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## Hepatocyte Nuclear Factor 3 $\beta$ Is Involved in Pancreatic $\beta$ -Cell-Specific Transcription of the *pdx-1* Gene

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**The mammalian homeobox gene *pdx-1* is expressed in pluripotent precursor cells in the dorsal and ventral pancreatic bud and duodenal endoderm, which will produce the pancreas and the rostral duodenum. In the adult, *pdx-1* is expressed principally within insulin-secreting pancreatic islet  $\beta$  cells and cells of the duodenal epithelium. Our objective in this study was to localize sequences within the mouse *pdx-1* gene mediating selective expression within the islet. Studies of transgenic mice in which a genomic fragment of the mouse *pdx-1* gene from kb  $-4.5$  to  $+8.2$  was used to drive a  $\beta$ -galactosidase reporter showed that the control sequences sufficient for appropriate developmental and adult specific expression were contained within this region. Three nuclease-hypersensitive sites, located between bp  $-2560$  and  $-1880$  (site 1), bp  $-1330$  and  $-800$  (site 2), and bp  $-260$  and  $+180$  (site 3), were identified within the 5'-flanking region of the endogenous *pdx-1* gene. Pancreatic  $\beta$ -cell-specific expression was shown to be controlled by sequences within site 1 from an analysis of the expression pattern of various *pdx-1*-herpes simplex virus thymidine kinase promoter expression constructs in transfected  $\beta$ -cell and non- $\beta$ -cell lines. Furthermore, we also established that this region was important in vivo by demonstrating that expression from a site 1-driven  $\beta$ -galactosidase reporter construct was directed to islet  $\beta$ -cells in transgenic mice. The activity of the site 1-driven constructs was reduced substantially in  $\beta$ -cell lines by mutating a hepatocyte nuclear factor 3 (HNF3)-like site located between nucleotides  $-2007$  and  $-1996$ . Gel shift analysis indicated that HNF3 $\beta$  present in islet  $\beta$  cells binds to this element. Immunohistochemical studies revealed that HNF3 $\beta$  was present within the nuclei of almost all islet  $\beta$  cells and subsets of pancreatic acinar cells. Together, these results suggest that HNF3 $\beta$ , a key regulator of endodermal cell lineage development, plays an essential role in the cell-type-specific transcription of the *pdx-1* gene in the pancreas.**

During mammalian pancreatic development, common multipotential endodermally derived precursors in the early embryo undergo a series of specific changes that lead to the differentiated exocrine and endocrine pancreas. The dorsal and ventral pancreatic primordia first appear as evaginations of the gut endoderm on day 9 postcoitum (p.c.) in the mouse. The first cells to express differentiated pancreatic hormone markers in these buds are found in embryos of about 20 somites, corresponding to 9.5 days p.c. (15, 18, 41, 55). The two buds grow independently, forming both exocrine and endocrine tissues, and finally merge on day 10.5 p.c. (41). Recent studies indicate that expression of the PDX-1 homeoprotein in a common precursor cell population is essential for the development of the endocrine and exocrine compartments of the pancreas, with pancreatic development becoming arrested at a very early post-bud stage in homozygous *pdx-1* mutant mice (1, 25, 35). PDX-1 was also recently shown to be essential for pancreatic development in humans (52). However, *pdx-1* not only is important in pancreatic development but is also expressed in the duodenum (18), and the morphology and differentiation of the rostral duodenum are affected in *pdx-1*<sup>-/-</sup> mice (35). The regulatory mechanisms that orchestrate the complex developmental transitions involved in cellular differentiation during embryogenesis have not been defined, but these studies clearly demonstrate that PDX-1 is essential for this process.

Endocrine cells of the adult pancreas are organized into the islets of Langerhans, which are distributed throughout the exocrine tissue. The four endocrine islet cell types,  $\alpha$ ,  $\beta$ ,  $\delta$ , and PP, express glucagon ( $\alpha$ ), insulin ( $\beta$ ), somatostatin ( $\delta$ ), or pancreatic polypeptide (PP) as their principal differentiated hormone products (reference 2 and references therein). Mammalian PDX-1 was first characterized as a transcription factor of the somatostatin (29, 31) and insulin (36, 39) genes by its ability to bind to and stimulate AT-rich *cis* elements within their transcription control regions. This gene product was referred to in these studies as the IPF-1 (36), STF-1 (29), or IDX-1 (31) protein, but the gene will be referred to here as *pdx-1* (for pancreatic and duodenal homeobox gene 1), the name given by the International Committee on Standardized Genetic Nomenclature for Mice. In the adult pancreas, PDX-1 is found within the nuclei of essentially all insulin-producing islet  $\beta$  cells (91%) and a subset of somatostatin (15%) producing  $\delta$  cells but is almost undetectable in other pancreatic cell types (18, 38–40).

Together, these data implicated PDX-1 as a vital regulator in a genetic program leading to the proper development of the pancreas and duodenum. However, the factors that control PDX-1 expression and activity during embryogenesis or in adult islet and duodenal cells are not understood. One approach to determining the molecular mechanisms influencing PDX-1 transcription is to identify those factors that commit specific cells to express this gene product. Since PDX-1 is expressed both in multipotential endodermally derived precursor cells in embryos and in fully differentiated cell types in adults, we postulated that transcription factors important for

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differentiation and lineage commitment pathways would be important in PDX-1 expression.

In this paper, we identify sequences within the mouse *pdx-1* gene that regulate pancreatic  $\beta$ -cell-type-specific transcription. Our analyses reveal that selective expression is controlled by the region from bp -2560 to -1880, which was also found to direct appropriate developmental and adult specific expression in transgenic animals. In addition, we demonstrate that a hepatocyte nuclear factor 3 (HNF3)-like element at bp -2007 to -1996 is important for the  $\beta$ -cell-type-specific activity of the -2560/-1880 bp region. We provide evidence that the positive-acting factor interacting with this element is HNF3 $\beta$ , a protein in the fork head/winged helix transcription factor family that is essential for endodermal cell lineage development (for a review, see reference 62). We propose that HNF3 $\beta$  acts in concert with an islet  $\beta$ -cell-enriched factor(s) to direct the selective expression of the *pdx-1* gene.

#### MATERIALS AND METHODS

***pdx-1* transgenic constructs and generation of transgenic mice.** The *pdx-1*- $\beta$ -galactosidase ( $\beta$ -gal) (*lacZ*) transgene construct XSlacZ contains 9.3 kb of genomic sequence, lying between an upstream *Xba*I site and a *Sma*I site within the homeobox in exon 2, fused in-frame to a  $\beta$ -gal cassette from pPD1.27lacZ, which encodes bacterial  $\beta$ -gal with simian virus 40 nuclear localization and polyadenylation signals (14). XSlacZPS was generated by adding the 3' 3.5-kb *Pst*I-*Sal*I fragment, containing sequences extending from just 5' of the stop codon to the 3' end of an approximately 15-kb lambda genomic clone (35). The PB-hsplacZ transgene contains 1 kb of 5' genomic sequence from the *Pst*I site at kb -2.7 to the *Bst*EII site at kb -1.7 upstream of *lacZ*. All the constructs were confirmed by restriction mapping and by partial-sequence analysis of the junctions. DNA was CsCl purified, and the entire insert was released from the vector by *Sal*I digestion, isolated by low-melting-temperature agarose gel electrophoresis, and purified by standard methods. A 1- to 5- $\mu$ l volume of a 3-ng/ $\mu$ l DNA solution was injected into the pronucleus of one-cell embryos from B6D2 females, which were then implanted into pseudopregnant ICR females. Some F<sub>0</sub> founder embryos were dissected 11.5 days p.c. (XSlacZPS) to analyze the *pdx-1*-driven  $\beta$ -gal expression pattern. Transgenic lines were subsequently generated for all three constructs.

**Detection of  $\beta$ -gal.** Embryos at 11.5 days p.c. (XSlacZPS) or digestive organs from 2-day-old pups (PB-hsplacZ) and adult pancreata were dissected in phosphate-buffered saline (PBS) and kept on ice for 10 min until fixation. The tissues were fixed in 4% paraformaldehyde at 4°C for 30 to 40 minutes, permeabilized for 15 to 30 minutes in 2 mM MgCl<sub>2</sub>-0.01% sodium deoxycholate-0.02% Nonidet P-40, in PBS, and reacted with 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside (X-Gal) substrate solution overnight at room temperature (6). Digestive organs from 2-day-old pups were incubated at 4°C to minimize background expression within the intestine. The tissues were then postfixed in 4% paraformaldehyde at 4°C and rinsed with PBS. Genotyping determined that X-Gal reactivity was detected only in transgenic embryos, demonstrating the specificity of the reaction for the transgene. Adult pancreatic tissue was processed, and 15- $\mu$ m cryostat sections were cut (6). Alternatively, pancreatic tissue was fixed in 4% paraformaldehyde in PBS for 1 h at 4°C; dehydrated for 1 h in a 70 to 100% ethanol series, twice for 1 h in isopropanol, and for 1 h in isopropanol-paralplast (50:50, vol/vol) at 56°C; and embedded in paralplast first for 1 h and then overnight at 56°C. Serial 5- $\mu$ m sections were taken and mounted on glass slides with Sta-on (Surgipath Medical Industries, Inc.).

**Genotyping.** Embryos and adults were genotyped by Southern blot analysis involving *Pvu*II digestion, with a 726-bp *Pvu*II internal *lacZ* fragment as a probe. DNA from extraembryonic membranes, neonatal brain tissue or adult tails was prepared and analyzed as described by Hogan et al. (20).

