Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 9127–9131, September 1995
Medical Sciences

Reduction of insulin gene transcription in HIT-T15 β cells chronically exposed to a supraphysiologic glucose concentration is associated with loss of STF-1 transcription factor expression

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Communicated by Donald Steiner, University of Chicago, Chicago, IL, June 16, 1995

ABSTRACT **Chronic exposure of HIT-T15 β cells to elevated glucose concentrations leads to decreased insulin gene transcription. The reduction in expression is accompanied by diminished binding of a glucose-sensitive transcription factor (termed GSTF) that interacts with two (A+T)-rich elements within the 5′ flanking control region of the insulin gene. In this study we examined whether GSTF corresponds to the recently cloned insulin gene transcription factor STF-1, a homeodomain protein whose expression is restricted to the nucleus of endodermal cells of the duodenum and pancreas. We found that an affinity-purified antibody recognizing STF-1 supershifted the GSTF activator complex formed from HIT-T15 extracts. In addition, we demonstrated a reduction in STF-1 mRNA and protein levels that closely correlated with the change in GSTF binding in HIT-T15 cells chronically cultured under supraphysiologic glucose concentrations. The reduction in STF-1 expression in these cells could be accounted for by a change in the rate of STF-1 gene transcription, suggesting a posttranscriptional control mechanism. In support of this hypothesis, no STF-1 mRNA accumulated in HIT-T15 cells passaged in 11.1 mM glucose. The only RNA species detected was a 6.4-kb STF-1 RNA species that hybridized with 5′ and 3′ STF-1-specific cDNA probes. We suggest that the 6.4-kb RNA represents an STF-1 mRNA precursor and that splicing of this RNA is defective in these cells. Overall, this study suggests that reduced expression of a key transcriptional regulatory factor, STF-1, contributes to the decrease in insulin gene transcription in HIT-T15 cells chronically cultured in supraphysiologic glucose concentration.**

The glucose toxicity hypothesis proposes that chronic hyperglycemia in humans with type 2 diabetes exacerbates pancreatic islet β-cell defects in glucose-induced insulin secretion (1–3). Recently, we suggested that one potential mechanism by which chronic hyperglycemia might adversely affect pancreatic β-cell function is by decreasing the rate of insulin gene transcription (4). This proposal is based, in part, on our observation that chronic exposure of HIT-T15 β cells under supraphysiologic glucose concentrations (11.1 mM) markedly decreases insulin mRNA levels and insulin gene promoter activity (4, 5). In contrast, chronically culturing HIT-T15 cells at lower glucose concentrations (0.8 mM) preserves these functions (4, 5). The loss of insulin gene transcription in cells continually grown in 11.1 mM glucose was accompanied by decreased binding of a glucose-sensitive transcription factor (GSTF). This factor was shown to be necessary for transcription mediated by two (A+T)-rich elements located within the 5′ flanking transcriptional control region of the human insulin gene (4). The function and sequence of these cis-acting elements, which are located in the human gene between nucleotides −217 and −210 and between nucleotides −84 and −77, are conserved among all characterized mammalian insulin genes (6–9).

The gene encoding the pancreatic β-cell protein that forms the predominant base-pair −217 to −210 and −84 to −77 element binding activity has recently been independently isolated in three laboratories from rats [termed STF-1 (10) and IDX-1 (11)] and mice [IPF-1 (12)]. This factor will be referred to here as STF-1. STF-1 is a homeoprotein closely related to the *Xenopus* XIHbox 8 protein (9). XIHbox 8 and STF-1 are selectively expressed in cells of the pancreas and duodenum (9, 11, 13). STF-1 nuclear staining is present in adult mouse islet cells in 92% of the insulin-producing β cells, 15% of the somatostatin-producing islet δ cells, and 3% of the glucagon-producing α cells (9). These results, coupled with the observation that (over)expression of STF-1 can specifically activate transcription from reporter constructs containing the 5′ flanking region of the insulin gene (ref. 12; R.S., unpublished observations), suggest that the STF-1 transcription factor is an important regulator of insulin gene expression *in vivo*. Interestingly, recent studies have demonstrated that pancreas formation is preserved in mice carrying a null mutation in STF-1 (ref. 14; C.V.E.W., unpublished observations).

