

# The Pancreatic Islet Factor STF-1 Binds Cooperatively with Pbx to a Regulatory Element in the Somatostatin Promoter: Importance of the FPWMK Motif and of the Homeodomain

BERNARD PEERS,<sup>1</sup> SEEMA SHARMA,<sup>1</sup> TED JOHNSON,<sup>1</sup> MARK KAMPS,<sup>2</sup> AND MARC MONTMINY<sup>1\*</sup>

*The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, California 92037,<sup>1</sup> and Department of Pathology, University of California at San Diego, La Jolla, California 92093<sup>2</sup>*

Received 6 June 1995/Returned for modification 20 July 1995/Accepted 28 September 1995

**A number of homeodomain proteins have been shown to regulate cellular development by stimulating the transcription of specific target genes. In contrast to their distinct activities in vivo, however, most homeodomain proteins bind indiscriminately to potential target sites in vitro, suggesting the involvement of cofactors which specify target site selection. One such cofactor, termed extradenticle, has been shown to influence segmental morphogenesis in *Drosophila melanogaster* by binding cooperatively with certain homeodomain proteins to target regulatory elements. Here we demonstrate that STF-1, an orphan homeodomain protein required for pancreatic development in mammals, binds cooperatively to DNA with Pbx, the mammalian homolog of extradenticle. Cooperative binding with Pbx requires a pentapeptide motif (FPWMK) which is well conserved among a large subset of homeodomain proteins. The FPMWK motif is not sufficient to confer Pbx cooperativity on other homeodomain proteins, however; the N-terminal arm of the STF-1 homeodomain is also essential. As cooperative binding with Pbx occurs on only a subset of potential STF-1 target sites, our results suggest that Pbx may specify target gene selection in the developing pancreas by forming heterodimeric complexes with STF-1.**

The adult pancreas consists of both endocrine and exocrine components which are thought to arise from common precursor cells in the developing gut. Such precursor cells express a homeodomain protein referred to as STF-1 (14, 20), IPF-1 (19), IDX (17), or XIHbox 8 (22), the importance of which is illustrated by knockout studies in which targeted disruption of the STF-1 gene leads to congenital absence of the pancreas (10). Although STF-1 is initially expressed in both exocrine and endocrine cells of the developing pancreas, its production is progressively restricted to insulin- and somatostatin-producing islet cells (7). In these cells, STF-1 action appears to be important for maintaining high-level expression of both somatostatin and insulin genes (14, 17, 19, 20, 22).

STF-1 recognizes two well-defined islet-specific elements on the insulin promoter, termed FLAT and P. When bound to these sites, STF-1 stimulates insulin transcription in concert with E47, a helix-loop-helix protein which recognizes two E-box elements termed *Far* and *Nir*. Similarly, STF-1 regulates somatostatin expression in islet cells via two islet-specific elements, termed TSEI and TSEII (14, 17).

Homeodomain proteins such as STF-1 have been found to play an important role in development by establishing cell or segmental identity (5, 8, 13, 16). In contrast to their specific and distinct effects in vivo, most homeodomain proteins exhibit low and overlapping DNA binding specificity in vitro. However, recent studies have implicated certain protein cofactors as determinants of homeodomain DNA binding specificity in vivo (8, 13). In *Drosophila melanogaster*, for example, extradenticle has been shown to modulate the activity of homeotic proteins without altering their pattern of expression (21, 24). Rather, extradenticle appears to promote target gene selection by enhancing the DNA binding specificity of certain homeodo-

main proteins (2, 25). Indeed, extradenticle is highly conserved in vertebrates, sharing extensive sequence similarity (71%) with the human proto-oncogene Pbx1 (23).

In the present study, we show that the TSEII element of the somatostatin promoter recognizes a heteromeric complex composed of STF-1 and Pbx in pancreatic islet cells. Our results suggest that specificity of STF-1 action in the pancreas may in part be dictated by the ability of potential target promoter sites to recognize the STF-1-Pbx heteromeric complex.

## MATERIALS AND METHODS

**Plasmid constructions and expression of proteins.** The STF-1 deletion constructs shown in Fig. 3B have been described previously (20). The Pbx1 cDNA (generous gift of C. Murre) (18) and the STF-1 deletion mutants  $\Delta$ 1-70,  $\Delta$ 1-115,  $\Delta$ 1-139, and  $\Delta$ 216-284 were produced with the Promega TNT rabbit reticulocyte lysate-coupled transcription-translation system, according to the protocol of the manufacturer. The STF-1 full-length protein and the STF-1 deletion mutants Hox 140-215, 1-140, and 110-215 (see Fig. 3A) were expressed in *Escherichia coli* with the bacterial expression vector pGEX3X. Recombinant proteins were purified as described previously (14) and eluted from the glutathione-Sepharose beads by digestion with 4  $\mu$ g of factor Xa for 16 h at room temperature.

The glutathione S-transferase fusion proteins described in Fig. 4 were also expressed in *E. coli* with the pGEX3X vector. The sequences coding for the rat Isl1 homeodomain (12) (amino acids [aa] 176 to 248), for the hamster cdx3 homeodomain (6) (aa 143 to 253), and for the STF-1 homeodomain (aa 140 to 215) were amplified by PCR and fused in frame in the pGEX3X plasmid. For the Pim-homeodomain fusion proteins, the STF-1 coding sequence from residues 110 to 139, encompassing the FPWMK motif, was ligated in frame upstream of the coding sequence of the Isl1 homeodomain (aa 176 to 248), of the cdx3 homeodomain (aa 143 to 253), and of the STF-1 homeodomain (aa 140 to 215) and inserted in the pGEX3X vector. Bacterial expression vectors coding for chimeric STF-1-cdx3 fusion proteins in Fig. 5 were constructed by introducing an *EcoRI* restriction site at position 19 (helix 2) and a *BglII* site at position 46 (helix 3) of both homeodomains without changing the respective amino acid sequences. Chimeric STF-1-cdx3 proteins were then produced by ligation of the corresponding sequences from both homeodomains with these two restriction sites. Swapping of the N-terminal arms of the STF-1 and cdx3 homeodomains was performed by PCR amplification. The glutathione S-transferase fusion proteins were expressed and purified as described previously (14) and eluted from the

\* Corresponding author.

beads with 5 mM reduced glutathione. Prior to gel shift analysis, all STF-1 polypeptides were analyzed for proper expression by Coomassie staining of sodium dodecyl sulfate-polyacrylamide gels.