**Cell culture and transfections.** Monolayer cultures of HIT T-15 2.2.2 and  $\beta$ TC3 cells were maintained as described previously (39, 57). Baby hamster kidney (BHK) and NIH 3T3 cells were grown in Dulbecco modified Eagle medium containing 10% (vol/vol) calf serum and 50  $\mu$ g each of streptomycin and penicillin per ml. The *pdx-1*-pTk constructs were transfected by either the calcium phosphate coprecipitation procedure (HIT T-15 and BHK) (56) or the electroporation procedure ( $\beta$ TC3) (42). The precipitates (11  $\mu$ g total) used for the HIT T-15 and BHK transfections contained 1  $\mu$ g of *pdx-1*-pTk and pRSV LUC, while 10  $\mu$ g of each of these plasmids (60  $\mu$ g total) was used with  $\beta$ TC3 cells. The Rous sarcoma virus (RSV) enhancer-driven luciferase (LUC) expression plasmid, pRSV LUC (10), was used as a recovery marker. At 4 h after addition of the calcium phosphate DNA precipitate, the cells were treated with 20% glycerol for 2 min and harvested 40 to 48 h after transfection. The chloramphenicol acetyltransferase (CAT) activity from the reporter plasmid was normalized to the LUC activity of the cotransfected internal control plasmid. LUC and CAT enzymatic assays were performed as described by De Wet et al. (10)

and Nordeen et al. (34), respectively. Each experiment was carried out three to five times with at least two different plasmid preparations.

**Transfection constructs.** *pdx-1* sequences were isolated from a complete lambda clone insert (plasmid 572) containing a genomic fragment from approximately kb -6.8 to +8.2. All of the mouse *pdx-1* gene promoter sequences were cloned directly upstream of the herpes simplex virus thymidine kinase (HSV TK) promoter region in the CAT expression vector, pTK(An) (23). The 5' and 3' *pdx-1*-pTk expression constructs were formed using existing restriction enzyme sites. The 5' restriction enzyme, followed by the 3' restriction enzyme, was the convention used in naming these pTk constructs. The internal deletion mutant, PstBstpTk  $\Delta$ -2031/-1996, was generated by removing sequences from -2031 (*Sph*I) to -1996 (*Bst*NI) from PstBstpTk. The site-directed mutants of PstBstpTk were generated by PCR by the method of Saiki et al. (44). The following 5' oligonucleotides were used for mutagenesis: PDX-1 binding site mutant (PDX-1m), 5' CCAATTTACAAAATGCATGCCAGTCGACCAGAAG 3'; M1, 5' CCAAAAATGCATGCAATTAGCAACTCCTTGCTAAGCAAACATC CGGG 3'; M2, 5' CCAAAAATGCATGCAATTAGACCAGAAGGTTAGCCCTA AAACATCCTGGGGTGTGG 3'; M3, 5' CCAAAAATGCATGCAATTAG ACCAGAAGTGAAGCCCAACGAATGGGG TG TGGGT TAGGC 3'; M4, 5' CCAAAAATGCATGCAATTAGACCAGAAGGTTAGCAAGCAACATCCT GGG 3'; M5, 5' CCAAAAATGCATGCAATTAGACCAGAAGTGCT AATACCACATCCTGGGGTGTGG 3'; M6, 5' CCAAAAATGCATGCAATTAGACCAGAAGTGC TAAGCAAACAGAATGGGG TG TGGGT TAGGC 3'. The mutated nucleotides are underlined. A 3' oligonucleotide containing CAT gene sequences (5' AGCTCCTGAAAATCTCGCCAAGT 3') was also used in these PCRs. Each of the constructs was verified by DNA sequencing.

**Electrophoretic mobility shift assays.** Nuclear extracts were prepared from  $\beta$ TC-3, HIT T-15, NIH 3T3, and BHK cells by the method of Sadowski and Gilman (43), except that the cells were lysed by Dounce homogenization (20 strokes with pestle A). Human islet extracts were prepared by the methods described by Schreiber et al. (47). Binding reactions (20- $\mu$ l reaction mixtures) were conducted in the binding buffer [20 mM HEPES (pH 7.9), 20 mM KCl, 50 mM NaCl, 1 mM dithiothreitol, 1  $\mu$ g of poly(dI-dC), 10% (vol/vol) glycerol] of Wang et al. (53). Approximately 4  $\mu$ g of extract protein was used per gel mobility shift sample. Double-stranded oligonucleotides to detect the *pdx-1* -2017/-1991 element and the USF factor binding were end labeled with [ $\alpha$ -<sup>32</sup>P]dATP and the Klenow fragment of *Escherichia coli* DNA polymerase I and used as probes. The conditions for the competition analyses were the same, except that the specific competitor DNAs were included in the mixture (in the amounts detailed in the figure legends) prior to addition of extract. The double-stranded oligonucleotides were as follows: wild type, -2017 ATTAGTGCTAAGCAAAACA TCCTGGGG -1991; M4, 5' ATTAGGTAGAAGCAAAACATCCTGGGG 3'; M5, 5' ATTAGTGCTAATACCACATCCTGGGG 3'; M6, 5' ATTAGTGC TAAGCAAACAGAATGGGG 3'; and USF, 5' TAGGTGATAGGCCAGTGTA CCGGGTGTTC 3' (16). The mutated nucleotides are underlined. The rabbit anti-mouse HNF3 $\beta$  (3  $\mu$ l) or control preimmune polyclonal serum (3  $\mu$ l) was preincubated with extract protein for 10 min at room temperature prior to initiation of the DNA-binding reactions. The HNF3 $\beta$  antibody was raised against amino acids 1 to 117 of the mouse HNF3 $\beta$  protein (30); this antiserum appears to be HNF3 $\beta$  specific (30). The samples were subjected to electrophoretic separation on a 6% nondenaturing polyacrylamide gel at 150 V for 2 h under high-ionic-strength polyacrylamide gel electrophoresis conditions (39). The gel was then dried, and labeled DNA-protein complexes were localized by autoradiography.

**Isolation of nuclei and nuclease-HS-site analysis.** Nuclei were isolated from  $\beta$ TC-3 and NIH 3T3 cells as described previously (21), except that the cells were broken by Dounce homogenization (20 strokes with pestle A). The nuclear pellet was resuspended in nuclease digestion buffer (10 mM Tris [pH 7.4], 50 mM NaCl, 0.5 mM spermidine, 0.15 mM spermine, 0.25 M sucrose) at 25 absorbance units at 260 nm ( $A_{260}$  U)/ml. Nuclease digestions (5  $A_{260}$  U of nuclei in 0.5 ml) were started by the addition of micrococcal nuclease (MNase; Pharmacia) or DNase I (DPFF grade; Worthington), MgCl<sub>2</sub> (10 mM), and CaCl<sub>2</sub> (1 mM). After incubation for 5 min at 25°C, the reaction was stopped by adding sodium dodecyl sulfate (1%) and EDTA (25 mM), and genomic DNA was isolated by the method of Herrmann and Frischauf (19). The purified DNA (20  $\mu$ g/lane) was digested to completion with *Eco*RI and *Pst*I for analysis of 5' and 3' hypersensitive (HS) sites, respectively. The fragmented DNA was resolved by electrophoresis on a 1% agarose gel and transferred to a Zeta probe membrane (Bio-Rad) as described by the supplier. The membrane was probed with the mouse *pdx-1* *Pst*I-*Eco*RI fragment (10<sup>9</sup> cpm/ $\mu$ g) at nucleotides +1154 (*Pst*I) to +1954 (*Eco*RI). High-stringency conditions used for probing and washing the membrane were as described by the supplier. The labeled DNA bands were localized by autoradiography.

**Immunohistochemistry.** Adult mouse liver and pancreatic tissues were embedded and sectioned as described above. The sections were deparaffinized and rehydrated with distilled water. Immunoperoxidase staining was carried out essentially as described by Jetton et al. (24), except that endogenous peroxidase activity was quenched with 0.6% H<sub>2</sub>O<sub>2</sub> in methanol for 30 min. Primary antibodies were used at the following dilutions: guinea pig anti-insulin (Linc), 1:500, and rabbit anti-mouse HNF3 $\beta$  (a gift from Brigid Hogan), 1:10 and 1:50. The primary antibodies were incubated overnight at 4°C. Secondary antibodies, either donkey anti-guinea pig-horseradish peroxidase or goat anti-rabbit-horseradish

