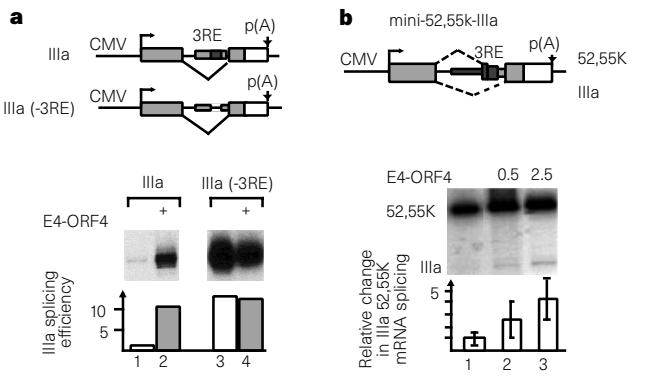

Figure 4 E4-ORF4 regulates L1 splicing in transiently transfected HeLa cells. Conditions for DNA transfection and S1 analysis were as described[19]. **a**, E4-ORF4 activation of IIIa splicing is dependent on 3RE. IIIa mRNA levels in HeLa cells transfected with pAdCMV-IIIa or pAdCMV-IIIa (–3RE) and empty vector (lanes 1 and 3) or pCMV–E4-ORF4 (lanes 2 and 4). Top, schematic representation of the transcription units. Bottom, quantification of IIIa splicing (results from two experiments, arbitrary units). **b**, E4-ORF4 induces a shift in L1 alternative RNA splicing. 52,55K and IIIa mRNA levels in HeLa cells transfected with pAdCMV-mini-52,55K-IIIa and empty vector (lane 1) or increasing amounts of pCMV-E4-ORF4 (lanes 2 and 3). Top, schematic representation of the transcription unit and its alternative splicing pathways. Bottom, quantification of the effect of E4-ORF4 on the ratio of IIIa/52,55K mRNA splicing (arbitrary units), with the results without E4-ORF4 set as one. Error bars indicate standard deviation, from seven experiments.

Taken together, our results suggest that the adenovirus E4-ORF4 protein functions as a virus-encoded alternative RNA splicing factor, regulating L1 IIIa 3′ splice-site usage both *in vitro* and *in vivo* by modulating the activity of SR proteins. Previous results suggest that the change in L1 3′ splice-site usage results from decreased 52,55K splicing combined with an increased IIIa splicing[16,17]. Notably, E4-ORF4 activates IIIa splicing *in vivo* without having much effect on 52,55K splicing (Fig. 4b). Thus E4-ORF4 alone is unable to account for the complete shift in L1 alternative splicing[7].

We have shown that reversible SR protein phosphorylation may be an important mechanism controlling alternative RNA splicing in mammalian cells. Thus it is possible that alternative RNA splicing might be regulated by common pathways used in signal transduction, and so it might be important in cell-cycle control, differentiation and development. □

Methods

DNA. Plasmid maps and sequences are available on the World-Wide Web at www.bmc.uu.se/IMIM/res/GA.html.

Complex assembly. Briefly, 0.5 μg of SR-HeLa were incubated with or without 4.5 pmol of His–E4-ORF4 in 15 μl HeLa-NE in buffer D for 15 min at 30 °C. Subsequently, ATP (2 mM), $MgCl_2$ (2.5 mM), creatine phosphate (20 mM), and substrate RNA[8] (15 fmol) were added (final concentrations), and the reaction was incubated at 30 °C for 15 min. Heparin (2.5 μg) was added and complexes separated on a 4.25% polyacrylamide/glycerol gel[16]. His–E4-ORF4 was purified from *Escherichia coli* as described by the manufacturer (Novagen), except that pH 8.2 buffers were used.

Metabolic labelling of cells. Equal numbers (1.4×10^9 cells) of uninfected or adenovirus-infected HeLa cells (16 h post-infection) were resuspended (10^7 cells ml^{-1}) in phosphate-free spinner medium supplemented with 2.5% newborn calf serum. Cells were labelled with 2.5 mCi of [^{32}P]orthophosphate (Amersham) for 12 h. SR proteins were purified by double-salt precipitation[9].