**Transfections.** Transfection experiments were performed in GC cells as previously described (20) with calcium phosphate coprecipitation. Cells were harvested 24 h after glycerol shock and assayed for luciferase activity after normalizing to  $\beta$ -galactosidase ( $\beta$ -Gal) activity derived from a cotransfected Rous sarcoma virus- $\beta$ -Gal control plasmid. All experiments were performed in triplicate.

**Gel mobility shift assays.** Gel mobility shift assays were performed exactly as described previously (20) with 0.1 ng of an end-labeled double-stranded oligonucleotide whose sequences are presented in Fig. 2C. For off-rate analysis, the TSEII probe was first incubated with the proteins at room temperature for 30 min, and then a 1,000-fold excess of unlabeled TSEII oligonucleotide was added and aliquots were loaded at various time points on a running gel. In supershift assays, Tu-6 nuclear cell extract was preincubated with 1  $\mu$ l of Pbx (11) or STF-1 (20) antiserum for 15 min at room temperature before adding the TSEII probe. Pbx antiserum does not discriminate among members of the Pbx family (Pbx1, -2, and -3).

## RESULTS

In pancreatic tumor cell lines, expression of the pancreatic islet hormone genes somatostatin and insulin depends on the homeobox factor STF-1. STF-1 regulates somatostatin expression in Tu-6 cells by binding to two tissue-specific regulatory elements termed TSEI and TSEII (14). Using a  $^{32}$ P-labeled TSEII site oligonucleotide probe in gel mobility shift assays of crude Tu-6 nuclear extract, we detected three specific protein DNA complexes, referred to as C1, C2, and C3 (Fig. 1A). Complexes C1 and C2 were observed only in pancreatic islet cell extracts, C3 being detected at comparable levels in nonislet lines such as HeLa cells. Complex C3 appeared to contain the ubiquitous Oct-1 protein, as revealed by supershift assay with Oct-1 antiserum. By contrast, complexes C1 and C2 contained STF-1 protein, as determined by their sensitivity to addition of STF-1 antiserum.

Complex C2 increased exponentially as a function of extract concentration whereas complex C1 increased linearly in gel mobility shift assays. Recombinant full-length STF-1 protein generated a single complex migrating at the same position as complex C1 (Fig. 1A, lane labeled "STF"), suggesting that complex C1 contains only STF-1 protein while complex C2 represents a high-affinity heteromeric STF-1 complex. In this regard, off-rate studies indicated that the half-life for complex C1 was less than 1 min compared with a half-life of about 15 min for the heteromeric STF-1 complex C2 (Fig. 1A, right panel).

To confirm that the C2 complex does not correspond to a homodimeric form of STF-1, we tested increasing concentrations of recombinant STF-1 protein in gel mobility shift assays with the TSEII probe (Fig. 1B). No more slowly migrating complexes were observed with high amounts of STF-1 protein, indicating that complex C2 must contain an additional protein component which strongly stabilizes binding of STF-1 to DNA. To test whether this component was ubiquitously expressed, we added crude Jurkat (or HeLa) nuclear extracts to reactions containing recombinant STF-1 protein. Under these conditions, formation of complex C2 was readily observed and was dependent on addition of recombinant STF-1 (Fig. 1B). The off-rate for the C2 complex in reconstituted extracts was comparable to that in Tu-6 extracts (about 15 min), indicating that a ubiquitous factor stabilizes the binding of STF-1 to the TSEII site (Fig. 1B, right).

Recent reports of a *Drosophila* homeobox protein termed extradenticle, which binds cooperatively with other homeodomain proteins to target promoter sites (2, 25), prompted us to test whether the mammalian homolog of extradenticle, termed Pbx (23), might be contained in complex C2. When added to crude Tu-6 nuclear extracts, Pbx antiserum specifically blocked

