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Cloning and properties of the *Caenorhabditis elegans* TATA-box-binding protein

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ABSTRACT **The nematode *Caenorhabditis elegans* has become an organism of choice for the study of developmental processes at the genetic level. We have undertaken to develop an *in vitro* system to study transcription in *C. elegans*. As a first step we report here the cloning of the cDNA encoding the *C. elegans* TATA-box-binding protein (CeTBP). We used "touchdown PCR" to generate a specific DNA probe derived from the C-terminal region conserved in all TBP genes cloned to date. Several clones encoding an extended open reading frame were isolated from a phage λ cDNA library. The complete amino acid sequence of CeTBP deduced from the cDNA reveals a protein of 37 kDa with an extended sequence similarity in the C-terminal region with all other TBP cDNAs sequenced so far. The N-terminal region of CeTBP (amino acids 1–153), however, does not show any homology with TBPs from other organisms. Interestingly, the N-terminal portion of the molecule contains three short direct repeats. Purified recombinant CeTBP binds specifically to the TATA box sequence, interacts with transcription factors TFIIA and TFIIB, and is able to substitute for the TFIID basal activity when assayed by *in vitro* transcription in both HeLa and *C. elegans* nuclear extracts. CeTBP is therefore a basal transcription factor.**

The nematode *Caenorhabditis elegans* has emerged as a model organism for the study of developmental biology at the genetic level. Several aspects of nematode development have been extensively studied, and recent reports indicate that transcriptional control of gene expression might play a crucial role in sex determination (1–3) and the generation of touch-specific neurons (4, 5). Although transcriptional regulation is likely to play a critical role in a number of developmental processes, little is known about the mechanisms that control transcription in *C. elegans*. We have recently developed an *in vitro* transcription system derived from *C. elegans* embryos (to be reported elsewhere). As part of our effort to develop a defined system to study the biochemistry of transcription in the nematode we have decided to clone the *C. elegans* TATA-box-binding protein (CeTBP) gene.

The TATA-box-binding protein (TBP) was originally identified as a component of a phosphocellulose fraction of human nuclear cell extracts and was designated TFIID (reviewed in ref. 6). This fraction was shown to be required for transcription *in vitro* by RNA polymerase II from a promoter containing a TATA box. It has recently been shown that the TFIID fraction is composed of TBP and at least seven TAFs (TBP-associated factors) (7, 8). cDNAs encoding TBP sequences from various origins including human, *Drosophila*, yeast, and several plant species have been cloned (9–18). Sequence comparisons have revealed a highly conserved 180-aa residue C-terminal domain which has been shown to bind specifically to the TATA sequence and supports basal transcription *in vitro* (6). The role of the N-terminal region of TBP is unknown, although it might be involved in species-specific activated transcription (17).

Despite its small size, TBP is a complex protein that binds specifically to DNA and is apparently involved in interactions with many different types of factors, including general transcription components such as TFIIA and TFIIB, the acidic tail of RNA polymerase II, several TAFs, as well as activators and inhibitors of transcription (reviewed in refs. 19 and 20). Moreover, the universal requirement for TBP in transcription by all three RNA polymerases (reviewed in refs. 21 and 22) regardless of the presence of a cognate TATA box DNA-binding site suggests that TBP is probably involved mechanistically in different ways depending on the composition of the protein complex. The central role of TBP in transcription and its multifunctional properties prompted us to clone and characterize cDNAs from other species, including the nematode *C. elegans*.*

Here we report the isolation and sequence of the cDNA encoding CeTBP. We also tested the ability of CeTBP to bind specifically to the TATA box sequence, to interact with TFIIA and TFIIB, and to sustain basal transcription *in vitro* in both heterologous and homologous systems.