In this study, we show that the GSTF–DNA complex detected with a base-pair −217 to −210 element probe in the gel mobility shift assay was supershifted with antibodies that recognize the STF-1 protein. The reduced GSTF binding activity in HIT-T15 cells chronically passaged in supraphysiologic glucose concentrations was associated with decreased expression of the 2.3- and 1.9-kb STF-1 mRNAs and STF-1 protein. Interestingly, prolonged supraphysiological glucose concentrations did not affect the rate of STF-1 gene transcription. These results suggest that GSTF corresponds to the STF-1 transcription factor and that the reduced level of insulin gene transcription in these cells is due, at least in part, to abnormal posttranscriptional processing of STF-1.

MATERIALS AND METHODS

Cultured Cells. HIT-T15 cells were routinely cultured in RPMI 1640 medium (GIBCO/BRL) containing 11.1 mM glucose and supplemented with 10% (vol/vol) fetal bovine serum (15). Beginning at passage 70, the cells were split into medium containing either 11.1 mM or 0.8 mM glucose and continuously cultured through passage 140 as described (5). The HIT-T15 cells are defined as early (E = passages 70–81) or late (L = passages 126–140) passage. NIH 3T3 L1 cells were grown in Dulbecco modified Eagle medium (GIBCO/BRL) containing 10% (vol/vol) fetal calf serum and 50 μg each of penicillin and streptomycin per ml.

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Abbreviations: GSTP, glucose-sensitive transcription factor; CAT, chloramphenicol acetyltransferase.

Nuclear Extracts and Electrophoretic Mobility Shift Assays. Nuclear extracts were prepared from HIT-T15 cells as described (16). Double-stranded oligodeoxynucleotides to detect GSTF binding to human insulin sequence (−230 to −201, CCCCTGGTTAAGACTCTAATGACCCGCTGG) were end-labeled with [α-^{32}P]dCTP and *Escherichia coli* Klenow DNA polymerase I. The binding and electrophoresis conditions were performed as described (17) except that 12 ng each of poly(dI-dC) and poly(dA-dT) per μl were added to the reaction buffer. The affinity-purified XIHbox 8 or Mox-1 antibody (4 μl) was added with the HIT-T15 extract (5 μg) to the DNA binding reactions and incubated for 20 min at room temperature.

Homeoprotein Antibodies. The N-terminal XIHbox 8 antibody was raised against the first 75 amino acids of XIHbox 8 as a GST/XIHbox 8 fusion protein in the pGEX system (18). The C-terminal XIHbox 8 antibody was raised to amino acids 181–271 (19). The Mox-1 antibodies were raised to amino acids 2–139 as a glutathione *S*-transferase (GST) fusion protein (20). Fusion proteins were induced and purified, rabbits were immunized, and antibodies were affinity purified using techniques described previously (21).

DNA Constructs. GAL4:VP16 (22), $(GAL4)_5$ E1bCAT (23), and pSG 1–147 (23) plasmids have previously been described. GAL4:STF-1 was constructed by the PCR using a full-length rat STF-1 cDNA (18) and the following primers: 5′-GCGCGGATCCAGATGAATAGTGAGGAG-3′ and 5′-CGCGGGTACCGGGGGTTCCTGCGGTCG-3′ (the underlined nucleotides correspond to STF-1 coding sequences). The resulting fragment was digested with *Bam*HI (5′) and *Kpn* I (3′) and ligated into these sites in the GAL4 expression plasmid, pSG 1-147 (23), to create the in-frame GAL4 fusion protein. The construct was verified by DNA sequencing.

Cell Transfections and Chloramphenicol Acetyltransferase (CAT) Assays. HIT-T15 cells were subcultured for 2 days in RPMI 1640 medium containing 11.1 mM glucose at a density of 1.2×10^6 cells per well (35 mm). Duplicate wells were cotransfected with the $(GAL4)_5$ E1bCAT reporter gene (0.5 μg) and a GAL4 fusion plasmid (1 μg) using the liposome-mediated techniques described previously (4). Cells were harvested 30 hr after transfection. CAT enzymatic assays were performed as described (24).

