mers (Dekker, New York, 1986).
28. J. L. Bredas and G. B. Street, *Acc. Chem. Res.* **18**, 309 (1985).
29. S. Kivelson and A. J. Heeger, *Synth. Met.* **22**, 371 (1988).
30. J. Lei, Z. Cai, C. R. Martin, *ibid.* **46**, 53 (1992).
31. A. G. MacDiarmid, in *Conjugated Polymers and Related Materials,* W. R. Salaneck, I. Lundstrom, B. Ranby, Eds. (Oxford Univ. Press, Oxford, 1993), pp. 73–97.
32. W. Fosong, T. Jinsong, W. Lixiang, Z. Hongfang, M. Zhishen, *Mol. Cryst. Liq. Cryst.* **160**, 175 (1988).
33. R. V. Parthasarathy and C. R. Martin, *Chem. Mater.* **6**, 1627 (1994).
34. W. Cahalane and M. M. Labes, *ibid.* **1**, 519 (1989).
35. C. G. J. Koopal, R. J. M. Nolte, B. De Ruiter, *J. Chem. Soc. Chem. Commun.* **1991**, 1691 (1991).
36. M. Granstorm and O. Inganas, *Synth. Met.* **55–57**, 460 (1993).
37. R. P. Burford and T. Tongtam, *J. Mater. Sci.* **26**, 3264 (1993).
38. C. R. Martin, L. S. Van Dyke, Z. Cai, W. Liang, *J. Am. Chem. Soc.* **112**, 8976 (1990).
39. C. R. Martin, *Adv. Mater.* **3**, 457 (1991).
40. W. Liang and C. R. Martin, *J. Am. Chem. Soc.* **112**, 9666 (1990).
41. J. Lei, V. P. Menon, C. R. Martin, *Polym. Adv. Technol.* **4**, 124 (1992).
42. V. P. Menon and C. R. Martin, in preparation.
43. J. C. Wittmann and P. Smith, *Nature* **352**, 414 (1991).
44. Z. Cai and C. R. Martin, *J. Electroanal. Chem.* **300**, 35 (1991).
45. C. R. Martin, *Acc. Chem. Res.*, in press.
46. R. Pool, "101 Uses for Tiny Tubules," *Science* **247**, 1410 (1990).
47. C. K. Preston and M. J. Moskovits, *J. Phys. Chem.* **97**, 8495 (1993).
48. J. T. Masden and N. Giordino, *Phys. Rev. B* **36**, 4197 (1987).
49. T. M. Whitney, J. S. Jiang, P. C. Searson, C. L. Chien, *Science* **261**, 1316 (1993).
50. G. E. Possin, *Rev. Sci. Instrum.* **41**, 772 (1970).
51. T. Asada, Japanese Patent 310,401 (1960).
52. R. D. Patel, M. G. Takwale, V. K. Nagar, V. G. Bhide, *Thin Solid Films* **115**, 169 (1984).
53. S. Kawai and R. Ueda, *J. Electrochem. Soc.* **112**, 32 (1975).
54. I. F. Cheng, L. D. Whiteley, C. R. Martin, *Anal. Chem.* **61**, 762 (1989).
55. K. Uosaki, K. Okazaki, H. Kita, H. Takahashi, *ibid.* **62**, 652 (1990).
56. S. K. Chakarvarti and J. Vetter, *J. Micromech. Microeng.* **3**, 57 (1993).
57. ———, *Nucl. Instrum. Methods Phys. Res. B* **62**, 109 (1991).
58. C. J. Miller, C. A. Widrig, D. H. Charych, M. Majda, *J. Phys. Chem.* **92**, 1928 (1988).
59. C. A. Huber *et al.*, *Science* **263**, 800 (1994).
60. H. C. van de Hulst, *Light Scattering by Small Particles* (Dover, New York, 1981).
61. K. B. Oldham and C. G. Zoski, *J. Electroanal. Chem.* **256**, 11 (1988).
62. V. P. Menon and C. R. Martin, in preparation.
63. A. M. Kressin, V. V. Doan, J. D. Klein, M. J. Sailor, *Chem. Mater.* **3**, 1051 (1991).
64. M. Sundaram, S. A. Chalmers, P. F. Hopkins, A. C. Gossard, *Science* **254**, 1326 (1991).
65. O. Zhou *et al.*, *ibid.* **263**, 1744 (1994).
66. This work would not have been possible without the efforts of a number of hardworking and highly motivated graduate students and postdocs: V. P. Menon, C. A. Foss Jr., Z. Cai, J. Lei, W. Liang, R. V. Parthasarathy, D. R. Lawson, C. J. Brumlik, G. L. Hornyak, L. S. Van Dyke, L. D. Whiteley, I. F. Cheng, and R. M. Penner. Financial support from the Office of Naval Research is also gratefully acknowledged. We also wish to thank the Colorado State University Electron Microscopy Center.

RESEARCH ARTICLE

Reconstitution of Transcription Factor SL1: Exclusive Binding of TBP by SL1 or TFIID Subunits

Lucio Comai, Joost C. B. M. Zomerdijk, Holger Beckmann, Sharleen Zhou, Arie Admon,* Robert Tjian†

RNA polymerase I and II transcription factors SL1 and TFIID, respectively, are composed of the TATA-binding protein (TBP) and a set of TBP-associated factors (TAFs) responsible for promoter recognition. How the universal transcription factor TBP becomes committed to a TFIID or SL1 complex has not been known. Complementary DNAs encoding each of the three TAF_Is that are integral components of SL1 have now been isolated. Analysis of subunit interactions indicated that the three TAF_Is can bind individually and specifically to TBP. In addition, these TAF_Is interact with each other to form a stable TBP-TAF complex. When TBP was bound first by either TAF_I110, 63, or 48, subunits of TFIID such as TAF_{II}250 and 150 did not bind TBP. Conversely, if TBP first formed a complex with TAF_{II}250 or 150, the subunits of SL1 did not bind TBP. These results suggest that a mutually exclusive binding specificity for TBP intrinsic to SL1 and TFIID subunits directs the formation of promoter- and RNA polymerase–selective TBP-TAF complexes.

Although the regulation of transcription in eukaryotes has been studied intensively for more than 20 years, it has only recently become possible to attempt a detailed mechanistic analysis of the protein-DNA and protein-protein interactions that govern this essential cellular process. The identification and biochemical characterization of transcription factors that assemble into multi-subunit complexes responsible for promoter recognition and regulation have presented problems which are now yielding to analysis. In the past 5 years, substantial progress has been made in the definition of regulatory factors that direct transcription by each of the three RNA polymerases (I, II, and III), which are dedicated to the synthesis of ribosomal, messenger, and transfer RNAs, respectively (*1–3*). The basal complex responsible for RNA polymerase II transcription is well characterized with most components identified, purified, and cloned (reviewed in *4*). In addition, many of the ancillary transcription factors that govern RNA polymerase I and III have been identified, although only a few of the genes encoding these transcription factors have been cloned, and little is known about how they interact with each other in order to assemble into functional complexes (*3*).

Originally thought to be exclusively an RNA polymerase II transcription factor, the TATA binding protein (TBP) instead serves as a universal subunit that participates in transcription by all three RNA polymerases (*5–9*). Studies of the RNA polymerase II basal factor TFIID revealed that TBP is actually associated with at least eight distinct subunits or TAFs (TBP-associated factors), which together form a complex essential for promoter recognition and for mediating activation by enhancer bound regulatory factors (*6, 7*). Subsequent studies revealed that the RNA polymerase I transcription factor SL1 also consists of TBP and contains three TAFs of estimated molecular mass of 110, 63, and 48 kD, respectively (*8, 10*). A similar arrangement of TAFs and TBP has been documented for transcription factors that are effective in RNA polymerase III transcription (*3*). Thus, it became of considerable interest to dissect the nature of each of these three apparently structurally and functionally distinct TBP-TAF complexes.

