Cell, Vol. 94, 1–4, July 10, 1998, Copyright ©1998 by Cell Press

The TAFs in the HAT

Minireview

Kevin Struhl and Zarmik Moqtaderi
Department of Biological Chemistry
and Molecular Pharmacology
Harvard Medical School
Boston, Massachusetts 02115

TFIID, a multiprotein complex comprising the TATA-binding protein (TBP) and approximately 10 associated factors (TAFs), is a key component of the RNA polymerase II (pol II) transcription machinery (Burley and Roeder, 1996). The TBP subunit specifically recognizes TATA elements, which are found in most eukaryotic promoters, and TFIIB, the protein primarily responsible for connecting TFIID to the pol II holoenzyme. Thus, TBP contributes significantly to the overall level of transcription, and it is important for positioning the pol II machinery at the correct location with respect to the mRNA initiation site. Certain TAF subunits of TFIID directly interact with initiator or downstream promoter elements, and these interactions are particularly important for transcription from promoters lacking conventional TATA elements. In addition, TAFs play some role in the response of the basal machinery to transcriptional activator proteins, although their specific functions in this regard are not well understood.

Considerable work over the last decade has led to a simple and clarifying view that TFIID (not TBP) is the functional entity in vivo that associates with promoters of all protein-coding genes and is universally required for the initiation of mRNA synthesis. TFIID specifically functions in association with the pol II machinery. It differs from TBP complexes (e.g., SL-1, TFIIIB, $SNAP_c$) that function with the pol I or pol III machinery as well as TBP complexes (such as those containing Mot1 or NC2) that function as negative regulators of pol II transcription (Lee and Young, 1998). The TAF subunits of TFIID are not found in any of these other TBP complexes. Thus, it has been believed that these TAFs exist and function solely in association with TBP in the context of the TFIID, wherein they confer the transcriptional specificity of the complex.

Recently, this textbook view of TFIID has been assaulted on several fronts. First, in the *Drosophila* nervous system, there is a distinct TFIID-like complex composed of a TBP-related factor (TRF) and a novel set of associated factors (nTAFs) that interacts with a subset of genes in vivo (Hansen et al., 1997). Hence, the TRF-nTAF complex is likely to be a functional equivalent of TFIID at specific promoters, in a manner loosely analogous to the multiple σ factors in prokaryotes. Human cells also contain a TBP-related protein, hTLF (Wieczorek et al., 1998), suggesting that multiple TFIID-like complexes might be commonly utilized in multicellular eukaryotes. Second, biochemical fractionation of an extract from human cells has uncovered TFTC, a novel complex containing a subset of TAFs but apparently lacking TBP (Wieczorek et al., 1998). Although its physiological significance is unknown, TFTC functions similarly to TFIID in vitro on TATA-containing and TATA-lacking promoters, and it can respond to activators. It is unclear whether TFTC contains a TBP-related factor (or a modified form of TBP) or whether the subset of TAFs (perhaps with additional proteins) are sufficient for promoter recognition and assembly of the pol II machinery. Third, the Gcn4 activation domain interacts with a subset of TAFs, suggesting the existence of a TAF-containing complex distinct from TFIID (Drysdale et al., 1998).

In this issue of *Cell*, two papers dramatically alter the textbook view by demonstrating that a subset of the TAFs in TFIID are also integral components of histone acetylase complexes (Grant et al., 1998; Ogryzko et al., 1998). Though completely unexpected, this finding is provocative and highly significant especially because of the striking parallels between the TAFs that are present in the human PCAF and yeast SAGA histone acetylase complexes. At one level, the presence of TAFs in histone acetylase complexes provides yet another example of the mechanistic connection between the pol II machinery and chromatin modifying activities (Struhl, 1998). At another level, these new results significantly complicate our understanding of the physiological functions of TAFs, given that any particular function could be exerted either in the context of the TFIID or the histone acetylase complex. Finally, the fact that several of the TAFs in the histone acetylase complexes interact to form a histone-like octamer provokes evolutionary and mechanistic speculations. Before considering the above implications in more detail, however, it will be useful to review what is currently known about the biochemical roles of TAFs in the context of TFIID and about the transcriptional roles of TAFs in vivo.

