Transcription of the Acanthamoeba TATA-binding Protein Gene

A SINGLE TRANSCRIPTION FACTOR ACTS BOTH AS AN ACTIVATOR AND A REPRESSOR*

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Transcription of the Acanthamoeba TATA-binding protein (TBP) gene is regulated by TBP promoter-binding factor (TPBF), a previously described transactivator that binds as a tetramer to the TBP Promoter Element (TPE) and stimulates transcription up to 10-fold in vitro. Here we report that TPBF also functions as a transcription repressor by binding to a negative cis-element, located between the TATA box and the transcription initiation site. The negative element, referred to as the nTPE, is structurally similar to the TPE, and its disruption increases the transcription potency of the TBP promoter. TPBF binds to the nTPE, as demonstrated by mobility shift assays. However, the binding affinity of TPBF for the nTPE is about 10-fold lower than for the TPE. When placed upstream of the TATA box, the nTPE has very little effect on transcription. However, it inhibits transcription when placed at several positions downstream of the TATA box. Mechanistic studies with the TBP promoters suggest that binding of TPBF to the nTPE not only prevents TBP from binding to the TATA box but also displaces bound TBP, thereby inhibiting further assembly of the preinitiation complex. These results suggest a mechanism in which the cellular TPBF concentration controls the level of TBP gene transcription and show that a single factor can be stimulatory, inhibitory, or neutral depending on the sequence and the context of its binding site.

Modulation of gene expression at the level of transcription initiation is a major regulatory strategy for eukaryotic cells to control their responses to intra- or extracellular stimuli. Similarly, the level of production of housekeeping genes is also tightly regulated at the level of promoter efficiency. Transcription initiation on eukaryotic promoters involves the sequential addition of individual transcription factors through protein-DNA and/or protein-protein interactions (1). While accurate initiation of transcription from most eukaryotic class II promoters requires RNA polymerase II as well as a set of general transcription factors that includes TFIID,1 TFIIB, TFIIF, and TFIIE, also known as activators or repressors. Sequence-specific transcription factors bind to promoter elements via DNA-binding motifs and modulate transcription positively or negatively through direct or indirect (via coactivators) communication with the general transcription machinery (3). Most promoter elements are recognized by one single transcription factor. However, there are several examples in which one promoter element can be recognized by multiple factors or seemingly unrelated DNA elements can be recognized by a single factor (4–9).

TBP is a highly conserved eukaryotic basal transcription factor that is required for transcription by all three RNA polymerases both in vitro and in vivo (10–12). TBP can associate with distinct sets of proteins (TBP-associated factors) thereby forming the complexes TIF (13), TFIIID (14), and TFIIIB (15, 16) required for RNA polymerase I, II, and III transcription, respectively.

Due to the central role that TBP plays in eukaryotic transcription, changes to cellular TBP levels would impact all biological events occurring during cell growth and differentiation. It is thus important to understand the regulatory mechanisms that control TBP gene transcription. Although the genomic DNAs encoding TBP have been cloned from various organisms (17–20), regulation of TBP gene expression is far from fully understood. We have previously performed detailed promoter mapping studies to investigate how TBP gene transcription is regulated in Acanthamoeba. Two major cis-elements that are necessary for efficient transcription were identified. One is the TATA box, which is required for basal transcription. The other major control element is the TBP promoter element (TPE), a 23-base pair element located between positions −94 and −72 of the TBP gene promoter, which can stimulate transcription up to 10-fold in vitro (21). A regulatory protein called TPBF, which specifically binds to the TPE, was previously purified from Acanthamoeba (21, 22), and the cDNA encoding TPBF was subsequently isolated (23).

TPBF is a novel tetrameric DNA-binding protein. It contains a C-terminal coiled-coil domain, which drives tetramerization. The pattern of protein-DNA contacts between tetrameric TPBF and TPE, which resembles the proposed model for the interaction between the p53 tetramer and its target DNA (24), is distinct from that produced by other coiled-coil transcription factors (22). Our domain mapping studies also suggest that TPBF, like p53, has an apparently large central region involved in specific DNA binding. TPBF is likely to bind to other Acanthamoeba promoter elements such as that of the polypeubiquitin gene, which contains a near perfect TPE (25).

