18. J. D. Bernal, *Proc. R. Soc. London, Ser. A* **62**, 537 (1949).
19. C. Ponnamperuma, A. Shimoyama, E. Friebele, *Origins Life* **12**, 98 (1982).
20. B. Young and T. R. Cech, *J. Mol. Evol.* **29**, 480 (1989).
21. R. Lohrmann and L. E. Orgel, *ibid.* **11**, 17 (1978).
22. L. Rodriguez and L. E. Orgel, *ibid.* **32**, 101 (1991).
23. A. Kornberg and T. A. Baker, *DNA Replication* (Freeman, New York, ed. 2, 1991), pp. 310–320.
24. K. J. Luebke and P. B. Dervan, *J. Am. Chem. Soc.* **113**, 7447 (1991).
25. D. T. Ashirbekova *et al.*, *Sov. J. Bioorg. Chem.* **15**, 166 (1989).
26. We thank R. Stribling for informing us of the utility of the anion-exchange HPLC column. Supported by NSF grant CHE 9000187.

14 April 1992; accepted 9 July 1992

Cloning of a Subunit of Yeast RNA Polymerase II Transcription Factor b and CTD Kinase

Opher Gileadi, William J. Feaver, Roger D. Kornberg

Yeast RNA polymerase II initiation factor b copurifies with three polypeptides of 85, 73, and 50 kilodaltons and with a protein kinase that phosphorylates the carboxyl-terminal repeat domain (CTD) of the largest polymerase subunit. The gene that encodes the 73-kilodalton polypeptide, designated *TFB1*, was cloned and found to be essential for cell growth. The deduced protein sequence exhibits no similarity to those of protein kinases. However, the sequence is similar to that of the 62-kilodalton subunit of the HeLa transcription factor BTF2, suggesting that this factor is the human counterpart of yeast factor b. Immunoprecipitation experiments using antibodies to the *TFB1* gene product demonstrate that the transcriptional and CTD kinase activities of factor b are closely associated with an oligomer of the three polypeptides. Photoaffinity labeling with 3′-*O*-(4-benzoyl)benzoyl-ATP (adenosine triphosphate) identified an ATP-binding site in the 85-kilodalton polypeptide, suggesting that the 85-kilodalton subunit contains the catalytic domain of the kinase.

Initiation of transcription by RNA polymerase II requires several accessory protein factors whose roles remain, for the most part, to be elucidated. Recognition of the TATA element of a promoter is effected by the binding of a factor termed TFIID, which may be assisted by TFIIA; other factors and RNA polymerase then bind to the complex. RNA polymerase II exists in cells in two forms that differ in the degree of phosphorylation of the CTD of the largest subunit. Phosphorylation of the CTD is thought to occur at every round of initiation and to be required for that process or for the transition to elongation (*1*–*5*); therefore, the CTD kinase might be an essential initiation factor. Yeast polymerase II initiation factor b copurifies with a CTD kinase (*6*, *7*). Factor b also copurifies with polypeptides of 85, 75, and 50 kD. We report the results of chemical cross-linking and molecular cloning experiments that identify the likely catalytic subunit and establish the association of transcription factor and CTD kinase with a single oligomeric molecule composed of the three subunits.

The photoactivatable ATP derivative 3′-*O*-(4-benzoyl)benzoyl-ATP (BzATP) (*8*) was used to identify ATP-binding subunits of factor b. When we incubated highly purified factor b with [α-^{32}P]BzATP and exposed the mixture to ultraviolet (UV) irradiation, the 85-kD polypeptide was labeled with ^{32}P (Fig. 1). The labeling was prevented by an excess of nonradioactive ATP and was dependent on UV irradiation. The 85-kD polypeptide therefore contains an ATP binding site and might be the catalytic subunit of the CTD kinase. Another possibility is that the 85-kD polypeptide has the DNA-dependent adenosine triphosphatase activity associated with factor b (*7*). The relation between the two activities is presently unclear.