Western Blot Analysis. HIT-T15 nuclear extracts (30 μg per lane) were resolved on a 12.5% SDS/polyacrylamide gel and electrotransferred onto Immobilon poly(vinylidene difluoride) membrane (Millipore). The membrane was first incubated for 2 hr at room temperature in 5% nonfat dry milk and a blocking solution containing 0.2% Nonidet P-40, 50 mM Tris·HCl (pH 8.0), 80 mM NaCl, 2 mM $CaCl_2$, and 3 mM sodium azide and then incubated with the primary affinity-purified anti-N-terminal XIHbox 8 antibody (1:1000 dilution) overnight. After two washes in the blocking solution, the membranes were probed with alkaline phosphatase-conjugated goat anti-rabbit antibody (diluted 1:1000) for 2 hr. The membrane was then washed three times in blocking solution and twice in a solution containing 50 mM Tris·HCl (pH 8.0), 80 mM NaCl, and 2 mM $CaCl_2$. Immunoreactivity was visualized by diaminobenzidine staining (Bio-Rad).

RNA Isolation and Northern Blot Analysis. Total RNA from 5×10^6 cells was isolated by the guanidinium isothiocyanate method (25). RNA expression levels were measured from 15 μg of total cellular RNA by Northern blot analysis on 1.5% agarose gels containing 6.7% formaldehyde. Integrity and relative amounts of RNA were checked by ethidium bromide staining of the rRNA. The RNA was electrotransferred onto a nylon membrane filter (Micron Separations, Westboro, MA). Prehybridization and hybridization protocols were performed in 50% formamide at 42°C with cDNA probes labeled with [α-^{32}P]dCTP by random priming (26). The membrane was washed three times at room temperature in 2× SSC/0.1% SDS and washed twice at 60°C in 0.2× SSC/0.1% SDS. The blots were probed with cDNAs isolated from the following: pHFβA-1, a human β-actin cDNA clone (27); and pSKII 900, a rat STF-1 cDNA clone (10). The 5′ and 3′ STF-1 probes contained sequences from +331 to +717 and +714 to +1182, respectively.

Transcriptional Run-On Analysis. Nuclear run-on assays were performed on an equivalent number of nuclei isolated from NIH 3T3 L1 cells, early-passaged HIT-T15 cells (HIT[11.1E]), and late-passaged HIT-T15 cells grown in the presence of 11.1 (HIT[11.1L]) or 0.8 mM glucose (HIT[0.8L]). The concentration of nuclei, as isolated according to Sasaki *et al.* (28), was measured by diluting an aliquot into 10% (wt/vol) SDS and measuring the absorbance at 260 nm. Newly synthesized RNA was labeled by incubating the nuclei (4.0 A_{260} units per reaction) in the presence of 100 μCi of [α-^{32}P]UTP (1 Ci = 37 GBq) as described (28, 29). Approximately 10^7 cpm of each [^{32}P]RNA run-on product was hybridized to the following DNAs (0.5 μg each) applied to nitrocellulose filters: Bluescript II; a probe for β-actin, pHFβA-1 (27); and a probe for STF-1, pSKII 900 (10). Hybridization and washing conditions were as described by McKnight and Palmiter (29). The amount of [^{32}P]RNA hybridized per lane was determined by densitometric scanning of the autoradiographs.

RESULTS

GSTF Corresponds to the STF-1 Transcription Factor. To determine whether GSTF corresponded to the STF-1 transcription factor, we tested the effect of adding affinity-purified antibodies against the N-terminal region of XIHbox 8 protein on the HIT-T15 cell complexes formed with a human insulin gene probe spanning the −217- to −210-bp element. This N-terminal polypeptide antiserum was raised to a region of XIHbox 8 (amino acids 1–75) that is distinct from the homeodomain region but highly conserved with the STF-1 protein (9). Previous studies have shown that the XIHbox 8 N-terminal antiserum specifically supershifts the STF-1 protein–DNA complex from HIT-T15 cell extracts (9). In contrast, an antiserum raised to the nonconserved C-terminal region of XIHbox 8 (amino acids 181–271) does not recognize STF-1 (9). Affinity-purified antibodies raised to the C-terminal region of XIHbox 8 and to an unrelated murine mesoderm-specific homeoprotein, Mox-1, were used as negative controls in the gel shift experiments.

