THE JOURNAL OF BIOLOGICAL CHEMISTRY
© 2001 by The American Society for Biochemistry and Molecular Biology, Inc.
Vol. 276, No. 27, Issue of July 6, pp. 25582–25588, 2001
Printed in U.S.A.

c-Jun Binds the N Terminus of Human $TAF_{II}250$ to Derepress RNA Polymerase II Transcription *in Vitro**

Received for publication, January 11, 2001, and in revised form, April 18, 2001
Published, JBC Papers in Press, April 20, 2001, DOI 10.1074/jbc.M100278200

Tricia N. Lively‡, Heather A. Ferguson, Shelly K. Galasinski, Anita G. Seto, and James A. Goodrich§

From the Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado 80309-0215

c-Jun is an oncoprotein that activates transcription of many genes involved in cell growth and proliferation. We studied the mechanism of transcriptional activation by human c-Jun in a human RNA polymerase II transcription system composed of highly purified recombinant and native transcription factors. Transcriptional activation by c-Jun depends on the TATA-binding protein (TBP)-associated factor (TAF) subunits of transcription factor IID (TFIID). Protein-protein interaction assays revealed that c-Jun binds with high specificity to the largest subunit of human TFIID, $TAF_{II}250$. The region of $TAF_{II}250$ bound by c-Jun lies in the N-terminal 163 amino acids. This same region of $TAF_{II}250$ binds to TBP and represses its interaction with TATA boxes, thereby decreasing DNA binding by TFIID. We hypothesized that c-Jun is capable of derepressing the effect of the $TAF_{II}250$ N terminus on TFIID-driven transcription. In support of this hypothesis, we found that c-Jun increased levels of TFIID-driven transcription *in vitro* when added at high concentrations to a DNA template lacking activator protein 1 (AP-1) sites. Moreover, c-Jun blocked the repression of TBP DNA binding caused by the N terminus of $TAF_{II}250$. In addition to revealing a mechanism by which c-Jun activates transcription, our studies provide the first evidence that an activator can bind directly to the N terminus of $TAF_{II}250$ to derepress RNA polymerase II transcription *in vitro*.

Regulation of mRNA synthesis in eukaryotes is a complex process involving gene specific activators, coactivators, and general transcription factors. Understanding how this multitude of factors sets levels of transcription of individual genes is the goal of much research. Emerging evidence indicates that transcriptional activators have the potential to stimulate transcription via many different mechanisms. For example, activators can exert their effects by binding components of the transcription machinery, including subunits of the TFIID[1] and mediator complexes (for review, see Refs. 1 and 2) as well as by recruiting chromatin modifying complexes (for review, see Ref. 3).

Whereas unique combinations of activators and coactivators function at different promoters, RNA polymerase II and the general transcription factors (TFIIA, -B, -D, -E, -F, and -H) are thought to function at most mRNA-encoding genes (for review, see Ref. 4). The TFIID complex plays a central role in core promoter recognition and the assembly of preinitiation complexes regardless of the manner by which the remaining basal transcriptional components are recruited, either via a stepwise pathway or as a holoenzyme (5). TFIID is composed of the TATA-binding protein (TBP) and multiple TBP-associated factors (TAFs) whose sizes range from 18 to 250 kDa (for review, see Ref. 6). Subunits of TFIID contact core promoter elements including the TATA box, initiator, and downstream promoter element (7–10).

At some promoters, the recruitment of TFIID limits the overall level of transcription *in vitro*. Efficient recruitment of TFIID can be achieved by upstream activators that interact with TFIID components (11–13). In addition, activators can elicit a conformational change in the TFIID-TFIIA-promoter complex, as observed by DNase I footprinting (14, 15). Although direct interactions between TBP and activators have been noted, the primary functional targets of activators within TFIID are the TAF subunits. TAFs were coined coactivators in recognition of their requirement for activated transcription in some *in vitro* systems (16, 17). Recently, the ability of TAFs to adopt multifunctional roles as components of distinct complexes including TFIID, Spt-Ada-Gen5-acetyltransferase (SAGA), p300/CBP-associated factor (PCAF), and TBP-free TAF_{II} complex (TFTC) has been demonstrated (for review, see Ref. 1). The validity of the coactivator function of TAFs has been firmly established from examples in yeast, *Drosophila*, and human systems.

Although TAFs can function as coactivators, regulated transcription of some promoters is not dependent on TAFs *in vitro* and *in vivo* (18–22). The mediator and other complexes are also targets of transcriptional activators and serve to coactivate transcription (for review, see Ref. 2). In the context of chromatin, nucleosome-remodeling complexes and histone acetyltransferases such as cAMP-response element-binding protein (CREB)-binding protein (CBP) function to coactivate transcription (3). It is likely that activators can stimulate transcription by many different mechanisms depending upon the conditions and the context of the promoter at which the activator binds.

One mechanism by which activators can function is by re-

* This research was supported by National Institutes of Health Public Health Service Grant GM-55235, a Leukemia Society of America Special Fellowship, and a Scholarship from the Pew Charitable Trusts (to J. A. G.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Supported in part by a National Institutes of Health Predoctoral Training Grant T32 GM07135 and a Graduate Student Fellowship from the Colorado Institute for Research in Biotechnology, which is state-funded by the Colorado Advanced Technology Institute.

§ To whom correspondence should be addressed: Dept. of Chemistry and Biochemistry, University of Colorado, Campus Box 215, Boulder, CO 80309-0215. Tel.: 303-492-3273; Fax: 303-492-5894; E-mail: james.goodrich@colorado.edu.

[1] The abbreviations used are: TFII-, transcription factor for RNA polymerase II; TBP, TATA-binding protein; TAF, TBP-associated factor; AP-1, activator protein 1; VP16, herpesvirus protein 16; GST, glutathione *S*-transferase; PAGE, polyacrylamide gel electrophoresis; AdMLP, adenovirus major late promoter; y-, yeast; d-, *Drosophila*; DTT, dithiothreitol; PAGE, polyacrylamide gel electrophoresis; HA, hemagglutinin.

