



**HAL**  
open science

## TAF7 (TAFII55) Plays a Role in the Transcription Activation by c-Jun

Christine Munz, Eleni Psichari, Dimitris Mandilis, Anne-Claire Lavigne, Maria Spiliotaki, Thomas Oehler, Irwin Davidson, Lászlo Tora, Peter Angel, Alexander Pintzas

► **To cite this version:**

Christine Munz, Eleni Psichari, Dimitris Mandilis, Anne-Claire Lavigne, Maria Spiliotaki, et al.. TAF7 (TAFII55) Plays a Role in the Transcription Activation by c-Jun. *Journal of Biological Chemistry*, 2003, 278 (24), pp.21510 - 21516. 10.1074/jbc.m212764200 . hal-03108309

**HAL Id: hal-03108309**

**<https://hal.science/hal-03108309v1>**

Submitted on 13 Jan 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## TAF7 (TAF<sub>II</sub>55) Plays a Role in the Transcription Activation by c-Jun\*

Received for publication, December 16, 2002, and in revised form, April 2, 2003  
Published, JBC Papers in Press, April 3, 2003, DOI 10.1074/jbc.M212764200

Christine Munz,<sup>a,b</sup> Eleni Psichari,<sup>c,d</sup> Dimitris Mandilis,<sup>c</sup> Anne-Claire Lavigne,<sup>c,e,f</sup>  
Maria Spiliotaki,<sup>c,g</sup> Thomas Oehler,<sup>a,h</sup> Irwin Davidson,<sup>e</sup> Laszlo Tora,<sup>e</sup> Peter Angel,<sup>a,i</sup>  
and Alexander Pintzas<sup>c,j</sup>

From the <sup>a</sup>Division of Signal Transduction and Growth Control, Deutsches Krebsforschungszentrum, 69120 Heidelberg, Germany, the <sup>c</sup>Laboratory of Signal Mediated Gene Expression, Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, 48 Vassileos Constantinou Avenue, 116 35 Athens, Greece, and the <sup>e</sup>Institut de Genetique et de Biologie Moleculaire et Cellulaire, CNRS/INSERM/ULP, 67404, Illkirch Cedex BP 10142, France

**c-Jun is a member of the AP-1 family of transcription factors regulating expression of specific target genes in a variety of cellular processes including proliferation, stress response, and tumorigenicity. In the present study we have analyzed the mechanism of c-Jun function as a transactivator with respect to members of the basal transcription machinery, TATA-binding protein-associated factors (TAFs). We show that one member of the family, human TAF7 (formerly TAF<sub>II</sub>55), physically interacts with c-Jun through two independent interaction domains, within the N- and C-terminal part of c-Jun. Interaction *in vitro* correlates with enhanced transactivation function of c-Jun in HEK293 and COS cells in the presence of increasing amounts of TAF7. TAF7 interacts preferentially with DNA-bound phosphorylated c-Jun, suggesting that TAF7 represents a novel c-Jun co-activator mediating activation of AP-1 target genes in response to extracellular signals.**

The AP-1 family of leucine zipper proteins is composed of Fos (c-Fos, FosB, Fra-1, and Fra-2), Jun (c-Jun, JunB, and JunD), and ATF proteins, which can form a variety of functional dimers (for review see Ref. 1). Both gain-of-function and loss-of-function approaches in tissue culture and in mice have established specific and essential functions of the various sub-

units in a number of processes ranging from cell survival, proliferation, oncogenic growth, cell death, neuronal excitation, and chronic inflammation to the cellular defense against toxicity (for reviews see Refs. 2–7). During this broad spectrum of important cellular processes, AP-1 activity is subject to rigorous and complex regulation at transcriptional and post-transcriptional levels. Regulatory signals by growth factors, oncoproteins, proinflammatory cytokines, and environmental stresses are transmitted to AP-1 by various signaling pathways, including those utilizing specific members of the mitogen-activated protein kinase family, such as extracellular signal-regulated kinases, JNKs,<sup>1</sup> and p38 (8).

AP-1 factors represent prototypes of signal-modulated transcriptional regulators. Much is known about the events that lead to their activation. Both the signal transduction pathways and the molecular events regulating the transcriptional potential of these factors are well described. Particularly, phosphorylation sites and their function are in many cases well defined, like N-terminal phosphorylation of c-Jun at serines 63 and 73 by the Jun N-terminal kinases (9). c-Jun interacts with many other transcription factors, which modulate AP-1-dependent activity, such as steroid hormone receptors (10), Smads (11), and Cbfa/runt (12).

The interaction of c-Jun with co-factors of transcription bearing intrinsic acetyltransferase activity, like CBP (13), TAF1 (formerly TAF<sub>II</sub>250) (14), as well as the SWI/SNF chromatin remodeling complex (15) and basal transcription factors, like TBP and TFIIB (16), provides evidence for a cross-talk between c-Jun and the basal transcription/chromatin remodeling machinery.

Despite this detailed knowledge on signaling cascades and interacting factors, neither the mechanism of how hyperphosphorylation is “translated” into increased RNA synthesis nor the players involved in the process of transactivation, potentially including components of the basal transcription machinery, have been identified conclusively.

Among the activities involved in transcriptional regulation of eukaryotic protein-coding genes the factor TFIID plays a particularly central role. Mammalian TFIID consists of TBP and 13 TBP-associated factors (TAFs) (for review see Refs. 17–19) and several TFIID subpopulations comprising a subset of TAFs

\* This work was supported by the European Union through the Training and Mobility of Research network FMRX-CT96-0044 and the Biomed-2 Program, by the Greek Secretariat of Research and Technology, and by grants from the Deutsche Forschungsgemeinschaft and CNRS of France. 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.