There has been rapid progress in characterizing the structure and function of the TFIID subunits which established that, through specific TAF-TBP and TAF-TAF contacts, a complex is formed that binds to DNA elements of core RNA polymerase II promoters including the TATA box and downstream initiator sequences (*11–16*).

The authors are in the Howard Hughes Medical Institute, Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley, CA 94720–3204, USA.

*Present address: Department of Biology, Technion Institute, Haifa 32000, Israel.
†To whom correspondence should be addressed.

Thus, TAFs in the TFIID complex not only participate in specific protein-protein interactions, but some subunits (that is, $TAF_{II}150$) also exhibit sequence-specific DNA binding activities (*16*). In addition, various TAFs within the TFIID complex have been shown to interact selectively with different classes of transcriptional regulators, and distinct TAFs are required to mediate transcriptional enhancement by different activators (*16–19*). It is probable, therefore, that the TBP-TAF complexes involved in RNA polymerase I and III transcription will also provide essential functions such as promoter selectivity and transcriptional regulation.

Accordingly, we have attempted to characterize the biochemical properties of the TAFs associated with the RNA polymerase I transcription factor SL1. We have shown earlier that SL1 plays a critical role during transcription of ribosomal RNA by RNA polymerase I (*20–22*). First, SL1 was shown to be required for accurate and efficient initiation of transcription in vitro from the ribosomal promoter. Second, SL1 appears to serve as the core complex for the communication of signals between the upstream binding factor (UBF) and RNA polymerase I (*22–24*). Third, SL1 is responsible for the species specificity of transcription by RNA polymerase I (*22*). Finally, the cooperative DNA binding interactions between UBF and SL1 as well as the recognition of species-specific core promoter elements by SL1, suggest that one or more of the subunits comprising this essential transcription factor must be responsible for promoter recognition. In addition, we anticipate that regulation of ribosomal RNA transcription in response to amino acid starvation and other physiological cues, is likely to be mediated by way of SL1 in a manner analogous to the function of TFIID in the integration of regulatory signals from multiple upstream enhancer binding proteins. Thus, it was necessary to dissect the subunit structure and biochemical properties of SL1. With a view to learning the mechanisms governing transcriptional regulation by the RNA polymerase I transcription complex using a defined reconstituted in vitro reaction with purified components, we have recently isolated from human cells the RNA polymerase I promoter-specific factor UBF and obtained complementary DNA (cDNA) clones encoding this sequence-specific DNA binding protein (*24, 25*). In the presence of purified recombinant UBF and partially purified SL1, RNA polymerase I can be programmed in vitro to accurately initiate large amounts of transcription from the human ribosomal RNA promoter (*22, 24*). However, the purification and biochemical characterization of SL1 eluded us until we realized that this transcription factor contains TBP as an integral subunit (*8*). We were thus able to obtain highly purified preparations of SL1 by immunoaffinity chromatography. Only very small quantities of purified SL1 were obtainable by conventional purification procedures however, and this hampered detailed functional and structural analysis of SL1. To overcome this obstacle, it was necessary to isolate the genes encoding the subunits of SL1 and to overproduce them in recombinant form. In particular, we sought to provide evidence for direct interactions between the TAF subunits of SL1 and TBP. We also wanted to establish the network of TAF-TAF interactions required to assemble SL1 and to decipher the mechanisms by which TBP is directed to assemble into SL1 as opposed to TFIID complexes.

We now report the molecular cloning of cDNAs encoding each of the three TAFs associated with the RNA polymerase I transcription factor SL1. The products expressed from the cloned cDNAs are identi-

A

TAF_I110

```
MDFPSSLRPALFLTGPLGLSDVPDLSFMCSWRDALTLPEAQPQNSENGAL  50
HVTKDLLWEPATPGPLPMLPPLIDPWDPGLTARDLLFRGGYRYRKRPRVV 100
LDVTEQISRFLLDHGDVAFAPLGKLMLENFKLEGAGSRTKKKTVVSVKKL 150
LQDLGGHQPWGCPWAYLSNRQRRFSILGGPILGTSVASHLAELLHEELVL 200
RWEQLLLDEACTGGALAWVPGRTPQFGQLVYPAGGAQDRLHFQEVVLTPG 250
DNPQFLGKPGRIQLQGPVRQVVTCTVQGESKALIYTFLPHWLTCYLTPGP 300
FHPSSALLAVRSDYHCAVWKFGKQWQPTLLQAMQVEKGATGISLSPHLPG 350
ELAICSRSGAVCLWSPEDGLRQIYRDPETLVFRDSSSWRWADFTAHPRVL 400
TVGDRTGVKMLDTQGPPGCGLLLFRLGAEASCQKGERVLLTQYLGHSSPK 450
CLPPTLHLVCTQFSLYLVDERLPLVPMLKWNHGLPSPLLLARLLPPPRPS 500
CVQPLLLGGQGGQLQLLHLAGEGASVPRLAGPPQSLPSRIDSLPAFPLLE 550
PKIQWRLQERLKAPTIGLAAVVPPLPSAPTPGLVLFQLSAAGDVFYQQLR 600
PQVDSSLRRDAGPPGDTQPDCHAPTASWTSQDTAGCSQWLKALLKVPLAP 650
PVWTAPTFTHRQMLGSTELRREEEEGQRLGVLRKAMARGQLLLQRDLGSL 700
PAAEPPPAPESGLEDKLSERLGEAWAGRGAAWWERQQGRTSEPGRQTRRP 750
KRRTQLSSSFSLSGHVDPSEDTSSPHSPEWPPADALPLPPTTPPSQELTP 800
DACAQGVPSEQRQMLRDYMAKLPPQRDTPGCATTPPHSQASSVRATRSQQ 850
HTPVLSSSQPLRKKPRMGF                                  869
```

B

TAF_I48

```
MSDFSEELKGPVTDDEEVETSVLSGAGMHFPWLQTYVETVAIGGKRRKDF  50
AQTTSACLSFIQEALLKHQWQQAAEYMYSYFQTLEDSDSYKRQAAPEIIW 100
KLGSEILFYHPKSNMESFNTFANRMKNIGVMNYLKISLQHALYLLHHGML 150
KDAKRNLSEAETWRHGENTSSREILINLIQAYKGLLQYYTWSEKKMELSK 200
LDKDDYAYNAVAQDVFNHSWKTSANISALIKIPGVWDPFVKSYVEMLEFY 250
GDRDGAQEVLTNYAYDEKFPSNPNAHIYLYNFLKRQKAPRSKLISVLKIL 300
YQIVPSHKLMLEFHTLLRKSEKEEHRKLGLEVLFGVLDFAGCTKNITAWK 350
YLAKYLKNILMGNHLAWVQEEWNSRKNWWPGFHFSYFWAKSDWKEDTALA 400
CEKAFVAGLLLGKGCRYFRYILKQDHQILGKKIKRMKRSVKKYSIVNPRL 450
```