Gel shift analysis was performed with cell extracts from early-passage HIT-T15 cells (HIT[11.1E]) and late-passage HIT-T15 cells grown in the presence of 11.1 mM (HIT[11.1L]) or 0.8 mM glucose (HIT[0.8L]). As described previously (4), GSTF binding activity was significantly reduced in HIT[11.1L] extracts when compared to HIT[11.1E] and HIT[0.8L] (Fig. 1, compare lane 2 with lanes 1 and 3). The GSTF binding complex has previously been shown by oligodeoxynucleotide competition analysis to correspond to the −217 to −210 element activator (4, 30). The loss in GSTF expression was concomitant with the decrease in insulin gene transcription in HIT[11.1L] cells (4). Addition of XIHbox 8 N-terminal antiserum to HIT-T15 cell extracts supershifted the GSTF complex (Fig. 1, compare lane 4 with lane 3). In contrast, GSTF complex formation was not affected by either the C-terminal XIHbox 8 or Mox-1 antiseurm (Fig. 1, compare lanes 6 and 5 with lane 3). These results suggest that the GSTF binding complex contains the STF-1 transcription factor and that the binding activity or expression of STF-1 is reduced to HIT-T15 cells chronically passaged in the presence of 11.1 mM glucose.

Western Blot Analysis for STF-1. The reduction in STF-1 binding activity in HIT[11.1L] cells suggested that STF-1 protein levels were limiting in these cells. As a result, we examined the levels of the STF-1 protein in HIT[11.1E], HIT[11.1L], and HIT[0.8L] cells by Western blot analysis using

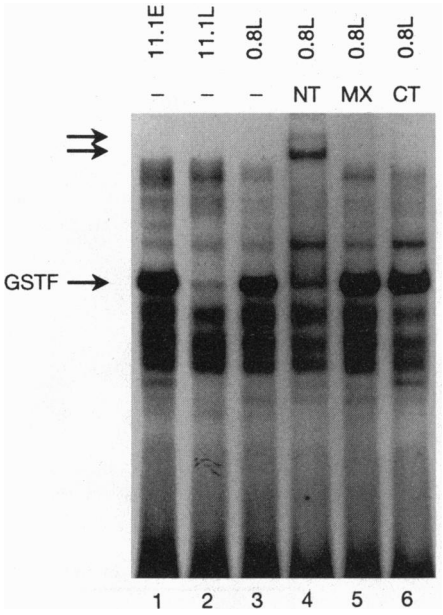


FIG. 1. Supershift of the GSTF complex with an affinity-purified anti-N-terminal XIHbox 8 antibody. Equal concentrations (5 μg) of HIT[11.1E], HIT[0.8L], and HIT[11.1L] protein extract were analyzed for GSTF binding (lanes 1–6) using a human insulin (−230/−201) probe. The effect of the affinity-purified Mox-1 and N- and C-terminal XIHbox 8 antibody (4 μl) on GSTF complex formation was analyzed. The GSTF complex is labeled and the supershifted complexes are indicated with arrows. Lane 1, HIT[11.1E] alone; lane 2, HIT[11.1L] alone; lane 3, HIT[0.8L] alone; lane 4, HIT[0.8L] plus N-terminal XIHbox 8 antibody; lane 5, HIT[0.8L] plus Mox-1 antibody; lane 6, HIT[0.8L] plus C-terminal XIHbox 8 antibody.

the antibody against the N terminus of XIHbox 8. When equal amounts of nuclear protein were analyzed, the levels of STF-1 protein were reduced in HIT[11.1L] cells to ≈1% of that observed in HIT[11.1E] or HIT[0.8L] cells (Fig. 2). These data indicate that the reduction in insulin gene transcription in HIT[11.1L] cells is associated with a decrease in STF-1 expression.

