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# Molecular cloning of a cDNA encoding human SPH-binding factor, a conserved protein that binds to the enhancer-like region of the U6 small nuclear RNA gene promoter

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## ABSTRACT

**Many vertebrate small nuclear RNA gene promoters contain an SPH motif in their distal control regions that can confer transcriptional stimulation by RNA polymerase II or RNA polymerase III. Using the human U6 gene SPH motif as a probe, we isolated a cDNA encoding human SPH-binding factor (hSBF) from a HeLa cell expression library. The coding region of hSBF is almost identical to ZNF143, a 626 amino acid, seven zinc finger protein of previously unknown function. Furthermore, the predicted amino acid sequence of hSBF is highly homologous to *Xenopus laevis* and mouse Staf proteins, that bind to SPH motifs and stimulate transcription of selenocysteine tRNA gene promoters. Recombinant hSBF expressed *in vitro* or from *Escherichia coli* bound specifically to the human U6 gene SPH motif as shown by DNase I footprinting and electrophoretic mobility shift assays using various mutant SPH sites as competitors. Antibodies prepared against recombinant hSBF inhibited assembly of native SBF–DNA complexes. Immunodepleted HeLa S100 transcription extract no longer supported elevated levels of transcription by RNA polymerase III from a U6 promoter containing an SPH motif, whereas addition of recombinant hSBF protein to the immunodepleted extract reconstituted stimulated transcription.**

## INTRODUCTION

Vertebrate small nuclear RNA (snRNA) gene promoters are among the most active known in growing cultured cells. In order to synthesize up to several million new copies of a snRNA during every cell generation, a new transcript must be produced from each promoter every 2–4 s (1,2). The distal or enhancer-like regions of vertebrate snRNA gene promoters are responsible for a 4- to 100-fold increase in transcription *in vitro* or in cells (3–7). The distal region is located ~200 bp upstream of the start site and, typically, contains two adjacent elements. One is almost invariably an octamer motif, that is bound by the ubiquitous Oct-1 transcription factor (4,6,8–11). It has become apparent that the other site is often an SPH motif (6,12–15). Because of the high activity, relative simplicity and conservation of the snRNA enhancer-like region, it is an excellent model to investigate eukaryotic transcriptional activation.

Some aspects of the mechanism of stimulation of snRNA gene promoters by Oct-1 have been elucidated. Oct-1, or a fragment of this protein that includes the DNA-binding POU domain, recruits the binding of a multisubunit factor that contacts the proximal sequence element (PSE) within the vertebrate snRNA promoter (16–19). This PSE-binding factor is known as $SNAP_c$, PTF or PBP. Indeed, Oct-1 interacts with the largest subunit of the PSE-binding factor, SNAP190 (20). Furthermore, the presence of an octamer motif with a basal promoter will stimulate the formation of Sarkosyl-resistant preinitiation complexes on the human U6 promoter (7).

Much less is known about the mechanism of transcriptional stimulation via the SPH element of snRNA distal regions. The SPH element can function independently, but the highest level of activation requires both OCT and SPH elements (6,7,12,13,21,22). In the case of the human U6 promoter, the degree of stimulation effected by these two elements is similar (7). In addition, partial characterization of HeLa cell and chicken SPH-binding factor (SBF) has demonstrated that a polypeptide of ~85 kDa binds to the SPH motif (14). Focusing on the SPH motifs present in selenocysteine tRNA gene promoters, cDNA clones have been isolated from *Xenopus laevis* and mouse that encode the corresponding transcription factor, known as Staf (23,24). Significantly, *Xenopus* Staf also binds to SPH motifs in a number of snRNA gene promoters and can stimulate transcription of U1 and U6 genes, as well as the selenocysteine tRNA gene (15,25).

In this report, we describe the isolation of a cDNA clone that encodes human SBF. The deduced protein sequence of hSBF is almost identical to that of ZNF143, a putative zinc finger protein of unknown function (26), and hSBF is highly homologous to the Staf proteins. We demonstrate the specificity of recombinant hSBF

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for binding the SPH element and the ability of this protein to stimulate transcription from a human U6 snRNA promoter *in vitro*.

## MATERIALS AND METHODS

### Screening of λ gt11 cDNA expression library

A λ gt11 HeLa S3 cDNA library (Clontech no. HL3021b, lot no. 37208) was screened according to established procedures (27–29) using a concatenated, radiolabeled double-stranded oligonucleotide corresponding to the human U6 SPH motif [NONOCT(long); 14]. Approximately 5.5 × $10^5$ phages were screened at a density of 1.5 × $10^4$ per filter. Two positive clones (λSBF1 and λSBF2) were selected that withstood four rounds of probe binding. Inserts were excised by restriction with *Eco*RI and subcloned into the pGEM3Zf(+) plasmid vector (Promega). The insert from λSBF2 was sequenced entirely on both strands using the dideoxy method with multiple internal primers. This partial cDNA sequence of human SPH-binding factor has been deposited in GenBank under accession no. AF071771.

