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TBP – DNA interactions in the minor groove discriminate between A:T and T:A base pairs

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ABSTRACT

In this report, we test the hypothesis that TBP binds DNA promiscuously due to its manner of recognition of the DNA minor groove. The experiment performed was to select TBP-binding sequences from a pool of random double stranded oligonucleotides. Sixty two clones from this pool were sequenced. Surprisingly, the results show that TBP has a marked preference for stably binding one sequence (TATATAA) over all others, yet only four classes of TATA box were selected. The features of the selected sequences allow definition of a binding consensus for TBP. The DNA binding properties of TBP to the four TATA variants was examined, the results being in accord with the observed selection frequencies. However, the nature of TBP – DNA binding is strongly affected by ionic strength. We infer that recognition of DNA via the minor groove can be highly selective even where A:T and T:A discrimination is required. Models for how this might be accomplished are discussed.

INTRODUCTION

Sequence-specific protein–DNA interactions have been viewed as occurring by way of the major groove because of the extensive differences in hydrogen bonding potential of individual base pairs (1,2). The TATA binding protein, TBP, is an essential eukaryotic transcription factor (3) that binds DNA through minor groove interactions with DNA sequences resembling 5′ TATAAAA 3′ (4,5). In accord with the apparently limited opportunity for proteins to distinguish bases in the minor groove, TBP may be able to functionally interact with numerous DNA sequences (6–9).

TBP plays a pivotal role in the assembly of transcription preinitiation complexes (3,10–12). Of the core factors, TBP alone is able to specifically recognize and bind DNA. Although its DNA binding specificity may be modified by TAFs *in vivo* (see 13,14), TBP DNA binding specificity is relevant to the larger question of whether minor groove interactions can accomplish the specificity required of a DNA binding transcription factor.

In this report, we test the hypothesis that TBP binds DNA promiscuously due to its manner of recognition of the DNA minor groove. The experiment performed was to select and clone TBP-binding sequences from a pool of random double stranded oligonucleotides. Surprisingly, our results show that TBP has a marked preference for stably binding the sequence (TATATAA) over three other TATA-like sequences selected. Recognition of DNA via the minor groove can therefore be highly selective even where A:T and T:A discrimination is required.

MATERIALS AND METHODS

Preparation of TBP

Acanthamoeba TBP, expressed in *E.coli* (15), was purified to ~90% homogeneity by successive chromatography steps on DEAE-cellulose, heparin–sepharose and mono-S FPLC. The concentration of active TBP was determined essentially as described elsewhere (16). All comparisons of DNA binding were made with the same batch of TBP.

Preparation of random, double strand DNA probes

An eighty four base oligonucleotide containing forty randomized positions (Figure 1) was chemically synthesized using standard procedures. To generate double strand DNA from the randomized oligonucleotide, the 84 mer was annealed to a synthetic primer (primer 2) followed by synthesis of the complementary strand with the klenow fragment of DNA polymerase I under standard conditions (17). Double stranded DNA was subsequently purified from an 8% polyacrylamide gel. The sequences of primers and the randomized template are shown in Figure 1. While it is unlikely that the starting oligonucleotide pool contains every possible sequence (4^{40} sequences), by sequencing several clones we found that no bases were over-represented at any position within the randomized portion.

Selection of TBP DNA binding sites

Electrophoretic mobility shift (EMS) assays were performed by binding TBP to the 84 mer in a buffer containing 20 mM Hepes (pH7.9), 5mM $MgCl_2$, 5mM DTT, 150mM KCl, 4μg poly(dG–dC)/ml, 0.1% Triton and 10% glycerol. Approximately 40 ng of the 84 bp duplex and 20 nmol TBP were incubated in 20 μl at 30°C for 30 minutes. Free and bound DNA were separated on a 4% polyacrylamide gel in 0.5×TGEM

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buffer. The gel was prerun at 4 °C for 1 h and run for 2 h at 200 V after loading. After autoradiography, the bound DNA was cut out from the gel, electroeluted and used for subsequent cycles.

PCR amplification of selected DNA

PCR amplification of selected DNA was done by standard procedures. Because of the internal degeneracy of the 84 bp template DNA, which hinders correct re-annealing after PCR, and the high affinity of TBP for single stranded DNA, DNA was denatured, annealed with the two primers and the DNA made double stranded with Klenow fragment. The DNA was subsequently treated with mung bean nuclease to remove any remaining single stranded DNA. The amplified template was gel purified and electroeluted. After six rounds of selection, DNA was amplified and digested with *Xho*I and *Xba*I. Digested DNA was then cloned into pBluescript and sequenced.

DNase 1 footprinting

Footprinting assays were performed essentially as described previously (15), except that binding reactions were the same as for selection as described above. Additions of competitor or other variations are noted in the appropriate figure legends.

RESULTS

Selection of TBP binding sites from a random DNA population

TBP binding sites were selected from an oligonucleotide pool containing random, double stranded DNA sequences using electrophoretic mobility separation of free and TBP-bound oligonucleotides (18,19), as diagrammed in Figure 1. After each round of selection, DNA that bound TBP was amplified by PCR and the selection repeated. This process resulted in increasing efficiency of TBP binding to DNA within the selected pool through four or five rounds (Figure 1). In addition, an upper band appears in the gel shift assay of selected sequences, due to the presence of molecules that contain two TATA boxes. The population of DNA molecules obtained after six rounds of selection was sampled by cloning. Sixty two such clones were sequenced, of which fifty four contain a single TATA box (Table 1).

