Preinitiation complex assembly: potentially a bumpy path

Julia M Cox, Alexis R Kays, Jennifer F Sanchez and Alanna Schepartz*

During 1996 and 1997, several chemical issues that arise in the early stages of preinitiation complex (PIC) formation were resolved. Kinetics experiments indicated that both TBP dimerization and DNA bending influence the rate of TBP–TATA box assembly. Affinity cleavage experiments indicated that TBP lacks the specificity to nucleate assembly of a properly oriented PIC. Finally, high-resolution structures provided the atomic detail of early intermediates in PIC formation.

Addresses
Department of Chemistry, PO Box 208107, 225 Prospect Street, Yale University, New Haven, CT 06520-8107, USA
*Correspondence: Alanna Schepartz
e-mail: alanna@milan.chem.yale.edu

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Abbreviations

PIC	preinitiation complex
polII	RNA polymerase II
TAFs	TBP-associated factors
TBP	TATA-binding protein
TF	transcription factor

Introduction

Gene expression in eukaryotes is regulated in large part by control of the rate of transcription initiation (for a recent review see [1]). For genes that encode proteins, this initiation requires assembly of a preinitiation complex (PIC) on a core promoter. This PIC consists of RNA polymerase II (pol II) and transcription factors TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH (reviewed in [2••,3]). This supramolecular complex, which in humans comprises at least 35 polypeptide chains and has a molecular weight of two million Daltons, performs the synthetic chemistry of template-directed mRNA synthesis in response to environmental changes, metabolic needs or signals that regulate development and the cell cycle.

Two pathways can describe PIC formation

Early biochemical experiments indicated that PIC formation can begin when TFIID binds its DNA target site, the TATA box (consensus sequence TATA a/t A a/t N, where N represents any nucleotide) [4]. In higher eukaryotes, TFIID consists of the TATA binding protein (TBP), which binds DNA as a monomer, and at least eight additional TBP-associated factors (TAFs) (reviewed in [5]) that are believed necessary for enhanced transcription ('activation') in response to transcriptional activators and coactivators (reviewed in [6–8]; although this issue is continually being evaluated [9,10]). Once assembled, the TFIID–TATA box complex functions as a scaffold for the subsequent binding of TFIIA, TFIIB, TFIIF–pol II, TFIIE and TFIIH (reviewed in [2••,8]). The TBP subunit of TFIID can bind the TATA box even in the absence of TAFs, however, and can assemble a PIC capable of basal levels of transcription. Recent experiments indicate that PIC assembly can also follow a simpler pathway in which a pol II holoenzyme [11,12] containing pol II and various general factors and coactivators (reviewed in [13,14]), binds a preformed TBP/TFIID–TATA box complex. Both the stepwise and holoenzyme pathways begin with a preformed TBP/TFIID–TATA box complex and end with a fully functional PIC stabilized by an extensive array of noncovalent, intermolecular interactions. The difference between the two pathways relates not to the identities of these intermolecular contacts but rather to the order in which they form.

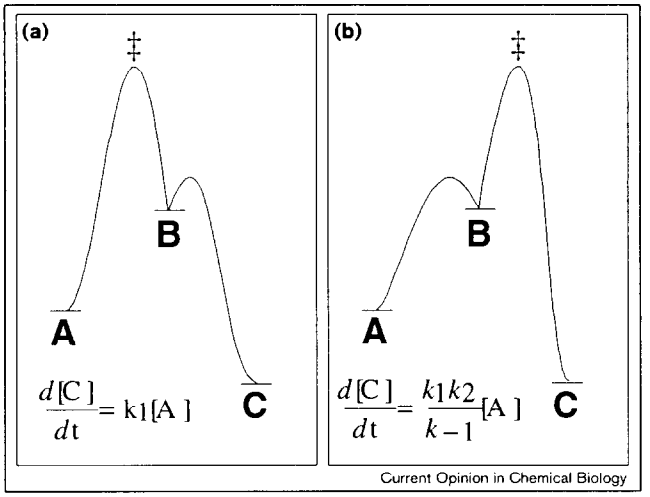
The importance of the early steps

Regardless of the pathway by which the PIC assembles, the first steps on TATA-box-containing promoters are the same: promoter DNA becomes accessible through processes that remodel chromatin (reviewed in [15–17]) and TBP (with or without associated TAFs) binds the TATA box. *In vivo* and *in vitro* experiments performed in the presence and absence of transcriptional activators indicate that the rate of association of TBP and a TATA box can limit the overall rate of transcription [18–22]. These experiments emphasize the importance of early steps to the overall kinetics of a multistep pathway, even if later steps are accessed through higher activation barriers and are therefore formally rate limiting (Figure 1). This review will concentrate on the chemistry of these early steps in PIC formation, with a special focus on constructing a potential energy surface for the initial — and perhaps defining — steps in preinitiation complex formation.

Why does TBP bind DNA slowly?