C

TAF_I63

```
IPAFPAGTVLQPFPEAALATRVTVPAVEAPAAPRLDLEESEEFKERCTQC  50
AAVSWGLTDEGKYYCTSCHNVTERYQEVTNTDLIPNTQIKALNRGLKKKN 100
NTEKGWDWYVCEGFQYILYQQAEALKNLGVGPELKNDVLHNFWKRYLQKS 150
KQAYCKNPVYTTGRKPTVLEDNLSHSDWASEPELLSDVSCPPFLESGAES 200
QSDIHTRKPFPVSKASQSETSVCSGSLDGVEYSQRKEKGIVKMTMPQTLA 250
FCYLSLLWQREAITLSDLLRFVEEDHIPYINAFQHFPEQMKLYGRDRGIF 300
GIESWPDYEDIYKKTIEVGTFLDLPRFPDITEDCYLHPNILCMKYLMEVN 350
LPDEMHSLTCHVVKMTGMGEVDFLTFDPIAKMAKAVKYDVQAVAIIVVVL 400
KLLFLMDDSFEWSLSNLAEKHNEKNKKDKPWFDFRKWYQIMKKAFDEKKQ 450
KWEEARAKYLWKSEKPLYYSFVDKPVAYKKREMVVNLQKQFSTLVDSTAT 500
AGKKSPSSFQFNWTEEDTDRTCFHGHSLQGVLKEKGQSLLTKNSLYWLST 550
QKFCRW                                               556
```

Fig. 1. Amino acid sequences were deduced from the (**A**) TAF_I110, (**B**) TAF_I48, and (**C**) TAF_I63 cDNAs. Peptide sequences obtained from microsequencing of Lys-C or trypsin fragments are underlined. (B) The two imperfect direct repeats [10 out of 25 (closed arrows above the sequence) and 10 out of 26 (open arrows above the sequence) amino acid identity] in TAF_I48 are indicated. (C) Numbering of TAF_I63 is arbitrary. Cysteine and histidine residues in the two putative Zn fingers in TAF_I63 are highlighted in bold letters. Abbreviations for the amino acid residues are in (*26*).

cal to the 110-, 63-, and 48-kD subunits of SL1. We performed various protein-protein interaction assays to determine which TAFs contact TBP and which subunits interact with each other. Our results suggest that the stable SL1 complex involves multivalent contacts between TBP and TAFs as well as between TAFs. We have also tested the ability of SL1 and TFIID subunits to interact simultaneously with TBP. The interaction of SL1 subunits with TBP precludes binding of this universal subunit to TFIID subunits and vice versa, thus providing a mechanism for directing the formation of TBP-TAF complexes with distinct transcriptional properties.

Cloning and expression of TAF_I48, 63, and 110. In order to isolate cDNAs that encode the three TAF_Is, we purified the human SL1 complex from approximately 500 liters of HeLa cells by heparin-agarose and S-Sepharose chromatography followed by TBP affinity chromatography with an antibody to TBP. The three TAF_Is were eluted by treating the antibody affinity resin with 1 M guanidine-HCl. The eluted polypeptides were subjected to SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and the separated fractions were transferred to polyvinylidene difluoride (PVDF) membrane. The membranes were stained with Ponceau S, and the regions of the membrane containing each of the three TAF_Is were excised and digested with either the protease Lys-C or trypsin. Proteolytic peptides were separated by reversed-phase high-pressure liquid chromatography (HPLC) and subjected to microsequencing. Amino acid sequences derived from peptides corresponding to each of the TAF_Is were then used to design either degenerate oligonucleotide primers for polymerase chain reactions with first-strand cDNAs or guessmer probes for cDNA library screening.

For the cloning of TAF_I48, two unique guessmer probes were used to screen a human cDNA library, from which four independent isolates were obtained (*26*). The longest cDNA insert contained a 1.5-kb open reading frame flanked by an in-frame stop codon and a poly(A)$^+$ (polyadenylated) tail at the 3′ end. The open reading frame encodes a polypeptide of 450 amino acid residues with a predicted molecular mass of 53 kD (Fig. 1). All of the peptides obtained from the microsequence analysis of the smallest subunit (TAF_I48) were found within this open reading frame confirming that this clone encodes the 48-kD protein.

For the cloning of TAF_I63, we designed degenerate oligonucleotide primers derived from one peptide of 15 amino acid residues in order to amplify the internal DNA fragment by PCR on first-strand HeLa cDNA (*26*). A 45-residue oligonucleotide corresponding to the nucleotides located within the amplified sequence was subsequently used to screen a human teratocarcinoma cDNA library, and 14 positive phages were isolated. The longest cDNA clone was 2.5 kb and contained a poly(A)$^+$ tail at the 3′ end. The coding region for TAF_I63 continued uninterrupted to the 5′ end of the cDNA clone and did not start with a methionine, an indication that this clone lacked 5′ end sequences. The sequence predicts an open reading frame of 556 amino acids and specifies a polypeptide with a molecular mass of 64 kD, which contains all of the peptides obtained by microsequencing (Fig. 1). Because the deduced size of the recombinant protein is close to the estimated molecular mass of the endogenous TAF_I63, the cDNA may be missing codons for only a few amino acids at the NH_2-terminus.

The cDNA for the third component of the SL1 complex, TAF_I110, was cloned by a PCR-based strategy similar to that used for the cloning of TAF_I63, which involves the peptide indicated in (*26*). A teratocarcinoma cDNA library was screened with an oligonucleotide derived from the amplified probe. Partial cDNAs were then used as probes to identify in a HeLa cDNA library a full-length cDNA clone containing a 3.9-kb open reading frame preceded by an in-frame stop codon and a 3′ end poly(A)$^+$ tail. All of the amino acid sequences for peptides derived from TAF_I110 were found within this open reading frame. A protein of 869 amino acids with a predicted molecular mass of 95 kD was deduced from the cDNA sequence (Fig. 1).

None of the TAF_I proteins showed any sequence similarity to the recently characterized TAF_{II} polypeptides. The deduced amino acid sequences of TAF_I48, 63, and 110 (Fig. 1) did not reveal significant similarities to any proteins in the database, which suggests that these TAFs represent previously undescribed proteins. Inspection of the deduced amino acid sequence of TAF_I48 revealed two stretches near the NH_2-terminus that were imperfectly repeated at the COOH-terminus of the protein. In addition, TAF_I63 contains two putative Zn fingers (C-X_2-C-X_{14}-C-X_2-C and C-X_2-H-X_{18}-H-X_3-C; C is cysteine and H is histidine).

To establish that these three cDNAs are integral components of SL1, we produced recombinant proteins using *Escherichia coli* and baculovirus expression vectors. Proteins for TAF_I63 and 110, expressed in *E. coli*, and TAF_I48 produced with recombinant baculovirus in insect cells, were used to generate polyclonal antibodies in rabbits (*27*). These antibodies recognized the endogenous TAF_Is present in the SL1 complex (Fig. 2A). Moreover, the electrophoretic mobilities of the recombinant proteins appeared indistinguishable from those of the endogenous TAF_Is, which confirms that rTAF_I48 and 110 are full-length proteins, and that rTAF_I63 is most likely nearly full-length. To provide further evidence that each of the expressed recombinant proteins was a bona fide TAF_I, we used antibodies to TAF_I48, 63, and 110 (anti-TAF_I48, anti-TAF_I63, and anti-TAF_I110) which immunoprecipitated the endogenous native SL1 complex from a partially purified fraction and confirmed the presence of