<sup>b</sup> Present address: Roche Diagnostics, 68305 Mannheim, Germany.

<sup>d</sup> Present address: Institut Curie, 26, rue d’Ulm, 75248 Paris Cedex 05, France.

<sup>f</sup> Present address: IPBS 205 route de Narbonne 31077 Toulouse, France.

<sup>g</sup> Present address: Medical School, University of Athens, Mikras Asias 75, Athens, Greece.

<sup>h</sup> Present address: Promega GmbH, 68199 Mannheim, Germany.

<sup>i</sup> To whom correspondence may be addressed: Deutsches Krebsforschungszentrum, Division of Signal Transduction and Growth Control (B-0800), Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany. Tel.: 49-6221-42-4570; Fax: 49-6221-42-4554; E-mail: p.angel@dkfz.de.

<sup>j</sup> To whom correspondence may be addressed: Laboratory of Signal Mediated Gene Expression, Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, 48, Vas. Constantinou Ave., 116 35 Athens, Greece. Tel.: 30210-7273753; Fax: 30210-7273755; E-mail: apintz@eie.gr.

<sup>1</sup> The abbreviations used are: JNK, c-Jun N-terminal kinase; bZIP, basic region and leucine zipper domain; CBP, cAMP-responsive element-binding protein-binding protein; GST, glutathione S-transferase; MMS, methyl methanesulfonate; TBP, TATA binding protein; TAF, TBP-associated factor(s); CAT, chloramphenicol acetyltransferase; RSV, Rous sarcoma virus.

may exist in mammalian cells (18).

Evidence that TAF<sub>II</sub>s function as co-activators in eukaryotic cells has come from the investigation of the role of hsTAF11 (formerly TAF<sub>II</sub>28), hsTAF10 (formerly TAF<sub>II</sub>30), and hsTAF4 (formerly TAF<sub>II</sub>135) in transcriptional activation by several transactivators, mainly by nuclear receptors (20–22). Further evidence that TAFs are required for transcriptional activation *in vivo* derives from genetic studies in *Drosophila* (23), *Caenorhabditis elegans* (24), and mouse (25). Thus, the currently available information indicates that TAFs act as specific co-activators in metazoans. Moreover, several studies (26–31) suggested that TFIID subunits are important in the regulation of cell cycle (TAF1, TAF2, TAF5, and TAF10) and apoptosis (TAF4b and TAF6 $\delta$ ).

Here we provide evidence that hsTAF7 is involved in the transcriptional activity of c-Jun. We show the importance of their synergistic interaction, which is mediated through the N- and C-terminal domains of c-Jun.

#### EXPERIMENTAL PROCEDURES

**Recombinant Plasmids**—hTAF<sub>II</sub>55 (TAF7) expression vectors have been described previously (21). RSV-c-Jun (32), –73/63 collCAT, thymidine kinase-CAT (3), 5xcoll TRE luc TATA-luc (53), GST-vJun  $\Delta$ 6–91 (33), c-Jun 1–87, c-Jun  $\Delta$ 6–194,  $\Delta$ 194–223,  $\Delta$ 6–223,  $\Delta$ 146–221, GHF-cJ (34), GLZ (35), c-Jun1–166 (36), c-Jun-ser63/73, GSTc-Jun1–79 (37), and GSTc-Jun1–166 (36) have been described. GSTc-JunZip containing amino acids 223–331 was constructed by cloning a *Bgl*II/*Eco*RI fragment of RSV-c-Jun  $\Delta$ 194–223 (34) into *Bam*HI/*Eco*RI-digested pGEX-3X. c-J basicD covering amino acids 1–6 and 221–288 was created by PCR using c-Jun $\Delta$ 146–221 as a template. Insect cell baculovirus expression vectors for TAFs and TBP have been previously described in Ref. 32.

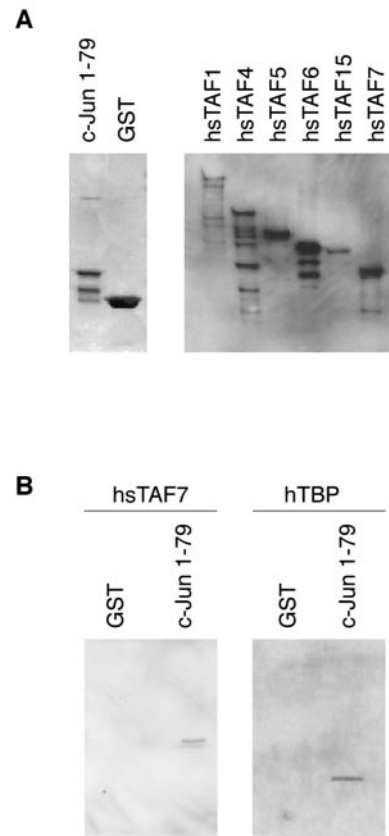
**Antibodies and Oligonucleotides**—The following antibodies have been previously described:  $\alpha$ -TAF7 (34),  $\alpha$ -B10 (38),  $\alpha$ -c-Jun (Signal Transduction Laboratories),  $\alpha$ -p-c-Jun (New England Biolabs), and  $\alpha$ -H-Ras (Santa-Cruz). Expression of baculovirus-expressed recombinant TAFs was monitored using the following antibodies:  $\alpha$ -TAF1, Santa Cruz Biotechnology,  $\alpha$ -TAF4 (22),  $\alpha$ -TAF5 (32),  $\alpha$ -TAF6 (26), and  $\alpha$ -TAF7 (38). The oligonucleotides used are the following: TRE (5'-AGCTAAAGTGGTGATCATCACTAT-3') and mt TRE (5'-AGCTAAAGTGGCGATCGTTCATAT-3').

