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Hormonal Regulation of an Islet-Specific Enhancer in the Pancreatic Homeobox Gene *STF-1*

SEEMA SHARMA,[1,2]* ULUPI S. JHALA,[2] TED JOHNSON,[2] KEVIN FERRERI,[3] JAMES LEONARD,[4] AND MARC MONTMINY[2]*

Department of Biology, University of California, San Diego, La Jolla, California 92037[1]; Department of Cell Biology, Harvard Medical School, Research Division, Joslin Diabetes Center, Boston, Massachusetts 02215[2]; VivoRx, Inc., Santa Monica, California 90404[3]; and Strang-Cornell Research Laboratory, New York, New York 10021[4]

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The homeobox protein STF-1 appears to function as a master control switch for expression of the pancreatic program during development. Here we characterize a composite enhancer which directs STF-1 expression to pancreatic islet cells via two functional elements that recognize the nuclear factors HNF-3β and BETA-2. In keeping with their inhibitory effects on islet cell maturation, glucocorticoids were found to repress STF-1 gene expression by interfering with HNF-3β activity on the islet-specific enhancer. Overexpression of HNF-3β suppressed glucocorticoid receptor-mediated inhibition of the *STF-1* gene, and our results suggest that the expansion of pancreatic islet precursor cells during development may be restricted by hormonal cues which regulate *STF-1* gene expression.

The vertebrate pancreas is composed of exocrine and endocrine compartments which appear to be derived from a common endodermal precursor. Within the endocrine pancreas, a pluripotent cell expressing a number of islet hormones appears to undergo progressive restriction until each of the individual islet cell populations is achieved (6). By contrast with the endocrine pancreas, the exocrine pancreas is composed of a relatively homogeneous population of acinar cells which arise from the pancreatic ducts and which secrete a number of digestive enzymes.

The homeobox gene *STF-1* (9), also referred to as *IDX-1* (11), *IPF-1* (7), and *pdx-1* (13), appears to serve as a master control switch for expression of both the exocrine and endocrine pancreatic programs, as revealed by gene disruption studies in which targeted deletion of the *STF-1* gene leads to a null pancreas phenotype (7, 13). First detected in dorsal endoderm cells at embryonic day 8.5, STF-1 is initially expressed in both exocrine and endocrine cells (6). As pancreatic morphogenesis proceeds, STF-1 production is eventually restricted to β and δ cells of the islets, where it appears to regulate expression of the insulin and somatostatin genes, respectively (6).

In a previous report, we noted that an *STF-1* promoter construct extending 6500 bases upstream of the transcriptional initiation site could direct expression of a β-galactosidase (β-Gal) reporter gene to pancreatic islet and duodenal cells of transgenic mice (17). Appropriate expression of the *STF-1* gene was found to rely in part on a proximal E box which binds the ubiquitous factor USF-1, and tissue-specific regulation appeared to center on a distal enhancer located 6 kb upstream (17). Here we examine the mechanism by which this distal enhancer targets STF-1 expression to islet cells of the adult pancreas. Our analysis indicates that two endodermal factors, HNF-3β and BETA-2, act synergistically to induce STF-1 expression in pancreatic islet cells. Remarkably, exposure of pancreatic islet cells to glucocorticoids was found to block *STF-1* gene expression by specifically interfering with HNF-3β activity, an effect which could be rescued by overexpressing HNF-3β in treated cells. As glucocorticoids have been shown to inhibit islet cell maturation in cultures of fetal pancreas (10, 15), our results suggest that hormonal cues may function importantly in limiting expansion of the β-cell population during development by restricting STF-1 expression.

* Corresponding author. Phone: (617) 735-1926. Fax: (617) 735-1928.

MATERIALS AND METHODS

Reporter clones and transient transfections. Distal regions of the *STF-1* gene were inserted 120 bases upstream of a *STF-1*–luciferase (STF Luc) fusion gene by using standard cloning techniques. The insulin-chloramphenicol acetyltransferase (CAT) reporter plasmid contained sequences from −332 to +34 of the rat insulin I gene linked to CAT. HIT-T15 cells were obtained from the American Type Culture Collection (ATCC) and cultured according to ATCC guidelines from passages 60 to 76. Plasmids were transfected into HIT and COS cells by using calcium phosphate precipitation. Luciferase values were quantitated on a Monolight luminometer and normalized to β-Gal activity derived from a cotransfected cytomegalovirus (CMV)–β-gal internal control plasmid. For glucocorticoid treatment, cells were cultured in medium containing charcoal-stripped serum and either ethanol or 10^{-7} M dexamethasone for various amounts of time (Northern and Western blotting). For transfection assays, dexamethasone was added for 18 to 24 h after glycerol shock.

Gel shift assays and DNase I protection assays. For electrophoretic mobility shift assays, double-stranded oligonucleotides were labeled by using a [^{32}P]dCTP fill-in reaction with Klenow fragment. Five micrograms of nuclear extract was incubated with 0.5 ng of labeled oligonucleotide and 1 μg of nonspecific competitor DNA for 30 min on ice and subjected to nondenaturing polyacrylamide gel electrophoresis. For supershift assays, nuclear extracts were preincubated with antiserum for 30 min to 1 h on ice prior to addition of labeled probe. Anti-HNF-3α, anti-HNF-3β, and anti-HNF-3γ antibodies were kindly provided by S. Duncan and J. Darnell. Anti-BETA-2 antibody was a generous gift of M. J. Tsai. Anti-E2A antibody was purchased from Santa Cruz Biochemicals. DNase I protection assays were performed as previously described (17). Recombinant HNF-3α was the gift of K. Zaret.