By 1995, we knew much about TBP–TATA box complex formation, including the following 3 observations: human TBP forms a dimer with a nanomolar dissociation constant in the absence of DNA [23]; association of TBP with DNA follows second-order kinetics and proceeds at least three orders of magnitude more slowly than the diffusion limit [24–27]; and the 80° bend in the DNA seen in the structurally characterized TBP–TATA box complexes [28,29] is modulated by changes in TATA box sequence in a way that correlates with TBP–TATA box dissociation rates [30]. Yet several key questions remained. Is the slow association of the TBP–TATA box complex due to a high barrier for dissociation of the TBP dimer, a high barrier for DNA bending, or both of these effects? How do the remaining components of TFIID and other factors influence these barrier heights? Kinetic and equilibrium

Figure 1



Two hypothetical potential energy diagrams for the reaction sequence:

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C$$

that illustrate how the overall reaction rate can be influenced by the rates of steps that precede the rate-limiting step (denoted here with the symbol ‡). In diagram **(a)**, the rate-limiting step separates A and B, and the overall reaction rate depends on the rate constant k_1. In diagram **(b)**, the rate-limiting step separates intermediates B and C and the overall reaction rate depends on rate constants k_1, k_2, and k_{-1}.

results described recently can be consolidated with earlier data to formulate a potential energy diagram for TBP binding to the TATA box as the initial step in preinitiation complex formation.

One early equilibrium step that occurs prior to DNA binding is dissociation of the TBP dimer observed with yeast [31••], human [23], and archaeal [32•] TBP. Studies published recently attest to the physiological relevance of the TBP dimer first observed by X-ray crystallography [33,34] and demonstrate how the TBP monomer–dimer pre-equilibrium affects the kinetics of TBP–TATA box interactions. In addition, TBP is not found as a free molecule in the nucleus, but is complexed tightly with TAFs in the form of TFIID as noted above (reviewed in [5,35]). Taggart and Pugh [31••] demonstrated that TFIID formed stable dimers *in vitro* (K_d = 10nM) and detected TBP dimers within the highly crowded environs of growing HeLa cells. More to the point, the dimers dissociated slowly into monomers ($k_{off} = 10^{-3}$ sec^{-1} for TBP) and dissociation was essential for binding to DNA [31••,36••]. These studies indicate that at its estimated nuclear concentration (1 μM), the TBP dimer occupies a relevant position on the potential energy surface leading to a functional PIC. Taggart and Pugh [31••] propose a mechanism in which slow TBP dimer dissociation precedes a rapid DNA-binding step that was intimated as early as 1993 [37] and proposed by Parkhurst *et al.* [38••] (discussed below).

The relationship between TBP–TATA box complexation and DNA bending was investigated by Parkhurst *et al.* [38••] using human TBP. They made use of an oligonucleotide duplex containing the Adenovirus major late promoter (AdMLP) TATA box, labeled at opposite ends with tetramethylrhodamine and fluorescein, in order to monitor the concentration- and time-dependent changes in TATA box conformation induced upon TBP binding. The crystal structure of the human TBP–TATA box complex was reported in 1996 [39] and supported Parkhurst *et al.*'s findings. The bend induced in the TATA box by human TBP decreases the distance between the dyes as indicated by an increase in the extent of Förster resonance energy transfer (FRET) [40] between them. Analysis of fluorescein emission changes during dissociation as a function of time revealed that DNA bending and binding occurred with a second-order rate constant of 2.4×10^6 M^{-1} s^{-1}, a value within one order of magnitude of rate constants measured previously with other TBPs [25–27]. Importantly, the fluorescein emission change observed as the binding reaction proceeded was identical to that observed when the components were mixed and allowed to reach equilibrium. Parkhurst *et al.* concluded [38••] that for the range of TBP concentrations examined (at the nanomolar level), DNA binding and bending occur simultaneously. Because the observed kinetics are second order, these results rule out a two-step mechanism characterized by rate-limiting bending of a preformed TBP–TATA box complex [25]. These experiments were performed under conditions where the TBP monomer concentration exceeded the DNA concentration. Consequently, they define a rate constant for the association of monomeric TBP with DNA in the absence of a competing monomer–dimer equilibrium.