**Cell Culture and Transfection**—COS and HEK 293 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and used for transfections. For transient expression assays, the cells were seeded at 30% confluency and transfected by the calcium phosphate method. 2  $\mu$ g of the  $\beta$ -galactosidase expression plasmid pCH110 (Pharmacia Corp.) was used to normalize for transfection efficiencies, and the total amount of DNA was kept constant at 20  $\mu$ g/plate with the use of pSG5 plasmid (Ref. 40). 18 h after the transfection, the precipitate was removed, and the cells were further incubated in Dulbecco's modified Eagle's medium with 10% fetal bovine serum for 30 h. In the cases of c-Jun-induced hyperphosphorylation, MMS was added 36 h after the transfection at a concentration of 1 mg/ml, and the cells were incubated for 2 h (39).

**Total Cell Extracts, *In Vitro* Translation, and Bacterial and Baculovirus Expression of Recombinant Proteins**—Total cell extracts that were used for Western blot and immunoprecipitations were prepared as described (34, 39, 40). *In vitro* translation was performed using the TNT T7 coupled reticulocyte lysate system (Promega), as described previously (12, 41).

**Immunoprecipitation and Pull-down Assays**—Immunoprecipitation assays were carried out using 40  $\mu$ g of protein A-Sepharose (Sigma), mixed with 1  $\mu$ g anti-c-Jun antibody in non-denaturing immunoprecipitation buffer (20% glycerol, 0.05 M Tris, pH 7.9, 1 mM dithiothreitol, 0.1 M KCl, 0.1% Nonidet P-40) and incubated for 2 h at 4 °C with rotary shaking. The resin with the bound antibody was collected by centrifugation (6500 rpm, 1 min) and washed three times for 5 min with 1 ml of ice-cold immunoprecipitation buffer. 100  $\mu$ g of total cell extracts and 4 ng/ $\mu$ l of DNA were added, and the mixture was further incubated for 4 h at 4 °C with rotary shaking. The resin was then washed and collected by centrifugation as above. The pellet was resuspended in 2 $\times$  SDS sample buffer and assayed by Western blot analysis. Expression and purification of GST fusion proteins and GST pull-down assays were performed as described previously (36, 41).

**Western Blot Assays**—30  $\mu$ g of total cell extracts separated by SDS



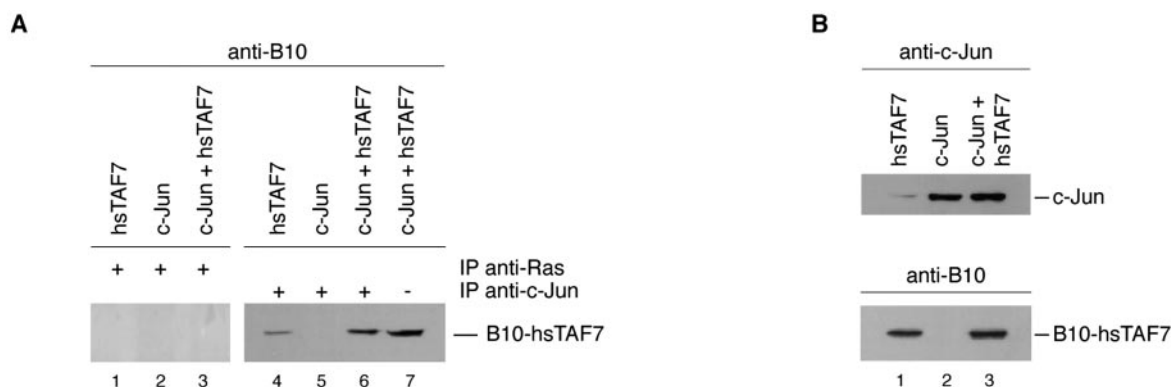
**FIG. 1. c-Jun interacts with hsTAF7 *in vitro*.** A, GST c-Jun 1–79 (left panel) was expressed in bacteria and purified by affinity chromatography. hsTAFs (right panel, as indicated) and hTBP were expressed in SF9 cells using recombinant baculovirus expression vectors. The samples from each preparation were subjected to SDS-PAGE and either stained with Coomassie (GST or GST-c-Jun) or analyzed by Western blot analysis using specific  $\alpha$ -TAF antibodies as described under “Experimental Procedures.” B, hsTAFs and hTBP were mixed with c-Jun 1–79 *in vitro* and subjected to GST pull-down assay as described under “Experimental Procedures.”

electrophoresis on 15% polyacrylamide gels were transferred on a nitrocellulose membrane. The blots were incubated with the corresponding rabbit antisera. The immunocomplexes were visualized using the kit of enhanced chemiluminescent substrate (Pierce), and quantitation was performed by densitometry of x-ray films.

**CAT and Luciferase Assays**—Total cell extracts were prepared as described above, and expression of CAT and luciferase reporter genes was measured as described previously (36, 42). The experiments were repeated at least three times.

#### RESULTS

**c-Jun Interacts with TAF7 (TAF<sub>II</sub>55) *In Vitro* and *In Vivo***—To identify cellular proteins involved in mediation of the transactivation function of c-Jun, we searched for the potential interaction partners of c-Jun in TFIID, using recombinant baculovirus expression vectors to express TAFs and TBP (Fig. 1A, right panel). First, *in vitro* protein-protein interactions were performed using baculovirus expressed, recombinant human TBP and TAFs (TAF1, TAF4, TAF5, TAF6, TAF7, and TAF15) and bacterially expressed recombinant GST-c-Jun (1–79; Fig. 1A) encoding the minimal transactivation domain of c-Jun. In agreement with previous reports (16), recombinant TBP was bound by immobilized GST-c-Jun (Fig. 1B, right panel). Similarly, TAF7, but none of the TAFs tested here, showed a detectable physical interaction with c-Jun (Fig. 1B and data not shown). Under these conditions, TAF7 also interacted with the GST control. However, this interaction was much weaker compared with that with GST-c-Jun1–79.



