

TFIID: What's in a name?

Resolving the differences between the cloned protein and the natural activity

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The cloning of TFIID marked a significant turning point in the research effort to dissect the initiation of eukaryotic gene expression. Now the experimental goal is to rebuild the natural TFIID complex from the recombinant protein up. This effort is uncovering a number of surprises that shift the focus to the remarkable fine-tuning and custom assembly which may occur at the promoters of individual genes.

Introduction

With the proliferation of cloned and sequenced eukaryotic genes in the late 1970s, the TATA sequence emerged as a widely conserved motif in many genes transcribed by RNA polymerase II. Using reverse genetics, pioneers in the developing field of eukaryotic promoter analysis in the early 1980s rapidly identified the TATA box sequence as an indispensable element controlling basal mRNA synthesis and the site of mRNA initiation (1).

In parallel, biochemical analyses *in vitro* showed that specific RNA polymerase II (pol II) transcription could be reconstituted from a small number of chromatographic fractions (2-6). These activities, now recognized as the general transcription factors, were named TFIIA, TFIIB, TFIID, TFIIE and TFIIF by Robert Roeder and his colleagues. TFIID contained an activity which bound to the TATA box (7,8). Moreover, this unique binding activity appeared to be the first step in the commitment of a template to form an active transcription complex *in vitro* (5,7-9).

It is now two years since the cloning of TATA box binding proteins was first reported (10-12,13-15), and the differences between the recombinant protein and the native TFIID activity are not fully understood. These differences include questions of identity (Is the activity of the cloned proteins completely equivalent to native TFIID activity?) and questions of nomenclature (Are the cloned proteins subunits of TFIID or are they "TATA-binding proteins"?). Speculation continues, as well, on the role of the conserved domains within the cloned TFIIDs and the potential role of TFIID in the expression of TATA-less genes and genes transcribed by polymerases other than pol II (16).

A recent paper by Robert Tjian and colleagues (reviewed below) addresses the first three issues with some provocative observations on the isolation and composition of native *Drosophila* TFIID. Four other new publications return the experimental focus to the *in vivo* role of the highly conserved C-terminal domain of yeast, human, and *Drosophila* recombinant TFIID (17-20). These gene replacement experiments show that human recombinant TFIID cannot substitute for the endogenous yeast TFIID. Surprisingly, the species specificity resides not within the divergent N-terminus of cloned TFIID, as predicted, but primarily within subdomains of the C-terminus. All of these issues were addressed and

extended by results presented at the Cold Spring Harbor transcription conference held in August of this year (21).

How does TFIID mediate transcriptional enhancement of upstream activators?

The first reports on the cloning of TFIID were quick to point out that the recombinant protein, unlike the native TFIID chromatographic fraction, was unable to mediate activation *in vitro* by upstream binding factors such as SP1 (11,22-24). Some results indicated that the cloned protein was able to mediate activation, but that this property was dependent upon the purity of the other general transcription factors used in the functional assay (compare 23 and 24), the species specificity of the recombinant protein in the HeLa assay system (24), or the presence of reconstituted chromatin templates (25). However, the reported levels of activation achieved by the recombinant TFIID were very low (3- to 8-fold) compared to native TFIID activity (20-fold or greater).

A number of investigators have now reported similar results with different transcriptional activators (26-28). They predict that missing components of natural TFIID or independent factors copurifying with natural TFIID, variously termed coactivator or mediator proteins, are the direct targets of activators (see reference 29 and the discussion below). Dynlacht *et al.* report the first biochemical isolation of TFIID activity from *Drosophila* embryo nuclear extracts. They present convincing data that the proteins are tightly bound to the endogenous TATA box binding protein and, after dissociation (Figure 1), they are capable of reconstituting the transcriptional enhancement of the *Drosophila* upstream activator NTF-1.

Review of: Brian D. Dynlacht, Timothy Hoey, and Robert Tjian. (1991) Isolation of Coactivators Associated with the TATA-Binding Protein that Mediate Transcriptional Activation. Cell 66: 563-576.

