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Human transcription factor IIIC box B binding subunit

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ABSTRACT **Transcription factor IIIC (TFIIIC) is a multisubunit basic TF for RNA polymerase III. It initiates transcription complex assembly on tRNA and related genes by binding to the internal box B promoter element and is also required for transcription of 5S rRNA and other stable nuclear and cytoplasmic RNAs transcribed by polymerase III. In mammalian cells, regulation of TFIIIC activity controls overall polymerase III transcription in response to growth factors and viral infection. Here, we report the cloning and sequencing of a full-length cDNA (and genomic DNA from the transcription initiation region) encoding the box B binding subunit of human TFIIIC, the 243-kDa α subunit. Specific antisera raised against the encoded protein super shifts a TFIIIC–box B DNA complex during an electrophoretic mobility shift assay and immunodepletes TFIIIC transcriptional activity from a partially purified TFIIIC fraction, proving that the cDNA encodes a component of TFIIIC. The human protein shows surprisingly little similarity to the box B binding subunit of yeast TFIIIC.**

tRNA transcription in eukaryotes (1) requires an internal promoter region lying entirely downstream of the transcription start site. The internal control region consists of two conserved ≈10-bp elements, boxes A and B. The position of box A sets the start site for transcription and the sequence of box B regulates the strength of the promoter such that the closer to consensus the stronger the promoter (2). A multisubunit transcription factor (TF) termed TFIIIC binds to the intragenic control region, stably sequestering the template (1, 3). In yeast the TFIIIC–DNA complex then nucleates the assembly of the complete transcription complex composed of the "TATA" box binding protein, the TFIIB-related *BRF1* gene product (4–6), and the TFIIIB 90-kDa polypeptide and RNA polymerase III (pol III) (7). These basic steps in tRNA transcription are probably conserved in higher cells.

Yeast TFIIIC purifies as a single multisubunit protein composed of six polypeptides of 145 (132), 135 (120), 95 (74), 90, 62, and 55 kDa (ref. 1; numbers in parentheses refer to molecular masses calculated from the sequences of the cloned genes). Genes encoding the three largest TFIIIC subunits from yeast have recently been cloned (8–10).

In contrast to the yeast system, activities in a partially purified human TFIIIC fraction required for tRNA transcription separate into two components called TFIIIC1 and TFIIIC2 (11). RNA pol III TFs from *Bombxy mori* also resolve into several components including two activities separated from a partially purified TFIIIC fraction called TFIIIC and TFIIID (12). The human TFIIIC2 fraction most closely resembles yeast TFIIIC in that it also binds to the box B promoter element with high affinity (11, 13, 14), can stably sequester a template (15), and is a multisubunit protein composed of five copurifying polypeptides of 230, 110, 100, 80, and 60 kDa (14, 16, 17). In both the yeast and human systems, the largest polypeptide can be specifically cross-linked to box B DNA (13, 16–18). Because the protein we have referred to as TFIIIC2 (16) has many of the functional properties of yeast TFIIIC, we will generally refer to the protein previously called TFIIIC2 (11, 16) as simply TFIIIC. Nonetheless, we continue to observe a requirement for TFIIIC1 activity for transcription of tRNA genes and the adenovirus *VAI* gene, which has a homologous promoter.

TFIIIC is the target of both positive and negative regulation in mammalian cells. Pol III transcription of transiently transfected tRNA genes is increased in cells infected with adenovirus (19, 20) and in cells expressing the pseudorabies virus immediate early protein (20). In contrast, poliovirus infection substantially decreases pol III transcription of cellular genes (21). The activities of extracts prepared from virus-infected cells reflect these types of regulation when assayed *in vitro*. Also, nuclear extracts prepared from rapidly proliferating HeLa cells have much higher activity for *VAI* transcription compared to extracts of HeLa cells cultured in the absence of growth factors (22). Pol III TFs that are modified by viral infection and growth factors chromatograph in the 0.35–0.6 M KCl fraction C on phosphocellulose (23–25).