The selected population contains just four classes of TATA element (Table 1). The sequence TATATAA occurs in 35/54 clones sequenced, TATATATA, or longer runs of alternating A/T, occur in 14/54 clones, TATAAATA occurs in 4/54 clones and the TATA sequence found most commonly at natural promoters—TATAAAA, only occurred once. No clones contain C or G at any position within the TATA box. Although we have classed alternating A/T as a single sort of TATA element, because these range in size from eight to twelve residues, the selection of fourteen such clones may be biased, since these clones essentially contain overlapping binding sites for TBP.

The TATA elements of selected clones bind TBP

We confirmed the identity of each of the four types of TATA box by using DNase 1 footprinting, which shows that the TATA region of each clone is responsible for TBP binding, not a cryptic TATA sequence in the same clone (Figure 2). This analysis also shows that in cases where two TATA boxes were obtained within a single clone, both are functional, but differ in the extent of protection afforded by TBP (Figure 2, lanes 9 and 10). Clones

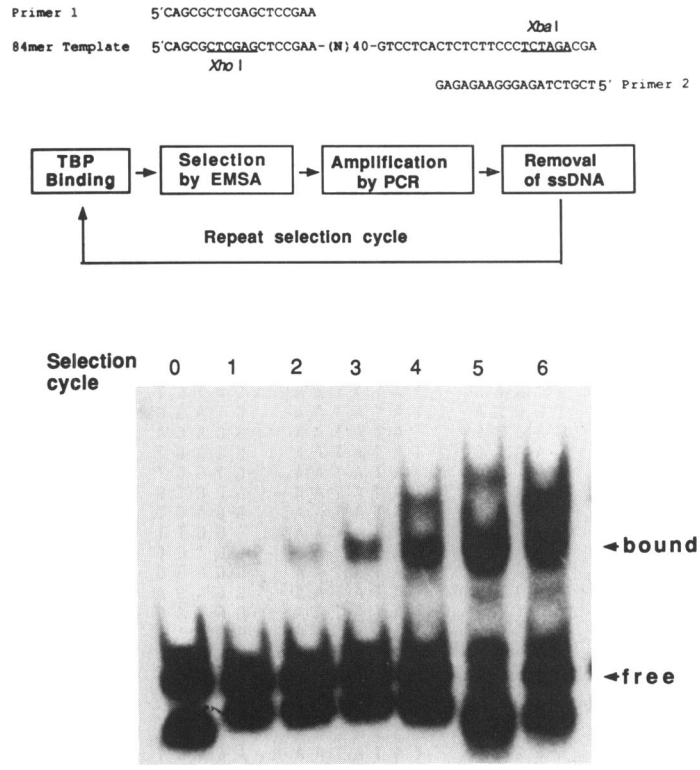

Figure 1. Selection of TBP DNA binding sequences. General scheme for the *in vitro* selection of TBP-binding sequences, showing the sequence of the oligonucleotides used for *in vitro* selection of TBP binding sequences and subsequent PCR. The results of EMS assays of TBP DNA-binding after selection in each cycle are shown.

containing two TATA boxes were not scored in the frequency analysis above.

Identification of preferred sequences adjacent to the TATA element

Because the sequence TATATAA occurred in 35/54 clones sequenced, we were able to determine, by aligning the TATATAA sequences, whether any bases outside the A−T rich region were preferentially selected (Table 2). This analysis shows clearly that there are preferences for particular base pairs on either side of the TATA box, suggesting that base pairs flanking the TATA element *per se*, can have a measurable effect on TBP binding. A consensus from the data of Table 2 is as follows:

5′ A/G G A/G G/C T A T A T A A G G/C 3′.

The other classes of TATA element shown in table 1 also display distinct preferences for certain bases outside the TATA box, but because of the limited number of clones we have not attempted to derive a consensus for each. We note that this experiment is akin to the principle behind chemical interference assays (4,5), but in the present case, we are discriminating among naturally occuring bases.

K_{eq} measurements for TBP bound to different TATA sequences

The over-representation of TATATAA in our pool suggests that TBP binds this sequence preferentially. Moreover, because of the electrophoretic mobility shift method used for selection, the

Table 1. TBP DNA binding sequences selected by EMS. Four classes of TATA boxes were isolated by the selection procedure as shown. The sequences of all clones are shown