This paper is available on line at http://www.jbc.org

lieving the effects of factors that repress basal transcription. Although this is certainly true of activators in the context of chromatin, mechanisms of transcriptional repression and derepression have also been observed in the absence of chromatin. For example, the binding of TFIID to core promoters is internally repressed by the largest subunit of TFIID, $TAF_{II}250$ (23). Most of the work on the mechanism of this repression has been carried out with $dTAF_{II}230$ and $yTAF_{II}145$, the *Drosophila* and yeast homologs of human $TAF_{II}250$. Repression is mediated by a small domain (~150 amino acids) in the N termini of these proteins that binds directly to TBP and inhibits its ability to bind TATA boxes (23, 24). Furthermore, the N-terminal domain of $dTAF_{II}230$ can repress TBP-driven transcription when added in excess of TBP in an *in vitro* transcription system (23). Mutational analysis identified the concave DNA binding surface of TBP as a binding site of the N terminus of $dTAF_{II}230$ (25). In NMR studies, amino acids 11–77 of $dTAF_{II}230$ appeared to mimic DNA by binding to the DNA binding cleft of TBP (26). A second region of $dTAF_{II}230$ (amino acids 118–143) interacts with the convex surface of TBP and further stabilizes the $dTAF_{II}230$-TBP interaction (27). The importance of $TAF_{II}250$ repression to transcriptional regulation in cells and the potential for targeting the N terminus of $TAF_{II}250$ as a means to derepress transcription are currently under study. TFIIA, which aids TFIID in binding core promoters, can prevent $TAF_{II}250$ from inhibiting TBP-DNA binding *in vitro* (28). In addition, $yTAF_{II}145$ and yTFIIA bind competitively to TBP (24). The acidic activation domain of VP16 also binds the underside of TBP and can block the interaction of $dTAF_{II}230$ with TBP (25, 29). Activators have not, however, been shown to bind directly to the N terminus of $TAF_{II}250$ to remove its repressive effects on TBP-DNA binding.

AP-1 transcription factors of the Jun and Fos families activate many genes including those involved in cell growth and proliferation (for review, see Ref. 30). AP-1 proteins each contain an extended α-helical dimerization/DNA binding domain of ~62 amino acids that consists of a leucine zipper found C-terminal of a basic region (31). Dimers bind DNA through sequence-specific contacts between amino acids in the basic regions and the major groove of the DNA helix (32). Members of the Jun family can form homo- and heterodimers. Members of the Fos family cannot form homo- or heterodimers among themselves but can heterodimerize with Jun proteins. Although there is high sequence homology in the basic leucine zipper domains of AP-1 proteins, there is much less homology in other regions, which contain activation domains. The activation domains of c-Jun and c-Fos, which are essential for transcriptional activation *in vitro*, have been characterized functionally (33–35) but not structurally and are known to bind other proteins such as the coactivator CBP (36). Despite a wealth of studies, the mechanisms by which c-Jun and c-Fos stimulate transcription are not well understood.

Here we have investigated the mechanism by which human c-Jun activates transcription using a highly purified human RNA polymerase II transcription system. Activation of transcription by recombinant c-Jun was found to be dependent on TAFs. Interactions between c-Jun and TAFs were identified and characterized using protein-protein affinity chromatography, which revealed that c-Jun binds to the N terminus of human $TAF_{II}250$. The function of c-Jun in derepressing transcription driven by TFIID and the ability of c-Jun to block $TAF_{II}250$ repression of TBP transcription and DNA binding were characterized *in vitro*. Our results reveal a unique interaction between c-Jun and $TAF_{II}250$ that results in derepression of TFIID-driven transcription *in vitro*. These studies lead to a novel model for activation in which c-Jun interacts with $TAF_{II}250$ to relieve repression of TFIID-DNA binding, thereby contributing to transcriptional activation.

EXPERIMENTAL PROCEDURES

Plasmids—pET-Jun, a plasmid for expressing full-length human c-Jun, was a gift from T. Hoey (Tularik, Inc.). In creating this plasmid an *Nco*I site was generated that changed the fourth base pair in the c-Jun-coding region from AT to GC, resulting in a point mutation in the second amino acid of c-Jun (Thr to Ala). pGEX-Jun was created by digesting pET-Jun with *Nco*I and *Eco*RV and subcloning the c-Jun cDNA into the *Nco*I and *Stu*I sites of pGEX-2TKN (a kind gift of S. Ruppert and R. Tjian).

Plasmids for expressing TAFs (pTβ-hTAF250, pTβ-dTAF40, pTβ-dTAF30α, and pTβ-dTAF30β) were gifts from R. Tjian. HA-$hTAF_{II}250$ was expressed in SF9 cells as previously described (37). pTβ-hTAF250-(1–560) and pTβ-hTAF250-(1–163) were created by digestion of pTβ-hTAF250 with *Eco*RI and *Sma*I, respectively, followed by gel purification of the linearized plasmid and ligation. pGEX-hTAF250-(1–163) was created by digestion of pTβ-hTAF250-(1–163) with *Nde*I and *Eco*RI and insertion of the DNA fragment into pGEX-2TKN.

Plasmid $p(AP\text{-}1)_5$-E1b-CAT was created by inserting five direct copies of a double-stranded oligonucleotide containing the AP-1 element from the human metallothionein IIA promoter (−105 to −96) into the *Xba*I site of plasmid E1b-CAT (38). Plasmid $p(AP\text{-}1)_5$-E1b-G-less, used as template DNA in the *in vitro* transcription assays, was created by inserting a 377-base pair G-less cassette (excised from $p(GAL4)_5$-E1b-G-less (a kind gift from M. G. Peterson and R. Tjian) with *Sac*I) into the *Sac*I site of $p(AP\text{-}1)_5$-E1b-CAT. Plasmid $p(GAL4)_5$-AdMLP-G-less (39), which contains five GAL4 sites upstream of the adenovirus major late core promoter (AdMLP; −53 to +10) and a 380-base pair G-less cassette, was generated by subcloning a *Hin*dIII-*Eco*RI fragment isolated from plasmid G5-MLP-G-less (a kind gift from Mike Carey) into the *Hin*dIII and *Eco*RI site of pBluescript-KS(+).

Expression and Purification of Recombinant c-Jun and GST Fusion Proteins—Recombinant full-length human c-Jun was expressed in *Escherichia coli* BL21:DE3. Cultures (500 ml) were grown in LB containing 0.3 mM ampicillin at 37 °C until the $A_{600\ nm}$ was 0.4. Expression was induced with 0.5 mM isopropyl-1-thio-β-D-galactopyranoside for 2 h at 37 °C. Cells were harvested and resuspended in 10 ml of a solution containing 20 mM Tris (pH 7.9), 1 mM EDTA, 100 mM NaCl, 1 mM DTT, 0.2 mM phenylmethylsulfonyl fluoride, and sonicated 4 times for 15 s. Samples were centrifuged for 30 min in a Sorval SS34 rotor at 18,000 rpm and 4 °C. Precipitated material (inclusion bodies) containing c-Jun was resuspended in 10 ml of 5 mM DTT and sonicated 2 times for 30 s. Samples were centrifuged for 10 min in a Sorval SS34 rotor at 15,000 rpm and 4 °C. Insoluble material was washed three more times by resuspending in 10 ml of 5 mM DTT, followed by centrifugation. Pellets from the final wash were resuspended in 5 ml of buffer A (20 mM Tris (pH 7.9), 1 mM EDTA, 5 mM DTT, and 7 M urea) containing 0.1 M NaCl. Soluble protein was loaded on an 8-ml column packed with sulfopropyl-Sepharose fast flow (Amersham Pharmacia Biotech) and washed with three column volumes of buffer A containing 0.1 M NaCl followed by three column volumes of buffer A containing 0.2 M NaCl. c-Jun was then eluted with buffer A containing 0.3 M NaCl and subjected to three sequential dialyses in buffer B (20 mM Tris (pH 7.9), 0.1 mM EDTA, 10% glycerol, 5 mM DTT) containing the following additions: 1) 1 M urea and 1 M NaCl; 2) 1 M NaCl; 3) 0.1 M NaCl. After dialysis, the purified c-Jun was separated into aliquots and stored at −80 °C.

