TATA-flanking Sequences Influence the Rate and Stability of TATA-binding Protein and TFIIIB Binding

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The kinetics of TATA-binding protein (TBP) and TFIIIB binding were measured on a series of promoter constructs that had varying sequences within and flanking the TATA box. The flanking sequences were found to influence TBP stability even though they do not contact the protein. This occurs by altering the decay rate rather than the association rate. TFIIIB association is accompanied by protein-protein cooperativity as indicated by the simultaneous release of both proteins in challenge experiments. The sequence of the TATA box and the sequences that flank it can influence the kinetics of the TFIIIB-TBP-DNA complex. TFIIIB can contribute to tighter TATA binding in two ways. It always slows the decay rate of TBP, but it can also increase the rate of association at promoters with certain combinations of TATA and flanking sequences. The results imply that the interplay between the TATA box and flanking elements leads to variations in the kinetics of preinitiation complex formation that may account for the observed effects of all of these diverse sequences on transcription.

The proper temporal and spatial expression of a protein encoding gene in eukaryotes requires the formation of a large, multiprotein preinitiation complex at the gene promoter. The process begins with sequence-specific DNA recognition of the promoter. Two primary recognition elements, TATA and initiator (Inr), have been extensively studied (reviewed in Ref. 1). Each of these elements is recognized by specific proteins as follows: TATA by the TATA-binding protein (TBP) and Inr by several proteins including TBP-associated factors. In addition to recognition, these elements also play regulatory roles. TATA has been shown to play a role in setting the rate of transcription initiation (2, 3), whereas Inr can play a role in activation (4).

Although much is now known about the roles of TATA and Inr, comparatively little is known about possible roles for other sequences within the core promoter. Ultraviolet cross-linking experiments have shown that several transcription factors are in intimate contact with the DNA throughout the core promoter region. TFIIA, IIB, IIE, IIF, TBP-associated factors, and polymerase II subunits all cross-link to regions between -50 and +15 relative to the transcription start site (5–8), suggesting that these sequences have the potential to influence the binding or functionality of these factors. Although no core consensus sequence exists outside the TATA and Inr elements, there are clear nucleotide preferences, with the majority of promoters being GC-rich (9).

Two recent studies have investigated the roles of non-TATA, non-Inr sequences. A GC-rich sequence upstream from TATA (the BRE) can enhance TFIIIB binding to the TBP-DNA complex and stimulate basal transcription (10). Sequences both upstream and downstream from TATA can affect transcription; these can alter both basal and activated levels independently (9). The latter observations were based on “block-swapped” promoters in which blocks of sequences were exchanged among promoters, and transcription and factor binding were assayed. Bandshift analyses showed that the 10-base pair blocks flanking the TATA box play roles in both TBP and TFIIIB binding. For example, replacing either (or both) of these blocks in the more GC-rich adenovirus ML promoter with the corresponding AT-rich sequences from the E4 promoter lowered the level of TBP binding.

Two aspects of these results were unexpected. First, there were no prior indications of a role for the block downstream from TATA in transcription. Second, the TBP binding results are difficult to explain as there is no prior evidence that TBP contacts sequences that flank TATA; crystallographic and UV cross-linking studies have suggested that TBP makes contacts only within the TATA box region (11–13). Of course the effects on TBP binding and on transcription could be closely related.

