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# TATA Box Mimicry by TFIID: Autoinhibition of Pol II Transcription

Minireview

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In eukaryotes, RNA polymerase II (pol II) is responsible for transcribing nuclear genes encoding the messenger RNAs and several small nuclear RNAs. Like RNA polymerases I and III, pol II cannot recognize its target promoter directly and initiate transcription in the absence of accessory proteins. Instead, this large multisubunit enzyme relies on both general transcription factors, or GTFs, and transcriptional activators and cofactors (both positive and negative) to regulate transcription from class II promoters. The primary DNA anchor of this complicated macromolecular machine is transcription factor IID, or TFIID, a 700 kDa complex composed of the TATA box–binding protein (TBP) and a set of phylogenetically conserved, pol II–specific TBP-associated factors, or $TAF_{II}$s (reviewed in Burley and Roeder, 1996). DNA binding by human TFIID was first demonstrated with the adenovirus major late promoter (AdMLP). DNase I footprinting studies of the AdMLP and selected human gene promoters revealed sequence-specific interactions between human TFIID and the TATA element, which are primarily mediated by TBP (reviewed in Patikoglou and Burley, 1997). In contrast, protection both upstream and downstream of the TATA box is largely sequence independent, displays a nucleosome-like pattern of DNase I hypersensitivity, varies radically among promoters, and can be induced by some activators (reviewed in Burley and Roeder, 1996).

In vivo, the transcription initiation process can be usefully divided into two mechanistic phases, referred to as antirepression and net activation (Figure 1A). At any given time, most class II nuclear genes are transcriptionally silent or repressed. Like activation (reviewed in Roeder, 1996), the mechanisms underlying transcriptional repression and its reversal are manifold. The subject of this minireview is an elegant autoinhibitory strategy characterized at the molecular level by the laboratories of Ikura and Nakatani using NMR spectroscopy (Liu et al., 1998 [this issue of *Cell*]). Their technically impressive structure determination proves that the N-terminal portion of the largest pol II–specific *Drosophila melanogaster* TBP-associated factor ($dTAF_{II}230$) recognizes the DNA-binding surface of *Saccharomyces cerevisiae* TBP, forming a stable 1:1 complex that inhibits TATA box binding (see Figure 6 in (Liu et al., 1998). Evidence for autoinhibition of TATA element recognition by TFIID first emerged nearly a decade ago, with Nakatani's demonstration that the DNA-binding properties of TFIID and TBP could be distinguished in the context of weak promoters (Nakatani et al., 1990). Subsequently, work with recombinant $TAF_{II}$s (reviewed in Liu et al., 1998) and reconstituted TFIID complexes (Verrijzer et al., 1995; Guermah et al., 1998) documented that certain *Drosophila* and human $TAF_{II}$s ($dTAF_{II}230$ and $hTAF_{II}250$) interfere with formation of a stable TBP-TATA element complex. Deletion studies with $dTAF_{II}230$ mapped the autoinhibitory activity to the first 81 amino acids (reviewed in Liu