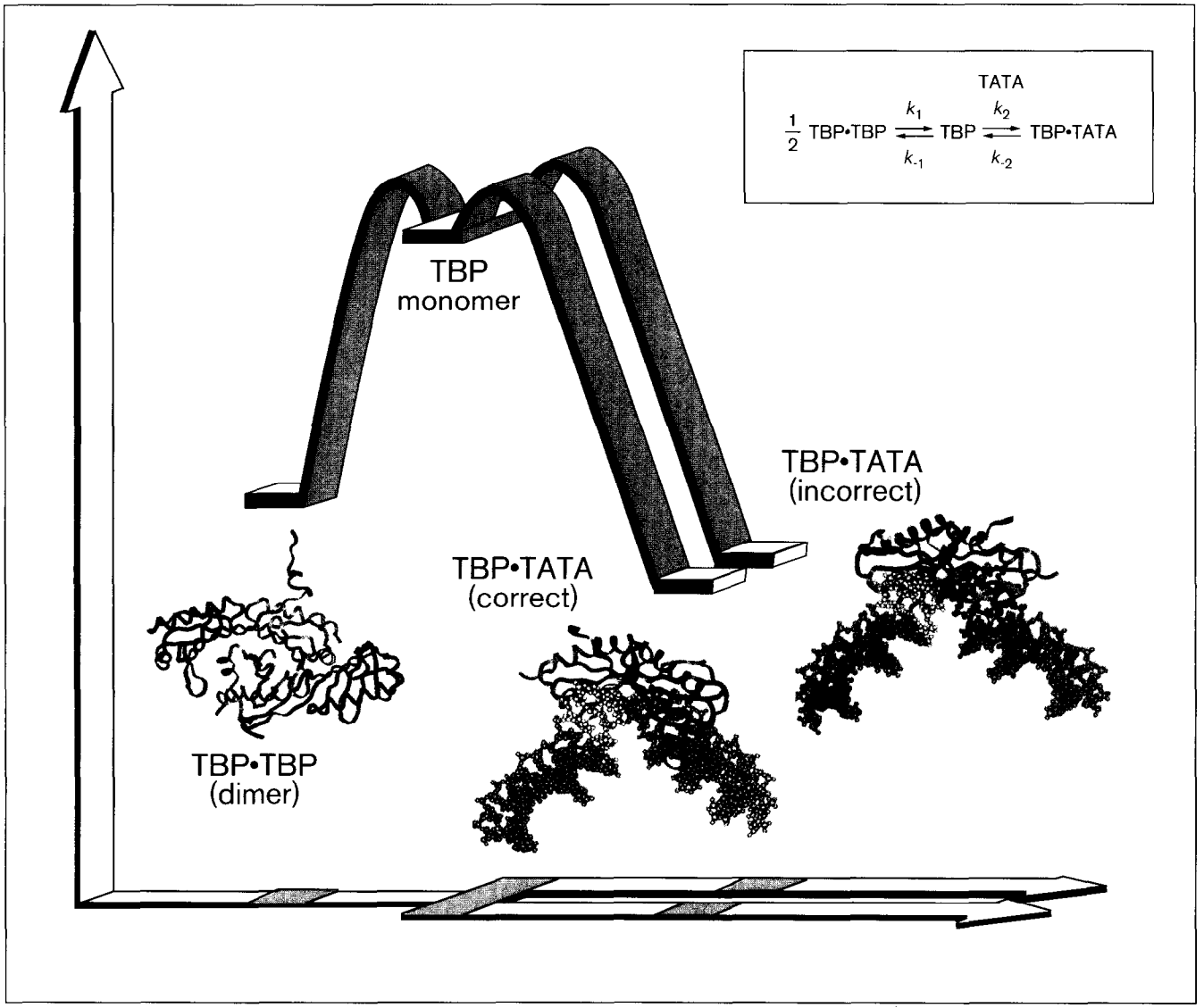
Consideration of the kinetic data discussed above indicates that TBP dimerization and DNA bending/binding both contribute to the observed slow association kinetics (Figure 2). DNA binding by monomeric TBP proceeds with a rate constant of approximately 5×10^5 M^{-1} s^{-1} [25–27,38••]. This rate constant is comparable to that observed for dimerization of monomeric TBP, 1×10^5 M^{-1} s^{-1} (calculated from the dissociation equilibrium and rate constants, 10 nM and 1×10^{-3} s^{-1}, respectively [36••]). Thus, in the absence of other factors, monomeric TBP is faced with two equivalent barriers (Figure 2): overcoming one barrier accesses the final TBP–TATA box complex; overcoming the other barrier returns to the inactive TBP dimer. The equivalence of these barriers permits speculation that they are both easily manipulated by additional factors to regulate transcription initiation. These factors could facilitate the binding of TFIID by inhibiting TFIID dimerization or perhaps by prebending DNA [6,41]. Indeed, recent experiments support this idea: TBP

mutants that dimerized poorly sustained increased levels of basal transcription in yeast cells (BF Pugh, personal communication), suggesting that an increase in TBP monomer concentration led to a concomitant increase in the probability that the TBP–TATA box complex formed.

Does TBP bind the TATA box in a single orientation?

Interwoven with the kinetics of TBP–TATA box interactions is the orientation of the binary and higher order complexes formed in solution. Early structural studies on free TBP revealed a molecular fold containing two subdomains related by an approximate twofold symmetry axis (Figure 2) [33,34,42]. Later structural studies on binary TBP–TATA box complexes demonstrated that the symmetry extends to the interface with the TATA box [28,29,39]. The amino acids of TBP used to recognize the eight base pair TATA box are as much as 89% (in human TBP) conserved between the two subdomains, yet all eukaryotic TBP–TATA box crystal structures show the protein bound in a single orientation consistent with assembly of uniquely oriented PIC. This orientation shows the carboxy-terminal TBP subdomain interacting with the more conserved TATA half site and the amino-terminal subdomain interacting with the less conserved a/t A a/t N half site (where N is any nucleotide).