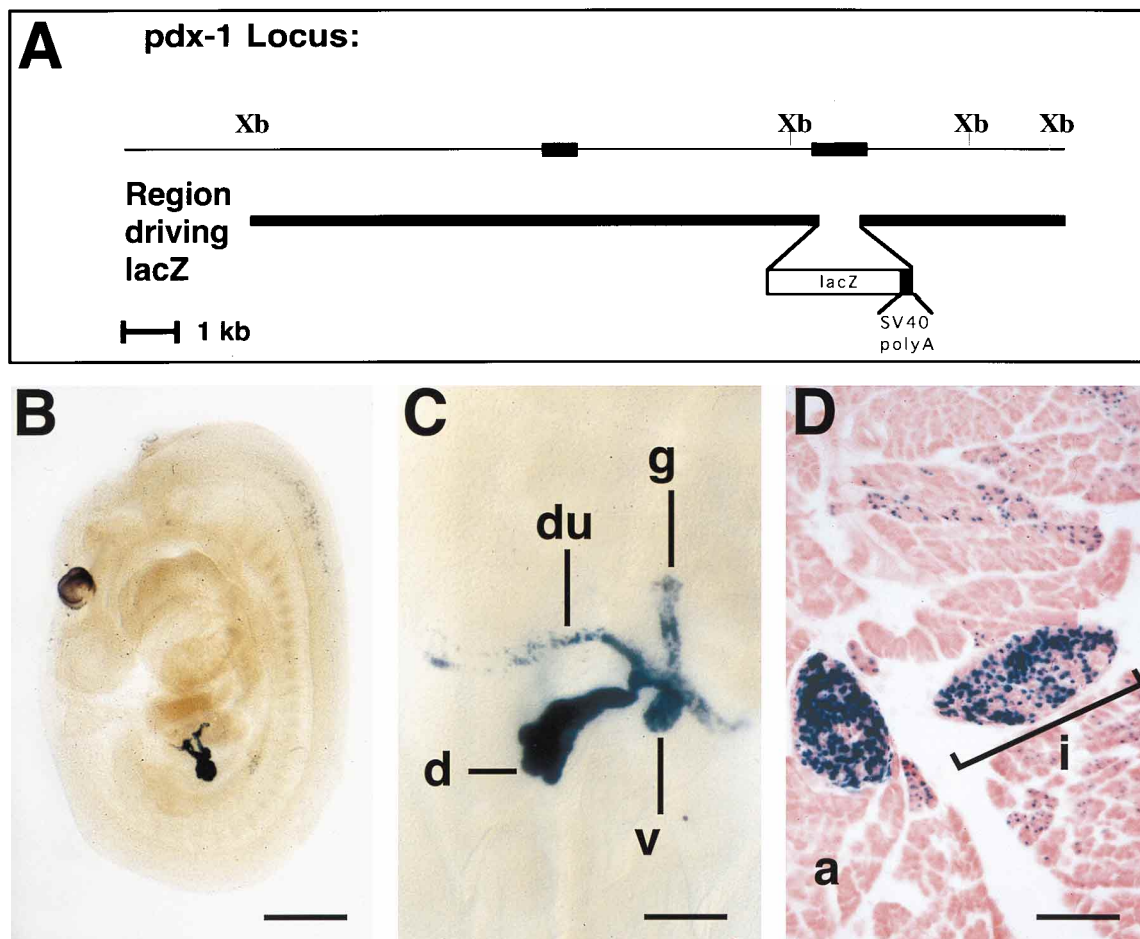


FIG. 1. Expression of a *pdx-1-lacZ* fusion gene in transgenic mice. (A) Diagrammatic representation of the *pdx-1* locus. Thin lines indicate noncoding sequences, and boxes represent the first and second exons. The homeodomain in exon 2 is depicted by the hatched box. The lower line represents the genomic sequences used to generate the *lacZ*-containing transgene, XSlacZPS. (B) Lateral view of a whole day 11.5 p.c. transgenic embryo stained with X-Gal to visualize the *pdx-1-lacZ* expression domain (blue). Expression is present within a restricted region of the developing posterior foregut. The melanized eye is visible to the upper left. The anterior is toward the top. Bar, 330  $\mu$ m. (C) Higher-magnification view of panel B, showing  $\beta$ -gal staining in the dorsal and ventral pancreatic buds and the rostral duodenum. Bar, 100  $\mu$ m. (D) In sectioned adult pancreatic tissue, *pdx-1-lacZ* expression is seen in the majority of islet  $\beta$ -cells and at lower intensity within acinar cells of some pancreatic lobes, but not others. Bar, 50  $\mu$ m. Abbreviations: Xb, *Xba*I; d, dorsal pancreatic bud; v, ventral bud; du, duodenum; g, gallbladder and cystic duct; a, acinar cells; i, islet.

peroxidase (Jackson Immunoresearch; 1:500), were incubated for 1 h at room temperature. Immunoperoxidase was detected with diaminobenzidine- $H_2O_2$  for 2 to 5 min. The samples were viewed under bright-field illumination and photographed with Kodak Ektachrome 64T film.

## RESULTS

***pdx-1* promoter activity in transgenic mice.** A transgenic approach was used to determine whether a 15-kb lambda genomic clone contained *cis*-acting sequences sufficient to recapitulate normal *pdx-1* expression. A construct replacing most of the second exon, including the homeobox, with a *lacZ* cassette was used to generate a reporter transgene plasmid, XSlacZPS (Fig. 1A), in which  $\beta$ -gal activity was controlled by *pdx-1* sequences. Three of nine  $F_0$  embryos sacrificed at 11.5 days p.c. for  $\beta$ -gal staining were transgenic and showed equivalent staining patterns (Fig. 1B). The  $\beta$ -gal staining was detected in a restricted region of the developing foregut corresponding to the dorsal and ventral pancreatic buds and the endoderm of the developing rostral duodenum (Fig. 1C). Transgenic mouse pups were also used to establish lines corresponding to the same *pdx-1-lacZ* construct. The adult pan-

creatic expression of the  $\beta$ -gal reporter in these lines occurred principally in the islets, although staining was also detected within acinar cells in some, but not all, pancreatic lobes (Fig. 1D). Multiple lines gave the same expression pattern (data not shown). The activity of the *pdx-1-lacZ* transgene reproduces to a large degree the expression pattern observed after recombination of *lacZ* into the endogenous locus (35). We conclude from these results that the regulatory elements necessary for appropriate development- and differentiation-specific transcription are present in the kb  $-4.5$  to  $+8.2$  region of the *pdx-1* gene, especially in relation to the adult cell pattern.

**Identification of nuclease-HS sites within the 5' region of the *pdx-1* gene in  $\beta$  cells.** Genes that are transcriptionally active or potentially active are contained in a chromatin conformation different from that of inactive genes (5, 51, 58). The binding of nonhistone proteins, such as transcription factors, contributes to the altered nucleosomal organization in active genes (11, 17). The binding of these factors renders these regions susceptible to digestion by DNase I and micrococcal nuclease (MNase). We probed for DNase I and MNase HS sites within the *pdx-1* gene in a PDX-1-expressing mouse islet

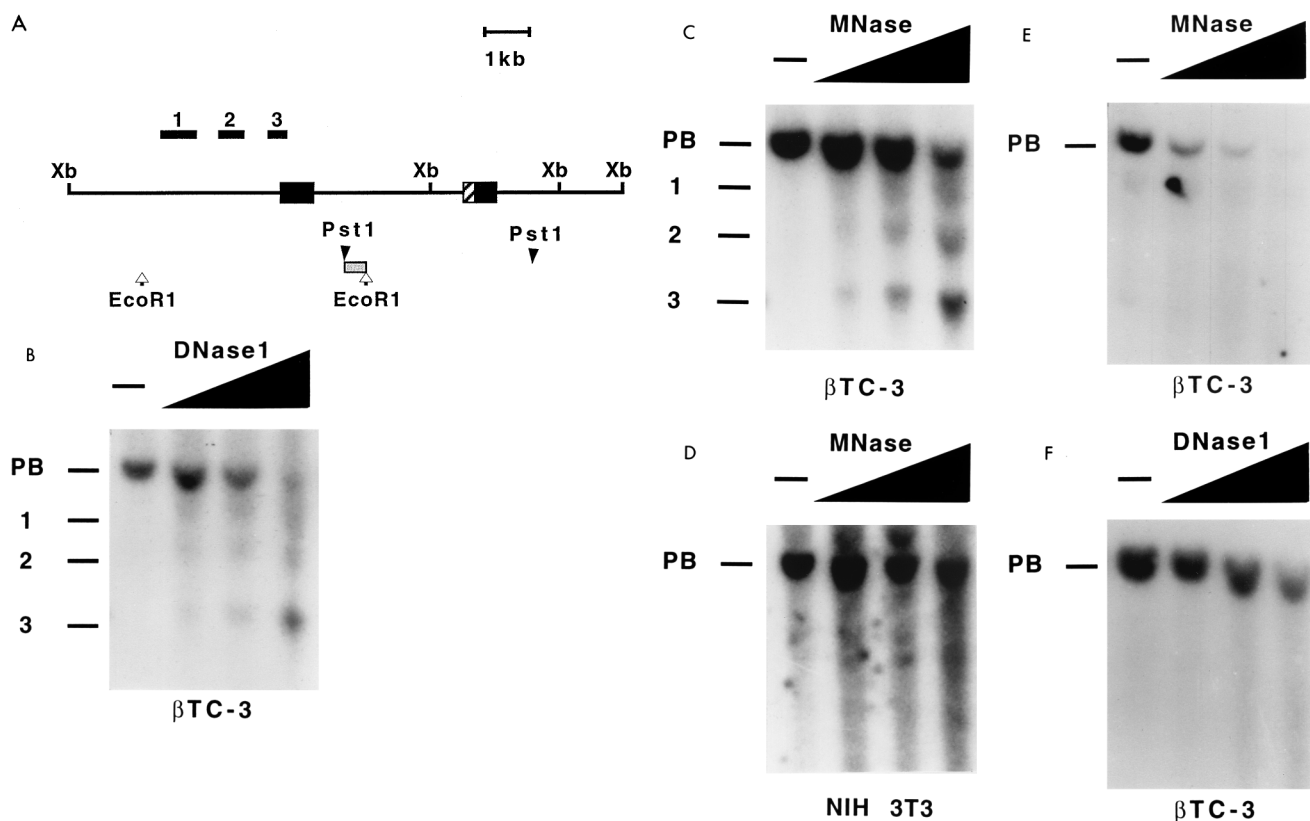


FIG. 2. Identification of 5' flanking nuclease-HS regions in the mouse *pdx-1* gene. (A) Genomic organization of the *pdx-1* gene. The location of the exons (black boxes) and flanking regions of *pdx-1* are shown. The locations of the *EcoRI* and *PstI* restriction sites are shown. The *PstI-EcoRI* fragment at bp +1154 to +1954 was used as the probe (gray box). Nucleotide numbering is relative to the S1 transcription start site in the rat *pdx-1* gene (48); the sequences of the rat and mouse *pdx-1* genes within this region are highly conserved (data not shown). The 5' and 3' HS sites were determined by *EcoRI* and *PstI* digestion, respectively. The HS sites in  $\beta$ TC3 cells were located at bp -2560 to -1880 (site 1), -1330 to -800 (site 2), and -260 to +180 (site 3). (B to F)  $\beta$ TC-3 (B, C, E, and F) and NIH 3T3 (D) nuclear DNA was either not digested (-) or digested with 2, 4, and 8 U of DNase I per ml (B and F) or 20, 50, and 100 U of MNase per ml (C to E). *EcoRI* (B to D) and *PstI* (E and F) restriction enzyme digests are shown. Analysis of 5' and 3' HS sites is shown in panels B to D and panels E and F, respectively. The exposure time in panels B, E, and F was 24 h; that in panels C and D was 48 h. The degrees of MNase and DNase I digestion were comparable in all cell types as determined by ethidium bromide staining of the agarose gels. The 5' HS sites in  $\beta$ TC-3 cells are labeled as described in the text. Abbreviations: Xb, *Xba*I; PB, parent band.