The gene encoding the 75-kD subunit of factor b was cloned (*9*). Factor b was purified to near homogeneity (*6*), and the 75-kD polypeptide was subjected to tryptic digestion. The NH_2-terminal sequences of two peptides were obtained by sequential Edman degradation. Polymerase chain reaction with oligonucleotide primers derived from the peptide sequences gave an amplified fragment of 1 kb, which was used to probe yeast genomic libraries. Sequence analysis of the resulting genomic clones (Fig. 2) disclosed an open reading frame (ORF) of 1926 nucleotides encoding a polypeptide of 642 amino acids with a predicted molecular size of 73 kD. The sequences of both tryptic peptides were found within the ORF (Fig. 2). Analysis of yeast RNA (*10*) revealed a set of transcripts of 2.4 to 2.5 kb starting 11 to 35 bases upstream of the initiation codon. We have termed the gene *TFB1* (transcription factor b, subunit 1); the protein is designated TFB1.

The deduced protein sequence of TFB1 does not show any of the elements conserved in protein kinases, so the gene product is evidently not the catalytic subunit of the CTD kinase, as suggested by the affinity-labeling experiments. The 73-kD polypeptide may function in the regulation of kinase activity or in the interaction of the kinase with other components of the transcription machinery. The sequence of the polypeptide is unrelated to any other sequence in the Genbank database (release 70), nor does it contain any recognizable sequence motifs. However, in its native size and subunit composition, yeast factor b resembles the rat liver initiation factor δ (*11*–*13*) and the HeLa factor BTF2 (*14*, *15*). Moreover, the deduced amino acid sequence encoded by *TFB1* is similar to that of a 62-kD subunit of BTF2 (Fig. 2). The most significant region of similarity (42% identity, 62.5% similarity; $z = 14.7$ SD) (*16*) is in the region between amino acids 234 and 297; the similarity outside this region is only marginally significant (19% identity, 32% similarity; $z = 3$ SD).

For purposes of genetic analysis, we prepared a yeast strain with most of the coding sequence of *TFB1* (between the Acc I site corresponding to Ser^{31} and the Sal I-Acc I site corresponding to Asp^{528}) deleted. Sequences flanking the *TFB1* gene were inserted in the integrative *LEU2* vector pRS305 (*17*). After transformation of the diploid strain YPH501 (*17*), the replacement of *TFB1* sequences with plasmid sequences was confirmed by DNA blot hybridization. The heterozygous cells were then sporulated and tetrads were manually dissected (*10*). Only two spores of each true tetrad germinated and formed colonies on rich medium (and were both *leu*$^-$), which demonstrates that the *TFB1* gene is essential for viability.

To establish the relation between the 73-kD polypeptide encoded by the ORF and the polypeptides, transcription factor activity, and CTD kinase activity of factor b, antibodies were raised to the product of the cloned gene (*18*). The resulting antiserum reacted with a polypeptide of ~75 kD in a partially purified preparation of factor b (Fig. 3A); serum obtained from the same rabbit before immunization did not react with this polypeptide. To assess the association of the subunits and the biochemical activities, we covalently attached antibodies from immune and preimmune sera to Affi-Gel–protein A beads and used the antibodies to bind proteins from fractions containing factor b. Starting with a relatively crude fraction, a single step of immunoaffinity purification resulted in an eluted preparation containing almost exclusively the 85-, 73-, and 50-kD polypeptides (Fig.

Department of Cell Biology, Sherman Fairchild Center, Stanford University Medical School, Stanford, CA 94305.

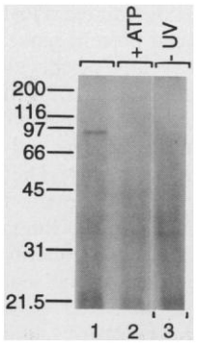
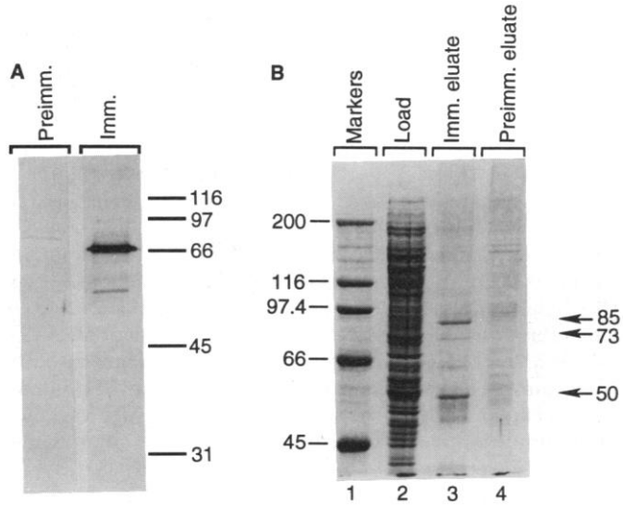