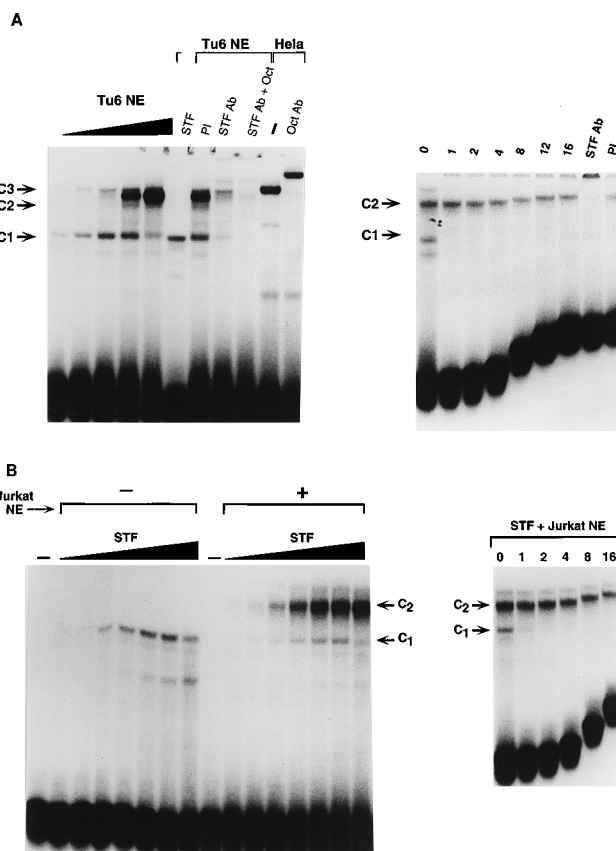


FIG. 1. The pancreatic homeobox factor STF-1 binds cooperatively to a cell-specific regulatory element in the somatostatin promoter along with a ubiquitous nuclear factor. (A) Left panel, gel mobility shift assay of crude nuclear extracts from pancreatic islet Tu-6 cells with a double-stranded somatostatin TSEII oligonucleotide extending from -303 to -281. C1, C2, and C3, protein DNA complexes as indicated. The crescendo bar indicates increasing amounts of nuclear extract. STF, recombinant STF-1 protein only; Tu-6 NE and HeLa NE, nuclear extract from Tu-6 and HeLa cells, respectively. PI, preimmune antiserum. STF-1 Ab, STF-1 antiserum raised against the C-terminal part of STF-1 protein. STF-1 Ab + Oct, STF-1 antiserum plus Oct-1 binding site (Sph1 motif) of simian virus 40. Oct Ab, monoclonal Oct-1 antiserum (gift of W. Herr) raised against human Oct-1 protein. Right panel, off-rate analysis of complexes formed with somatostatin TSEII probe and Tu-6 nuclear extract (preincubated with unlabeled Oct-1 oligonucleotide to analyze only C1 and C2 complexes) as described in Materials and Methods. Complexes C1 and C2 are as indicated. Time (in minutes) after addition of 1,000-fold excess of unlabeled TSEII competitor DNA is indicated above each lane. PI, preimmune serum; STF-1 Ab, STF-1 antiserum added to gel shift assay. (B) Left panel, gel mobility shift analysis of recombinant STF-1 protein, with the somatostatin TSEII oligonucleotide as probe. STF-1 binding activity was tested in the absence (-) or presence (+) of heterologous nuclear extract from Jurkat cells, which do not contain detectable levels of STF-1 protein by Western blot (immunoblot) assay. Crescendo bars indicate increasing amounts of recombinant STF-1 protein. Complexes C1 and C2 are as indicated. Right panel, off-rate of complexes C1 and C2 in reconstituted extracts containing recombinant STF-1 plus Jurkat nuclear extract. Time points (in minutes) after addition of unlabeled TSEII competitor (1,000-fold excess) are indicated above each lane.

formation of complex C2, but this antiserum had no effect on the formation of C1, which contains only STF-1 (Fig. 2A, left panel, lane labeled "PBX Ab"). Moreover, coinubation of Pbx and recombinant STF-1 proteins resulted in formation of the heteromeric C2 complex in gel shift assays (Fig. 2A, center). The stability of this Pbx-STF-1 complex, evaluated by off-rate analysis, was comparable to that of the endogenous C2 complex in Tu-6 nuclear extracts (Fig. 2A, right panel).