### Production of recombinant protein

*In vitro transcription/translation*. The *Eco*RI insert from pGEM/SBF2 was subcloned into the pET5a vector (Promega). Translation of the T7 transcript from this template is expected to yield a protein of 568 amino acids including the hSBF sequence from V76 to D626 (C-terminus). Synthesis of this protein *in vitro* was carried out using the manufacturer's protocol with a $T_N$T T7 Quick transcription/translation system (Promega).

*Expression in bacteria*. *Escherichia coli* BL21(DE3)pLysS competent cells were transformed with pET5a/SBF2 DNA and production of recombinant protein was induced by addition of IPTG to 1 mM for 3 h at 37°C. Pelleted cells were resuspended in 0.5 M NaCl, 20 mM Tris (pH 7.5), 10% glycerol, 1 mM EDTA, 0.1% NP40, 2 mM dithiothreitol, lysed by sonication and centrifuged at 8000 *g* for 15 min at 4°C. The insoluble pellet containing recombinant hSBF(76–626) was washed once with 1 M urea, 0.1 M Tris (pH 7.5), 20 mM dithiothreitol and then dissolved with 7 M urea, 0.1 M Tris (pH 7.5), 0.1 mM $ZnCl_2$, 20 mM dithiothreitol by continuous agitation for 30 min at room temperature. The solution was cleared by centrifugation at 8000 *g* for 15 min and the supernatant was dialyzed overnight at 4°C against 0.1 M KCl, 20 mM HEPES (pH 7.9), 20% glycerol, 0.1 mM $ZnCl_2$, 2 mM dithiothreitol.

### Production of polyclonal antisera against hSBF and western blots

Antisera were generated from two rabbits injected with recombinant hSBF(76–626) that was gel purified and emulsified along with the slice of polyacrylamide. Sera were assayed initially by ELISA and western blotting and then by inhibition of SBF–DNA complexes in an electrophoretic mobility shift assay (EMSA).

For the western blots, ~100 µg of total protein from a HeLa S100 extract were fractionated on a 7% polyacrylamide–SDS gel and electrophoretically transferred to a nitrocellulose filter. The filter was incubated with a 1:1000 dilution of anti-hSBF antiserum and visualized by a colorimetric assay using Western Blue stabilized substrate for alkaline phosphatase (Promega).

### DNA binding assays

*EMSA*. For the experiment shown in Figure 3, a radiolabeled probe containing both the OCT and SPH elements from the human U6 distal region (NPLUSO probe) was prepared by PCR as described (14). For the experiment shown in Figure 4, a double-stranded oligonucleotide probe containing the U6 SPH element [NONOCT(long); 14] was end-labeled using [γ-$^{32}$P]ATP and T4 polynucleotide kinase. DNA–protein complexes were separated on 4% polyacrylamide/Tris–acetate/EDTA gels as described previously (22). The amounts of radioactivity in the hSBF–DNA complexes were quantified using a Fujix BAS2000 Phosphorimager (Fuji).

*DNase I footprinting*. The radiolabeled probe for footprinting was the NPLUSO probe described above for the electrophoretic mobility shift assays. Binding, DNase I digestion and gel electrophoresis were carried out as described previously (14).

### *In vitro* transcription in HeLa S100 extract

S100 extracts were prepared from HeLa spinner cells as described (30). In order to remove endogenous SBF, the extract was immunodepleted using polyclonal anti-hSBF antiserum. Protein A–Sepharose resin was preincubated with immune serum, preimmune serum or no serum for 1 h at room temperature. After washing twice with phosphate-buffered saline and three times with buffer D (0.1 M KCl, 20 mM HEPES, pH 7.9, 20% glycerol, 0.1 mM EDTA, 2 mM dithiothreitol), the antibody–protein A–Sepharose resin was incubated with the S100 extract (20 µl resin/200 µl S100) for 2 h at 4°C with gentle agitation, followed by a brief centrifugation to separate the depleted extract.

Transcription reactions were carried out with 50 ng of plasmid DNA containing the U6/CFREE reporter plus 450 ng of pGEM3Zf(–) DNA. Final reactions (25 µl) contained 40 mM KCl, 8 mM HEPES (pH 7.5), 2 mM $MgCl_2$, 2 mM dithiothreitol and ~100 µg of protein from a HeLa cell S100 extract. After preincubation at 30°C for 90 min, transcription proceeded with the addition of nucleoside triphosphates to final concentrations of 0.5 mM ATP, 0.5 mM UTP, 20 µM unlabeled GTP and 10 µCi [α-$^{32}$P]GTP (800 Ci/mmol; DuPont/NEN), along with α-amanitin to 2 µg/ml. After further incubation at 30°C for 30 min, nucleic acids were isolated by phenol/$CHCl_3$ extraction and ethanol precipitation. RNAs were separated by electrophoresis on 12% polyacrylamide/8.3 M urea gels, visualized by autoradiography or quantitated using a Fujix BAS2000 PhosphorImager (Fuji).