Biochemical Properties of TAFs in the TFIID Complex

In vitro, TAFs play a critical role in core promoter function, primarily because individual TAFs directly and specifically interact with initiator and downstream promoter elements that are found in a significant number of eukaryotic promoters (Burley and Roeder, 1996; Burke and Kadonaga, 1997). Although these TAF–promoter interactions are weaker and less specific than the TBP-TATA interaction, they are critical for transcription from promoters lacking TATA elements; in such TATA-less promoters, transcription is observed in reactions with TFIID, but not TBP. In addition, these TAF–promoter interactions contribute to the level of transcription from TATA-containing promoters. Thus, TAFs provide part of the promoter recognition surface of TFIID. TAFs might also affect core promoter function by interacting with or by affecting the activity of components of the basic transcription machinery.

TAFs are likely to play some role in the response of the pol II machinery to activators, because in various in vitro reactions, TFIID can support activated transcription, whereas TBP can not (Burley and Roeder, 1996). Moreover, in some situations, TAFs are required for activator-dependent recruitment of TFIIA and TFIID to promoters or for activator-dependent recruitment of pol II holoenzyme components. Although there are many possible mechanisms by which TAFs can affect the response to activators, one popular model is that TAFs

function as direct targets of activation domains (Verrijzer and Tjian, 1996). In support of this idea, isolated TAF subunits can interact with specific activation domains. Furthermore, reconstituted subcomplexes containing TBP and a subset of TAFs can mediate cooperative binding and activator-stimulated transcription in a manner consistent with the isolated activation domain–TAF interactions. From these observations, it has been proposed that individual TAFs within the TFIID complex selectively mediate the response to activator proteins.

Although the activator–TAF interaction model of activation is attractive, it is not without problems. First, in yeast and mammalian in vitro transcription reactions, efficient activation can occur in the absence of TAFs (Koleske and Young, 1995; Oelgeschlager et al., 1998), indicating that TAFs are not essential for activation and that other targets are sufficient. However, these results are not inconsistent with activator–TAF interactions contributing to activation; activator targets could be redundant and/or multiple targets could be utilized to enhance transcription synergistically. Second, there is no evidence that TFIID can support activated transcription in reactions reconstituted with purified components of the basic pol II machinery (Burley and Roeder, 1996). On the contrary, TFIID-dependent activation in vitro is observed only in the presence of other "coactivators" such as PC2 or PC4. These observations are noteworthy because straightforward models invoking activator–TAF interactions predict that TFIID should be sufficient to support at least some degree of activation in vitro. Third, there is no evidence that activation domains can contact TAFs in the context of the intact TFIID complex. For example, the Gcn4 activation domain, which functions in yeast and mammalian cells, specifically interacts with the pol II holoenzyme and the SAGA complex, which contains a subset of TAFs, but not with TFIID. Thus, the observed interactions of isolated TAF subunits with activation domains might involve TAF protein surfaces that are not accessible in the context of intact TFIID.

For these reasons, a convincing case for TAFs as direct targets of activators has yet to be made, and the issue must be considered to be an open question. Moreover, some activators might function via TAFs, whereas others might utilize non-TAF targets. While TAFs are likely to play some role in the activation process given the distinct biochemical behaviors of TFIID and TBP, this role could be indirect. For example, TAFs could interact with the direct target of activators, thereby stabilizing this target within the preinitiation complex. Alternatively, if activation requires a highly stable association of the pol II machinery with the promoter, additional promoter interactions mediated by the TAFs might account for the activation-specific differences between TFIID and TBP.