MATERIALS AND METHODS

Isolation of CeTBP cDNA Clones. We used three degenerate oligonucleotide primers (see Fig. 1) derived from the conserved C-terminal region of TBPs to amplify a *C. elegans* specific TBP DNA fragment. Primer 1, 5′-GATGTGAAGTTCCC(C, T, or A)AT(C or T)(C or A)G-3′; primer 2, 5′-GGGAACAGCTC(A, G, or T)GG(C or T)TC(A or G)TA-3′; and primer 3, 5′-ACAATCTT(G, A, or T)GG(C or T)TT(C or G)ACCAT-3′. Primer 1, primer 3, and *C. elegans* genomic DNA were used in "touchdown PCR" (23). A fragment of the expected size (116 bp) was amplified and isolated to serve as a template in a PCR mixture containing primer 1 and primer 2 (primer 2 is a primer internal to the amplified 116-bp fragment). An expected fragment of 80 bp was isolated, and the sequence revealed that it was a bona fide member of the TBP family. A 31-nucleotide-long primer (5′-ACTTGAAGGATTGTGCATTACTCACTCTCA-3′) was synthesized to serve as a probe for the screening of a cDNA library. The oligo(dT)-primed cDNA library was constructed with poly(A)$^+$ RNA isolated from mixed stages of the Bristol N2 *C. elegans* strain. cDNAs longer than 0.5 kb were selected and ligated to λ ZAP/*Eco*RI arms. The [γ-^{32}P]ATP-labeled 31-mer was used to probe 560,000 recombinant phages. The nitrocellulose filters were hybridized with the labeled oligonucleotide in 6× SST/0.25% milk powder/50 mM sodium phosphate, pH 7.3, at 52°C for 16 hr (1× SST = 0.15 M NaCl/0.015 M Tris·HCl, pH 7.5). The filters were then washed at 55°C for 15 min, twice in 4× SST, twice in 2× SST,

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Abbreviations: TBP, TATA-box-binding protein; CeTBP, *C. elegans* TBP; hTBP, human TBP; TAFs, TBP-associated factors.

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. L07754).