$\beta$ -cell line,  $\beta$ TC-3, and a nonexpressing mouse fibroblast cell line, NIH 3T3. Most of the region of the *pdx-1* gene that controlled appropriate developmental and islet  $\beta$ -cell-specific-expression in transgenic mice was analyzed in these experiments (Fig. 2).

Nuclease-HS sites were detected in  $\beta$ TC-3 cells at bp -2560 to -1880 (termed site 1), -1330 to -800 (site 2), and -260 to +180 (site 3); DNase I (Fig. 2B) and MNase (Fig. 2C) resulted in the same nuclease digestion pattern. The entire 3' region included in this analysis (approximately bp +175 to +5000) was resistant to nuclease attack in  $\beta$ TC-3 cells (Fig. 2E and F). HS sites were also not detected within either the 5' (Fig. 2D) or 3' (data not shown) region of the *pdx-1* gene in NIH 3T3 cells.

These results localized potential sequences controlling transcription of the *pdx-1* gene in  $\beta$ TC-3 cells to the 5' flanking region. Since the HS sites lie within the region of the *pdx-1* gene that defined selective expression in transgenic mice, we infer that the -2560 to -1880, -1330 to -800, and/or -260 to +180 sequences are used by endogenous factors essential in directing islet  $\beta$ -cell-specific expression.

**Sequences between bp -2560 and -1880 control  $\beta$ -cell-type-specific expression.** As a first step toward identifying *cis* elements within *pdx-1* that can direct selective expression in  $\beta$  cells, *pdx-1* sequences were subcloned directly upstream of the

HSV TK minimal promoter sequences in the CAT expression plasmid, pTk(An). Their activity was compared after transfection in two pancreatic  $\beta$ -cell lines, HIT T-15 and  $\beta$ TC-3, and the nonpancreatic BHK cell line. *pdx-1* is expressed only in the  $\beta$ -cell lines (39). We reasoned that *pdx-1*-pTk constructs containing sequences that mediate  $\beta$ -cell-specific expression would be more active in the  $\beta$ -cell lines. CAT activity from each transfected construct was normalized to the activity obtained from a cotransfected RSV LUC expression plasmid.

The *pdx-1* sequences analyzed with the pTk(An) vector spanned the region shown to be sufficient for selective expression in transgenic mice (Fig. 3). The extent of  $\beta$ -cell-specific activation was expressed as the ratio of *pdx-1*-pTk activity in either HIT T-15 or  $\beta$ TC-3 cells to that in BHK cells. The only constructs that were more active in  $\beta$  cells were those containing 5' flanking sequences, such as XbSacpTk and XbBstpTk. None of the 3'-flanking-sequence-driven constructs were expressed selectively (Fig. 3, compare PstHindpTk, XbXbpTk, or XbXb3'pTk with XbSacpTk). These findings supported our proposal that the sequences controlling *pdx-1* expression were spanned by the 5' flanking nuclease-HS sites. However, we also found that *pdx-1*-pTk constructs containing only HS site 2 (XmnSacpTk), HS site 3 (BglPst[A]pTk), or both HS sites 2 and 3 (BstSacpTk) were equally active in *pdx-1*-expressing and nonexpressing cells (Fig. 3). A recent study of the rat *pdx-1*

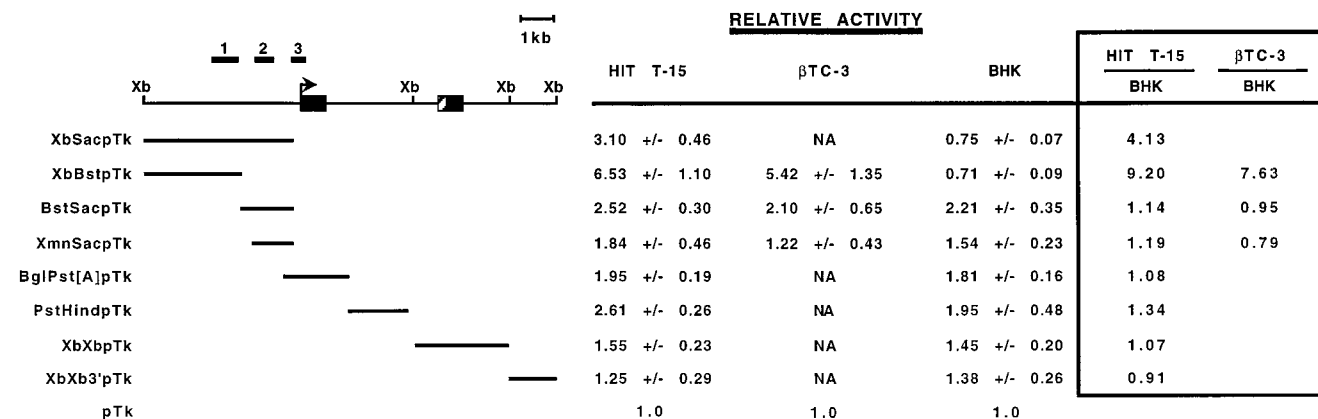


FIG. 3. Sequences in the 5' flanking region impart  $\beta$ -cell-specific expression. A schematic of the mouse *pdx-1* gene with the nuclease-HS sites at nucleotides  $-2560$  to  $-1880$  (site 1),  $-1330$  to  $-800$  (site 2), and  $-260$  to  $+180$  (site 3) is shown. The thick line spans the *pdx-1* sequences present in the *pdx-1*-pTk chimera. Each construct was named for the 5'-end followed by the 3'-end restriction enzyme site in the *pdx-1* gene, except BglPst[A]pTk, whose *pdx-1* sequences were in the antisense orientation. The *pdx-1*-pTk constructs were transfected into HIT T-15,  $\beta$ TC-3, and BHK cells. The CAT activity in each sample was normalized to the LUC activity from the cotransfected pRSV LUC plasmid. Results are represented relative to pTK(An)/CAT activity  $\pm$  standard deviation. The normalized pTK(An)/CAT activity in HIT T-15,  $\beta$ TC-3, and BHK cells was  $30,382 \pm 3,342$ ,  $5,457 \pm 1,350$ , and  $22,996 \pm 3,500$  cpm, respectively. The ratio of *pdx-1*-pTk activity in  $\beta$  cells (i.e., HIT T-15 or  $\beta$ TC-3) to that in BHK cells represents the fold  $\beta$ -cell-specific activation. The activity of the 3' flanking region constructs was the same when *pdx-1* gene sequences were cloned in their antisense orientation (data not shown). Abbreviations: Bgl, *Bgl*II; Bst, *Bst*EII; Hind, *Hind*III; Pst, *Pst*I; Sac, *Sac*I; Xb, *Xba*I; Xmn, *Xmn*I.

gene also indicates that HS site 3 does not contain positive-acting elements driving selective expression (48). Furthermore, we have found that the sequences of the mouse *pdx-1* gene within site 3 are closely related to those in the rat (data not shown) and that the number and locations of the transcription start sites are similar (59).

In contrast to the HS site 2- and 3-driven constructs, the HS site 1-driven construct, XbBstpTk, mediates more efficient transcription from the TK promoter in HIT T-15 and  $\beta$ TC3 cells (Fig. 3). However, XbBstpTk and XbSacpTk were also less active than the HS site 2- or 3-driven construct in BHK cells (Fig. 3), indicating that HS 1 sequences may function as a site of repression in nonexpressing cells. We conclude that sequences spanned by nuclease-HS site 1 at bp  $-2560$  to  $-1880$  are sufficient to support pancreatic  $\beta$ -cell-specific transcription.

To identify more precisely the sequences involved in controlling  $\beta$ -cell-type expression, we generated a series of 5' and 3' flanking deletion mutants spanning the region containing HS site 1 (Fig. 4). Compared to XbBstpTk, selective expression in HIT T-15 versus that in BHK cells was lost by deletion of HS site 1 (Fig. 4, compare XbPvupTk, XbSphpTk, or XbKpnpTk with XbBstpTk). In contrast, a construct that completely spanned this site (PstBstpTk) was the most effectively expressed *pdx-1*-pTk chimera. There was a two- to threefold drop in the  $\beta$ -specific activation when sequences from the 5' flanking region were deleted from PstBstpTk (Fig. 4, compare KpnBstpTk, StuBstpTk, or XmnBstpTk with PstBstpTk), indicating that essential *cis* elements were being removed in the process. The same preferential activation pattern was also observed with PstBstpTk in  $\beta$ TC3 (data not shown). These studies strongly indicated that HS site 1 sequences alone could direct  $\beta$ -cell-specific activation.

**The bp  $-2560$  to  $-1880$  region directs islet  $\beta$ -cell expression in transgenic animals.** To determine the relevance of the  $\beta$ -cell-specific regulatory sequences identified in  $\beta$ -cell lines to *pdx-1* regulation in vivo, *lacZ* transgenes spanning HS site 1 were generated. The first contained a 2.8-kb *Xba*I-*Xho*I fragment from the 5' end of the XSlacZPS transgene, and the second (PB-hsplacZ) corresponded to the *Pst*I-*Bst*EII region shown in Fig. 4. The *Pst*I-*Bst*Eii region is found within the

*Xba*I-*Xho*I fragment. Transgenic embryos carrying the *Xba*I-*Xho*I-hsplacZ transgene were analyzed at 14.5 days p.c. Expression of  $\beta$ -gal from the *lacZ* transgene was detected only in pancreatic islets and not in any other cell population, including other cell types in which *pdx-1* is normally expressed (data not shown). A line of mice carrying the PB-hsplacZ transgene was also generated, and analysis of tissues from 2-day-old pups from this line revealed  $\beta$ -gal expression in all islets (Fig. 5A) as well as the pyloric sphincter and the common bile duct. Within the pancreas itself, insulin colocalized with  $\beta$ -gal expression, demonstrating that these were indeed  $\beta$  cells (Fig. 5B). PstBst-driven expression of  $\beta$ -gal was also localized to  $\beta$  cells in a 10-month-old adult founder male (data not shown), suggesting that this region can drive reporter expression to islet  $\beta$  cells in early embryos, neonates, and adults. We conclude that the evidence for tissue- and cell-type-specific regulation of *pdx-1* conferred by PstBst in cell lines is borne out in vivo and that the PstBst region interacts with factors essential for controlling  $\beta$ -cell-specific transcription of the *pdx-1* gene.