Fig. 1. Affinity labeling of factor b with an ATP derivative. Highly purified factor b was incubated with [α-^{32}P]BzATP and exposed to UV irradiation. Proteins were separated by gel electrophoresis and visualized by autoradiography (*26*). UV irradiation was omitted in lane 3; excess ATP was present in the reaction analyzed in lane 2. The labeled band at 85 kD coincided with the position of the 85-kD polypeptide of factor b in the stained gel (*10*). Molecular size markers are shown at the left (in kilodaltons).

Fig. 2. Alignment of the deduced amino acid sequence of TFB1 with that of the gene encoding the 62-kD subunit of BTF2 (*15*). Vertical lines mark identical residues; dots mark similar residues (I, L, V, M, F, Y, and W; D and E; K and R; N and Q; S and T). TFB1 sequences obtained by Edman sequencing of tryptic peptides are underlined. The entire nucleotide sequence of *TFB1* has been deposited in GenBank and assigned accession number M95750. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

```
TFB1  MSHSGAAIFE KVSGIIAINE DVS---PAE- LTWRSTDGDK VHTV-VLSTI DKLQATPASS   55
BTF2  MATSSEEVLL IVKKVRQKKQ DGALYLNAER IAHAPEGKDR FTISHNYADI KCQKISPEGK   60
TFB1  EKNMLRLIGK VDESKK-RKD NEGNEVVPKP QRHNFSFNNR TVNDNIKNTL QQIISRYKDA  114
BTF2  AKIQLQLVLN AGDTTNFNFS NESTAVKER- ---------- ---DAVKDLL QQLLPKF---  103
TFB1  DIYEEKRRRE ESAQNTETPN SSSSVTAGTP TPNLDTPQLN NGAPLINTAK LDDSLSKEKL  174
BTF2  ---------- ---------- ---------- ---------- ---------- ------KRKA  107
TFB1  LTNLKLQQSL LKGNKVLNKV FQETVINAGL PPSEFWSTRI PLLRAFALST S---QKVGP-  230
BTF2  NKELEEKNRN LQEDPVLFQL YKDLVVSQVI SAEEFWANRL NVNATDSSST SNNKQDVGIS  167
TFB1  YNVLSTIKPV ASSENKVNVN LSREKILNIF ENYPIVKKAY TDNVPKNFKE PEFWARFFSS  290
BTF2  AAFLADVRPQ TDGCNGLRYN LTSDIIESIF RTYPAVKNKY AENVPHNNTE KEFWTRFFQS  227
TFB1  KLFRKLRGEK INQNDRGDVI IDRYLTLDQE FDRKDDDNLL HPVKKIIDLD GNIQDDPVVR  350
BTF2  HYFNRDR--- -LNTGSKDLF AECAKIDEKG LKTNVSLGVK NPLLDLTALE DKPLDEGYGI  283
TFB1  GNRPDFTNQP GVDINGNSDG TVDILKGNNR LSEKNINA-L KNEYSRTNLQ NKSNITNDEE  409
BTF2  SSVPSASNSK SIKERSNA-- --AIIKRFNH HSANVLAAGL RKQEAQNEQT SEPSNNDGNS  339
TFB1  DEDNDERNEL KIDDLNESYK TNYAIIHLKR NAHEKTTDND AKSS------ ADSIKNADLK  463
BTF2  GDADCFQPAV KRAKLQESIE YE----DLGK NNSVKTIALN LKKSDRYYHG PTPIQSLQYA  395
TFB1  VSNQQN--LQ QLSLVNDNLI NKLDLNQYVP NNEVSNKINK RVITAIKINA KQAKHNNVNS  521
BTF2  TSQDIINSFQ SIRQENEAYT PK--LTQVLS SSAASS---- -TITAL---- ---------S  435
TFB1  ALQSFVDNTS QANELEVKST LPIDLLESGR NLHTTCGEFL KHFYIHFQSG EQKQASTVKK  581
BTF2  PGGALNQGGT QQ---AINQN VPNDIQSELK HLYYAVGELL RHFWSCFPVN TPFLEEKVVK  492
TFB1  LYNHLKDC-I EKLNELFQDV LNGDGESNSN TCTAYLKPVL NSITLATHKY DEYFNEYNNN  640
BTF2  NKSNLERFQV TKLCPFQEKI ------RRQY LSTNLVSHIE ENLQTAYNKL HTWQSRRLNK  546
TFB1  SN- 642
BTF2  KTA     549
```