It is important to further investigate the mechanism of human pol III transcription to determine which aspects are controlled in virus-infected mammalian cells and which are regulated in coordination with cell growth and replication. To this end, we have cloned the 230-kDa box B DNA binding subunit of TFIIIC.§ This cDNA clone and immunological reagents prepared with it should be useful in elucidating how human TFIIIC functions in pol III transcription and the molecular mechanisms underlying its regulation.

MATERIALS AND METHODS

Purification of TFIIICα. Nuclear extract (26) was prepared from 300 liters of HeLa cells. A phosphocellulose column was loaded at 0.1 M KCl/buffer A (26) and washed with 0.4 M KCl/buffer A; TFIIIC activity was eluted with 0.7 M KCl/buffer A (fraction C′) and precipitated with 57% ammonium sulfate. The precipitate was dissolved in buffer Z (20 mM Hepes, pH 7.9/10% glycerol/0.1 μM $ZnCl_2$) and applied to an S400 gel filtration column run in 0.8 M KCl/buffer Z. Fractions with peak electrophoretic mobility shift assay (EMSA) activity were pooled (≈180 mg) and dialyzed against 0.09 M KCl/buffer Z. The following components were added: dithiothreitol (DTT) to 5 mM, $MgCl_2$ to 5 mM, Nonidet P-40 to 0.1%, and poly[d(I-C)]·poly[d(I-C)] to 10 μg/ml before application to a 4-ml box B oligonucleotide affinity column (16). The column was washed with 0.2 M KCl/buffer Z and

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Abbreviations: pol III, RNA polymerase III; EMSA, electrophoretic mobility shift assay; DTT, dithiothreitol; BSA, bovine serum albumin; TF, transcription factor; HMG, high mobility group.

§The sequence reported in this paper has been deposited in the GenBank data base (accession no. U02619).

the TFIIIC activity was eluted with 0.7 M KCl/bovine serum albumin (BSA, 0.1 mg/ml)/buffer Z (fraction BB1). This fraction was diluted to 0.09 M KCl with buffer Z containing 0.1% Nonidet P-40, 1 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride, BSA (0.1 mg/ml), and dI·dC (5 μg/ml) before incubation at 4°C for 18–24 h with 4 ml of fresh affinity matrix. The elution was performed as in the first pass except that the elution buffer contained insulin (0.1 mg/ml) as carrier instead of BSA (fraction BB1A).

Protein Sequencing. Fraction BB1A (16 μg) was separated on a SDS/7% polyacrylamide gel and electroblotted onto 0.2-μm (pore size) nitrocellulose. The region of the blot containing the 230-kDa polypeptide was directly treated with trypsin. Eluted peptides were separated by reverse-phase HPLC and subjected to protein sequencing (27).

Cloning the cDNA for TFIIICα. The sequence of the longest peptide obtained was VVDEGLIPGDGLGAAGLDS. One degenerate primer (CGGGGATCCA/GTGNAA/GNCCNGCNGCNCC) was made to anneal to the sense strand of the cDNA (corresponding to the C-terminal amino acid sequence GAAGLDS) and the other (CGGGGATCCGTNGTNGAT/CGAA/GGGNT/CTNAT) was made to anneal to the antisense strand (corresponding to the N-terminal amino acid sequence VVDEGLI). HeLa cell poly(A)$^+$ RNA (87 μg) was used as template for reverse transcription in a solution of 43.5 μg of random hexamer primers, 50 mM Tris·HCl (pH 7.5), 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, all four dNTPs (each at 500 μM), BSA (0.1 mg/ml), and 30,000 units of Moloney murine leukemia virus reverse transcriptase (BRL) in a final volume of 4.3 ml at 37°C for 1 h. After digestion with 8 μg of RNase A (Boehringer Mannheim) at 37°C for 30 min, phenol/chloroform extraction, and ethanol precipitation, random hexamer primers were removed (30). Recovered cDNA (20 μg) was used as a template in a PCR with the degenerate oligonucleotide primers described above. The sequence between the primers was determined to be CCCAGCCCATCTCCAGGGAT. This sequence was used to probe a size-selected human λgt10 library (31). Two overlapping cDNA clones were isolated by standard cloning techniques (29).