TATATAA			
1.	G G G A G	T A T A T A A	G C G C G
2.	G C A G G	T A T A T A A	G T C A G
3.	T C G A C	T A T A T A A	G C A A G
4.	C A G G C	T A T A T A A	G G G T C
5.	A G G A C	T A T A T A A	A G A T C
6.	A A C A C	T A T A T A A	G G A G C
7.	G C C T G	T A T A T A A	G C C A G
8.	A A C A C	T A T A T A A	G G A G C
9.	G C C T G	T A T A T A A	G C C A G
10.	C T A A G	T A T A T A A	G C C C A
11.	G G G G G	T A T A T A A	G G C T T
12.	A T G A C	T A T A T A A	G C A T T
13.	G C G A G	T A T A T A A	C A T G T
14.	T G G A A	T A T A T A A	G T A G G
15.	G A G T G	T A T A T A A	G C A C A
16.	C A G A C	T A T A T A A	G G G G T
17.	G A T A G	T A T A T A A	G T C C T
18.	C C G A C	T A T A T A A	G C G T G
19.	G A A G A	T A T A T A A	G G A G C
20.	G G G G G	T A T A T A A	C G G T T
21.	T A A G G	T A T A T A A	G C T C G
22.	G A G G G	T A T A T A A	G G C G G
23.	A A G A C	T A T A T A A	G G A G A
24.	T C T A G	T A T A T A A	G G G A G
25.	T G G C C	T A T A T A A	G G C T A
26.	A A A G G	T A T A T A A	G G G G G
27.	C A G G G	T A T A T A A	G G G C T
28.	C A G A C	T A T A T A A	G G G G T
29.	A G G G G	T A T A T A A	G C A T G
30.	G T G T G	T A T A T A A	A C C C A
31.	G G G A G	T A T A T A A	A C C C T
32.	T T C A C	T A T A T A A	G G T C T
33.	A A C G C	T A T A T A A	G C A T T
34.	C G C T A	T A T A T A A	G G T A T
35.	A A G G A	T A T A T A A	G G G A A
Alternating A/T			
36.	C G A G C	T A T A T A T A	G G A T T
37.	G A T G C	T A T A T A T A	C T T C T
38.	G T A G G	T A T A T A T A T	G A A C G
39.	A A G A G	T A T A T A T A T A	C C A G A
40.	A A C T G	T A T A T A T A	C A C A T
41.	T T C A C	T A T A T A T A	G T G T C
42.	A G T G G	T A T A T A T A	C T C G G
43.	G A A G A	T A T A T A T A T A T	C G C T C
44.	A G T A C	T A T A T A T A	C T C T T
45.	T T T A C	T A T A T A T A	C T C T C
46.	G A G T C	T A T A T A T A	G C T A G
47.	G T C G G	T A T A T A T A T A	C C A G T
48.	G T T C T	T A T A T A T A T A	G T C C T
49.	G C T G G	T A T A T A T A	G G G A C
TATAAATA			
50.	G T G A G	T A T A A A T A	C G T C G
51.	C A G G G	T A T A A A T A	C T T C A
52.	C A G A G	T A T A A A T A	C T G A G
53.	T A G G G	T A T A A A T A	C T C C G
TATAAAA			
54.	A A G A G	T A T A A A A	G A G A G

Table 2. The frequency of occurrence of individual bases within clones containing a TATATAA sequence are shown. The consensus deduced from these data is also shown

	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12
A	9	15	5	17	4	0	35	0	35	0	35	35	3	1	11	7	6
G	13	9	21	12	18	0	0	0	0	0	0	0	30	18	10	10	12
C	7	7	7	1	13	0	0	0	0	0	0	0	2	13	10	9	7
T	6	4	2	5	0	35	0	35	0	35	0	0	0	3	4	12	9
	N	a/g	G	A/G	G/C	T	A	T	A	T	A	A	G	G/C	N	N	N

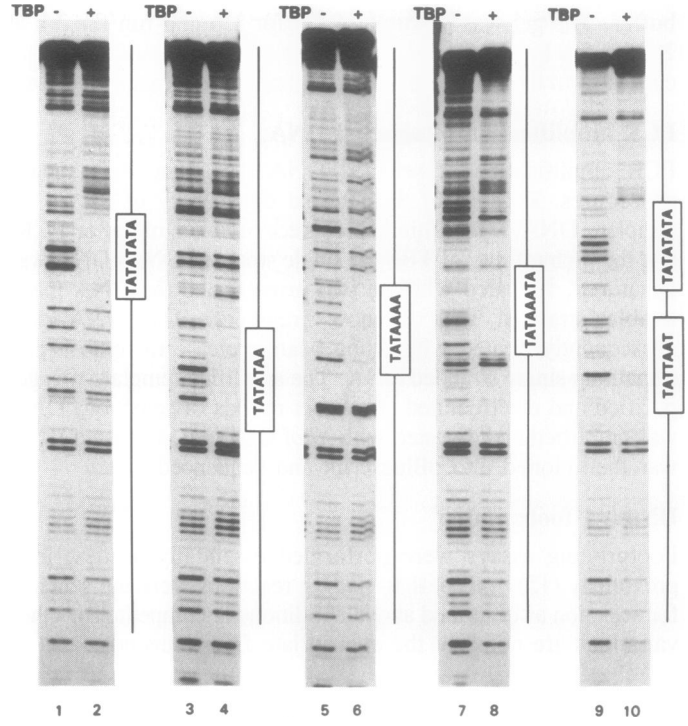

Figure 2. DNase 1 footprinting of selected TATA elements. Individual representative clones were used in TBP DNase footprinting assays. The sequence of each TATA element and the protected region(s) are indicated. A footprint assay for a double-TATA box is also shown.

result implies that TBP binds this sequence more stably than other TATA-like sequences (20). In order to test this notion, we determined the equilibrium binding constant of TBP bound to TATATAA with that for TBP binding to the three other variants of the TATA box. K_{eq} for different types of TATA sequences was estimated by DNase footprinting at varying concentrations of TBP (Figure 3). The point at which 50% binding is observed provides a value for K_{eq}. This analysis was corrected for the concentration of active TBP, and all values were determined at least twice. Values for K_{eq} obtained for the various TATA variants are shown in Table 3. TBP has a higher affinity for the sequence TATATAA (1.1×10^{-9}M) as compared to TATAAAA 3.7×10^{-9}M), TATATATA (1.4×10^{-9}M) and TATAAATA (1.6×10^{-9}M). Thus, the sequence TATATAA is the most efficiently bound by TBP, and TATAAAA is the least avidly bound.