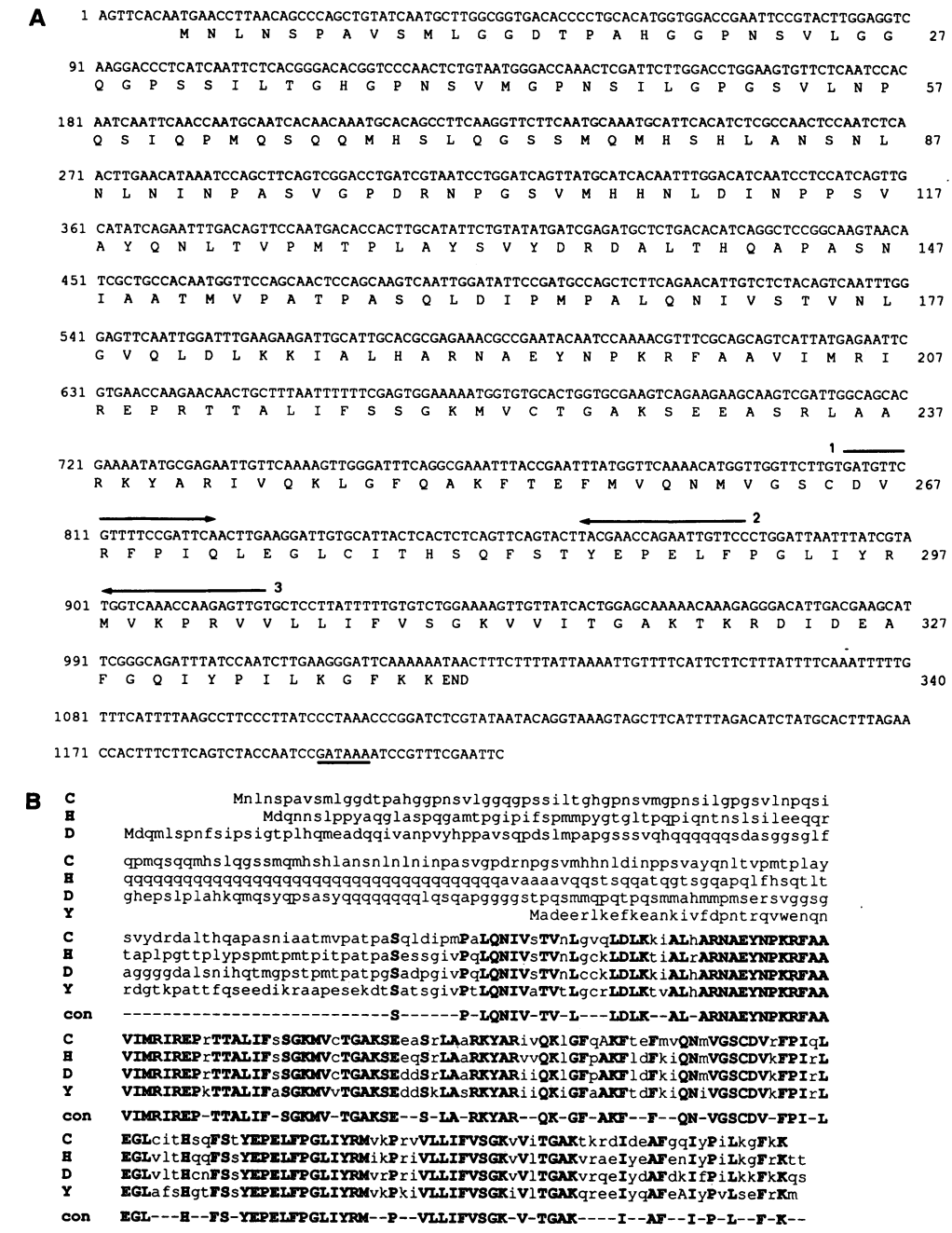

FIG. 1. cDNA sequence of CeTBP and comparison of predicted amino acid sequence to TBPs from various organisms. (*A*) cDNA and predicted amino acid sequence of CeTBP. The position and orientation of the oligonucleotide primers used in the PCR cloning of CeTBP are indicated by numbered arrows. Underlined is the potential polyadenylylation signal (GATAAA); no poly(A) stretch was found in the cDNA clones. (*B*) Comparison of the amino acid sequences of *C. elegans, Drosophila*, human, and yeast TBPs. Amino acids that are identical in *C. elegans* (C), *Drosophila melanogaster* (D), human (H), and yeast (Y) are denoted by capital letters and also indicated on the conserved (con) line.

and once in 1× SST. The filters were dried and exposed to x-ray film at −80°C with intensifying screens for 12 hr. Six clones were obtained and further analyzed. The inserts from the six positive phages were isolated by *in vivo* excision according to the protocol of the λ ZAP cloning kit supplier (Stratagene). DNA sequences of the positive clones were obtained by partial digestion of the inserts with BAL-31 and subcloning of overlapping fragments. The cDNAs were sequenced on both strands with Sequenase (United States Biochemical).

Since the CeTBP mRNA is trans-spliced to SL1, we used the oligonucleotide primers SL1 (5′-GTTTAATTAC-CCAAGTTTG-3′) and AsA (5′-GAGGGTCCTTGACCTC-CAAG-3′) to amplify the 5′ end of CeTBP, using standard PCR conditions.

***In Vitro* Transcription.** *In vitro* transcription reactions were performed in 20 μl, containing 1–2 μl of HeLa cell nuclear extract (50–100 mg of protein per ml), 200 ng of G-less DNA template [pS-TI(C_2AT) or MLC$_2$AT], 15 mM Hepes at pH 7.6, 60 mM KCl, 7% (vol/vol) glycerol, 3 mM $MgCl_2$, 4 mM spermidine, 0.7 mM dithiothreitol, 10 units of RNase T1, 0.625 mM ATP, 0.625 mM CTP, 12.5 μM UTP, 0.1 mM 3′-*O*-methyl-GTP, 0.5 μl of [^{32}P]UTP (3000 Ci/mmol; 1 Ci = 37 GBq), and 1 μl of RNasin (Promega). The components were assembled on ice and extracts were added last. The reactions were allowed to take place for 30 min at 30°C and

they were stopped by the addition of 380 μl of a buffer containing 1% SDS, 0.25 M NaCl, 5 mM EDTA, 20 mM Tris·HCl at pH 7.5, tRNA at 100 μg/ml, and proteinase K at 100 μg/ml. Proteins were digested for 15 min at 37°C and extracted once with phenol/chloroform. The nucleic acids were precipitated and resuspended in formamide dyes to be loaded on denaturing 4% or 6% acrylamide gels.

For the TBP transcription restoration assay, the HeLa nuclear extract was heat treated at 47°C for 15 min to inactivate the endogenous TBP. Recombinant TBP (between 50 and 200 ng per reaction) was added to the transcription reaction before the heat-treated extract, which was added last.

In vitro transcription with *C. elegans* nuclear extracts were performed essentially as described for HeLa nuclear extracts except for the following important modifications: transcriptions were performed for 1 hr at 25°C in a final KCl concentration of 15 mM and optimal heat inactivation of endogenous CeTBP was obtained by treatment of the extract for 15 min at 33°C.

The HeLa nuclear extract was a gift from Grace Gill (University of California, Berkeley) and the preparation of the *C. elegans* nuclear extracts will be described elsewhere.