One possibility for the role of these sequence blocks is that surrounding the AT-rich TATA element with GC-rich sequences (as in the ML case) could make the TATA box more distinctive as TBP scans the DNA for the correct binding site. At an AT-rich promoter the TBP search might be slowed if nearby TATA-like sequences are present, and this would be reflected by a lower rate of association. A different possibility is that AT-rich and GC-rich sequences take on unique structures nearby sequences (as in the ML case) could make the TATA box more distinctive as TBP scans the DNA for the correct binding site. At an AT-rich promoter the TBP search might be slowed if nearby TATA-like sequences are present, and this would be reflected by a lower rate of association. A different possibility is that AT-rich and GC-rich sequences take on unique structures nearby sequences (as in the ML case) could make the TATA box more distinctive as TBP scans the DNA for the correct binding site. At an AT-rich promoter the TBP search might be slowed if nearby TATA-like sequences are present, and this would be reflected by a lower rate of association. A different possibility is that AT-rich and GC-rich sequences take on unique structures nearby sequences (as in the ML case) could make the TATA box more distinctive as TBP scans the DNA for the correct binding site. At an AT-rich promoter the TBP search might be slowed if nearby TATA-like sequences are present, and this would be reflected by a lower rate of association. A different possibility is that AT-rich and GC-rich sequences take on unique structures.
done previously. The outcome provides an explanation in terms of TFII B having different effects on the rates of formation and rates of decay of these complexes depending on promoter context. Overall, the data suggest how blocks that flank TATA can influence the kinetics of factor binding in unique ways and thus enhance the potential for diversity in transcriptional regulation.

**MATERIALS AND METHODS**

Nucleic Acids and Proteins—Probes for gel shifts were made by annealing pairs of 40-nucleotide-long oligonucleotides (Ouperon) with 20 base pairs of complementarity at their 3' ends (see Ref. 9). Equimolar (1 nmoI) amounts of each oligonucleotide were annealed in 20 mM Tris-Cl, pH 7.5, 10 mM MgCl₂, 100 mM NaCl in a 25-μl volume by rapid heating to 95 °C and slowly cooling to room temperature in a polymerase chain reaction thermocycler (MJ Research). The overhanging ends were filled by mixing the 25-μl annealing reactions with 7.5 mM dithiothreitol (DTT), 33 μM each dNTP, and 5 units of Klenow enzyme (Life Technologies, Inc.) in a final volume of 50 μl and incubating for 1 h at room temperature. DNA was precipitated with sodium acetate and ethanol, dissolved in 8 mM urea, 0.5× TBE and purified on 12% urea-polyacrylamide gel electrophoresis (8 mM urea, 29:1 ratio of acrylamide to bisacrylamide, 0.5× TBE). The highest molecular weight bands were visualized by UV shadowing and excised from the gel. The gel slices were crushed to beads and soaked overnight in 1 ml of 0.3 M NaCl, 10 mM Tris-Cl, pH 7.4, 1 mM EDTA at 37 °C. The beads were then spun down in a centrifuge and the supernatant recovered. DNA was again precipitated and dissolved in Tris-Cl, pH 8.0. The concentration of DNA was determined by heating the DNA to 95 °C to denature and taking the absorbance at 260 nm. The absorbance was multiplied by 33 μg/ml to determine the total concentration of single strands. The probes were labeled by end labeling with [γ-32P]ATP and 3 units T4 polynucleotide kinase (Promega) in 25 μl in 1× TBE and purified on 12% urea-polyacrylamide gel electrophoresis (see above) to verify the Klenow extension. These probes contain the ML and/or E4 promoter sequences from -41 to +18 flanked by restriction sites.

Full-length, His-tagged human TBP (15) was provided by A. Berk (UCLA). Full-length, C-HMK-tagged human TFII B (16) was provided by M. Carey (UCLA).