**Identification of an HNF3-like element involved in regulating  $\beta$ -cell-specific expression.** In cell culture, selective expression from PstBstpTk was eliminated either by 5'-end deletions to the Sph1 site or by 3'-end deletions to the same site (Fig. 4, compare PstSphpTk and SphBstpTk with PstBstpTk). Thus, both the 5' and 3' regions contain key *cis* elements required for preferential expression in  $\beta$  cells. Given its relatively small size, we have initially focused on characterizing the importance of the SphBst region, which spanned nucleotides  $-2030$  to  $-1924$ , in mediating transcriptional activation in  $\beta$  cells.

When the region from bp  $-2031$  to  $-1996$  was deleted from PstBstpTk, a threefold decrease in HIT T-15 cell activity was reproducibly observed (Fig. 6B). Block mutations were made throughout this region to identify the control element(s) removed as a result of the internal deletion mutation (Fig. 6B). Mutations toward the 3' end of the bp  $-2031$  to  $-1996$  region reduced PstBstpTk activity (Fig. 6, compare M2 and M3 with PstBstpTk), while mutations at the 5' end had little or no effect (Fig. 6, compare PDX-1m and M1 with PstBstpTk). Mutations within an AT-rich element at bp  $-2031$  to  $-1915$  had no effect on PstBstpTk activity (Fig. 6B), although these sequences

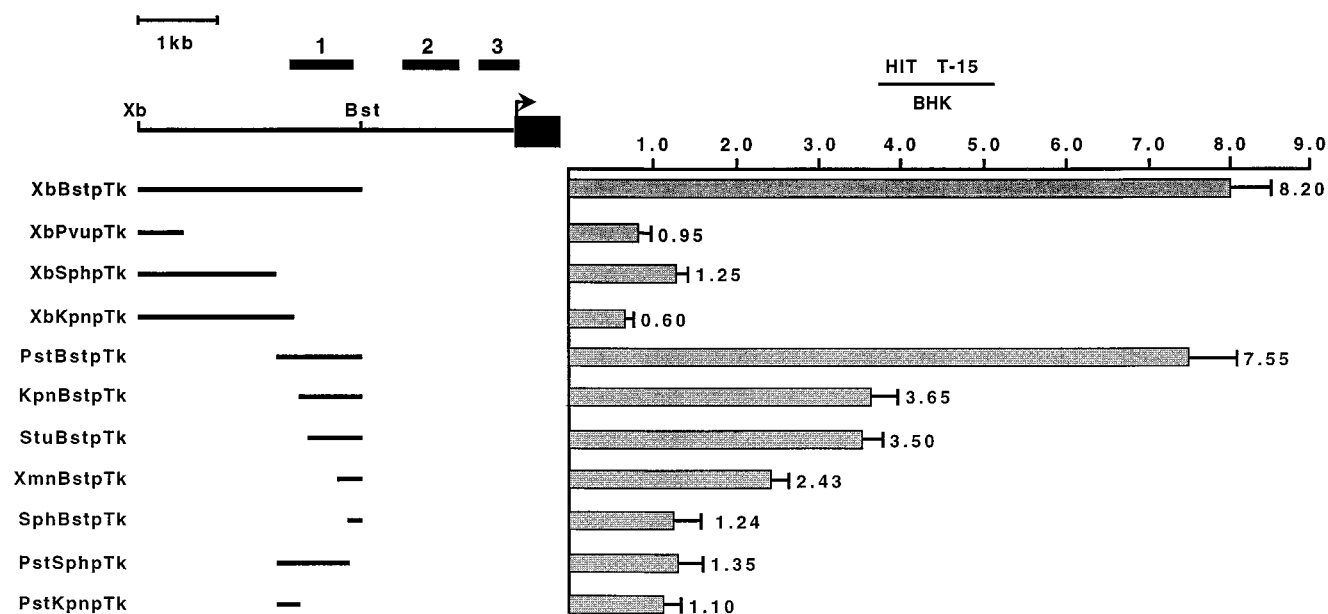


FIG. 4. Selective expression properties of the nuclease-HS site 1 sequences. A map of the mouse *pdx-1* gene sequences in the pTk(An) CAT expression vectors is shown. The thick line spans the region present in the construct. Each chimera was named by the 5'-end followed by the 3'-end restriction site used in *pdx-1*-pTk construction. A diagram of the 5' flanking region of the *pdx-1* gene with the bp -2560 to -1880 (site 1), -1330 to -800 (site 2), and -260 to +180 (site 3) nuclease-HS sites is shown. The activities were calculated as described in the legend to Fig. 4. The ratio of the *pdx-1*-pTk to pTk activity in HIT T-15 divided by that in BHK cells is presented as the mean  $\pm$  standard deviation of results from at least three independent transfections. Abbreviations: Bst, *Bst*EII; Xb, *Xba*I.

could bind effectively to PDX-1 in vitro (Fig. 6B, compare PstBstpTk to PDX-1m).

The region defined as mutationally sensitive by the PstBstpTk M2 and M3 mutants in Fig. 6B had substantial similarity to a consensus HNF3-binding site, with 11 of the 12 nucleotides at bp -2006 to -1994 matching a consensus HNF3 site (Fig. 6A). Analysis of small block mutations spanning these sequences strongly indicated that the HNF3-like site was required for maximal activity in HIT T-15 cells. Thus, two mutations that reside outside or within a less conserved portion of this element had little effect on activity (Fig. 6B, compare PstBstpTk M4 and PstBstpTk M6 with PstBstpTk), whereas a core region mutation reduced PstBstpTk activity to the level of that in the bp -2031 to -1996 internal deletion mutant (Fig. 6B, compare PstBstpTk M5 with PstBstpTk  $\Delta$ -2031/-1996). This HNF3 site mutant also reduced PstBstpTk activity in  $\beta$ TC-3 cells (Fig. 6C). In contrast, neither wild-type nor HNF3 site mutant PstBst region sequences influenced pTk activity in either non-*pdx-1*-expressing liver H4IIE (Fig. 6C) or BHK (data not shown) cells. These results strongly indicate that the HNF3-like site at bp -2006 to -1994 binds a factor required for HS site 1-driven expression in  $\beta$  cells.

#### HNF3 $\beta$ binds to the bp -2006 to -1994 element in $\beta$ cells.

The HNF3 family of transcription factors contain a conserved 110-amino-acid DNA-binding domain (62). Mammalian proteins in this family include factors enriched in both liver and pancreatic cells, like HNF3 $\alpha$ , HNF3 $\beta$ , and HNF3 $\gamma$  (32). The gel mobility shift assay was used to test the cell type specificity of the factors interacting with the HNF3-like element at bp -2006 to -1994 using nuclear extracts from  $\beta$ TC3, HIT T-15, H4IIE (a rat hepatoma cell line), BHK, and NIH 3T3 cells, and pancreatic islets. A single common protein-DNA complex was detected in  $\beta$  and liver cells, as well as human islet extracts, but not in NIH 3T3 or BHK extracts (Fig. 7A). The binding activity of the ubiquitously distributed USF transcription factor (16) in each of these extracts served as an internal control in these

experiments, and similar levels were detected in  $\beta$ TC3, HIT T-15, BHK, and NIH 3T3 cells (Fig. 7A).

The specificity of protein-DNA binding to the bp -2017 to -1991 probe was first determined by competition assays with the wild-type bp -2017 to -1991 element and the PstBstpTk M4, M5, and M6 mutants in HIT T-15 extracts. The M5 mutant did not compete, while both M4 and M6 competed efficiently (Fig. 7B). In addition, binding of the wild type and M4, M5, and M6 mutant probes in  $\beta$ TC-3 extracts was directly compared (Fig. 7C). The results of these experiments were similar and indicated that only the M5 mutant eliminated binding to the bp -2017 to -1991 element. Together with the transfection results, we conclude that the activator of HNF3-like element at bp -2006 to -1994 was a nuclear protein present in both liver and islet  $\beta$  cells.

To examine the potential involvement of HNF3 $\beta$  on bp -2006 to -1994 element-driven activity, we tested whether an antibody specific for mouse HNF3 $\beta$  affected the formation of the bp -2017 to -1991 complex in gel shift assays with H4IIE,  $\beta$ TC3, and human islet extracts. When anti-HNF3 $\beta$  antibody was preincubated with extract and then added to the bp -2017 to -1991 probe, the protein complex was quantitatively supershifted in  $\beta$  extracts (Fig. 8). This is a specific reaction, since the supershifted complex was not formed by adding preimmune serum. These results point to HNF3 $\beta$  as the  $\beta$ -cell nuclear protein that binds and activates bp -2006 to -1994 element-driven expression in vivo.