## RESULTS

### Molecular cloning of cDNA encoding human SPH-binding factor

In order to isolate a cDNA clone that encodes human SBF, we screened a HeLa λ gt11 expression library with a radiolabeled, concatenated oligonucleotide containing the human U6 snRNA gene SPH motif. Two independent plaques were obtained after screening ~550 000 phage. Limited sequencing of the first candidate indicated that we had obtained a partial cDNA encoding human Sµbp-2, a protein that binds to the immunoglobulin µ switch region (31). However, it is unlikely that hSBF and Sµbp-2 are the same since the predicted molecular weight of Sµbp-2 (~110 000) is significantly larger than that of hSBF (~85 000), a value that was determined by protein–DNA crosslinking (14). Partial sequencing

of a second candidate (λSBF2) showed that it was highly homologous to human ZNF143 (26) and *Xenopus laevis* Staf (23). Complete sequencing of the λSBF2 insert (GenBank accession no. AF071771) demonstrated that it contained a partial cDNA, including nucleotides that encode amino acids 76–626 of the ZNF143 coding sequence, with a single nucleotide difference leading to an E→Q change at amino acid 549. The deduced hSBF sequence contains seven putative CCHH zinc fingers in the central region of the primary structure. Our partial cDNA clone encodes two of the four 15mer repeats noted previously in the sequences of Staf from *Xenopus* and mouse (23,24). In the 3′-untranslated region (3′-UTR), the HeLa-derived hSBF sequence is truncated relative to ZNF143 with divergent sequence prior to the polyadenylation site of hSBF.

In order to obtain the remainder of the coding sequence of HeLa cell SBF, we employed RT–PCR with a sense strand primer corresponding to the start codon region of ZNF143, an antisense primer including hSBF sequence just 3′ to the C-terminal 15mer repeat and total RNA isolated from HeLa cells. The sequence obtained was identical to that of the N-terminal region of ZNF143 (results not shown). Human SBF is highly homologous to *Xenopus* Staf (463 out of 626 residues are identical = 74%; 23) and the recently cloned mouse Staf (608 out of 626 residues are identical = 97%; 24).

## DNA-binding specificity of recombinant hSBF

In order to investigate the DNA-binding properties of hSBF encoded by the cDNA clone, we constructed a bacterial expression plasmid containing the hSBF sequence. Recombinant hSBF was expressed in bacteria and partially purified by urea solubilization out of inclusion bodies (Fig. 1A). This protein preparation bound the SPH motif of the human U6 distal region in a DNase I footprint assay (Fig. 1B). Furthermore, we noticed two details of the DNase I protection pattern of recombinant SBF that matched what was found with a HeLa cell fraction of SBF (see fig. 6 in 14), namely an extended region of protection 5′ to the SPH motif and a hypersensitive band at the junction of the OCT and SPH elements.

In addition, we expressed hSBF(76–626) after transcription/translation *in vitro* and used the protein in an EMSA (Fig. 2). A strong hSBF–DNA complex was detected on a human U6 distal region probe containing both octamer and SPH motifs. This complex was formed only upon addition of a transcription/translation lysate containing the pET/hSBF(76–626) plasmid and not with addition of a pET5a control plasmid (compare lanes 5–7 with lanes 2–4 in Fig. 2A). Furthermore, the hSBF–DNA complex was competed effectively by addition of unlabeled hU6 SPH oligonucleotide (Fig. 2A, lanes 12 and 13), but not by addition of OCT oligonucleotide (Fig. 2A, lanes 10 and 11). A minor complex was formed on the probe with both pET5a control and pET/hSBF(76–626) samples that was competed upon addition of unlabeled OCT oligonucleotide. It is possible that this complex resulted from a small amount of Oct protein in the reticulocyte lysate. To further analyze the specificity of *in vitro* translated hSBF(76–626) protein in EMSA experiments, we added various plasmid DNA competitors that contained mutations in the SPH motif (Fig. 2B). These mutations have been shown to compete less effectively than the wild-type sequence for hSBF–DNA complex formation in gel mobility shift experiments using HeLa S100 extract (14; bottom row of Fig. 2C). All the mutations were