Several previous observations suggested, but did not prove, that TBP gene expression is subject to negative control by a TPBF-binding element between positions −5 and −19 of the TBP gene promoter. First, in vitro transcription of the TBP gene is surprisingly efficient given the low abundance of TBP
mRNA in vitro (17). Second, analyses of 3’ deletions showed that removal of sequences between positions 5 and 19 of the TBP gene promoter results in a 10-fold increase in transcription efficiency (26). Third, DNase I footprinting showed that at high concentrations TPBF can bind to several sites within the TBP gene promoter including the region between the TATA box and the start site (22). Finally, the addition of recombinant TPBF to extracts partly depleted of TPBF failed to show the expected stimulation of transcription in vitro.

Here we report the identification and characterization of another major cis-element, the nTPE, which is located between the TATA box and the transcription initiation site. The nTPE exerts a strong negative effect on TBP gene transcription in vitro. The nTPE is structurally similar to the TPE and is also specifically bound by TPBF. Comparison of TPBF binding affinities suggests that TPBF can sequentially bind to these two elements and thereby modulate TBP gene expression. By adding TPBF back into TPBF-free nuclear extracts, we show that the TPBF concentration determines the level of TBP gene transcription. Finally, we suggest a mechanism of TPBF-induced repression by showing that TPBF, when bound to the nTPE, actively displaces TBP from the TATA box. A working model of how TPBF regulates TBP gene expression is presented.

EXPERIMENTAL PROCEDURES

Oligonucleotides—The oligonucleotides used in this research were as follows: TPEu, 5′-AACAGGCAGGAAAACACCGATCGC-3′; TPEb, 5′-CCGAGACTTCGTTTCTTTTCACTGGTGT-3′; nTPEu, 5′-ACAGGACGTTAGAACAGGATAGG-3′; nTPEb, 5′-CCGAGACTTCGTTTCTTTTCACTGGTGT-3′; nTPEm, 5′-AACAGGACGTTAGAACAGGATAGG-3′; TPE, 5′-GCCAGACTTCGTTTCTTTTCACTGGTGT-3′; RTA4, 5′-ACGGTGAACCTGGCTTGGCTT-3′; RTA5, 5′-ACGGTGAACCTGGCTTGGCTT-3′; TPE-nTPE probe containing a double-stranded TPE DNA into the EcoRV site of the pSK(−) vector. Similarly, a wild type control construct was generated by using primers TPEu and nTPEm. Plasmid TATA1 was made by inserting a synthetic TATA box (−TATATAAG−) into the Escherichia coli promoter and KpnI sites, respectively. The same approach was used to generate plasmids TATA1-nTPEa, TATA1-nTPEb, and TATA1-nTPEm.

Affinity Purification of Anti-TPBF Serum—1 mg of purified, recombinant TPBF was fractionated on an SDS-polyacrylamide gel and transferred electrothermally to a nitrocellulose membrane. The membrane was quickly stained with 0.2% Ponceau S in 1% acetic acid. The TPBF strip was washed three times using 10 ml of phosphate-buffered saline. The strip was placed in a 0.5 ml centrifuge tube and incubated with 5 ml of crude rabbit anti-TPBF serum at 4 °C overnight (23). After the serum was removed, the strip was washed three times using 20 ml of phosphate-buffered saline. Antibodies were eluted by incubation with 1 ml of 0.1 M glycine (pH 2.5), 100 mM NaCl at 4 °C for 30 min. Purified antibodies were quickly neutralized to pH 7.0 by adding 6 μl NaOH.

Immunodepletion of TPBF from Acanthamoeba Nuclear Extract—Affinity-purified TPBF antibodies were coupled to CNBr-activated Sepharose 4B (Pharmacia) under the conditions suggested by the manufacturer. Immunodepletion was carried out in a minicolumn containing 1 ml of antibody-coupled Sepharose 4B resin at 4 °C. The column was equilibrated with CB100 (20 mM HEPES (pH 7.5), 0.2 mM EDTA, 10% glycerol, 0.2 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 0.1 mM N-tosyl-L-phenylalanine chloromethyl ketone, 2 μg/ml leupeptin, 2 μg/ml pepstatin, 1 mM DTT, and 100 mM KCl). 1 ml of Acanthamoeba nuclear extract (~5 mg in CB100) was loaded, and the flow-through was collected and subjected to another cycle of affinity chromatography. TPBF was quantitatively removed as confirmed by immunoblotting (23).

