genes and function
1 5–9 (1997)

REVIEW

Selective roles for TATA-binding-protein-associated factors *in vivo*

Kevin Struhl

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, U.S.A.

ABSTRACT

Transcription factor TFIID, a central component of the eukaryotic RNA polymerase II transcription machinery, is a multiprotein complex containing the TATA-binding protein (TBP) and TBP-associated factors (TAFs). *In vitro*, TAFs are required for the response to activator proteins, but they are dispensible for basal transcription. However, recent work in yeast cells indicates that TAFs are not generally required for transcriptional activation, but rather have selective effects on gene expression. Molecular mechanisms for these observations are considered.

Expression and regulation of eukaryotic protein-coding genes depend upon activator proteins that bind enhancer elements and stimulate transcription by RNA polymerase (Pol) II [1–4]. The Pol II transcription machinery is complex, containing >50 polypeptides and having a molecular mass comparable with that of a ribosome. Biochemical analyses indicate that activators can interact directly with many components of the Pol II machinery and can affect multiple steps in the assembly of an active transcription complex. However, the molecular mechanisms of transcriptional activation *in vivo*, particularly the physiological significance and relative importance of specific protein–protein interactions and mechanistic steps, remain to be clarified.

Assembly of an active transcription complex on a promoter typically begins with the binding of transcription factor (TF)IID to the TATA element. As initially isolated from flies [5] and humans [6,7], TFIID is a complex containing the TATA-binding protein (TBP) and approximately 10 TBP-associated factors (TAFs). Yeast TFIID complexes have also been characterized [8,9], and yeast homologues of nearly all human and *Drosophila* TAFs have been identified. Binding of TBP (and presumably TFIID) to the TATA element and the consequent distortion in the DNA [10,11] are necessary for stable association of TFIIB [12] and TFIIA [13,14] with the promoter. TAFs may also contribute to assembly by interacting with promoter DNA [15–17] and with other basic Pol II factors [18,19]. In addition, TBP (and presumably TFIID) binds very poorly to chromatin templates [20]; hence, the binding step may be limiting for transcription *in vivo*. For these reasons, TFIID has been an attractive candidate as a target for transcriptional activators.

A variety of *in vitro* transcription experiments strongly implicate TAFs as being specifically involved and generally required for the response to activator proteins [1]. First, reactions containing TFIID support the response to a variety of transcriptional activators, whereas those containing TBP are competent only for basal transcription. Secondly, TAFs are required for activator proteins to stimulate formation of a TFIID–TFIIA–TATA-element complex [21,22], a step that can be rate-limiting for transcription *in vitro* [23]. Thirdly, different classes of activation domains interact with distinct TAFs, and the presence of the relevant TAF correlates with the activator's ability to enhance

transcription [24,25]. Fourthly, multiple contacts between activation domains and TAFs can strongly increase TFIID binding to the TATA element and synergistically activate transcription [26]. Fifthly, TAFs affect the assembly of an activator-dependent transcription complex at a step after recruitment of TFIID and TFIIB to the promoter [27].

In yeast cells, TBP is required for transcription of all Pol II genes [28], and recruitment of TBP to the TATA element is an important step in transcriptional activation. Activation domains can stimulate recruitment of TBP to promoters *in vivo* [29], and artificial recruitment of TBP by physical connection to a promoter-bound protein bypasses the need for an activation domain [30–32]. An efficient TBP–TATA interaction is essential for the response to strong activators, because weak TATA elements are functionally saturated at lower levels of activation [33]. Genetic screens have identified TBP mutants specifically defective in the response to acidic activators *in vivo*. These mutants are impaired for interacting with TATA elements [34,35] or TFIIA [36], a protein that stabilizes the TBP–TATA interaction.

