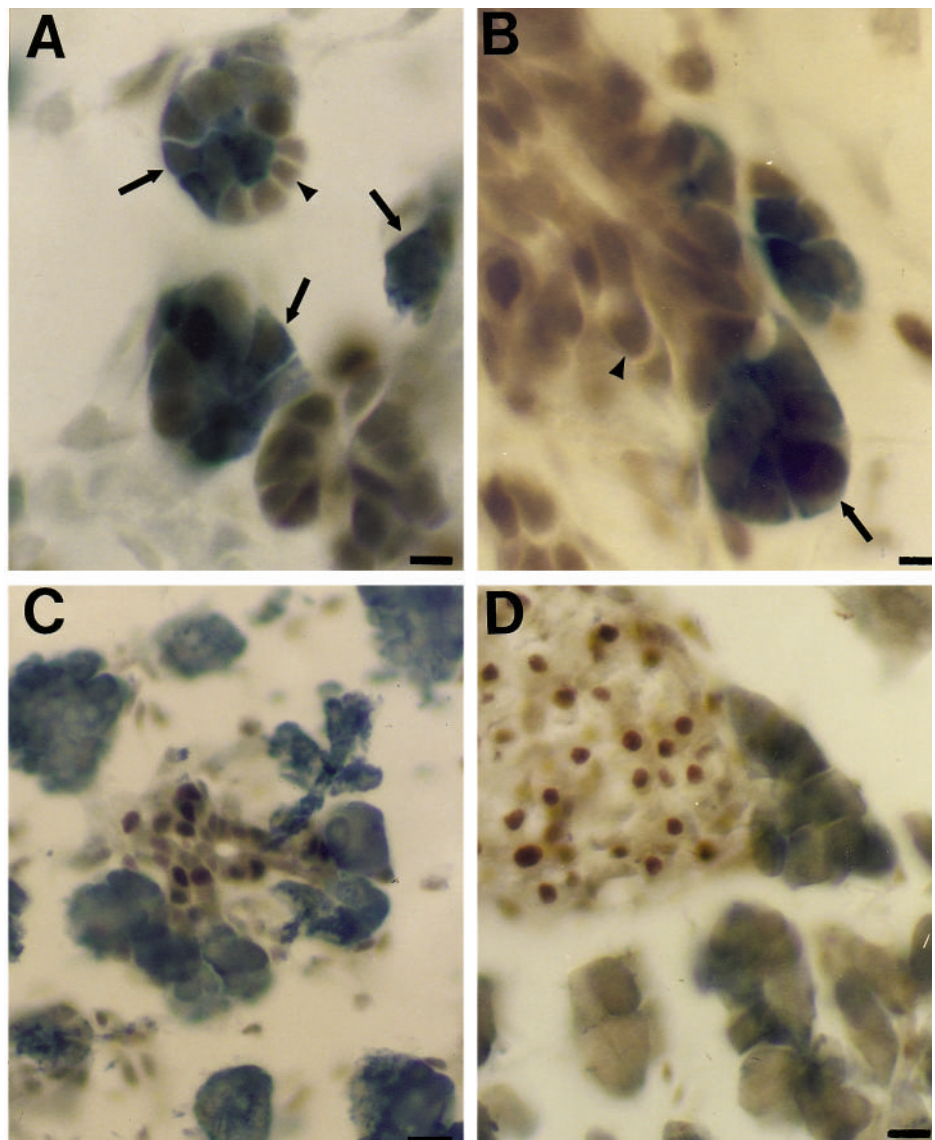
We found that STF-1 expression first appeared in a narrow transverse band of endoderm in the duodenum at E8.5 and preceded that of the islet hormones. At this stage the embryo has just completed the process of turning, and the tubular shape of the gut becomes apparent (Kaufman, 1992). In addition, we show that during development, STF-1 was transiently expressed in cells of the pancreatic duct and in endocrine cells with each islet hormone as well as in acinar cells with the exocrine protein amylase. The distribution of STF-1<sup>+</sup> cells became gradually restricted and, in the mature pancreas, STF-1 immunoreactivity was localized to  $\beta$  cells and to small subsets of the other endocrine non- $\beta$  cells of the islet. Collectively, these observations suggest that in mouse, as in *Xenopus*, STF-1 provides a spatial and temporal marker of endodermal commitment to a pancreatic and subsequently, to an endocrine  $\beta$  cell fate.