TATA-binding protein vs. TFIID: nomenclature

This study begins by clarifying the nomenclature employed in this set of experiments. The authors distinguish the cloned protein as the TATA-binding protein (TBP) and they reserve the term "TFIID" to specify the natural activity prepared by chromatography. This decision clearly indicates that the authors do not find the two factors to be identical. Since both the cloned protein and the chromatographic activity support basal transcription from a minimal promoter (i.e., containing a TATAbox but no upstream activator sites), the critical difference is the ability to support transcriptional activation.

The original definition of TFIID activity, however, was confined to basal transcription exclusively from the core promoter of the adenovirus major late (AdML) promoter (2). Indeed, the functional assay used to track purification of TFIID (reconstitution of AdML promoter basal transcription from heat-inactivated nuclear extracts) did not account for any activation properties (8). It was not until the AdML promoter was studied in more detail that a cooperative DNA binding effect between TFIID and an upstream activator (USF) was inferred (7).

This contrast between the original definition of TFIID and the current focus on activation properties is still reflected in the variety of ways TFIID nomenclature appears in publications. For example, Meisterernest *et al.* (25) use superscripts to indicate natural or recombinant TFIID, while Kambadur *et al.* (26) use no distinctions. Although the nomenclature of TFIID was intensely discussed at a separate session during the 1991 Cold Spring Harbor transcription meeting, no consensus was reached on the

appropriate system for naming this factor.

At this time Promega has adopted the nomenclature used in the Dynlacht *et al.* paper for our preparation of the human recombinant TFIID protein (Promega Cat.# E3081). Thus, we identify the product as TFIID (TATA-binding protein). The distinction between an activity and an individual recombinant protein is a necessary one for Promega because we offer other purified recombinant factors which are components of a naturally occurring complex. For example, Promega offers bacterially expressed human c-jun, although we do not supply the corresponding transcriptional activity, AP1.

For the remainder of this article, TFIID will be referred to only as a natural activity. Recombinant TFIID will be noted as the TATA-binding protein (rTBP). The endogenous, or cellular, protein will be called the natural TATA-binding protein (nTBP). While this nomenclature admittedly places too much emphasis on the DNA binding properties of this general transcription factor, it also is the most direct description which simultaneously preserves the distinction between the individual protein and an, as yet, poorly defined natural complex.

TATA-binding protein vs. TFIID: chromatographic differences

For many laboratories interested in the regulation of transcription initiation, a major experimental obstacle in analyzing the fate of the endogenous TATA-binding protein (nTBP) in any system is the inability to follow that protein through a fractionated extract.

Dynlacht *et al.* use affinity-purified polyclonal and monoclonal antibodies directed against *Drosophila* rTBP to allow them to ask where the endogenous nTBP is fractionating in the extracts ([Figure 1](#)). By Western blot analysis, these authors show that all of the detectable nTBP is contained in the 0.3M KCl eluate from a Q-Sepharose column (Q.3), a fraction first identified by Kadonaga and colleagues (30) through reconstitution experiments as a candidate fulfilling at least some of the properties expected of *Drosophila* TFIID activity.

This fraction reconstitutes transcription from the minimal BCAT promoter (a CAT reporter plasmid driven only by a TATA consensus sequence) in a manner indistinguishable from *Drosophila* rTBP expressed in bacteria. By sedimentation analysis, this nTBP-containing fraction migrates as a complex with a molecular weight of 350kDa. In contrast, rTBP bands at its predicted molecular weight of 45kDa in glycerol gradients. This finding parallels the earlier observations of Reinberg and Roeder (5), who noted that HeLa TFIID activity elutes from high-salt gel filtration columns with an apparent molecular weight of 120,000 to 140,000, whereas the electrophoretic molecular weight of the recombinant human TATA-binding protein is 37kDa (11,14). This observation is also consistent with the observation of Gilmour *et al.* (20) that the *Drosophila* endogenous TATA-binding activity assayed by DNase I footprinting covers a large region of the heat shock gene, *hsp70*. Like the TFIID pattern on the AdML promoter, Gilmour, *et al.* (31) saw protection from -44 to +35 on *hsp70* promoter (with +1 as the start site of transcription).