**Gel Shifts**—Electrophoretic mobility shift assays were performed as described previously (9). Briefly, 1 fmol of labeled probe was mixed with the indicated amounts of TBP and TFII B in a 10-μl reaction that contained 50 mM HEPES, pH 7.9, 12% glycerol, 60 mM KCl, 0.12 mM EDTA, 0.6 mM DTT, 8 mM MgCl₂, 50 ng of poly(dI-dC), and 500 ng of bovine serum albumin (Sigma). Proteins were bound at 30 °C for the indicated times and loaded directly onto prechilled 5% native gels (59:1 acrylamide:bisacrylamide, 5% glycerol, 2 mM MgCl₂, 1 mM DTT, 0.5× TBE) and run in prechilled 0.5× TBE, 2 mM MgCl₂ buffer. Gels were run in Bio-Rad mini-Protein 3 units at 400 V for 30 min. The temperature of the inner tank and gels never exceeded 25 °C. The gels were dried and exposed to PhosphorImager screens (Molecular Dynamics) for 16 h. Time course experiments were initiated by adding protein and probe to reaction mixtures containing all the remaining components (see above). Time points were initiated at intervals so that they could all be loaded on the gel at the same time, ensuring that any dissociation of complexes in the gel would be constant for all samples.

**On-rate Determination**—The indicated amounts of protein were bound to DNA for various times before loading on gels. The appropriate shifted complexes were quantitated using ImageQuant software (Molecular Dynamics). The raw data (counts) were fitted by nonlinear least squares regression to Equation 1,

\[ F_I = F_{\text{final}} (1 - e^{-kt/2}) \]

(Eq. 1)

using the SigmaPlot (SPSS, Inc.) program for PC. \( k_{\text{on}} \) and \( F_{\text{final}} \) are the calculated first-order rate constant and calculated fraction bound at the completion of the reaction, respectively. In many cases the raw data at each time point were normalized to \( F_{\text{final}} \) for plotting. The half-time of association is given by Equation 2,

\[ t_{1/2} = \ln(2)/k_{\text{on}} \]

(Eq. 2)

**RESULTS**

TBP binding was determined by monitoring the reciprocal of the \( k_{\text{obs}} \) determined at each concentration of TBP versus the reciprocal of the TBP concentration. \( k_{\text{obs}} \) was then taken as the reciprocal of the slope of this plot.

**Off-rate Determination**—The indicated amounts of protein were bound to DNA for 1 h and then challenged with 20 ng/ml poly(dI-dC) for the indicated times. As above, each time point was initiated at intervals and then challenged after 1 h so that all time points could be loaded simultaneously. Addition of poly(dI-dC) prior to probe results in no detectable binding (data not shown). The bands were quantitated and fit by nonlinear least squares regression to Equation 3,

\[ F_I = F_{\text{obs}} (e^{-kt/2}) \]

(Eq. 3)

where \( F_I \) is the calculated starting fraction bound and \( k_{\text{off}} \) the calculated rate constant. The half-life of the complex is given by \( t_{1/2} = \ln(2)/k_{\text{off}} \), and the dissociation constant for TBP is given by \( K_{d} = k_{\text{off}}/k_{\text{on}} \).

**DISCUSSION**

We showed previously that replacing either or both of the 10-base pair GC-rich blocks flanking the ML TATA box with the more mixed sequence blocks from the adenovirus E4 promoter led to a significant decrease in the level of TBP binding (9). TBP does not contact these sequences directly, leaving the source of the effect unknown. To help distinguish between various models (see Introduction), we measured whether the altered binding was due to changes in on-rate or off-rate or both.

The on-rate behavior may be related to the limiting amounts of DNA used in these assays.

On-rates for all four probes were determined at 20 nM TBP, and the values are shown in Table I. Only promoter M-E12 shows a difference at 20 nM TBP, and this effect is small. The half-time to saturation is \( 5 \) min. The bands were quantified by PhosphorImager analysis and fit to Equation 1, see under “Experimental Procedures.” The data and best fit curve are plotted in Fig. 2B. This reaction appears to follow first-order kinetics with a \( k_{\text{obs}} \) of \( 2.7 \times 10^{-3} \) s⁻¹. The concentration of TBP in this experiment (20 nM) is comparable to the published values (14, 18, 19). The apparent two-phase kinetics that might be expected if dissociation of TBP dimers are rate-limiting (17). This first order behavior may be related to the limiting amounts of DNA used in these assays.