GST-Jun was expressed in *E. coli* strain XA90. Cultures were grown in LB containing 0.3 mM ampicillin at 37 °C until the $A_{600\ nm}$ was 0.4. Expression was induced with 0.5 mM isopropyl-1-thio-β-D-galactopyranoside for 3 h at 37 °C. Cells were harvested and resuspended in a solution containing 20 mM Tris (pH 7.9), 20% glycerol, 1 mM EDTA, 5 mM $MgCl_2$, 0.1 M NaCl, 5 mM DTT, 0.2 mM phenylmethylsulfonyl fluoride and sonicated 4 times for 15 s. Insoluble material was washed with 5 mM DTT as described for c-Jun above. Pellets from the final wash were resuspended in a solution containing 20 mM Tris (pH 7.9), 1 mM EDTA, 5 mM DTT, and 6 M guanidine-HCl, and protein was extracted by overnight nutation at 4 °C. After centrifugation to remove the remaining insoluble material, extracts were diluted to give final concentrations of 200 ng/μl of GST-Jun. Samples were subjected to three sequential dialyses in buffer B containing 0.1 M NaCl and guanidine-HCl at 2, 1, and 0.5 M. The guanidine-HCl was then slowly lowered to 50 mM by pumping (over 10 h) buffer B containing 0.1 M NaCl into the third dialysis listed above. After dialysis, samples containing GST-Jun were separated into aliquots and stored at −80 °C. Just before use in protein-protein interaction assays, GST-Jun was further purified by incubation

with glutathione-Sepharose for 1 h at 4 °C followed by two washes with 10 column volumes of buffer B containing 1 M NaCl and 0.1% Nonidet P-40 and three washes with the appropriate protein-protein interaction assay buffer (see below and figure legends). The affinity resin contained ~1 mg of protein/ml of beads.

GST-TAF_{II}250-(1–163) and GST were expressed in *E. coli* strain XA90 as described for GST-Jun above. Harvested cells were resuspended in 1/50 culture volume of lysis buffer containing 20 mM Tris (pH 7.9), 20% glycerol, 1 mM EDTA, 0.1 M NaCl, 1 mM DTT, and 0.2 mM phenylmethylsulfonyl fluoride. Samples were sonicated 4 times for 15 s and centrifuged for 30 min in a Sorval SS34 rotor at 18,000 rpm and 4 °C. Supernatants from the centrifugation were incubated with glutathione-Sepharose at 4 °C for 1 h with constant nutation. Resin was washed two times with 10 column volumes of buffer B containing 1 M NaCl and 0.1% Nonidet P-40. For use in protein-protein interaction assays, the resin was washed three times with ten column volumes of the appropriate protein-protein interaction assay buffer (see below and figure legends). For elution, resin was washed five times with 10 column volumes of buffer C (100 mM Tris (pH 7.9), 120 mM NaCl). Protein was eluted by incubating resin at 4 °C for 10 min in 1 column volume of buffer C containing 20 mM glutathione (reduced) with constant nutation. Eluates were dialyzed overnight at 4 °C in a buffer containing 20 mM Tris (pH 7.9), 10% glycerol, 1 mM EDTA, 0.1 M NaCl, and 1 mM DTT. Samples were separated into aliquots and stored at −80 °C.

Protein-Protein Interaction Assays—Each reaction contained 1 μg of GST-Jun or GST immobilized on 10 μl of glutathione-Sepharose beads (Amersham Pharmacia Biotech). Immobilized proteins and extracts containing target proteins were incubated with micrococcal nuclease at 30 °C for 10 min. Target proteins were then incubated with GST or GST-Jun beads at 4 °C for 2 h with constant nutation in TGEMD buffer (20 mM Tris (pH 7.9), 10% glycerol, 1 mM EDTA, 5 mM $MgCl_2$, and 1 mM DTT) containing 0.1% Nonidet P-40, 0.2 mM phenylmethylsulfonyl fluoride, and 0.1 M NaCl. After incubation, beads were washed at least 3 times with 10–15 bead volumes of buffer. Protein was eluted from the beads in SDS sample buffer and resolved by SDS-PAGE.

In Vitro Transcription—General transcription factors (TBP, TFII-B, -E, -F, and -H) and RNA polymerase II were expressed and purified as previously described (40). Human TFIID was immunopurified (39). Recombinant human TFIIA was prepared as previously described (41, 42). The amounts of general factors and buffer conditions used for transcription were as previously described (39). The amounts of the TFIID (10 ng) and TBP (0.5 ng) used in transcription reactions contained equal moles of TBP, as determined by quantitative Western analysis with polyclonal antibody directed against TBP (39).

To study transcriptional activation (see Fig. 1*B*) c-Jun was incubated with p(AP-1)$_5$-E1b-G-less for 5 min on ice. After the addition of general transcription factors, reactions (20 μl) were transferred to 30 °C for 25 min before adding nucleoside triphosphates. RNA synthesis was allowed to proceed for 20 min at 30 °C. To study derepression (see Fig. 4), c-Jun was incubated with TBP or TFIID in 13-μl reactions for 10 min on ice. p(GAL4)$_5$-AdMLP-G-less template DNA and the remaining general transcription factors where then added to reactions in a final volume of 20 μl.

To study transcriptional repression (see Fig. 5*A*) GST-TAF_{II}250-(1–163) and GST were added to transcription reactions containing p(GAL4)$_5$-AdMLP-G-less template DNA before the addition of TBP, TFIIB, TFIIF, and RNA polymerase II. In Fig. 5*B*, c-Jun was incubated with GST-TAF_{II}250-(1–163) (*lanes 3* and *4*) in 3 μl for 13 min on ice. p(GAL4)$_5$-AdMLP-G-less template DNA was then added, followed immediately by the addition of the general transcription factors to a final volume of 20 μl.