Fig. 3. Antibodies to TFB1 recognize the 73-kD polypeptide and immunoprecipitate all three polypeptides of factor b. (**A**) Reaction of antibodies to TFB1 with the 73-kD component of yeast initiation factor b. Extensively purified factor b (50 μl) was fractionated by SDS-PAGE, blotted onto a PVDF membrane, and incubated with preimmune or immune serum (*27*). (**B**) Immunoaffinity purification of factor b subunits (*28*). Factor b purified through two chromatographic steps from whole-cell extract was applied to antibody-agarose columns formed from immune or preimmune serum. The columns were washed extensively with high salt (0.8 M potassium acetate) and detergents [Triton X-100 (0.5%) and sodium deoxycholate (0.5%)], and bound material was eluted at pH 3. Lane 1, markers; lane 2, column load; lane 3, immune eluate; lane 4, preimmune eluate. Molecular size markers are shown in kilodaltons.



3B). The 73-kD polypeptide was only partially released from the antibody beads under the conditions used; harsher elution conditions released more of this polypeptide (*10*). The binding of the three polypeptides was stable to washing with 1 M ammonium sulfate and sodium deoxycholate (0.5%). These results establish the strong physical association between the three polypeptides of factor b and identify them as subunits of a single molecule.

In separate experiments (Fig. 4A), material flowing through the immune column was depleted of factor b transcriptional activity and retained only 26% of the activity in the starting fraction. Material bound to the column and eluted at pH 3 exhibited a small amount of activity (about 4% of that originally applied). In contrast, essentially all of the activity was found in the flow through and none in the eluted fraction from the preimmune column. The low recovery of activity in the eluate of the immune column may reflect the fact that only a small fraction of the 73-kD subunit was released from the column. However, material eluting at pH 2.1, which contained similar amounts of all three subunits, was nearly inactive (*10*), probably owing to irreversible dissociation or denaturation of the subunits.

Partially purified fractions from yeast (such as that used in Figs. 3B and 4A) contain abundant protein kinases and substrates, which makes it difficult to specifically detect the kinase activity associated with factor b. To observe immunodepletion of CTD kinase activity, we repeated the immunoaffinity chromatography experiment with more highly purified factor b (Fig. 4B). The immunoaffinity protocol was the same as before, except that the factor b preparation was passed sequentially over the preimmune and immune columns. The load, flow through, and eluted fractions were then assayed for protein kinase activity in the absence and presence of purified RNA polymerase II. When the fraction loaded on the immunoaffinity columns was incubated with [γ-^{32}P]ATP and the proteins were separated by SDS–polyacrylamide gel electrophoresis (PAGE) and visualized by autoradiography, two major bands were obtained. These bands do not cochromatograph with the transcriptional activity of factor b (*7*). In the presence of RNA polymerase II, an additional band was obtained, slightly lower in mobility than the unphosphorylated form of the largest polymerase subunit. This band corresponds to the largest subunit, multiply phosphorylated, predominantly on the CTD (*7*). The labeling of the band by the fraction that flowed through the immune column was only 36% of that obtained with the starting preparation. The phosphorylation of the polymerase subunit by the eluted fraction was about 0.1% of that obtained with the starting material. In contrast, the material that flowed through the preimmune column retained 81% of the CTD kinase activity of the starting preparation, and no detectable