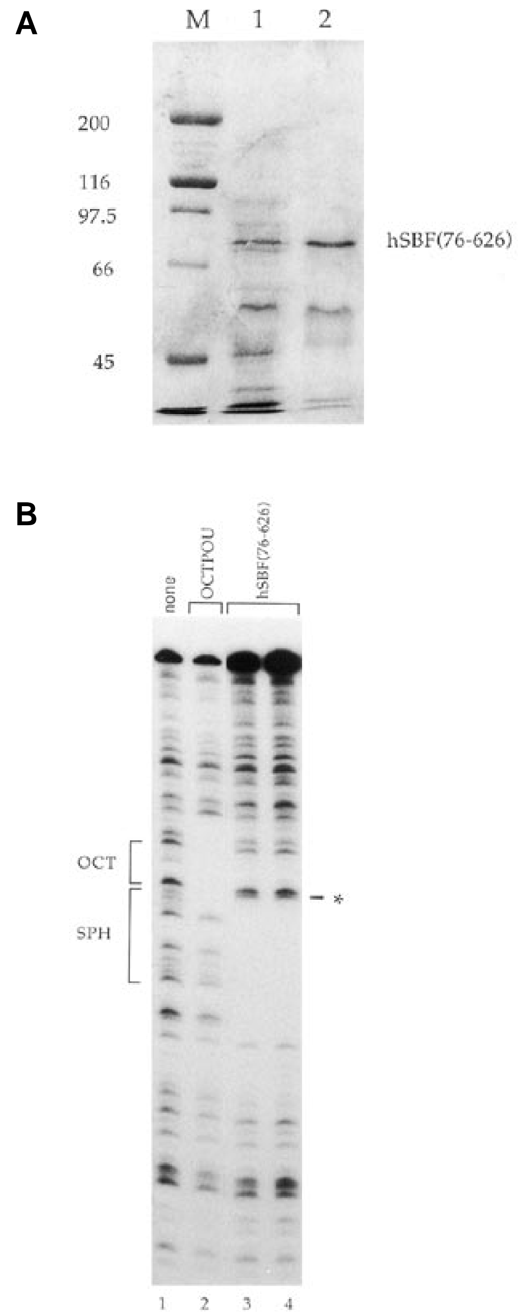


**Figure 1.** Recombinant hSBF(76–626) binds to the human U6 SPH motif. (**A**) SDS–PAGE showing expression of hSBF(76–626) in *E.coli*. Lane 1 shows total protein from cells lysed after 3 h IPTG induction at 37°C and lane 2 shows partially purified protein recovered from inclusion bodies after urea solubilization and removal of urea by dialysis. (**B**) Binding of human U6 SPH motif by recombinant hSBF(76–626) detected by DNase I footprinting. Lane 1 shows the pattern of digestion with naked DNA probe, lane 2 shows protection by a recombinant protein containing the Oct-1 POU domain and lanes 3 and 4 show the cleavage patterns after incubation with recombinant hSBF(76–626).

relatively less effective competitors for binding of the recombinant hSBF protein than the wild-type SPH element (Fig. 2C). Significantly, the RM2-8 mutant was the most effective competitor among the mutants in experiments using both the *in vitro* translated hSBF (30% complex remaining with 1 μg competitor) and the