**FIG. 2. c-Jun interacts with hTAF7 *in vivo*.** *A*, COS cells were transiently transfected with hsTAF7 (lanes 1 and 4), c-Jun (lanes 2 and 5), or hsTAF7 and c-Jun (lanes 3, 6, and 7) as indicated at the top. Extracts of these transfected cells were immunoprecipitated (IP) with an anti-Ras (lanes 1–3) or anti-c-Jun antibody (lanes 4–6). Nonimmunoprecipitated extracts were loaded on lane 7. Western blot analysis followed, using the anti-B10 antibody, which recognizes the B10 epitope of the transfected hsTAF7. *B*, Western blot analysis, showing the expression levels of c-Jun and hsTAF7 after the transfection, using the anti-c-Jun (upper panel) and anti-B10 (lower panel) antibodies, respectively. The lanes correspond to the same transfections described for *A*.

These data show that under these conditions in addition to TBP, only one of the analyzed TAF family members, TAF7, was able to interact with the N-terminal region of c-Jun *in vitro*. Note that under different interaction conditions another TFIID component, TAF1, can also bind to c-Jun *in vitro* (14).

To confirm the physical interaction between TAF7 and c-Jun proteins in living cells, immunoprecipitation assays combined with Western blotting were performed using extracts from transiently transfected COS cells (Fig. 2*A*). The cells received the B10-hTAF<sub>1155</sub> plasmid expressing human TAF7 protein tagged with the B10 epitope (lanes 1 and 4), the RSV c-Jun expression vector encoding wild-type c-Jun (lanes 2 and 5), or both (lanes 3, 6, and 7). After transfection total cell extracts were prepared and expression of c-Jun (Fig. 2*B*, upper panel), and B10-tagged TAF7 (Fig. 2*B*, lower panel) was confirmed by Western blot. To demonstrate the c-Jun/TAF7 interaction, c-Jun-containing protein complexes were immunoprecipitated using an anti-c-Jun antisera (Fig. 2*A*, lanes 4–6), and the amount of co-precipitated TAF7 was determined by Western blot, using the anti-B10 antibody. As shown in Fig. 2*A* (lane 4), significant amounts of TAF7 interacting with endogenous c-Jun can be co-precipitated, which is further amplified in cells overexpressing c-Jun (Fig. 2*A*, lane 6). Immunoprecipitation of the same samples using an anti-Ras antisera as a negative control showed that TAF7 was not co-precipitated unspecifically under these conditions (Fig. 2*A*, lanes 1–3). These data show that TAF7 specifically interacts with c-Jun both *in vitro* and *in vivo*.

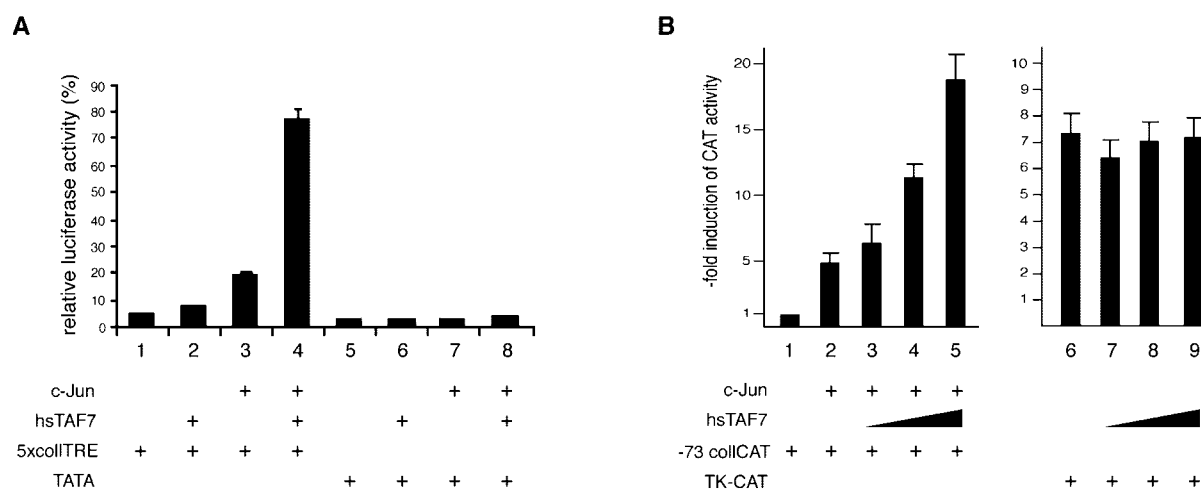
**c-Jun-dependent Transcriptional Activation Is Increased by TAF7**—To determine whether co-expression of TAF7 further increases c-Jun-dependent activity of AP-1-dependent promoters, transactivation experiments with c-Jun-responsive promoters fused to luciferase or CAT reporters were performed in COS and HEK 293 cells. In COS cells transfected with the standard AP-1-dependent reporter gene 5xcoll TRE-luc containing five synthetic AP1 sites, c-Jun had an almost 4-fold effect on transactivation of the reporter that was further enhanced about 4-fold upon ectopic overexpression of TAF7 (Fig. 3*A*). Expression of the TATA-luc reporter lacking AP-1 sites, which was used as a negative control, was affected by neither c-Jun nor TAF7 nor overexpression of both. These data show that the TAF7-induced enhancement of 5xcoll-TRE-luc reporter gene expression was specifically mediated through c-Jun rather than by a general effect on components of the basal transcriptional machinery. Similar to the 5xcoll-TRE-luc construct expression of the “classical” AP-1-dependent reporter

–73/63 CollCAT was increased about 5-fold by c-Jun (Fig. 3*B*, lanes 1 and 2), which was further enhanced in the presence of increasing amounts of TAF7 (Fig. 3*B*, lanes 3–5). When we analyzed the promoters/enhancer units of thymidine kinase or RSV-LTR, whose high basal activity is not affected by c-Jun overexpression,<sup>2</sup> no major changes in activity of these regulatory units were detected (Fig. 3*B*, lanes 6–9, and data not shown). These data show that the series of transcription factors regulating the activity of these potent promoters were not affected by TAF7. Co-transfection of expression vectors encoding other TAFs (*i.e.* TAF12) did not influence the activation of the reporters by c-Jun (data not shown), confirming our data obtained by *in vitro* interaction of recombinant proteins. These data demonstrate that TAF7 interacts with c-Jun and positively influences the transcriptional activation by c-Jun.