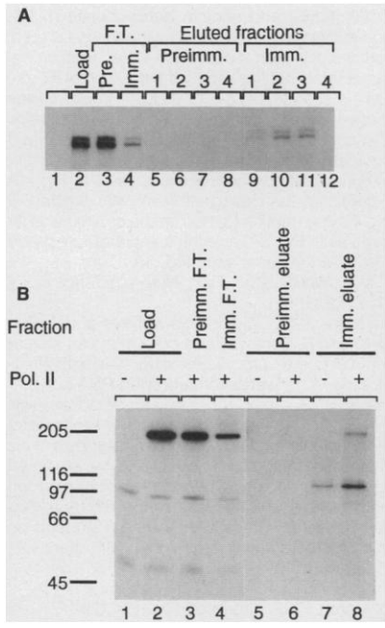


Fig. 4. Antibodies to TFB1 specifically immunoprecipitate both transcriptional and CTD kinase activities of factor b. (**A**) Interaction of factor b transcriptional activity from a crude yeast fraction with an anti-TFB1 antibody column (*28*). Affinity chromatography was performed as in Fig. 3B, except that the detergent wash was omitted. The flow through (F.T.) was collected, and bound material was eluted at pH 3. All fractions were assayed for factor b activity in transcription, and an autoradiograph of the region of gel containing specific transcripts is shown. Lane 1, no factor b; lane 2, crude yeast fraction (2 μl) loaded on the columns; lanes 3 and 4, flow through (3 μl) from preimmune and immune columns, respectively; lanes 5 through 8, eluted fractions (5 μl) from the preimmune column; lanes 9 to 12, eluted fractions (5 μl) from the immune column. (**B**) Interaction of CTD kinase activity with an anti-TFB1 column (*28*). A highly purified factor b preparation (2 ml) was applied to a preimmune column. The flow through was then applied to an immune column, and the column was washed and eluted. Fractions (0.05 μl in lanes 1 through 4 and 1 μl in lanes 5 through 8) were assayed for protein kinase activity as described (*7*) in the presence (lanes 2 through 4, 6, and 8) or absence (lanes 1, 5, and 7) of purified RNA polymerase II (Pol. II) (25 ng) (*29*). Molecular size markers are shown at the left (in kilodaltons).

activity was eluted. Because CTD phosphorylation was not a linear function of the amount of factor b under these assay conditions, the results are only qualitative. It is possible that the catalytic subunit of the CTD kinase is only loosely bound to the other subunits of factor b, which could account for the low recovery in the eluate of the immune column. This is unlikely because similar kinase activity was recovered when the antibody column was washed under milder conditions (0.1 M potassium acetate) (*10*).

The data presented here demonstrate the association of the 85-, 73-, and 50-kD polypeptides, factor b activity in transcription, and CTD kinase activity, an association that was previously suggested on the basis of cochromatography (*6*, *7*). The two catalytic activities evidently reside in the same molecular assembly; whether the activities are identical remains to be established. The kinase catalytic domain may reside in the 85-kD subunit, so the 73- and 50-kD polypeptides may have other catalytic, regulatory, or structural roles. Factor b differs from a CTD kinase (termed CTK) purified and cloned from yeast (*19*, *20*) in subunit composition and chromatographic properties, and purified CTK did not substitute for factor b in transcription in vitro (*10*). Factor b and the product of the *TFB1* gene do not resemble the molecularly defined HeLa factors TFIIA, B, D, E, or F in subunit organization or in amino acid sequence, although evidence for CTD phosphorylation points to the existence of a functional homolog of factor b in the human system. Yeast factor b does resemble initiation factor δ from rat liver in size and in copurifying with a DNA-dependent ATPase activity (*11*). Recently, factor δ was found to have a CTD kinase activity as well (*13*). The HeLa initiation factor BTF2 resembles both rat liver δ and yeast factor b in size and subunit composition. The data reported here showing a sequence similarity between a subunit of BTF2 and TFB1 suggest that all three factors have similar functions in transcription.