Physiological Functions of TAFs in Transcription

The physiological functions of TAFs can only be assessed in living cells, preferably under conditions in which genes are examined in their normal chromosomal context and the numerous components of the pol II machinery are present at normal intracellular levels. Protein–protein interactions, reactions, or mechanisms that occur in vitro may or may not be relevant or significant in vivo. Conversely, the interpretation of genetic experiments in terms of molecular mechanisms is virtually

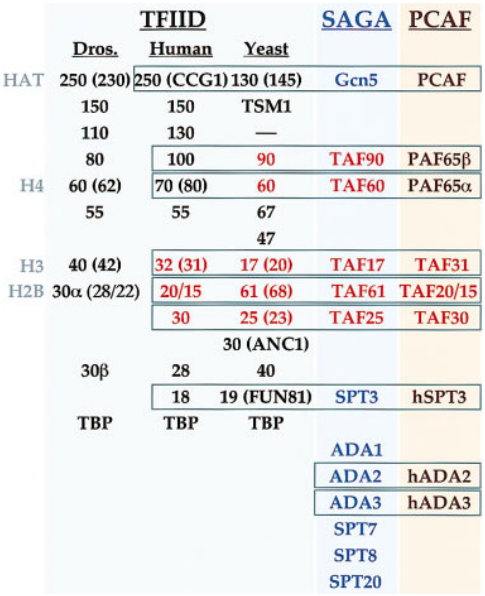

Figure 1. TAFs in the TFIID, SAGA, and PCAF Complexes

The complexes and their unique components are color coded: TFIID, black; SAGA, blue; PCAF, brown. TAFs common to more than one complex are shown in red. Parallel components across complexes are designated by horizontal boxes.

impossible in the absence of biochemical and structural information about the relevant components.

In yeast cells, TAFs are essential for cell growth, but depletion of a variety of individual TAFs does not significantly affect transcriptional activation of the vast majority of genes (Moqtaderi et al., 1996; Walker et al., 1996). As transcription of essentially all yeast genes requires activator proteins (i.e., the intact promoter is typically much more active than promoter derivatives containing only the TATA and initiator elements), this result indicates that TAFs are not generally required for activation. This conclusion does not exclude the possibility that TAFs are targets for a limited subset of activators or that the currently untested TAFs have more general effects. Alternatively, if activators contact multiple components of the pol II machinery, individual TAFs could be nonessential for activation even if they are potential targets (Struhl, 1996).

Although TAF depletion does not generally affect transcription, gene-specific and TAF-specific effects are clearly observed (Lee and Young, 1998). Depletion of certain TAFs can differentially affect *his3* TATA-element utilization or can selectively affect genes in a manner dependent on the core promoter, not the enhancer. TAF mutations in *Drosophila* or hamster cells also cause selective effects on gene expression, although the molecular bases of these effects are not well understood. Finally, TAF mutations can arrest the cell-cycle at distinct stages, indicating that individual TAFs have distinct biological roles and affect distinct subsets of genes. At present, all the evidence suggests that these selective effects on gene expression reflect core promoter functions of TAFs, but activator-specific functions remain possible.

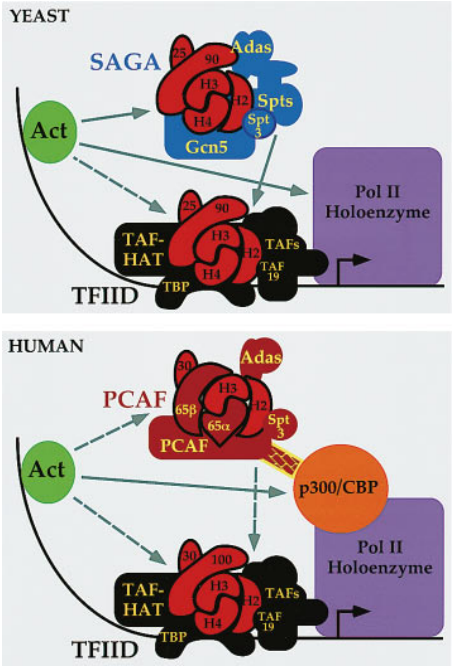

Figure 2. Potential Roles of the Yeast SAGA and Human PCAF Complexes in Transcription