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Second-order rate constants for TBP binding were determined by plotting the reciprocal of the \( k_{\text{obs}} \) determined at each concentration of TBP versus the reciprocal of the TBP concentration. \( k_{\text{obs}} \) was then taken as the reciprocal of the slope of this plot.

**On-rate Determination**—The indicated amounts of protein were bound to DNA for 1 h and then challenged with 20 ng/ml poly(dI-dC) for the indicated times. As above, each time point was initiated at intervals and then challenged after 1 h so that all time points could be loaded simultaneously. Addition of poly(dI-dC) prior to probe results in no detectable binding (data not shown). The bands were quantitated and fit by nonlinear least squares regression to Equation 3,
calculated rate constant ($k$) of the bands in A were quantified, fit to Equation 1, and plotted. The calculated rate constant ($k_{obs}$) for this experiment is $2.7 \times 10^{-3} s^{-1}$. The half-time is 4.3 min.

Fig. 2. Time course of TBP binding. A, the ML promoter probe (0.1 nM) was mixed with TBP (20 nM) as described. Time points were initiated at intervals so that all samples could be loaded on the gel at the same time. Arrows indicate the bound complex and free probe. B, the bands in A were quantified, fit to Equation 1, and plotted. The calculated rate constant ($k_{obs}$) for this experiment is $2.7 \times 10^{-3} s^{-1}$. The half-time is 4.3 min.

different from the parent ML promoter. We conclude that the TATA-flanking blocks have little effect on the association rate constant for TBP binding.

Since the TATA-flanking blocks have no apparent effect on the on-rate of TBP binding, we tested whether they affect the off-rates. We chose to use TBP at 5 nM since that is the concentration used in the prior study (9). TBP-DNA complexes were formed at 30°C for 60 min to ensure that binding had reached equilibrium and then challenged with 20 ng/μl poly(dI-dC). Addition of this competitor prior to adding TBP prevented binding to the probe so it serves as an effective sink for dissociated TBP (data not shown). Examples of off-rate experiments are shown in Fig. 3A. The data from several such experiments were fit to Equation 3 and averaged. The average $k_{off}$ for the parent ML promoter is $1.3 \times 10^{-4} s^{-1}$. This rate and the dissociation constant ($K_d$) of $1 \times 10^{-8} M$ (Table I) are both consistent with published data (14, 18, 19).

The experiment was repeated using promoters in which the flanking sequence blocks had been swapped. A sample autoradiograph comparing two promoters is shown in Fig. 3A with the data analysis in Fig. 3B. The difference between the two curves (closed circles, ML; open circles, M-E12) is approximately a factor of 2. A similar 2-fold difference was seen with the other block-swapped promoters (compare off-rates in Table I). The half-life of TBP on the ML promoter is nearly 90 min while the half-lives for the other promoters range from 40 to 50 min. This indicates that TBP is less stably bound to the block-swapped promoters than to the ML parent and demonstrates a kinetic role for both flanking sequences. We conclude that the sequences that flank TATA affect the stability of TBP binding without altering the rate at which TBP locates the TATA box.

TFIIDB Affects Both the On-rate and the Off-rate with the On-rate Being Sequence-dependent—The sequence of the block upstream from TATA (block 1) is known to influence TFIIDB binding (10). In general, TFIIDB binding enhances the binding of TBP to the TATA box. It is not known whether these stabilizing effects are due to faster on-rate, slower off-rate, or both. In the series of block-swapped promoters studied here, the sequence of the ML block 1 is expected to bind TFIIDB better than block 1 from E4 as it provides a much better match to the GC-rich consensus (10). The effect of TFIIDB on TBP binding is known not to be uniform among this promoter set (9). The greatest contribution of TFIIDB to TBP binding is made with promoter M-E2, which has the ML sequence upstream from TATA and the E4 sequence downstream. To learn why this sequence-specific effect occurs and to locate the general kinetic sources of the TFIIDB effect on TBP binding to TATA, we established rate assays for formation of the ternary complex containing TBP, TFIIDB, and TATA DNA.