REFERENCES AND NOTES

1. D. L. Cadena and M. E. Dahmus, *J. Biol. Chem.* **262**, 12468 (1987).
2. J. M. Payne, P. J. Laybourn, M. E. Dahmus, *ibid.* **264**, 19621 (1989).
3. P. J. Laybourn and M. E. Dahmus, *ibid.* **265**, 13165 (1990).
4. J. A. Arias, S. R. Peterson, W. S. Dynan, *ibid.* **266**, 8055 (1991).
5. J. D. Chesnut, J. H. Stephens, M. E. Dahmus, *ibid.* **267**, 10500 (1992).
6. W. J. Feaver, O. Gileadi, R. D. Kornberg, *ibid.* **266**, 19000 (1991).
7. W. J. Feaver *et al.*, *Cell* **67**, 1223 (1991).
8. N. Williams, S. H. Ackerman, P. S. Coleman, *Methods Enzymol.* **126**, 667 (1986).
9. Yeast RNA polymerase II transcription factor b was purified as described, subjected to SDS-PAGE, and transferred to a nitrocellulose filter. The piece of filter bearing the 75-kD polypeptide was excised, and tryptic peptides were isolated and subjected to automated Edman microsequence analysis (*21*) by W. S. Lane, Harvard Microchemistry Facility. The two peptide sequences YDEYFNEYNN and YLTLDQEFD were obtained, and the following degenerate oligonucleotides corresponding to these peptides were synthesized: GCTCTAGAAGCTTRTAYTCRTTRAARTAYTCRTC, and a mixture of CCCTCGAGTCGACNCTNGAYCARGARTTYGA and CCCTCGAGTCGACNTTRGAYCARGARTTYGA (R is A or G; Y is C or T; and N is A, T, G, or C). Polymerase chain reactions (*22*) were performed for 30 cycles of 1 min at 94°C, 2 min at 52°C, and 3 min at 72°C, with 100 pmol of each degenerate oligonucleotide and 0.1 μg of yeast DNA. An amplified fragment of 1 kb was purified by gel electrophoresis and used to probe yeast genomic libraries in the plasmid pBluescript II (Stratagene). Five independent clones were isolated, and all contained overlapping sequences. Two clones were used for sequencing by the dideoxy method with a Sequenase kit (U.S. Biochemicals). The entire coding sequence was obtained from both strands, with a combination of exonuclease III deletions, standard subcloning techniques, and custom-made primers. Sequence assembly and analysis were performed with the University of Wisconsin GCG programs and Geneworks (Intelligenetics).
10. O. Gileadi, unpublished data.
11. R. C. Conaway and J. W. Conaway, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 7356 (1989).
12. R. C. Conaway, K. Garrett, J. Hanley, J. W. Conaway, *ibid.* **88**, 6205 (1991).
13. H. Serizawa, R. C. Conaway, J. W. Conaway, *ibid.*, in press.
14. M. Gerard *et al.*, *J. Biol. Chem.* **266**, 20940 (1991).
15. L. Fischer *et al.*, *Science* **257**, 1392 (1992).
16. The statistical significance of a sequence alignment is estimated by the value $z = (q_1 - q_{av})/(SD)$, where q_1 is the quality score of the best alignment of TFB1 and BTF2 amino acid sequences, q_{av} is the average quality of 100 separate alignments of TFB1 sequences with randomly scrambled BTF2 sequences, and SD is the standard deviation, as determined by the GAP program of the University of Wisconsin GCG package.
17. R. S. Sikorski and P. Hieter, *Genetics* **122**, 19 (1989).
18. The entire ORF of the *TFB1* gene and the region encoding amino acids 102 to 642 were cloned separately into the vector pGEX-3X (*23*) and introduced into *Escherichia coli* strain XL-1 (Stratagene). Fusion proteins were purified by glutathione affinity chromatography (*23*), yielding mixtures of proteolytic fragments in which the full-length products were minor components. The purified mixtures were used to immunize rabbits at 21-day intervals, and sera were collected 10 days after each injection (Berkeley Antibody Co., Richmond, CA).
19. J. M. Lee and A. L. Greenleaf, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 3624 (1989).
20. _____, *Gene Expression* **1**, 149 (1991).
21. R. Lim, A. Zaheer, W. S. Lane, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5233 (1990).
22. R. K. Saiki *et al.*, *Science* **239**, 487 (1988).
23. D. B. Smith and K. S. Johnson, *Gene* **67**, 31 (1988).
24. E. Harlow and D. Lane, *Antibodies, a Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988).
25. P. M. Flanagan *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
26. We synthesized and purified the [α-^{32}P]BzATP as described (*8*), with the use of radiolabeled ATP diluted to a specific activity of 10 Ci/mmol. For affinity labeling, factor b (~50 ng) was purified to near homogeneity as described (*6*) and was incubated with labeled BzATP (10^5 dpm) in 50 μl of glycerol (20%), 20 mM Hepes (pH 7.5), 6 mM $MgSO_4$, 1 mM dithiothreitol (DTT), and 100 mM potassium acetate, at 20°C for 20 min, and irradiated for 3 min at 340 to 366 nm with a handheld UV lamp (UVSL-25 mineralight, Ultraviolet Products) through a Pyrex filter. The reactions were then precipitated with trichloroacetic acid (10%), washed with acetone, and analyzed by SDS-PAGE (10% gels) and autoradiography.
27. The factor b preparation used in Fig. 3A was prepared as described (*6*), except that the phenyl–high performance liquid chromatography (HPLC) step was omitted, yielding a final product of lower purity. The proteins were resolved by SDS-PAGE (7.5% gel) and transferred to a polyvinylidene difluoride (PVDF) membrane (Bio-Rad)