To determine whether Pbx and STF-1 can bind coopera-

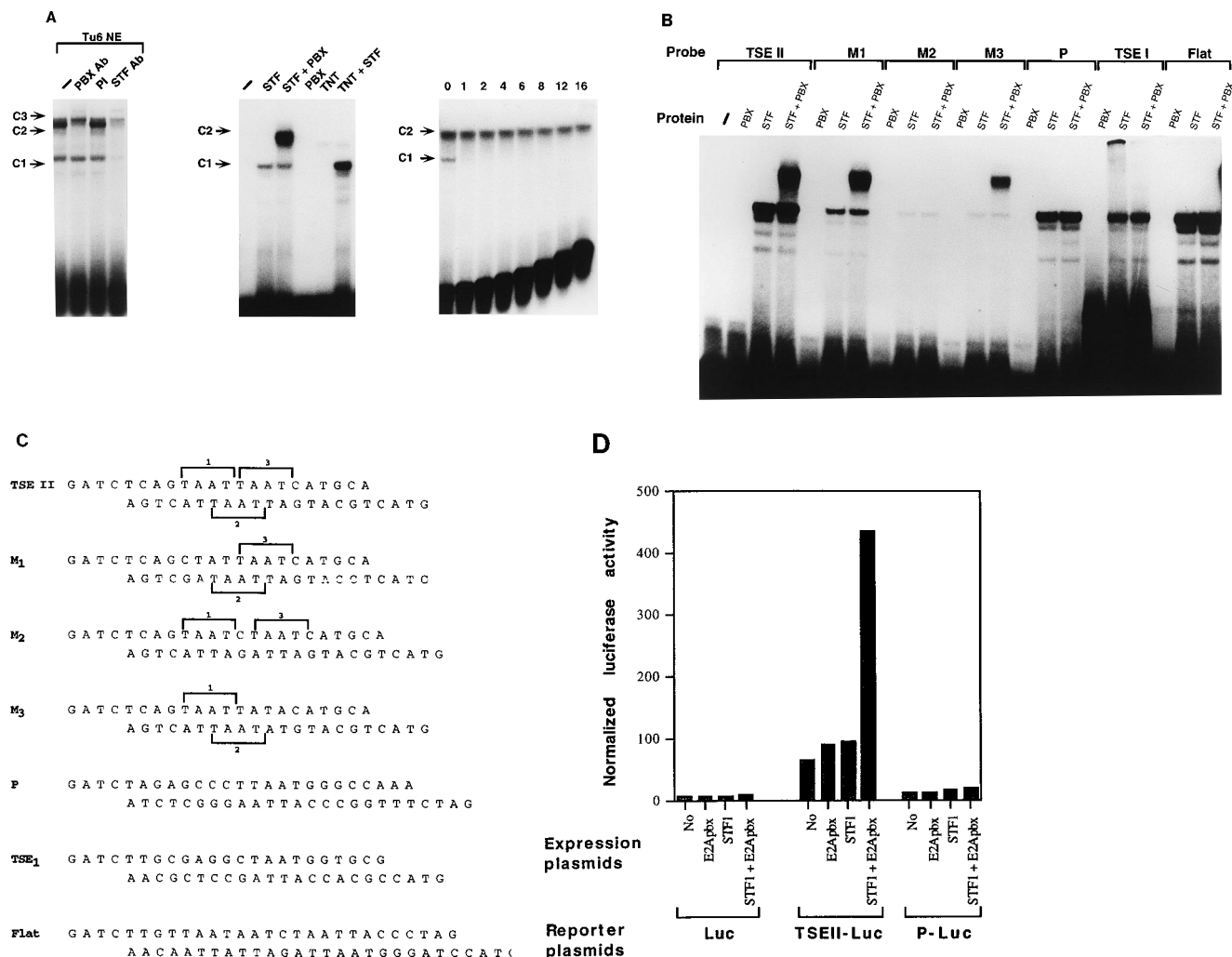


FIG. 2. The ubiquitous homeobox protein Pbx forms a heterodimeric complex with STF-1 on the somatostatin TSEII element. (A) Left panel, gel mobility shift assay of Tu-6 nuclear extract with TSEII oligonucleotide as probe. Complexes C1, C2, and C3 are as indicated. PBX Ab, Pbx antiserum included in reaction mixture. PI, preimmune serum. STF-1 Ab, STF-1 antiserum. Middle panel, effect of in vitro-translated Pbx protein (PBX) on STF-1 binding activity. Gel mobility shift assay used TSEII oligonucleotide plus STF-1 and/or Pbx as indicated above each lane. TNT, control lysate. Right panel, PBX stabilizes STF-1 binding to TSEII complex. Shown are results of off-rate analysis of STF-1 and STF-1-Pbx complexes with somatostatin TSEII oligonucleotide as probe. Time (in minutes) after addition of excess unlabeled TSEII oligonucleotide is as indicated. Complexes C1 and C2, corresponding to STF-1 monomer and STF-1-Pbx heterodimer, respectively, are as indicated. (B) Analysis of STF-1-Pbx heterodimer formation on somatostatin and insulin promoter sequences. TSEII, wild-type TSEII oligonucleotide. M1, M2, and M3, mutant TSEII oligonucleotides in which TAAT motifs were mutated as shown in panel C. P and FLAT, insulin I promoter elements which recognize STF-1 with high affinity. TSEI, somatostatin promoter element which binds STF-1. PBX, in vitro-translated Pbx protein added to binding reaction mixtures. STF, full-length recombinant STF-1 protein. (C) Sequence of wild-type and mutant oligonucleotides used in gel shift assays in panel B. Brackets indicate consensus TAAT motifs which were targeted for mutagenesis. (D) STF-1 and Pbx act cooperatively on a subset of promoter sites. Shown are results of transient transfection assay of GC cells with STF-1 and E2A-Pbx effector plasmids as indicated below each bar. Bar graph shows luciferase activity derived from reporter construct containing minimal growth hormone promoter alone (Luc), with two copies of the somatostatin TSEII element upstream of the growth hormone promoter (TSEII-Luc) or two copies of the insulin P element (P-Luc). Activities were normalized to cotransfected Rous sarcoma virus- $\beta$ -galactosidase control plasmid.

tively to other STF-1 target sites besides TSEII, we performed gel shift assays with the recombinant STF-1 and Pbx proteins on the somatostatin TSEI or insulin P and FLAT elements, sites which bind STF-1 and which are required for islet cell-restricted expression of the rat somatostatin and insulin I genes, respectively, in pancreatic cells (Fig. 2B and C). In contrast to the TSEII element (Fig. 2B, four leftmost lanes), no heteromeric STF-1-Pbx complex was observed on the TSEI, P, or FLAT element. Indeed, heteromeric STF-1 complexes are also undetectable when the TSEI, P, or FLAT element is used in gel shift assays of crude pancreatic cell extracts (data not shown) (20).

To determine whether STF-1 and Pbx can stimulate tran-

scription in a cooperative manner, we performed transient transfection assays with a reporter vector which contains two copies of the somatostatin TSEII site upstream of a minimal growth hormone promoter (Fig. 2D). To optimize regulatory contributions from the transfected Pbx expression plasmid, we employed an E2A-Pbx vector which expresses the activation domain of E2A fused to Pbx (15). When transfected separately into GC cells, STF-1 and E2A-Pbx expression vectors had negligible effects on TSEII luciferase reporter activity. Cotransfection of STF-1 and E2A-Pbx effector plasmids markedly increased TSEII reporter activity, however, suggesting that STF-1 can indeed cooperate with Pbx on the TSEII site. In the presence of STF-1, a Pbx effector plasmid lacking the E2A

*trans*-activation domain also induced the TSEII reporter activity about two- to threefold (not shown). By contrast, no such cooperativity between STF-1 and E2A-Pbx was observed in using a reporter plasmid which contained the insulin P element (P-Luc). These results suggest that STF-1 and Pbx can act synergistically only on STF-1 binding sites that are able to recognize the STF-1-Pbx heterodimer (Fig. 2B).

To identify sequences within the somatostatin TSEII element which were important for Pbx-STF-1 cooperativity, we constructed several mutant TSEII oligonucleotides (Fig. 2B and C). The TSEII site contains three TAAT motifs, the principal recognition motif for homeodomain proteins. Mutation of TAAT motif 1 or 3 (M1 and M3, plus strand) had minimal effects on cooperative binding between STF-1 and Pbx, but mutation of TAAT motif 2 (minus strand) completely abolished Pbx-STF-1 cooperativity, indicating the importance of that special TAAT motif for the STF-1-Pbx complex formation. Mutation in the third TAAT motif (M3) also affected formation of STF-1 monomeric C1 complex.

To identify residues in STF-1 which promote cooperative binding with Pbx, we tested a series of truncated STF-1 polypeptides in gel shift assays with the TSEII probe (Fig. 3A and B). Deletion of residues C-terminal to the homeobox domain had no effect on cooperative binding with Pbx ( $\Delta$ 216-284 mutant). And N-terminally truncated STF-1 polypeptides lacking the STF-1 *trans*-activation domain (aa 1 to 115) also retained ability to bind cooperatively to the TSEII site. But further deletion of the residues from aa 115 to 123 abolished cooperativity with Pbx, suggesting that a region outside the STF-1 homeodomain was important for heterodimer formation (Fig. 3A and B). Indeed, the homeobox region of STF-1 (Hox 140-215) formed a monomeric complex on the TSEII site but did not bind cooperatively with Pbx. But STF-1 polypeptides containing N-terminal sequences in addition to the STF-1 homeodomain (aa 110 to 215) showed cooperativity with Pbx, indicating that N-terminal residues flanking the STF-1 homeobox may form protein-protein contacts with Pbx upon binding to the TSEII site.