We looked first at the effect of TFIIDB on off-rates. The kinetic analysis is potentially complicated because either TBP or TFIIDB could be released first. However, if TBP were released first then TFIIDB would effectively be released concurrently because it cannot bind these DNA templates on its own (9). As shown below the dissociation also does not include a pathway in which TFIIDB only is released. Thus we can monitor dissociation by following the joint loss of TFIIDB and TBP. The experiment is similar to that described above, adding poly(dI-dC) to preformed complexes as a sink for released TBP. Although this has not been done previously for the TFIIDB-TBP-DNA complex, a similar experiment was used to measure the bulk off-rate of TFIIDB and TBP from a promoter containing the ML TATA box (20).

TFIIDB, TFIIDF, and labeled DNA were preincubated for 60 min to ensure that binding had reached equilibrium. The complexes were then challenged with poly(dI-dC). At no time during the decay phase of these reactions is a TBP-DNA intermediate observed (Fig. 4A), and TFIIDB does not bind to these promoters in the absence of TBP (9). This verifies that the poly(dI-dC) challenge measures the bulk off-rate of TFIIDB and TBP and demonstrates that these two proteins come off the DNA as a unit.

Fig. 4B shows that TFIIDB (open circles) decreases the rate of decay compared with TBP alone (closed circles). The TFIIDB-TBP-DNA complex decays at about half the rate of the TBP-DNA complex. The kinetic effect shows no promoter specificity; the rates of decay ($k_{off}$) for all four promoters are decreased similarly by TFIIDB with an average decrease of 2.3-fold (compare off-rates in Table II for TBP + TFIIDB to those in Table I for TBP alone). Two conclusions are drawn. One is that the well known ability of TFIIDB to enhance TBP binding has a source in the slowing of the TBP decay rate. The second is more surprising. Because TBP can bind TATA well in the absence of TFIIDB one would expect it to remain bound after TFIIDB dissociates. However, that was not observed. It appears that the TFIIDB stabilization is accompanied by a change in state that requires that the two proteins dissociate jointly.

These effects appear to be sequence-nonspecific. Therefore, one aspect of the prior data remains unexplained. The TFIIDB effect on TBP promoter binding is greater for M-E2 than at other promoters (9). As the data show that the source of this was not the off-rate (2.4-fold stimulation for M-E2 versus a 2.3-fold average), we investigated the on-rate.

TFIIDB-TBP-DNA complexes were assembled under the con-
ditions used previously (9): 5 nM TBP, 20 nM TFIIB and 0.1 nM DNA were mixed and incubated at 30 °C for various times. An example of an autoradiograph from such a time course is shown in Fig. 5. Lane 1 contains only TBP (60 min) as a marker. Arrows indicate the locations of TBP\( \text{z} \) DNA and TFIIB\( \text{z} \) TBP\( \text{z} \) DNA complexes. No band is seen in the position expected for the TBP\( \text{z} \) DNA binary complex, indicating that the ternary complex forms cooperatively.

A comparison of M-E2 and the ML parent is shown in the association rate curves of Fig. 5B, and normalized data for association with each of the four promoters are shown in Table II. TFIIB causes a very slight acceleration of binding on three of these promoters, but the effect on M-E2 is much greater, nearly 3-fold (compare on-rates in Table II to those for 5 nM TBP in Table I). Combined with the 2-fold effect on off-rate, this accounts for roughly a 6-fold increase in binding on M-E2. This compares with the observed 8-fold increase in overall binding seen previously when TFIIB was added to TBP at this promoter (9). Thus in addition to a nonspecific stabilization against decay, TFIIB can assist TBP binding via a sequence-specific acceleration of binding rate. This latter occurs with only one of the four promoters studied.