Two-dimensional gel electrophoresis. *In vivo* ^{32}P-labelled SR-HeLa or SR-Ad proteins (20 μg) were separated, on Immobiline strips with a linear pH gradient 3–10 (Pharmacia), and on a 12% SDS–polyacrylamide gel, followed by autoradiography.

SR protein dephosphorylation. Reactions (25 μl) contained 42 mM imidazole-HCl, pH 7.2, 0.5 mM EGTA, 0.25% (v/v) 2-mercaptoethanol, 2.5 mg ml^{-1} BSA; ^{32}P-labelled SR-HeLa (2,000–3,000 c.p.m.) and combinations of 0.4% HeLa-NE, 0.27 μg His–E4-ORF4 and 20 nM okadaic acid. Samples were incubated at 30 °C for the indicated time and phosphate release was measured by binding to ammonium molybdate[18].

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Function of TAF_{II}-containing complex without TBP in transcription by RNA polymerase II

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Initiation of transcription of a gene from a core promoter region by RNA polymerase II requires the assembly of several initiation factors to form a preinitiation complex. Assembly of this complex[1,2] is thought to be nucleated exclusively by the sequence-specific binding of the TFIID transcription factor complex, which is composed of the TATA-binding protein (TBP) and TBP-associated factors (TAF_{II}s) (refs 3, 4), to the different promoters.

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Here we isolate and characterize a new multiprotein complex that does not contain either TBP or a TBP-like factor but is composed of several TAF_{II}s and other proteins. This complex can replace TFIID on both TATA-containing and TATA-lacking promoters in *in vitro* transcription assays. Moreover, an anti-TBP antibody that inhibits TBP- and TFIID-dependent transcription does not inhibit activity of this new complex. These results indicate that TBP-free RNA polymerase II mediated transcription may be able to occur in mammalian cells and that multiple preinitiation complexes may play an important role in regulating gene expression.

In human cells, distinct TFIID complexes exist[5–7]. They have been divided into two different subpopulations on the basis of the absence (in TFIIDα) or presence (in TFIIDβ) of a specific TAF_{II}, $hTAF_{II}30$ (refs 6, 8). To characterize TFIIDβ complexes, we developed a two-step immunopurification procedure using antibodies raised against human (h) $TAF_{II}30$ and TBP (Fig. 1a). We immunopurified complexes containing $hTAF_{II}30$ from the flow-through fraction of a single-stranded-DNA column (Fig. 1a) with an anti-$hTAF_{II}30$ monoclonal antibody (1H8). Bound complexes were eluted with an excess of the corresponding epitope peptide (producing eluate 1; Fig. 1a, b). The anti-$hTAF_{II}30$ antibody immune-depleted all $hTAF_{II}30$ from the input fraction (Fig. 1b). In agreement with earlier results[6,8], this immunoprecipitation divided the endogenous HeLa cell TFIID complexes into two subpopulations, TFIIDα and TFIIDβ, as the total cellular amount of two typical $hTAF_{II}$s, $hTAF_{II}100$ and $hTAF_{II}55$, was divided approximately in half (Fig. 1b, lanes 1, 2).

To ensure that the eluted $hTAF_{II}30$-containing complexes (eluate 1) contained only TFIIDβ, the E_1 fractions were again immunoprecipitated with an anti-TBP monoclonal antibody (2C1; ref. 6) and eluted with the corresponding peptide (producing eluate 2 or TFIIDβ; Fig. 1c). Although TBP was completely depleted from the input fraction by two rounds of anti-TBP immunoprecipitations (see the lack of TBP in supernatants 2 and 3 in Fig. 1a, c), surprisingly a portion of $hTAF_{II}30$ and several other TAF_{II}s, such as $hTAF_{II}135$, $hTAF_{II}100$, $hTAF_{II}80$, $hTAF_{II}55$ and $hTAF_{II}20$, remained in the supernatant fraction, as detected by immunoblotting (see supernatant 3; Fig. 1c). To test whether the lack of TBP in supernatant 3 was a result of partial proteolysis of hTBP, we checked for the presence of fragments of TBP in supernatant 3 by using six different anti-TBP antibodies (3G3, 1C2, 2C1, 4C2, 1D8, 1H10). These monoclonal antibodies recognize different epitopes either in the amino-terminal 180 amino acids of hTBP[6,9] or in the carboxy-terminal 20 amino acids of TBP. None of these anti-TBP antibodies recognized TBP in the supernatant 3 fraction (Fig. 1c, e; data not shown). These data indicate that there is probably no TBP in this fraction.