The genomic clone was obtained by using an 800-bp probe from the 5′ end of the cDNA to screen a human genomic cosmid library (32).

Production of Specific Anti-TFIIICα Antibody. The region corresponding to aa 1064–1365 (see Fig. 6) was subcloned into the 6His Qiagen (Chatsworth, CA) expression vector, pQE12, and the resultant ≈30-kDa protein was expressed, purified, and used to immunize rabbits.

EMSA Super-Shift Conditions. EMSA binding reactions were as described (13), except that the KCl concentration was increased to 0.095 M. S400 fraction was incubated with the 250-bp *Eco*RI–*Hin*dIII fragment of pVAIA (33). After a 30-min incubation, 1 or 2 μl of a 1:10 dilution of preimmune or immune serum was added to each reaction mixture, and the products were fractionated on a 1.4% agarose gel (34).

Immunodepletion of TFIIIC Transcriptional Activity. Equal volumes of serum (preimmune or immune) and protein A-Sepharose beads (Pharmacia) were mixed, incubated at room temperature for 30 min, and then washed with buffer A. The spin-dried beads with bound immunoglobulin were then incubated with an equal volume of the phosphocellulose C′ fraction (≈1 mg of protein per ml) with rocking overnight at 4°C. The supernatant was depleted once more for 2 h at 4°C and in *in vitro* transcription reactions as detailed (11).

DNA Sequencing and Analysis. Sequencing was performed with an Applied Biosystems model 373A DNA sequencer. Both strands of the protein coding region were completely sequenced.

Primer Extension and S1 Nuclease Mapping. Poly(A)$^+$ HeLa RNA (7 μg) was incubated at 55°C with either 5′-^{32}P-labeled oligonucleotide primer P1 (nt +217 to +259) or P2 (nt +14 to +56) (see Fig. 5) in 10 μl of 0.2 M NaCl for 3 h. This was diluted to 40 μl with 50 mM Tris, pH 8.3/40 mM KCl/6 mM $MgCl_2$/10 mM DTT/BSA (0.1 mg/ml)/20 units of RNAsin (Promega)/400 units of SuperScript II reverse transcriptase (BRL) and incubated for 60 min at 45°C.

S1 probe was prepared by hybridizing a 5′-^{32}P-labeled oligonucleotide primer complementary to the cDNA sequence at positions +149 to +173 to a denatured plasmid containing a genomic *Sma* I fragment extending from −136 to approximately +1 kb subcloned into the *Sma* I site of pBluescript. The Klenow fragment of DNA polymerase I and dNTPs were incubated with the annealed DNA and the

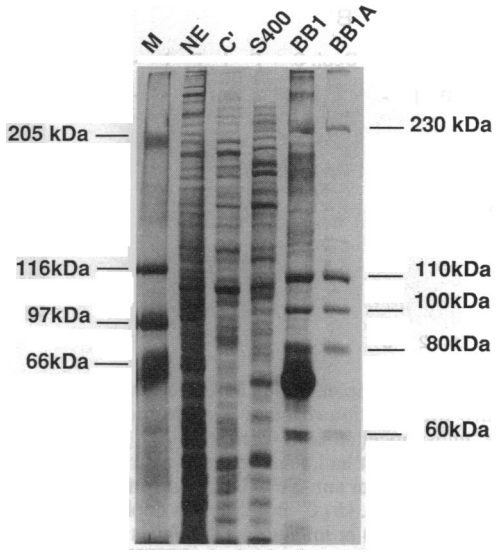

FIG. 1. Purification of TFIIIC. A silver-stained 5.5% polyacrylamide gel is shown. Lanes: M, molecular mass standards; NE, 1 μg of HeLa cell nuclear extract; C′, 1 μg of pooled 0.4–0.7 M KCl fraction from the phosphocellulose column; S400, 1 μg of the pooled S400 fractions with peak EMSA activity; BB1, the 0.2–0.7 M KCl eluate from the first box B oligonucleotide column, 1 μg including carrier BSA; BB1A, the second-pass box B eluate.