DNase I Footprinting—DNase I footprinting reactions were performed with a 183-base pair DNA fragment containing the AdMLP (−53 to +33) ^{32}P-labeled on the 5′-end of the nontemplate strand. The buffer conditions were identical to those used for transcription (before the addition of NTPs). When present, GST-TAF_{II}250-(1–163) and c-Jun were incubated together in a reaction volume of 2 μl for 10 min on ice. These mixtures were then added to reactions containing promoter DNA followed immediately by the addition of TBP at a final concentration of 6 nM in a final volume of 20 μl. After 10 min at 30 °C, 1 μl of a solution

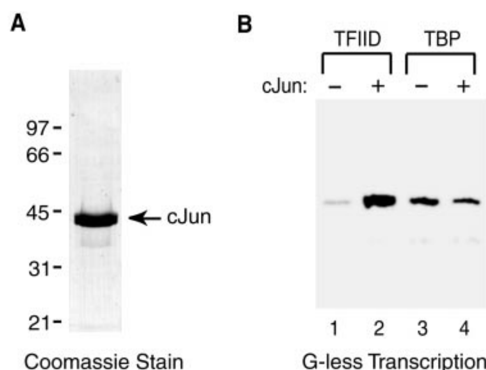

FIG. 1. **The TAF subunits of TFIID are required for activation of transcription by c-Jun in a RNA polymerase II transcription system reconstituted from highly purified transcription factors.** *A*, purified recombinant human c-Jun (2 μg) was subjected to SDS-PAGE and visualized by staining with Coomassie Brilliant Blue. *B*, c-Jun activates transcription in a highly purified *in vitro* transcription system containing TFIID but not TBP. Reactions in *lanes 1* and *2* contained 10 ng of immunopurified TFIID. Reactions in *lanes 3* and *4* contained 0.5 ng of recombinant TBP. 30 ng of recombinant c-Jun was added to the reactions in *lanes 2* and *4*. The DNA template was p(AP-1)$_5$-E1b-G-less, which contained five AP-1 sites upstream of the adenovirus E1b TATA box and a 377-base pair G-less cassette.

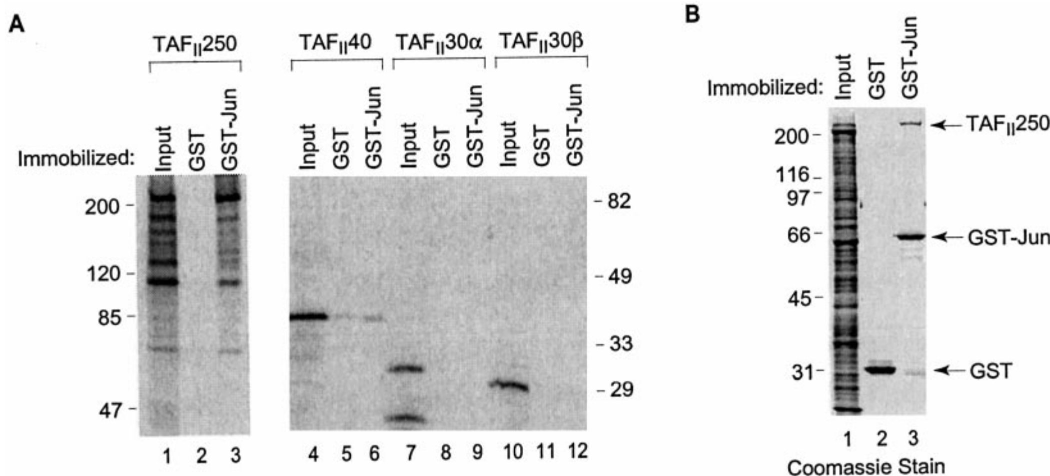

FIG. 2. **c-Jun binds TAF_{II}250 with high specificity.** *A*, protein-protein affinity chromatography was used to identify TAFs that interact with GST-Jun. ^{35}S-Labeled human TAF_{II}250 (*lanes 1–3*), *Drosophila* TAF_{II}40 (*lanes 4–6*), *Drosophila* $TAF_{II}30\alpha$ (*lanes 7–9*), and *Drosophila* $TAF_{II}30\beta$ (*lanes 10–12*) were expressed by *in vitro* translation and tested for interaction with immobilized GST-Jun or GST. 10% of the input ^{35}S-labeled proteins were included in *lanes 1*, *4*, *7*, and *10*. *B*, recombinant human TAF_{II}250 was specifically purified from cell lysates using GST-Jun affinity resin. HA-TAF_{II}250 was expressed in insect cells using a recombinant baculovirus. Portions of cell lysate containing TAF_{II}250 were incubated with GST-Jun and control GST resins. After extensive washing, protein bound to the affinity resins was resolved by SDS-PAGE and stained with Coomassie Brilliant Blue. An aliquot of insect cell lysate equal to 100% of the input was included in *lane 1*.

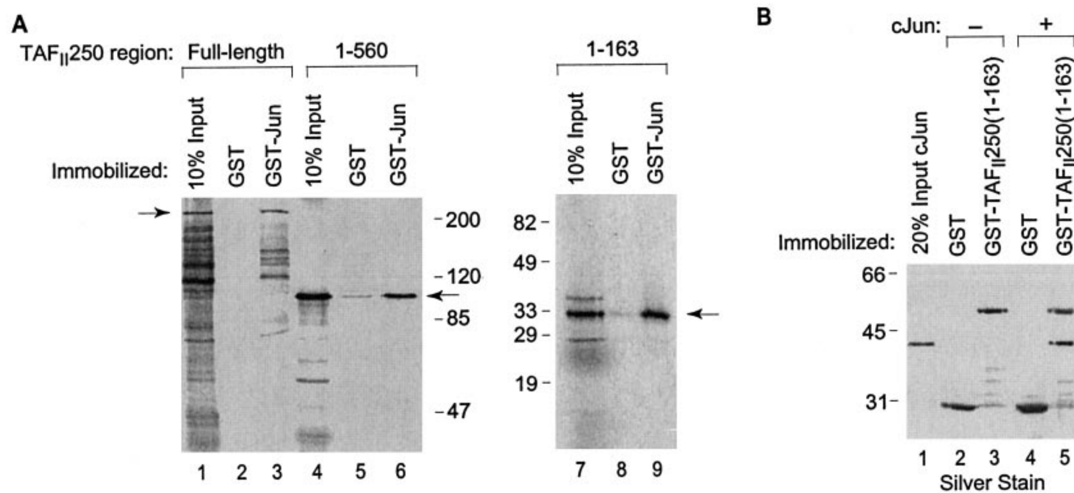

FIG. 3. **c-Jun binds the N-terminal 163 amino acids of $TAF_{II}250$.** *A*, ^{35}S-Labeled full-length $TAF_{II}250$ and two C-terminal deletions were expressed by *in vitro* translation and tested for interaction with immobilized GST-Jun and GST. Bound protein was analyzed by SDS-PAGE and autoradiography. *B*, immobilized GST-$TAF_{II}250$-(1–163) binds purified c-Jun. Immobilized GST-$TAF_{II}250$-(1–163) and control GST were incubated with c-Jun. Bound protein was analyzed by SDS-PAGE and silver staining (*lanes 4* and *5*). Purified GST and GST-$TAF_{II}250$-(1–163) are shown in *lanes 2* and *3*, respectively.

containing 0.15 units/μl of DNase I (Promega) and 10 mM $CaCl_2$ was added to each reaction. Reactions were stopped, and DNA products were analyzed as described (39).