Two lines of evidence suggest that supernatant 3 contains a multiprotein complex and is not simply a product of TFIIDβ dissociation. First, all the proteins present in supernatant 3 must be associated directly or indirectly with $hTAF_{II}30$, as they have been specifically immunopurified with an anti-$hTAF_{II}30$ antibody; and second, not only TAF_{II}s but several other protein species, not seen normally in TFIID complexes, were also specifically present in this TFIIDβ-free supernatant (Fig. 1d). We called this new multiprotein complex TBP-free TAF_{II}-containing complex (TFTC).

We further purified the different proteins present in TFTC by gel-filtration chromatography. Analysis of the fractions by either western blotting or silver staining (data not shown) indicated that TFTC elutes as a single peak with an apparent native relative molecular mass of between 600K and 1,300K (data not shown), indicating that TFTC is probably a multiprotein complex.

Many TAF_{II}s, including $hTAF_{II}20$, $hTAF_{II}30$, $hTAF_{II}55$, $hTAF_{II}80$, $hTAF_{II}100$ and $hTAF_{II}135$, are apparently common to both TFTC and TFIIDβ. In contrast, $hTAF_{II}250$ and $hTAF_{II}28$, which strongly interact with TBP, were absent from the TFTC fraction (Fig. 1c). Moreover, recombinant TAF_{II}s ($hTAF_{II}18$, $hTAF_{II}20$, $hTAF_{II}31$, $hTAF_{II}80$, $hTAF_{II}100$ and $hTAF_{II}135$) can assemble into a stable complex, containing stoichiometric amounts of each of these TAF_{II}s. Thus, TBP, $hTAF_{II}250$ and $hTAF_{II}28$ are not required for TFTC assembly (data not shown). The TAF_{II}s in TFTC are also associated with a number of non-TAF protein species with apparent relative molecular masses of 47K, 50K, 85K, 120K, 145K, 165K, 190K and 290K (Fig. 1d). Western blot analysis showed that none of these polypeptides correspond to human proteins that can replace