Stability of TBP–DNA complexes

We next determined the half life for TBP–DNA complexes at various TATA boxes, in order to examine the possibility that the values for k_{eq} reflect differences in stability of the TBP–DNA complexes. In this case, excess unlabeled competitor DNA was added to TBP–DNA complexes, and the fraction of TBP remaining bound after various intervals was assessed by footprinting (Figure 4). The relative stability of the various complexes determined from this analysis is as follows: TATATAA > TATATATA > TATAAATA > TATAAAA. We infer that the stability of TBP bound at these TATA elements largely accounts for differences in k_{eq}, as well as the selection frequencies for each type.

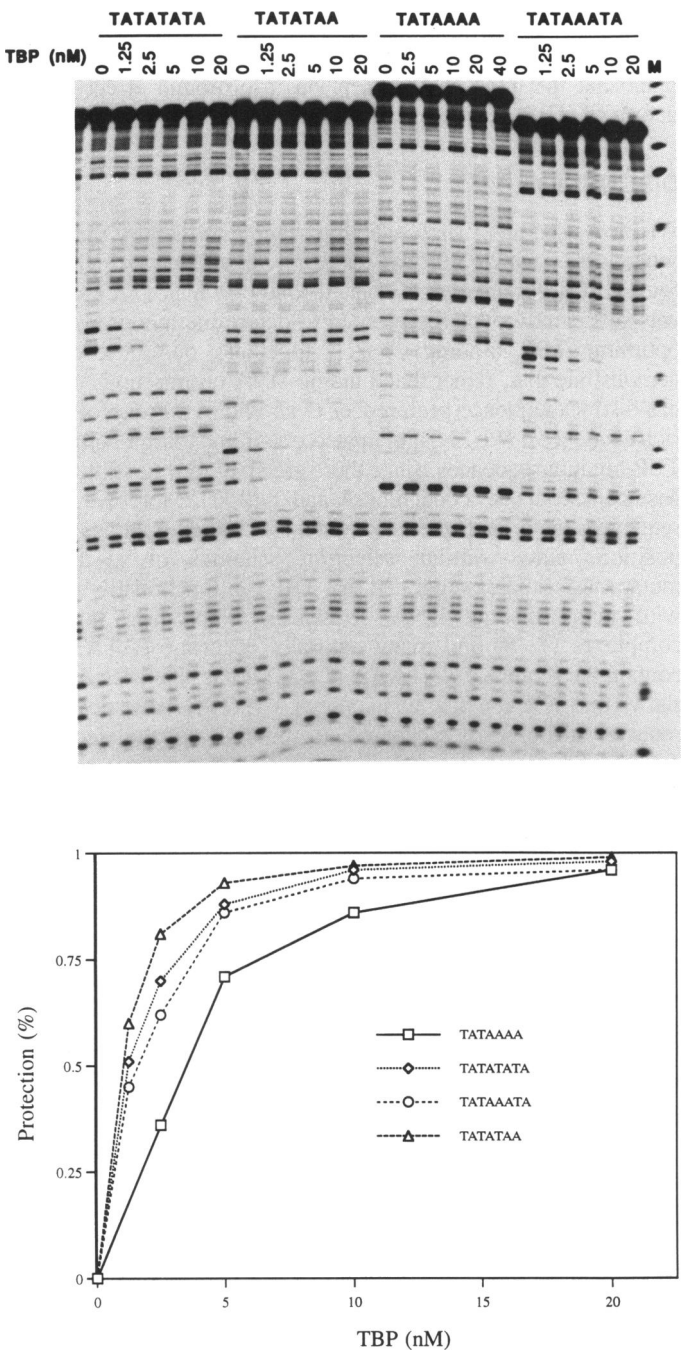

Figure 3. K_{eq} measurements for TBP binding to selected sequences. DNase 1 footprinting analysis of TBP binding. Increasing amounts of TBP were incubated with end-labeled DNA containing a TATA sequence as shown above each series of experiments. Autoradiograms were scanned by densitometry, and the data corrected for film response in order to determine the fractional occupancy of binding sites at the various TBP concentrations. The values were plotted and used to determine K_{eq} for each type of TATA element. K_{eq} is taken as the concentration of active TBP required to give 50% binding.

Table 3. Summary of k_{eq} and $t_{1/2}$ values for TBP at each TATA element. Note; the value for TATATAA is from the data of figure 5.

Sequence	Frequency	K_{eq} (M)	$t_{1/2}$
TATATAA	35/54	1.1×10^{-9}	16 min.
TATATATA	14/54	1.4×10^{-9}	14 min.
TATAAATA	4/54	1.6×10^{-9}	12 min.
TATAAAA	1/54	3.7×10^{-9}	9 min.