**Kinetic Interplay between TATA and the Flanking Blocks**—

These data indicate two roles for TFIIB as follows: a general strengthening of bound TBP against decay, and a sequence-dependent enhancement of the rate of TBP binding to TATA. The
M-E2 promoter that has the enhanced on-rate has a unique combination of elements upstream and downstream from TATA. Its upstream block from the ML promoter has a resemblance to the consensus TFIIB-binding site (BRE). Its downstream block from the E4 promoter weakens TBP binding (9). How TFIIB can selectively enhance the binding on-rate for promoter M-E2 is not clear, but two possibilities can be considered. One is that the unique combination of ML block 1 with E4 block 2 creates a novel site that binds TFIIB rapidly. Alternatively, the loosely bound TBP, destabilized by the E4 block 2 sequence, may be configured in a manner that can bind TFIIB rapidly if a strong BRE is present. We sought to mimic this second possibility by directly weakening TBP binding via TATA mutation within the parent ML promoter. The question is, will the BRE, silent when embedded in the ML but not the M-E2 promoter, now also show function in the ML context with a nonconsensus TATA box?

To examine this possibility a new series of promoters was constructed (see Fig. 6A). The 2T mutation replaces the two adenoses at positions –27 and –28 in the ML TATA box with thymidines (underlined Ts in Fig. 6A) in the context of the parent ML promoter (to form the ML-2T promoter). The substitutions were chosen to reduce TBP affinity without causing the drastic decreases in binding associated with “weak” TATA boxes. This mutation decreases the lifetime of TBP binding (21) without affecting the association rate (19). The reduction in affinity is comparable to that caused by the M-E1 substitution. We measured the on- and off-rates for TBP and TBP + TFIIB on this mutant parent and the series of block-swapped promoters also containing the TATA mutation (Fig. 6A).

Initial control experiments confirmed that the 2T mutation led to more rapid decay of TBP from binary complexes (see Fig. 6B, upper row and TBP off-rates in Table III). The rate of TBP decay from ML-2T is comparable to the rate from M-E1, indicating that the 2T mutation does indeed destabilize TBP binding. Further destabilization was not seen for the block-swapped 2T promoters indicating that the effects of TATA and the adjacent sequences are not cumulative in this context. Next, TFIIB was added to the mutant promoter, and the rate of decay of ternary complexes was determined (Fig. 6B, lower row). The TATA mutation led to destabilization of ternary complexes (Table III) as it had for the binary complexes at the same promoter. Yet again this effect was not seen when the flanking sequences were changed.

Next the effect of TFIIB on the on-rate was measured at the ML-2T promoter with the weakened TATA box (Fig. 6C, upper row for binary complex and lower row for ternary complex). The data in Table III show that the ternary complex forms nearly 3-fold faster than the binary complex. As this acceleration was not seen when the TATA box retained the wild-type sequence, we infer that the TATA mutation makes the adeno-ML promoter susceptible to a stimulating effect of TFIIB on the rate of ternary complex formation. This acceleration by TFIIB was eliminated when the BRE was replaced with an AT-rich sequence (2T-E1 and 2T-E12 in Table III, Ratios). It was retained when the BRE was retained (promoter 2T-E2). Overall, the data favor the possibility that there are at least two requirements for the TFIIB effect via on-rate. First, TBP binding must be weakened, either by TATA mutation (as shown here) or by inserting a weakening sequence block downstream (as shown above). Second, a strong TFIIB-binding site must lie upstream of TATA. The implications of obtaining similar effects on gen-

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**Fig. 5.** Time course of ternary complex formation. A, TBP (5 nM), TFIIB (20 nM), and probe M (0.1 nM) were mixed and incubated for the indicated times. Lane T contains only TBP and DNA (1-h time point). The binary and ternary complexes are indicated. B, data from several on-rate time courses for all four promoters were normalized and averaged. The half-time to saturation is 7.7 min for M-E2 and roughly 15 min for the others. Closed circles, ML; open circles, M-E1; closed triangles, M-E2; open triangles, M-E12.