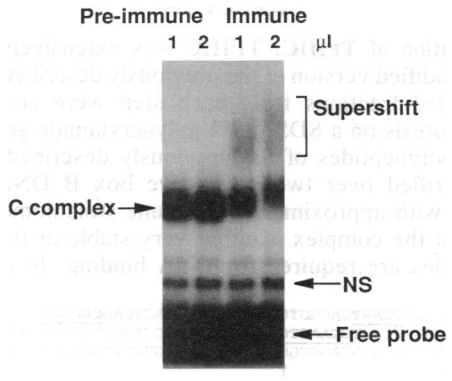

FIG. 2. Super-shifting C complex with antiserum to a polypeptide fragment encoded by the cloned cDNA. An autoradiogram of an EMSA is shown. Reaction products were analyzed in which 2 μg of the S400 fraction was incubated with 20 fmol of the ^{32}P-labeled *VAI* gene fragment. Either preimmune or immune antiserum was added subsequent to binding, as indicated. The positions of the TFIIIC–*VAI* DNA complex (C complex) (13), the super-shifted TFIIIC–*VAI* DNA–IgG complexes (Supershift), a nonspecific protein–DNA band (NS), and the free probe are marked. The *VAI* gene utilized in both the EMSA and the *in vitro* transcription reactions was an *Xba* I–*Bal* I fragment from the adenovirus 2 genome (nt 10,579–10,812) containing a point mutation in box A that increases transcription (33); the fragment was subcloned into the *Xba* I–*Sma* I sites of plasmid pUC87.

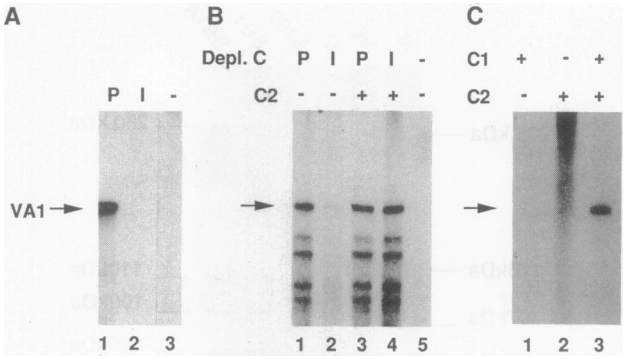

FIG. 3. Immunodepletion of TFIIIC activity by specific antisera. (*A*) *In vitro* transcription reaction mixtures contained a *VAIA* gene template, phosphocellulose B fraction (5 μg), and phosphocellulose C′ fraction (4 μg) that had been depleted with either the preimmune antiserum (P, lane 1) or anti-TFIIICα antiserum (I, lane 2). B fraction alone is in lane 3 (−). (*B*) C′ fraction was depleted with preimmune IgG (P, lanes 1 and 3) or immune IgG (I, lanes 2 and 4). Lanes 3 and 4 were further supplemented with 2 μl of TFIIIC BB1A fraction (C2, Fig. 1). Lane 5 (−) contains B fraction only. All reaction mixtures were supplemented with B fraction and *VAIA* template. Bands below the major transcripts were likely due to nuclease digestion. (*C*) TFIIIC1 activity is required for transcription in reactions with highly purified TFIIIC. Transcription mixtures contained B fraction, *VAIA* plus 1 μg of TFIIIC1 Mono Q fraction (C1, lane 1), 2 μl of TFIIIC BB1A fraction (C2, lane 2), or both the TFIIIC1 Mono Q fraction plus the TFIIIC BB1A fraction (lane 3).

primer-extended product was digested with *Sst* I. After denaturation and separation on a 3.5% polyacrylamide gel, the 300-bp single-stranded probe was eluted from the gel. This probe has ≈40 bp of polylinker sequence 3′ of the genomic DNA sequences. Probe (0.1 pmol) was hybridized to 5 or 40 μg of HeLa cell cytoplasmic poly(A)$^+$ RNA, 40 μg of tomato poly(A)$^+$ RNA, 1 or 0.1 fmol of *in vitro*-transcribed TFIIICα RNA in 30 μl of 0.5 M NaCl/10 mM Tris·HCl, pH 7.5/2 mM EDTA for 3 h at 60°C. S1 buffer (300 μl) (35) was added to the annealing reaction mixture, and 133 units of S1 was added and incubated at 37°C for 30 min.