Surprisingly, recent experiments strongly suggest that TAFs are not generally required for transcriptional activation in yeast cells [37–39]. Specifically, depletion or inactivation of seven different TAFs (TAF130, TAF90, TAF68, TAF60, TAF47, TAF19 and Tsm1) do not significantly affect activation by four acidic activators: Gcn4, Ace1, Gal4 and Hsf. In contrast, depletion of TBP or TFIIB by the same experimental protocol results in a rapid and dramatic decrease in transcriptional activation. Since depletion of TAF, TFIIB and TBP results in similar kinetics of growth arrest and cell inviability, the lack of a transcriptional phenotype upon TAF depletion is not due to TAFs being less limiting in the cell than TFIIB or TBP. Furthermore, transcriptional activation occurs when the number of TAF molecules is at least 20-fold below the number of Pol II promoters, conditions where the vast majority of cells are dead. It is important to note that TAF130 is the only TAF in the yeast TFIID complex that strongly interacts with TBP [8], and it is required to nucleate the formation of the TFIID complex *in vitro* [25]. Thus TBP itself may be sufficient to mediate transcriptional activation.

The conclusion that TAFs are not generally required for transcriptional activation in yeast contrasts with numerous experiments indicating that TAFs are crucial for activated transcription *in vitro* [1,40]. One possible explanation for this apparent discrepancy is that yeast TAFs might be less important than their mammalian and *Drosophila* counterparts; in this regard, the yeast TFIID complex is less stable in extracts. This hypothesis is unlikely, because TAFs are strongly conserved among eukaryotes [40,41], TAF-dependent activation *in vitro* can be achieved with yeast components [8,9], and activation can occur in hamster cells in which TAF250 (yeast TAF130 homologue) has been thermally inactivated [42].

Instead, we have suggested that, for transcriptional activation in general, TAFs are functionally redundant with other factors that are absent (or inactive) in typical *in vitro* reactions [37]. Most *in vitro* transcription reactions are reconstituted with core Pol II (the 12-subunit enzyme), and hence are likely to lack components of the Pol II holoenzyme (e.g. Srb proteins, Gal11, Sin4 and the Swi/Snf complex) that are important for transcription in yeast cells [4,43]. Conversely, activated transcription in the apparent absence of TAFs can occur in reactions containing Pol II holoenzyme [44,45] or chromatin templates [46]. In accord with this functional redundancy *in vitro*, artificial recruitment of TBP [30–32], TAFs [39] and components of the Pol II holoenzyme [47,48] can bypass the need for an activation domain. Thus if natural activators interact with multiple components of the Pol II machinery, individual components such as TAFs are likely to be non-essential for activation, even if they are potential targets. These considerations have led to a triad model for transcriptional activation [4] in which interactions between activation domains and components of TFIID and the Pol II holoenzyme stabilize the formation of an active transcription complex. Although the complexity of TFIID and the Pol II holoenzyme provides at least 50 potential targets for activation domains, the various protein–protein interactions that underlie distinctions between activator proteins or promoters might reflect a common mechanism of transcriptional activation.

Although TAFs are not generally required for transcriptional activation, they selectively affect the transcription of specific genes *in vivo*. The clearest example is that depletion of TAF130 or TAF19 significantly reduces the level of the *trp3* and *his3* +1 transcripts [37]. This selective effect is not observed upon depletion of TAF60 or TAF90, indicating that individual TAFs have distinct transcriptional functions *in vivo*. Interestingly, the promoters responsible for *trp3* and *his3* +1 transcription contain suboptimal, non-consensus TATA elements, suggesting that TAF130 and TAF19

are important for transcription from promoters lacking conventional TATA elements. In this regard, the transcriptional patterns resulting from TAF130 or TAF19 depletion are remarkably similar to those observed in yeast cells containing human TBP as the sole functional source of TBP [49]; this similarity may reflect poor interactions between human TBP and yeast TAFs. In weak promoters lacking consensus TATA elements, it is likely that there are relatively few interactions stabilizing the Pol II machinery at the promoter. Thus TAFs may play an important role at such promoters, by interacting either with components of the basic Pol II machinery or with promoter DNA.