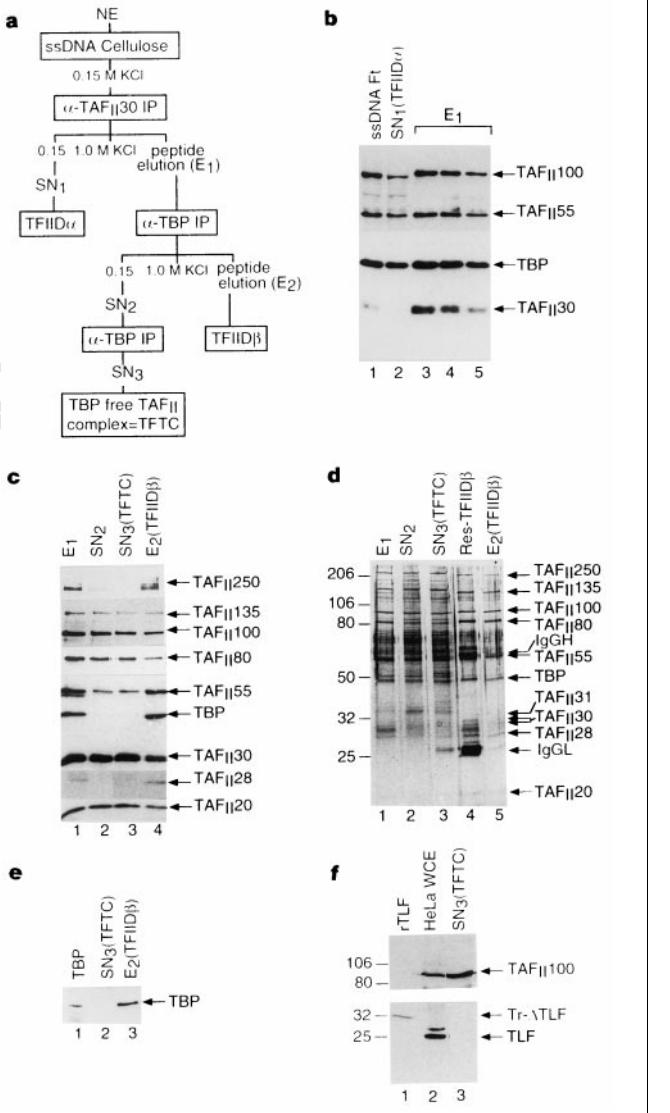

Figure 1 Purification of TFTC. **a**, Chromatography protocol used to purify TFTC. NE, nuclear extract; ss, single-stranded; α-, anti-; IP, immunoprecipitation; E, eluate; SN, supernatant. **b**, Immunoprecipitation of $hTAF_{II}30$-containing complexes. The flow-through fraction of the ssDNA cellulose column (ssDNA Ft) was subjected to immunoprecipitation with an anti-$hTAF_{II}30$ monoclonal antibody and bound complexes were eluted. We analysed input, eluate (E_1) and supernatant fractions by immunoblotting. **c**, E_1 fractions were re-immunoprecipitated twice with the anti-TBP monoclonal antibody (see **a**) and TFIIDβ was eluted (E_2). Fractions were analysed by immunoblotting with the different anti-TAF_{II} antibodies. **d**, Fractions from the double immunoprecipitation and the resin-bound TFIIDβ complex (Res-TFIIDβ) were analysed by silver staining. **e**, An anti-TBP monoclonal antibody raised against the 20 last amino acids of hTBP does not recognize TBP in TFTC. **f**, Lower panel, antibodies raised against hTLF recognize 1 ng thioredoxin-hTLF (lane 1) and endogenous hTLF in 50 μg HeLa cell extract (lane 2) but do not reveal hTLF in 5 μg TFTC; upper panel, immunoblot with an anti-$TAF_{II}100$ monoclonal antibody. WCE, whole-cell extract.