RESULTS

Purification of TFIIIC. TFIIIC was extensively purified using a modified version of the previously described protocol (16). Pooled fractions from each step were analyzed by electrophoresis on a SDS/5.5% polyacrylamide gel (Fig. 1). All five polypeptides of the previously described complex (16) copurified over two successive box B DNA affinity columns, with approximately the same stoichiometry, indicating that the complex is either very stable or that all five polypeptides are required for DNA binding. *In vitro* transcription of the adenovirus 2 *VAI* gene using this highly purified fraction of TFIIIC was absolutely dependent upon a TFIIIB/pol III fraction and a TFIIIC1 fraction (see Fig. 3*C*). The molecular masses of the copurifying polypeptides appeared to be 230, 110, 100, 80, and 60 kDa, corresponding well to earlier studies (16, 17)

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GCGGGGCCCCGGACCAATGACGGTGGAGCTCCACTTACTGAGCCCGCCCCCGGGGAGTG
ACGTCATGCCGGCGCGCTGGAACTCCTCCTCCGAACCCTGGGTCTACCCACCTCCCCGGG
GCCCCGGATCGTTGTGCGCAGGCGCGATGGACCAAGGCCCTTGGCGGTGCGTTGCGCACC
   +1                  ↑
CCCGGGGCGCCGCGACTGAAGTAGCAATGGACGCGCTGGAGTCGTTGTTGGACGAAGTCG
   ↑                  ↑ M D A L E S L L D E V
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FIG. 4. Nucleotide sequence of genomic DNA encoding the 5′ end of TFIIICα mRNA. The nucleotide corresponding to the 5′ end of the longest cDNA clone is designated +1, and the amino acid sequence initiating at the first ATG is indicated. Primer-extension and S1 nuclease analysis of HeLa poly(A)$^+$ cytoplasmic RNA mapped a heterogeneous group of 5′ ends in the interval from position +1 to approximately position +20 (see Fig. 5), indicated by arrows. A minor group of RNAs extending to approximately −40 (marked by an arrow) were detected by primer extension. The boldface underlined sequences indicate a potential CAAT box, SP1 binding site, and a putative box B. The sequence of the consensus box B is GGTTCGANTCC (1).

Cloning a cDNA Encoding TFIIICα. Amino acid sequence was obtained from the 230-kDa polypeptide α subunit in the highly purified fraction of TFIIIC and used to design a PCR-based strategy for isolating cDNA clones. Analysis of the hybridizing clones obtained revealed two that encoded ≈7 kb of sequence with 0.5 kb of overlap.

Proof of the Clone's Identity. Specific antiserum was raised against an ≈30-kDa polypeptide encoded by an internal portion of the cDNA clone expressed in *Escherichia coli*. Affinity-purified immunoglobulin detected only an ≈230-kDa polypeptide in Western blots of the C′ fraction (data not