In Vitro Transcription and Primer Extension—Transcription and primer extension assays were performed essentially as described (21, 23). Briefly, 200 μg of supercoiled plasmid templates were incubated with 50 μg of Acanthamoeba nuclear extracts in 50–μl reactions containing 20 mM HEPES (pH 7.5), 10 mM MgCl2, 1 mM DTT, 15 mM potassium acetate, 0.02 mM EDTA, 2% glycerol, 1 unit of RNasin (Life Technologies, Inc.), and 0.4 mM each NTP. Reactions were carried out at 30 °C for 1 h. Transcripts were extracted with phenol-chloroform twice, precipitated with ethanol, and dissolved in 10 μl of annealing buffer containing 20 mM Tris-HCl (pH 8.3), 400 mM KCl, and 50,000–100,000 cpm of 32P-labeled primer. Annealing was performed by slowly cooling from 65 °C to room temperature. Primer extensions were started by adding 4 μl of 10 × RT buffer (500 mM Tris-HCl (pH 8.3), 60 mM MgCl2, 25 mM DTT), 4 μl of dNTPs (2.5 mM each), 1 unit of RNasin, and 20 units of Superscript II into a total volume of 40 μl, followed by incubation for 1 h at 45 °C. Primer extension products were precipitated and analyzed by electrophoresis in a 6% polyacrylamide, 8 μm urea gel in TBE buffer (27).

Purification of Recombinant TPBF and TBP—Purifications of recombinant TPBF and TBP proteins were performed essentially as described elsewhere (23, 28). Briefly, the pET3a vector containing the TPBF or TBP cDNA was freshly transformed into Escherichia coli strain LE392. The E. coli cells were grown to an A500 of 0.8 and infected with acE6 (Novagen). Cells were harvested after 3.5 h of infection, and the cells were lysed by sonication. For TPBF purification, the cell lysate was subjected to nickel affinity chromatography. TPBF was eluted with 300 mM imidazole (pH 7.5). Fractions containing TPBF were pooled and concentrated using a Centricon 10 spin column (Amicon). Purified proteins were stored at −70 °C in buffer containing 20 mM HEPES (pH 7.5), 10% glycerol, 0.1 mM EDTA, and 0.2 mM phenylmethylsulfonyl fluoride.

For the production of recombinant Acanthamoeba TBP, the cell lysate was applied to DEAE-cellulose (Whatman) equilibrated with column buffer (20 mM HEPES (pH 7.5), 10% glycerol, 0.1 mM EDTA, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1% Triton X-100) containing 200 mM KCl. The flow-through was collected and applied to an Affi-gel heparin column (Bio-Rad) equilibrated with column buffer containing 200 mM KCl. TPBF was eluted with column buffer containing 500 mM KCl. Active fractions, as judged by mobility shift assay, were pooled together and dialyzed against column buffer containing 100 mM KCl. TPBF was purified to ~80% homogeneity.

Gel Mobility Shift Assays and Probe Preparations—Approximately 0.5 ng of 32P-labeled probe was mixed with the indicated amount of protein in each reaction. The binding reactions were carried out in a total volume of 15 μl containing 20 mM HEPES (pH 7.5), 50 mM KCl, 7.5 mM MgCl2, 0.2 mM EDTA, 10% glycerol, and 0.5 mM DTT at 30 °C for 20 min. In the experiments shown in Fig. 6, HindIII treatment was done by adding 10 units of enzyme and incubating for an additional 10 min. Probes containing single TPE or nTPE elements were produced by annealing appropriate complementary oligonucleotides and end-labeling using T4 polynucleotide kinase. Full-length TBP promoter was made by PCR amplification from the plasmid template TBP-97 (21) using primers TPEu and nTPE. TPE-nTPE probe containing a HindIII site was made by inserting TPE and nTPE elements into the EcoRV and HindIII sites of the pSK(−) vector, respectively, and then amplified using primers TPEu and nTPEu. All PCR products were gel-purified and labeled with 32P. Probe TPE-nTPE labeled with 32P at either the TPE side or the nTPE side was generated by PCR using an appropriate 32P-labeled primer and its corresponding pair. Probe TATA-nTPE was generated by PCR amplification of TBP-35 template (21) using primers KS and nTPEb.