**c-Jun Contains Two Binding Sites for TAF7**—Previous findings showed an interaction between TBP or p300 with both the N- and C-terminal halves of c-Jun (27, 43). These findings prompted us to test whether in addition to the N-terminal domain the DNA-binding domain of c-Jun could also participate in an interaction with TAF7. To answer this question, GST pull-down assays followed by Western blot analysis were performed using protein extracts from 293 cells transiently transfected with a hsTAF7 expression vector. In agreement with the data on *in vitro* interaction of recombinant hsTAF7 and c-Jun N terminus (Fig. 1), using a GST fusion protein containing the N-terminal part of c-Jun (amino acids 1–166) as a bait, significant amounts of hsTAF7 could be pulled down (Fig. 4*A*, lane 3). A GST-c-Jun fusion protein containing amino acids 1–79 of c-Jun was less efficient. In contrast, GST alone did not pull down detectable levels of hsTAF7 (lane 1), confirming specificity of interaction. Interestingly, compared with the transactivation domain, the C-terminal part of c-Jun bZip containing the DNA-binding region was even more efficient in hsTAF7 pull down (lane 2). These data suggest that in addition to the N terminus, the C-terminal part of c-Jun is able to interact with hsTAF7.

The potent binding of hsTAF7 to the bZip sequence of c-Jun can be explained by direct interaction or by heterodimer formation of c-Jun with a hsTAF7-loaded dimerization partner. To distinguish between these two possibilities and to define the structural requirements of c-Jun required for hsTAF7 interaction in more detail, GST pull-down assays were performed with

<sup>2</sup> P. Angel and T. Oehler, unpublished observation.



**FIG. 3. Overexpression of hsTAF7 enhances transactivation by c-Jun in COS (A) and HEK 293 (B) cells.** A, 1.5  $\mu$ g of the 5xcoll TRE or TATA reporter plasmids were transfected with 4  $\mu$ g of RSV c-Jun and 0.3  $\mu$ g of B10-hsTAF7. The cells were harvested after 36 h and analyzed for luciferase activity. The results of three independent experiments ( $\pm$  S.D.) are shown by graphic representation. B, 5  $\mu$ g of -73/63 CollCAT or thymidine kinase-CAT reporter plasmids were transfected with 4  $\mu$ g of empty vector (lane 1) or RSV c-Jun (+) and 1, 2.5, or 5  $\mu$ g of RSV-hsTAF7 (lanes 3–5 and 7–9). Expression of the reporter genes in the absence of expression vectors was set as 1. The experiment was performed three times, and the averages ( $\pm$  standard deviations) are shown.

*in vitro* transcribed and translated c-Jun proteins (Fig. 4, C, lanes 1–5, and D, lanes 1, 2, 7, and 8) and GST fusion proteins containing the full-length hsTAF7 protein (Fig. 4B, lane 3). As shown in Fig. 4C,  $^{35}$ S-labeled wild-type and mutant c-Jun proteins containing an intact bZip region were efficiently bound to GST-hsTAF7 (lanes 6, 8, 10, 12, and 14). Even the minimal DNA-binding domain of c-Jun (amino acids 224–331) was precipitated as efficiently as the wild-type protein (lanes 6 and 12). As a negative control a GST fusion protein containing a small part of the N-terminal region of avian c-Jun, which completely lacks the transactivation domain, was used (Fig. 4, B, lane 6,  $\Delta$ 6–91, and C, lanes 7, 9, 11, 13, and 15). This protein precipitated the various Jun proteins with only very low efficiency. These data show that the region of c-Jun spanning amino acids 224–331 is able to interact with TAF7 independently of a dimerization partner. Moreover, this domain acts independently from the hsTAF7 interaction domain in the N terminus. This is further supported by the very efficient precipitation of a GHF1-c-Jun fusion protein composed of the DNA-binding domain of c-Jun and the transactivation domain of the transcription factor GHF1/Pit1 (Fig. 4D, lanes 5 and 6). Analysis of additional c-Jun mutants revealed that in the absence of the N terminus, the leucine zipper region is required for c-Jun/TAF7 interaction (Fig. 4D). Deletion of this region in a c-Jun mutant containing only the basic domain (c-Jun basicD; amino acids 221–288) led to a complete loss of complex formation (lanes 3 and 4). Moreover, the Jun-TAF7 interaction was similarly lost upon replacement of the c-Jun leucine zipper by the corresponding region of GCN4 (GLZ, lanes 11 and 12). Together these results suggest that dimerization is not sufficient for hsTAF7 binding but rather relies on c-Jun-specific sequences of the leucine zipper domain.