The fact that a subset of GLU<sup>+</sup> cells contained STF-1 from the time they first

**Table 1. Coexpression of STF-1 and pancreatic exocrine and endocrine markers during development**

	Glu/STF-1	Som/STF-1	PP/STF-1	Amy/STF-1
E9.5	11.4±0.9	ne	ne	ne
E13.5	9.5±0.78	ne	ne	41.3±3.1
E14.5	ND	46.5±2.9	ne	34.8±1.9
E16.5	ND	ND	ne	9.5±0.5
P1	ND	ND	40.2±3.5	ND
Adult*	2.68±0.09	15.2±1.2	8.6±0.35	0

At least six embryonic and newborn pancreata were processed for each antigen combination. Total number of cells scored was 234 cells at E9.5 and over 500 cells at other developmental stages. The number of cells expressing STF-1 and a pancreatic antigen is expressed as the mean percentage ±s.e.m. of the cells immunoreactive to the antigen. Abbreviations: ND, not determined; ne, not expressed (pancreatic exocrine or endocrine marker is not expressed at that developmental stage). \*Peshavaria et al. 1994.



**Fig. 4.** Exocrine cells transiently express STF-1 during development. (A,B) The colocalization of the homeoprotein and amylase at E15.5. At this stage many amylase<sup>+</sup> cells express STF-1 (arrows), also shown are STF-1<sup>+</sup> Amy<sup>-</sup> cells (arrowheads); Bar, 4  $\mu$ m. (C) Photomicrograph of an E17.5 pancreas shows that, at this stage, most exocrine cells do not contain STF-1. Also shown is a small cluster of STF-1<sup>+</sup>, amylase<sup>-</sup> cells. It is likely that these cells are islet cells. Bar, 40  $\mu$ m. (D) In adult pancreas, homeoprotein expression is restricted to islet cells. Bar, 15  $\mu$ m.

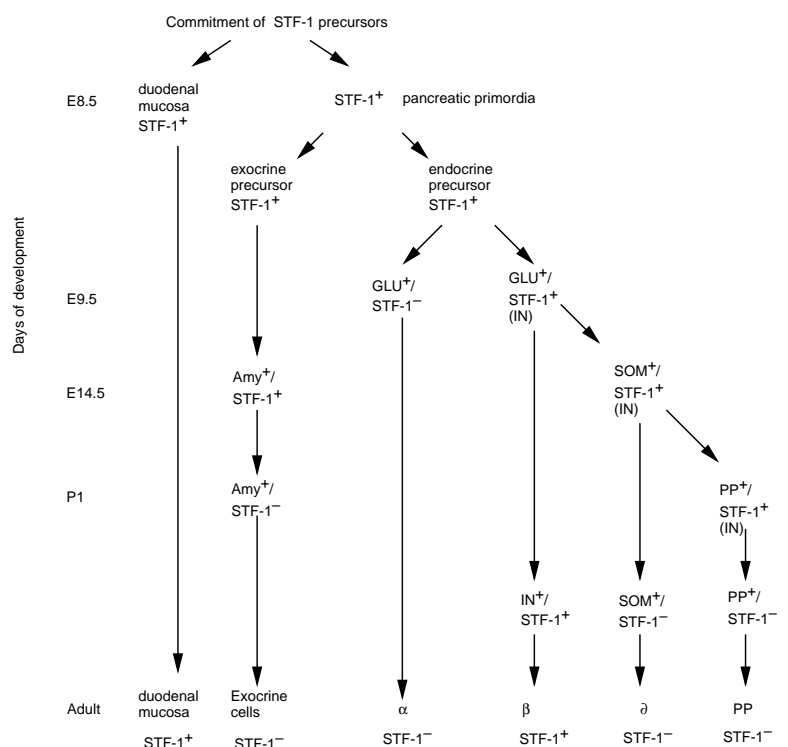
appeared supports our hypothesis that  $\alpha$  and  $\beta$  cells arose from a common pool of stem cells (Alpert et al., 1988; Teitelman et al., 1993) and also indicates that most  $\alpha$  cells extinguished STF-1 expression rapidly. Conceivably, the  $GLU^+/STF-1^+$  cells at E9.5 also contained IN and represent the precursors of the other islet cell types (Fig. 5). During midgestation, almost 50% of the newly differentiated  $SOM^+$  cells expressed STF-1, strongly suggesting that  $\delta$  cells were generated from precursors containing this homeoprotein. The fact that, in embryos,  $\delta$  cells coexpressed IN when they first appeared (Alpert et al., 1988), is consistent with the proposition that  $SOM$  precursor cells contained IN in addition to the homeoprotein (Fig. 5). Similarly, PP cells also probably arose from cells coexpressing STF-1 and IN (Fig. 5). Since only a fraction of embryonic non- $\beta$  cells expressed the homeoprotein, STF-1 expression may be incompatible with differentiation of mature  $\alpha$ ,  $\delta$ , PP and exocrine cell types. Alternatively, STF-1 expression may be lost because it plays no role in specific gene transcription in non- $\beta$  cells. In adults, we found that STF-1 was expressed by most  $\beta$  cells and by a subset of  $\alpha$ ,  $\delta$  and PP cells. It is tempting to speculate that the non- $\beta$  islet cells that contained STF-1 may represent immature cells which possess a 'precursor-like' potential.