Purification of Recombinant CeTBP. The coding portion of CeTBP was placed in the T7 RNA polymerase expression vector pET3a and the resulting plasmid, pSL2348, was used to transform *Escherichia coli* strain BL21pLysS. To purify the recombinant protein from bacteria induced by isopropyl β-D-thigalactoside, we followed the protocol described by Pugh and Tjian (24), omitting the last column.

Gel-Shift and Footprinting Reactions. These assays were performed essentially as described (25) except that poly(dG-dC)·poly(dG-dC) was substituted for poly(dI-dC)·poly(dI-dC). Both footprint and gel-shift probes contained the *C. elegans* hsp16-2 promoter, which includes both the TATA box region and the start site of transcription (26).

RESULTS AND DISCUSSION

Molecular Cloning of CeTBP. To clone cDNAs encoding CeTBP, we performed PCR on *C. elegans* genomic DNA, using a set of degenerate oligonucleotide primers selected on the basis of the yeast and *Drosophila* conserved protein sequences. The results of the PCR generated enough information to synthesize a 31-nucleotide-long probe specific for CeTBP. This oligonucleotide was used to screen a *C. elegans* cDNA library, and six positive phages were obtained. The inserts from these phages were isolated and sequenced (Fig. 1). A fragment of CeTBP cDNA containing the conserved region was used to probe *C. elegans* poly(A)$^+$ RNA. The CeTBP probe detects a messenger RNA about 1.5 kb in size (data not shown).

The DNA sequence of the CeTBP gene (Fig. 1*A*) predicts a long open reading frame encoding a polypeptide of 340 aa corresponding to a molecular mass of 37 kDa. Comparison of the deduced polypeptide sequences of the *C. elegans*, human, *Drosophila*, and yeast genes (Fig. 1*B*) reveals a strong homology in the C-terminal region. In the region encompassing aa 167–355 of CeTBP, there is a 83%, 82%, and 75% identity with the human, *Drosophila*, and yeast TBPs, respectively. In contrast to the conservation in the C-terminal region, the N-terminal region of CeTBP does not bear any strong homology with its counterpart in human or *Drosophila*. For instance, the polyglutamine stretch present in human and *Drosophila* TBPs is absent from *C. elegans*. However, the N-terminal sequence of CeTBP, like the TBPs of human and *Drosophila*, is rich in proline, methionine, and serine or threonine residues. Moreover, this region contains very few charged residues, in contrast to the C-terminal region, which is rich in basic residues. It is interesting to note the existence of a common PATPa/gS motif in the *C. elegans*, human, and *Drosophila* TBPs found in the exact same position, 14 aa upstream of the conserved C-terminal region.

Table 1. Sequences and locations of the small repeats in the N termini of TBPs from different organisms

Species	Peptide sequence	Location, aa residue nos.
C. elegans	SmLGGdtP	9–16
	SvLGGqgP	23–30
	SqQMHS	65–70
	SmQMHS	75–80
	NLnINPaSV	88–96
	NLdINPpSV	109–117
D. melanogaster	TPQSMM	114–119
	TPQSMM	123–128
H. sapiens	QsTSqQA	105–119
	QgTSgQA	113–119

Close inspection of the N-terminal region of CeTBP revealed the presence of three short direct repeats (see Table 1) ranging from 6 to 9 aa in length. Short repeats were also found in the human and *Drosophila* TBP N-terminal regions. A search for homologies in the protein data bases did not reveal other proteins bearing such repeats. It is unlikely that the presence of these repeats is accidental, but the role they might play in transcription is not clear. All the repeats share one feature, namely the presence of a serine residue which could serve as a site for post-translational modification.

Biochemical Properties of Recombinant CeTBP. To characterize CeTBP polypeptide biochemically we expressed the protein in *E. coli* with a T7 polymerase vector. CeTBP was purified by a combination of polymin P precipitation, phosphocellulose affinity chromatography, and selective ammonium sulfate precipitation. The resulting purified protein was analyzed on a silver-stained gel (Fig. 2*A*). The full-length CeTBP protein migrates at about 40 kDa, close to the predicted molecular mass of 37 kDa. The smaller products seen on the silver-stained gel are degradation products of CeTBP and are detected on Western blots by antibodies raised against the full-length protein (data not shown). The recombinant CeTBP migrates on a protein gel in a similar position to the endogenous protein present in *C. elegans* nuclear extracts (compare lanes 1 and 3 of Fig. 2*B*).