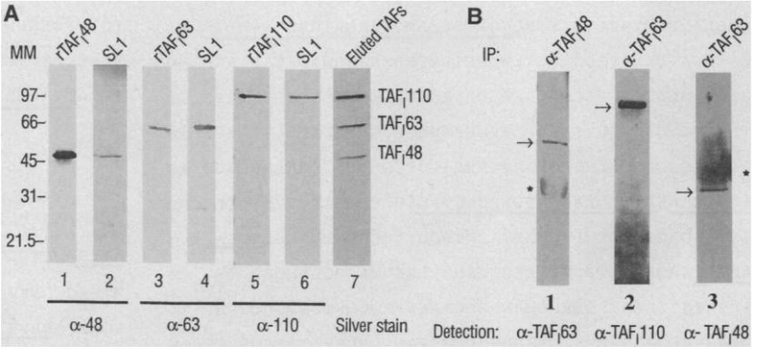

Fig. 2. (**A**) Recombinant TAF_I48, 63, and 110 are indistinguishable from the endogenous subunits associated with TBP in the SL1 complex. TAFs eluted with guanidine hydrochloride from TBP antibody immunopurified SL1 (lanes 2, 4, 6, and 7), and whole extracts from Sf9 insect cells infected with recombinant untagged TAF_I48, 63, and 110 baculoviruses were resolved by SDS-PAGE and analyzed by immunoblotting with polyclonal anti-TAF_I48 (lanes 1 and 2), anti-TAF_I63 (lanes 3 and 4), and anti-TAF_I110 (lanes 5 and 6). Lane 7 shows the eluted TAF_Is by silver staining. Preimmune sera did not react with any of the proteins on the blots (*28*). (**B**) Antibodies against TAF_I48, 63, and 110 immunoprecipitate the endogenous SL1 complex. Partially purified SL1 (Heparin-agarose; SP-Sepharose fraction) was immunoprecipitated with polyclonal an tibodies to TAF_I48 (lane 1) or TAF_I63 (lanes 2 and 3) (data for anti-TAF_I110 are not shown). The immunoprecipitatedproteins were resolved by SDS-PAGE and analyzed by immunoblotting with polyclonal antisera as indicated. The positions of each TAF_I are indicated by arrows; asterisks denote cross reactivity with immunoglobulin heavy chain.

each TAF_I and TBP by immunoprotein blot analysis (Fig. 2B) (*28*). These results taken together confirmed that we have isolated cDNAs encoding each of the three TAF_Is in the SL1 complex.

Protein-protein interactions between TAF_Is and TBP. The role of TAF_Is and TBP within the SL1 complex, was elucidated by identification of the specific protein-protein interactions involved in the assembly of SL1. Our studies of TAF_{II}s in the TFIID complex indicated that only some of the subunits directly contact TBP, whereas other subunits are brought into the complex by way of TAF-TAF interactions. To begin the investigation of subunit interactions within the SL1 complex we performed a series of experiments to define which TAFs contacts TBP. First we tested the ability of TBP to bind to epitope-tagged TAF_I48, 63, or 110 immobilized on antibody beads (*29*). In vitro–translated [^{35}S]Met-labeled TBP can bind independently to each of the three TAF_Is that form SL1 (Fig. 3). Furthermore, we show that under these conditions, TBP also stably associates with a TFIID subunit, TAF_{II}150, but not with TAF_{II}110, as had been shown previously (*16, 17*). To further establish the specificity of these interactions, we performed reciprocal assays in which glutathione-S-transferase (GST)–TBP fusion proteins were immobilized on glutathione beads and incubated with radiolabeled in vitro–translated TAF_I48, 63, and 110 (*30*). Again, each of the TAFs bound efficiently to GST-TBP but not to control GST beads (Fig. 3B). These interactions have been confirmed with the use of purified recombinant TAFs expressed in *E. coli* (*31*). Thus, in contrast to what is seen for the subunits of the TFIID complex, all of the subunits of SL1 appear to make contact with TBP.

TAF_I-TAF_I interactions. To further identify potential protein-protein contacts that stabilize the SL1 complex, we investigated TAF_I-TAF_I interactions. Each subunit of SL1 was tested for the ability to selectively bind to either of the other two TAF_Is by affinity resin assays. Individual FLAG epitope-tagged TAFs were immobilized on M2-antibody beads and incubated with insect cell extracts containing one of the other nontagged baculovirus-expressed TAFs. The formation of TAF-TAF heteromeric complexes was analyzed by SDS-PAGE and subsequent immunoblotting with antibodies to the captured TAF_I. These experiments revealed that TAF_I48 can bind directly to both TAF_I63 and 110 (Fig. 4, lanes 1 to 3). In a similar manner, TAF_I63 can stably associate with TAF_I48 and 110 (Fig. 4, lanes 4 to 6), and TAF_I110 can bind to TAF_I48 and TAF_I63 (Fig. 4, lanes 7 to 9). In contrast none of the TAF_Is interact with TAF_{II}s [(*16*) and below]. Identical results have been obtained with the use of purified recombinant proteins expressed in *E. coli* (*31*). In summary, our data indicate that each TAF can contact all other components of the SL1 complex. Thus, multivalent TAF-TAF and TBP-TAF interactions are involved in the assembly of a stable SL1 complex.

Mutually exclusive binding of SL1 and TFIID subunits to TBP. Biochemical characterization of the TFIID complex responsible for RNA pol II transcription suggested that the binding of TAF_{II}250 and 150 to TBP plays a crucial role in the assembly process. Here we show that each of the TAF_Is in the SL1 complex interact with TBP. This multiplicity of interactions with one single protein, TBP, raised the question of whether the binding of TAF_Is to form SL1 would prevent the binding of TAF_{II}s to assemble TFIID. To test this possibility, we expressed all of the relevant TAFs either as FLAG, hemagglutinin antigen (HA), or polyoma-myc (PM) (*32*) epitope-tagged molecules with the baculovirus expression system (*33*). First, TBP was bound to immobilized FLAG-tagged TAF_I48, 63, 110, or TAF_{II}150. Unbound TBP was washed away and captured TBP was then tested for its ability to bind TAF_{II}250. The resulting complexes were analyzed by SDS-PAGE and immunoblotting with antibodies to TBP and TAF_{II}250. As has been shown (*16*), TAF_{II}250 can bind to TBP alone, or to TBP in complex with TAF_{II}150 (Fig. 5A, lanes 7 to 9). However, when TBP was

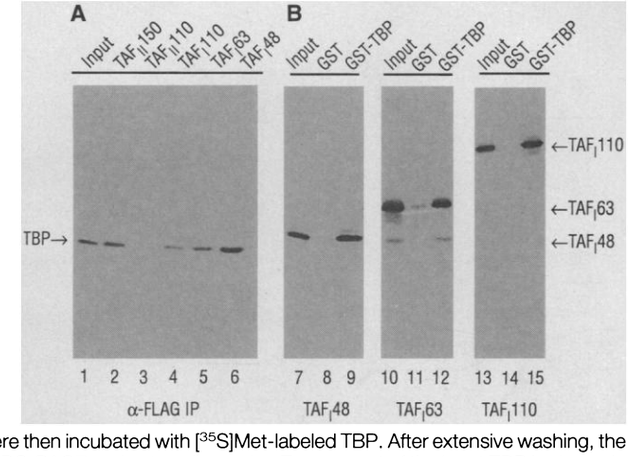

Fig. 3. TBP can bind TAF_I48, TAF_I63, and TAF_I110 individually. (**A**) (Lanes 1 to 6) FLAG epitope-tagged TAF_{II}150, TAF_{II}110, TAF_I110, TAF_I63, and TAF_I48 and (lanes 1 to 6) were immobilized on protein A–Sepharose beads conjugated covalently with monoclonal antibodies directed against the FLAG-epitope. Approximately equal amounts of TAFs were immobilized on the affinity resins as judged by SDS-PAGE and Coomassie blue staining. *Drosophila* TAF_{II}150 and TAF_{II}110 were used in these and the following experiments. The resins were then incubated with [^{35}S]Met-labeled TBP. After extensive washing, the bound TBP was analyzed by SDS-PAGE and autoradiography. Ten percent of the input TBP is shown in lane 1. (**B**) (Lanes 7 to 15) GST and GST-TBP were expressed in *E. coli* and purified on glutathione beads. In vitro–translated [^{35}S]Met-labeled TAF_I48 (lanes 7 to 9), TAF_I63 (lanes 10 to 12), and TAF_I110 (lanes 13 to 15) were incubated with GST and GST-TBP beads. After washing the beads extensively, the resulting protein complexes were resolved by SDS-PAGE and analyzed by autoradiography. Lanes 7, 10, and 13 show 10 percent of input protein.