RESULTS

Activation of Transcription by Human c-Jun in a Highly Purified in Vitro System Requires TAFs—To study the mechanism of c-Jun transcriptional activation, we developed a human RNA polymerase II transcription system reconstituted from highly purified recombinant and native general transcription factors. This system is useful for studying the roles of individual subunits of the general RNA polymerase II transcription machinery, including the TFIID TAFs, in basal and activated transcription. Recombinant human c-Jun was expressed in *E. coli* and purified to near homogeneity (Fig. 1*A*). To test the activity of c-Jun for activating transcription *in vitro* we created a DNA template, p(AP-1)$_5$-E1b-G-less, containing five AP-1 elements upstream of the adenovirus E1b TATA box and a 377-base pair G-less cassette. We detected basal transcription from this template *in vitro* (Fig. 1*B*) in reactions reconstituted with equal molar concentrations of either immunopurified native TFIID (*lane 1*) or recombinant TBP (*lane 3*). Interestingly, reactions reconstituted with TBP reproducibly showed higher levels of basal transcription than those containing TFIID even though equivalent molar concentrations of TBP and TFIID were used (39). This is likely to be due to activities present in the TFIID complex that inhibit its binding to DNA, such as the N terminus of $TAF_{II}250$ (23). c-Jun (30 ng) activated transcription ~10-fold in reactions reconstituted with TFIID (Fig. 1*B*, compare *lanes 1* and *2*) but did not stimulate transcription in reactions reconstituted with TBP (Fig. 1*B*, compare *lanes 3* and *4*). Thus, in a highly purified *in vitro* transcription system, TAFs are required for transcriptional activation by human c-Jun.

c-Jun Binds $TAF_{II}250$ with High Specificity—The finding that TAFs were required to mediate transcriptional activation by c-Jun in our *in vitro* transcription system led us to hypothesize that c-Jun interacted with one or more of the TAFs. To identify interactions between c-Jun and TAFs, we performed protein-protein affinity chromatography utilizing immobilized GST-Jun and *in vitro* translated ^{35}S-labeled TAFs. TAFs were incubated with GST-Jun or control GST resin, and after extensive washing, proteins bound to the resins were analyzed by SDS-PAGE and autoradiography. These studies revealed that $TAF_{II}250$ interacted with immobilized GST-Jun but not control GST (Fig. 2*A*, *lanes 1–3*). Other TAFs, for example $dTAF_{II}40$, $dTAF_{II}30\alpha$, and $dTAF_{II}30\beta$, did not interact with GST-Jun (Fig. 2*A*, *lanes 4–12*).

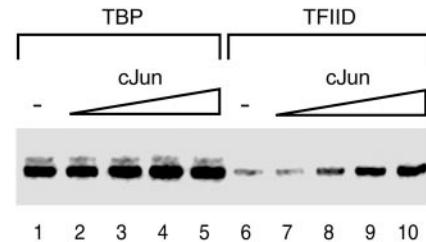

FIG. 4. **c-Jun derepresses TFIID-directed transcription from a template that lacks AP-1 sites.** Recombinant c-Jun was incubated with TBP (*lanes 2–5*) or TFIID (*lanes 7–10*) before adding p(GAL4)$_5$-AdMLP-G-less (consisting of the adenovirus major late core promoter upstream of a 380-base pair G-less cassette) and the remaining general transcription factors. The amounts of c-Jun added to the transcription reactions were 30 ng (*lanes 2* and *7*), 200 ng (*lanes 3* and *8*), 400 ng (*lanes 4* and *9*), and 700 ng (*lanes 5* and *10*).

To further characterize the affinity and specificity of the c-Jun-$TAF_{II}250$ interaction, we utilized extracts of insect cells containing HA-tagged $TAF_{II}250$ that was expressed from a recombinant baculovirus. Insect cell extracts were incubated with immobilized GST-Jun and control GST. After extensive washing, bound protein was resolved by SDS-PAGE and visualized by staining with Coomassie Brilliant Blue. As shown in Fig. 2*B*, the GST-Jun resin specifically purified recombinant HA-$TAF_{II}250$ from the crude insect cell extract. The numerous and abundant insect cell proteins in the extract were not retained on the GST-Jun resin.

c-Jun Binds the N-terminal Region of $TAF_{II}250$ That Is Involved in Transcriptional Repression—To gain insight into the role of the c-Jun-$TAF_{II}250$ interaction in transcriptional activation, we delimited the region of $TAF_{II}250$ that is bound by c-Jun. Two C-terminal deletions of $TAF_{II}250$ were expressed by *in vitro* translation and tested for interaction with immobilized GST-Jun: $TAF_{II}250$-(1–560) and $TAF_{II}250$-(1–163). As shown in Fig. 3*A*, $TAF_{II}250$-(1–560) and $TAF_{II}250$-(1–163) each bound GST-Jun at a level comparable with that observed for full-length $TAF_{II}250$ (amino acids 1–1893).

As an additional test of the ability of the N-terminal 163 amino acids of $TAF_{II}250$ to bind c-Jun, we expressed and purified a GST-$TAF_{II}250$-(1–163) fusion protein and incubated