The observation that many exocrine cells expressed STF-1 protein when they first differentiate supports a common origin for pancreatic exocrine and endocrine cells from STF-1<sup>+</sup> endodermal precursor cells present in the pancreatic duct. The origin of acinar and islet cells from a common stem cell population has been controversial. Since pancreatic islet cells display a large number of neural properties (Pearse, 1977; Alpert et al., 1988; Le Douarin, 1988; Teitelman, 1990) it was proposed that exocrine tissue derived from endoderm, while endocrine cells were generated by the neural crest (Pearse, 1977). However, previous experiments performed in chick-quail chimeras (Le Douarin, 1982) and mouse embryos (Pictet et al., 1976; Teitelman, 1990) and the present observations, support a common origin for both endocrine and exocrine pancreatic cells as indicated in our model (Fig. 5).

Since exocrine cells never expressed islet-specific cell markers (Teitelman et al., 1987), the subset of amylase<sup>+</sup> STF-1<sup>+</sup> precursor cells probably diverged towards an exocrine pathway of differentiation at the time they became specified. In agreement with this possibility, recent studies by Kruse et al. (1993) showed that an essential transcription control element within exocrine specific genes, including those of elastase I and amylase 2, functions as an endocrine-specific control element in transgenic animal. This element is also contained within the transcription control region of the insulin and somatostatin genes, and bears sequence similarity to the STF-1 binding site. It was proposed that this element may act during pancreatic determination prior to the divergence of the acinar and islet cell lineages.

Our present study also documents that most

epithelial cells of the duodenal mucosa expressed STF-1 throughout life. In contrast, Ohlsson et al. (1993) did not find homeoprotein<sup>+</sup> cells in gut. In agreement with our finding, however, Leonard et al. (1993) and Miller et al. (1994) reported STF-1 mRNA expression in duodenum of adults. In embryos, all cells lining the lumen of the duodenum were STF-1<sup>+</sup>. The distribution of STF-1<sup>+</sup> cells, however, changed during development due to modifications in the cytoarchitecture of the gut. During maturation, the duodenal mucosa forms numerous folds (villi), containing absorptive, secretory and enteroendocrine cells, and these folds are continuous with invaginations or crypts, which are formed mostly by stem cells (reviewed in Neutra, 1988). In adults, STF-1 expression was restricted to epithelial cells lining the villi but was absent from crypt cells. Since cells of the villi