**HNF3 $\beta$  is expressed in pancreatic cells.** The expression pattern of HNF3 $\beta$  in relation to *pdx-1* within adult mouse pancreatic tissue was determined immunohistochemically. While HNF3 $\beta$  is known to be expressed in the developing dorsal pancreatic bud at 10 days p.c. (1) and its mRNA has also been detected in adult pancreatic tissue and exocrine cell lines (9), the distribution of HNF3 $\beta$  protein in the adult pancreas has not been reported. We therefore compared the expression of HNF3 $\beta$  and *pdx-1*-driven  $\beta$ -gal in pancreatic tissue from



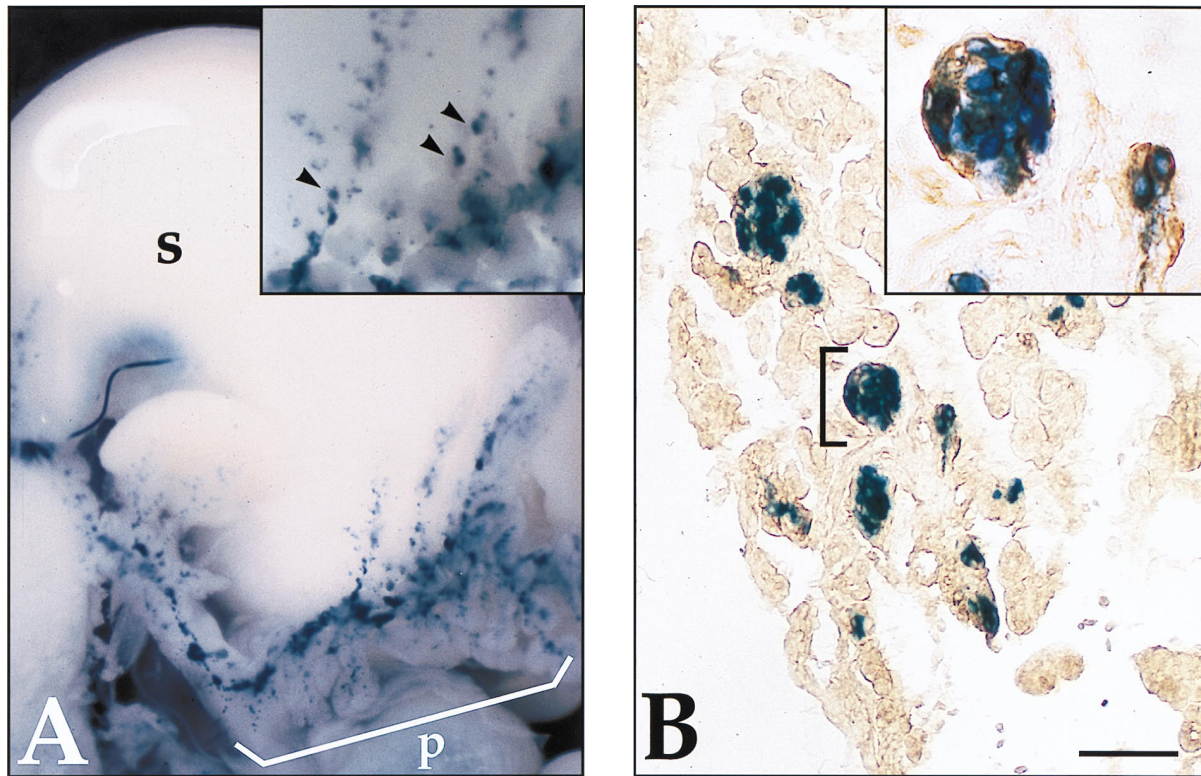


FIG. 5. Transgenic analysis of  $\beta$ -cell-specific regulatory regions. (A) Digestive organs of 2-day-old neonatal pups carrying a PB-hsplacZ transgene show  $\beta$ -gal expression (blue) in islets within the pancreas (bracket) and not within the exocrine cells, stomach, or duodenum. The inset shows islets at higher magnification (arrowheads). Expression is detected in islets close to the ductal epithelium and those located more distally. (B) PstBst- $\beta$ -gal expression colocalizes with insulin. The indicated islet is magnified in the inset, showing  $\beta$ -gal expression in blue overlapping insulin in brown. Bar, 45  $\mu$ m. Abbreviations: p, pancreas; s, stomach.

mice heterozygous for a *pdx-1-lacZ* fusion allele, *pdx-1<sup>lacZKO</sup>* (35). The pancreas of these animals is equivalent to that of *+/+* animals (35). As shown in Fig. 9A, HNF3 $\beta$  is expressed in islet cells, where *pdx-1*- $\beta$ -gal-positive cells are also found (Fig. 9E). HNF3 $\beta$  nuclear staining was also detected in acinar cells within some pancreatic lobes (Fig. 9C), whereas other lobes did not show expression (Fig. 9D). HNF3 $\beta$  was not detected in the spleen or pancreatic mesenchyme (data not shown). The variable acinar cell expression of HNF3 $\beta$  was similar to the transcription pattern of the *pdx-1*-driven *lacZ* from the endogenous locus (Fig. 9F and data not shown), as well as  $\beta$ -gal expression from the *pdx-1* transgene XSlacZPS (Fig. 1D). Thus, HNF3 $\beta$  is expressed in pancreatic cells that also express *pdx-1*, particularly in the endocrine compartment, consistent with the proposal that HNF3 $\beta$  is a bona fide regulator of *pdx-1* transcription in vivo.

## DISCUSSION

We have analyzed the basis for pancreatic islet  $\beta$ -cell-specific transcription of the mouse *pdx-1* gene, which encodes a homeodomain protein known to play an essential role during pancreatic development and for which strong circumstantial evidence that it is involved in islet  $\beta$  and  $\delta$  gene expression exists. Our results indicate that selective expression is directed by *pdx-1* 5' flanking sequences and that a significant level of control is exerted by the sequences between nucleotides  $-2560$  and  $-1880$ . A detailed mutational analysis of this region demonstrated that an HNF3-like element was important for activity. Thus, our data strongly suggest that at least some aspects of *pdx-1* transcription rely on HNF3 $\beta$ , a factor known from other

studies to be important in endodermal development (62). However, since HNF3 $\beta$  is not restricted to islet  $\beta$  cells, selective transcription of *pdx-1* likely results from HNF3 $\beta$  cooperating with another factor(s) having a more  $\beta$ -cell-restricted expression pattern.

Initially, we found that a transgenic  $\beta$ -gal reporter construct containing sequences from kb  $-4.5$  to  $+8.2$  of the mouse *pdx-1* gene recapitulated the endoderm-specific expression pattern of the endogenous *pdx-1* gene throughout development, from which we inferred that the *cis*-acting elements required for selective transcription were contained within these sequences. DNase I and MNase analyses on the endogenous *pdx-1* gene identified three HS sites, which spanned nucleotides  $-2560$  to  $-1880$  (site 1),  $-1330$  to  $-800$  (site 2), and  $-260$  to  $+180$  (site 3), which were found in *pdx-1*-expressing  $\beta$ TC-3 cells but not NIH 3T3 cells. These results indicated that the elements regulating *pdx-1* expression were located within these 5' flanking HS site sequences.

Among these three HS regions, we found that only the bp  $-2560$  to  $-1880$  site could direct  $\beta$ -cell-specific transcription from transiently transfected *pdx-1*-pTk CAT chimeras. Most importantly, these sequences also directed  $\beta$ -cell-specific transgene expression in vivo. While our transfection assays almost certainly cannot detect all of the factors regulating *pdx-1* transcription in vivo, these results indicated that site 2 or 3 sequences are less important in  $\beta$ -cell-specific expression. This proposal is supported by the occurrence of HS site 3 at bp  $-260$  to  $+180$  over the mouse *pdx-1* promoter and the observation that transcriptional activation from this region is apparently regulated by generally distributed factors (48). However,