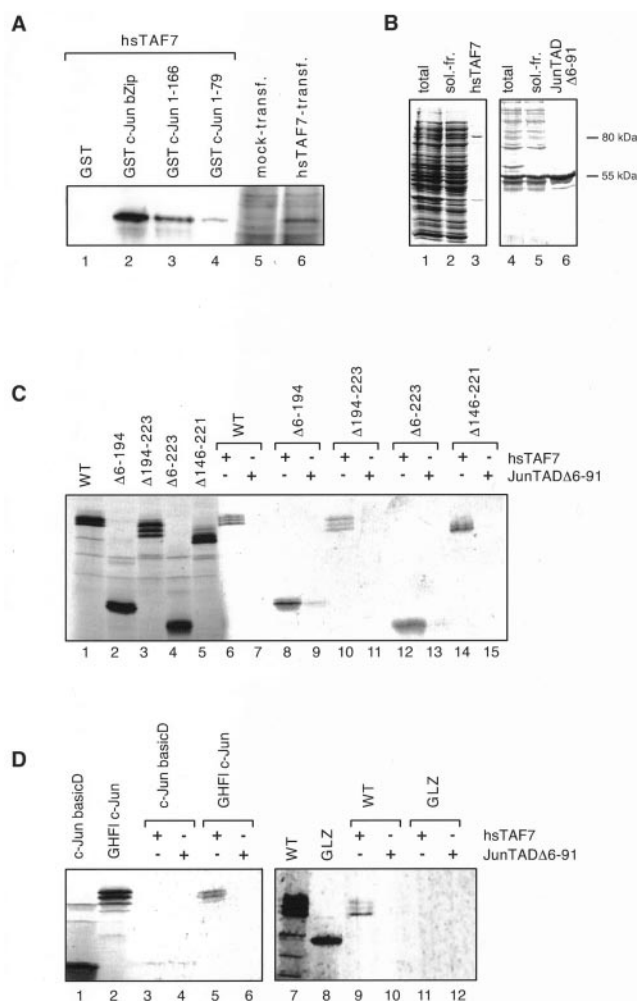
**hsTAF7 Interacts Preferentially with DNA-bound Phosphorylated c-Jun in Vivo**—Having defined hsTAF7 interaction regions in the N- and C-terminal parts of c-Jun, we wanted to know whether this interaction could be modulated by the phosphorylation events that regulate the activity of the c-Jun transactivation domain. To address this question, HEK 293 cells were transiently transfected with a c-Jun expression vector to increase c-Jun levels in the cell (Fig. 5A, lanes 3 and 4). To efficiently generate phosphorylated c-Jun, the cells were treated with the alkylating agent MMS, which is one of the most potent inducers of the JNK pathway (42, 39). When we

analyzed phosphorylation of c-Jun at serines 63 and 73 by Western blot using a phosphospecific antibody (lanes 5–8), a low level of spontaneous c-Jun phosphorylation was observed in transfected cells already in the absence of MMS treatment (lane 7), which was possibly induced in response to the transfection procedure. Phosphorylation was drastically increased in both untransfected (lane 6) and c-Jun overexpressing cells (lane 8) in response to treatment with MMS. A significant percentage of c-Jun exhibits a slower electrophoretic mobility, which is caused by hyperphosphorylation of c-Jun at serines 63 and 73, as well as threonines 89 and 91 (34, 36). These slower migrating forms are also detectable in MMS-treated c-Jun overexpressing cells using the antibody, which recognizes both unphosphorylated and phosphorylated forms of c-Jun (lane 4, marked by an asterisk). When we incubated GST-hsTAF7 protein with extracts from untreated c-Jun overexpressing cells, a significant amount of c-Jun was retained (Fig. 5B, lane 2). The amount of TAF7-bound c-Jun is not significantly increased when extracts from MMS-treated cells were used (lane 3). These data were obtained in the presence of an oligonucleotide comprising a mutated AP-1 binding site. However, when we included an excess of oligonucleotides representing a wild-type AP-1 binding site in the binding reaction, the level of TAF7-bound c-Jun was increased when comparing with untreated cells (lane 4). Thus, in the presence of wild-type AP-1 binding sites, the amount of co-precipitated Jun was strongly increased (compare lanes 3 and 5). Particularly, the slower migrating, hyperphosphorylated form of c-Jun became preferentially bound to GST-hsTAF7 (lane 5). These data strongly suggest that c-Jun and hsTAF7 interact in solution regardless of the phosphorylation status of c-Jun. DNA binding of Jun may induce a conformational change of the N terminus, making this transactivation domain susceptible for phosphorylation-dependent binding of TAF7.

## DISCUSSION

Transcriptional activation by sequence-specific activators can be mediated via TAFs, through interaction between transactivation domains and the TAFs (21, 44, 45). However, there is not much known about how and whether these interactions participate *in vivo* in the regulation of specific target genes.

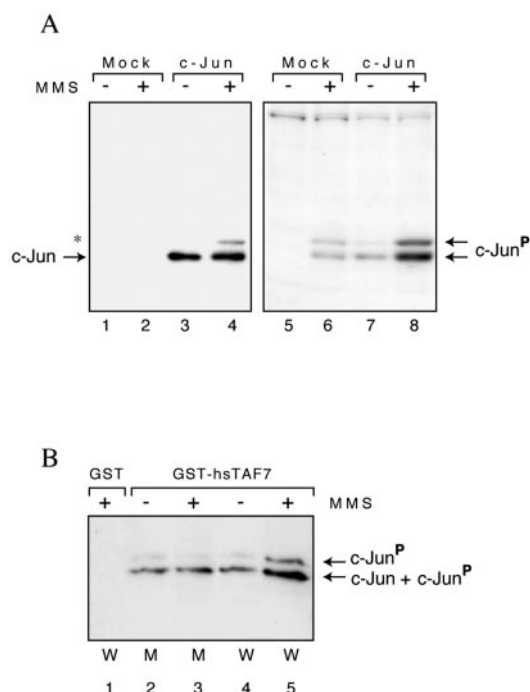
Here we describe novel interactions *in vitro* and in living cells between the hsTAF7 component of TFIID and the tran-