**Fig. 5.** Proposed model of pancreatic cell determination and differentiation. At around E8.5, expression of STF-1 endows a dorsoventral portion of endodermal cells with the positional status of caudal foregut-rostral midgut. Under the influence of unknown factors, dorsal and ventral pancreatic anlagen bud from this area, while the gut tube itself forms the duodenum. STF-1 expression throughout the dorsal, lateral and ventral duodenal epithelium lining the lumen of the gut is maintained throughout life. The dorsal and ventral pancreatic primordia arise at E9.5 and E10 respectively and initially all their cells are STF-1<sup>+</sup>. The first glucagon and insulin cells are seen at E9.5. At this stage the vast majority of the  $\beta$  cells are STF-1<sup>+</sup>, but only a subset of  $\alpha$  cells contain the homeoprotein. It is possible that the STF-1<sup>+</sup>GLU<sup>+</sup> cells also contain insulin and represent the precursors of the  $\beta$ ,  $\delta$  and PP cells. In agreement with this hypothesis, subsets of  $SOM^+$  and  $PP^+$  cells express insulin (Alpert et al., 1988) and STF-1 (present work). STF-1 is also expressed by exocrine cell precursors. At E14.5, the first amylase<sup>+</sup> cells arise and many of these cells contain STF-1. Homeoprotein expression outside the islets, however, declines rapidly, so that at E16.5, STF-1 signal is restricted to few ductal cells while the exocrine tissue is almost uniformly unstained. These pathways lead to the adult pancreatic expression pattern, in which STF-1 is found in 90% of  $\beta$ , and in small subsets of  $\delta$  (15%),  $\alpha$  (3%) and PP (9%) cells.

are constantly being renewed and cell replacement occurs by proliferation, upward migration and differentiation of the crypt stem cells, our findings indicate that cells of the duodenal epithelium of adults initiated STF-1 expression after they migrated into the villi and differentiated. As a consequence of this process of intestinal cell neogenesis and maturation, the duodenum was lined by STF-1<sup>+</sup> cells not only in embryos but also in adults. This suggests that STF-1 controls the expression of, as yet, unidentified molecules that are important for duodenal epithelial cell function.

The identity of the signals that initiate STF-1 expression in the gut and pancreas are unknown. It has been suggested that the notochord, which in early embryos is in close contact with midgut endoderm, may play a role in the induction of STF-1 expression (Ohlsson et al., 1993). This is an attractive hypothesis, since the notochord is a known source of inductive signals that may instruct surrounding tissues in a region specific manner during development (Holtfreter and Hamburger, 1955; Hemmati-Brivanlow et al., 1990). Our findings indicate that the first inductive signal initiated STF-1 expression in stem cells of both duodenum and pancreas. It is likely that the these two sets of precursors diverged at around E8, when commitment of endodermal cells to a pancreatic fate occurs (Wessels and Cohen, 1967). During maturation there were differences in the fate of STF-1 expression in gut and pancreas. Thus, while epithelial cells of the duodenum contained the homeoprotein throughout life, STF-1 expression in pancreas was transient in non- $\beta$  cells, persisting only in the insulin-producing cells. This observation suggest that STF-1 expression is down-regulated in all the cell types of the pancreatic cell lineage that do not synthesize insulin, and that other factor/s are required for its maintenance in  $\beta$  cells. According to this proposition, these molecule/s are present in  $\beta$  cells but not in ductal, exocrine or endocrine non- $\beta$  cells.

The signal/s that instruct endodermal cells to follow alternate pathways of differentiation remain to be determined. Tissue co-culture experiments have revealed that, following the initial commitment of the endoderm to a pancreatic fate, further differentiation of the pancreatic primordium requires interactions with mesoderm (Wessels and Cohen, 1967). It is now well established that the mesoderm secretes a variety of peptides implicated in growth and differentiation. Conceivably, factors secreted by mesodermal cells may be involved in a paracrine fashion in different aspects of pancreatic differentiation including the restriction of expression of STF-1 and insulin to the  $\beta$  cells of the pancreas.

This work was supported by NIH grants, by the Juvenile Diabetes Foundation International and in part by the Vanderbilt University Diabetes Research and Training Center Molecular Biology Core Laboratory and The Foundation for Medical Research. The authors thank M. Ehrlich (New York University) for her helpful comments.

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