Figure 2



Potential energy diagram for the reaction sequence shown in the box (top, right). The reaction coordinate is represented by the x axis; the free energy along the reaction coordinate is represented by the y axis. The amino- and carboxy-terminal subdomains of the conserved core of TBP are colored blue and pink, respectively. The 5′ and 3′ half sites of the eight base pair TATA box are colored yellow and green, respectively. The structure of the TBP dimer is taken from [33]; the structures of the TBP–TATA box complexes are taken from [29]. The heights of barriers leading to the two orientations of the TBP–TATA box complex are not known with precision.

Several theories have been proposed to explain why TBP binds in a single orientation to asymmetric TATA box sequences. The theme underlying each theory is that a difference in the ease of bending the 5′ and 3′ TATA box half sites (in eukaryotes, TATA and a/t A a/t N, respectively) is recognized or accommodated by a difference in the two TBP subdomains. This theory was first proposed by Kim *et al.* [29], who pointed out that alternating TA base steps in the 5′ half site should be easier to bend than nonalternating TA base steps in the 3′ half site. Stronger interactions are therefore necessary to bend/interact with the more rigid 3′ half site. Kim *et al.* [29] proposed that the greater positive electrostatic potential in the amino-terminal subdomain provides the enthalpic boost to bend the 3′ half site. Suzuki *et al.* [43••] proposed that the larger size of amino acid sidechains on the interior of the β sheet in the carboxy-terminal subdomain renders this half of TBP more curved (and presumably more rigid) than the amino-terminal subdomain and leads to preferential interaction of the carboxy-terminal subdomain with the more easily deformed 5′ half site. Juo *et al.* [44••] proposed that the presence of a proline residue within the carboxy-terminal subdomain of TBP demands a TA base pair at the first position in the 5′ half site, resulting in the orientation observed in the crystal. It is notable that TBP from the archaeal *Pyrococcus woesei* contains this proline residue in both the carboxy- and amino-terminal stirrup and lacks noticeable asymmetry in electrostatic charge potential [32], yet it exhibits oriented binding in the ternary complex containing DNA, archaeal TBP and the archaeal homolog TFB [45••].

The theories described above may explain why oriented binding is observed in crystals, but affinity cleavage results indicate that, in solution, less than 0.3 kcal mol^{-1} separates the energy of the observed conformer from one in which TBP is rotated 180°. Cox *et al.* [46••] prepared two TBP variants modified with a 1,10-phenanthroline DNA-cleaving agent on a residue within either the amino- or carboxy-terminal stirrup (Figure 2). Cleavage with this nondiffusible oxidizing agent is expected on only one side of the TATA box if TBP binds in a single orientation. The resulting molecules cleaved DNA on both sides of the TATA box with a modest preference (K_{eq} = 1.5–1.8) for the side expected based on data from crystallography [28,29,39]. No preference was observed when the asymmetric eight base pair TATA box in AdMLP was replaced by the symmetric sequence TATATATA. These experiments are consistent with modeling studies that revealed no unfavorable interactions when TBP was bound in the opposite orientation [47]. These experiments demonstrate that, in the absence of other proteins, TBP binds the TATA box as a mixture of orientational isomers that are related by a 180° rotation about the pseudo-dyad (the approximate symmetry axis) of the complex. Additional experiments indicated that TBP bound a distribution of sites displaced by up to three base pairs on either side of the eight base pair TATA box [46••].

The multiple axial and rotational conformations identified by this study, coupled with the known high nonspecific DNA affinity of TBP, indicates that TBP alone is unable to accurately position itself on a promoter. These results add an additional dimension to the potential energy diagram defined earlier (Figure 2). Monomeric TBP chooses between three options: forming a dimer, binding in the orientation required presumably for PIC assembly and accurate transcription initiation, and binding in the opposite orientation. These barriers are likely to be equivalent and therefore all three steps could be modulated to regulate transcription from different promoters.

How do other general factors influence TBP–TATA box structure and equilibria?

In the stepwise assembly pathway, the initial step of TBP binding to promoter DNA can be accompanied by the binding of TFIIA and is followed by the binding of TFIIB ([4]; reviewed in [2••]). The high resolution structure of the TBP–TATA box complex bound to TFIIA [48••,49••] or the archaeal TFIIB homologue TFB [45••], and the imaged structure of the yeast TFIIB–TFIIE–pol II complex [50] provide insight into factors influencing the next steps in the pathway. These structures complement the ternary TFIIB–TBP–AdMLP structure reported in 1995 [51] and provide a framework for understanding how properly oriented binding of the PIC is achieved.