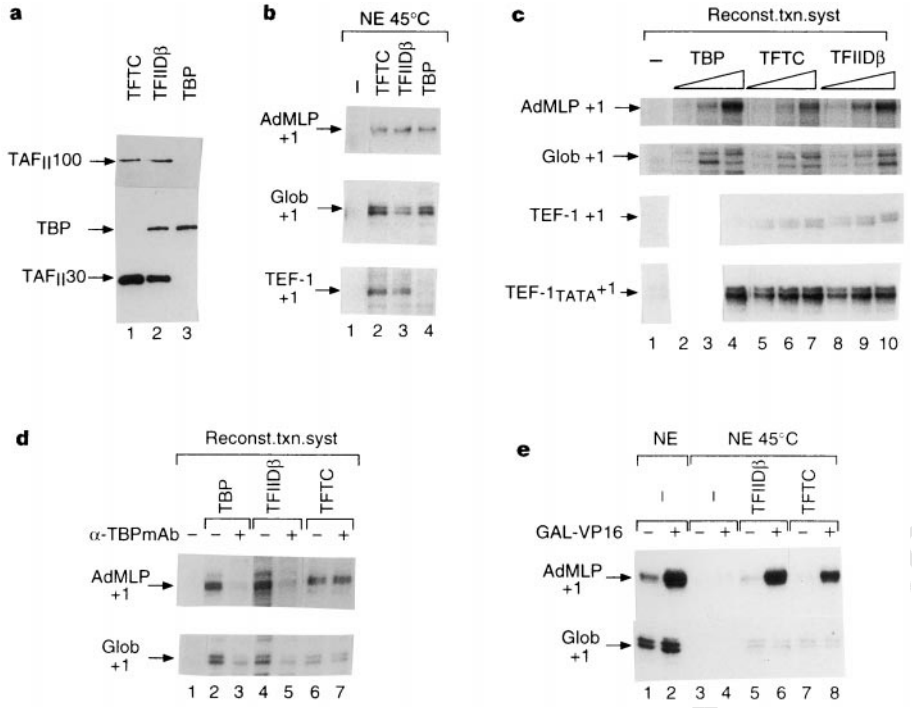

Figure 2 TFTC functions in basal and activated Pol II mediated transcription. **a**, Normalization of the different fractions by immunoblotting. **b–e**, Gel photographs show products of transcription from the relevant promoters. **b**, TFTC, TFIIDβ or TBP was preincubated with templates containing AdMLP, the β-globin promoter (Glob) or the TATA-less TEF-1 promoter before the addition of the heat-treated nuclear extract (NE 45 °C). **c**, Increasing amounts of TBP, TFTC or TFIIDβ were preincubated with the different templates before the addition of the highly purified basal factors (reconstituted transcription system, Reconst. txn. syst.). TEF-1_{TATA}, TEF-1 gene promoter with an added TATA box. **d**, Buffer or the anti-TBP monoclonal antibody (α-TBPmAb) was added to TBP, TFIID or TFTC before the addition of the template DNAs and the missing factors. **e**, TFTC or TFIIDβ was preincubated with the (17M)5/AdMLP and the β-globin templates in the presence or absence of the activator GAL-VP16 before addition of the heat-treated nuclear extract (NE 45 °C).

TFIID or TBP in basal RNA polymerase II (Pol II) mediated transcription, such as YY1 (ref. 10) or TAF170 (refs 11, 12) (data not shown). To determine whether TFTC contains a TBP-like factor(s), like the *Drosophila* TRF–nTAF complex[13], we identified a novel human TBP-like factor (hTLF; see Methods) and generated antibodies against it (Methods). hTLF has significant homology to the core domains of both hTBP (64% similarity) and *Drosophila* TRF[14] (60% similarity). Anti-hTLF antibodies recognize endogenous hTLF in HeLa cell extracts and recombinant TLF, but we found no hTLF in the immunopurified TFTC (Fig. 1f). Thus, TFTC does not contain any known TBP-like factor.

We then determined whether TFTC can function in Pol II mediated transcription. To ensure that comparable amounts of TFTC, TFIIDβ and TBP were used in the transcription reactions, we normalized these fractions by immunoblotting (Fig. 2a). TFTC, TFIIDβ or TBP was first preincubated with promoter-containing DNA templates, two of which contained both a TATA box and an initiator element (the adenovirus major late promoter (AdMLP) and the rabbit β-globin gene promoters[5,6]) and one of which was a TATA-less promoter that contained only an initiator element (the transcription-enhancer factor-1 (TEF-1) gene promoter[15]). A heat-inactivated nuclear extract[16] was then added to the reactions for 30 min at 25 °C and transcription started. Surprisingly, TFTC was as efficient in supporting transcription initiation from TATA-box-containing promoters as TFIIDβ or recombinant (r) TBP (Fig. 2b). Moreover, TFTC and TFIIDβ supported transcription initiation not only on TATA-containing promoters but also on the TATA-lacking promoter (Fig. 2b). In agreement with results showing that TAF_{II}s are required for transcription from TATA-less promoters[17], rTBP did not allow transcription from the TATA-less TEF-1 promoter (Fig. 2b). TFTC eluted from the gel-filtration column at between 600K and 1,300K also supported transcription initiation in this system (data not shown). These results indicate that TFTC can function in Pol II mediated transcription. Furthermore, heat treatment of the HeLa nuclear extracts seems to inactivate the TFTC activity (Fig. 2b, lane 1).

We then tested the TFTC activity in a highly purified, TBP/TFIID-free, transcription system (Fig. 2c) in which the general Pol II transcription factors were either purified recombinant proteins