in a solution of 10 mM 3-[cyclohexylamine]-1-propane-sulfonic acid (CAPS)-NaOH (pH 11.0) and 10% methanol (the TFB1 protein does not transfer efficiently in buffers commonly used for transfer to nitrocellulose). The membrane was incubated with a 1:200 dilution of preimmune or immune serum, and bound antibodies were visualized by means of alkaline phosphatase–conjugated secondary antibodies.

28. Preimmune or immune serum was mixed with an equal volume of Affi-Gel–protein A beads (Bio-Rad), the beads were washed, and the antibodies were covalently cross-linked to the protein A with dimethyl pimelimidate (*24*). The columns were washed in elution buffer [0.1 M glycine-HCl (pH 3), glycerol (20%)] and equilibrated in binding buffer [20 mM Hepes (pH 7.5), 0.1 M potassium acetate, glycerol (20%), 1 mM DTT, 1 mM EDTA, Triton X-100 (0.1%), and protease inhibitors (*6*)]. The factor b used in Fig. 3B was a partially purified preparation obtained by fractionation of a yeast whole-cell extract on Biorex 70 (Bio-Rad) and DEAE-Sephacel (Pharmacia) columns (*25*). The preparation (1.5 ml) was applied to a preimmune or immune antibody column (0.5 ml), and the flow through was reapplied to the column five times. The column was washed with 10 volumes of binding buffer followed by 50 volumes of binding buffer containing 0.8 M potassium acetate and 50 volumes of binding buffer containing Triton X-100 (0.5%) and sodium deoxycholate (0.5%). Bound proteins were eluted with several 0.25-ml portions of elution buffer. Each eluted fraction was immediately neutralized by the addition of 15 μl of 2 M tris-acetate, pH 8.0. The eluted fractions were concentrated by trichloroacetic acid precipitation, analyzed by SDS-PAGE, and stained with Coomassie blue. The same starting material and immunoaffinity procedure were used in Fig. 4A, except that the detergent wash was omitted. In Fig. 4B, the fraction loaded on the column was the same as in Fig. 3A, and the immunoaffinity procedure was the same as in Fig. 4A.

29. A. M. Edwards *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 87, 2122 (1990).

30. We thank J.-M. Egly and co-workers and J. Conaway and R. Conaway and co-workers for sharing unpublished results; N. Shavit for help with affinity labeling; A. Edwards for supplying RNA polymerase II; H. Goodson for help with tetrad analysis; and P. Patek and S. Martinez for synthesizing oligonucleotides. O.G. was supported by a Weizmann postdoctoral fellowship and by an American Cancer Society (California Division) postdoctoral fellowship. W.J.F. was supported by a Medical Research Council of Canada studentship. This research was supported by NIH grant GM-36659 to R.D.K.

12 March 1992; accepted 22 June 1992

Cloning of the 62-Kilodalton Component of Basic Transcription Factor BTF2

Laurent Fischer, Matthieu Gerard, Christian Chalut, Yves Lutz, Sandrine Humbert, Masamoto Kanno, Pierre Chambon, Jean-Marc Egly*

Cloning of the mammalian basic transcription factors serves as a major step in understanding the mechanism of transcription initiation. The 62-kilodalton component (p62) of one of these transcription factors, BTF2 was cloned and overexpressed. A monoclonal antibody to this polypeptide inhibited transcription in vitro. Immunoaffinity experiments demonstrated that the 62-kilodalton component is closely associated with the other polypeptides present in the BTF2 factor. Sequence similarity suggests that BTF2 may be the human counterpart of RNA polymerase II initiation factor b from yeast.