Endogenous *Drosophila* TATA-binding protein exists in a complex *In vitro*.

Dynlacht *et al.* also show the endogenous *Drosophila* nTBP to be in a complex by immunoprecipitation experiments which use the Q.3 fraction as starting material. This Q.3 fraction contains a very large number of proteins. However, after immunoprecipitation, at least six proteins are revealed by silver-stained gels. These proteins range in molecular weight from 32 to 150kDa. Significantly, the internal

control for the immunoprecipitations shows the nTBP comigrating with the recombinant protein expressed in bacteria. This result indicates that the nTBP within a complex is not post-translationally modified to any great extent.

These tightly bound TBP-associated proteins, or factors, (TAFs) contained in the immunoprecipitates (Figure 1) can be dissociated by treatment with 3M urea. Similarly, nTBP and TAFs are also dissociated if the Q.3 fraction is treated with 3M urea or heat treatment, although the complex is otherwise refractory to separation by other standard chromatographic techniques. For use in transcription reconstitution reactions, nTBP is prepared by chromatography through Q-Sepharose in the presence of urea; under these conditions, nTBP elutes with 0.18M KCl (Q.18/urea).

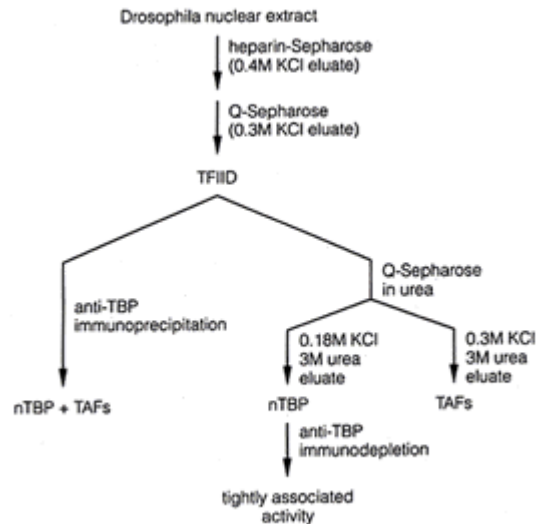


Figure 1. Fractionation of *Drosophila* TFIID into natural TBP and its associated factors (Dymlacht, *et al.*, 1991).

Are rTBP and nTBP functionally equivalent?

With respect to the support of transcriptional enhancement, rTBP and nTBP (each reconstituted with TAFs), differ in the same way as rTBP and TFIID activity. Endogenous nTBP is capable of supporting activation by the transcription factors NTF-1 and human SP1 if the TAFs are added to the basal transcription reaction. Recombinant TBP, however, cannot support this type of upstream activation. Instead, Dymlacht *et al.* report that an *additional* activity, presumably distinct from the ones represented in the TAF preparation, must be added to rTBP to elicit even a small degree of NTF-1 activation. This new activity is obtained by immunodepletion of the endogenous nTBP preparation (Q.18/urea). When combined with the TAFs, this activity reconstitutes NTF-1 activation with the nTBP.

In summary, this evidence argues for two classes of coactivators which, in the view of Tjian and colleagues, together comprise TFIID: the first class consists of the tightly bound TAF proteins dissociable by urea and the second class is the extremely tenacious activity present in the Q.18/urea TBP.

Discussion

The work in this paper represents a significant achievement in addressing the nature of the nTBP in an

extract and in identifying the first tangible candidates for coactivator proteins. Ironically, the rTBP is still less effective than the endogenous protein in establishing transcriptional activation; the NTF-1 reconstitution achieved with rTBP is not as convincing as the activation reconstituted with endogenous nTBP. Unfortunately, the comparisons of activation in the Dynlacht *et al.* experiments are rather qualitative, based on subjective evaluations of band intensities on an autoradiogram. In general, activation arguments would benefit from better quantification of the *in vitro* transcription data.