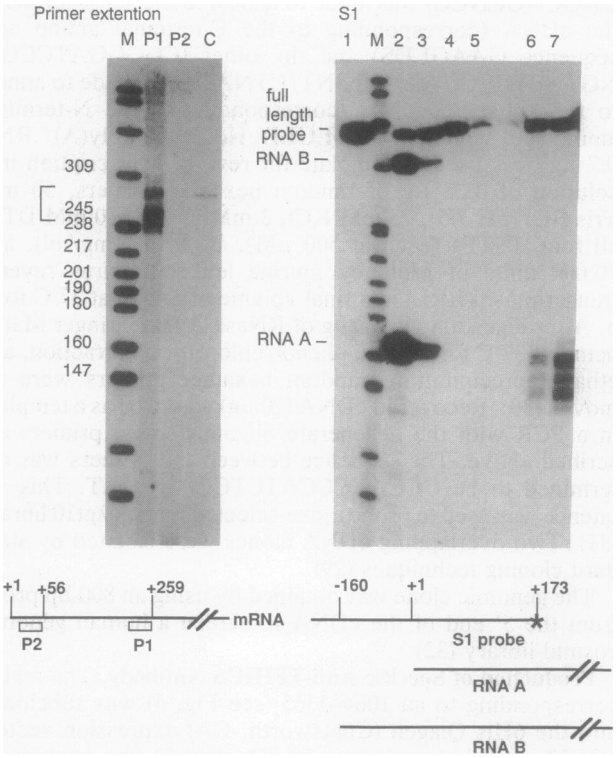

FIG. 5. Mapping the heterogeneous 5′ ends of TFIIICα mRNA. (*Left*) Primer extension: 7 μg of poly(A)$^+$ HeLa RNA was annealed with either oligonucleotide primer P1 or P2. The relative positions to which primers P1 and P2 hybridize along the mRNA are diagrammed at the bottom. Lane C shows a control primer-extension reaction products using a restriction fragment template that extends 300 nt beyond the 5′ end of the P1 primer. The sizes of the smallest molecular mass markers are 123 and 110 nt. The brackets indicate the range of primer-extension products found in lane P1. The ≈300-nt primer-extension products were minor bands on the original autoradiogram. Primer P2 was included to detect potential RNAs with 5′ ends extending further than 300 nt from the 5′ end of primer P1. None were detected. (*Right*) S1 nuclease analysis: the probe is diagrammed at the bottom. The probe was end-labeled (*) at position +173 relative to the 5′ end of our longest cDNA clone. Undigested full-length probe is in lane 1. HeLa cell poly(A)$^+$ cytoplasmic RNA (8 and 40 μg) is in lanes 6 and 7, respectively. S1 protection of control *in vitro*-transcribed RNAs A and B, with regions complementary to the probe extending to positions +1 and −160, were analyzed in lanes 2 (100 pmol) and 3 (10 pmol). The positions of the control S1-protected products are marked RNA A and RNA B. Incubation with 40 μg of tomato poly(A)$^+$ RNA and yeast tRNA (lanes 4 and 5, respectively). The lengths of the products protected by HeLa RNAs were estimated from the mobility of the S1-protected fragment generated by RNA A and the mobilities of the markers.