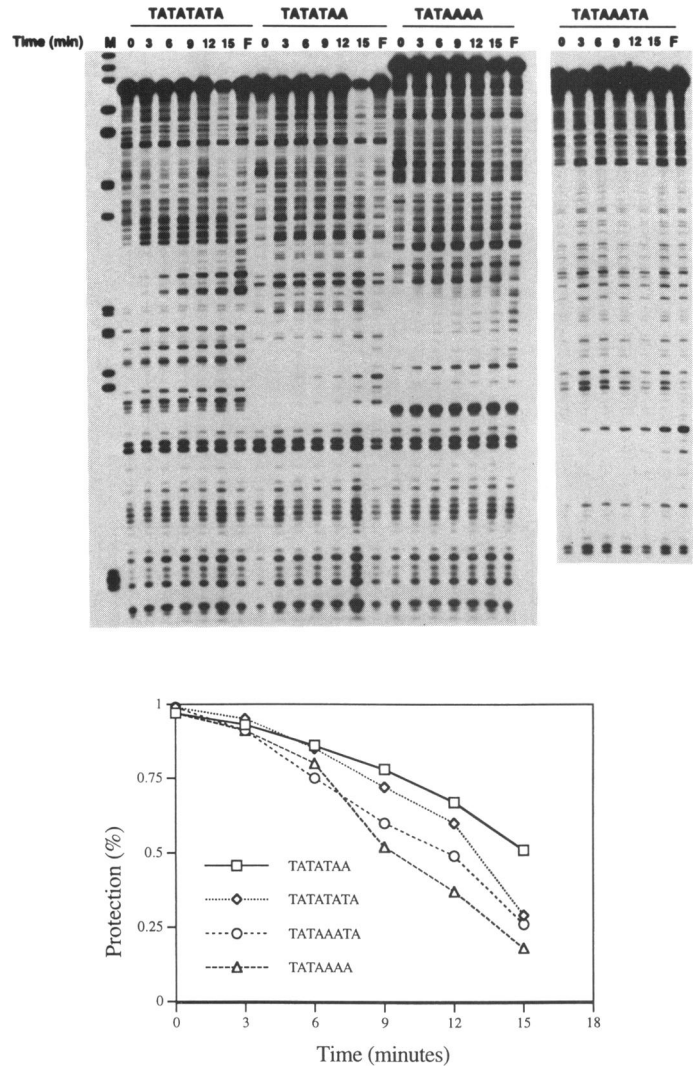

Figure 4. Measurement of the relative rates of dissociation of TBP from complexes with selected TATA elements. To measure the half life of TBP–DNA complexes, TBP was incubated with probes for 30 minutes to form TBP–DNA complexes. A large excess of cold TATAAAA containing oligonucleotide was then added to bind free TBP. A portion of each reaction was withdrawn for DNase I footprinting at the times indicated. Data were quantified and plotted to determine the approximate half life of TBP at each element.

TBP–DNA complexes are highly sensitive to the ionic environment

The range of values of half lives shown here for TBP complexes is substantially different from those reported for yeast TBP (20). One reason for this discrepancy is that in the experiments here, 150 mM KCl was used for the selections and assays, because at lower concentrations we observe avid, non-specific binding by TBP virtually everywhere within the footprinting probe employed (see Figure 5, lane 7). Such binding is easily competed, but implies a general and significant propensity of TBP to bind DNA randomly at low salt concentrations. We therefore determined the effect of salt concentration on the TBP dissociation rate. Figure 5 shows results obtained at 150 mM KCl or 80 mM

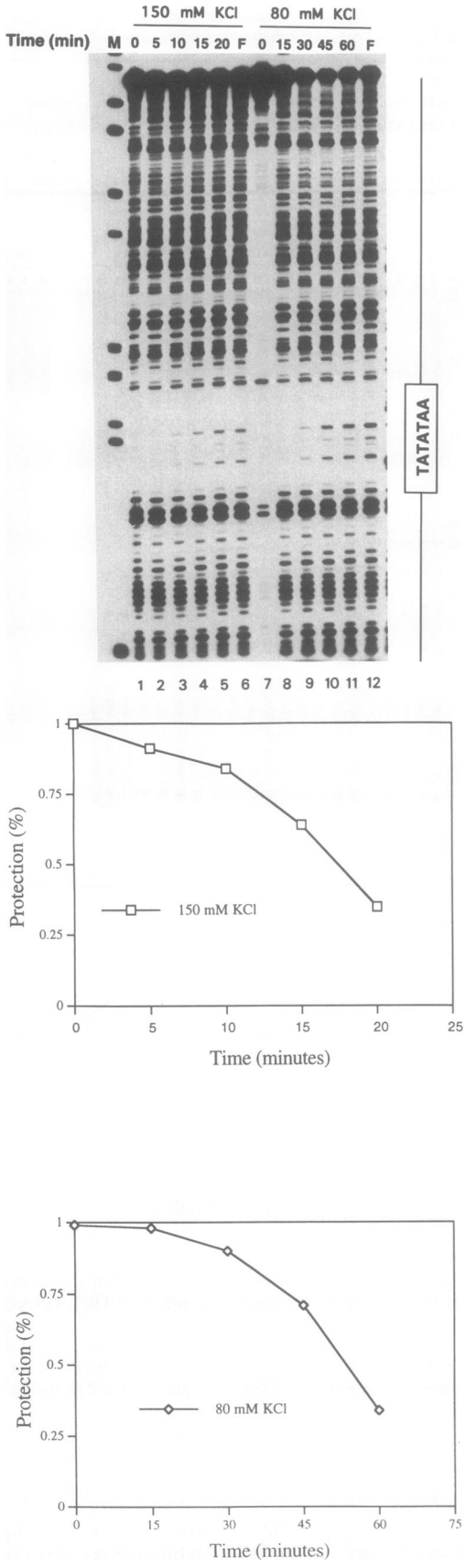

Figure 5. Effect of salt on the dissociation rate of TBP−DNA complexes. DNase 1 footprinting of TBP complexes with the sequence TATATAA was used to determine the dissociation rate of TBP at 80 mM KCl or 150 mM KCl, as indicated. Quantification was performed as described in the legend to figure 3.