The yeast SAGA complex (Ada and Spt proteins indicated) interacts (solid arrows) with TBP in the TFIID complex and with activator proteins (Act). The TAF subunit with histone acetylase activity is indicated as TAF-HAT and the histone-like TAFs are indicated as H2B, H3, and H4 in accord with their similarity to core histones. TAF subunits common to TFIID and SAGA are indicated in red. Interactions of activators with the pol II holoenzyme and TFIID (dashed arrow to indicate not yet demonstrated with the intact complex in vitro) are also indicated. The human PCAF complex is depicted in a similar manner, with the addition that both the N-terminal region of PCAF (yellow hatched rectangle) and activators are shown as interacting with p300/CBP, which is tightly associated with the pol II holoenzyme. By analogy with SAGA, potential (but not yet demonstrated) interactions of the PCAF complex with activators and TFIID are indicated by dashed arrows.

A Subset of TAFs in Histone Acetylase Complexes: Striking Parallels between the Human PCAF and Yeast SAGA Complexes

Understanding the physiological role of TAFs has become complicated by the new findings that a subset of TAFs are present in histone acetylase complexes that are distinct from TFIID (Figure 1). As initially described (Grant et al., 1997), the yeast SAGA complex contains Gcn5 histone acetylase as the catalytic subunit as well as various Ada (Ada1, -2, -3, -5) and Spt (Spt3, -7, -8) proteins. It is now clear that SAGA also contains the histone-like TAFs (TAF20, TAF60, and TAF68), TAF90, and TAF25 (Grant et al., 1998). However, SAGA clearly does not contain TAF130(145) and Tsm1, and it probably lacks TAF40 and TAF19.

The PCAF complex (Ogryzko et al., 1998) is remarkably similar to SAGA. PCAF itself is strongly related to Gcn5 (Yang et al., 1996), and it confers the catalytic activity of the complex. The PCAF complex contains human versions of Ada2, Ada3, and Spt3; homologs of the other Ada and Spt proteins are likely to be present, but this needs to be confirmed. Furthermore, the PCAF complex resembles SAGA in that it contains TAF20/15, TAF31, and TAF30, but not TAF250 or TAF130. Although the PCAF complex does not contain TAF80 or TAF100, it does contain highly related proteins, PAF65α and PAF65β, respectively. Thus, the SAGA and PCAF complexes are virtually identical with respect to their TAF components, except that the PCAF complex has specialized versions of two of the TAFs. In addition, human cells contain a distinct histone acetylase termed hGcn5, and the composition of the hGcn5 complex appears remarkably similar to that of the PCAF complex (Ogryzko et al., 1998). Thus, it is very likely that the SAGA, PCAF, and hGcn5 complexes are structurally and functionally homologous; in other words, the PCAF and hGcn5 complexes can be viewed as human versions of SAGA.

It is noteworthy that, like the SAGA and PCAF complexes, TFIID is a TAF-containing histone acetylase complex. These complexes share several TAFs in common, but TFIID contains a structurally distinct catalytic subunit (TAF130 in yeast), a sequence-specific DNA-binding subunit (TBP), and several TAFs that are not present in SAGA or PCAF. Conversely, the PCAF and SAGA complexes contain Ada and Spt proteins that are not found in TFIID. Thus, while the general and specific transcriptional roles of TFIID are clearly different from those of SAGA or PCAF, these complexes are structurally and functionally related.

Role of TAFs in the SAGA Complex

TAF function in the SAGA complex was examined in a yeast strain containing a temperature-sensitive mutation in the histone H2B-like TAF (Grant et al., 1998). Functional inactivation of the H2B-like TAF significantly reduces the integrity, and hence size, of the SAGA complex. The smaller complex retains the other TAFs, Gcn5, and the Ada proteins, but the level of Spt3 is significantly reduced. Like wild-type SAGA, the mutant complex interacts with TBP and acidic activation domains, and it retains the ability to acetylate free histones. However, the mutant complex has significantly reduced enzymatic activity on nucleosomal substrates, and it fails to support activation in vitro. These observations suggest that, in the context of SAGA, the H2B-like TAF functions in nucleosomal recognition or histone presentation to permit Gcn5 to acetylate nucleosomal substrates. As a consequence of this biochemical activity, the H2B-like TAF is required for SAGA- and Gcn5-dependent transcriptional activation.