**FIG. 4. Mapping of domains of c-Jun that interact with hsTAF7 *in vitro*.** *A*, HEK 293 cells were transiently transfected with RSV-hsTAF7, and cell extracts were prepared after 36 h. 500  $\mu$ g of extract was subjected to GST pull-down analysis using the various GST proteins indicated at the top (lanes 1–4), and the amount of pulled down hsTAF7 was determined by Western blot analysis. In lanes 5 and 6, in parallel, 50  $\mu$ g of cellular extracts from mock transfected and RSV-hsTAF7-transfected cells, respectively, were subjected to gel electrophoresis, and the amount of hsTAF7 in the extract was determined by Western blotting. *B*, expression and purification of GST-hsTAF7 and GST-JunTAD $\Delta$ 6–91 proteins. Bacteria expressing the GST proteins indicated at the top were lysed, the protein extracts were prepared, and a fraction of them were analyzed by SDS-PAGE followed by Coomassie staining on the gel. Lanes 1 and 4, total extracts; lanes 2 and 5, soluble fraction; lanes 3 and 6, affinity-purified proteins. The molecular masses of GST-hsTAF7 (80 kDa) and GST-JunTAD $\Delta$ 6–91 (50 kDa) are indicated. *C*, hsTAF7 interacts with the bZip domain of c-Jun. Various c-Jun deletion mutants were synthesized *in vitro* (lanes 1–5) in the presence of [<sup>35</sup>S]methionine and subjected to GST pull-down analysis (lanes 6–15) using GST-hsTAF7 (+). GST-Jun $\Delta$ 6–91 was used as a negative control. Pulled-down c-Jun proteins were subjected to SDS-PAGE and visualized by autoradiography. *D*, the c-Jun leucine zipper is a target for interaction with hTAF7 *in vitro*. [<sup>35</sup>S]Methionine-labeled c-Jun mutants were synthesized *in vitro* (lanes 1, 2, 7, and 8) and analyzed as described for *C*. WT, wild type; sol.-fr., soluble fraction.

scriptional activator c-Jun, a member of AP-1 family of transcription factors. Under these conditions c-Jun did not interact with other TAFs, and strong promoter/enhancer units, which are controlled in a c-Jun-independent manner, were not affected, showing selectivity of interaction.

TAF7 protein contains almost 40% of charged residues and has been shown to interact with Sp1, USF, YY1, and CTF transactivators in solution interaction studies but not with NF- $\kappa$ B p50 subunit (60). Thus, similar to other c-Jun-interact-



**FIG. 5. TAF7 interacts preferentially with DNA-bound, phosphorylated c-Jun.** *A*, HEK 293 cells were mock transfected (lanes 1, 2, 5, and 6) or transfected with a c-Jun expression vector (RSV c-Jun; lanes 3, 4, 7, and 8). After 36 h, the cells were left untreated (–) or treated with MMS (1 mg/ml) to induce c-Jun phosphorylation. 2 h later the cellular extracts were prepared, and the total amount of c-Jun (lanes 1–4) as well as the level of phosphorylated c-Jun at serines 63 and 73 (lanes 5–8) was determined by Western blot analysis using antibodies that recognize either both unphosphorylated and phosphorylated forms of c-Jun (left panel) or only the phosphorylated forms of c-Jun (right panel). The asterisk marks the more slowly migrating forms of hyperphosphorylated Jun detectable in MMS-treated c-Jun overexpressing cells (lane 4). *B*, cell extracts from untreated (–) or MMS-treated (+) HEK 293 cells, which were transiently transfected with a c-Jun expression vector (see lanes 3, 4, 7, and 8 in *A*) were subjected to GST pull-down analysis using GST (lane 1) or GST-hTAF7 (lanes 2–5) in the presence of wild-type (W) or mutant (M) AP-1 binding sites. Pulled down c-Jun proteins were visualized by Western blot analysis using the antibody, which recognizes both unphosphorylated and phosphorylated forms of c-Jun.

ing proteins involved in transcriptional regulation, such as CBP/p300 (41, 46, 47) and ASC proteins (48), this highly charged protein potentially represents a central component of transcription factor cross-talk that interacts under various cell and/or promoter context with multiple molecules sharing subtle commonality in structure.

TAF7 interacts with two independent sites in the N-terminal and C-terminal parts of c-Jun. The first one comprises the dimerization domain, adjacent to the basic DNA-binding domain, referred as the “leucine zipper” (49). In the absence of the N-terminal interaction domain, either deletion or replacement of the leucine zipper results in a complete loss of interaction. Previously, a number of other structurally unrelated cellular proteins including NF-AT, members of the Ets and Smad protein families, and p300 have been identified that interact with the DNA-binding domain of bZip proteins, including c-Jun (for review see Ref. 50). Nevertheless, c-Jun/TAF7 interaction significantly differs from complex formation of Jun with these proteins. First, c-Jun/TAF7 complexes were detectable in the absence of DNA, whereas Jun-NFAT interactions rely on the presence of adjacent binding sites. In contrast to Jun/NF-AT, interactions between Ets and Jun proteins are formed in a DNA-independent manner. However, interaction is mediated through the basic region of c-Jun (50). Similarly, the basic

domain of c-Jun is involved in p300 interaction (43). In the case of Smad proteins, amino acid substitutions, which interfere with homodimerization of Jun or ATF-2, also interfere with Smad interaction (51). Whether this interference is due to the requirement of specific amino acid sequences in the leucine zipper domain (as found for c-Jun/TAF7 interaction) or to the general loss of dimerization remains to be determined. Structural analysis of TAF7/c-Jun complexes will be required to determine in more detail the structural features of interaction.