KCl. At the lower salt concentration, the value for $t_{1/2}$ is 50 minutes. At 150 mM KCl this value is 16 minutes. It is therefore plain that the ionic environment has a profound effect on the nature of TBP−DNA interactions.

DISCUSSION

We have used an *in vitro* selection scheme to identify the DNA sequences to which TBP can efficiently bind. Surprisingly, just four TATA variants were isolated, one of which (TATATAA) was sufficiently over-represented to determine a consensus for optimum TBP binding. Taken together, our observations demonstrate that, rather than binding DNA promiscuously, TBP has distinct sequence preferences even within a group of A−T rich sequences. We had anticipated obtaining a large number of TBP-binding sequences, since there are sixty four possible seven base pair sequences containing A and T ($2^7/2$). Moreover, some sequences were expected to contain C residues at least one position, since similar selection schemes or systematic mutagenesis studies suggested that TBP can functionally interact with many distinct DNA sequences to produce initiation complexes (7−9) and many naturally occuring TATA boxes contain the sequence CATAAA (21,22) It has also been directly shown that TBP can bind non-consensus TATA boxes, albeit with reduced affinity as compared to binding to the sequence TATAAAA (6). Because most previous studies did not measure binding by TBP alone, it is possible that additional protein−protein interactions can stabilize the TBP complex at non-ideal sequences, for example those provided by interaction with TAFs or other general initiation factors. Indeed, we have found that human TFIIB improves the binding by TBP under conditions where TBP is otherwise limiting (J.-M.W. and E.B., unpublished data). Moreover, in cases where a promoter lacks a TATA box, TFIID is still required for transcription initiation to occur (see 13, 14). This result taken together with those mentioned above (7−9) strongly suggest that the avidity of TBP binding to a TATA box is not the sole determinant for transcription efficiency. We have tested the ability of all four types of TATA box to support transcription *in vitro*. While all are functional, there is no obvious connection between TBP binding avidity and transcription efficiency or direction (J.-M.W. and E.B., submitted).

Another explanation for why so few TATA variants were isolated might be that, in this study, we used *Acanthamoeba* TBP, which may have a different DNA binding specificity from yeast or human TBP. However, we consider this unlikely because of the extensive (>80%) amino acid identity in the carboxyl terminus 180 amino acids (15,23). The simplest explanation for our results is that TBP recognizes DNA in a highly selective manner. This result was also unexpected because the hydrogen bonding potential within the minor groove of all the selected sequences are virtually identical, as illustrated in figure 6a, which shows superimposed A:T and T:A base pairs [a presentation first used for A:U and U:A base pairs (1)]. For example, the position of thymidine O2 in an A:T base pair is within 0.4 Angstroms of N3 of adenine in a T:A base pair when the structures are superimposed. Comparison of the Van der Waals radii of these base pairs (Figure 6b) reveals a somewhat different picture. As for the superimposed stick figures, the overall minor groove surface for an A:T base pair is very similar to that of a T:A base pair, with the important exception that there is a pocket between the thymidine O2 and adenine 2H position. This pocket is located