More than one TFIID?

Citing unpublished data, the authors also mention that no activation could be reconstituted using a different upstream regulatory factor, *zen*, in combination with the urea or heat-isolated TAFs. Thus, the set of proteins which define TFIID activity for a given promoter configuration may be unique or part of a class of *promoter specific* TAFs. Jeffrey Parvin and Phil Sharp raised this possibility in a presentation at the August 1991 Cold Spring Harbor transcription meeting (21). In preliminary data, these investigators showed that the transcriptional strength of two different promoters in two different cell lines may be reversed *in vitro* by exchanging only the TATA motifs. The two motifs, obtained from the adenovirus major late promoter and the human immunoglobulin heavy chain gene promoter, appeared to have cell-type specific basal transcription factors assembled at their core TATA and initiator elements.

Using independently raised polyclonal antibodies against the human rTBP, H.T.M. Timmers and Phil Sharp presented preliminary data at Cold Spring Harbor showing that in HeLa nuclear extracts the human nTBP exists in two functionally active complexes. Interestingly, one complex is capable of supporting transcriptional enhancement by SP1 and the other is capable only of initiating basal mRNA synthesis.

Lastly, Gilmour *et al.* have identified four *Drosophila* proteins which make up the apparent TFIID activity binding to a large region on the promoter of the major heat shock protein gene, *hsp70* (31). They were identified as a complex by DNA-protein crosslinking induced by ultraviolet light. One protein of 42kDa is an obvious candidate as the endogenous *Drosophila* TBP. The remaining three proteins might be a subset of the six TAFs described by Dynlacht *et al.*

Protein complexes found *in vitro*: how to prove functionality

The use of reliable antibodies against the TBP is a crucial method in the identification of complexes which assemble at the TATA boxes. Dynlacht *et al.* used their antibodies in two types of experiments to establish that endogenous TBP is in a complex of proteins: Western blot analysis of glycerol gradient sedimentation and immunoprecipitation from fractions. A third line of evidence, immunoinhibition of transcription reactions, would be interesting to pursue in this analysis. Such inhibition would add further evidence that the complex of proteins Dynlacht *et al.* have isolated is indeed a functional one. Moreover, the methods of crosslinking (31) and immunoprecipitation can be applied together to resolve the composition of TFIID protein complexes both *in vitro* and *in vivo*. This method has already been used very successfully in the elucidation of nuclear RNA binding complexes in HeLa and *Drosophila* (32, and references therein).

Because artifactual protein-protein interactions are difficult to rule out *in vitro*, an informative additional experiment would be immunoprecipitation of the TFIID complex as it is formed on a promoter. The immunoprecipitations described in Dynlacht *et al.* are performed on fractions and not on reactants actively assembling on a TATA box.

Species specificity of TBPs

These results raise additional questions about the species specificity of the TATA-binding protein. Dynlacht *et al.* observe that *Drosophila* TATA-binding protein, when expressed in HeLa cells using a vaccinia vector, does not have any detectable proteins associated with it. Moreover, in the original identification of the *Drosophila* TFIID activity by Wampler *et al.* (30), the Q.3 fraction was unable to substitute for the HeLa TFIID activity in a reconstituted transcription reaction. Nevertheless, *Drosophila* TFIID appears capable of supporting transcriptional activation by human SP1, an activity not found in *Drosophila* (24). This result leads one to expect that at least one coactivator protein is shared between HeLa and *Drosophila*.

How might these species differences arise at the molecular level? A number of recent articles using yeast genetics indicate that the conserved C-terminal domain of the TATA-binding protein contains all of the information necessary to sustain cell viability. In these gene replacement experiments, the corresponding conserved segments of the *Drosophila* and human cloned TATA-binding proteins are lethal (17-20).