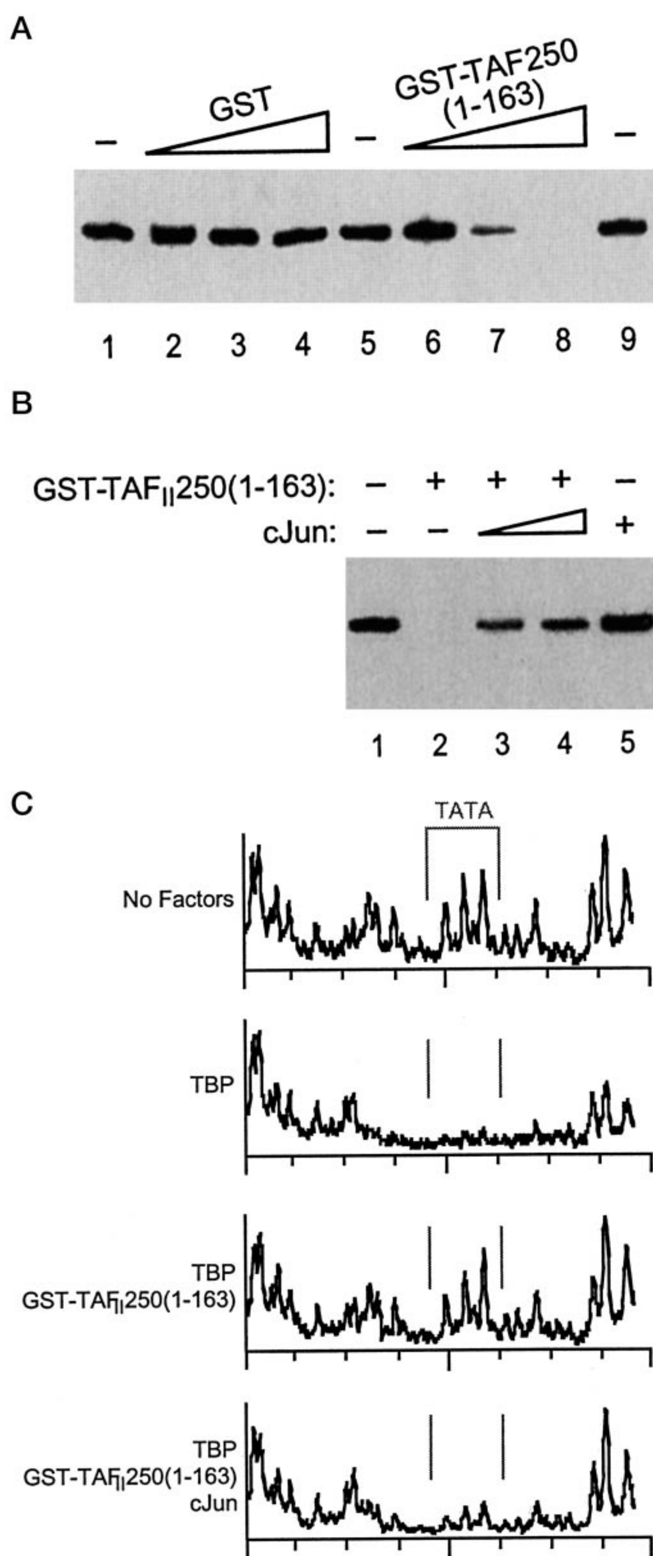

FIG. 5. **c-Jun blocks the repression of basal transcription and TBP-DNA binding caused by the N terminus of TAF$_{II}$250.** *A*, GST-TAF$_{II}$250-(1–163) specifically represses basal transcription *in vitro*. GST-TAF$_{II}$250-(1–163) and control GST were added to transcription reactions before the addition of the transcription factors TBP, TFIIB, TFIIF, and RNA polymerase II. The final concentrations of GST (*lanes 2–4*) and GST-TAF$_{II}$250-(1–163) (*lanes 6–8*) in reactions before the addition of nucleoside triphosphates were 10 nM (*lanes 2* and *6*), 50 nM (*lanes 3* and *7*), and 100 nM (*lanes 4* and *8*). *B*, c-Jun blocks transcriptional repression by GST-TAF$_{II}$250-(1–163). GST-TAF$_{II}$250-(1–163) was incubated alone (*lane 2*) or with c-Jun (*lanes 3* and *4*) before being added to reactions containing p(GAL4)$_5$-AdMLP-G-less, followed immediately by the addition of the general transcription factors. The amounts of c-Jun added to transcription reactions were 100 ng (*lane 3*) and 200 ng (*lanes 4* and *5*). *C*, c-Jun blocks the ability of GST-TAF$_{II}$250-(1–163) to repress DNA binding by TBP. GST-TAF$_{II}$250-(1–163) (18 ng) and c-Jun (530 ng) were incubated together before adding TBP and AdMLP promoter DNA. After treatment with DNase I, samples were subjected to denaturing PAGE, and the results were analyzed and quantitated by phosphor-imaging. Plots are shown of relative intensities of bands (*y* axis) *versus* position on gel (*x* axis) for four different reactions: no proteins, TBP alone, TBP with GST-TAF$_{II}$250-(1–163), and c-Jun with TBP and GST-TAF$_{II}$250-(1–163). Left to right in the plots represents top to bottom of the gel. The TATA box region is indicated with a *bracket*.

the immobilized GST-TAF$_{II}$250-(1–163) with purified c-Jun. The silver-stained SDS gel in Fig. 3*B* shows GST-TAF$_{II}$250-(1–163) bound c-Jun (*lane 5*). c-Jun was not retained on control GST resin (*lane 4*). Thus, purified c-Jun can bind directly to GST-TAF$_{II}$250-(1–163).

These results revealed that the region of TAF$_{II}$250 bound by c-Jun is the N-terminal domain, which is known to inhibit TBP-DNA binding and transcription. Based on this finding, we hypothesized that c-Jun binds the N terminus of TAF$_{II}$250 and inhibits its repression of TBP-DNA binding, thereby contributing to transcriptional activation.

c-Jun Derepresses Basal Transcription Directed by TFIID—It is possible that the lower level of basal transcription observed with TFIID compared with recombinant TBP (see Fig. 1) was due to the N-terminal region of TAF$_{II}$250 interacting with the DNA binding surface of TBP to repress the binding of TBP to TATA DNA. In this case, c-Jun may bind directly to the N terminus of TAF$_{II}$250 in TFIID to derepress transcription. To test this we used a DNA plasmid that lacks known AP-1 sites, p(GAL4)$_5$-AdMLP-G-less, which contains the adenovirus major late core promoter (−53 to +10) upstream of a 380-base pair G-less cassette. TFIID and TBP were incubated separately with increasing concentrations of c-Jun and then added to transcription reactions with p(GAL4)$_5$-AdMLP-G-less. As shown in Fig. 4, *lanes 7–10*, c-Jun stimulated transcription when preincubated with TFIID. The level of transcription with TFIID approached that observed with TBP at the highest concentrations of c-Jun. c-Jun did not increase the level of transcription when preincubated with TBP (Fig. 4, *lanes 2–5*).

c-Jun Overcomes Repression of TBP-DNA Binding and Transcription Caused by the N Terminus of TAF$_{II}$250—To demonstrate that the N terminus of TAF$_{II}$250 can repress *in vitro* transcription and to directly test whether c-Jun can inhibit TAF$_{II}$250-mediated transcriptional repression, we added purified GST-TAF$_{II}$250-(1–163) to transcription reactions. The ability of TAF$_{II}$250-(1–163) to repress basal transcription was determined by comparing the effects of GST-TAF$_{II}$250-(1–163) and GST on basal transcription in a minimal *in vitro* transcription system consisting of TBP, TFIIB, TFIIF, RNA polymerase II, and negatively supercoiled p(GAL4)$_5$-AdMLP-G-less template DNA. As shown in Fig. 5*A*, the addition of GST-TAF$_{II}$250-(1–163) repressed basal transcription by more than 95%, whereas GST did not affect transcription when titrated over the same concentration range. Thus, the N terminus of TAF$_{II}$250 can repress basal transcription in a highly purified minimal transcription system.