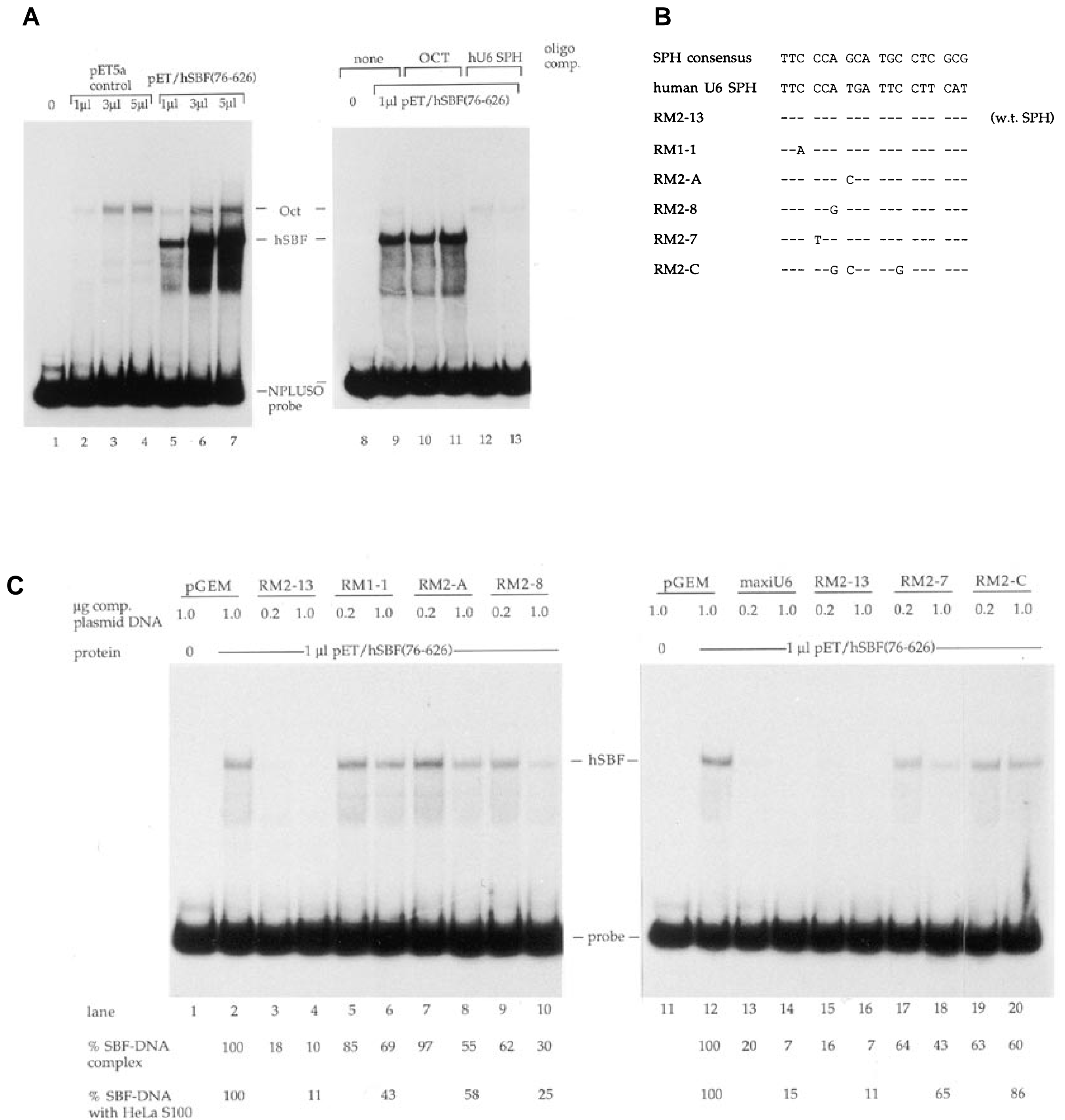


**Figure 2.** Binding of recombinant hSBF(76–626) expressed by *in vitro* transcription/translation is sensitive to mutations within the U6 SPH motif. (**A**) Electrophoretic mobility shift assay with *in vitro* translated protein. Protein was expressed from the pET/hSBF(76–626) plasmid DNA or a pET5a control plasmid lacking any insert, incubated with ~3 fmol of radiolabeled probe containing the human U6 SPH+OCT elements and separated by electrophoresis on 4% polyacrylamide non-denaturing gels. Bands labeled 'Oct' or 'hSBF' represent complexes that were competed by unlabeled OCT oligonucleotide (300- and 3000-fold molar excess in lanes 10 and 11, respectively) or unlabeled SPH oligonucleotide (300- and 3000-fold molar excess in lanes 12 and 13, respectively). (**B**) Sequences of sense strand of the human U6 SPH motif embedded in plasmid DNAs used for competition experiments. Each plasmid contained a human U6 maxigene with a set of mutations in the octamer motif (OCTMUT) and the specified mutations in the adjacent SPH motif (described in 14). (**C**) EMSA using *in vitro* expressed hSBF(76–626) plus ~3 fmol of radiolabeled U6 distal region probe containing OCT+SPH motifs, and various amounts of plasmid DNAs containing no U6 promoter (pGEM), wild-type U6 promoter (maxiU6) or promoters containing mutations in the SPH motif. Quantification using a PhosphorImager is tabulated in the row labeled '% SBF–DNA complex'. A quantitative comparison using the same mutant SPH competitors (1 µg of each) in EMSA experiments with HeLa S100 extract is supplied in the bottom row (14).

endogenous hSBF present in S100 extract (25% complex remaining with 1 µg of competitor). Quantitative comparison of the other mutants is complicated because all are relatively ineffective, leaving >40% of hSBF–DNA complex even after addition of 1 µg of plasmid DNA. In combination, DNase I footprinting and EMSA results show that the recombinant protein produced from the SBF2 cDNA clone binds to the human U6 SPH motif with the same specifity as the native SBF present in extracts from HeLa cells.

## Polyclonal antibodies to hSBF inhibited assembly of SBF–DNA complexes

To further verify the authenticity of the hSBF cDNA clone and to provide a biochemical reagent for studies of SBF function, we used recombinant, gel-purified hSBF(76–626) as an antigen to prepare rabbit polyclonal antibodies. Binding of a western blot containing total protein from a HeLa S100 extract with the anti-SBF antiserum identified a major band of ~79 kDa (Fig. 3A). At present, we do not know whether the closely spaced doublet indicates mild degradation of hSBF or altered native forms of the protein. The estimated molecular mass of 79 kDa on the western blot is fairly close to the size of 85 kDa that we determined previously by UV light-mediated protein–DNA crosslinking (14).