In the process of comparing residues in STF-1 with other *Drosophila* homeodomain proteins which bind cooperatively to DNA with extradenticle, the *Drosophila* homolog of Pbx, we noticed a pentapeptide motif, FPWMK (Table 1), which is located within this N-terminal region of STF-1 (aa 115 to 123) and which is conserved in many homeoproteins from a wide variety of metazoans (5). To assess the importance of this peptide motif for cooperativity with Pbx, we constructed a mutant STF-1 cDNA containing amino acid substitutions at each residue in the motif (FPWMK to AAGGQ) (Fig. 3C). When compared with wild-type STF-1 protein in gel mobility shift assays, the mutated STF-1 protein was deficient in its ability to cooperate either with recombinant Pbx or with endogenous Pbx from Jurkat extracts (Fig. 3C). By contrast, the mutant STF-1 protein showed wild-type binding activity on insulin P and FLAT elements, sites which do not form the heterodimeric complex. These results suggest that the Pbx interaction motif in STF-1, termed Pim, is indeed necessary for complex formation with Pbx.

To determine whether the Pim region was sufficient for complex formation with Pbx, we fused this motif to other homeobox proteins (Fig. 4). Fusion of the Pim region upstream of the homeobox for Isl1, a lim homeodomain protein which binds the somatostatin TSEII site with high affinity, did not promote complex formation with Pbx. Similarly, addition of the Pim region to the caudal-like factor *cdx3* did not induce formation of a Pbx-*cdx3* heterodimeric complex. The spacing between the Pim region and the homeobox is comparable for

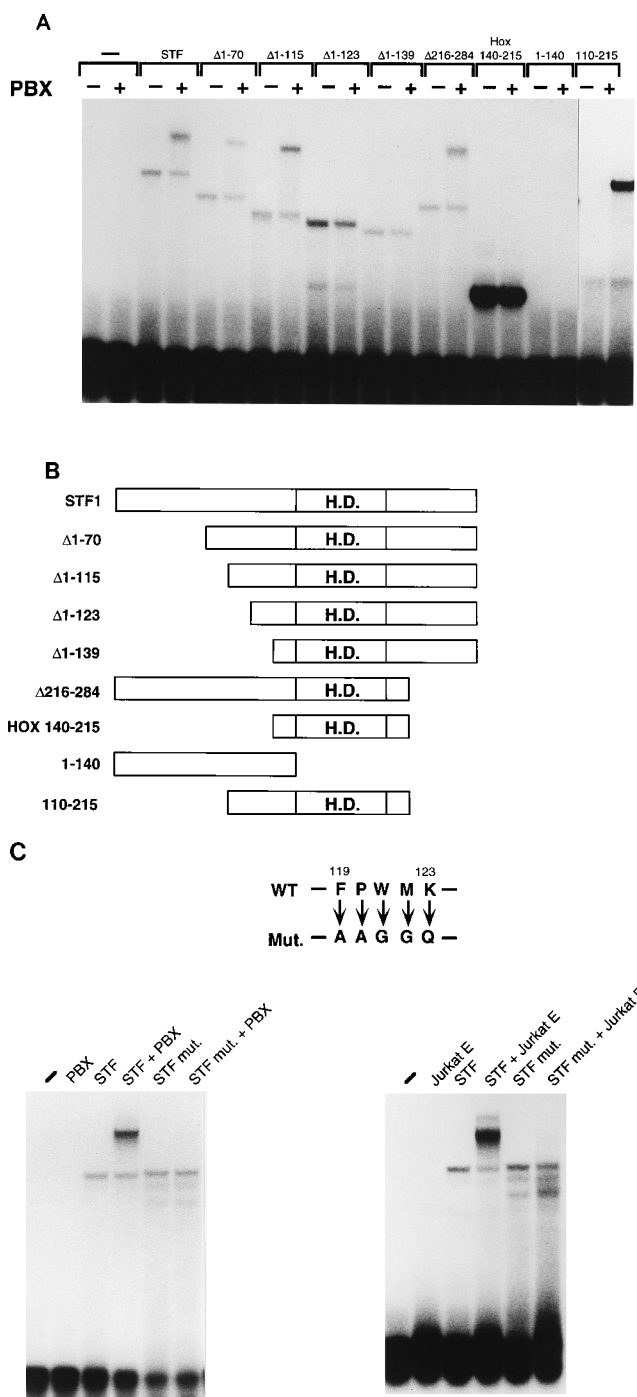


FIG. 3. A conserved pentapeptide motif in STF-1 is critical for cooperative binding with Pbx. (A) Analysis of STF-1 monomer (complex C1) and STF-1-Pbx heterodimer (complex C2) formation for wild-type and truncated recombinant STF-1 polypeptides. Deletion endpoints in mutant STF-1 polypeptides are indicated over each lane. For example,  $\Delta$ 1-70 indicates STF-1 polypeptide lacking residues 1 to 70. Hox 140-215, STF-1 homeodomain polypeptide. (B) Schematic representation of constructs used in gel shift assays above. H.D., STF-1 homeodomain (aa 140 to 215). Full-length STF-1 protein extends from aa 1 to 284. (C) Mutagenesis of the conserved pentapeptide motif destroys cooperativity with Pbx. Top, sequence of wild-type (WT) and mutant STF-1 protein with amino acid numbers indicated. Bottom, gel shift analysis of wild-type and mutant STF proteins alone and in combination with *in vitro*-translated Pbx (left) or Jurkat nuclear extract (right).

TABLE 1. Conservation of STF-1 pentapeptide motif in homeobox proteins<sup>a</sup>

Organism	Name (abbreviation)	Motif	Distance N-terminal from homeodomain (no. of residues)
Rat	STF-1	FPWMK	22
<i>D. melanogaster</i> (ANT and BX complex)	Labial (lab)	YKWMQ	120
	Proboscipedia (pb)	YPWMK	28
	Deformed (dfd)	YPWMK	17
	Sex combs reduced (Scr)	YPWMK	14
	Antennapedia (Antp)	YPWMR	8
	Ultrabithorax (Ubx)	YPWMA	50
	Abdominal-A (AbdA)	YPWMT	24
	<i>D. melanogaster</i> (orphan homeobox)	Caudal (cad)	FDWMK
<i>C. elegans</i> (HOM complex)	Mab5	FPWMK	8
	Lin-39	YPWMT	11
	Human (Hox complex)	Hox B1	FDWMK
Human (Hox complex)	Hox B2	FPWMK	43
	Hox B3	FPWMK	53
	Hox B4	YPWMR	15
	Hox B5	FPWMR	12
	Hox B6	YPWMQ	13
	Hox B7	YPWMR	5
	Hox B8	FPWMR	6
	Mammal (orphan homeobox)	Hox 11	FPWMK
m cdx1		YAWMR	11
m cdx2		CEWMR	15
m cdx4		YAWMR	
Sea urchin	Hox 1	YPWMK	11
	Consensus	$\begin{matrix} Y \\ F \end{matrix} P \begin{matrix} W \\ F \end{matrix} M \begin{matrix} K \\ R \end{matrix}$	

<sup>a</sup> A pentapeptide motif in STF-1 which is necessary for heterodimer formation with Pbx on the somatostatin TSEII site is conserved in a number of homeobox proteins. Different homeobox proteins are classified according to species and listed by name. For each protein, sequences related to motif in STF-1 are listed in single-amino-acid code, with distances N-terminal to the homeodomain indicated at the right.