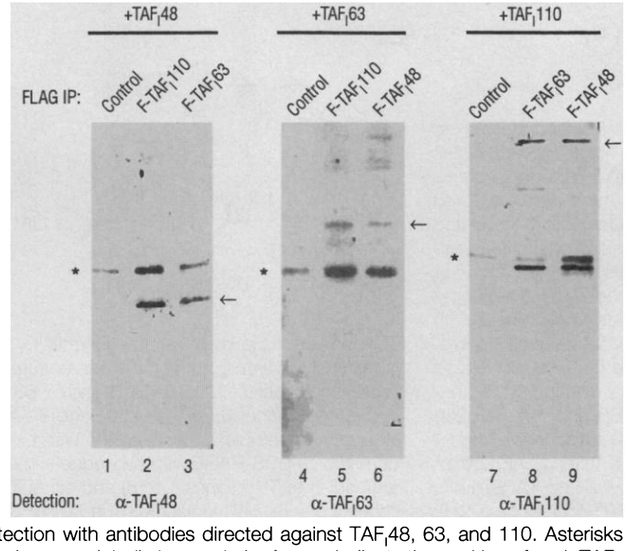

Fig. 4. Interaction of the three TAFs of SL1 with each other. FLAG epitope-tagged TAF_I48, TAF_I63, and TAF_I110 were immobilized on protein A–Sepharose beads coated with antibodies to the FLAG epitope. These resins and control FLAG antibody resins that have been preincubated with uninfected Sf9 extracts, were incubated with Sf9 cell extracts infected with recombinant baculoviruses expressing either TAF_I48 (lanes 1 to 3), 63 (lanes 4 to 6), or 110 (lanes 7 to 9). The beads were washed extensively and the resulting complexes were analyzed by SDS-PAGE with subsequent immunoblotting and detection with antibodies directed against TAF_I48, 63, and 110. Asterisks indicate cross-reactivity with the immunoglobulin heavy chain. Arrows indicate the position of each TAF_I.

associated with any one of the SL1 TAF_Is, we did not detect any binding of $TAF_{II}250$ to TBP (Fig. 5A, lanes 1 to 6). The addition of TAF_I48 to the same TBP-TAF_I63 or TBP-TAF_I110 complexes resulted in the efficient formation of triple complexes (Fig. 5A, lanes 10 to 13). To further establish this apparent mutually exclusive binding specificity of SL1 and TFIID subunits for TBP, we performed reciprocal interaction assays by first forming a TBP-TAF_{II} (150 or 250) complex and then adding the TAF_Is. Consistent with the interpretation of the results presented in Fig. 5A, when either $TAF_{II}150$ or 250 is first bound to TBP, TAF_I48, 63, and 110 did not associate with TBP (Fig. 5B, lanes 1 to 3 and 5 to 7). Triple complexes of TBP-$TAF_{II}250$-$TAF_{II}150$ are efficiently assembled under these conditions (Fig. 5B, lanes 4 and 8).

We also tested whether TAF_I and TAF_{II} would compete for binding to TBP free in solution. First, [^{35}S]Met-labeled TBP was mixed with FLAG-tagged TAF_I48 in the presence of increasing concentrations of $TAF_{II}250$. After several hours of incubation, TBP bound to TAF_I48 was co-immunoprecipitated with antibodies to FLAG and analyzed by SDS-PAGE and followed by autoradiography. Progressively less TBP was bound to TAF_I48 as increasing amounts of $TAF_{II}250$ were added to the reaction mixtures (Fig. 6A). By contrast, TAF_I110 had little effect on the amount of TBP bound to TAF_I48 (Fig. 6A). These data suggest that $TAF_{II}250$ and TAF_I48 compete for binding to TBP in a concentration-dependent manner. In a similar manner, increasing amounts of TAF_I110 led to a decrease in the amount of TBP bound to $TAF_{II}150$ (Fig. 6B). These results establish that at least in vitro, there is a mutually exclusive binding specificity for TBP of SL1 versus TFIID subunits. Thus, the initial binding of a TAF_I or TAF_{II} to TBP may determine the specificity of the subsequent assembly process leading to functional SL1 or TFIID complexes.

Central to understanding the mechanisms controlling transcription initiation in eukaryotic cells is the identification and biochemical characterization of factors mediating recognition and regulation at the various promoters (*34*). Human ribosomal RNA gene transcription is used for for such studies because of its relative simplicity. Biochemical studies indicated that RNA polymerase I and two additional factors, the UBF and the selectivity factor SL1 are minimally required for accurate initiation of