We next tested the ability of c-Jun to block transcriptional repression caused by GST-TAF$_{II}$250-(1–163). c-Jun was preincubated with GST-TAF$_{II}$250-(1–163) before adding the p(GAL4)$_5$-AdMLP-G-less template (lacking known AP-1 sites) and the remaining transcription factors. c-Jun overcame the repression of basal transcription observed with GST-TAF$_{II}$250-(1–163) (Fig. 5*B*, compare *lanes 3* and *4* with *lane 2*). These data demonstrate that c-Jun can bind the N terminus of

$TAF_{II}250$ to block its ability to repress basal transcription.

It was possible that the transcriptional effects presented above resulted from c-Jun binding the N terminus of $TAF_{II}250$ to inhibit its repression of TATA box binding by TBP. To test this, we performed DNase I footprinting with TBP using a DNA fragment containing the adenovirus major late core promoter but lacking AP-1 sites. GST-$TAF_{II}250$-(1–163) and c-Jun were added to determine their effects on TBP-DNA binding. Fig. 5*C* shows plots of relative intensities (*y* axis) *versus* position on the gel (*x* axis) for the region spanning the TATA box (left to right represents top to bottom on the gel). TBP alone protected bands in the TATA box region from DNase I digestion by greater than 90%. GST-$TAF_{II}250$-(1–163) almost completely repressed TBP-DNA binding. In the presence of GST-$TAF_{II}250$-(1–163), the intensities of bands in the TATA box region were decreased by less than 10% from the control reaction performed in the absence of transcription factors. Incubation of c-Jun with GST-$TAF_{II}250$-(1–163) blocked the repression of TBP binding, resulting in ~70% of the maximal protection in the TATA box region that was observed with TBP alone. Since the footprinting experiments were performed with recombinant TBP, GST-$TAF_{II}250$-(1–163), and c-Jun, we can rule out a requirement for other general transcription factors in repression by GST-$TAF_{II}250$-(1–163) and inhibition of this repression by c-Jun. Taken together, the transcription and DNase I footprinting experiments strongly support a model in which c-Jun binding to the N terminus of $TAF_{II}250$ inhibits repression of TBP-DNA binding and transcription initiation.

DISCUSSION

Here we have elucidated one mechanism by which c-Jun activates transcription. c-Jun binds the N terminus of $TAF_{II}250$ to derepress RNA polymerase II transcription. Not only did c-Jun derepress TFIID-directed transcription *in vitro*, but c-Jun was also capable of blocking repression of TBP-DNA binding by the N terminus of $TAF_{II}250$. Our results are the first to characterize a mechanism by which c-Jun activates RNA polymerase II transcription in a cell-free system. In addition, we provide the first evidence that activators can directly bind the N terminus of $TAF_{II}250$ to derepress transcription *in vitro*.

Derepression is emerging as a general mechanism for controlling levels of transcription in eukaryotic cells. This is not surprising since derepression is a prevalent mechanism for controlling gene expression in prokaryotes (43). The most widespread example of derepression in eukaryotes is thought to be the alteration of chromatin structure by histone acetyltransferases and ATP-dependent chromatin remodeling factors (3). In small nuclear RNA transcription the snRNA activating protein complex (SNAPc) has a built-in DNA binding damper that is deactivated by the transcriptional activator Oct-1 (44). Another general mechanism of transcriptional repression in eukaryotic cells results from the interaction of the N terminus of the largest TFIID subunit to the concave surface of TBP to prevent binding of TFIID to TATA boxes (23, 25). The finding that c-Jun binds the N terminus of $TAF_{II}250$ to derepress TFIID-driven transcription *in vitro* further supports the generality of derepression as a means of regulating eukaryotic transcription. We are left wondering whether other transcriptional activators also bind the N terminus of $TAF_{II}250$ to derepress transcription. We note that significant stimulation of transcription from the p(GAL4)$_5$-AdMLP-G-less template (which lacks known AP-1 sites) required 400 ng or more of c-Jun. These amounts are far greater than the amounts of transcriptional activators typically used in *in vitro* transcription experiments. For example, we observe significant activation from p(AP-1)$_5$-E1b-G-less with 15 ng of c-Jun. With this low amount of c-Jun we do not observe activation from any template that does not contain known AP-1 sites. Moreover, in early c-Jun activation experiments, we did not titrate c-Jun to high enough levels to observe AP-1 site-independent derepression. Perhaps other transcriptional activators that bind the N terminus of $TAF_{II}250$ to derepress transcription will be revealed through a combination of protein-protein interaction assays (with activators and the N terminus of $TAF_{II}250$) and *in vitro* transcription experiments aimed at testing for site-independent transcriptional stimulation in a TFIID-dependent transcription system.

Other models for derepression of TFIID binding have been studied. For example, it has been shown that VP16 and d$TAF_{II}230$ competitively bind the concave surface of TBP (25). A point mutation in TBP (L114K) that disrupted the TBP-VP16 interaction prevented VP16 activation *in vivo*. The same point mutation in TBP also disrupted interaction with d$TAF_{II}230$. In addition, when the N terminus of y$TAF_{II}145$ was replaced with an acidic activation domain, the fusion protein autoinhibited TFIID in yeast (29). A two-step "hand-off" model has been proposed to explain the function of acidic activation domains in derepressing DNA binding by TFIID (25, 29). In the first step, the acidic activation domain replaces the N terminus of d$TAF_{II}230$/y$TAF_{II}145$ by binding the underside of TBP. In the second step, TBP binds to the TATA box when the acidic activation domain releases its hold on TBP. This proposed mechanism of relieving the internal repression of TFIID binding is quite different from the mechanism of derepression by c-Jun that we have characterized here; however, both modes of derepression serve to increase TFIID-driven transcription.

Since TFIIA has been shown to stimulate TFIID binding to the TATA box and to compete with the largest subunit of TFIID for binding TBP (24, 28), it is possible that transcriptional activators could indirectly derepress $TAF_{II}250$ inhibition of TATA box binding by helping TFIIA bind TBP. Although TFIIA may play a role in c-Jun derepression, it is not required under all conditions. In our highly purified *in vitro* transcription system we found that c-Jun blocked the repression of transcription by GST-$TAF_{II}250$-(1–163) in the absence of TFIIA. Furthermore, using DNase I footprinting we found that c-Jun blocked the ability of GST-$TAF_{II}250$-(1–163) to repress TATA box binding by TBP. Thus, TFIIA and other general transcription factors are not required for the function of c-Jun in derepressing TBP binding *in vitro*.