It will be interesting to see the two types of experimental strategies discussed here, biochemical and genetic analysis, converge in addressing the complete role of TFIID. The special contribution of the genetic studies in yeast has been the construction of yeast/human hybrid TFIID genes. If these hybrid genes could be used to express stable proteins, for example, one could correlate the functional domains required for basal or activated transcription *in vitro*. Such experiments now seem especially possible in yeast with the recent development of a reconstituted *in vitro* transcription system reported by Roger Kornberg and colleagues at the Cold Spring Harbor meetings (21, abstract by H. Tschochner *et al.*).

References:

1. Breathnach, R. and Chambon, P. (1981) *Ann. Rev. Biochem.* **50**, 349.
2. Matsui, T., *et al.* (1980) *J. Biol. Chem.* **255**, 11992.
3. Samuels, M., Fire, A. and Sharp, P.A. (1982) *J. Biol. Chem.* **257**, 14419.
4. Sawadogo, M. and Roeder, R.G. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4394.
5. Reinberg, D. and Roeder, R.G. (1987) *J. Biol. Chem.* **262**, 3310.
6. Reinberg, D., Horikoshi, M. and Roeder, R.G. (1987) *J. Biol. Chem.* **262**, 3322.
7. Sawadogo, M. and Roeder, R.G. (1985) *Cell* **43**, 165.
8. Nakajima, N., Horikoshi, M. and Roeder, R.G. (1988) *Mol. Cell Biol.* **8**, 4028.
9. Buratowski, S., *et al.* (1989) *Cell* **56**, 549.
10. Cavallini, B., *et al.* (1989) *Proc. Natl. Acad. Sci. USA* **86**, 9803.
11. Peterson, M.G., *et al.* (1990) *Science* **248**, 1625.
12. Horikoshi, M., *et al.* (1989) *Nature* **341**, 299.
13. Fikes, J.D., *et al.* (1990) *Nature* **346**, 291.
14. Hoffman, A., *et al.* (1990) *Nature* **346**, 387.
15. Schmidt, M.C., *et al.* (1989) *Proc. Natl. Acad. Sci. USA* **86**, 7785.
16. Lobo, S., *et al.* (1991) *Genes & Develop.* **5**, 1477.
17. Gill, G. and Tjian, R. (1991) *Cell* **65**, 333.
18. Cormack, B., *et al.* (1991) *Cell* **65**, 341.
19. Reddy, P. and Hahn, S. (1991) *Cell* **65**, 349.
20. Poon, D., *et al.* (1991) *Mol. Cell Biol.* **11**, 4809.
21. *The Cold Spring Harbor Meeting on Cancer Cells: Regulation of Eukaryotic mRNA Transcription*. August 28-September 1, 1991. Cold Spring Harbor Laboratory. Arranged by Winship Herr, Robert Tjian and Keith Yamamoto.

22. Hoey, T., *et al.* (1990) *Cell* **61**, 1179.
23. Kao, C.C., *et al.* (1990) *Science* **248**, 1646.
24. Pugh, B.F. and Tijan, R. (1990) *Cell* **61**, 1187.
25. Meisterernest, M., Horikoshi, M. and Roeder, R.G. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 9153.
26. Kambadur, R., Culotta, V. and Hamer, D. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 9168.
27. Zhu, H., *et al.* (1991) *New Biologist* **3**, 455.
28. Bergh, S.L., *et al.* (1990) *Cell* **61**, 1199.
29. Lewin, B. (1990) *Cell* **61**, 1161.
30. Wampler, S., *et al.* (1990) *J. Biol. Chem.* **265**, 21223.
31. Gilmour, D., *et al.* (1990) *Mol. Cell Biol.* **10**, 4233.
32. Pinol-Roma, S., *et al.* (1990) *Meth. Enzymol.* **181**, 317.

Drosophila embryo and HeLa nuclear extract and several recombinant human transcription factors are available from Promega. Ordering information for these products is provided at the end of the article that starts on [page 5](#) of this issue of Promega Notes.