RESULTS

The TBP Gene Promoter Contains a Negative cis-Element between the TATA Box and the Transcription Initiation Site—Inspection of the DNA sequence between the TATA box and the transcription initiation site of the TBP gene promoter identifies a sequence that is similar to the upstream TPBF recognition site, the TPE, but inverted (Fig. 1A). In order to consolidate earlier results (see the Introduction) and to establish that this

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sequence functions as a negative element in the context of the native TBP gene promoter, we made a linker-scanning mutant in which the run of T nucleotides between nucleotides 214 and 28 of the TBP promoter are replaced with 5′-CGCGAGC-3′ (Fig. 1B, top). The promoter activity of the linker-scanning mutant was tested by in vitro transcription and was found to be 4-fold higher than that of a wild type promoter (Fig. 1B, bottom). This result clearly demonstrates that the sequence is a negative element in the context of the TBP promoter. The 4-fold effect of the linker-scanning mutagenesis is less than the 10-fold effect found with a simple deletion to 219 (26). This could be because the TPE-related sequence is not completely disrupted by linker scanning. However, as shown below, the nTPE is both necessary and sufficient for negative regulation of the TBP gene.

TPBF Acts as an Activator and a Repressor

The nTPE, a pair of complementary oligonucleotides corresponding to nucleotides −24 to +2 of the TBP promoter were synthesized. Mobility shift assays using purified recombinant TPBF clearly show an interaction between the nTPE and TPBF (Fig. 2B, lane 3). The interaction was specific, as suggested by both antibody supershift and competition experiments in which the TPE, but not a mutant TPE, prevent binding to the nTPE (Fig. 2B). A TPBF deletion mutant, in which the tetramerization region is removed, totally abolished the interaction, indicating that TPBF tetramerization is also necessary for nTPE binding. Moreover, the complex between TPBF and the nTPE has the same mobility as the complex between TPBF and the TPE. We infer that TPBF binds to the nTPE as a tetramer as well.

The Inhibitory nTPE Is a Much Weaker TPBF Binding Element than the Activating TPE—In order to further characterize the nTPE and understand its function in TBP gene expression, we compared the affinities of TPBF binding to the TPE and to the nTPE. A TPBF titration experiment was done with identical amounts of either the TPE or the nTPE in mobility shift assays (Fig. 3). Quantitative analyses of the intensity of the shifted bands indicated that 10-fold more TPBF was needed for the nTPE probe to achieve the same level of occupancy as the TPE probe. The affinity between TPBF and the TPE is therefore approximately 10-fold higher than that between TPBF and the nTPE.

The difference in binding affinities between TPBF and these two binding sites provides a hypothetical basis for regulation of TBP gene expression by TPBF. At low concentration, TPBF predominantly occupies the high affinity site that leads to transcription activation. At higher concentrations, it occupies
both binding sites and inhibits transcription. To directly test this hypothesis, we synthesized a probe containing both the TPE and the nTPE with a HindIII site between them (Fig. 4, top). When the probe was tested for TPBF binding using a mobility shift assay, we observed one single complex at low TPBF concentration (Fig. 4). With increasing amounts of TPBF, a second higher molecular weight complex appeared. A smooth transition from the lower molecular weight complex to the higher molecular weight complex was observed as the TPBF concentration increased. When the TPBF amount reached 100 ng, only the higher molecular weight complex, corresponding to occupancy of both sites, was present.

In order to determine the order of TPBF binding to the TPE and nTPE sites, we labeled the probe at either the TPE end or the nTPE end and incubated it with TPBF at a concentration that only generated the lower molecular weight complex (Fig. 4, lanes 2 and 9). When the binding reactions were treated with HindIII, the band between TPBF and the nTPE end-labeled probe completely disappeared (Fig. 4, lane 8), suggesting that the band was not due to binding to the nTPE. This was confirmed by the result that the band between TPBF and the TPE end-labeled probe was retained after HindIII digestion (Fig. 4, lane 1). A minor molecular weight change occurred because of trimming of the probe (Fig. 4, compare lanes 1 and 2). These data demonstrate that at low concentration TPBF only binds to the TPE site and that binding to both sites occurs only when the TPBF concentration reaches a certain threshold. We have failed to observe any evidence for cooperative binding by TPBF to the TPE and nTPE in any context (data not shown).