Activation Potential of STF-1. We also considered that chronically passaging HIT-T15 cells in 11.1 mM glucose might interfere with activation mediated by STF-1. To test this possibility, we analyzed the transactivation ability of chimeric protein containing the coding sequences of STF-1 fused to the DNA binding domain of the *Saccharomyces cerevisiae* transcription factor GAL4 in HIT[11.1E] and HIT[11.1L] cells. The transcription activity of the GAL4:STF-1 construct was compared to activation mediated by a GAL4 derivative of the well-characterized and ubiquitously active herpes virus 16 acidic activator, GAL4:VP16. The GAL4 expression plasmids were cotransfected with a CAT reporter plasmid bearing five copies of the GAL4 binding site linked to the E1b promoter [referred to as $(GAL4)_5$ E1b CAT]. GAL4:STF-1 and GAL4:VP16 stimulated $(GAL4)_5$ E1b CAT expression in the HIT-T15 cell lines (Fig. 3). These results indicate that STF-1 contains a transactivation domain that is functional within HIT[11.1E] and HIT[11.1L] cells. GAL4:STF-1 was only slightly less active in HIT[11.1L] cells when compared to HIT[11.1E] cells. In contrast, STF-1 protein levels were reduced in HIT[11.1L] cells to 1% of that observed in HIT[11.1E] cells (Fig. 2). Thus, we propose that the expression of the STF-1 protein, and not its transactivation potential, is severely altered to HIT[11.1L] cells.

Endogenous STF-1 Gene Transcription. To determine if the specific decrease in STF-1 protein levels was due to reduced STF-1 gene transcription, we measured the rate of STF-1 transcription with nuclei isolated from HIT[11.1E], HIT[11.1L], HIT[0.8L], and NIH 3T3 L1 cells (Fig. 4*A*). β-Actin transcription rates served as an internal control, as its mRNA expression does not vary between these cells (Fig. 5). The absence of STF-1 transcription in NIH 3T3 L1 nuclei is consistent with the distribution of this factor being limited to endocrine cell types (9–13). The rate of STF-1 transcription relative to the rate of β-actin transcription did not change significantly between HIT[11.1E], HIT[11.1L], and HIT[0.8L] cells (Fig. 4*B*). In contrast, insulin gene transcription was inhibited in HIT[11.1L] cells when compared to HIT[11.1E] cells (data not shown), which is consistent with the loss in insulin promoter activity and insulin mRNA expression (4, 5). These results indicate that the reduction in STF-1 protein levels in HIT[11.1L] cells is not do to a decrease in the rate of STF-1 gene transcription.

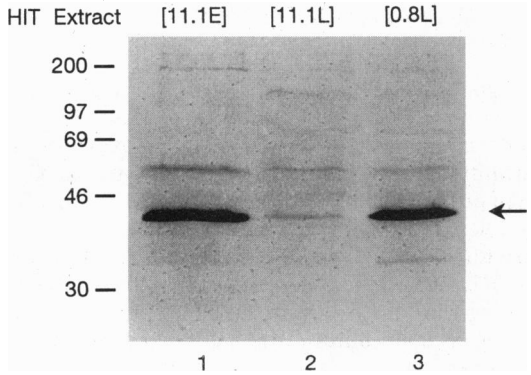


FIG. 2. Expression of STF-1 in HIT-T15 cells chronically cultured in 11.1 mM glucose. Each lane contains 30 μg of HIT[11.1E], HIT[0.8L], or HIT[11.1L] nuclear extract protein. The blot was probed with the affinity-purified N-terminal XIHbox 8 antibody. The position of the STF-1 protein is indicated by the arrow and the migration of various molecular mass (kDa) markers is shown. Densitometric scanning of the blot demonstrated that STF-1 immunoreactivity in HIT[11.1L] cells was <1% of that observed in HIT[11.1E] and HIT[0.8L] cells.

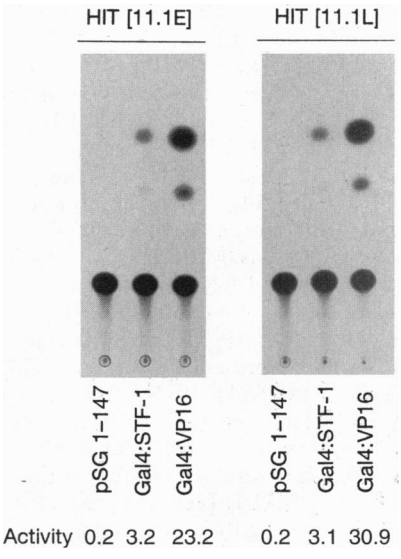


FIG. 3. Expression of GAL4:STF-1 in HIT[11.1E] and HIT[11.1L] cells. The GAL4 fusion constructs (GAL4:STF-1, GAL4:VP-16, and pSG 1-147) were cotransfected into HIT[11.1E] or HIT[11.1L] cells along with $(GAL4)_5$ Elb CAT. Cells were harvested 30 hr later, and the extracts were analyzed for CAT enzymatic activity. A representative thin-layer chromatogram showing the results is presented. The activity of each construct is expressed as the % conversion of the [^{14}C]chloramphenicol substrate to [^{14}C]chloramphenicol acetylated products.