Northern and Western blotting. Somatostatinoma/insulinoma Tu6 cells were cultured in ethanol or 10^{-7} M dexamethasone for various amounts of time. Total RNA was extracted by a guanidinium-phenol procedure. Fifteen micrograms of total RNA was run on agarose gels containing formaldehyde and transferred to a Zeta-Probe membrane. Random-primed *STF-1* and tubulin cDNAs were generated by using an Amersham random priming kit. Western blot assays were performed with whole-cell extracts from control and dexamethasone-treated cells, using STF-1 antiserum developed against amino acids 215 to 283 of STF-1.

RESULTS

Glucocorticoids repress *STF-1* gene expression via an Islet-specific enhancer. Previous reports showing that glucocorticoids repress islet cell maturation in vitro (10, 15) prompted us to examine whether this hormone may correspondingly inhibit

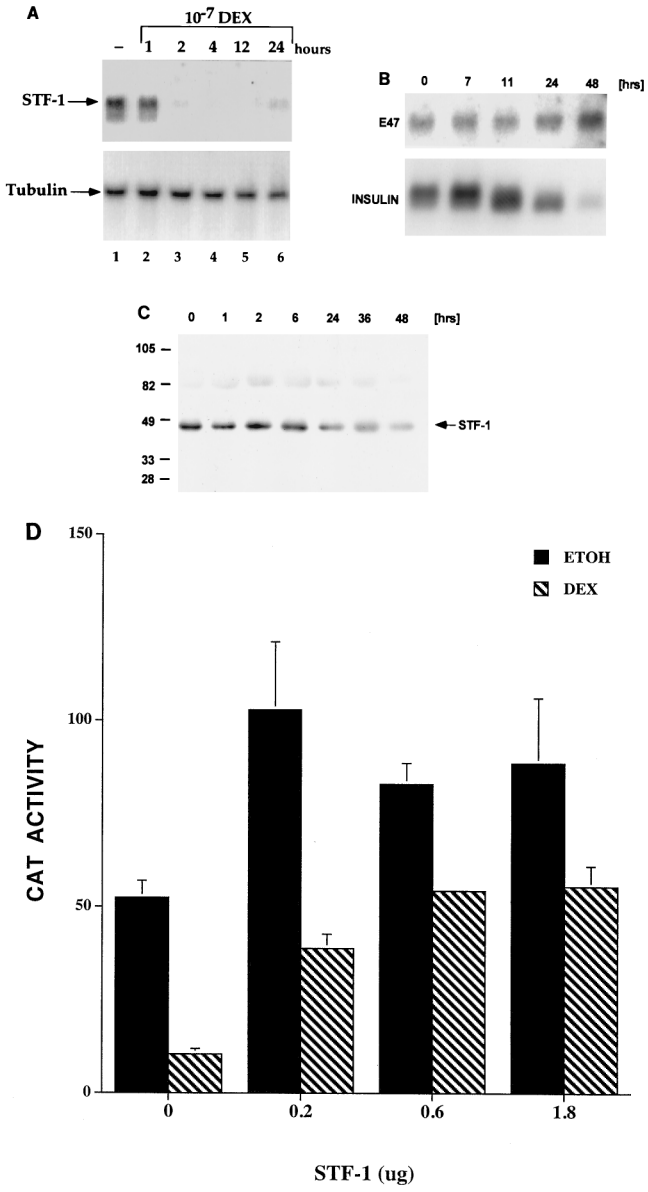

FIG. 1. Glucocorticoids repress *STF-1* and insulin gene expression in pancreatic islet cells. (A) Northern blot analysis of total RNA from control (−) or dexamethasone (DEX; 10^{-7} M)-treated rat pancreatic TU6 insulinoma cells for 1, 2, 4, 12, or 24 h. The top panel shows *STF-1* mRNA levels, as detected with a ^{32}P-labeled random-primed *STF-1* cDNA probe. The bottom panel shows tubulin mRNA levels in control and treated cells. (B) Northern blot assay of total RNA from HIT-T15 cells following treatment with 10^{-7} M dexamethasone for the times indicated above the lanes. Levels of insulin and E47 mRNAs are shown. (C) Western blot analysis of STF-1 protein levels in HIT-T15 cells following treatment with 10^{-7} M dexamethasone for times indicated above the lanes. An STF-1 immunoreactive band is indicated. (D) Overexpression of STF-1 suppresses dexamethasone-mediated inhibition of the insulin gene in HIT-T15 cells, as determined by a transient transfection assay of an insulin I-CAT reporter construct in control (ethanol [ETOH]-treated) or dexamethasone (DEX)-treated HIT-T15 cells. Cells were cotransfected with increasing amounts of STF-1 expression plasmid as indicated at the bottom. Error bars are indicated. CAT activity is expressed as percent chloramphenicol converted to acetylated forms after normalization to activity from an internal CMV–β-galactosidase control plasmid.