In addition, anti-SBF antisera from both rabbits that we inoculated effectively inhibited detection of hSBF–DNA complexes on a human U6 SPH oligonucleotide probe incubated with HeLa S100 extract (Fig. 3B, compare lanes 2 and 3 with lane 1 or lanes 5 and 6 with lane 4). We detected no discrete supershifted band in this assay, although a significant amount of probe was retarded at the origin of the gel when the antisera were added subsequent to complex formation (Fig. 3B, lanes 5 and 6).

## Recombinant hSBF-stimulated transcription from a U6 promoter *in vitro*

In previous work we have demonstrated that a human U6 promoter containing a single copy of an SPH motif was transcribed *in vitro* at an ~3.5-fold higher level than a promoter lacking any distal element (7). In order to examine further the capability of hSBF to stimulate polymerase III transcription of snRNA gene promoters, we used anti-SBF antiserum to immunodeplete the HeLa S100 transcription extract. The immune antiserum almost completely eliminated the ability of the extract to activate transcription of the +SPH template (NON10) when compared with the dl-84 template (Fig. 4A, compare lanes 6 and 7 with 2 and 3). Using a gel shift assay, the immunodepleted extract contained a barely detectable amount of hSBF (results not shown). Controls in which the HeLa S100 extract was treated without serum or with preimmune serum did not diminish the SPH-dependent transcriptional stimulation (Fig. 4A, lanes 4 and 5 and 8 and 9, respectively).

Next, we added back recombinant hSBF(76–626) to the immunodepleted extract and compared the transcription of the dl-84 and +SPH templates. Addition of the recombinant protein caused a marked increase in transcription, up to a 6-fold higher level with ~20 ng of protein (Fig. 4B, lanes 4–6, and C). We consistently found that the recombinant hSBF(76–626) also stimulated transcription of the dl-84 template that lacks an SPH motif (Fig. 4B, lanes 1–3, and C). However, the fold stimulation of the +SPH/U6 promoter was at least double that of the –SPH template (Fig. 4C).

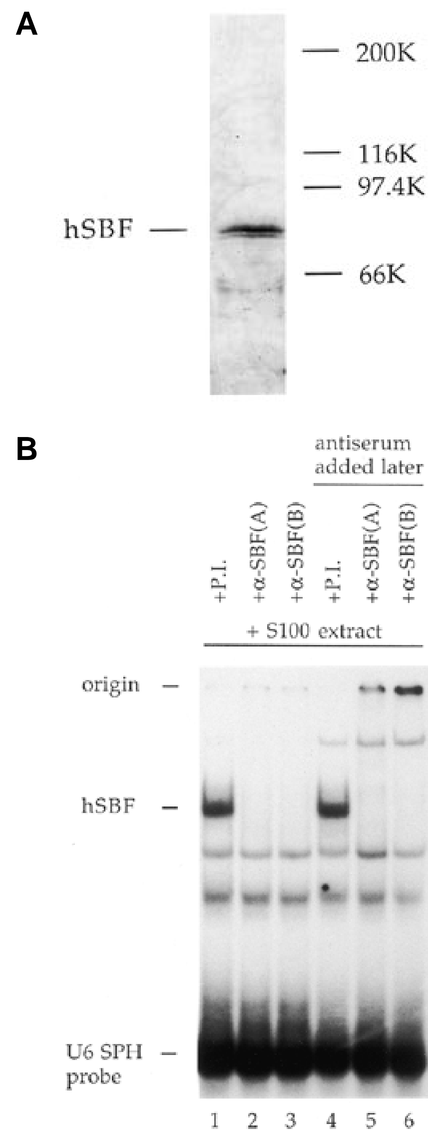


**Figure 3.** Polyclonal antibodies prepared against recombinant hSBF(76–626) bind specifically to native hSBF. (**A**) Western blot. Approximately 100 µg of total protein from a HeLa S100 extract were fractionated on a 7% polyacrylamide–SDS gel, electrophoretically transferred to nitrocellulose and probed with a 1:1000 dilution of serum. Antibody binding was visualized with a colorimetric assay. The positions of markers run on an adjacent lane are noted on the right side. (**B**) EMSA. Each lane contained ~2 fmol of radiolabeled SPH oligonucleotide probe and 4 µg of protein from a HeLa cell S100 extract. For samples in lanes 1–3, 0.5 µl of the designated antiserum was added at the same time as the S100 extract and incubated at 30°C for 30 min. For samples loaded in lanes 4–6, the SPH probe and S100 extract protein were preincubated at 30°C for 30 min, followed by a further 15 min incubation after the addition of 0.5 µl of the designated antiserum. Two antisera from rabbits separately injected with hSBF antigen (A and B) or preimmune serum (P.I.) were used in this experiment. The band marked 'hSBF' was the only one competed by addition of excess unlabeled SPH oligonucleotide in samples that lacked rabbit antisera (results not shown).