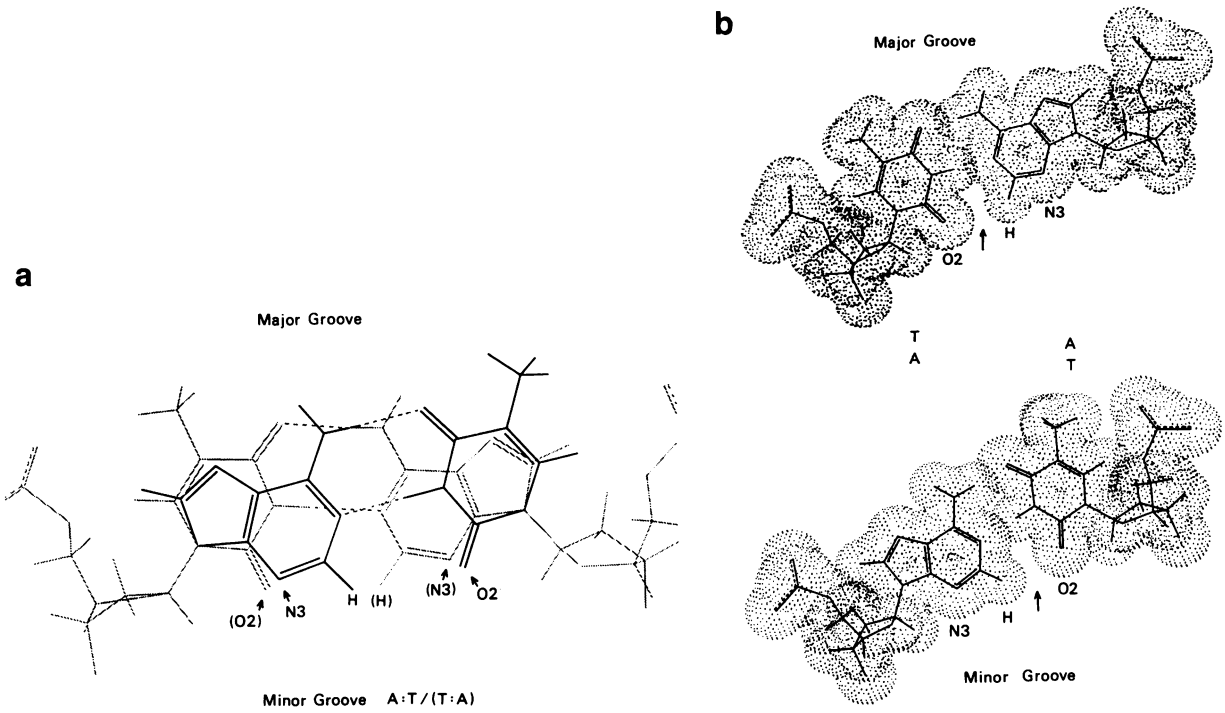

Figure 6. Minor groove structures of A:T and T:A base pairs. **a.** An A:T base pair is shown superimposed on a T:A base pair. **b.** Comparison of the Van der Waals surface of A:T and T:A base pairs. The arrows indicate a cleft that occupies a distinct position with respect to the DNA backbone, when A:T and T:A base pairs are compared. Both representations were produced in *Insight II* (Biosym Corp., San Diego, CA) as a canonical B DNA base pair.

in a distinct position, displaced by roughly 2 Angstroms relative to the sugar-phosphate backbone, when A:T and T:A base pairs are compared. This difference might contribute to the non-equivalence of A:T and T:A in their recognition by TBP, at least at some positions within the TATA box.

The consensus sequence identified here: 5′ A/G G A/G G/C TATATAA G G/C 3′; demonstrates that bases outside of the TATA element proper, can have a measurable influence on TBP binding (Table 2). This result is wholly consistent with those obtained by chemical interference studies (4,5), which showed enhanced binding by TBP to DNA methylated by dimethyl sulfate at the N7 position of a G residue at position −3 relative to the TATA box. In the present study, we observe a preference for G at −3 as well as other bases outside of the TATA box.

The crystal structure of a complex between TBP and DNA has recently been determined (24−26), and this structure explains some of the observations made here. In particular, the selection of a G residue at the 3′ end of the TATA consensus given above. In this case, the A−G step interacts with phenylalanine residues, that pry the bases apart, inducing a kink (25,26). It is thus possible that the A−G step is most favorable both for the interaction with phenylalanine and its ability to deform. An identical argument can be made for the selection of TAT as the first three residues in all cases, since the T−A step also interacts with kink-inducing phenylalanine residues, and the third T interacts closely and assymetrically with the protein. Interestingly, some of these interactions are in the vicinity of the surface pocket in A:T base pairs noted above. However, it remains unclear why a G residue at −3 relative to the TATA box was found in the majority of selected sequences, since the crystal structure does not show interactions in this region. We note however, that the oligos used for crystallization extend only to −1 or −2 of the TATA box (5′ GTATATAAAACGGT 3′ or 5′ GCTATAAAAGGGCA 3′). Similarly, it is unclear why the sequence TATATAA is a better binder than TATAAAA, since both make favorable, apparently similar contacts in the crystal structure.

The structure of A:T rich DNA has been determined, demonstrating that A:T and T:A base pairs in consecutive positions can impart particular geometric constraints on the structure of DNA as compared to random DNA (27,28). The TATA element, TATAAAA, may be the most rigid of those selected by our scheme, because of an expected effect of the four consecutive adenines, and the more avidly bound sequences TATATAA and TATATATA may be the most flexible. It is also possible that differences in the hydration patterns of different TATA sequences could contribute to binding avidity, since water is excluded from the contact region between TBP and DNA (24−26). However, since the stability of bound complexes appears to account for the overall variation in k_{eq} values, we do not think this likely.

It thus seems feasible to suggest that TBP binding specificity and the stability of the complex is dictated by particular direct interactions with chemical groups of the DNA minor groove, but that these contacts are optimized by the ability of a given sequence to adopt a particular structure. In the present case the sequence TATATAA may be the closest to an optimum configuration, but this configuration can be modified by adjacent bases on either side of the TATA box. This latter interpretation is made appealing by the observation that TBP induces a drastic bend and unwinding of the TATA box upon binding (25,26,29,30).

Such a hypothetical situation is not unlike that of 434, CAP and other repressors (31,32), where bases that do not make direct contact nonetheless influence protein−DNA interactions overall.

Such observations have led Crothers and co-workers to propose that certain dinucleotide pairs, particularly A−T steps, have a tendency to bend toward the minor groove, wheras others, such as G−C steps, bend in toward the major groove (32). This suggestion would explain the selection of Gs outside the TATA box, which are centered ten bases, or one DNA helical turn apart. Thus the sequence TATATAA could bend around TBP with the minor groove closest to the protein, and the flanking sequences with the major groove closest to the protein surface. However this model is not consistent with the evidence obtained by crystallography (25,26).

The values for k_{eq}, obtained here, are in general agreement with the relative values one might predict based upon the frequency of the sequences obtained by selection, and are in good general agreement with those obtained previously for different TATA elements complexed with yeast TBP (16). Because of the different conditions used here, it is difficult to directly compare these values, but we note a marked effect of ionic strength on the stability of TBP−DNA interactions and on the affinity of TBP for non-specific DNA. These observations are consistent with the observed deleterious effects of DNA phosphate ethylation on TBP binding (4,5), since electrostatic interactions are expected to be decreased at higher ionic strength. It seems probable that the weaker non-specific complexes observed at low salt are due solely to electrostatic interactions, wheras the specific complex, or that favored at high salt, is stabilized by the extensive hydrophobic interactions between the surface of TBP and DNA (25,26). One prediction of this observation is that the TBP complex should be salt-resistant, as described for the complex between TFIIIB and DNA (33). In fact we have observed TBP binding at KCl concentrations as high as 400 mM (J.-M.W and E.B., unpublished data).