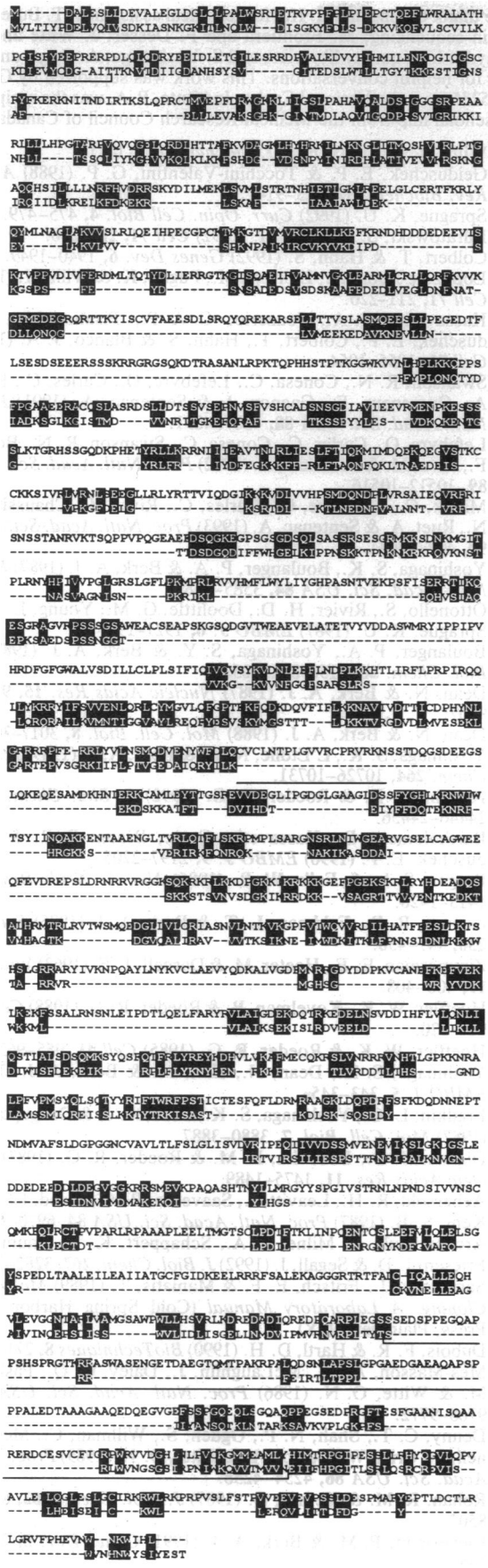

FIG. 6. Comparison of the amino acid sequences of human and yeast box B binding subunits of TFIIIC. The human sequence is shown on the upper line. The two sequences were aligned with the CLUSTAL alignment program; identical residues and those with a Dayhoff replacement matrix score of >8 are shown in white on a black background. The position of the putative HMG boxes in the yeast τ_{138} are bracketed. Sequences determined by peptide microsequencing are overlined.

shown). Immune and preimmune antisera were used in a TFIIIC EMSA (Fig. 2). Each sample contained labeled *VAI* DNA incubated with a TFIIIC S400 fraction and either preimmune or immune serum was added subsequent to the DNA binding reaction. Increasing amounts of preimmune serum did not alter the mobility of the specific TFIIIC complex (13), whereas the same amounts of immune serum resulted in a clear decrease in the intensity of the TFIIIC complex band and the generation of super-shifted complexes of antibody bound to the TFIIIC–*VAI* DNA complex. Immune serum alone did not alter the mobility of the probe (data not shown).

Further proof that the cDNA encoded a component of TFIIIC came from the ability of the specific antiserum to immunodeplete transcriptional activity from a TFIIIC fraction. While the immunodepletion procedure using preimmune immunoglobulin reduced C-fraction transcriptional activity somewhat, immunodepletion with immune immunoglobulin consistently reduced transcriptional activity to a far greater extent (Fig. 3*A*).

TFIIIC1 Activity Is Not Depleted. Highly purified TFIIIC (fraction BB1A) was sufficient to restore transcriptional activity to the immunodepleted fraction (Fig. 3*B*). As in earlier studies from this laboratory (11, 14, 16), *VAI* transcription required the addition of both TFIIIC1 and TFIIIC fractions in addition to the B fraction (Fig. 3*C*). TFIIIC1 was shown to be the limiting factor for *VAI* transcription in the *in vitro* system (11). Consequently, the experiment shown in Fig. 3*B* indicates that only TFIIIC was immunodepleted by the antibodies specific for the cloned polypeptide, leaving TFIIIC1 in the immunodepleted TFIIIC fraction free to perform transcription once the depleted TFIIIC was replaced.

Mapping the Heterogeneous 5′ Ends of TFIIICα mRNA. Since the results above indicated we had cloned a component of TFIIIC, we determined the sequence of the long cDNA clone. Analysis of the 3′ end showed a poly(dA) tail, indicating that we had cloned the 3′ end of the cDNA.

To analyze the 5′ end of the TFIIICα mRNA, we isolated additional cDNA clones extending 22 nt 5′ of the first AUG and a cosmid clone of genomic DNA encoding the 5′ end of the cDNA. None of the cDNA clones had stop codons in-frame with the first AUG. The genomic DNA revealed that an open reading frame extended 197 bp 5′ to the most 5′ AUG in our longest cDNA clones, but there were no potential AUG start codons in this region (Fig. 4). We used the genomic DNA to prepare a single-stranded DNA probe for S1 nuclease analysis and compared the S1-protected fragments to the products of primer-extension reverse transcription to confirm the mapping of 5′ ends (35).