The SAGA, PCAF, and TFIID histone acetylase complexes contain histone-like TAFs that form a sub-structure similar to the histone octamer (Xie et al., 1996). This histone-like feature of histone acetylases could reflect the fortuitous utilization of a common structural motif or it could arise as a consequence of evolutionary history (see below). However, it is tempting to speculate that the histone-like features of the TAFs are relevant for their functions in transcription and chromatin modification. Perhaps the histone-like TAFs interact with chromosomal histones in some fashion to facilitate histone acetylation in nucleosomes (SAGA and PCAF complexes) and/or to facilitate TFIID binding to promoters. Perhaps the histone-like TAF octamer can locally displace chromosomal histones; in this regard, the histone H3- and

H4-like TAFs interact with promoter DNA in the context of TFIID although it is unlikely that these DNA interactions resemble those of histones.

Physiological Functions of TAFs Revisited

A fundamental concept of eukaryotic gene regulation that has emerged over the past few years is that chromatin modifying activities are integral components of or are physically associated with the pol II machinery (Figure 2). For example, TFIID is a basic component of the pol II machinery that possesses histone acetylase activity (through one of the TAFs) and promoter recognition function (through TBP and several TAFs). The SAGA complex is not a component of the pol II machinery, and it does not directly interact with promoters. However, SAGA can interact with TBP (probably through Spt3) and with a variety of acidic activation domains, and mutations in individual SAGA components have various transcriptional effects in vivo. The PCAF complex is less well characterized in this regard, but PCAF itself interacts with p300/CBP (Yang et al., 1996), a transcriptional coactivator that interacts with numerous DNA-binding activator proteins and is tightly associated with the pol II holoenzyme (Nakajima et al., 1997).

For TAFs that are present in both TFIID and non-TFIID complexes, there is an obvious complication in elucidating TAF functions in vivo. When one observes a phenotypic effect arising from a TAF mutation, does the inferred physiological function result from this TAF being in the TFIID complex or the SAGA or PCAF (or hGcn5) complexes or some combination thereof? For example, if TAFs are targets of activation domains, do the targets exist in the context of TFIID or the SAGA or PCAF complexes? In this regard, the Gcn4 activation domain appears to interact (directly or indirectly) with TAFs within SAGA but not TFIID (Drysdale et al., 1998).

Mutational analysis in yeast suggests that SAGA selectively affects the transcription of a small subset of genes, but this has not been definitively resolved. Gcn5 histone acetylase activity plays a critical role in SAGA function, but it is unclear whether this enzymatic activity is sufficient to account for all SAGA functions in vivo. The possibility of additional functions is suggested by the fact that mutations in certain SAGA components (e.g., Ada1 and Ada5/Spt20) cause stronger phenotypic effects than mutations that eliminate or inactivate Gcn5; alternatively, the additional functions of Ada1 and Ada5/Spt20 might reflect the presence of these proteins in protein complexes distinct from SAGA. In the context of the SAGA complex, the TAFs might simply contribute to the nonessential and selective transcriptional functions of SAGA. However, the possibility that the TAFs perform additional, possibly essential, functions within SAGA can not be excluded. To resolve these questions, it will be necessary to generate mutations or other genetic situations involving TAFs that selectively affect the TFIID or SAGA complexes.