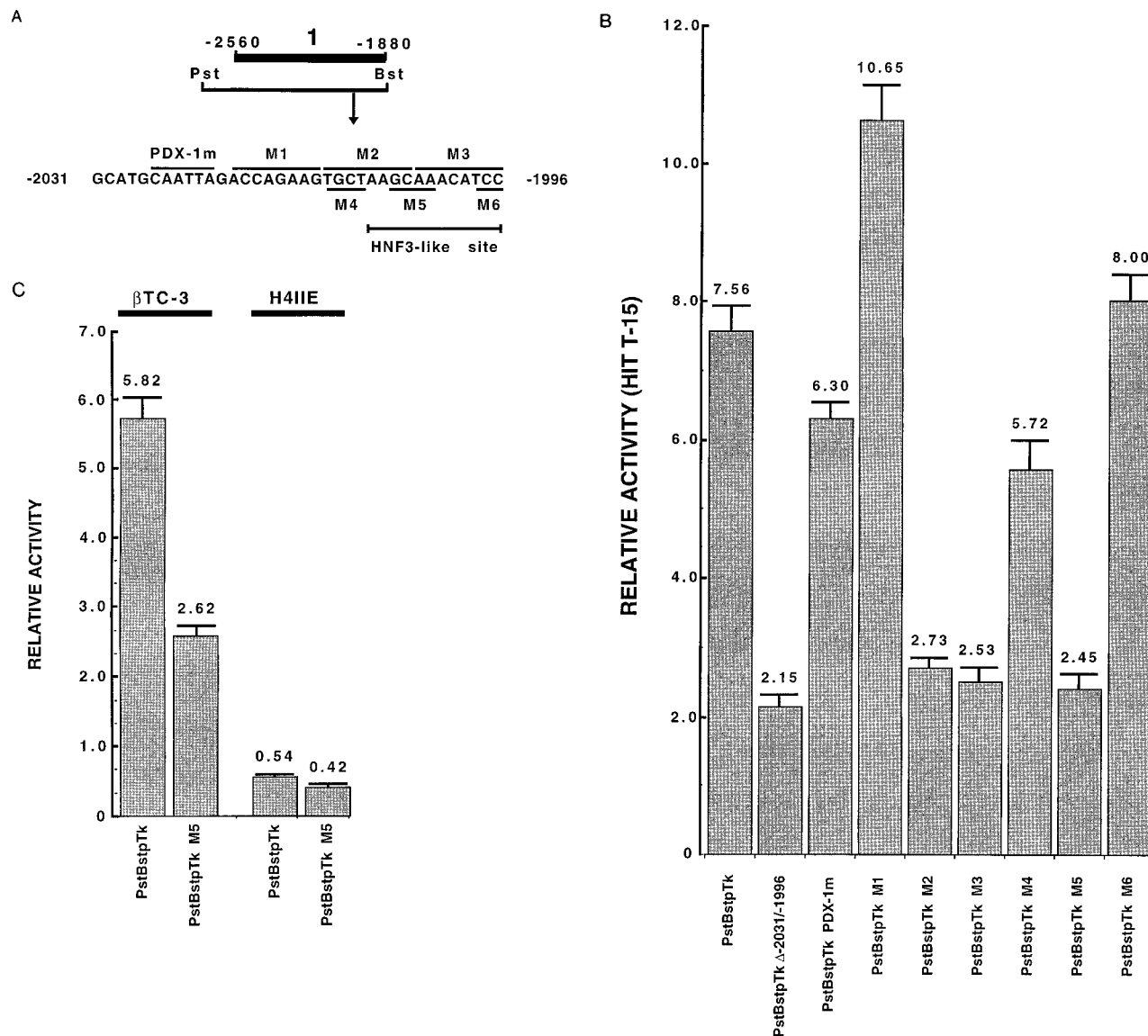


FIG. 6. An HNF3-like element is important in the activity of nuclease-HS site 1 in  $\beta$  cells. (A) The position of the nuclease-HS site 1 site within the PstBst region is shown diagrammatically. The sequence of the bp -2031 to -1996 region is indicated. Of the 12 nucleotides between bp -2007 and -1996, 11 match an HNF3 consensus binding site (VAWTRITTKRYTY, where V = A, C, or G; W = A or T; K = G or T; Y = pyrimidine, C, or T; R = purine, G, or A [Fig. 3C of reference 38]); the nonconsensus nucleotide in -2007 AAGCAAACATCC -1996 is underlined. The HNF3-binding site consensus sequence (VAWTRITTKRYTY, where V = A, C, or G; W = A or T; K = G or T; Y = pyrimidine, C, or T; R = purine, G, or A [Fig. 3C of reference 37]) was used in the alignment. The mutated nucleotides in PstBstpTk M1 through PstBstpTk M6 and PstBstpTk PDX-1m are indicated. The AT-rich sequences between bp -2031 and -2015 bind effectively and specifically to PDX-1 in vitro, and the mutation in PstBstpTk PDX-1m eliminates this binding activity (data not shown). The bp -2031 to -1996 region was deleted in PstBstpTk  $\Delta$ -2031/-1996. (B and C) Wild-type and mutant PstBstpTk activity in transfected HIT T-15 (B) and  $\beta$ TC-3 and H4IIE (C) cells. The ratio of the *pdx-1*-pTk to pTk activity is the mean  $\pm$  standard deviation of results from at least three independent transfections.

it is also possible that HS site 2 and other regions are required for *pdx-1* expression during development or in differentiated duodenal cells. A precedent for this comes from the finding that discrete regions of the mammalian HNF3 $\beta$  (45) and *Drosophila snail* (22) genes direct different spatial and cell-type-specific transcription patterns during embryogenesis. We are continuing our studies with transgenic mice to determine whether separate modules of the *pdx-1* gene direct different aspects of development- or differentiation-specific expression and how these correlate with the locations of HS sites 1, 2, and 3.

Maximal selective activity in transfection assays was obtained from a *pdx-1*-pTk chimera, PstBstpTk, spanning the bp

-2560 to -1880 nuclease-HS site. The activity of this construct was compromised by removing *pdx-1* sequences from either the 5' or 3' end of this region, although a construct containing sequences from bp -2158 to -1880 still had approximately one-third of the activity of PstBstpTk (XmnBstpTk in Fig. 4). Gel shift assays demonstrated that HNF3 $\beta$  was present in human islet and  $\beta$ -cell nuclear extracts that bound to the mutationally sensitive bp -2006 to -1994 element. Since HNF3 proteins bind DNA as a monomer via their winged helix DNA-binding domain (8), it is possible that HNF3 $\beta$  was the only protein present in the bp -2006 to -1996 element-binding complex detected in liver and  $\beta$ -cell extracts.

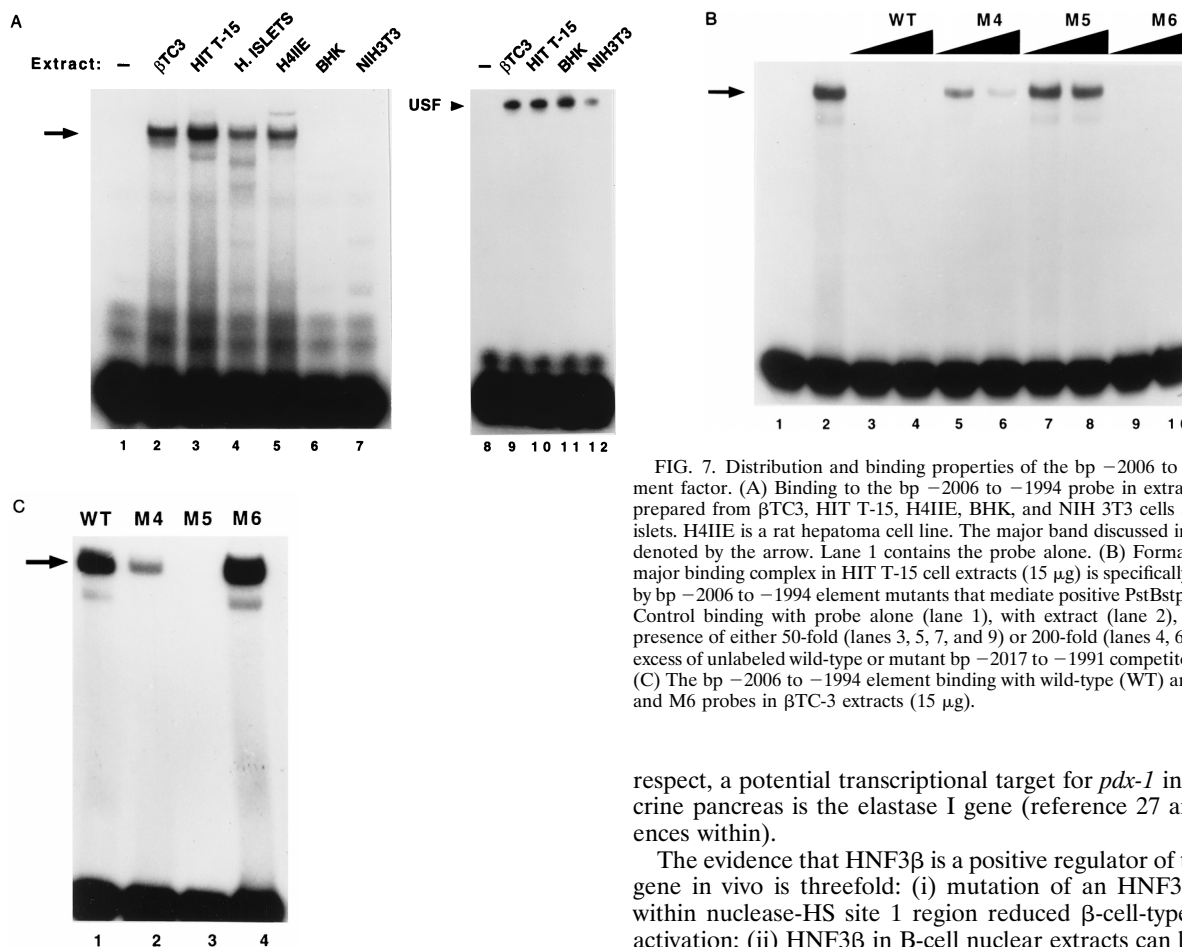


FIG. 7. Distribution and binding properties of the bp -2006 to -1994 element factor. (A) Binding to the bp -2006 to -1994 probe in extracts (15  $\mu$ g) prepared from  $\beta$ TC3, HIT T-15, H4IIE, BHK, and NIH 3T3 cells and human islets. H4IIE is a rat hepatoma cell line. The major band discussed in the text is denoted by the arrow. Lane 1 contains the probe alone. (B) Formation of the major binding complex in HIT T-15 cell extracts (15  $\mu$ g) is specifically competed by bp -2006 to -1994 element mutants that mediate positive PstBstpTk activity. Control binding with probe alone (lane 1), with extract (lane 2), and in the presence of either 50-fold (lanes 3, 5, 7, and 9) or 200-fold (lanes 4, 6, 8, and 10) excess of unlabeled wild-type or mutant bp -2017 to -1991 competitor is shown. (C) The bp -2006 to -1994 element binding with wild-type (WT) and M4, M5, and M6 probes in  $\beta$ TC-3 extracts (15  $\mu$ g).

HNF3 $\beta$  was also shown to be present in the nuclei of the majority of pancreatic islet cells and was detected uniformly within acinar cells of some pancreatic lobes, while it was not detected in other lobes. PDX-1, like HNF3 $\beta$ , was expressed in islets and was distributed in a similar pattern to HNF3 $\beta$  in acinar cells. Previous immunohistochemical analysis of *pdx-1* expression suggested a restriction to islets in the adult pancreas (18), since any low-intensity nonuniform staining over the acini, although nuclear, had been rejected as nonspecific background. However, we now propose that detection of *pdx-1* expression in pancreatic exocrine tissue, through the *pdx-1-lacZ* fusion allele, provides a more sensitive means of detecting bona fide low-level *pdx-1* expression. We note that Finegood et al. (13) observed incorporation of bromodeoxyuridine into a subset of pancreatic lobes in the adult rat, suggesting that en block formation of whole pancreatic lobes, containing both exocrine and endocrine cells, occurs normally during pancreatic growth and remodeling. Expression of *pdx-1* and HNF3 $\beta$  may therefore be correlated with the state of growth of a particular lobe. Similarly, the observed transient increase in the level of PDX-1 in the majority of exocrine cell nuclei following streptozotocin-induced  $\beta$ -cell injury (12) also suggests a link between PDX-1 and pancreatic growth during regeneration. It remains to be seen whether PDX-1 and/or HNF3 $\beta$  expression in acini is required for normal exocrine pancreas growth and differentiation and/or fulfills a maintenance function throughout the lifetime of the pancreas. In this

respect, a potential transcriptional target for *pdx-1* in the exocrine pancreas is the elastase I gene (reference 27 and references within).