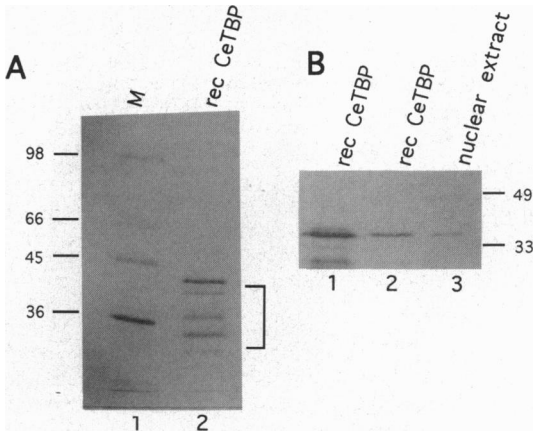

FIG. 2. Purification of CeTBP from *E. coli*. (*A*) SDS/10% polyacrylamide gel of purified CeTBP. Lanes: 1, molecular mass standards (kDa); 2, recombinant CeTBP. The bracket indicates the distinctive degradation products observed when CeTBP is expressed in *E. coli*. (*B*) Comparison of recombinant and native CeTBP by Western blotting. Lanes: 1 and 2, recombinant CeTBP, respectively 100 ng and 10 ng; lane 3, *C. elegans* embryo nuclear extract. CeTBP was detected on the Western blot with polyclonal antibodies raised in rabbits against recombinant CeTBP.

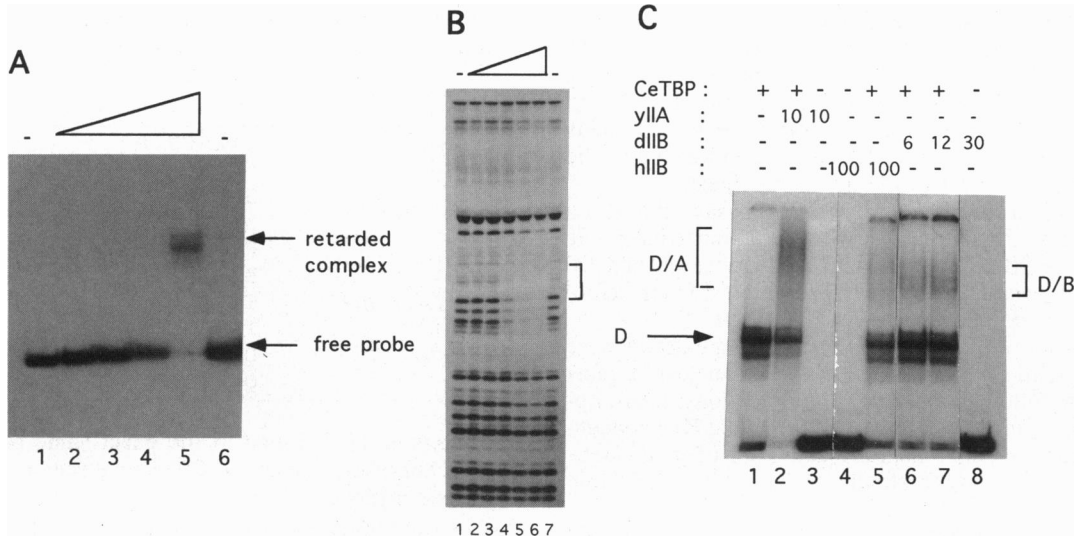

FIG. 3. Recombinant CeTBP binds specifically to the TATA box and interacts with general transcription factors. (*A*) Gel-shift assay. Lanes: 1 and 6, no CeTBP; 2, 3, 4, and 5 contained, respectively, approximately 4, 8, 20, and 100 ng of CeTBP. (*B*) Footprint. Lanes 1 and 7, no CeTBP; lanes 2, 3, 4, 5, and 6 contained, respectively, about 4, 8, 20, 40, and 100 ng of recombinant CeTBP. The position of the TATA box (TATAAATA) is indicated by a bracket. (*C*) Gel-shift assay. Lanes: 1, 100 ng of CeTBP; 2, 100 ng of CeTBP plus 10 ng of recombinant yeast TFIIA (yIIA); 3, 10 ng of yIIA only; 4, 100 ng of recombinant human TFIIB (hIIB); 5, 100 ng of CeTBP plus 100 ng of hIIB; 6, 100 ng of CeTBP plus 6 ng of *Drosophila* TFIIB (dIIB); 7, 100 ng of CeTBP plus 12 ng of dIIB; and 8, 30 ng of dIIB only. The arrow indicates the position to which the CeTBP/DNA complex migrates (D). The position of migration of the TFIIA/CeTBP complex is denoted by D/A and the position of migration of the TFIIB/CeTBP complex is denoted by D/B.