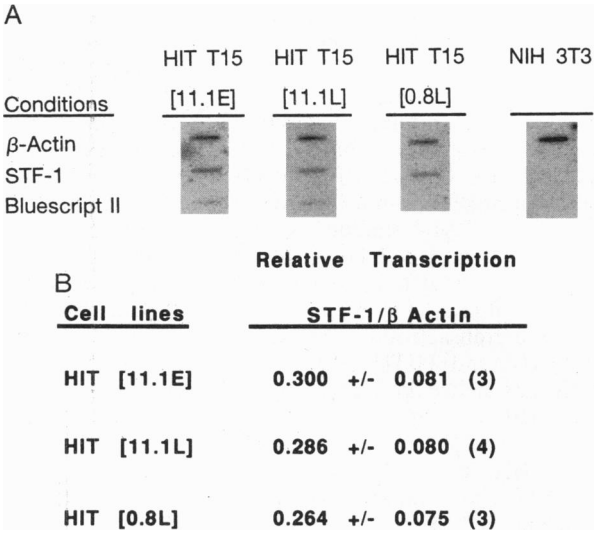
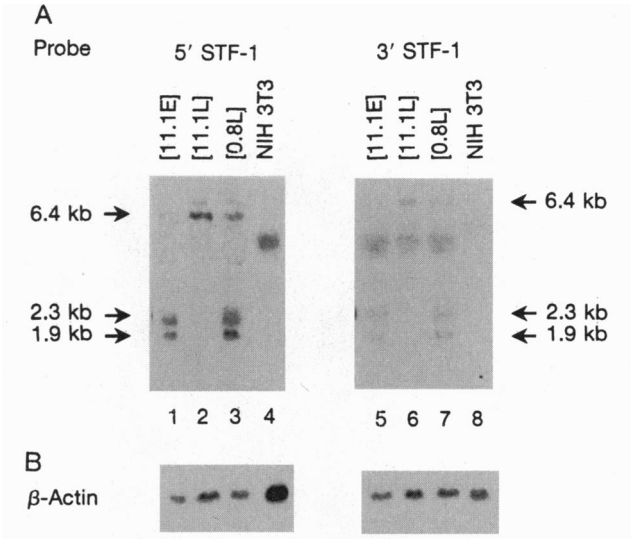


FIG. 4. STF-1 transcription in HIT[11.1L] cells. (*A*) Nuclear run-on transcription analyses were performed on nuclei isolated from HIT[11.1E], HIT[11.1L], HIT[0.8L], and NIH 3T3 L1 cells (see text). (*B*) The relative transcription rates (±SEM) of STF-1 to β-actin were measured by densitometric scanning of the autoradiogram. Values in parentheses correspond to the number of independent experiments performed.