In vitro transcription of genes requires RNA polymerase B (II) and transcription factors TFIIA, TFIIB, TFIID (*1*), TFIIE, TFIIF (*2–4*), and BTF2 (*5*). The BTF2 transcription factor was purified and found to coelute or to cosediment with five polypeptides ranging from 35 to 90 kD after five chromatographic steps and one glycerol gradient (*5*). Transcription factor BTF2 is absolutely required for accurate in vitro transcription from a minimal promoter (containing the cap site and the TATA box) and sediments with a molecular size of 250 kD (*5*). Other transcription factors have also been identified and purified and include TFIIG, TFIII (*6*), TFIIH (*7*), and δ (*8*). It is unclear whether BTF2 corresponds to one or more of these factors (*5–8*). We now report the cloning and properties of the 62-kD component (p62) of BTF2.

We designed three degenerate oligonucleotide probes on the basis of the sequences of tryptic peptides from the purified p62 and used them for the screening of a HeLa cell cDNA library (*9*). Three independent cDNA clones were isolated and sequenced. All of them contained a predicted open reading frame (ORF) encoding a polypeptide of 548 amino acids with a calculated molecular size of 62.030 kD and an isoelectric point (pI) of 8.82 (Fig. 1). The encoded polypeptide contained the three oligopeptides that we microsequenced. We found no similarity with any of the sequences present in protein and nucleic acid databases. No known DNA binding motifs, kinase motifs, or nucleotide binding sites were found. When overexpressed in *Escherichia coli* (*10*) or in insect cells (*11*), the recombinant polypeptide (rp62) had the same electrophoretic mobility on SDS–polyacrylamide gel electrophoresis (PAGE) as the p62 from BTF2. The recombinant protein from either source did not substitute for the BTF2 activity in a HeLa cell in vitro transcription system lacking BTF2 (*12*), and no stimulation was observed when rp62 was added to an in vitro transcription system containing limiting amounts of BTF2. This result indicates that rp62 alone is not sufficient to restore BTF2 activity and that other BTF2 polypeptides may be required.

Several lines of evidence indicate that p62 is associated with BTF2 activity. First, a monoclonal antibody (M.Ab3c9) to purified rp62 (*13*), recognized a 62-kD polypeptide (p62) that cofractionated with the BTF2 activity throughout the purification (Fig. 2, A to D) (*5*, *12*, *14*). Second, inhibition of in vitro transcription indicated that p62 is a component of the BTF2 activity. Increasing amounts of either purified M.Ab3c9 or a control antibody M.AbC (*13*) were incubated for 1 hour at

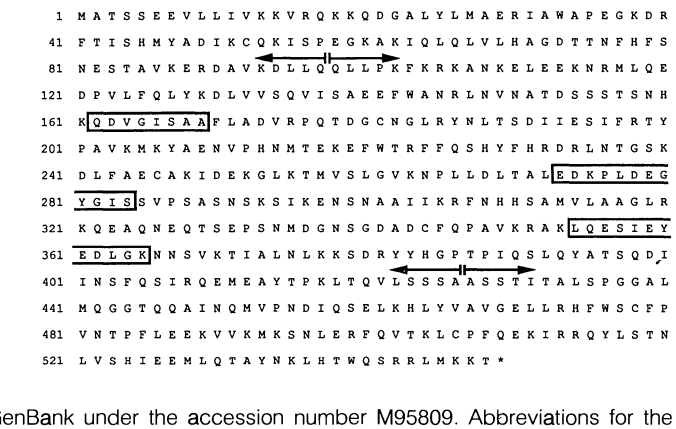


Fig. 1. Analysis of the human BTF2 p62 polypeptide sequence. Positions of the amino acids are indicated on the left. The three peptides that were microsequenced from the purified 62-kD polypeptide are boxed. Two imperfect amino acid repeats [KDLLQ/QLLPK (residues 93 to 102) and LSSSA/ASSTI (residues 422 to 431)] are shown by inverted bold arrows. The nucleotide sequence of the BTF2 p62 subunit can be found in GenBank under the accession number M95809. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

Laboratoire de Génétique Moléculaire des Eucaryotes du Centre National de la Recherche Scientifique, Unité 184 de Biologie Moléculaire et de Génie Génétique de l'Institut National de la Santé et de la Recherche Médicale, Faculté de Médecine, 11 rue Humann, 67085 Strasbourg Cédex, France.

*To whom correspondence should be addressed.