To verify the integrity of the DNA-binding activity of the recombinant CeTBP, we performed both gel-shift and footprinting reactions using the *C. elegans* hsp16-2 promoter (26) as a probe (Fig. 3). Fig. 3*A* shows that CeTBP is able to bind to a 100-bp fragment of DNA containing the hsp16-2 TATA box in a gel-shift assay. DNase I footprinting analysis (Fig. 3*B*) confirms that the recombinant protein interacts specifically with the TATA box sequence and protects a 20-bp stretch present in the hsp-16 promoter fragment. The recombinant CeTBP also protects the adenovirus major late promoter TATA box from DNase I digestion in a fashion indistinguishable from its *Drosophila* counterpart (data not shown). We surmised that since the homology in the C-terminal part of TBPs is so strong that CeTBP could interact with the general factors TFIIA and TFIIB from other species. The gel-shift assay in Fig. 3*C* illustrates that CeTBP interacts

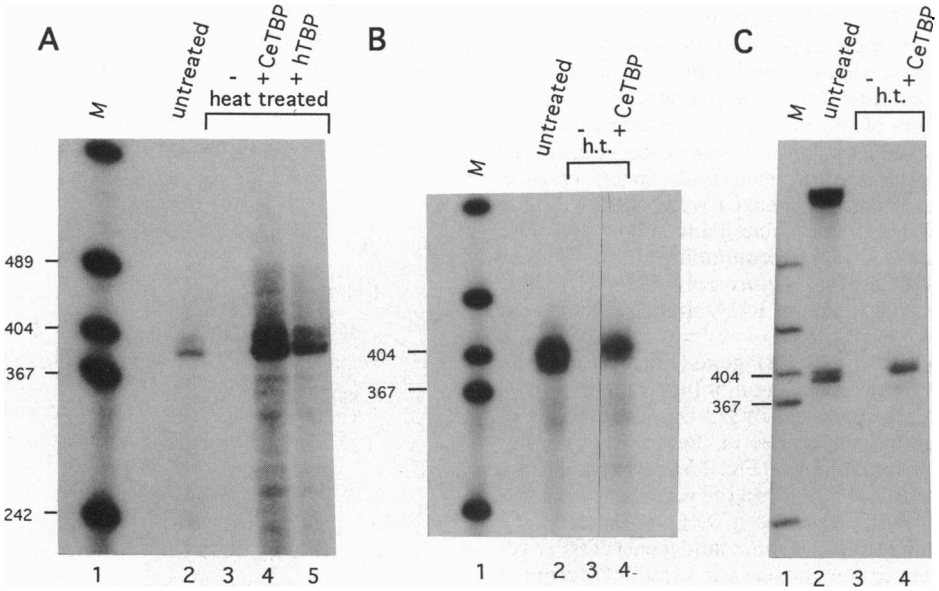

FIG. 4. Recombinant CeTBP is active for basal transcription. (*A*) Transcription of the TI promoter in HeLa nuclear extract. Lanes: 1, molecular mass markers (nucleotides); 2, untreated HeLa nuclear extract; 3, heat-treated HeLa nuclear extract; and 4 and 5, heat-treated HeLa nuclear extract supplemented with approximately 100 ng of recombinant CeTBP and 50 ng of recombinant hTBP, respectively. (*B*) Transcription of the adenovirus major late promoter in HeLa nuclear extract. Lanes: 1, molecular mass markers; 2, untreated HeLa nuclear extract; 3, heat-treated HeLa nuclear extract; and 4, heat-treated HeLa extract supplemented with approximately 100 ng of recombinant CeTBP. (*C*) Restoration of transcription of the TI promoter by CeTBP in a *C. elegans* nuclear extract. Lanes: 1, molecular mass markers; 2, untreated *C. elegans* nuclear extract; 3, heat-treated *C. elegans* nuclear extract; 4, heat-treated *C. elegans* nuclear extract supplemented with approximately 100 ng of recombinant CeTBP. The strong high molecular mass signal observed in lane 2 is not related to transcription, because transcription reactions in the absence of DNA template generate the same unspecific signal. Moreover, heat treatment of the extract nearly abolishes the generation of this signal.