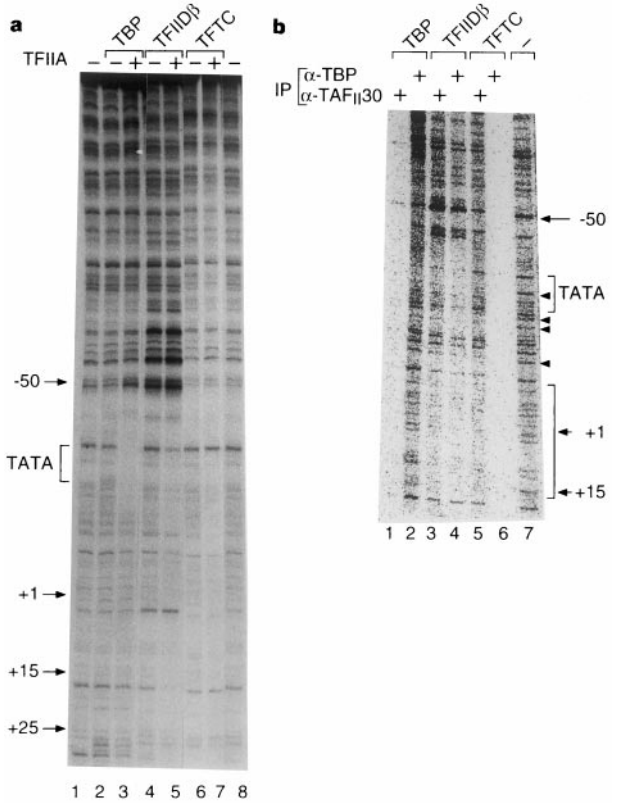

Figure 3 TFTC recognizes the AdMLP. **a**, Complexes were formed on the AdMLP probe in the absence or presence of purified endogenous TFIIA[26]. Under these conditions, TFIIA alone did not bind to the AdMLP (data not shown). **b**, TBP, TFIIDβ or TFTC was incubated with either the anti-TBP (α-TBP) monoclonal antibody (3G3) or the anti-$hTAF_{II}30$ (α-$TAF_{II}30$ monoclonal antibody) (2F4) and the different complexes were allowed to bind to the AdMLP probe. Complexes were immobilized, washed and briefly digested with DNase I (ref. 18). Control digestions were performed with the probe alone (–). Arrowheads and the vertical square brackets indicate regions protected by TFTC and numbers are relative to the transcription start site. IP, immunoprecipitation.

(TFIIB, TFIIEα, TFIIEβ and TFIIF) or extensively purified factors from HeLa cell nuclear extracts (TFIIA, TFIIH and Pol II). Increasing amounts of TFTC, TFIIDβ and TBP were tested for their ability to support basal transcription in this system. TFTC can initiate transcription on the AdMLP and the β-globin promoter in a similar manner to TFIIDβ and TBP, but with three times lower efficiency (Fig. 2c). In this system, transcription initiation from the TATA-less TEF-1 gene promoter in the presence of TFTC was weak but as efficient as with TFIIDβ (Fig. 2c). Furthermore, when we introduced a TATA box into the TEF-1 gene promoter (TEF-1_{TATA}; ref. 15), both TFTC and TFIIDβ allowed more efficient transcription initiation than from the TATA-less promoter (Fig. 2c). Thus, TFTC can support transcription initiation in this TBP-free transcription system from both TATA-containing and TATA-less promoters.

To eliminate the possibility that trace amounts of TFIID or TBP contaminated the purified transcription factors and/or TFTC, we included an anti-TBP monoclonal antibody (1C2) in the transcription reactions. This antibody inhibits TBP- and TFIID-dependent Pol II transcription from TATA-containing promoters *in vitro*[9]. It also inhibited TBP- and TFIID-dependent transcription from both the AdMLP and the globin promoter in the highly purified transcription system (Fig. 2d). In contrast, it did not inhibit TFTC-dependent transcription (Fig. 2d). Thus TFTC is a new transcription factor that functions in a TBP-independent manner in transcription initiation.

Next we examined whether TFTC can mediate transcriptional activation. We first preincubated TFTC and TFIIDβ with the AdMLP template containing five binding sites for the transcriptional activator GAL4 (and the rabbit β-globin promoter as a control) in the presence or absence of GAL-VP16. We then added a heat-inactivated nuclear extract to the reactions for 30 min at 25 °C and transcription was started. Under these conditions, TFTC was as efficient in mediating transcriptional activation by GAL-VP16 as TFIIDβ (Fig. 2e).