STF-1 expression. Northern blot analysis of RNAs prepared from control and dexamethasone-treated HIT-T15 islet cells revealed a 10-fold reduction in levels of STF-1 mRNA within 2 h of treatment (Fig. 1A). By contrast, expression of the tubulin and E47 genes was unaffected by glucocorticoid induction, demonstrating the specificity of this repression (Fig. 1A and B). In agreement with Northern blot data, STF-1 protein levels were also reduced following exposure to glucocorticoids, although the time course of STF-1 protein disappearance by Western blot assay (half-life of 24 h) was delayed relative to that of *STF-1* mRNA (Fig. 1C).

Previous studies demonstrating that STF-1 regulates insulin gene expression via two TAAT motifs in the insulin promoter prompted us to test whether dexamethasone inhibits insulin gene expression via an STF-1-dependent mechanism. Northern blot analysis of total RNA from HIT-T15 cells revealed that mRNA levels for insulin were indeed reduced four- to fivefold after 24 to 48 h of exposure to 10^{-7} M dexamethasone (Fig. 1B). Insulin promoter activity, as measured by transient-transfection assay in HIT-T15 cells, was also inhibited about 8- to 10-fold by dexamethasone treatment, revealing that glucocorticoids block insulin expression, at least in part, at the level of transcription (Fig. 1D). Cotransfection of an STF-1 expression vector into dexamethasone-treated HIT-T15 cells restored insulin promoter activity in a dose-dependent manner (Fig. 1D). Taken together, these results support the notion that glucocorticoids inhibit insulin gene expression in HIT-T15 cells by blocking *STF-1* gene expression.

In a previous study, we found that an *STF-1*–β-Gal fusion gene containing 6,500 bases of the *STF-1* 5′ flanking region was appropriately expressed in pancreatic islet cells of transgenic mice (18). To evaluate whether glucocorticoids inhibit STF-1 expression at the level of transcription, we performed transient-transfection assays with a −6500 STF Luc reporter in HIT-T15 insulinoma cells (Fig. 2A). Dexamethasone treatment blocked expression of the STF Luc reporter plasmid about 10-fold, revealing that glucocorticoids may interfere with *STF-1* promoter activity (Fig. 2A).

Two regions of the *STF-1* promoter appear to be essential for islet-restricted expression: a proximal element located at bp −100 which binds the ubiquitous factor USF-1, and a distal islet-specific enhancer located between kb −6.5 and −3 of the *STF-1* promoter (18). To determine whether glucocorticoids repress *STF-1* gene expression via this distal enhancer, we performed transient-transfection experiments in HIT-T15 cells, using a series of STF-1 reporter plasmids. Promoter constructs containing a 530-bp fragment extending from −6200 to −5670 of the *STF-1* gene were fully repressed by dexamethasone treatment, but a minimal *STF-1* promoter construct containing the ubiquitous USF-1 recognition site was not (Fig. 2A). The same 530-bp region of the *STF-1* gene was about 5- to 10-fold more active in HIT-T15 cells than in COS-7 cells, demonstrating that this fragment contains islet cell-specific activity (Fig. 2B).

The endodermal factors HNF-3β and BETA-2 regulate STF-1 expression via an islet-specific enhancer. Inspection of the nucleotide sequence within the cell-specific *STF-1* enhancer revealed consensus motifs for E-box-binding proteins (−5974) and for HNF-3 (−5920) (Fig. 2C). The E-box motif, termed the B element, is identical in sequence to the NIR and FAR elements within the rat insulin I and II promoters (4). Also, HNF-3 site, referred to as the H element, coincides with the HNF-3 consensus binding site in 9 of 12 positions (3).

To determine whether the B and H elements are recognized by islet-specific nuclear proteins, we performed DNase I protection assays using a ^{32}P-labeled *STF-1* enhancer fragment extending from −6100 to −5870. Nuclear extracts prepared from HIT-T15 cells were found to contain DNA binding activities over both the B (E-box motif) and H (HNF-3 motif) sites (Fig. 3A; compare lanes 1 and 2). The B element was