with yeast TFIIA (lane 2), human TFIIB (lane 5), and *Drosophila* TFIIB (lanes 6 and 7) as judged by the presence of a diffuse but specific super-shifted complex that migrates slower than the CeTBP/DNA complex. Except for CeTBP none of the proteins tested binds to the DNA probe on its own (lanes 3, 4, and 8). These results suggest that at least some of the surfaces for interaction between TBP and TFIIA or TFIIB are conserved between the species analyzed here.

As an additional test of the purified recombinant CeTBP we determined its ability to support basal transcription *in vitro*. In such assays CeTBP not only must bind to the TATA box but also must interact appropriately with other components of the general transcription machinery to initiate transcription.

We first tested whether CeTBP was able to restore basal transcription to a HeLa nuclear extract depleted of endogenous human TBP (hTBP). It has been shown previously (27) that treatment of a HeLa nuclear extract for 15 min at 47°C selectively kills the hTBP present in the extract and presumably disrupts the TFIID complex. Furthermore, addition of recombinant hTBP restored basal activity to the heat-treated extract. Fig. 4*A*, lane 2, shows basal transcription from the TI (28) promoter in a HeLa extract. TI is a synthetic promoter which has an initiator sequence located 20 bp downstream of the adenovirus major late TATA box and drives the transcription of a G-less RNA product 400 nucleotides in length. Lane 3 shows the dramatic effect on basal transcription when the HeLa extract is heat treated: no detectable transcripts are visible. Lanes 4 and 5 represent the characteristic results after addition to heat-treated extract of purified recombinant CeTBP and hTBP, respectively. Transcription is restored by these recombinant proteins, showing that they are functional in replacing the endogenous TBP. Transcription is not only restored but significantly improved by addition of either CeTBP or hTBP. This observation can be explained by two different phenomena. First, TBP may be limiting in the extracts because addition of recombinant protein to an untreated extract can result in increased basal transcription (data not shown). Second, it is possible that the heat treatment inactivates a nonspecific inhibitor of transcription, thereby increasing the observed basal activity when recombinant TBP is added back in the transcription assay.

A similar restoration experiment was performed using the adenovirus major late (AdML) promoter. In contrast to the TI basal promoter, the AdML promoter (29) contains a binding site for the upstream stimulatory factor (USF) in addition to the TATA box and the initiator sequence. USF stimulates transcription of the AdML promoter up to 10-fold *in vitro* (30); therefore the transcription signals observed with an untreated HeLa nuclear extract correspond to activated transcription (Fig. 4*B*, lane 2). Lane 3 shows the disruption of transcription by heat treatment. Lane 4 is the result of complementing the heat-treated extract with recombinant CeTBP. The protein restores transcription to the extract but not to a level comparable to that of the untreated extract as was observed in the experiments using the basal TI promoter. This is not surprising, since it has been shown that native TFIID in multicellular organisms has several activities that the cloned TBP does not, such as the ability to respond to upstream activators.

We have recently developed an *in vitro* transcription system using nuclear extracts derived from *C. elegans* embryos, and therefore we decided to verify that the *C. elegans* recombinant TBP was also functional in this homologous nuclear extract. We assayed the TI promoter *in vitro* (Fig. 4*C*). The presence of a transcript of the correct size in lane 2 shows that untreated *C. elegans* nuclear extract supports basal transcription. Heat treatment of this extract resulted in the loss of transcriptional activity (lane 3), and lane 4 shows that addition of recombinant CeTBP to the heat-treated *C. elegans* nuclear extract restored basal transcription to a level similar to that of the untreated nuclear extract. These experiments establish that we have cloned the CeTBP gene and that the purified recombinant protein supports basal transcription in both heterologous and homologous nuclear extracts depleted of endogenous TBP activities.

This paper is, to our knowledge, the first to report the cloning of a functional basal transcription factor from the nematode *C. elegans*. The cloning of the CeTBP may serve as a valuable tool both for the study of transcription in multicellular eukaryotes in general and for the establishment of a defined *in vitro* transcription system in *C. elegans*. It should now be possible to combine the biochemical analysis of transcription factors, especially the interaction between TBP, TAFs, and activators or repressors, with a complementary genetic approach.

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