The idea that TAFs have selective transcriptional effects is supported by the observation that cells containing temperature-sensitive TAF mutations arrest at particular points in the cell cycle upon a shift to the restrictive temperature [38,39]. Arrest of TAF90 and Tsm1 mutant strains occurs at the G_2–M boundary, whereas arrest of TAF130 strains arrests in the G_1 phase. Interestingly, a hamster cell line containing a temperature-sensitive mutation in TAF250, the homologue of yeast TAF130, also undergoes G_1 arrest upon shifting to the restrictive temperature [42]. The similar phenotypes conferred by yeast TAF130 and hamster TAF250 mutations suggest that this TAF may directly affect genes involved in progression through G_1; however, specific genes have not yet been identified and indirect effects cannot be excluded. Nevertheless, the distinct cell-cycle effects strongly argue that the various TAFs perform specific transcriptional roles.

With one exception (see below), yeast cells contain homologues of all known human and *Drosophila* TAFs, and these yeast TAFs are associated in a TFIID complex [8,9,41]. Each yeast TAF is essential for cell growth, presumably because of its selective effects on transcription. The essential function(s) of an individual TAF might be due to one or more of the following explanations. First, certain TAFs (e.g. TAF130 and TAF19) might be important for transcription from a subclass of promoters with a common feature (e.g. weak TATA elements). Secondly, a TAF could subtly affect transcription of many genes, such that the cumulative effects lead to cell inviability. Thirdly, although TAFs are not generally required for activation, an individual TAF might be required for a subset of activators that affect one or more essential genes (e.g. an activator involved in cell-cycle progression). In this regard, the failure of glutamine-rich activation domains to stimulate transcription in yeast [50,51] may reflect the lack of a homologue of *Drosophila* TAF110 [41], a target for the glutamine-rich activation domains *in vitro* [24,25].

In summary, the initial analyses of TAF functions *in vivo* have led to the surprising conclusion that TAFs are not generally required for transcriptional activation, but rather play selective roles. A more detailed understanding of the physiological roles of individual TAFs awaits further genetic and molecular analysis.

REFERENCES

1 Tjian, R. and Maniatis, T. (1994) Transcriptional activation: a complex puzzle with few easy pieces. Cell **77**, 5–8
2 Zawel, L. and Reinberg, D. (1995) Common themes in assembly and function of eukaryotic transcription complexes. Annu. Rev. Biochem. **64**, 533–561
3 Carey, M.F. (1995) Transcriptional activation – a holistic view of the complex. Curr. Biol. **5**, 1003–1005
4 Struhl, K. (1996) Chromatin structure and RNA polymerase II connection: Implications for transcription. Cell **84**, 179–182
5 Dynlacht, B.D., Hoey, T. and Tjian, R. (1991) Isolation of coactivators associated with the TATA-binding protein that mediate transcriptional activation. Cell **66**, 563–576
6 Tanese, N., Pugh, B.F. and Tjian, R. (1991) Coactivators for a proline-rich activator purified from the multisubunit human TFIID complex. Genes Dev. **5**, 2212–2224
7 Zhou, Q., Lieberman, P.M., Boyer, T.G. and Berk, A.J. (1992) Holo TFIID supports transcriptional stimulation by diverse activators and from a TATA-less promoter. Genes Dev. **6**, 1964–1974
8 Reese, J.C., Apone, L., Walker, S.S., Griffin, L.A. and Green, M.R. (1994) Yeast TAF_{II}s in a multisubunit complex required for activated transcription. Nature (London) **371**, 523–527
9 Poon, D., Bai, Y., Campbell, A.M., Bjorklund, S., Kim, Y.J., Zhou, S., Kornberg, R.D. and Weil, P.A. (1995) Identification and characterization of a TFIID-like multiprotein complex from *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S.A. **92**, 8224–8228
10 Kim, Y., Geiger, J.H., Hahn, S. and Sigler, P.B. (1993) Crystal structure of a yeast TBP-TATA box complex. Nature (London) **365**, 512–520