The nTPE Inhibits Transcription When Placed Further Downstream of the TATA Box—To further investigate how the nTPE inhibits transcription and to demonstrate that the nTPE is sufficient for repression, we analyzed the effect of the nTPE on transcription when it is positioned at various sites within a heterologous promoter. The promoter that we used contains a synthetic TATA box, which is able to direct RNA polymerase II transcription in both orientations. In previous studies we showed that the positive element, the TPE, is able to stimulate downstream transcription and inhibit transcription toward it in this promoter context (29). Here, we tested whether the nTPE has similar effects on bidirectional transcription as those of the TPE. We subcloned the nTPE DNA fragment into three different sites (Fig. 5, top), which are 15, 36, and 55 base pairs downstream of the TATA box, respectively, and analyzed the effect of the nTPE on transcription. We employed a primer derived from the pSK(−) vector sequence and assayed the transcription activity of the constructs by primer extension. As shown in Fig. 5, the nTPE was able to repress transcription toward it in any of these three positions as efficiently as the TPE (Fig. 5, compare lanes 12–14 with lanes 9–11). However, unlike the TPE, which is able to stimulate downstream transcription severalfold (Fig. 5, compare lane 1 with lanes 2–4), the nTPE was not able to either activate or repress downstream transcription in any of these positions (Fig. 5, compare lane 1 with lanes 5–7). These data demonstrate that 1) the
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Fig. 6. TPBF is the only protein responsible for activation and repression. A, basal transcription remains unchanged after TPBF depletion. Immunodepletion, Western blotting, and in vitro transcription assays were performed as described under “Experimental Procedures.” On the left and in the middle are Western blots assaying TPBF and TBP levels, respectively, in nuclear extract before and after immunodepletion. On the right is the transcription assay of the TATA1 promoter (diagramed in Fig. 5), using nuclear extract before and after immunodepletion. The indicated amounts of TPBF were included to promoter (diagramed in Fig. 5), using nuclear extract before and after immunodepletion. The indicated amounts of TPBF were included to promoter (diagramed in Fig. 5), using nuclear extract before and after immunodepletion. The templates assayed and the primers being used to detect the transcripts are indicated at the top and bottom, respectively. Template TBP-97 is the 5′ deletion of the TBP promoter up to nucleotide −97. Template TATA1-TPEa is diagrammed in Fig. 5. RTA3 detects downstream transcription, while RTA4 detects transcription toward the TPE. Whether nuclear extract was TPBF-depleted or not and how much TPBF was added are indicated above each lane. Specific products are shown by triangles. C, TPBF is responsible for nTPE-induced repression in a native TBP promoter, TBP-35. Lanes 1 and 2 show transcription activities from a control nuclear extract and a TPBF-depleted nuclear extract, respectively. Lanes 3–7 show transcription activities after the indicated amounts of recombinant TPBF were added back into the TPBF-depleted nuclear extract. Transcripts were detected by primer extension with primer TBP.

TPBF Is Necessary and Sufficient for Activation and Repression—Although TPBF can bind to both the TPE and the nTPE, it was possible that other proteins were also responsible for their effects on transcription. To directly examine the effect of TPBF on activation and repression, we established a TPBF-free Acanthamoeba nuclear extract. As shown in Fig. 6A, we depleted TPBF from a nuclear extract using an affinity-purified anti-TPBF column. Immunodepletion completely removed TPBF (Fig. 6A, left) while not affecting the level of TBP or basal transcription (Fig. 6A, middle and right). We then compared levels of activated transcription supported by the TPBF-depleted nuclear extract with levels supported by normal nuclear extract from promoters containing a TPE. For both the natural TBP promoter and the TATA1-TPEa promoter, depletion of TPBF from the nuclear extract completely abolished the elevated transcription level that was observed with the control nuclear extract (Fig. 6B, lanes 1 and 2 and lanes 5 and 6). The direct involvement of TPBF in transcription repression was also investigated by assaying transcription toward the TPE in the TATA1-TPEa promoter (Fig. 6B, lanes 9–12). We detected at least a 5-fold transcription recovery after TPBF was removed from nuclear extract (Fig. 6B, compare lanes 9 and 10).