In conclusion, TBP binds DNA with a significant ability to discriminate among A:T and T:A base pairs by interactions with the DNA minor groove. Plainly, some feature other than the mere presence of A:T base pairs determines the DNA binding specificity of TBP. As noted in the foregoing, it seems possible that specificity is determined by the combined effects of overall geometry, rigidity and by influences of flanking DNA sequence, in addition to specific recognition by TBP of chemical groups within the minor groove. It seems quite feasible to anticipate that other proteins, such as IHF (34,35), which also specifically recognize DNA bases in the minor groove will share this property.

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REFERENCES

1. Seeman, N.C., Rosenberg, J.M. and Rich, A. 1976 *Proc. Natl. Acad. Sci. USA* 73, 804−808.
2. Schleif, R. 1988 *Science* 241, 1182−1187.
3. Sharp, P.A. 1992 *Cell* 68, 819−821.
4. Starr, D.B. and Hawley, D.K. 1991 *Cell* 67, 1231−1240.
5. Lee, D.K., Horikoshi, M. and Roeder, R.G. 1991 *Cell* 67, 1241−1250.
6. Wiley, S.R., Kraus, R.J. and Mertz, J.E. 1992 *Proc. Natl. Acad. Sci. USA* 89, 5814−5818.
7. Singer, V.L., Wobbe, C.R. and Struhl, K. 1990 *Genes Dev.* 4, 636−645.
8. Chen, W. and Struhl, K. 1988 *Proc. Natl. Acad. Sci. USA* 85, 2691−2695.
9. Wobbe, C.R. and Struhl, K. 1990 *Molec. Cell. Biol.* 10, 3859−3867.
10. Zawel, L. and Reinberg, D. 1993 *Prog. Nucleic Acids Res. Molec. Biol.* 44, 67−108.
11. Rigby, P. 1993 *Cell* 72, 7−10.
12. Greenblatt, J. 1991 *Cell* 66, 1067−1070.
13. Zhou, Q., Lieberman, P.M., Boyer, T.G. and Berk, A.J. 1992 *Genes Dev.* 6, 1964−1974.
14. Zhou, Q., Boyer, T.G. and Berk, A.J. 1993 *Genes Dev.* 7, 180−187.
15. Wong, J.M., Liu, F. and Bateman, E. 1992 *Gene* 117, 91−97.
16. Hahn, S., Buratowski, S., Sharp, P.A. and Guarente, L. 1989 *Proc. Natl. Acad. Sci. USA* 86, 5718−5722.
17. Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. (1989) Current protocols in molecular biology, Greene Publishing Associates and Wiley Interscience, New York
18. Oliphant, A.R., Brandl, C.J. and Struhl, K. 1989 *Mol. Cell. Biol.* 9, 2944−2949.
19. Blackwell, T.K. and Weintraub, H. 1990 *Science* 250, 1104−1110.
20. Hoopes, B.C., LeBlanc, J.F, and Hawley, D.K. 1992 *J. Biol. Chem.* 267, 11539−11547.
21. Breathnach, R. and Chambon, P. 1981 *Ann. Rev. Biochem.* 50, 349−383.
22. Bucher, P. and Trifonov, E.N. 1986 *Nucleic Acids Res.* 14, 10009−10026.
23. Nikolov, D.B., Hu, S.-H., Lin, J., Gasch, A., Hoffmann, A., Horikoshi, M., Chua, N.-H., Roeder, R.G. and Burley, S.K. 1992 *Nature (London)* 360, 40−46.
24. Klug, A. 1993 *Nature (London)* 365, 486−487.
25. Kim, Y., Geiger, J.H., Hahn, S. and Sigler, P. 1993 *Nature (London)* 365, 512−520.
26. Kim, J.L., Nikolov, D.B. and Burley, S.K. 1993 *Nature (London)* 365, 520−527.
27. Drew, H.R., McCall, M.J. and Calladine, C.R. 1988 *Ann. Rev. Cell. Biol.* 4, 1−20.
28. Travers, A.A. 1989 *Ann. Rev. Biochem.* 58, 427−452.
29. Horikoshi, M., Bertuciolli, C., Takada, R., Wang, T., Yamamoto, T. and Roeder, R.G. 1992 *Proc. Natl. Acad. Sci. USA* 89, 1060−1064.
30. Kuddus, R. and Schmidt, M.C. 1993 *Nucleic Acids Res.* 21, 1789−1796.
31. Harrison, S.C. and Aggarwal, A.K. 1990 *Ann. Rev. Biochem.* 59, 933−969.
32. Gartenberg, M.R. and Crothers, D.M. 1988 *Nature (London)* 333, 824−829.
33. Kassavetis, G.A., Braun, B.R., Nguyen, L.H. and Geiduschek, E.P. 1990 *Cell* 60, 235−245.
34. Yang, C.-C. and Nash, H.A. 1989 *Cell* 57, 869−880.
35. Pabo, C.O. and Sauer, R.T. 1992 *Ann. Rev. Biochem.* 61, 1053−1095.