STF-1 and both the Pim-Is11 and Pim-cdx3 fusion proteins, suggesting that the distance from the homeobox domain may not explain their inability to cooperate with Pbx. Additionally, the chimeric proteins (Pim-Is11 and Pim-cdx3) appeared to bind DNA with wild-type affinity, indicating that lack of complex formation may not reflect incorrect folding. Taken together, these results suggest that, although the Pim region is required for formation of a heteromeric complex with Pbx, additional residues within the STF-1 homeodomain itself may be required. To characterize such residues, we replaced segments within the cdx3 homeodomain with the corresponding segments of the STF-1 homeodomain (Fig. 5). When tested in the context of the Pim-cdx3 fusion protein (Fig. 4), the N-terminal arm of STF-1 could reconstitute complex formation with Pbx, but other regions of the STF-1 homeodomain (helix 1, 2, or 3) showed no such activity. These results demonstrate that both the Pim motif and the N-terminal arm of STF-1 are necessary for complex formation with Pbx.

## DISCUSSION

extradenticle, the *Drosophila* homolog of Pbx, appears to be essential for proper activation of a subset of target genes such as *wingless*, *teashirt*, and *decapentaplegic* during development (24). extradenticle appears to induce these target genes by binding cooperatively to DNA with different homeotic proteins (2, 25). In this study, we found that Pbx, the human homolog of extradenticle, forms a heterodimeric complex with STF-1 on the somatostatin TSEII element. Compared with STF-1 alone, the STF-1-Pbx complex is highly stable, as measured by the decreased off-rate of this complex in gel mobility shift assays. It is important to note, however, that our results do not discriminate among various members of the Pbx family (Pbx 1, -2, and

-3), all of which apparently have the capacity to bind cooperatively with Hox proteins (3).

Formation of a STF-1-Pbx heterodimer on the somatostatin TSEII site requires a pentapeptide motif (FPMWK) which is conserved in a number of homeotic proteins, not only in vertebrates, but also in *D. melanogaster* (5) and *Caenorhabditis elegans*. Thus, the *C. elegans* homeotic protein Mab5 or Lin-39, which contains this motif, may similarly cooperate with the protein Ceh-20, which appears to be homologous to Pbx (1). Using an artificial target sequence to induce Pbx-Hox heterodimer formation, Chang et al. have noted the importance of this conserved motif for cooperative binding with Pbx to DNA (3). Our results suggest that the effects of Pbx are not restricted to proteins in the Hox complex but include orphan homeobox proteins such as STF-1.

Although Chang et al. found that fusion of the conserved YPMWK motif to Hox A10 was sufficient to promote cooperativity with Pbx (3), we were unable to induce cooperativity when this motif was transferred to islet cell homeodomain proteins such as Is11 and cdx3. These results indicated that additional residues within the homeodomain itself may be required for formation of the STF-1-Pbx complex. In this regard, we found that the flexible N-terminal arm of the STF-1 homeodomain (aa 145 to 153) was essential for cooperativity with Pbx. The N-terminal arm has been shown to confer functional specificity on homeodomain proteins such as Antennapedia, although the underlying mechanism remains uncharacterized (4, 26). Structural studies have revealed that the N-terminal arm is located within the minor groove of the DNA, where it may impart subtle differences in DNA binding or in protein-protein interactions. Although our results do not discriminate between these models, it is tempting to speculate that the formation of a STF-1-Pbx complex may rely in part on the