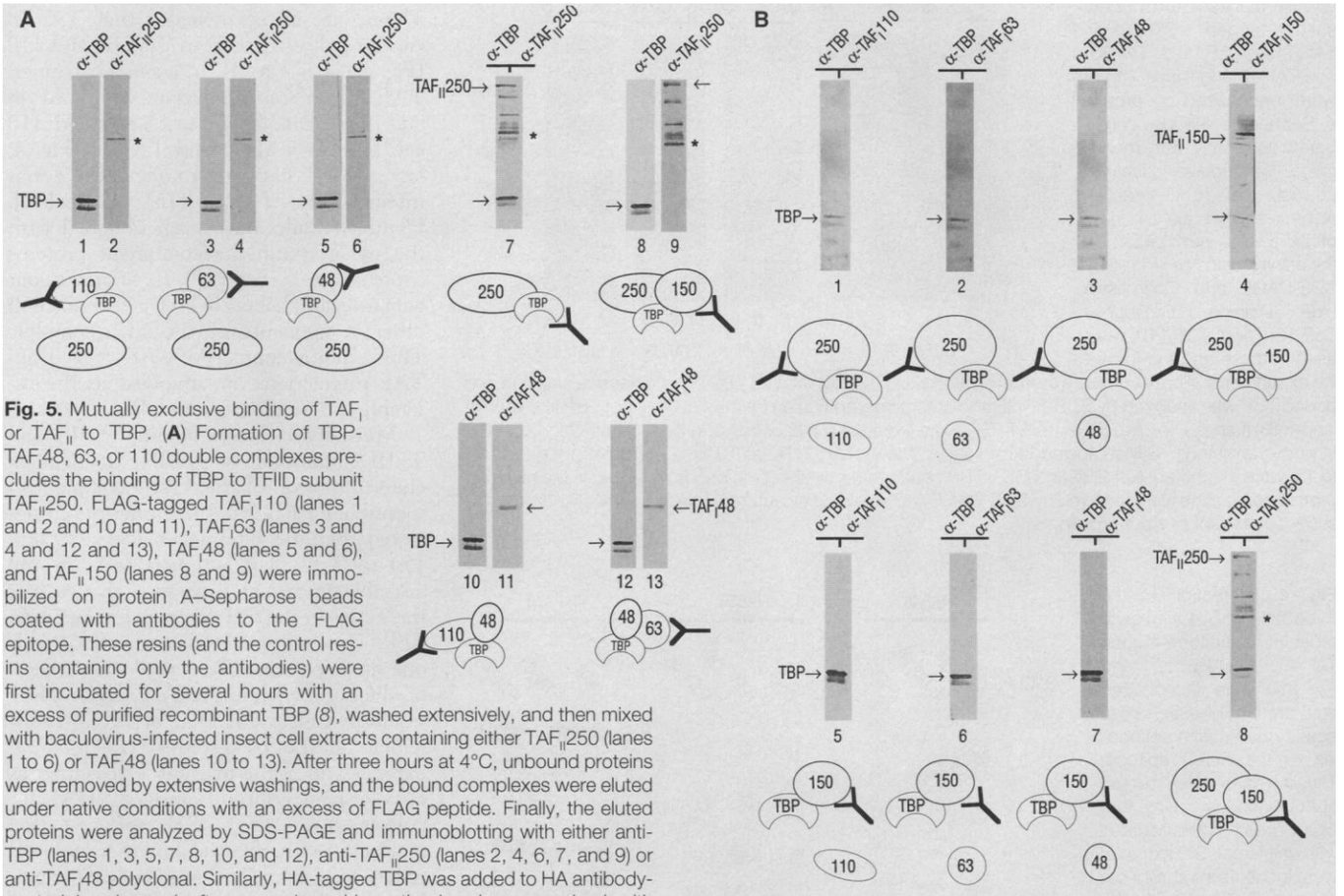

Fig. 5. Mutually exclusive binding of TAF_I or TAF_{II} to TBP. (**A**) Formation of TBP-TAF_I48, 63, or 110 double complexes precludes the binding of TBP to TFIID subunit $TAF_{II}250$. FLAG-tagged TAF_I110 (lanes 1 and 2 and 10 and 11), TAF_I63 (lanes 3 and 4 and 12 and 13), TAF_I48 (lanes 5 and 6), and $TAF_{II}150$ (lanes 8 and 9) were immobilized on protein A–Sepharose beads coated with antibodies to the FLAG epitope. These resins (and the control resins containing only the antibodies) were first incubated for several hours with an excess of purified recombinant TBP (*8*), washed extensively, and then mixed with baculovirus-infected insect cell extracts containing either $TAF_{II}250$ (lanes 1 to 6) or TAF_I48 (lanes 10 to 13). After three hours at 4°C, unbound proteins were removed by extensive washings, and the bound complexes were eluted under native conditions with an excess of FLAG peptide. Finally, the eluted proteins were analyzed by SDS-PAGE and immunoblotting with either anti-TBP (lanes 1, 3, 5, 7, 8, 10, and 12), anti-$TAF_{II}250$ (lanes 2, 4, 6, 7, and 9) or anti-TAF_I48 polyclonal. Similarly, HA-tagged TBP was added to HA antibody-coated beads, and after several washings the beads were mixed with $TAF_{II}250$ containing Sf9 extracts. After incubation at 4°C, the resulting proteins complexes were washed extensively, and bound material was eluted under native conditions with an excess of HA peptide. Products were analyzed by SDS-PAGE and immunoblotting with anti-TBP and anti-$TAF_{II}250$ (lane 7). Asterisks indicate a cross reactive band present also in the control beads with antibody only (*28*). (**B**) Binding of TBP to either $TAF_{II}250$ or $TAF_{II}150$ prevents the subsequent association of any of the TAF_Is. First, HA-tagged $TAF_{II}250$ (lanes 1 to 4) and FLAG-tagged $TAF_{II}150$ (lanes 5 to 8) were immobilized on HA antibodies and FLAG antibodies covalently linked to protein A–agarose beads, respectively. Then, molar excess of recombinant TBP was added and after incubation at 4°C, the unbound TBP was removed by extensive washing. Finally, the beads were incubated with Sf9 cell extracts containing either TAF_I110 (lanes 1 and 5), 63 (lanes 2 and 6), 48 (lanes 3 and 7), $TAF_{II}150$ (lane 4), or $TAF_{II}250$ (lane 8). After the unbound material was washed off, complexes were eluted with excess HA or FLAG peptides and analyzed by SDS-PAGE with subsequent immunoblotting and detection with antibodies to TBP (lanes 1 to 8) and either TAF_I110 (lanes 1 and 5), TAF_I63 (lanes 2 and 6), TAF_I48 (lanes 3 and 7), $TAF_{II}150$ (lane 4) or $TAF_{II}250$ (lane 8). Arrows indicate the position of TBP and TAF_{II}s. Asterisks indicate a cross-reactive band present also in the control beads with antibody only (*28*).

transcription from the human ribosomal RNA promoter (*20, 21*). UBF recognizes and binds DNA in a sequence-specific manner with the use of HMG-box DNA binding motifs, which make contact with both the upstream and core elements of the ribosomal DNA promoter (*21, 22, 24, 25*). The binding of UBF to the template is thought to help recruit the selectivity factor SL1 to the template. However, direct interactions between UBF and the subunits of SL1 have yet to be defined. With the cloning of the SL1 subunits reported here, we can address specific questions concerning mechanisms governing pol I transcription.

We previously showed that there is a cooperative DNA binding interaction between UBF and SL1 that results in an extended deoxyribonuclease I (DNase I) footprint at the ribosomal DNA promoter (*21, 23, 24*). Moreover, ultraviolet (UV) crosslinking experiments with endogenous SL1 suggested that TAF_I48 and TAF_I63 may be in close proximity to the DNA at the promoter (*35, 36*). Consistent with this observation is the finding reported here that TAF_I63 contains two putative Zn fingers which may be involved in binding the ribosomal DNA promoter and that may contribute to the species-specific properties of SL1. Indeed, the interaction of SL1 with a DNA element (SSE) within the ribosomal RNA promoter appears to play a major role in dictating species-specificity of ribosomal DNA transcription (*23*).

Our TBP-TAF_I and TAF_I-TAF_I interactions studies indicate that each TAF_I in the SL1 complex is potentially competent to bind to each of the other TAF_Is (but not TAF_{II}s) and TBP. However, we do not know how the complex is assembled in vivo and whether each subunit enters the complex following an ordered process. For example, the results (Fig. 6) suggest that although TAF_I48 and TAF_I110 can each independently bind TBP, they appear to partially compete for binding when mixed together. Thus, it is possible that some of the interactions that occur in vitro may not be physiologically relevant in the endogenous SL1 complex.

Recently, a set of genes has been identified with a genetic approach that are essential for pol I transcription in *Saccharomyces cerevisiae* (*37*). It was suggested that two of these cloned genes, *RRN6* and *RRN7*, might be putative TAF_Is although their association with TBP is relatively weak and is lost after several chromatographic steps. Sequence comparison between the human TAFs and RRN6/7 do not show any significant similarities. In spite of the lack of structural similarities, there could be functional homologies between human TAF_Is and RRN6/7 which could be demonstrated by the ability to complement the *RRN* yeast mutants with the human TAF_I clones. However, such an approach may not work even if the TAFs and the RRN proteins carry out analogous functions because of the evolutionary distance between these two species. For example, even though there is a high degree of structural similarity between *Drosophila* $TAF_{II}150$ and the *Saccharomyces cerevisiae* gene TSM-1, $dTAF_{II}150$ cannot substitute for TSM-1 in a yeast complementation assay (*16*). Similarly, human and *Drosophila* TBP do not complement yeast TBP mutants in vivo (*38*). Alternatively RRN 6 and 7 proteins may be auxiliary factors that have not yet been characterized in the human system. Indeed, UBF and SL1 most likely represent the minimal set of factors needed in addition to RNA pol I to direct ribosomal RNA transcription in human cells.