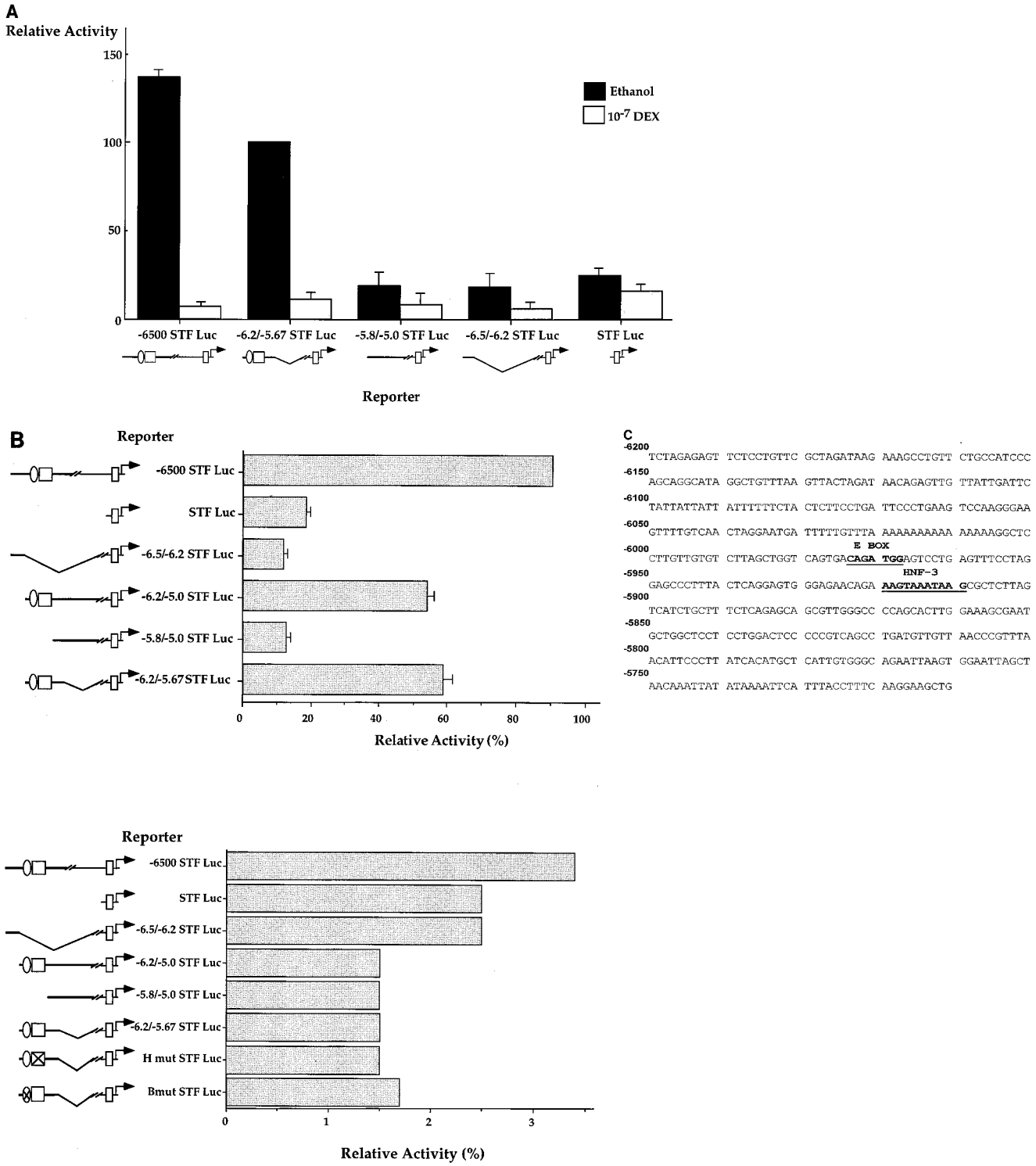

FIG. 2. Glucocorticoids repress STF-1 expression via an islet-specific enhancer in the 5′ flanking region of the *STF-1* gene. (A) Activities of *STF-1* promoter constructs following treatment of HIT-T15 cells with dexamethasone (DEX; 10^{-7} M) or control ethanol vehicle for 18 to 24 h. The activity of the −6.2/−5.67 STF Luc reporter is set at 100%. All constructs were evaluated in the context of an STF Luc vector which contains 120 bases of 5′-proximal flanking region. Inclusive *STF-1* sequences inserted into the STF Luc reporter are indicated by nucleotide numbers. For example, −6.5/−6.2 STF Luc contains STF-1 sequences from −6500 to −6200 fused to the proximal 120 bases of the STF-1 5′ flanking region. Distal regions which contain activating elements are indicated by ovals and squares. The minimal *STF-1* promoter is indicated by rectangles. Standard error bars are shown. Assays were repeated at least three times. STF-1 reporter activity throughout was normalized to transfection efficiency by using a cotransfected CMV–β-Gal control plasmid. (B) Top, transient-transfection assay of STF-1 reporter constructs in HIT-T15 cells. The activity of −6500 STF Luc, which contains 6500 bases of 5′ flanking sequence was set at 100%. Each assay was repeated in duplicate at least four times. Standard errors are shown. Bottom, activities of STF Luc reporter plasmids following transient transfection into COS-7 cells. Promoter activity was normalized to CMV–β-Gal control activity, allowing for direct comparison with STF-1 reporter activity in HIT cells. (C) Nucleotide sequence of the minimal islet-specific enhancer in the *STF-1* gene. Nucleotide positions relative to the transcriptional start site are indicated. HNF-3 and E-box binding motifs are labeled.