TFIIA, TFIIB, and TFB interact with TBP and promoter DNA

The structure of the ternary TFIIA–TBP–CYC1 TATA complex shows residues at the edge of the TFIIA β-sandwich domain interacting with residues within the TBP β2 strand that immediately precedes the TBP amino-terminal stirrup. These interactions position TFIIA on the amino-terminal (downstream) TBP subdomain; however, the severe bend in the DNA allows TFIIA to interact with the phosphodiester backbone upstream of the TATA box [48••,49••]. The structures of the heterologous TFIIB–TBP–AdMLP [51] and archaeal TFB–TBP–TATA box [45••] complexes show a complex set of interactions between TFIIB/TFP and the TBP carboxyl-terminal stirrup. It is interesting that, despite the relatively large protein surfaces that are buried upon ternary complex formation, alanine scanning mutagenesis experiments suggest that both the TFIIA–TBP and TFIIB–TBP interfaces [52] are characterized by 'hot spots' [53], small clusters of amino acid residues that contribute the majority of favorable binding energy.

In contrast to eukaryotic complexes, the higher degree of symmetry inherent in the archaeal complex provides even less insight into factors influencing the orientation of archaeal TBP on promoter DNA [45••]. A striking difference of the archaeal complex is that TBP is found bound to DNA in the opposite orientation relative to all eukaryotic complexes. The consensus boxA sequence of archaeal promoters (consensus sequence c/t TTA t/a ANN,

where N is any nucleotide), however, closely resembles an inverted eukaryotic TATA box. The structure of this complex raises questions as to whether archaeal TBP recognizes promoter DNA in a sequence-specific fashion and whether the archaeal factors are arranged differently with respect to the polymerase. Further study is needed to determine how archaeal TBP recognizes DNA, and how TFB and polymerase affect this recognition.

Affinity cleavage experiments performed within TFIIA–TBP–TATA box and TFIIB–TBP–TATA box complexes revealed that TFIIA and TFIIB each contribute to formation of an oriented PIC, but are not sufficient individually to anchor the complex in a single orientation [46••]. Affinity cleavage of DNA in the ternary complex containing TBP, TFIIB, and the AdMLP reflected a greater preference for the 'correct' orientation than that seen for TBP alone ($K_{eq}=4$ versus $K_{eq}=1.5$–1.8, an increase in specificity of about 0.5 kcal mol^{-1}). Moreover, the distribution of cleavage sites in the presence of TFIIB was narrower than in its absence, indicating that TFIIB also positioned TBP more accurately on the 8 base pair TATA box. Similar results were obtained with TFIIA [46••]. These results suggest that TFIIA and TFIIB enhance the orientational preference and axial positioning of TBP on the promoter either by exploiting sequence-specific differences in the TATA box or the flanking DNA, or by enhancing the intrinsic selectivity of TBP itself.

Evidence for sequence-specific DNA binding by TFIIB: a new core promoter element?

Recent evidence suggests that the effect of TFIIB on the orientation of bound TBP may arise, at least in part, from sequence-specific recognition of flanking DNA by TFIIB [54••]. *In vitro* selection experiments identified a seven base pair sequence (5′–g/c g/c g/a CGCC–3′) which increased the stability of a TFIIB–TBP–AdMLP complex by threefold (0.7 kcal • mol^{-1} at 30°C) when located upstream of the AdMLP TATA box. Fluorescence anisotropy and cross-linking data provided evidence for a direct TFIIB–DNA interaction ($K_d=0.2\,\mu M$) in the absence of TBP. Similar *in vitro* selection experiments with TFIIA–TBP–TATA box complexes are in progress; however, no interactions between TFIIA and TBP are detected by filter-binding methods [55]. These results suggests that TFIIA could enhance the orientational specificity of TBP by a different mechanism, perhaps by exaggerating differences at the TBP-TATA box interface as discussed earlier [29,43••,44••]. Further study is needed to elucidate the precise effects of TFIIA, TFIIB, and other PIC components on orientation equilibrium and kinetics and to determine whether these effects and the eventual orientation vary between different promoters.

Onward to the final PIC

The remaining intermediates in PIC assembly are not accessible by current high resolution methods. Chemical cross-linking methods, however, have advanced our understanding of the overall topology of the complex and conformational changes that may occur during assembly [56–58]. The emerging picture of PIC assembly includes TFIIA-mediated changes in the orientation of distal DNA and wrapping of the DNA around a multiprotein core. Lagrange *et al.* [56] detected cross-links between TFIIB and DNA at positions further from the TATA box only when TFIIA was present. Oelgeschläger *et al.* [57] reported cross-links between several TAF_{II}s and DNA downstream of the TATA box only in the presence of TFIIA. Both studies are consistent with a conformational change within TFIID induced upon the binding of TFIIA. In addition, both Lagrange *et al.* [56] and Kim *et al.* [58] present evidence for wrapping of DNA within the assembled PIC. The insight provided by these chemical studies will help pave the way towards a defined topology of the PIC.