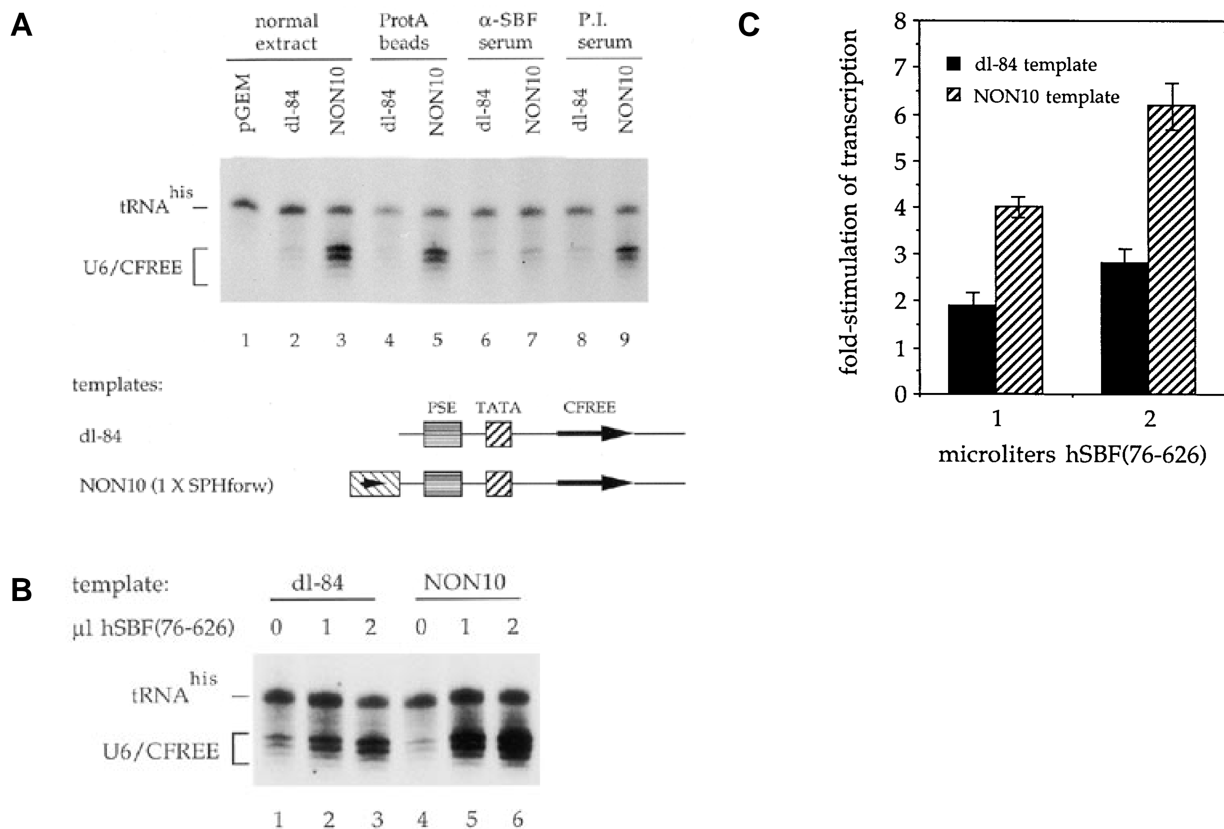


**Figure 4.** *In vitro* transcription reactions using human U6 promoter templates in hSBF-depleted HeLa S100 extracts. (**A**) HeLa S100 extract was depleted of hSBF using anti-SBF antiserum or mock-depleted without addition of any serum (ProtA beads) or with addition of preimmune serum (P.I. serum). Untreated S100 extract or each treated extract was used to compare the transcriptional efficacy with 50 ng of a proximal-only U6 promoter template (dl-84) or 50 ng of a template containing a single copy of the SPH element ligated to the U6 proximal promoter (NON10). Bands labeled 'U6/CFREE' are transcripts derived from the U6 promoter. The band marked 'tRNA$^{his}$' resulted from guanylyltransferase radiolabeling of endogenous RNA in the extract and served as a convenient recovery control for each sample. (**B**) Stimulation of U6 promoter transcription by addition of recombinant hSBF(76–626) to SBF-immunodepleted S100 extracts. All reactions contained immunodepleted extract such as characterized in lanes 6 and 7 from (A). Reactions analyzed in lanes 1–3 contained 50 ng of dl-84/CFREE plasmid DNA, whereas RNAs in lanes 4–6 resulted from reactions containing 50 ng of NON10 (+SPH) plasmid DNA. (**C**) Quantitation of transcription reactions. Transcripts that had been separated by electrophoresis on polyacrylamide gels were quantified with a Fujix BAS2000 PhosphorImager (Fuji). After background subtraction, the amount of each U6/CFREE group of bands was normalized according to the tRNA$^{his}$ band intensity in that lane and compared with the signal from each promoter when hSBF(76–626) was omitted from the transcription reaction. Each bar represents the average of two independent experiments and the error bar shows the range from each experiment.