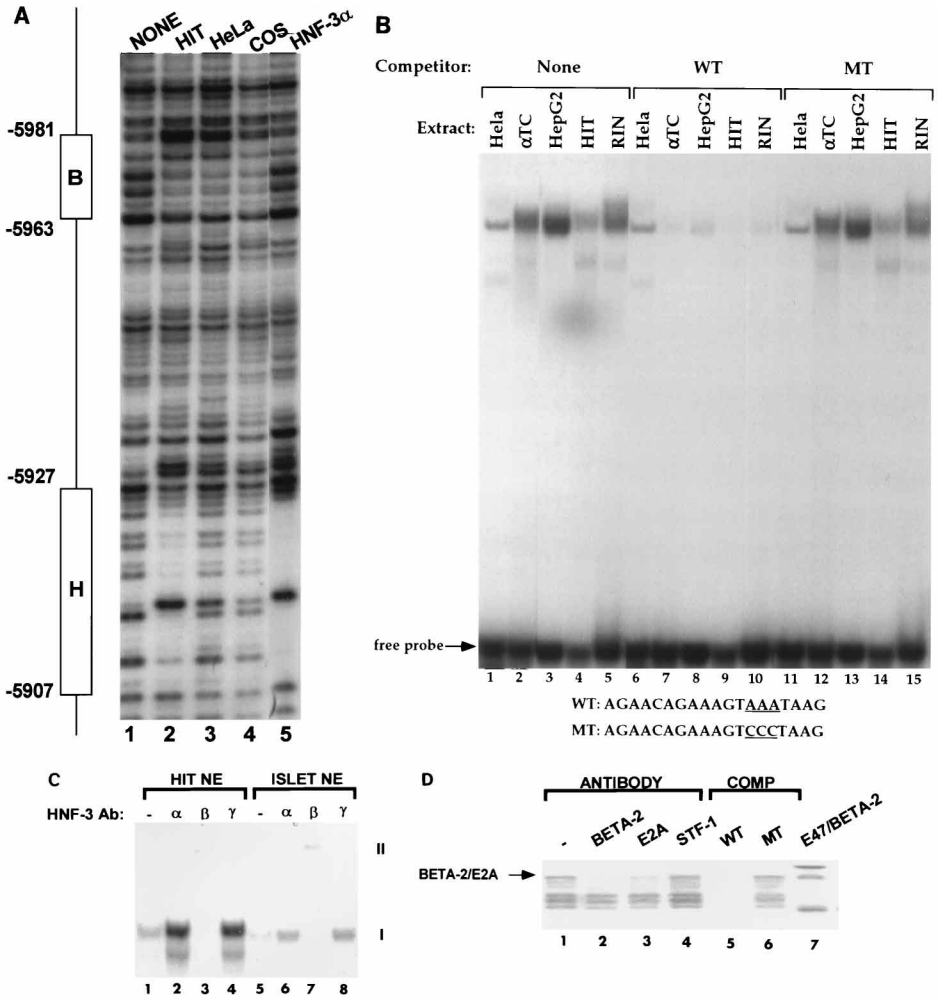

FIG. 3. The pancreatic islet-specific enhancer in the *STF-1* gene contains binding sites for HNF-3β and BETA-2. (A) DNase I protection assays of crude nuclear extracts from HIT-T15 islet cells, HeLa cells, or COS-7 cells, using a ^{32}P-labeled STF-1 probe extending from −5870 to −6100 of the rat *STF-1* promoter. NONE, control reaction without added extract; HNF-3α, reaction using recombinant protein. (B) Gel mobility shift assay of crude nuclear extracts from HeLa, HepG2, glucagon-producing αTC, or insulin-producing HIT and RIN cell lines. Assays were performed with a ^{32}P-labeled STF-1 oligonucleotide probe containing the H element. Nucleotide sequences of wild-type (WT) and mutant (MT) H elements are shown below. A 100-fold excess of unlabeled wild-type or mutant competitor DNA was added as indicated above the lanes. (C) Gel mobility shift assay of crude nuclear extract from HIT insulinoma (HIT NE) and primary cultured adult rat islet (ISLET NE) cells, using a ^{32}P-labeled STF-1 H-element probe extending from −5907 to −5927. HNF3α, -β, or -γ antiserum was added to reactions as indicated above the lanes. −, no antiserum added. I and II, protein-DNA and antibody (Ab) supershifted complexes, respectively. (D) Gel mobility shift assay of crude nuclear extract from HIT-T15 cells, using a ^{32}P-labeled B-element probe extending from −5963 to −5981 of the *STF-1* promoter. Addition of BETA-2, E2A, or STF-1 antiserum to binding reactions is indicated above the lanes. Addition of a 100-fold excess of unlabeled wild-type (WT; 5′-TCAGTGACAGATGGAGTCCT-3′) or mutant (MT; 5′-TCA GTGAAAGACGGAGTCCT-3′) competitor (COMP) DNA is also indicated. Binding activity derived from reticulocyte lysate programmed with BETA-2 and E-47 cDNAs is shown in lane 7. The top band in lane 7 is indigenous to reticulocyte lysate.

comparably protected in DNase I protection assays with nuclear extracts from HIT, HeLa, and COS-7 cells, perhaps due in part to binding of the ubiquitous factor E47. By contrast, protection of the H element was observed only with extracts from HIT cells (Fig. 3A; compare lanes 2 to 4). A prominent hypersensitive site which interrupted the H-element footprint was also noted in reactions containing purified recombinant HNF-3α protein, leading us to speculate that a member of the HNF-3 family of regulators binds to the STF-1 H element in HIT-T15 cells (Fig. 3A; compare lanes 2 and 5).

To further characterize nuclear factors which recognize the H element on the *STF-1* enhancer, we performed gel mobility shift assays using a ^{32}P-labeled STF-1 H-element probe. One major low-mobility complex was observed in reactions containing HIT or RIN nuclear extracts, and formation of this complex was specifically inhibited by adding a 100-fold molar excess of unlabeled wild-type but not mutant H-element competitor DNA (Fig. 3B; compare lanes 4 and 5, 9 and 10, and 14 and 15). No specific protein-DNA complexes were observed in reactions containing HeLa nuclear extracts, indicating that the H element may recognize nuclear factors with a restricted expression pattern (Fig. 3B; compare lanes 1 and 6). The presence of an H-element-specific complex in HepG2 hepatoma extracts running at the same mobility as the RIN–HIT-T15 complex, however, suggested that the H-element binding protein may be generally expressed in cells of endodermal origin (Fig. 3B; compare lanes 3 to 5).