Conclusions and future directions

Research reported in the past two years has increased our understanding of several chemical issues that arise in the early stages of PIC formation. The debate over whether TBP dimerization or DNA bending constitutes the rate-limiting step for promoter binding was ended by kinetics experiments indicating that both steps can contribute to the formation of TBP–TATA box complexes. This conclusion permits speculation that one or both of these steps could be regulated by interactions with other factors in the cell, an idea currently being investigated. The mystery of why the highly symmetric TBP molecule binds in one direction to an asymmetric TATA box was superseded by the observation that both bound orientations are populated in solution. In addition, X-ray crystallography provided a look at ternary complexes containing TFIIA–TBP–TATA [48••,49••], TFB–TBP–TATA [45••] and TFIIB–TFIIE–pol II [50]. Despite this progress, many interesting and difficult questions remain. For example, what effect do TAFs have on the kinetic barriers defined with TBP alone? Can interactions between upstream activators and TBP or TAFs lower the barrier for TFIID–TATA box assembly so that later steps become rate limiting? Do all PIC complexes possess the same orientational specificities? How is the PIC assembled at promoters that lack TATA boxes? Because activator function is intimately related to recruitment of TBP/TFIID, a better understanding of the mechanism of TBP–TATA box association should allow the chemical detail of activation mechanisms to be understood. Improved kinetic methods and high resolution structural studies of the relevant assemblies may ultimately provide sufficient guidance for a chemical understanding of both regulated gene expression and to what extent the stepwise and holoenzyme assembly pathways are used at various pol II promoters.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Goodbourn S (Ed): *Eukaryotic gene transcription.* 1996, Oxford: New York.

2. •• Roeder RG: **The role of general initiation factors in transcription by RNA polymerase II.** *Trends Biochem Sci* 1996, **21**:327-334.

This outstanding review provides a primer for anyone interested in the events leading to transcription from RNA polymerase II promoters *in vivo* and *in vitro*.

3. Tjian R: **The biochemistry of transcription in eukaryotes: a paradigm for multisubunit regulatory complexes.** *Phil Trans R Soc Lond B* 1996, **351**:491-499.

4. Buratowski S, Hahn S, Guarente L, Sharp PA: **Five intermediate complexes in transcription initiation by RNA polymerase II.** *Cell* 1989, **56**:549-561.

5. Burley SK, Roeder RG: **Biochemistry and structural biology of transcription factor IID (TFIID).** *Annu Rev Biochem* 1996, **65**:769-799.

6. Tjian R, Maniatis T: **Transcriptional activation: A complex puzzle with few easy pieces.** *Cell* 1994, **77**:5-8.

7. Verrijzer CP, Tjian R: **TAFs mediate transcriptional activation and promoter selectivity.** *Trends Biochem Sci* 1996, **21**:338-342.

8. Ranish JA, Hahn S: **Transcription: basal factors and activation.** *Curr Opin Genet Dev* 1996, **6**:151-158.

9. Walker SS, Reese JC, Apone LM, Green MR: **Transcription activation in cells lacking TAFIIs.** *Nature* 1996, **282**:185-188.

10. Moqtaderi Z, Bai Y, Poon D, Weil PA, Stwhl K: **TBP-associated factors are not generally required for transcriptional activation in yeast.** *Nature* 1996, **282**:188-191.

11. Kim Y-J, Björkland S, Li Y, Sayre MH, Kornberg RD: **A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II.** *Cell* 1994, **77**:599-608.

12. Koleske A, Young RA: **A RNA polymerase II holoenzyme responsive to activators.** *Nature* 1994, **368**:466-469.

13. Björklund S, Kim Y-J: **Mediator of transcriptional regulation.** *Trends Biochem Sci* 1996, **21**:335-337.

14. Koleske AJ, Young RA: **The RNA polymerase II holoenzyme and its implications for gene regulation.** *Trends Biochem Sci* 1995, **20**:113-116.

15. Steger DJ, Workman JL: **Remodeling chromatin structures for transcription: what happens to the histones?** *Bioessays* 1996, **19**:875-884.

16. Struhl K: **Chromatin structure and RNA polymerase II connection: implications for transcription.** *Cell* 1996, **84**:179-182.

17. Grunstein M: **Histone acetylation in chromatin structure and transcription.** *Nature* 1997, **389**:349-352.

18. Klein C, Struhl K: **Increased recruitment of TATA-binding protein to the promoter by transcriptional activation domains *in vivo*.** *Science* 1994, **266**:280-2.

19. Chatterjee S, Struhl K: **Connecting a promoter-bound protein to TBP bypasses the need for a transcriptional activation domain.** *Nature* 1995, **374**:820-822.

20. Klages N, Strubin M: **Stimulation of RNA polymerase II transcription initiation by recruitment of TBP *in vivo*.** *Nature* 1995, **374**:822-823.

21. Colgan J, Manley JL: **TFIID can be rate limiting *in vivo* for TATA-containing, but not TATA-lacking, RNA polymerase II promoters.** *Genes Dev* 1992, **6**:304-315.

22. Lieberman PM, Berk AJ: **A mechanism for TAFs in transcriptional activation: activation domain enhancement of TFIID-TFIIA–promoter DNA complex formation.** *Genes Dev* 1994, **8**:995-1006.

23. Coleman RA, Pugh BF: **Evidence for functional binding and stable sliding of the TATA binding protein on nonspecific DNA.** *J Biol Chem* 1995, **270**:13850-13859.