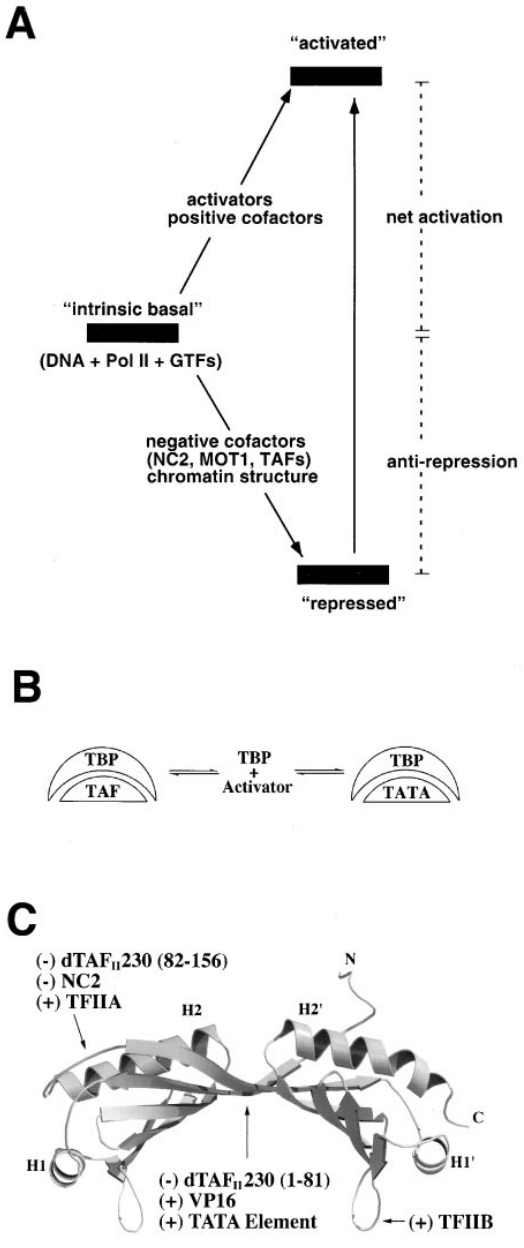


Figure 1. Autoinhibition of TFIID

(A) Decomposition of pol II transcription initiation into antirepression and net activation.

(B) Two-step model for reversal of the autoinhibitory effects of TFIID, involving formation of an intermediate complex composed of TBP and a transcriptional activator, such as VP16.

(C) Ribbon drawing of saddle-shaped TBP, showing binding surfaces for portions of $dTAF_{II}230$, NC2, VP16, TFIIA, TFIIB, and the TATA element. $\alpha$ helices H1, H2, H1′, and H2′, and the N and C termini of TBP are labeled.

et al., 1998). Once deprived of its primary anchor to the promoter, TFIID is no longer able to direct accretion of the large number of accessory factors and pol II that are required for transcription initiation and gene expression is repressed.

The Ikura/Nakatani study reveals that the N terminus of dTAF$_{II}$230 exists in isolation as an unstructured random coil polypeptide chain, which undergoes a disorder-to-order transition on interaction with the hydrophobic underside of the saddle-shaped TBP. The induced-fit structure of the dTAF$_{II}$230 fragment bears a remarkable resemblance to the doubly-kinked, unwound, and smoothly bent structure of the TATA element common to all TBP-DNA complexes (reviewed in Patikoglou and Burley, 1997). The folded structure of the TAF$_{II}$ consists of three $\alpha$ helices and a $\beta$ hairpin, with a well-defined hydrophobic core. Unlike most globular proteins, however, the N terminus of dTAF$_{II}$230 is amphipathic. Its solvent-exposed surface is hydrophilic, while its convex TBP-binding surface is hydrophobic (like the widened minor groove face of the TATA box). The similarity to the TBP-deformed TATA element extends even further. Not only is the convex upper surface hydrophobic, it is also lined on each side by negatively charged side chains that mimic the phosphate groups of the TATA box (see Figure 6 in Liu et al., 1998). With our growing database of X-ray crystal structures containing TBP and DNA (plant, yeast, or human TBP plus DNA; plant TBP and human TFIIB plus DNA, archaebacterial TBP, and TFIIB plus DNA; yeast TBP and TFIIA plus DNA), we know that the deformed structure of the TBP-bound TATA box is essentially independent of the TATA element sequence and is phylogenetically conserved (reviewed in Patikoglou and Burley, 1997). Therefore, it seems likely that molecular mimicry of the TBP-bound structure of the TATA box by human TAF$_{II}$250, *Drosophila* TAF$_{II}$230, and yeast TAF$_{II}$145 represents a conserved mechanism of TFIID autoinhibition, which directly blocks TATA element recognition and thereby prevents transcription initiation from some promoters (see below).

It should come as no surprise that proteins mimic nucleic acids in other biological contexts. The molecule responsible for inhibiting uracil-DNA glycosylase presents a leucine-bearing $\beta$ strand to the DNA-binding groove of the enzyme (Mol et al., 1995; Savva and Pearl, 1995), effectively mimicking flipped-out uracil nucleotides derived from the U-A and A-U base pairs that occur normally in various bacteriophages. An even more impressive example of nucleic acid mimicry is exploited by the eubacterial translation machinery. An elongation factor (EF-G) resembles the tRNA structure in the ternary complex of EF-Tu-GTP and tRNA (Nyborg et al., 1996). There are also examples of target-induced disorder-to-order transitions during assembly of the eukaryotic transcription machinery. The basic regions of leucine zipper and helix-loop-helix proteins undergo random coil-to-$\alpha$ helix conformational changes on binding to their DNA targets but not in the presence of nonspecific DNA (reviewed in Patikoglou and Burley, 1997). Similar effects have been observed when an activator recognizes a coactivator. Wright and coworkers showed that the transactivation domain of CREB undergoes a random coil-to-$\alpha$ helix conformational change on binding to the KIX domain of the coactivator CBP (Radhakrishnan et al., 1997).