The HNF-3 family of nuclear activators consists of three genes (α, β, and γ) which bind to DNA via a highly conserved winged helix domain (8). To determine whether the H-element binding activity in HIT extracts corresponded to an HNF-3 family member, we performed gel mobility shift studies using

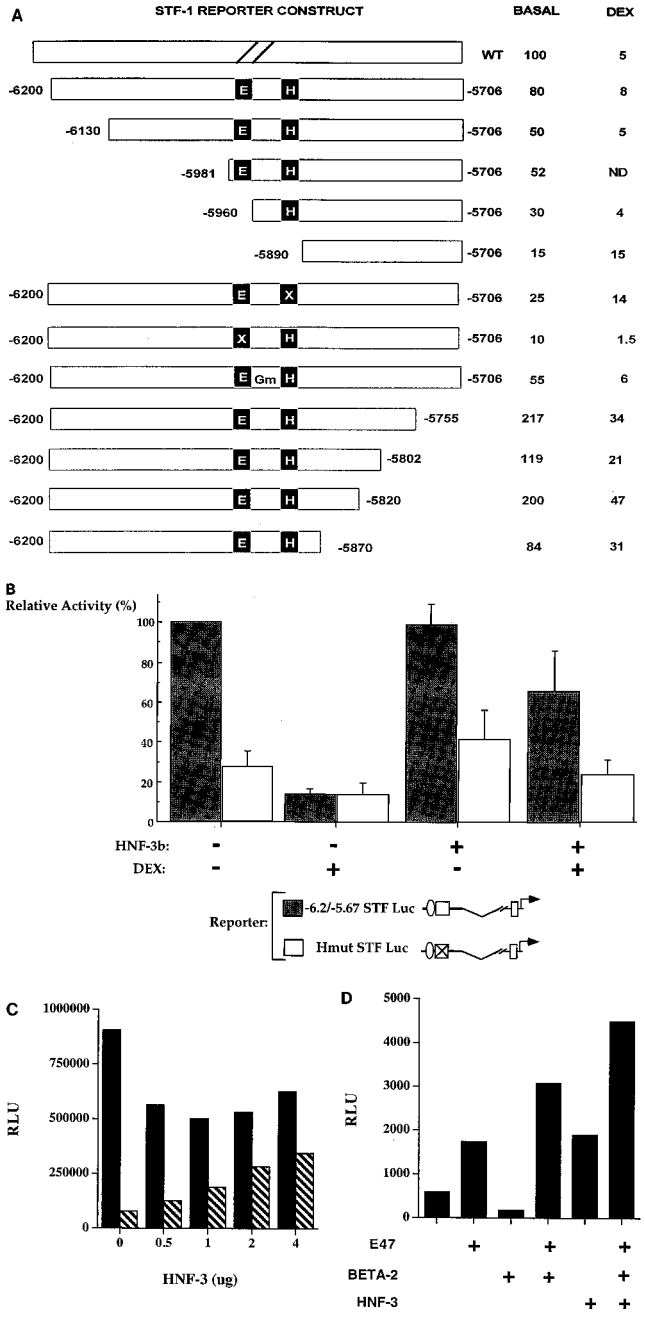

FIG. 4. E-box and HNF-3 binding sites mediate *STF-1* enhancer activity in HIT-T15 cells. (A) Relative luciferase activity derived from STF Luc reporter constructs following transfection into control (■) or dexamethasone (DEX)-treated ▨ HIT-T15 cells. Values from a representative assay are shown. All reporters contained *STF-1* enhancer sequences fused to a minimal STF Luc reporter plasmid containing 120 bases of the proximal *STF-1* promoter region. The activity of each construct in control and dexamethasone-treated cells is expressed relative to that of the full-length *STF-1* promoter plasmid (wild type [WT]; 100%) extending from −6500 to +1. Relative positions of E-box (E) and HNF-3 (H) binding sites are indicated. Constructs containing mutation in the E box (−5973; CAGATG/AAGACG) and HNF-3 sites (−5917; TAAAT/TCCCT) are indicated by X's in the appropriate boxes. A construct containing point mutations over a potential GRE site (Gm; −5937 [GAACA/TAATA]) is also indicated. Point mutations in E-box and H-element mutations are identical to those used in gel mobility shift assays (Fig. 3). (B) Effect of HNF-3β overexpression on wild-type (dark bars) and H-element mutant (Hmut; light bars) STF-1 reporter activity in control (−) and dexamethasone-treated (+) HIT-T15 cells. The activity of the wild-type −6.2/−5.7 STF Luc construct containing minimal islet cell-specific enhancer (−6200 to −5700) in control cells is set at 100%. Standard error bars are shown. Experiments were repeated at least four times. (C) Effect of increasing levels of HNF-3β effector plasmid on wild-type STF-1 (−6500 STF Luc) reporter activity in HIT-T15 cells. Results of a representative transfection assay are shown. The amount of HNF-3β effector plasmid is indicated below each bar. The total amount of effector plasmid in each assay for panels C and D was kept constant by balancing with an empty CMV expression vector. ■, control (ethanol-treated) cells; ▨, dexamethasone-treated cells. Activity was normalized to that of an internal CMV–β-Gal control. (D) Representative transfection assay of HeLa cells, using the −6.2/−5.7 STF Luc reporter plasmid. Cells were cotransfected with eukaryotic expression plasmids for E47, BETA-2, and HNF-3 (6 μg) as indicated at the bottom. STF-1 reporter activity is expressed in relative light units (RLU) after normalization to internal CMV–β-Gal control plasmid activity.