By using DNase I footprinting, we tested where TFTC, TFIID and TBP contact the AdMLP. DNA–protein complexes were formed and cleaved by DNase I (Fig. 3a). Under the conditions used for *in vitro* transcription, there was no protection of the TATA-box region in the absence of endogenous TFIIA by TBP, TFIIDβ or TFTC (Fig. 3a, lanes 2, 4, 6); however, there were several hypersensitive sites seen with TFIIDβ alone (Fig. 3a, lane 4). Binding of TBP and TFIIDβ to the AdMLP was stimulated by TFIIA (Fig. 3a, lanes 3, 5). A weak extended protection, from −27 to roughly +15, was also observed when both TFTC and TFIIA were incubated with the probe (Fig. 3a; compare lanes 6, 7). To determine whether TFTC alone can bind to the promoter, we used the DNase I footprinting method with immobilized proteins[18]. Under these conditions, TFTC on its own bound specifically to the AdMLP (Fig. 3a, lane 5) and resulted in a protection similar to that obtained when its binding was stimulated by TFIIA. The antibodies did not immunoprecipitate protein-bound promoter fragments in reactions in which their corresponding epitopes were missing (Fig. 3b, lanes 1, 6), indicating that the binding of TFTC to the promoter was specific. In both cases, the protection and the hypersensitive-site patterns obtained with TFTC were different from those obtained with TFIIDβ. Thus, TFTC can bind to the AdML promoter and its binding is different from that of TFIID.

So far, TBP-independent Pol II transcription has only been reported with either the YY1 transcription factor[10] or the *Drosophila* TBP-related factor[13]. Our results indicate that TBP-independent transcription may be more common than originally thought and may change our understanding of the regulation of eukaryotic gene expression. As TFTC recognizes sequences around the initiator element on the AdMLP, the TAF_{II}s present in TFTC may be important in promoter recognition. $TAF_{II}250$, $TAF_{II}135$, $TAF_{II}100$, $TAF_{II}55$ and $TAF_{II}31$ (or $TAF_{II}30$) are involved in the interactions between TFIID and the promoter DNA downstream of the AdMLP TATA box[19]. Thus, these TAF_{II}s, but not $TAF_{II}250$, may have a similar role in the TFTC. The functional data obtained with TFTC further demonstrate that TAF_{II}s are vital not only in recognizing TATA-containing and TATA-less promoters but also in recruiting the other general transcription factors into the preinitiation complex[20–22]. Moreover, basal factors seem to stabilize the binding of TFTC to the promoter. Alternatively, the unidentified protein species in TFTC may also contribute to site-specific binding of TFTC to the promoter and in formation of the preinitiation complex. As there is no TBP, $TAF_{II}250$ or TLF in TFTC, the binding of TFTC to the AdMLP did not induce any hypersensitive sites or strong protections around the TATA box, but resulted in a protection around the initiator region of the promoter. Our results indicate that TBP-free Pol II mediated transcription can occur in eukaryotic cells and that multiple preinitiation complexes, assembled with different TFIIDs or TFTC(s), are important in regulation of gene expression. □

Methods

Immunization and monoclonal antibody production. To generate the anti-$hTAF_{II}30$ antibody (1H8) that allowed the preparation of TFIID*β* by peptide elution, we injected mice with 100 μg of the synthetic peptide encompassing the first 20 amino acids of $hTAF_{II}30$, coupled to ovalbumin. These injections were performed intraperitoneally three times at 2-week intervals. Spleen cells were fused with Sp2/0.Ag 14 myeloma cells and, at day 10 culture, we tested supernatants on endogenous $hTAF_{II}30$ by western blot analysis. Supernatants were also tested with HeLa cell nuclear extracts for immunoprecipitation and subsequent elution of the antigen with the epitope peptide.

The different anti-TBP monoclonal antibodies, the anti-$TAF_{II}30$ antibody (2F4), the anti-$TAF_{II}20$ antibody (22TA), the anti-$TAF_{II}28$ antibody (15TA), the anti-$TAF_{II}55$ antibody (19TA), the anti-$TAF_{II}100$ antibody (2D2) and the anti-$TAF_{II}135$ antibody (20TA) were described in refs 5, 6, 8, 9, 20, 23. The anti-$TAF_{II}250$ antibody antibody was from Santa Cruz Biotechnology, and the anti-$TAF_{II}80$ polyclonal antibody was from Transduction Laboratories.