Comparison of the structures of the TBP/TAF and TBP/DNA complexes provides new insights into the mechanism(s) of action of transcriptional activators, such as herpes simplex virus VP16 and adenovirus E1A, which both target TBP. A leucine-to-lysine (Leu114→Lys) substitution on the DNA-binding surface of yeast TBP is defective for VP16-dependent activation in vitro (Kim et al., 1994) and in vivo (Lee and Struhl, 1995), but not for basal transcription. (Basal transcription is an in vitro biochemical phenomenon defined as the level of transcription supported by a minimal set of factors, including TBP, TFIIB, TFIIF, TFIIE, TFIIH, and pol II.) The very same mutation and others in spatially proximal residues block the interaction between yeast TBP and the N terminus of dTAF$_{II}$230 (Nishikawa et al., 1997). Moreover, VP16 and the TAF$_{II}$ fragment compete with one another for binding to TBP (Nishikawa et al., 1997). Together, these data are consistent with the "hand-off" model illustrated schematically in Figure 1B and predicted by Nishikawa et al. (1997). In its ground state, TFIID cannot effectively participate in transcription from some promoters (i.e., those with weak TATA elements) because of the autoinhibitory effects of the TAF$_{II}$ N terminus on DNA binding. Competition between a transcriptional activator bound upstream of a core promoter and the TAF$_{II}$ fragment could lead to displacement of the inhibitory portion and tethering of TFIID in the vicinity of a given TATA box. Finally, a "hand-off" step during which the TATA element replaces the activator and binds to the underside of the molecular saddle, would yield a stable TFIID–promoter complex on which a functional preinitiation complex could be assembled (Figure 1B).

It is remarkable that binding of dTAF$_{II}$230 to the underside of TBP is stabilized by a second conserved N-terminal region (residues 82–156). Based on results obtained with the corresponding region of the yeast homolog of dTAF$_{II}$230 (Kokubo et al., 1998), this portion of dTAF$_{II}$230 is thought to interact with $\alpha$ helix H2 on the upper surface of TBP (Figure 1C) and compete with the positive cofactor TFIIA for a set of conserved positively charged residues. Thus, bipartite TBP binding by dTAF$_{II}$230 may provide a basis for synergism of activators and the coactivator TFIIA in reversing the autoinhibitory effects of TFIID during transcription initiation (Figure 1C). TFIID (TAF$_{II}$) interactions with other core promoter elements may also contribute to stable binding to the TATA element (reviewed in Roeder, 1996).

How does the effect of the N terminus of dTAF$_{II}$230 on TBP compare with previously established mechanisms by which pol II transcription initiation is repressed? The most abundant repressors of gene expression are the histones. Packaging of promoter DNA with H2A, H2B, H3, and H4 into nucleosomes prevents TFIID or TBP binding to the TATA element. Conversely, occupation of the promoter by TFIID or TBP precludes DNA packaging. The molecular basis of this mutual exclusion derives from the fact that TBP and the histone octamer employ precisely opposite strategies to bind A/T-rich DNA. In the nucleosome core particle, narrowed minor groove faces of A/T-rich segments are preferentially approximated to the surface of the protein octamer and bent

away from the major groove (reviewed in Luger et al., 1997). When TBP binds DNA, the widened minor groove face of the TATA box interacts with the underside of the molecular saddle and the double helix is bent toward the major groove (reviewed in Patikoglou and Burley, 1997). TFIID or TBP recognition of the TATA element can also be reversed by MOT1 (Auble et al., 1997), which is an ATP-dependent transcription inhibitor that destabilizes the TBP–DNA complex. TFIIB is the next general transcription factor to enter the preinitiation complex. This molecular recognition step represents another target for negative regulation of mRNA synthesis. Negative coactivator 2 (NC2 or DR1/DRAP1) recognizes TBP-bound promoters and inhibits entry of TFIIB, leading to transcriptional repression (reviewed in Lee and Young, 1998). Like the interaction of $dTAF_{II}230$ (residues 82–156) with the convex surface of the TBP, NC2 competes with TFIIA for conserved basic residues in $\alpha$ helix H2 (Kim et al., 1995; Bryant et al., 1996).

Structural biologists have made considerable progress toward defining the architecture of various transcription complexes. The quest to study even larger multiprotein–DNA complexes continues, and we should soon see three-dimensional structures of NC2 plus TBP plus DNA, $TAF_{II}$–TBP–DNA assemblies, and binary complexes of TBP with various transcriptional activators. The challenge facing molecular biologists is to use this wealth of structural detail to go beyond the static pictures provided by X-ray crystallography and NMR spectroscopy and characterize the kinetic and thermodynamic properties of these large transcriptionally active nucleoprotein complexes.

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