specific antisera against each of the HNF-3 members. Although each of the three HNF-3 proteins was detected in nuclear extracts of HIT-T15 insulinoma cells by Western blot analysis (not shown), only HNF-3β antiserum was found to block formation of the major protein-DNA complex in assays using the same extract with an H-element probe (Fig. 3C, lanes 1 to 4). Identical results were obtained in gel shift assays of nuclear extracts prepared from primary cultures of adult rat islet cells (Fig. 3C, lanes 5 to 8).

The B element within the *STF-1* enhancer contains a consensus E-box motif which is identical to E-box elements within the insulin promoter (4). Previous work demonstrating that the islet-specific factor BETA-2 activates insulin promoter activity by binding to these insulin promoter elements as a heterodimer with the ubiquitous factor E47 (12) prompted us to test for BETA-2–E47 heterodimer formation on the STF-1 B element. In gel mobility shift assays of HIT nuclear extracts, a ^{32}P-labeled B-element probe was observed to form four specific DNA-protein complexes which could be competed by addition of an unlabeled wild-type oligonucleotide but not a mutant B-element oligonucleotide (Fig. 3D; compare lanes 1, 5, and 6). The slowest complex migrated at the same position as a recombinant E2A–BETA-2 heterodimeric complex (Fig. 3D; compare lanes 1 and 7). Although an unrelated antiserum had no effect on B-element binding activities (Fig. 3D, lane 4), BETA-2 and E2A antisera specifically inhibited formation of the slowest complex (Fig. 3D, lanes 2 and 3), revealing that the BETA-2–E47 heterodimer does indeed recognize the B element in HIT nuclear extracts.

To evaluate the importance of HNF-3β and E2A–BETA-2 recognition sites in mediating *STF-1* enhancer activity as well as dexamethasone repression in HIT-T15 cells, we constructed a series of 5′ and 3′ deletion mutants within the 530-bp *STF-1* enhancer and fused these enhancer fragments to a minimal STF Luc reporter plasmid containing 120 bases of promoter-proximal sequence. Enhancer constructs containing a 110-nucleotide region extending from −5981 to −5870 had activities comparable to that of the wild-type −6500 STF Luc construct (Fig. 4A). Constructs containing deletions past the E- and H-box motifs were far less active, demonstrating the importance of these sites in mediating *STF-1* enhancer activity. Reporter plasmids containing the 110-nucleotide enhancer segment were also repressed by dexamethasone, although the extent of inhibition by dexamethasone appeared to diminish when sequences from −5706 to −5870 were removed. Substitution mutagenesis of the E-box motif (−5973; CAGATG/AAGACG) reduced STF Luc reporter activity five- to eightfold but did not appear to affect inhibition by dexamethasone. By contrast, mutagenesis of the HNF-3 binding site (−5917; TAAAT/TCCCT) significantly blocked inhibition by dexamethasone, indicating that this site may be specifically involved in glucocorticoid repression (Fig. 4A).

Within the 110-nucleotide *STF-1* enhancer, we identified a

single glucocorticoid response element (GRE) half-site consensus motif (−5927; AGAACA) close to the HNF-3 site (−5920). In gel mobility shift and footprint assays, however, this site did not bind detectably to a recombinant glucocorticoid receptor DNA binding domain polypeptide (not shown). Moreover, mutagenesis of the putative GRE half-site (GAAC A/TAATA) had no effect on the ability of dexamethasone to repress *STF-1* enhancer activity in transient-transfection assays of HIT-T15 cells, indicating that glucocorticoids may regulate STF-1 expression via an indirect mechanism (Fig. 4A).

Glucocorticoids repress STF-1 expression by blocking HNF3-β activity on the islet-specific enhancer. To evaluate whether dexamethasone inhibits STF-1 expression by disrupting HNF-3β or BETA-2–E47 activity on the distal enhancer, we performed transient-transfection assays with HNF-3β or BETA-2 and E47 effector plasmids. Overexpression of either HNF-3β (Fig. 4B) or BETA-2 and E47 (not shown) had minimal effects on STF-1 reporter expression in unstimulated HIT-T15 cells (Fig. 4B; compare bars 1 and 5). HNF-3β was found to suppress the inhibitory effects of dexamethasone on wild-type STF-1 reporter activity (Fig. 4B; compare bars 1, 3, and 7), but activators such as BETA-2–E47, STF-1, and HNF-4 did not rescue STF-1 promoter activity in dexamethasone-treated cells (not shown). In titration experiments with increasing amounts of effector plasmid, HNF-3β was found to rescue −6500 STF Luc reporter activity in a dose-dependent manner (Fig. 4C). HNF-3β did not potentiate the activity of a mutant STF-1 reporter plasmid containing a mutation in the H element, however, suggesting that the suppressive effects of this activator occurred via its recognition site in the *STF-1* enhancer (Fig. 4B; compare bars 2, 4, 6, and 8).