Evolutionary Considerations

The striking phenomenon of histone acetylases with histone-like substructures that play critical (though distinct) roles in eukaryotic gene regulation raises evolutionary questions. We propose the following speculative scenario that begins with an Archea-like organism containing TBP and histones lacking N-terminal tails. Following the split between Archea and eukaryotes, we imagine a eukaryotic ancestor with histone tails and a histone acetylase that might conceivably be associated with the histones; such an organism would have the ability to modify nucleosome structure. Subsequently, gene duplications and evolutionary divergence results in two sets of histones; the standard nucleosomal histones and the histone-like TAFs that associate with the histone acetylase while losing the ability to form nucleosomes. Next, the primitive histone acetylase complexes diverge into two types, which are distinguished by the catalytic subunit (Gcn5 or TAF-HAT). The TAF-HAT type acquired the ability to interact with TBP, additional TAF subunits, and promoter DNA, whereas the Gcn5 type acquired Ada and Spt subunits to facilitate the interaction with (and hence modification of) nucleosomal histones. Further divergence after the yeast–human split generated novel TAF-like proteins (e.g., PAF65α and PAF65β) that are specific to the human PCAF and hGcn5 complexes. In considering these ideas, it would be of interest to examine very primitive eukaryotes for the presence of histones, TAFs, Spt and Ada proteins, and Gcn5-like histone acetylases.

Selected Reading

Burke, T.W., and Kadonaga, J.T. (1997). Genes Dev. *11*, 3020–3031.

Burley, S.K., and Roeder, R.G. (1996). Annu. Rev. Biochem. *65*, 769–799.

Drysdale, C.M., Jackson, B.M., Klebanow, E.R., Bai, Y., Kokubo, T., Swanson, M., Nakatani, Y., Weil, A., and Hinnebusch, A.G. (1998). Mol. Cell. Biol. *18*, 1711–1724.

Grant, P.A., Duggan, L., Cote, J., Roberts, S.M., Brownell, J.E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C.D., Winston, F., Berger, S.L., and Workman, J.L. (1997). Genes Dev. *11*, 1640–1650.

Grant, P.A., Schieltz, D., Pray-Grant, M.G., Steger, D.J., Reese, J.C., Yates, J.R., and Workman, J.L. (1998). Cell *94*, this issue, 45–53.

Hansen, S.K., Takada, S., Jacobson, R.H., Lis, J.T., and Tjian, R. (1997). Cell *91*, 71–83.

Koleske, A.J., and Young, R.A. (1995). Trends Biochem. Sci. *20*, 113–116.

Lee, T.I., and Young, R.A. (1998). Genes Dev. *12*, 1398–1408.

Moqtaderi, Z., Bai, Y., Poon, D., Weil, P.A., and Struhl, K. (1996). Nature *382*, 188–191.

Nakajima, T., Uchida, C., Anderson, S., Parvin, J., and Montminy, M. (1997). Genes Dev. *11*, 738–747.

Oelgeschlager, T., Tao, Y., Kang, Y.K., and Roeder, R.G. (1998). Mol. Cell *1*, 925–931.

Ogryzko, V.V., Kotani, T., Zhang, X., Schlitz, R.L., Howard, T., Yang, X.-J., Howard, B.H., Qin, J., and Nakatani, Y. (1998). Cell *94*, this issue, 35–44.

Struhl, K. (1996). Cell *84*, 179–182.

Struhl, K. (1998). Genes Dev. *12*, 599–606.

Verrijzer, C.P., and Tjian, R. (1996). Trends Biochem. Sci. *21*, 338–342.

Walker, S.S., Reese, J.C., Apone, L.M., and Green, M.R. (1996). Nature *382*, 185–188.

Wieczorek, E., Brand, M., Jacq, X., and Tora, L. (1998). Nature *393*, 187–191.

Xie, X., Kokubo, T., Cohen, S.L., Mirza, U.A., Hoffman, A., Chait, B. T., Roeder, R.G., Nakatani, Y., and Burley, S.K. (1996). Nature *380*, 316–322.

Yang, X.-J., Ogryzko, V.V., Nishikawa, J., Howard, B.H., and Nakatani, Y. (1996). Nature *382*, 319–324.