24. von Hippel PH, Berg OG: **Facilitated target location in biological systems.** *J Biol Chem* 1989, **264**:675-678.

25. Hoopes BC, LeBlanc JF, Hawley DK: **Kinetic analysis of yeast TFIID·TATA Box complex formation suggests a multi-step pathway.** *J Biol Chem* 1992, **267**:11539-11547.

26. Perez-Howard GM, Weil PA, Beechem JM: **Yeast TATA binding protein interaction with DNA: fluorescence determination of oligomeric state, equilibrium binding, on rate, and dissociation kinetics.** *Biochemistry* 1995, **34**:8005-8017.

27. Petri V, Hsieh M, Brenowitz M: **Thermodynamic and kinetic characterization of the binding of the TATA binding protein to the adenovirus E4 promoter.** *Biochemistry* 1995, **34**:9977-9984.

28. Kim JL, Nikolov DB, Burley SK: **Co-crystal structure of TBP recognizing the minor groove of a TATA element.** *Nature* 1993, **365**:520-527.

29. Kim Y, Geiger JH, Hahn S, Sigler PB: **Crystal structure of a yeast TBP/TATA-box complex.** *Nature* 1993, **365**:512-520.

30. Starr DB, Hoopes BC, Hawley DK: **DNA bending is an important component of site-specific recognition by the TATA binding protein.** *J Mol Biol* 1995, **250**:434-436.

31. •• Taggart AKP, Pugh BF: **Dimerization of TFIID when not bound to DNA.** *Science* 1996, **272**:1331-1333.

This paper presents convincing evidence that the transcription factor TFIID forms a stable dimer in the absence of DNA and that TATA-binding protein (TBP)/TFIID dimers are present even inside the highly crowded environs of a growing HeLa cell.

32. • DeDecker BS, O'Brien R, Fleming PJ, Geiger JH, Jackson SP, Sigler PB: **The crystal structure of a hyperthermophilic archaeal TATA-box binding protein.** *J Mol Biol* 1996, **264**:1072-1084.

The crystal structure of TBP from the hyperthermophilic archaea *Pyrococcus woesei* is analyzed and compared to the corresponding eukaryotic TBP structures. The structure is notable because of the extensive symmetry between the carboxy- and amino-terminal subdomains.

33. Nikolov DB, Hu SH, Lin J, Gasch A, Hoffmann A, Horikoshi M, Chua NH, Roeder RG, Burley SK: **Crystal structure of TFIID TATA-box binding protein.** *Nature* 1992, **360**:40-46.

34. Chasman DI, Flaherty KM, Sharp PA, Kornberg RD: **Crystal structure of yeast TATA-binding protein and model for interaction with DNA.** *Proc Natl Acad Sci USA* 1993, **90**:8174-8178.

35. Burley SK: **The TATA binding protein.** *Curr Opin Struct Biol* 1996, **6**:69-75.

36. •• Coleman RA, Pugh BF: **Slow dimer dissociation of the TATA binding protein dictates the kinetics of DNA binding.** *Proc Natl Acad Sci USA* 1997, **94**:7221-7226.

This paper made use of a novel assay to provide evidence that slow dissociation of the human TBP dimer limited the rate of TBP–TATA box association. The results were used to formulate and test different mechanisms for the DNA binding reaction.

37. Kuddus R, Schmidt MC: **Effect of the non-conserved N-terminus on the DNA binding activity of the yeast TATA binding protein [published erratum appears in Nucleic Acids Res 1993 Jun 25;21(12):2962].** *Nucleic Acids Res* 1993, **21**:1789-1796.

38. •• Parkhurst KM, Brenowitz M, Parkhurst LJ: **Simultaneous binding and bending of promoter DNA by the TATA binding protein: real time kinetic measurements.** *Biochemistry* 1996, **35**:7459-7465.

Fluorescence experiments using a tetramethylrhodamine and fluorescein labeled duplex oligonucleotide were used to investigate the kinetics of binding and bending by yeast TBP. The time-dependent measurements obtained by stopped-flow fluorimetry showed that binding and bending of the DNA occur simultaneously and ended a long-standing debate.

39. Nikolov DB, Chen H, Halay ED, Hoffman A, Roeder RG, Burley SK: **Crystal structure of a human TATA box-binding protein/TATA element complex.** *Proc Natl Acad Sci USA* 1996, **93**:4862-4687.

40. Förster T: **Zwischenmolekulare energiewanderung und fluorescenz.** *Annu Phys* 1948, **2**:55.

41. Parvin JD, McCormick RJ, Sharp PA, Fisher DE: **Pre-bending of a promoter sequence enhances affinity for the TATA-binding factor.** *Nature* 1995, **373**:724-727.

42. Nikolov D, Burley SK: **2.1 Å resolution refined structure of a TATA box-binding protein (TBP).** *Nature Struct Biol* 1994, 1:621-637.