**Fig. 6.** Kinetic interplay between TATA and the flanking sequences. A, the 2T series of promoters differ from ML in the substitution of two thymidines for the adenoses at positions –27 and –28 (underlined). The consensus sequence for the BRE is shown (10). The block swaps are also illustrated as in Fig. 1. B, decay of binary (upper) and ternary (lower) complexes for the ML-2T promoter. C, association of binary (upper) and ternary (lower) complexes for ML-2T.
TATA-flanking Blocks Influence Factor Binding

Table III
Kinetics of factor binding to 2T series of promoters

| Promoter | Off-rates (× 10^⁻³ s⁻¹) | On-rates (× 10^⁻³ s⁻¹) | On-rate ratio |
|----------|-------------------------|------------------------|--------------|
|          | TBP | TBP + HB | TBP | TBP + HB |          |
| ML-2T    | 2.1 | 1.3     | 7.2 | 20       | 2.8       |
| 2T-E1    | 2.0 | 1.2     | 7.0 | 11       | 1.6       |
| 2T-E2    | 2.8 | 1.0     | 8.5 | 21       | 2.5       |
| 2T-E12   | 2.8 | 1.1     | 6.8 | 8        | 1.2       |

* Ratio of rate of ternary complex formation to rate of binary complex formation.


discussion

Many promoters have been sequenced so far, and within an organism no two have identical core DNA sequences (Eukaryotic Promoter Database base, see Ref. 9). In general, core promoter sequences are GC-rich, but there is very considerable diversity. Prior studies have indicated that elements both upstream and downstream from TATA can influence the level of transcription and its regulation (9, 10). The above results help to understand the origin of these effects. The flanking sequences are shown to affect the manner in which preinitiation complex assembly is originated to recruit RNA polymerase. Although the TATA-flanking elements do not contact TBP directly, their sequences are shown to influence the rate at which TBP is released from TATA. In addition certain combinations of element types can influence the rate at which TFIIB associates with the bound TBP. The nature and implications of these results are discussed below.

**TBP Binding and Flanking Sequences**—The data show that the TATA-flanking sequences influence the decay rate of bound TBP but not the rate at which TBP locates and binds the TATA box. The GC-rich flanking sequences of the adenovirus ML promoter increase the lifetime of the bound protein by roughly a factor of 2. It seems likely that among naturally occurring promoters there will be flanking sequences that yield both greater and lesser quantitative effects. Two possible sources of this effect were considered (see Introduction). The lack of effect on the association rate makes it unlikely that surrounding the TATA box with GC-rich sequences highlights it, making it easier for TBP to locate it and bind. The measurable effect on decay rate makes it more likely that flanking the AT-rich region with GC-rich blocks creates unique structures at the edges of the TATA element, i.e. kinks or bends, which stabilize TBP once it is bound.

Structural studies suggest how this might occur. When TBP binds a consensus TATA box, i.e. the ML TATA, it bends the DNA roughly 90° (11, 13, 21), and the stability of binding correlates with changes in the bend angle at the promoter (21, 22). The minor grooves at the TATA box boundaries are widened (13), and when artificial heteroduplex bubbles are placed at these boundaries TBP binding is stabilized (14). The ML promoter sequences that flank TATA both contain homopolymeric G runs which juxtapose to the homotetrameric A run within the ML TATA box. Such homopolymeric runs may form rigid helices (23, 24) forcing discontinuities at the borders (25). Thus, it seems that promoters with flanking elements that juxtapose such sequences could create bent structures that assist TBP binding.