Several primer-extension products were observed (Fig. 5 *Left*) extending from approximately positions +1 to +20, relative to the 5′-most sequence cloned as cDNA, designated +1 in Fig. 4. S1 analysis (Fig. 5 *Right*) revealed several protected products with 5′ ends mapping from approximately positions +1 to +20. These results indicate that the major TFIIICα mRNAs have heterogeneous 5′ ends mapping in this region. The agreement between the S1 and primer-extension analyses argues strongly against the occurrence of a further 5′ exon in these major TFIIICα mRNAs (35). In addition, primer extension detected a minor group of RNAs with an additional ≈40 nt at their 5′ ends. Corresponding 5′ ends were not detected by S1 mapping. We believe this is because of the low abundance of these mRNAs. However, we cannot rule out the possibility that these minor RNAs contain an addi-

tional ≈40 nt 5′ exon. Since the S1 and primer-extension reactions both pointed to the first nucleotide of our longest cDNA clones as a major 5′ end, we designated this base pair in the genomic sequence as +1.

Sequence Analysis. The protein sequence predicted by TFIIICα cDNA is shown in Fig. 6. Tryptic peptide sequences obtained from the isolated polypeptide are indicated. No matches were found with any sequence in the current data base. Neither were any convincing structural motifs identified. The homologous yeast TFIIIC 138-kDa subunit, which binds the box B promoter element, has been proposed to contain two high-mobility-group (HMG) box DNA binding motifs; the first composed of aa 1–68 and the second composed of the very C-terminal 60 residues (9). When aligned using the CLUSTAL (36) program, however, the yeast and human homologues showed surprisingly little homology, even in the proposed HMG box regions (Fig. 6). This calls into question the significance of the proposed HMG sequences. However, there is precedent for a lack of sequence conservation between yeast and higher eukaryotic pol III TFs. TFIIIA from yeast has surprisingly low homology to that of *Xenopus* (28). Only the coordinating histidine and cysteine residues essential for the TFIIIA zinc fingers are conserved. Analysis of the genomic sequence revealed several potential regulatory elements (Fig. 5) and a weak homology to the consensus tRNA box B sequence, which might function in an autoregulatory loop controlling TFIIICα transcription.

DISCUSSION

To rigorously study the molecular mechanisms by which pol III transcription is regulated and to glean a more thorough understanding of the molecular mechanism by which pol III transcription proceeds in mammalian cells, we have cloned a cDNA encoding the box B DNA binding subunit of human TFIIIC. We call the encoded polypeptide TFIIICα. To confirm that we cloned the correct cDNA, we raised specific antisera against a 30-kDa portion of the encoded protein expressed in *E. coli*. This antisera super-shifted a specific TFIIIC–box B DNA complex in an EMSA (Fig. 2) and specifically depleted an activity required for transcription from a partially purified TFIIIC fraction (Fig. 3*A*). The depleted activity was replaced by our most highly purified preparation of TFIIIC (Fig. 3*B*). These results confirm that the cloned cDNA encodes a component of TFIIIC.

The cDNA contains a single long open reading frame of 2209 codons, by assuming initiation at the first AUG (Fig. 4). The encoded polypeptide is ≈60% larger than the previously cloned *Saccharomyces cerevisiae* TFIIIC box B binding subunit (9) to which it has only limited sequence similarity (Fig. 6). The sequence of the human polypeptide does not show significant similarity to other sequences in the current data base. We have not detected sequence similarity to any known DNA binding structure. Indeed, although the large TFIIIC subunit of both the yeast and human proteins specifically cross-links to the region of the box B promoter element, it is not known whether the isolated large subunits can specifically bind DNA in the absence of other TFIIIC subunits.

In previous work on the purification of TFIIIC (11, 14, 16), it was found that the phosphocellulose C fraction from human cells contained an activity in addition to TFIIIC required for transcription of tRNA genes and the adenovirus 2 *VAI* gene. Here again, we found that TFIIIC was easily separated from TFIIIC1 since immunoprecipitation of TFIIIC from a phosphocellulose C′ fraction with anti-TFIIICα antibody left TFIIIC1 activity in the supernatant (Fig. 3). The cDNA clone of the α subunit and specific antibody raised against the encoded protein should be useful in analyzing the mechanisms controlling TFIIIC activity in mammalian cells.

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