Northern Analysis for STF-1 mRNA. Given the evidence that the rate of STF-1 gene transcription was not altered in HIT[11.1L] cells, we analyzed whether there was a change in STF-1 RNA expression between HIT[0.8L], HIT[11.1E], and HIT[11.1L] cells. The steady-state STF-1 RNA levels were analyzed by Northern analysis with distinct 5′ and 3′ cDNA probes. The amount of STF-1 RNA expression from each cell line was normalized to β-actin mRNA levels, as expression of β-actin did not vary between these HIT-T15 cell lines (Fig. 5*B*). Similar amounts of the 2.3- and 1.9-kb STF-1 mRNA were detected in HIT[0.8L] and HIT[11.1E] cells (Fig. 5*A*). In contrast, the levels of these STF-1 mRNAs were greatly reduced in HIT[11.1L] cells. The amount of the 2.3- and 1.9-kb STF-1 mRNA in HIT[11.1L] cells was ≈3–4% that found in HIT[0.8L] and HIT[11.1E] cells (Fig. 5*C*). The reduction in STF-1 mRNA levels correlates closely with the decreased expression of the STF-1 protein in HIT[11.1L] cells (Fig. 2). Interestingly, a 6.4-kb STF-1 RNA was detected by hybridization to the 5′ and 3′ STF-1 cDNA probes in each of the HIT-T15 cell lines (Fig. 5*A*). A comparably sized STF-1 RNA was also found in the mouse β-cell line βTC-6 and rat islets (data not shown) as well as by Miller *et al.* (11) in various islet cell lines. However, STF-1 RNA species are absent in nonendocrine cell lines that do not express STF-1 (9–13), as exemplified by our analysis of NIH 3T3 L1 RNA (Figs. 4*A* and 5*A*). The 6.4-kb RNA may represent a precursor STF-1 RNA. The steady-state level of total STF-1 RNA in HIT[11.1L] cells was less than in HIT[11.1E] or HIT[0.8L] cells (Fig. 5*C*) and occurred without a change in the rates of STF-1 gene transcription (Fig. 4). These results indicate that the loss of STF-1 gene expression in HIT[11.1L] cells may result from alterations in STF-1 RNA stability and/or processing.

DISCUSSION

When HIT-T15 cells are chronically cultured in supraphysiologic glucose concentrations, a nuclear protein–DNA complex, termed the GSTF, can no longer be detected by electrophoretic mobility shift assay (4). This is associated with a marked attenuation of insulin gene transcription, expression, cell content, and exocytosis (4, 5). The studies described herein



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STF-1 RNA Species	Relative Levels of STF-1 Message			
	Total	6.4 kb	2.3 kb	1.9 kb
HIT [11.1L]	1.0	0.92	0.046	0.037
		(1.0)	(0.05)	(0.04)
HIT [11.1E]	2.5	0.32	1.3	0.87
		(1.0)	(4.03)	(2.7)
HIT [0.8L]	2.85	0.57	1.18	1.1
		(1.0)	(2.05)	(1.91)

FIG. 5. Expression of STF-1 RNA in the HIT-T15 cell lines. Northern blot analyses were performed on total RNA (15 μg) isolated from HIT[11.1E], HIT[0.8L], HIT[11.1L], and NIH 3T3 L cells. Lanes 1 and 5, HIT[11.1E]; lanes 2 and 6, HIT[11.1L]; lanes 3 and 7, HIT[0.8L]; lanes 4 and 8, NIH 3T3 L1. The blots were hybridized with 5′ (lanes 1–4) and 3′ (lanes 5–8) STF-1-specific probes in *A*. Sizes of the STF-1 RNAs were determined from endogenous 28S and 18S rRNA transcripts. The 2.3- and 1.9-kb STF-1 mRNAs are labeled and the 6.4-kb STF-1 RNA is indicated with an arrow. The blot was exposed to autoradiography for 96 hr. (*B*) The probe from *A* was released by a high-temperature wash, and the filter was hybridized with a β-actin probe. The blot represents an 18-hr exposure. (*C*) Relative steady-state levels of the total STF-1 RNAs and the individual 6.4-, 2.3-, and 1.9-kb RNAs were determined by densitometric scanning of the autoradiogram and expressed after normalization to β-actin.

were designed to ascertain whether GSTF is related to STF-1, a recently isolated pancreatic transcription factor (10–12). Our results indicate that the GSTF–DNA complex is supershifted by an affinity-purified antibody directed against the XIHbox 8 protein (a protein immunologically related to STF-1); furthermore, the levels of STF-1 protein and mRNA are reduced in parallel with the reduction in GSTF binding. Thus, we propose that GSTF is identical to STF-1 and that its loss from HIT-T15 cells during chronic exposure to supraphysiologic glucose concentrations contributes to the marked decrease in insulin gene transcription.

Two principal sites of control of expression of the insulin gene are the (A+T)-rich elements found between nucleotides −217 and −210 and between nucleotides −84 and −77 (4, 7–9, 31). The STF-1 protein forms the predominant β-cell binding activity recognizing these sites (10–12). The observations that STF-1 is present in the nucleus of essentially all islet β cells (9, 13) and can stimulate expression from constructs containing these insulin (A+T)-rich elements (ref. 12; R.S., unpublished

observations) indicate that this factor directly regulates insulin transcription. The present studies provide additional support for this hypothesis.