To further establish that TPBF is directly responsible for activation and repression, we added purified recombinant TPBF to the TPBF-depleted nuclear extract. We were able to successfully recover both activation and repression with the TATA1-TPEa promoter by adding 100 or 200 ng of recombinant TPBF (Fig. 6B, lanes 7, 8, 11, and 12). However, we were unable to recover activation from a wild type TBP promoter by adding the same amounts of recombinant TPBF (Fig. 6B, lanes 3 and 4). These contradictory results prompted us to analyze the role of TPBF in a minimal TBP promoter (TBP-35) that retains the nTPE (Fig. 6C). Transcription of TBP-35 increased when TPBF was removed from nuclear extracts by immunodepletion (Fig. 6C, lanes 1 and 2). The increase was eliminated by adding back 50 ng of recombinant TPBF (Fig. 6C, lanes 3–5). The activity of TBP-35 was further repressed by adding 100 or 200 ng of TPBF (Fig. 6C, lanes 6 and 7). Overall, the above data demonstrate the presence of both positive and negative TPBF response elements in the TBP gene promoter and show that adding back TPBF has different effects on activation and repression, depending on the context of its binding site.

TPBF Gene Expression Is Regulated by a Combination of TPBF-induced Activation and Repression—Given these results, it seemed likely that the expression level of the TBP gene is determined by the sum of TPBF-induced activation and repression. To test this possibility, we performed a TPBF titration experiment on the TBP promoter with TPBF-depleted
nuclear extract. The addition of up to 75 ng of recombinant TPBF gave a significant level (3-fold) of transcription recovery from the TPBF-depleted nuclear extract (Fig. 7). However, maximum recovery was achieved with only 10 ng of recombinant TPBF. Greater amounts of TPBF started to inhibit transcription, which eventually dropped to levels lower than basal transcription (Fig. 7). TPBF is thus able to inhibit both activated transcription and basal transcription. We infer that TPBF modulates TBP gene transcription in a concentration-dependent manner.

TPBF Prevents Binding and Can Displace TBP from the TBP Promoter—Because the nTPE element is located between the TATA box and the transcription initiation site, an attractive mechanism of repression by TPBF would involve blocking the assembly of the basal transcription machinery. In order to determine whether TPB and TPBF could simultaneously occupy the TATA box and the nTPE, respectively, a TBP promoter fragment containing only the TATA box and the nTPE site was used in a mobility shift assay. 50 ng of TPBF was incubated with the probe for 15 min, followed by the addition of either 50 or 100 ng of TBP and another 15-min incubation. There was no change between TPBF alone and TPBF incubated with TBP (Fig. 8, compare lane 2 with lanes 3 and 4), suggesting that TBP cannot bind to the TATA box when TPBF is bound to the nTPE.

Reversing the order of addition, we first incubated either 50 or 100 ng of TBP with the probe for 15 min and then added 50 ng of TPBF and incubated for another 15 min. In the absence of TPBF, a distinct complex generated by the binding of TBP to the TATA box was visible (Fig. 8, lanes 5 and 6). The complex completely disappeared with the addition of TPBF (Fig. 8, lanes 7 and 8). The absence of a supershifted complex suggests that the complex between TBP and the probe was disrupted by the binding of TPBF. These data indicate that TPBF, once bound to the nTPE, is able to prevent TBP binding and, furthermore, that it is able to disrupt the interaction between TBP and the TATA box.

DISCUSSION

We have shown that both activation and repression of the *Acanthamoeba* TBP gene promoter are mediated by the transcription factor TPBF. TPBF functions as an activator when bound to a cis-element located upstream of the TATA box. It functions as a repressor when bound to the lower affinity sequence located between the TATA box and the transcription initiation site. These observations permit construction of a general model describing how the cellular TBP level is controlled (Fig. 9). At a relatively low TPBF level, the TBP gene is highly expressed due to occupancy of the high affinity TPE. As the TPBF level increases, the lower affinity nTPE becomes occupied. This leads to a decrease in the rate of TBP gene expression by counteracting the effect of activating TPBF. If the TPBF level reaches a certain point, TBP gene expression can be completely shut off.

In addition, these data show that the DNA sequence of the TPBF binding site determines whether bound TPBF can activate transcription. In this respect, TPBF is similar to MyoD, which also activates only when bound to particular DNA se-
As the TPBF level increases, as shown at the top, the TPBF gene is highly expressed due to TPBF occupancy of the high affinity TPE site. At the low TPBF concentration, as shown at the bottom, the lower affinity nTPE site is occupied. This leads to a decrease of TBP gene expression by counteracting the effect of activating TPBF.

Fig. 9. Model of regulation of TBP gene expression. At a relatively low TPBF concentration, as shown at the top, the TBP gene is highly expressed due to TPBF occupancy of the high affinity TPE site. The lower affinity nTPE site is occupied. This leads to a decrease of TBP gene expression by counteracting the effect of activating TPBF.