To determine whether HNF-3 and BETA-2 could act cooperatively on the *STF-1* enhancer, we performed transient-transfection assays using HeLa cells (Fig. 4D). A BETA-2 expression vector had no stimulatory effect on its own, whereas an E47 expression plasmid enhanced STF-1 reporter activity three- to fourfold. Cotransfection of both BETA-2 and E47 vectors increased *STF-1* enhancer activity about five- to sixfold, in keeping with the ability of these proteins to function as heterodimers. An HNF-3β expression vector also induced STF-1 reporter expression three- to fourfold when transfected into HeLa cells. Also, cotransfection of all three expression plasmids (HNF-3β, BETA-2, and E47) potentiated STF-1 enhancer activity 9- to 10-fold, revealing that these nuclear factors may indeed regulate STF-1 expression in a cooperative manner.

DISCUSSION

In this study, we found that glucocorticoids potently repressed the expression of the *STF-1* gene by blocking the activity of a distal islet-specific enhancer which recognizes two endodermal factors, HNF-3β and BETA-2–E47. Although mutations in either the HNF-3β or BETA-2–E47 sites disrupted basal *STF-1* promoter activity in HIT-T15 cells, only mutations in the HNF-3β site appeared to block glucocorticoid inhibition. Moreover, the unique ability of an HNF-3β expression vector to rescue *STF-1* enhancer activity in glucocorticoid-treated cells reveals that HNF-3β is indeed a target for hormonal regulation.

The HNF-3 family of activators has been shown to function in the specification of endodermal structures such as the liver (5), and our results support the notion that HNF-3β is additionally required for islet cell development. HNF-3β is expressed at early stages of pancreatic morphogenesis in cells which also appear to express STF-1 (1). The HNF-3s are structurally related to histone H5, and like H5, these nuclear factors are thought to regulate target gene expression via a chromatin remodeling mechanism (3). In this regard, binding of HNF-3 to promoter sites in hepatic precursor cells can be detected prior to transcriptional induction, indicating that HNF-3 may prime target genes for expression by opening chromatin structure and thereby providing promoter access to other transcription factors (5).

HNF-3 is expressed in a number of endodermal structures in addition to the pancreas, suggesting that other regulatory factors must cooperate with HNF-3 on the *STF-1* distal enhancer. In this regard, we found that an E-box motif located adjacent to the HNF-3 site on this enhancer was critical for *STF-1* gene expression. This E-box motif recognizes the β-cell-specific factors BETA-2 and E47 (12), and mutations which disrupt binding of BETA-2 and E47 correspondingly reduce *STF-1* gene expression. The ability of BETA-2 and E47 to stimulate STF-1 reporter expression supports the notion that these factors are required for *STF-1* enhancer activity. In contrast to the wide distribution of HNF-3 protein in endodermal structures, BETA-2 expression is restricted to duodenal and pancreatic islet cells of the gut, sites in which STF-1 is also produced (12). Thus, STF-1 expression may be restricted to a subset of endodermal structures which express both HNF-3β and BETA-2.

How then do glucocorticoids inhibit *STF-1* gene expression? Glucocorticoids have been shown to directly affect the ability of HNF-3 to bind to its recognition site on the *tat* gene via a hit-and-run mechanism (16). In this scenario, ligand-bound glucocorticoid receptor (GR) is proposed to disrupt chromatin structure, thereby providing access for HNF-3 to bind its cognate site. Although the *STF-1* enhancer contains a putative GRE adjacent to the HNF-3 binding site, mutagenesis of that element does not abolish glucocorticoid inhibition, suggesting that GR molecules may not inhibit HNF-3 activity by competing for occupancy on the *STF-1* promoter. Furthermore, extracts prepared from control and dexamethasone-treated cells contain comparable levels of HNF-3 binding activity by gel mobility shift assay (18), suggesting that dexamethasone may not alter either the level of HNF-3β expression or its DNA binding activity in vitro via posttranslational modification. GR has recently been shown to stimulate target gene expression via the coactivators CBP and SRC-1 (2, 14). Although it is intriguing to speculate that GR may interfere with HNF-3 action by competing for one of these coactivators, preliminary transfection studies indicate that neither CBP nor SRC-1 expression vectors can rescue *STF-1* enhancer activity in response to dexamethasone. Genomic footprinting studies should reveal whether glucocorticoids interfere with HNF-3β by altering local chromatin structure surrounding the *STF-1* enhancer.

Glucocorticoids have been shown to inhibit neonatal islet cell maturation in vitro while conversely promoting differentiation of acinar cells (10, 16). Our results suggest that the inhibitory effects of dexamethasone on islet cell maturation and on insulin gene expression may in part reflect a corresponding block in STF-1 production. A surge in glucocorticoid levels has been noted during weaning, and this increase in circulating glucocorticoid levels is thought to induce the massive expansion in the exocrine pancreatic component which is observed after birth. It will be of interest to determine how down regulation of STF-1 expression further influences the expansion of pancreatic islets during development and, perhaps more importantly, how these hormones may influence insulin production in diabetic patients on chronic glucocorticoid therapy.

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