43. •• Suzuki M, Allen MD, Yagi N, Finch JT: **Analysis of co-crystal structures to identify the stereochemical determinants of the orientation of TBP on the TATA box.** *Nucleic Acids Res* 1996, **24**:2767-2773.
In this paper, the authors discuss several possible reasons to explain why TBP binds to the TATA box in the single orientation observed in crystal structures.

44. •• Juo ZS, Chiu TK, Leiberman PM, Baikalov I, Berk AJ, Dickerson RE: **How proteins recognize the TATA box.** *J Mol Biol* 1996, **261**:239-254.
This paper presents arguments as to why the TBP·TATA box complex forms in the manner observed by crystallography.

45. •• Kosa PF, Ghosh G, DeDecker BS, Sigler PB: **The 2.1-Å crystal structure of an archaeal preinitiation complex: TATA-box-binding protein/transcription factor (II)B core/TATA-box.** *Proc Natl Acad Sci USA* 1997, **94**:6042-6047.
TBP from *Pyrococcus woesei* is crystallized in complex with promoter DNA and the TFB, the archaeal homolog of transcription factor (TF)IIB. The comparative analysis of the details of this complex and other higher order eukaryotic structures raises issues of evolutionary divergence and the implications of conservation to extend our understanding of the physical biochemistry of transcriptional initiation.

46. •• Cox JM, Hayward MM, Sanchez JF, Gegnas LD, van der Zee S, Dennis JH, Sigler PB, Schepartz A: **Bidirectional binding of TBP to the TATA box.** *Proc Natl Acad Sci USA* 1997, **94**:13475-13480.
This paper demonstrates that TBP binds to the TATA box in two orientations in solution and provides evicence that TBP alone does not define pre-initiation complex orientation.

47. Miaskiewicz K, Ornstein RL: **DNA binding by TATA-box binding protein (TBP): a molecular dynamics simulation.** *J Biomol Struct Dyn* 1996, **13**:593-600.

48. •• Geiger JH, Hahn S, Lee S, Sigler PB: **Crystal structure of the yeast TFIIA/TBP/DNA complex.** *Science* 1996, **272**:830-836.
This paper provides in-depth analysis of the TFIIA–TBP–TATA box ternary complex. Detailed accounts of TBP–TFIIA contacts are reported and correlated with mutagenesis studies of the same interface.

49. •• Tan S, Hunziker Y, Sargent DF, Richmond TJ: **Crystal structure of a yeast TFIIA/TBP/DNA complex.** *Nature* 1996, **381**:127-134.
In this paper, the ternary TBP–TFIIA–TATA box complex is critically analyzed. On the basis of TFIIA contacts to the phosphate backbone of DNA, both upstream and within the TATA box, it is suggested that TFIIA may be sensitive to major groove width, and, therefore, to the sequence of the upstream flanking DNA.

50. Leuther KK, Bushnell DA, Kornberg RD: **Two-dimensional crystallography of the TFIIB- and IIE-RNA polymerase II complexes: Implications for start site selection and initiation complex formation.** *Cell* 1996, **85**:773-779.

51. Nikolov DB: **Crystal structure of a TFIIB-TBP-TATA element ternary complex.** *Nature* 1995, **377**:119-128.

52. Tang H, Sun X, Reinberg D, Ebright RH: **Protein-protein interactions in eukaryotic transcription initiation: Structure of the preinitiation complex.** *Proc Natl Acad Sci USA* 1996, **93**:1119-1124.

53. Clackson T, Wells JA: **A hot spot of binding energy in a hormone-receptor interface.** *Science* 1995, **267**:383-386.

54. •• Lagrange T, Kapanidis AN, Tang H, Reinberg D, Ebright RH: **New core promoter element in RNA-polymerase-II-dependent transcription: Sequence-specific DNA binding by Transcription Factor IIB.** *Genes Dev.* 1998, in press.
Careful *in vitro* selection experiments provide evidence for sequence-specific interactions between TFIIB and DNA upstream of the TATA box.

55. Weideman CA, Netter RC, Benjamin LR, McAllister JJ, Schmiedekamp LA, Coleman RA, Pugh BF: **Dynamic interplay of TFIIA, TBP and TATA DNA.** *J Mol Biol* 1997, **271**:61-75.

56. Lagrange T, Kim TK, Orphanides G, Ebright YW, Ebright RH, Reinberg D: **High resolution mapping of nucleoprotein complexes by site-specific protein-DNA photocrosslinking: organization of the human TBP-TFIIA-TFIIB-DNA quaternary complex.** *Proc Natl Acad Sci USA* 1996, **93**:10620-10625.

57. Oelgeschläger T, Chiang C-M, Roeder RG: **Topology and reorganization of a human TFIID-promoter complex.** *Nature* 1996, **382**:735-738.

58. Kim T-K, Lagrange T, Wang Y-H, Griffith JD, Reinberg D, Ebright RH: **Trajectory of DNA in the RNA polymerase II transcription preinitiation complex.** *Proc Natl Acad Sci USA* 1997, **94**:12268-12273.