TBP has also been shown to dimerize *in vitro* and *in vivo* either alone or when part of TFIID (45, 46). Dimers form through interactions between the concave surfaces of two molecules of TBP, resulting in a complex that is not capable of binding DNA. The coordination of TBP dimerization and the N terminus of $TAF_{II}250$ interacting with TBP is not understood. Perhaps these two distinct mechanisms of repressing TFIID-DNA binding function together to ensure that the repressed state is maintained in the absence of interaction with TFIIA and transcriptional activators.

Acknowledgments—We thank Natalie Ahn, Lin Chen, Jennifer Kugel, and Irene Ota for helpful discussions and comments on the manuscript. J. A. G. is grateful to Robert Tjian for generous support, especially during the early stages of this work.

REFERENCES

1. Albright, S. R., and Tjian, R. (2000) *Gene* **242,** 1–13
2. Malik, S., and Roeder, R. G. (2000) *Trends Biochem. Sci.* **25,** 277–283
3. Workman, J. L., and Kingston, R. E. (1998) *Annu. Rev. Biochem.* **67,** 545–579
4. Orphanides, G., Lagrange, T., and Reinberg, D. (1996) *Genes Dev.* **10,** 2657–2683
5. Carey, M. F. (1995) *Curr. Biol.* **5,** 1003–1005
6. Verrijzer, C. P., and Tjian, R. (1996) *Trends Biochem. Sci.* **21,** 338–342
7. Kaufmann, J., and Smale, S. T. (1994) *Genes Dev.* **8,** 821–829
8. Weis, L., and Reinberg, D. (1992) *FASEB J.* **6,** 3300–3309
9. Verrijzer, C. P., Chen, J.-L., Yokomori, K., and Tjian, R. (1995) *Cell* **81,** 1115–1125

10. Burke, T. W., and Kadonaga, J. T. (1997) *Genes Dev.* **11,** 3020–3031
11. Wang, W., Gralla, J. D., and Carey, M. (1992) *Genes Dev.* **6,** 1716–1727
12. Sauer, F., Hansen, S. K., and Tjian, R. (1995) *Science* **270,** 1783–1788
13. Lieberman, P. M., and Berk, A. J. (1994) *Genes Dev.* **8,** 995–1006
14. Horikoshi, M., Carey, M. F., Kakidani, H., and Roeder, R. G. (1988) *Cell* **54,** 665–669
15. Chi, T., and Carey, M. (1996) *Genes Dev.* **10,** 2540–2550
16. Pugh, B. F., and Tjian, R. (1990) *Cell* **61,** 1187–1197
17. Dynlacht, B. D., Hoey, T., and Tjian, R. (1991) *Cell* **55,** 563–576
18. Lee, T. I., Causton, H. C., Holstege, F. C., Shen, W. C., Hannett, N., Jennings, E. G., Winston, F., Green, M. R., and Young, R. A. (2000) *Nature* **405,** 701–704
19. Moqtaderi, Z., Bai, Y., Poon, D., Weil, P. A., and Struhl, K. (1996) *Nature* **383,** 188–191
20. Oelgeschlager, T., Tao, Y., Kang, Y. K., and Roeder, R. G. (1998) *Mol. Cell* **1,** 925–931
21. Walker, S. S., Reese, J. C., Apone, L. M., and Green, M. R. (1996) *Nature* **383,** 185–188
22. Wu, S. Y., Kershnar, E., and Chiang, C. M. (1998) *EMBO J.* **17,** 4478–4490
23. Kokubo, T., Gong, D.-W., Yamashita, S., Horikoshi, M., Roeder, R. G., and Nakatani, Y. (1993) *Genes Dev.* **7,** 1033–1046
24. Kokubo, T., Swanson, M. J., Nishikawa, J. I., Hinnebusch, A. G., and Nakatani, Y. (1998) *Mol. Cell. Biol.* **18,** 1003–1012
25. Nishikawa, J., Kokubo, T., Horikoshi, M., Roeder, R. G., and Nakatani, Y. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 85–90
26. Liu, D., Ishima, R., Tong, K. I., Bagby, S., Kokubo, T., Muhandiram, D. R., Kay, L. E., Nakatani, Y., and Ikura, M. (1998) *Cell* **94,** 573–583
27. Kotani, T., Miyake, T., Tsukihashi, Y., Hinnebusch, A. G., Nakatani, Y., Kawaichi, M., and Kokubo, T. (1998) *J. Biol. Chem.* **273,** 32254–32264
28. Ozer, J., Mitsouras, K., Zerby, D., Carey, M., and Lieberman, P. M. (1998) *J. Biol. Chem.* **273,** 14293–14300
29. Kotani, T., Banno, K., Ikura, M., Hinnebusch, A. G., Nakatani, Y., Kawaichi, M., and Kokubo, T. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 7178–7183
30. Karin, M., Liu, Z., and Zandi, E. (1997) *Curr. Opin. Cell Biol.* **9,** 240–246
31. Turner, R., and Tjian, R. (1989) *Science* **243,** 1689–1694
32. Glover, J. N., and Harrison, S. C. (1995) *Nature* **373,** 257–261
33. Abate, C., Luk, D., Gagne, E., Roeder, R. G., and Curran, T. (1990) *Mol. Cell. Biol.* **10,** 5532–5535
34. Bohmann, D., and Tjian, R. (1989) *Cell* **59,** 709–717
35. Sutherland, J. A., Cook, A., Bannister, A. J., and Kouzarides, T. (1992) *Genes Dev.* **6,** 1810–1819
36. Lee, J. S., See, R. H., Deng, T., and Shi, Y. (1996) *Mol. Cell. Biol.* **16,** 4312–4326
37. Chen, J.-L., Attardi, L. D., Verrijzer, C. P., Yokomori, K., and Tjian, R. (1994) *Cell* **79,** 93–105
38. Lillie, J. W., and Green, M. R. (1989) *Nature* **338,** 39–44
39. Galasinski, S. K., Lively, T. N., Grebe de Barron, A., and Goodrich, J. A. (2000) *Mol. Cell. Biol.* **20,** 1923–1930
40. Kugel, J. F., and Goodrich, J. A. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 9232–9237
41. Ozer, J., Moore, P. A., Bolden, A. H., Lee, A., Rosen, C. A., and Lieberman, P. M. (1994) *Genes Dev.* **8,** 2324–2335
42. Sun, X., Ma, D., Sheldon, M., Yeung, K., and Reinberg, D. (1994) *Genes Dev.* **8,** 2336–2348
43. Struhl, K. (1999) *Cell* **98,** 1–4
44. Mittal, V., Ma, B., and Hernandez, N. (1999) *Genes Dev.* **13,** 1807–1821
45. Coleman, R. A., and Pugh, B. F. (1995) *J. Biol. Chem.* **270,** 13850–13859
46. Coleman, R. A., Taggart, A. K. P., Benjamin, L. R., and Pugh, B. F. (1995) *J. Biol. Chem.* **270,** 13842–13849