11 Kim, J.L., Nikolov, D.B. and Burley, S.K. (1993) Co-crystal structure of TBP recognizing the minor groove of a TATA element. Nature (London) **365**, 520–527
12 Nikolov, D.B., Chen, H., Halay, E.D., Usheva, A.A., Hisatake, K., Lee, D.K., Roeder, R.G. and Burley, S.K. (1995) Crystal structure of a TFIIB-TBP-TATA-element ternary complex. Nature (London) **377**, 119–128
13 Geiger, J.H., Hahn, S., Lee, S. and Sigler, P.B. (1996) Crystal structure of the yeast TFIIA/TBP/DNA complex. Science **272**, 830–836
14 Tan, S., Hunziker, Y., Sargent, D.F. and Richmond, T.J. (1996) Crystal structure of a yeast TFIIA/TBP/DNA complex. Nature (London) **381**, 127–134
15 Purnell, B.A., Emanuel, P.A. and Gilmour, D.S. (1994) TFIID sequence recognition of the initiator and sequences farther downstream in *Drosophila* class II genes. Genes Dev. **8**, 830–842
16 Kaufmann, J. and Smale, S.T. (1994) Direct recognition of initiator elements by a component of the transcription factor IID complex. Genes Dev. **8**, 821–829
17 Verrijzer, C.P., Yokomori, K., Chen, J.-L. and Tjian, R. (1994) *Drosophila* $TAF_{II}150$: Similarity to yeast gene TSM-1 and specific binding to core promoter DNA. Science **264**, 933–941
18 Goodrich, J.A., Hoey, T., Thut, C.J., Admon, A. and Tjian, R. (1993) Drosophila $TAF_{II}40$ interacts with both a VP16 activation domain and the basal transcription factor TFIIB. Cell **75**, 519–530
19 Ruppert, S. and Tjian, R. (1995) $TAF_{II}250$ interacts with RAP74: implications for RNA polymerase II initiation. Genes Dev. **9**, 2747–2755
20 Imbalzano, A.N., Kwon, H., Green, M.R. and Kingston, R.E. (1994) Facilitated binding of TATA-binding protein to nucleosomal DNA. Nature (London) **370**, 481–485
21 Lieberman, P.M. and Berk, A.J. (1994) A mechanism for TAFs in transcriptional activation: activation domain enhancement of TFIID-TFIIA-promoter DNA complex formation. Genes Dev. **8**, 995–1006
22 Chi, T., Lieberman, P., Ellwood, K. and Carey, M. (1995) A general mechanism for transcriptional synergy by eukaryotic activators. Nature (London) **377**, 254–257
23 Wang, W., Gralla, J.D. and Carey, M. (1992) The acidic activator GAL4-AH can stimulate polymerase II transcription by promoting assembly of a closed complex requiring TFIID and TFIIA. Genes Dev. **6**, 1716–1727
24 Hoey, T., Weinzierl, R.O.J., Gill, G., Chen, J.-L., Dynlacht, B.D. and Tjian, R. (1993) Molecular cloning and functional analysis of Drosophila TAF110 reveal properties expected of coactivators. Cell **72**, 247–260
25 Chen, J.-L., Attardi, L.D., Verrijzer, C.P., Yokomori, K. and Tjian, R. (1994) Assembly of recombinant TFIID reveals differential coactivator requirements for distinct transcriptional activators. Cell **79**, 93–105
26 Sauer, F., Hansen, S.K. and Tjian, R. (1995) Multiple TAF_{II}s directing synergistic activation of transcription. Science **270**, 1783–1788
27 Choy, B. and Green, M.R. (1993) Eukaryotic activators function during multiple steps of preinitiation complex assembly. Nature (London) **366**, 531–536
28 Cormack, B.P. and Struhl, K. (1992) The TATA-binding protein is required for transcription by all three nuclear RNA polymerases in yeast cells. Cell **69**, 685–696
29 Klein, C. and Struhl, K. (1994) Increased recruitment of TATA-binding protein to the promoter by transcriptional activation domains *in vivo*. Science **266**, 280–282
30 Chatterjee, S. and Struhl, K. (1995) Connecting a promoter-bound protein to the TATA-binding protein overrides the need for a transcriptional activation region. Nature (London) **374**, 820–822
31 Klages, N. and Strubin, M. (1995) Stimulation of RNA polymerase II transcription initiation by recruitment of TBP in vivo. Nature (London) **374**, 822–823
32 Xiao, H., Friesen, J.D. and Lis, J.T. (1995) Recruiting TATA-binding protein to a promoter: transcriptional activation without an upstream activator. Mol. Cell. Biol. **15**, 5757–5761
33 Iyer, V. and Struhl, K. (1995) Mechanism of differential utilization of the *his3* T_R and T_C TATA elements. Mol. Cell. Biol. **15**, 7059–7066
34 Arndt, K.M., Ricupero-Hovasse, S. and Winston, F. (1995) TBP mutants defective in activated transcription *in vivo*. EMBO J. **14**, 1490–1497
35 Lee, M. and Struhl, K. (1995) Mutations on the DNA-binding surface of TBP can specifically impair the response to acidic activators *in vivo*. Mol. Cell. Biol. **15**, 5461–5469
36 Stargell, L.A. and Struhl, K. (1995) The TBP-TFIIA interaction in the response to acidic activators *in vivo*. Science **269**, 75–78
37 Moqtaderi, Z., Bai, Y., Poon, D., Weil, P.A. and Struhl, K. (1996) TBP-associated factors are not