Because a subset of the TAFs in TFIID ($TAF_{II}150$ and $TAF_{II}250$), and all three TAFs in the SL1 complex bind strongly to TBP, we expected to find some common structural motifs between them. We were surprised that there were no obvious similarities between the subunits of SL1 and any of the TAFs in the TFIID complex. Our results suggest that RNA pol I and pol II specific TAFs either bind to different domains of TBP or that the binding specificity resides in some subtle structural features of TAFs that cannot be readily discerned by merely inspecting primary amino acid sequences. Studies from several laboratories indicate that various point mutations in TBP differentially affect transcription by RNA pol I and II, suggesting that TAF_{II} and TAF_I interact with distinct sites on the surface of TBP (*3, 39*). Our results indicate that the association of TAF_{II} and TAF_I to TBP is mutually exclusive. Taken together, these two sets of results imply that some kind of steric hindrance or conformational change may prevent TAFs integral to distinct complexes (that is, SL1 and TFIID) from binding simultaneously to TBP, even though these subunits may contact different surfaces on TBP. Consistent with this idea are the observations that TBP in the SL1 complex does not bind to the TATA box and is incompetent to direct basal or activated transcription by RNA polymerase II (*40*). Furthermore, in the cell other factors, like nuclear or nucleolar localization and compartmentalization events, may contribute to the formation of distinct TBP-TAF complexes. The availability of recombinant TAFs should help address some of these questions and allow a more detailed functional dissection of the transcription reaction in eukaryotes.

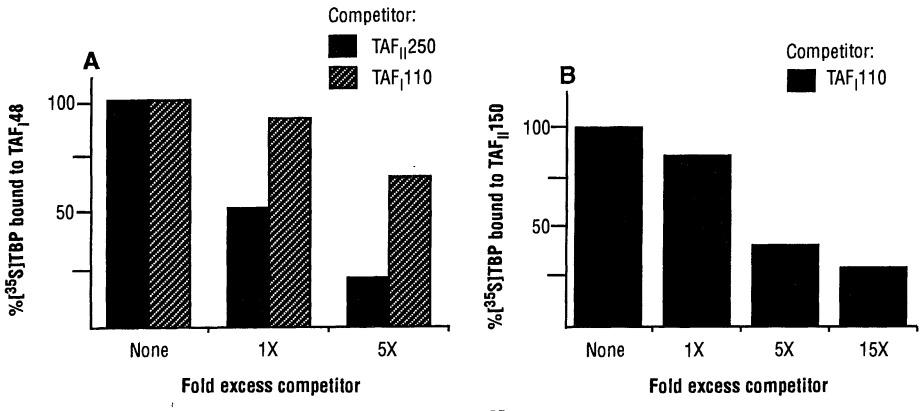

Fig. 6. TAF_I and TAF_{II} compete for TBP in solution. (**A**) [^{35}S]Met-labeled TBP was incubated, in a constant reaction volume, with an Sf9 cell extract containing FLAG-tagged TAF_I48 and increasing amounts of an extract containing the competitors either $TAF_{II}250$ or TAF_I110. Each reaction mixture had the same final protein concentration. After several hours of incubation at 4°C, TAF_I48 was immunoprecipitated from the reaction mixture by antibodies to FLAG covalently linked to protein A–Sepharose, and bound material was eluted under native conditions with FLAG peptide. Eluted TBP was analyzed by SDS-PAGE and quantified with a Phosphoimager (Molecular Dynamics). (**B**) [^{35}S]Met-labeled TBP was incubated with FLAG-tagged $TAF_{II}150$ and increasing amounts of TAF_I110. After several hours of incubation, TBP bound to $TAF_{II}150$ was analyzed by immunoprecipitation with anti-FLAG, elution with FLAG peptide, SDS-PAGE, and subsequently quantified with a Phosphorimager. Amount of ^{35}S-labeled TBP bound, was expressed in percent bound protein relative to binding of TBP to TAF_I48 or $TAF_{II}150$ in the absence of competitor. Concentrations of TAFs in the extracts were estimated by Coomassie staining.

REFERENCES AND NOTES

1. R. H. Reeder, *Regulation of Transcription by RNA Polymerase I*, in *Transcriptional Regulation*, S. McKnight and K. R. Yamamoto, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1992), pp. 315–348.
2. G. Gill and R. Tjian, *Curr. Opin. Genet. Dev.* **2**, 236 (1992).
3. N. Hernandez, *Genes Dev.* **7**, 1291 (1993).
4. L. Zawel and D. Reinberg, *Curr. Opin. Cell Biol.* **4**, 488 (1992).
5. B. F. Pugh and R. Tjian, *Cell* **61**, 1187 (1990).
6. B. D. Dynlacht, T. Hoey, R. Tjian, *ibid.* **66**, 563 (1992).
7. N. Tanese, B. F. Pugh, R. Tjian, *Genes Dev.* **5**, (1992).
8. L. Comai, N. Tanese, R. Tjian, *Cell* **68**, 965 (1992).
9. B. R. Cormack and K. Struhl, *ibid.* **69**, 685 (1992); C.