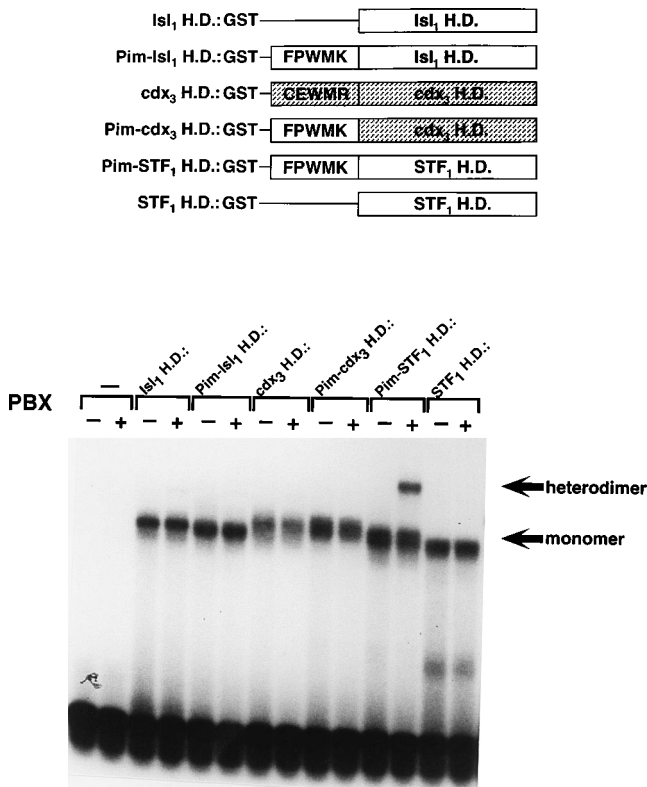


FIG. 4. The conserved Pbx interaction motif (Pim) is necessary but not sufficient to promote cooperative binding between Pbx and homeobox proteins. Top, schematic diagram of recombinant glutathione *S*-transferase (GST) fusion proteins used in gel shift assays below. Isl1 H.D., homeodomain of the lim domain factor Isl1. Pim-Isl1 H.D., STF-1-Isl1 fusion protein containing STF-1 Pim region (aa 110 to 138) fused to the Isl1 homeodomain. cdx3 H.D., homeodomain of the pancreatic homeobox protein cdx3 (aa 143 to 253). Pim-cdx3, fusion protein containing STF-1 Pim region (aa 110 to 138) fused to the cdx3 homeodomain (aa 176 to 253). Pim-STF-1, STF-1 polypeptide containing the Pim region (aa 110 to 138) fused to the STF-1 homeodomain (aa 140 to 215). STF-1 H.D., STF-1 homeodomain alone without the Pim region. Bottom, gel mobility shift assay with the somatostatin TSEII site as <sup>32</sup>P-labeled probe. – and + indicate absence and presence, respectively, of reticulocyte lysate programmed with Pbx RNA. Cooperativity with Pbx was tested with homeodomain fusion proteins shown over each lane. Monomeric and heterodimeric complexes are as labeled.

ability of the N-terminal arm to form specific minor groove contacts.

Formation of the STF-1–Pbx heterodimer occurs on only a subset of potential STF-1 target sites. Our results suggest that this preference may form the basis for target site selection in developing islet cells. We have previously noted, for example, that STF-1 induces both insulin and somatostatin expression, albeit in distinct cell types ( $\beta$  and  $\delta$ , respectively) within the pancreatic islet. In  $\beta$  cells, STF-1 appears to induce insulin expression by acting cooperatively with the helix-loop-helix protein E47. By contrast, STF-1 appears to promote somatostatin expression in  $\delta$  cells by binding cooperatively to the TSEII site with Pbx. These observations suggest that the commitment of cells within the islet lineage to express either insulin or somatostatin may depend on the relative expression of E-box binding versus Pbx-type proteins.

A combinatorial mechanism for developmental regulation, like the one envisioned here for pancreatic development, has also been described for *Saccharomyces cerevisiae*. In this regard, the homeodomain protein Mat $\alpha$ 2 cooperates with Mat $\alpha$ 1

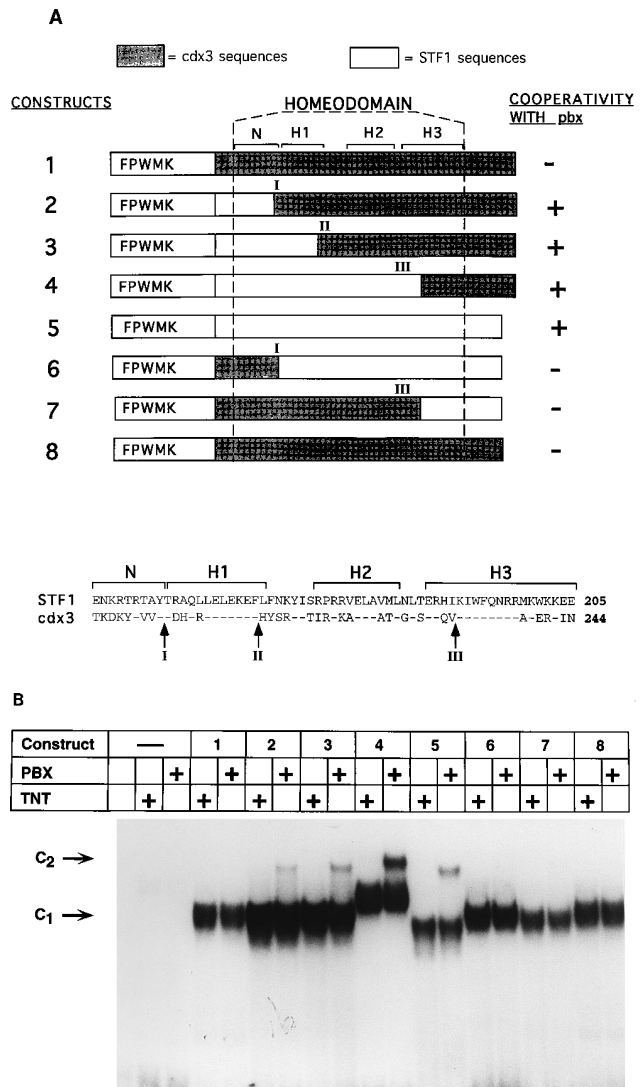


FIG. 5. The N-terminal arm of the STF-1 homeodomain is necessary for cooperativity with Pbx. Shown are results of gel shift analysis of Pim-cdx3 fusion constructs containing the STF-1 Pim motif (aa 110 to 138) plus various regions of the STF-1 homeodomain substituted in place of the cdx3 homeodomain. (A) Structure and activity of STF-1–cdx3 fusion constructs. cdx3 sequences are shaded; STF-1 sequences are in white. Relative positions of N-terminal arm (N) and helices 1, 2, and 3 (H1, H2, and H3) are as indicated. Cooperative binding with Pbx in gel shift assays is indicated (+, –) on right. Amino acid alignment of STF-1 and cdx3 homeodomains is shown below, with the amino acid number of the C-terminal residue shown on the right. Dashes indicate amino acid identity between STF-1 and cdx3. Arrows point to amino acid endpoints (I, II, and III) used for fusion constructs. (B) Gel shift assay of recombinant STF-1–cdx3 fusion proteins. Construct, numbers refer to constructs depicted in schematic diagram (A). Presence of in vitro-translated Pbx (PBX) or unprogrammed reticulocyte lysate (TNT) in binding reaction mixtures is as indicated. C1 and C2, complexes corresponding to STF-1 monomer and Pbx–STF-1 heterodimer, respectively.

in diploid  $a/\alpha$  cells to bind *hsg* operators and to repress haploid-specific genes. But in haploid  $a$  or  $\alpha$  cells, Mat $\alpha$ 2 appears to cooperate with a different activator, MCM1, and to thereby activate a distinct genetic program (9).

The presence of a conserved motif which permits interaction between Pbx and certain homeobox proteins may explain in part the global effects of this regulator in development. The somatostatin gene is expressed in a number of tissues besides pancreas, including brain, stomach, and medullary thyroid tis-

sues. Although factors which direct somatostatin expression in these tissues have not been identified, our results predict that such proteins may stimulate somatostatin expression on the TSEII element by forming heterodimeric complexes with Pbx.