Transcription repression can be achieved by a variety of distinct mechanisms. Repressors can inhibit transcription of their target genes by inhibiting the functions of activators by simply competing for the binding sites on the promoters (33–35). Repressors can also inhibit transcription through down-regulation of the DNA-binding or transcription activity of activators by forming complexes with them (36, 37). By targeting activators, these classes of repressors by and large only inhibit activated transcription. There is a third class of repressors that directly target a component(s) of the basal transcription machinery rather than inhibiting specific transcription activators. This class of repressor can destabilize or inhibit the formation of initiation complexes by targeting basal factors such as TFIID/TBP (38–41), TFIIB (5, 42), polymerase II (43), and TFIIE (44). For example, Dr1 prevents TFIIA and/or TFIIB from being recruited to the preinitiation complex by itself binding to TBP (40). The human cytomegalovirus immediate early protein 2 (IE2) (43) reportedly inhibits transcription by binding to an element located between the TATA box and the initiation site and blocking the recruitment of RNA polymerase II to the promoter. TPBF belongs to this third class of repressor. It functions by inhibiting the first essential step during transcription initiation, the association of TBP with the TATA box. While the exclusion of TBP from the TATA box is likely due to steric hindrance, it is somewhat surprising that TPBF is able to displace TBP from the TATA box. This mechanism of displacement gives TPBF the ability to dismantle an already assembled initiation complex and shut off TPBF gene transcription completely, which might be necessary during some developmental stages in Acanthamoeba.

The ability of regulatory proteins to act as either repressors or activators depending on the context of their binding site relative to other promoter elements is emerging as an important theme in transcription regulation (45, 46). λ repressor provides an example of a phage repressor that, at low concentrations, can activate transcription from the cl promoter by binding to the O2 and O3 sites within the operator. At higher concentrations, it inhibits transcription by binding to the lower affinity O3 site in the operator (47). Indeed, since λ repressor does not bind cooperatively to O3, this mechanism is strikingly analogous to that used within the Acanthamoeba TPBF gene promoter.

In eukaryotic systems, several regulatory proteins can act as activators or repressors depending on context or concentration, such as herpes simplex virus ICp4 (5), Drosophila Kruppel (44, 48), the human Kruppel-related factor YY1 (8, 9), and p53 (49). Various mechanisms are involved in the action of these factors. For example, YY1 activates transcription by binding to the initiator sequence of the adenovirus P5 promoter (9), but YY1 can act as a repressor by binding to an upstream element (8). While the action of YY1 depends on the context of its binding sequence, Drosophila Kruppel determines activation or repression solely based on its concentration. Monomeric Kruppel interacts with TFIIB to activate transcription, whereas dimeric Kruppel, as a result of an increase in concentration, interacts with TFIIE to repress transcription (44). Our work with TPBF describes another protein of this type with additional novel features. TPBF appears to be the first eukaryotic transcription factor whose action is determined by both its concentration and the sequence context of its binding sites. TPBF binds to two structurally similar elements with different affinities. Our data show that a strong interaction between TPBF and its binding site is required for transactivation, while only a modest interaction is sufficient for repression (Fig. 5). Although both TPBF-induced activation and repression are likely to be carried out through its interaction with basal factors, different mechanisms are clearly involved. While the mechanism of activation by TPBF is somewhat unclear, the close proximity of the nTPE to the TATA box suggests a mechanism for repression. Association between TBP and the TATA box, which is the first step during transcription initiation, is impeded by the binding of TPBF to the nTPE. Deciding between activation and repression as a consequence of the strength of interaction between transcription factors and their responding elements, as well as the location of these elements with respect to the TATA box could be a regulatory mechanism employed widely by cells.

In summary, we have described a eukaryotic promoter that is regulated in a fashion formally analogous to the λ repressor.
system. Since the rate of TBP gene expression is controlled by the cellular concentration of TPBF, it will also be of interest to determine how the level of TPBF is regulated, particularly since TPBF is likely to be involved in transcription of other genes, such as polyubiquitin (25). One working model is that TPBF gene expression is controlled at the basal level, specifically by the concentration of TPBF. An increase in TPBF concentration would stimulate TPBF transcription, which in turn would cause repression of the TBP gene by TPBF.

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