The evidence that HNF3 $\beta$  is a positive regulator of the *pdx-1* gene in vivo is threefold: (i) mutation of an HNF3-like site within nuclease-HS site 1 region reduced  $\beta$ -cell-type-specific activation; (ii) HNF3 $\beta$  in B-cell nuclear extracts can bind specifically to this site; and (iii) HNF3 $\beta$  is detected in PDX-1-producing cells in vivo. Unfortunately, the role of HNF3 $\beta$  in the expression of *pdx-1* in vivo cannot be resolved from the phenotype of HNF3 $\beta$  homozygous null mutant mice, since these mice die in early embryogenesis prior to the outgrowth and differentiation of the pancreatic endoderm (3, 54). During the review of this paper, another report was published that describes the importance of HNF3 $\beta$  in rat *pdx-1* gene tran-

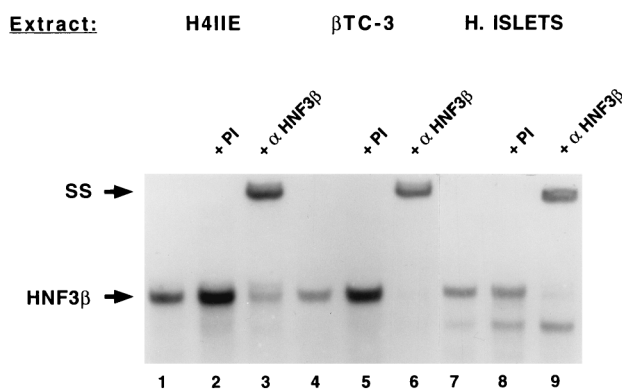


FIG. 8. HNF3 $\beta$  antibody recognizes the bp -2006 to -1994 element-binding complex. Binding reactions were carried out with H4IIE (lanes 1 to 3),  $\beta$ TC-3 (lanes 4 to 6), or human islet (lane 7 to 10) extracts. Extracts (4  $\mu$ g) were incubated for 10 min at room temperature with HNF3 $\beta$  antibody before the addition of probe. The HNF3 $\beta$  and antibody-supershifted (SS) complex are labeled. Lanes: 1, 4, and 7, extract plus probe; 2, 5, 8, plus 3  $\mu$ l of preimmune (PI) serum; 3, 6, and 9, plus 3  $\mu$ l of HNF3 $\beta$  antibody.

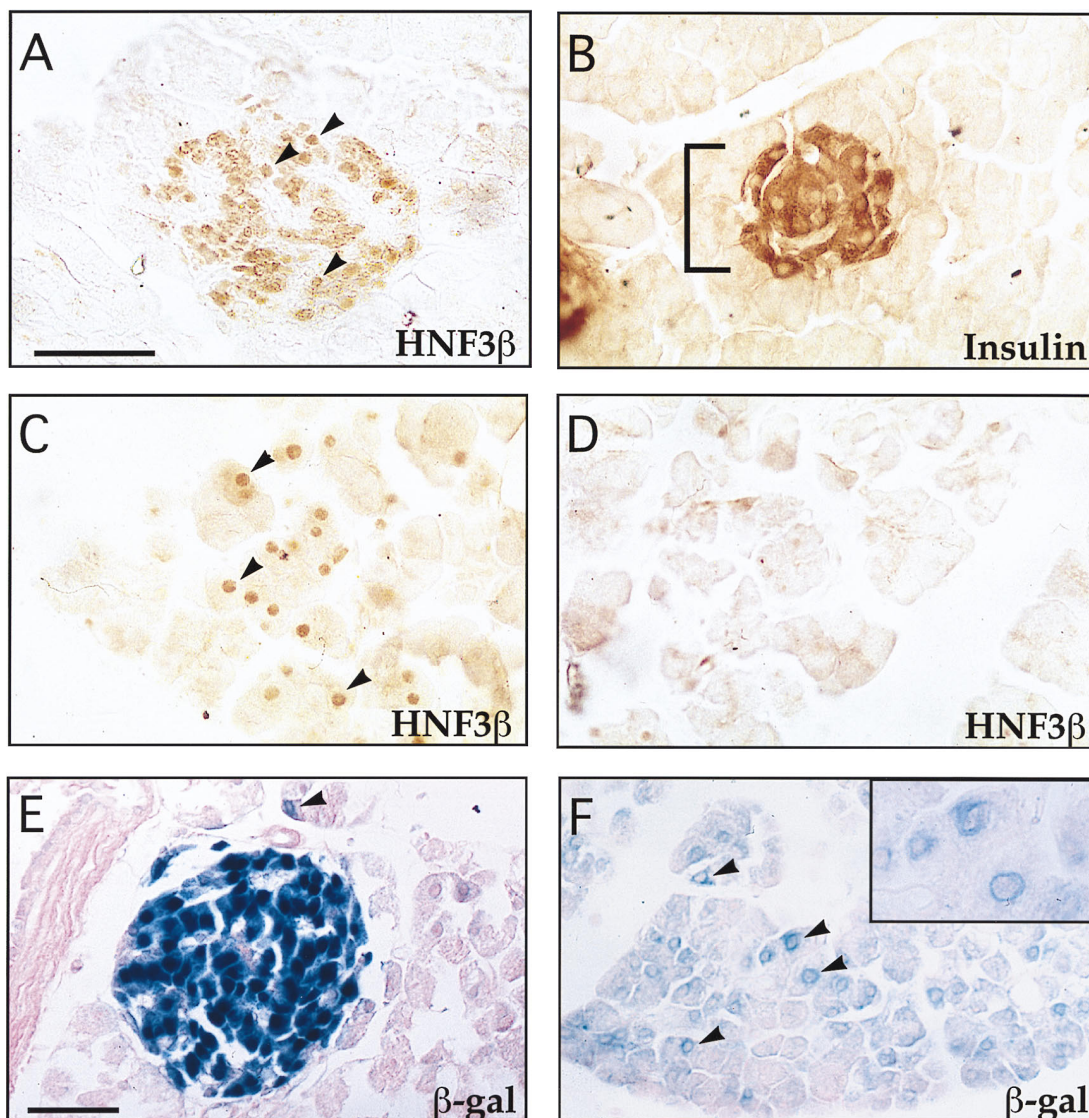


FIG. 9. HNF3 $\beta$  staining in adult pancreas. Immunohistochemistry was used to detect HNF3 $\beta$  protein on sections of pancreatic tissue from *pdx-1<sup>lacZKO</sup>* (35) adult heterozygotes. (A) Nuclear HNF3 $\beta$  is detected in endodermally derived cells within the pancreas, including islet  $\beta$  cells (arrowheads). (B) Insulin expression in  $\beta$  cells is localized to the cytoplasm (compare with panel A). (C) Subpopulations of acinar cells also express HNF3 $\beta$  (arrowheads), as do the majority of ductal epithelial cells (not shown). The intensity of HNF3 $\beta$  staining in the pancreas is similar to that in endodermal cells of the liver (not shown). (D) Population of acinar cells that do not express HNF3 $\beta$ . (E)  $\beta$ -gal expression from the endogenous *pdx-1* locus in *pdx-1<sup>lacZKO</sup>* adult heterozygotes is present in the majority of islet  $\beta$  cells, resembling the pattern of HNF3 $\beta$  expression in islets (compare with panel A). (F) *pdx-1*- $\beta$ -gal expression was also detected at lower levels in subpopulations of acinar cells (arrowheads in panels E and F). The inset shows representative positive nuclei at higher magnification. Bracket in panel B indicates an islet. Bar in panel A, 30  $\mu$ m (for panels A to D); bar in panel E, 30  $\mu$ m (for panels E and F).

scription (49). Interestingly, the location of the HNF3 $\beta$  binding site in the rat gene and the sequences surrounding this site are very different from the mouse *pdx-1* gene characterized here. Further characterization of the mouse, rat, and human *pdx-1* genes should provide insight into the key factors that cooperate with HNF3 $\beta$  to direct selective expression.

The rather general distribution of HNF3 $\beta$  during embryogenesis (4, 32, 45), the inactivity of PstBstpTk in liver H4IIE cells, and the observation that a mutation of the HNF3 $\beta$  control element within PstBstpTk altered the level, but not the cell specificity, of expression suggest that HNF3 $\beta$  is one of several effectors that generate the correct, cell-type-specific expression pattern of the *pdx-1* gene in vivo. The bp -2560 to -1880 region contains a consensus basic helix-loop-helix (bHLH) binding sequence, CANNTG, implicating  $\beta$ 2/NeuroD (28, 33)

and PTF1 (26) factors in control of  $\beta$ -cell and acinar cell-specific transcription, respectively.  $\beta$ 2/NeuroD appears to be important in the expression of the rat *pdx-1* gene (49). Since *pdx-1* expression in  $\beta$  cells is eliminated in Pax4 homozygous mutant mice (50), this factor may also be an important regulator. Higher-resolution mapping within the bp -2560 to -1880 region will be required to identify the other key factors involved in selective stimulation of the mouse *pdx-1* gene.

Mutations in the HNF1 $\alpha$  (61) and HNF4 $\alpha$  (60) genes, which encode transcription factors that were originally isolated because of their ability to activate liver-specific genes, have been shown to contribute to maturity-onset diabetes of the young. The biological effects of mutant forms of HNF1 $\alpha$  and HNF4 $\alpha$  are unknown, although it is presumed that they reduce either insulin production or secretion (7). Our results suggest that

mutations in HNF3 $\beta$  or accessory proteins in the transcription complex with HNF3 $\beta$  that cause a decrease in PDX-1 transcription factor synthesis would reduce insulin production and also lead to disease.

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