Identification of hTLF and antibody production. A computer scan of the available expressed-sequence-tag (EST) database revealed 11 human gene products that encode different portions of the same protein, which has significant homology to the C-terminal domains of both hTBP and dTRF. This protein has been called human TBP-like factor as it is 64% similar and 41% identical to the hTBP core and 60% similar and 38% identical to the dTRF core[14] (sequence alignment can be obtained upon request). One of the EST complementary DNA clones (accession number AA412574) was obtained from the IMAGE Consortium[24] and inserted in the *Hin*dIII–*Not*I sites of the pET32b vector, in frame with the thioredoxin protein, and the recombinant fusion protein (rTr-ΔTLF) was overexpressed in *Escherichia coli*. Next the synthetic peptide EIRLPEFTKNNRPHYASYEPELH, from amino acids 114–135 of hTLF, was coupled to ovalbumin. Mice were injected intraperitoneally, three times at 2-week intervals, with 100 μg synthetic peptide. The polyclonal mouse sera were tested on endogenous hTLF, on hTLF-transfected COS cell extracts and on *E. coli*-expressed rTr-ΔTLF by western blot analysis.

Immunoprecipitation. Monoclonal antibodies for immunoprecipitation were purified for ascites fluids using caprylic acid precipitation followed by precipitation with 50% ammonium sulphate, and were dialysed. Immunoprecipitation was performed as described[5]. HeLa cell nuclear extract was first passed through a single-stranded-DNA cellulose column to eliminate proteins that bind non-specifically to the resin used later in immunoprecipitations. Fractions were immunoprecipitated with monoclonal antibodies bound to protein G–Sepharose (Pharmacia), as indicated in the figures. The complexes bound to protein G–Sepharose/antibody were washed three times with IP buffer (25 mM Tris-HCl, pH 7.9, 10% (v/v) glycerol, 0.1% NP40, 0.5 mM dithiothreitol and 5 mM $MgCl_2$) containing 0.5 M KCl and two times with IP buffer containing 100 mM KCl. After washing, bound proteins were eluted with a 200–1,0000-fold excess of the corresponding epitope peptide and analysed by SDS–PAGE. The gels were either silver-stained or transferred to nitrocellulose membrane and probed with the indicated antibodies.

***In vitro* transcription.** *In vitro* transcription reactions were assembled using heat-treated HeLa cell nuclear extract (45 °C for 15 min) or the highly purified

general Pol II transcription factors[7,20,25,26]. All protein fractions were dialysed against buffer B (25 mM Tris-HCl, pH 7.9, 50 mM KCl, 0.5 mM dithiothreitol, 0.1 mM EDTA and 20% glycerol (v/v)). 25-μl reactions contained either 25 ng 17M/5pAL7 and pG1 (ref. 5) or 100 ng pTEF(Δ-138) or pTEF(Δ-138_{TATA})[15], with aliquots of TFTC, TFIIDβ and recombinant TBP[5]. Where indicated, 200 ng purified anti-TBP monoclonal antibody 1C2 was also included in the reactions before the other factors were added. GAL-VP16-activated transcription was performed as described[5]. After the preincubation steps (30 min), transcription was initiated by addition of nucleoside triphosphates to 0.5 mM and $MgCl_2$ to 5 mM. Transcriptions were incubated at 25 °C for 45 min. Correctly initiated transcripts from the different promoters were analysed by quantitative S1 nuclease analysis[15,27].

DNase I footprinting. DNase I footprinting was performed as described[18,19]. The labelled AdMLP-containing probes were amplified by polymerase chain reaction on either the 17M5/pAL7 (ref. 28) (Fig. 3a) or the pM677 (ref. 29) (Fig. 3b) templates. For the footprinting experiments, ten times more TBP, TFIIDβ, and TFTC was used than in the transcription reactions.

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corrections

Structure of the αβ tubulin dimer by electron crystallography

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Nature **391,** 199–203 (1998)

In this Letter, the numbers for the secondary structure elements involved in Taxol binding are incorrect (page 202, second-to-last paragraph of main text). The sentences giving the correct numbers are, "In our model, the C-3′ is near the top of helix H1 (that is, between β:15–25), and the C2 group near H6 and the H6–H7 loop (that is, between β:212–222). The main interaction of the taxane ring is at L275, at the beginning of the B7–H9 loop." □

Spatial and temporal organization during cardiac fibrillation

Richard A. Gray, Arkady M. Pertsov & José Jalife

Nature **392,** 75–78 (1998)

The *x*-axis of Fig. 1d was mislabelled: the frequency values should instead read 0, 10, 20, 30, 40 Hz. □