## DISCUSSION

We have isolated a cDNA that encodes the transcription factor hSBF, that binds to the SPH element of a human U6 snRNA gene distal region. Antibodies prepared against the recombinant protein expressed from the open reading frame recognized specific hSBF–DNA complexes formed with native protein in HeLa cell extracts and eliminated the capacity of such extracts to stimulate transcription by RNA polymerase III from U6 promoters that contained an SPH element. Addition of recombinant hSBF reconstituted transcriptional activation *in vitro*.

The hSBF coding sequence is almost identical to that of ZNF143 (1 nt change), first isolated in a general screen for human cDNAs containing zinc finger sequences (26). The gene for ZNF143 is located on chromosome 11 at position 11p15.3–15.4. Furthermore, the hSBF amino acid sequence is highly homologous to that of the recently cloned mouse Staf (97%; 24) and *Xenopus laevis* Staf (74%; 23), proteins named according to their ability to bind to SPH elements in, and activate, the promoters of selenocysteine tRNA genes. These proteins are homologous to another human zinc finger protein known as ZNF76, originally proposed as the human homolog of Staf (23,32). On a northern blot, ZNF76 is not expressed in HeLa cells (32), so it is unlikely that our previous work analyzing the DNA-binding and transcriptional properties of hSBF in HeLa cells can be ascribed to ZNF76. Nevertheless, the potential for a family of human proteins related to hSBF/ZNF143 raises interesting questions regarding differential regulation of promoters containing SPH elements.

Based on comparison to the ZNF143 sequence, our HeLa cell-derived cDNA clone contains only a partial sequence starting at a valine codon at position 76. We carried out RT–PCR with total HeLa cell RNA to obtain additional HeLa cell cDNA sequence for hSBF and the sequence matched that of ZNF143 exactly. However, we have been unable to obtain the sequence of the 5′-untranslated region (5′-UTR) of the HeLa cell cDNA after several attempts with a 5′ RACE procedure. We have noticed that the 10 in-frame codons immediately upstream of the first ATG in the ZNF143 and mouse Staf sequences match those found in the open reading frame of *Xenopus* Staf except for a single glycine to alanine substitution (23). Such conservation of deduced amino

acid sequence within the 5′-UTRs raises the possibility that the hSBF/ZNF143 and mouse Staf genes might have multiple transcriptional start sites or the primary transcript could be alternatively spliced, in order to produce multiple proteins differing at their N-termini.

On a related issue, the 3′-UTR of the HeLa-derived hSBF cDNA clearly deviates from that of ZNF143, which was isolated from a human insulinoma cDNA library (26). The hSBF 3′-UTR is much shorter with a distinct polyadenylation site marked by a stretch of A residues at the 3′-end and an appropriately placed AAUAAA signal sequence. After diverging from the hSBF sequence, the 3′-UTR for ZNF143 extends over 1600 nt farther with no clear polyadenylation site at its 3′-end. Analysis of the genomic sequence of hSBF/ZNF143 will provide information regarding alternative mRNA formation at either end of the message.

Using an extract depleted of endogenous hSBF, we have demonstrated transcriptional stimulation of the human U6 promoter by recombinant hSBF(76–626) *in vitro* (Fig. 4). Our hSBF protein preparation also increased transcriptional levels from a promoter lacking the SPH motif, although to a significantly lesser amount. Perhaps, at the concentrations used in these experiments, hSBF has the capacity to bind and stabilize the U6 transcription complex by protein–protein interactions. However, anti-hSBF antibody depletion does not significantly reduce transcription of the U6 basal promoter. These seemingly contradictory results could be resolved if the hSBF target is not limiting in the *in vitro* assay or if effective depletion of the target would require formation of a multiprotein complex on the promoter.

Previous reports have employed oocyte injection and *Drosophila* cell transfection assays with Staf proteins to show activation of selenocysteine tRNA and U6 snRNA gene promoters by RNA polymerase III and U1 snRNA and thymidine kinase promoters by RNA polymerase II (15,24,25). Furthermore, while this manuscript was under review, work was published demonstrating transcriptional stimulation by ZNF143 and ZNF76 of selenocysteine tRNA and U1 promoters in *Xenopus* oocytes (33). We are using the *in vitro* transcription assay with the human U6 promoter in order to investigate the function of this important activator protein.

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