We assessed the tendency of promoters to include this extreme juxtaposition using data from the Eukaryotic Promoter Database. Approximately 5% of these promoters were found to have pairs of juxtaposed tetrameric and homopolymeric (separated by no more than one base pair), roughly 10 times the expected frequency. At the other extreme there are expected to be promoters whose flanking sequences resist bending (9) at the TATA boundaries that would be expected to bind TBP less tightly than the average. Overall, this should lead to considerable diversity in TBP binding affinity, complementing the diversity within the TATA box itself. Indeed, promoters with the same affinity for TBP could have different properties as a consequence of this effect; AT-rich flanking sequences can enhance the sensitivity to induction (9), whereas nonconsensus TATA box sequences can decrease the continuous re-initiation events that determine promoter strength (2, 3, 26).

**TFIIB Binding**—The data show new aspects of TFIIB binding that complement knowledge concerning the central role of TFIIB in preinitiation complex assembly (27–32). In light of prior knowledge it is not surprising that TFIIB stabilizes bound TBP against decay at all promoters. That is, TFIIB cross-links to both flanking blocks 1 and 2 (6) and can recognize sequence elements within block 1 (10). However, two observations were surprising. First, the decay of the ternary complex was cooperative in that TBP and TFIIB dissociated essentially simultaneously. This is surprising because TFIIB has a far lower affinity for DNA than does TBP and thus decay would be expected to initially dissociate TFIIB leaving TBP bound. The observation implies that TBP may be bound differently to DNA in the ternary complex than in binary complex. A conformational change in this main DNA binding protein may influence transcription, especially since evidence has accumulated that the DNA within preinitiation complexes may have a defined topological superstructure (6, 7, 33–35).

A second surprising observation was that sequences flanking the TATA box can influence the rate at which ternary complexes assemble. This rate is enhanced only when the stronger ML BRE sequence is present upstream of TATA and TBP binding is weak. The data show that weakening can occur either by making the TATA box nonconsensus or by placing the more AT-rich E4 sequence downstream from TATA. The source of this more rapid rate may have its origin in an unstably bound less bent form of the TBP-DNA complex present when the TATA is nonconsensus (21, 22) or the promoter contains AT-rich DNA downstream (see above). TFIIB may bind the strong upstream BRE sequence and drive the TBP-binding reaction to completion. This would be in addition to the sequence nonspecific stabilization of TBP against decay. The sequence dependence of the on-rate means that it will differ among promoters that could have regulatory implications as forming a complex with bound TFIIB is central to transcriptional regulation.

Overall then the current data indicate that the diversity of sequences that flank TATA could have multiple influences in contributing to the diversity of transcription through altering how preinitiation complexes assemble at promoters. The affinity for TBP will be set by both TATA and flanking sequences, and this will influence many central properties of the promoter. There will be a further influence of the flanking sequences on how TFIIB works. Their effect on TBP binding could range from weak (upstream sequence AT-rich) to very strong (GC-rich sequence upstream but not downstream). These effects are likely to be relevant directly to basal transcription that relies centrally on the ternary complex than in binary complex. A conforma- tional change in this main DNA binding protein may influence transcription, especially since evidence has accumulated that the DNA within preinitiation complexes may have a defined topological superstructure (6, 7, 33–35).

Overall then the current data indicate that the diversity of sequences that flank TATA could have multiple influences in contributing to the diversity of transcription through altering how preinitiation complexes assemble at promoters. The affinity for TBP will be set by both TATA and flanking sequences, and this will influence many central properties of the promoter. There will be a further influence of the flanking sequences on how TFIIB works. Their effect on TBP binding could range from weak (upstream sequence AT-rich) to very strong (GC-rich sequence upstream but not downstream). These effects are likely to be relevant directly to basal transcription that relies centrally on the formation of the TFIIB-TBP-DNA complex. They should also be relevant to activated transcription (9), but the manner in which this occurs and the effect of initiator sequences remains to be determined. Basically, one can view the core promoter as a contiguous sequence of nearly 100 base pairs (4–6, 8, 10, 36), all parts of which are designed to work together to give appropriate amounts of properly regulated transcription.

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