The marked reduction in the steady-state levels of the 2.3-kb and 1.9-kb forms of STF-1 mRNA in HIT-T15 cells chronically cultured in a supraphysiological glucose concentration could not be accounted for by a decrease in the rate of STF-1 gene transcription. However, it is interesting to note that there is an apparent accumulation of a 6.4-kb RNA that hybridizes to two distinct and nonoverlapping STF-1 cDNA probes, suggesting that this 6.4-kb RNA species is a STF-1 precursor RNA. This hypothesis is supported by recent results demonstrating that the mRNA for the mouse STF-1 gene is specified by two exons of approximately 0.7 and 1.5 kb in length that are separated by a 3800-bp intron (C.V.E.W., unpublished data). Since the organization of the mouse STF-1 and *Xenopus* XIHbox 8 genes is conserved (C.V.E.W., unpublished data), it is reasonable to speculate that the STF-1 gene will be organized in similar fashion in the hamster. If this proposal is correct, then our results indicate that the 6.4-kb RNA species represents unspliced STF-1 precursor RNA. This, coupled with the observation that the rate of STF-1 gene transcription is unchanged in HIT cells chronically cultured in high glucose concentrations, suggests that a defect in the posttranscriptional processing of STF-1 RNA contributes to the observed decrease in the 2.3- and 1.9-kb STF-1 mRNA forms.

Although it has long been appreciated that glucose stabilizes insulin mRNA (32–36) and positively regulates insulin gene transcription (32, 37–41), it has more recently been shown that under certain conditions glucose can also decrease insulin gene transcription and expression (4, 5). The experiments herein indicate that the reduction in insulin gene expression induced by chronically passaging HIT cells in 11.1 mM glucose is linked to the STF-1 transcription factor. Acute induction of the insulin gene transcription appears to be mediated by a distinct set of insulin gene transcription factors, the RIPE3b1 (41) and ICE activators (40, 41), and possibly by STF-1 (39, 40, 42). In separate experiments we have observed that RIPE3b1, but not ICE, activator function is reduced in HIT-T15 cells chronically exposed to supraphysiologic glucose concentrations (30). Unfortunately, as the RIPE3b1 activator factor has not yet been isolated, we do not know the basis for the loss in RIPE3b1 activity. Nonetheless, these findings suggest that the reduction in insulin gene transcription mediated by chronic exposure to supraphysiologic glucose concentrations results from specific and selective deleterious effects on the expression of the RIPE3b1 and STF-1 activators.

Chronic hyperglycemia in humans with type 2 diabetes may play a secondary role in decreasing glucose-induced expression in β cells (3). The data presented here raise the possibility that defects in STF-1 expression could contribute to this adverse event. If so, the deleterious effects of chronic exposure to supraphysiologic glucose concentrations on STF-1 expression may not only be manifested upon the insulin gene but also upon other genes regulated by these factors in the β cell. Possible targets for STF-1 control include the glucokinase (43) and islet amyloid polypeptide genes (7), both of which appear to be regulated by the same factors that control expression from the (A+T)-rich base-pair -214 to -211 and -82 to -78 elements of the insulin gene.

In conclusion, we have shown that chronic exposure of HIT-T15 β cells to supraphysiologic glucose concentrations leads to decreased expression of the STF-1 transcription factor by altering the posttranslational processing of STF-1 RNA. The lack of STF-1 may contribute to the decrease in insulin gene transcription observed in these cells and may provide a mechanism for adverse glucotoxic effects on insulin gene expression in pancreatic β cells.

We are grateful to Dr. Ron Emenson for constructive criticism of the manuscript and to Marc Montminy for providing the rat STF-1 clone, pSK900. This work was supported by National Institutes of Health Grants DK R01-38325 (to R.P.R.) and DK-42502 (to R.S.) and by National Research Service Award Fellowship F32-DK-08742 (to L.K.O.). Partial support was also derived from the Vanderbilt University Diabetes Research and Training Center Molecular Biology Core Laboratory (Public Health Service Grant P60 DK20593 from the National Institutes of Health).

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