generally required for transcriptional activation in yeast. Nature (London) **382**, 188–191

38 Walker, S.S., Reese, J.C., Apone, L.M. and Green, M.R. (1996) Transcription activation in cells lacking TAF_{II}s. Nature (London) **382**, 185–188

39 Apone, L.M., Virbasius, C.A., Reese, J.C. and Green, M.R. (1996) Yeast $TAF_{II}90$ is required for cell-cycle progression through G_2/M but not for general transcription activation. Genes Dev. **10**, 2368–2380

40 Burley, S.K. and Roeder, R.G. (1996) Biochemistry and structural biology of transcription factor IID (TFIID). Annu. Rev. Biochem. **65**, 769–799

41 Moqtaderi, Z., Yale, J.D., Struhl, K. and Buratowski, S. (1996) Yeast homologues of higher eukaryotic TFIID subunits. Proc. Natl. Acad. Sci. U.S.A. **93**, 14654–14658

42 Wang, E.H. and Tjian, R. (1994) Promoter-selective transcriptional defect in cell cycle mutant ts13 rescued by $hTAF_{II}250$. Science **263**, 811–814

43 Koleske, A.J. and Young, R.A. (1995) The RNA polymerase II holoenzyme and its implications for gene regulation. Trends Biochem. Sci. **20**, 113–116

44 Koleske, A.J. and Young, R.A. (1994) An RNA polymerase II holoenzyme responsive to activators. Nature (London) **368**, 466–469

45 Kim, Y.-J., Bjorklund, S., Li, Y., Sayre, M.H. and Kornberg, R.D. (1994) A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. Cell **77**, 599–608

46 Workman, J.L., Taylor, I.C.A. and Kingston, R.E. (1991) Activation domains of stably bound GAL4 derivatives alleviate repression of promoters by nucleosomes. Cell **64**, 533–544

47 Barberis, A., Pearlberg, J., Simkovich, N., Farrell, S., Reinagel, P., Bamdad, C., Sigal, G. and Ptashne, M. (1995) Contact with a component of the polymerase II holoenzyme suffices for gene activation. Cell **81**, 359–368

48 Jiang, Y.W. and Stillman, D.J. (1992) Involvement of the *SIN4* global transcriptional regulator in the chromatin structure of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12**, 4503–4514

49 Cormack, B.P., Strubin, M., Stargell, L.A. and Struhl, K. (1994) Conserved and nonconserved functions of yeast and human TATA-binding proteins. Genes Dev. **8**, 1335–1343

50 Kunzler, M., Braus, G.H., Georgiev, O., Seipel, K. and Schaffner, W. (1994) Functional differences between mammalian transcription activation domains at the yeast *GAL1* promoter. EMBO J. **13**, 641–645

51 Ponticelli, A.S., Pardee, T.S. and Struhl, K. (1995) The glutamine-rich activation domains of human Sp1 do not stimulate transcription in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **15**, 983–988