#### ACKNOWLEDGMENTS

This work was supported by the Foundation for Medical Research (FMR). M.M. is an FMR investigator. B.P. was supported by a post-doctoral fellowship from the Juvenile Diabetes Foundation.

We thank C. Murre for the pSP6-Pbx1 expression vector.

#### REFERENCES

- Burglin, T. R., and G. Ruvkun. 1992. New motif in PBX genes. *Nature Genet.* **1**:319–320.
- Chan, S., L. Jaffe, M. Capovilla, J. Botas, and R. S. Mann. 1994. The DNA binding specificity of *ultrabithorax* is modulated by cooperative interactions with *extradenticle*, another homeoprotein. *Cell* **78**:603–615.
- Chang, C., W. Shen, S. Rozenfeld, H. J. Lawrence, C. Largman, and M. L. Cleary. 1995. Pbx proteins display hexapeptide-dependent cooperative DNA binding with a subset of Hox proteins. *Genes Dev.* **9**:663–674.
- Furukubo-Tokunaga, K., S. Flister, and W. Gehring. 1993. Functional specificity of the Antennapedia homeodomain. *Proc. Natl. Acad. Sci. USA* **90**:6360–6364.
- Gehring, W. J., M. Affolter, and K. Burglin. 1994. Homeodomain proteins. *Annu. Rev. Biochem.* **63**:437–526.
- German, M. S., J. Wang, R. B. Chadwick, and W. J. Rutter. 1992. Synergistic activation of the insulin gene by a LIM-homeo domain protein and a basic helix-loop-helix protein: building a functional insulin minienhancer complex. *Genes Dev.* **6**:2165–2176.
- Guz, Y., M. R. Montminy, R. Stein, J. Leonard, L. W. Gamer, C. V. E. Wright, and G. Teitelman. 1995. Expression of murine STF-1, a putative insulin gene transcription factor, in Beta cells of pancreas, duodenal epithelium and pancreatic exocrine and endocrine progenitors during ontogeny. *Development* **121**:11–18.
- Hayashi, S., and M. P. Scott. 1990. What determines the specificity of action of drosophila homeodomain proteins? *Cell* **63**:883–894.
- Johnson, A. 1992. A combinatorial regulatory circuit in budding yeast, p. 975–1006. *In* S. L. McKnight and K. R. Yamamoto (ed.), *Transcriptional regulation*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Jonsson, J., L. Carlsson, T. Edlund, and H. Edlund. 1994. Insulin-promoter-factor-1 is required for pancreas development in mice. *Nature (London)* **371**:606–609.
- Kamps, M. P., T. Look, and D. Baltimore. 1991. The human t(1;19) translocation in pre-B ALL produces multiple E2A-Pbx fusion proteins with differing transforming potentials. *Genes Dev.* **5**:358–368.
- Karlsson, O., S. Thor, T. Norberg, H. Ohlsson, and T. Edlund. 1990. Insulin gene enhancer binding protein Isl-1 is a member of a novel class of proteins containing both a homeo- and a Cys-His domain. *Nature (London)* **344**:879–882.
- Krumlauf, R. 1994. Hox genes in vertebrate development. *Cell* **78**:191–201.
- Leonard, J., B. Peers, T. Johnson, K. Ferreri, S. Lee, and M. R. Montminy. 1993. Characterization of somatostatin transcription factor-1, a novel homeobox factor that stimulates somatostatin expression in pancreatic cells. *Mol. Endocrinol.* **7**:1275–1283.
- Lu, Q., D. Wright, and M. Kamps. 1994. Fusion with E2A converts the Pbx1 homeodomain protein into a constitutive transcriptional activator in human leukemias carrying the t(1;19) translocation. *Mol. Cell. Biol.* **14**:3938–3948.
- McGinnis, W., and R. Krumlauf. 1992. Homeobox genes and axial patterning. *Cell* **68**:283–302.
- Miller, C. P., R. E. McGehee, and J. F. Habener. 1994. IDX-1: a new homeodomain transcription factor expressed in rat pancreatic islets and duodenum that transactivates the somatostatin gene. *EMBO J.* **13**:1145–1156.
- Monica, K., N. Galili, J. Nourse, D. Saltman, and M. Cleary. 1991. PBX2 and PBX3, new homeobox genes with extensive homology to the human proto-oncogene PBX1. *Mol. Cell. Biol.* **11**:6149–6157.
- Ohlsson, H., K. Karlsson, and T. Edlund. 1993. IPF1, a homeodomain-containing transactivator of the insulin gene. *EMBO J.* **12**:4251–4259.
- Peers, B., J. Leonard, S. Sharma, G. Teitelman, and M. R. Montminy. 1994. Insulin expression in pancreatic islet cells relies on cooperative interactions between the helix loop helix factor E47 and the homeobox factor STF-1. *Mol. Endocrinol.* **8**:1798–1806.
- Peifer, M., and E. Wieschaus. 1990. Mutations in the Drosophila gene *extradenticle* affect the way specific homeo domain proteins regulate segmental identity. *Genes Dev.* **4**:1209–1223.
- Peshavaria, M., L. Gamer, E. Henderson, G. Teitelman, C. V. E. Wright, and R. Stein. 1994. XIHbox 8, an endoderm-specific Xenopus homeodomain protein, is closely related to a mammalian insulin gene transcription factor. *Mol. Endocrinol.* **8**:806–816.
- Rauskolb, C., M. Peifer, and E. Wieschaus. 1993. *extradenticle*, a regulator of homeotic gene activity, is a homolog of the homeobox-containing human proto-oncogene pbx1. *Cell* **74**:1101–1112.
- Rauskolb, C., and E. Wieschaus. 1994. Coordinate regulation of downstream genes by *extradenticle* and the homeotic selector proteins. *EMBO J.* **13**:3561–3569.
- van Dijk, M., and C. Murre. 1994. *extradenticle* raises the DNA binding specificity of homeotic selector gene products. *Cell* **78**:616–624.
- Zeng, W., B. Andrew, L. Mathies, M. Horner, and M. Scott. 1993. Ectopic expression and function of the Antp and Scr homeotic genes: the N terminus of the homeodomain is critical to functional specificity. *Development* **118**:339–352.