M. Schultz, R. H. Reeder, S. Hahn, *ibid*., p. 697; S. M. Lobo, M. Tanaka, M. L. Sullivan, N. Hernandez, *ibid*. **71**, 1029 (1992); A. K. P. Taggart, T. S. Fisher, B. F. Pugh, *ibid*., p. 1015; R. J. White and S. P. Jackson, *ibid*., p. 1041.
10. D. Eberhard, L. Tora, J.-M. Egly, I. Grummt, *Nucleic Acids Res.* **21**, 4180 (1993).
11. B. D. Dynlacht, R. O. J. Weinzierl, A. Admon, R. Tjian, *Nature* **363**, 176 (1993).
12. S. Ruppert, E. H. Wang, R. Tjian, *ibid.* **362**, 175 (1993).
13. R. O. J. Weinzierl, B. D. Dynlacht, R. Tjian, *ibid*., p. 511; R. O. J. Weinzierl, S. Ruppert, B. D. Dynlacht, N. Tanese, R. Tjian, *EMBO J.* **12**, 5303 (1993).
14. T. Kokubo *et al*., *Genes Dev.* **7**, 1033 (1993); T. Kokubo *et al*., *Mol. Cell Biol.* **13**, 7859 (1993); T. Kokubo, D.-W. Gong, R. G. Roeder, M. Horikoshi, Y. Nakatani, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 5896 (1993); T. Kokubo, S. Yamashita, M. Horikoshi, R. G. Roeder, Y. Nakatani, *ibid.* **91**, 3520 (1994).
15. K. Hisatake *et al*., *Nature* **362**, 179 (1993).
16. C. P. Verrijzer, K. Yokomori, J.-L. Chen, R. Tjian, *Science* **264**, 933 (1994).
17. T. Hoey *et al*., *Cell* **72**, 247 (1993).
18. J. A. Goodrich, T. Hoey, C. J. Thut, A. Admon, R. Tjian, *ibid.* **75**, 519 (1993).
19. J.-L. Chen, L. D. Attardi, C. P. Verrijzer, K. Yokomori, R. Tjian, *ibid.* **79**, 93 (1994).
20. R. M. Learned, T. K. Learned, M. M. Haltiner, R. Tjian, *ibid.* **45**, 847 (1986); R. M. Learned, S. Cordes, R. Tjian, *Mol. Cell. Biol.* **5**, 1358 (1985).
21. S. P. Bell, R. M. Learned, H.-M. Jantzen, R. Tjian, *Science* **241**, 1192 (1988).
22. S. P. Bell, H.-M. Jantzen, R. Tjian, *Genes Dev.* **4**, 943 (1990).
23. S. P. Bell, C. S. Pikaard, R. H. Reeder, R. Tjian, *Cell* **59**, 489 (1989).
24. H.-M. Jantzen, A. Admon, S. P. Bell, R. Tjian, *Nature* **344**, 830 (1990).
25. H.-M. Janzen, A. M. Chow, D. S. King, R. Tjian, *Genes Dev.* **6**, 1950 (1992).
26. HeLa cells (~500 liters, 5×10^5 cells per milliliter) were harvested by centrifugation, and nuclear extracts were prepared by standard procedure (*3*). SL1 was immunopurified from nuclear extracts (*3*). TAFs were eluted with 1 M guanidine-hydrochloride and precipitated with 100 percent trichloroacetic acid (TCA) containing deoxycholate (4 mg/ml), subjected to SDS-PAGE, and transferred to a polyvinylidene difluoride membrane (Millipore). The membrane was stained with Ponceau S (Sigma), and TAFs bands were excised and digested with Lys-C or trypsin. After elution from the membrane, peptides were separated by reversed-phase chromatography and subjected to automated microsequencing. TAF_I48 was cloned with the use of two partially degenerate oligonucleotides, 5′-CAGTGGCARCARGCTGCT-GARTAYATGTA-3′ (R is an equimolar mix of A and G; Y is an equimolar mix of C and T) corresponding to the peptide sequence NH_2-QWQQAAEYMY-COOH, and 5′-ATTGCTGTSGCYCAIGAYGTST-TCAACCA-3′ (S is an equimolar amount of C and G; I= Inosine) based on the peptide NH_2-NAVAQD-VFNH-COOH. These oligonucleotides were used to screen approximately 1×10^6 recombinant clones from a λgt10 teratocarcinoma cDNA library (provided by A. Wilson and W. Herr, Cold Spring Harbor Laboratory) with standard techniques. Fourteen related cDNA clones were isolated; the inserts of three recombinant phages were subcloned into Bluescript KS (Stratagene) and subjected to dideoxy chain termination sequencing. For the cloning of TAF_I63, first-strand cDNA was generated by reverse transcription of HeLa poly(A)$^+$ RNA. This cDNA pool was used in a touchdown PCR reaction [R. H. Don, P. T. Cox, B. J. Wainwright, K. Baker, J. S. Mattick, *Nucleic Acids Res.* **19**, 4008 (1991)] with fully degenerate oligonucleotides (17 nt) derived from the extreme amino- and carboxy-termini of peptide NH_2-EKPLYYSFVD-KPVAYY-COOH. The specific intrapeptide-amplified fragment was then used to screen the teratocarcinoma cDNA library and four independent cDNA clones were isolated. Further analysis of the four positive cDNA clones was performed as described for TAF_I48. To clone TAF_I110, we used a similar intrapeptide PCR-based strategy with a completely degenerate set of oligonucleotides (20 nt) derived from both ends of the peptide NH_2-KQWQPTLLQAMQVEK-COOH. The amplified fragment was then used to screen a teratocarcinoma cDNA library, and all the subsequent steps in the characterization of TAF_I110 cDNA clones were carried out as described for TAF_I48 with the exception that another screen of a HeLa λZAP cDNA library (*12*) was necessary to obtain full-length cDNA clones. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
27. With a PCR-based strategy, an Nde I site was generated at the initiating methionine codon of the cDNA of TAF_I48 and TAF_I110. With the same strategy, an Nde I site was engineered in front of the partial cDNA clone of TAF_I63 which generates a methionine in front of the open reading frame. The coding regions of TAF_I48, TAF_I63, and TAF_I110 were then subcloned into pET vectors [A. H. Rosenberg *et al*., *Gene* **56**, 125 (1987)] for expression in *E. coli* and into baculovirus expression vectors pVL1392 or an HA epitope-containing version hereof (Pharmingen) (*12*). The experimental methods for expression in *E. coli* and Sf9 cells have been as described (*16, 19*). Proteins produced with these two systems were gel-purified and injected subcutaneously in rabbits to generate antibodies. Approximately 80 μg of proteins were used for each injection.
28. L. Comai and R. Tjian, unpublished results.
29. For co-immunoprecipitations, protein A–Sepharose beads coated with antibody to FLAG protein (Kodak) (about 20 μl of a 50 percent slurry) were incubated with Sf9 insect extracts expressing FLAG-tagged TAFs at 4°C in TM buffer containing 400 mM KCl and 0.1 percent NP-40. Unbound protein was washed away and beads were equilibrated in TM buffer containing 200 mM KCl and 0.1 percent NP-40 before adding [^{35}S]Met-labeled TBP. The subsequent steps were performed as described (*16*).
30. The in vitro transcription–translation vectors for the TAF_Is were constructed by subcloning the coding sequences with the engineered Nde I sites into pTβSTOP vectors (*24*). The [^{35}S]Met-labeled proteins were produced with the TnT-coupled transcription-translation system (Promega). FLAG-tagged TAF_Is were constructed according to a modified version of the pVL1392 baculovirus expression vector that contains a methionine and a FLAG (DYKDDDK) epitope in front of a single Nde I site (*19*). GST-TBP "pull-down" assays were performed as described (*18*), except that all the reactions were carried out in TM buffer [50 mM tris-HCl (pH 7.9), 12.5 mM $MgCl_2$, 10 percent glycerol, 0.5 mM EDTA, 1 mM dithiothreitol] containing 200 mM KCl and 0.1 percent NP-40.
31. H. Beckmann and R. Tjian, unpublished results.
32. NH_2-terminal polyoma middle T antigen and myc epitopes in the baculovirus expression vector pVL1392 were generated by insertion of a double-stranded oligonucleotide, encoding a methionine followed by polyoma (EYMPMEG) and myc (EQKLI-SEEDLN) epitopes.
33. Expression constructs FLAG-tagged $TAF_{II}150$ and HA-tagged $TAF_{II}250$ were described (*12, 16*). Assembly of partial complexes and protein-protein interactions were performed as described (*19*) with the exception that all the reactions were done in TM buffer containing 150 mM KCl and 0.1 percent NP-40.
34. P. J. Mitchell and R. Tjian, *Science* **245**, 371 (1989).
35. S. Bell and R. Tjian, unpublished results.
36. U. Rudloff, D. Eberhard, L. Tora, H. Stunnenberg, I. Grummt, *EMBO J.* **13**, 2611 (1994).
37. D. A. Keys *et al*., *Genes Dev.* **8**, 2349 (1994).
38. G. Gill and R. Tjian, *Cell* **65**, 333 (1991); B. P. Cormack, M. Strubin, A. S. Ponticelli, K. Struhl, *ibid*., p. 341.
39. T. K. Kim and R. G. Roeder, *J. Biol. Chem.* **269**, 4891 (1994).
40. L. Comai, N. Tanese, R. Tjian, unpublished results.
41. We thank K. Goodrich for the automated sequencing; L. Sun for technical support; D. King for help with the HPLC and peptides synthesis; J.-L. Chen, S. Ruppert, R. Weinzierl, and P. Verrijzer for various materials; J.-L. Chen, J. Goodrich, D. Rio, S. Ruppert, E. Wang, and C. P. Verrijzer for helpful discussions and comments on the manuscript. Supported by a Walter Winchell–Damon Runyon Cancer Research Fund Fellowship (DR-1130) (L.C.), by a long-term EMBO fellowship (J.C.B.M.Z.), by a Jane Coffin Child Memorial Fund for Cancer Research (H.B.), and in part by an NIH grant (R.T.).

29 September 1994; accepted 15 November 1994