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 TFII B [12] and TFII A [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 TFIIID 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 TFIIID and TFIIIB 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 vitro [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: Gal4, Ace1, Gal4 and Hsf. In contrast, depletion of TBP or TFIIIB by the same experimental protocol results in a rapid and dramatic decrease in transcriptional activation. Since depletion of TAF, TFIIIB 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 TFIIIB 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 TFIIID complex that strongly interacts with TBP [8], and it is required to nucleate the formation of the TFIIID 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 TFIIID 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 TFIIID and the Pol II holoenzyme stabilize the formation of an active transcription complex. Although the complexity of TFIIID 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 G2−M boundary, whereas arrest of TAF130 strains arrests in the G1 phase. Interestingly, a hamster cell line containing a temperature-sensitive mutation in TAF250, the homologue of yeast TAF130, also undergoes G1 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 G1; 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.

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