What are the possible functions of TAF7 binding to the leucine zipper region of c-Jun? A potential mechanism of cooperativity between c-Jun leucine zipper domain and TAF7 includes the stabilization of specific heterodimers, thus providing selective dimerization between specific AP-1 subunits in the cell. Interestingly, synergistic interactions of Jun complexes with non-bZIP DNA-binding proteins, like NF-AT, Ets, and Smad family members are mediated through their DNA-binding domains, extending the recognition specificity of the complex, as compared with the individual components (for review see Ref. 50). An additional function of TAF7 binding to the bZIP domain of c-Jun may reside in prevention of this domain for binding to negatively acting factors, such as steroid hormone receptors (10).

Although ternary complexes containing Jun and non-bZIP proteins stably associate with DNA, at present it is still unclear whether or not TAF7 remains associated with the bZIP region of c-Jun upon DNA binding. We have preliminary unpublished data that *in vitro* interaction between TAF7 and a recombinant c-Jun mutant lacking the N terminus (c-Jun $\Delta$ 6–223) is reduced in the presence of wild-type but not mutant AP-1 binding sites. Clearly, following c-Jun-mediated recruitment of TAF7 to AP-1 binding sites, liberation of TAF7 from the bZIP domain of c-Jun upon DNA binding will enhance the local concentration of TAF7 and support its interaction with the N-terminal transactivation domain of c-Jun. Our GST pull-down assays suggest that the site of interaction between TAF7 and the transactivation domain of c-Jun is localized between amino acids 1 and 166. Although deletion of amino acids 119–166 did not affect complex formation,<sup>3</sup> loss of the region between amino acids 79 and 118 reduced Jun/TAF7 interaction. Interestingly, these sequences overlap with the previously described  $\alpha$ 1 and  $\epsilon$  regions (52) also known as region III (33), which functionally cooperate with upstream sequences containing the JNK phosphorylation sites Ser<sup>63</sup> and Ser<sup>73</sup>. Neither the nature of the factor(s) binding to these regions nor the mechanisms of how these factors mediate the transactivation potential of c-Jun have been defined. Possibly, TAF7 directly binds to two sites located between amino acids 1–79 and 79–116. However, we consider this possibility unlikely, because these regions of c-Jun did not interact with TAF7 produced in bacteria or by *in vitro* transcription/translation (data not shown). Only recombinant TAF7 produced in insect cells or protein extracts from mammalian cells expressing TAF7 yielded efficient co-precipitation. These data suggest that one more additional factor associating with TAF7 is required for complex formation. This bridging factor might bind directly to c-Jun. On the other hand, it is also possible that post-translational modification of TAF7 contributes to c-Jun/TAF7 interaction.

Importantly, binding of TAF7 to the TAD of Jun was greatly enhanced in extracts from MMS-treated cells in the presence of wild-type but not mutant AP-1 binding sites. Under these conditions, the majority of Jun appears to be in a DNA-bound status (53). We propose a model where changes in the DNA-binding domain of c-Jun upon DNA binding leads to conforma-

tional change in the N terminus supporting binding of TAF7 to the transactivation domain of c-Jun. Several examples of conformational changes of transcription factors have been reported that are important for their activity, e.g. Ets (54, 55), ATF-2 (56), and c-Jun (57), where auto-inhibition by *cis*-acting modules is relieved by DNA-binding, post-translational modifications or the binding to other co-regulatory proteins. Binding of TAF7 to the N terminus of c-Jun is most efficient on c-Jun molecules, which underwent strong hyperphosphorylation at multiple serine and threonine residues in response to stress signaling. Enhanced binding of TAF7 might keep the c-Jun protein in an “open” active conformation by unmasking inhibitory sequences. By that, binding of additional factors, such as the phosphoisomerase Pin1, which specifically binds to hyperphosphorylated c-Jun and whose activity is required for JNK-mediated, c-Jun-dependent transcription of cyclin D1 (58), might be supported. Alternatively, phosphorylation-dependent interaction of c-Jun with other activators or co-factors of transcription with histone acetyltransferase activity, like p300/CBP (59), facilitates opening of chromatin structures. Subsequent interaction of c-Jun with TFIID components, like TAF7, stabilizes TFIID and enables more efficient initiation of transcription process. Although the role of TAFs in transcription is not entirely understood, additional interactions of c-Jun with TAF7 may well contribute to transactivation through AP-1 responsive elements. These inducible functional interactions with co-factors like TAFs can allow extracellular signal regulated transactivators, like c-Jun, to integrate these signals in basal transcription machinery in a promoter-specific manner.

## REFERENCES

- Angel, P., and Karin, M. (1991) *Biochim. Biophys. Acta.* **1072**, 129–157
- Angel, P., Szabowski, A., and Schorpp-Kistner, M. (2001) *Oncogene* **20**, 2413–2423
- Herdegen, T., and Waetzig, V. (2001). *Oncogene* **20**, 2424–2437
- Jochum, W., Passegue, E., and Wagner, E. F. (2001) *Oncogene* **20**, 2401–2412
- Mechta-Grigoriou, F., Gerard, D., and Yaniv, M. (2001) *Oncogene* **20**, 2378–2389
- Shaulian, E., and Karin, M. (2001) *Oncogene* **20**, 2390–2400
- Vogt, P. (2001) *Oncogene* **20**, 2365–2377
- Karin, M. (1995) *J. Biol. Chem.* **270**, 16483–16486
- Binetruy, B., Smeal, T., and Karin, M. (1991) *Nature* **351**, 122–127
- Herrlich, P. (2001) *Oncogene* **20**, 2465–2475
- Qing, J., Zhang, Y., and Derynck, R. (2000) *J. Biol. Chem.* **275**, 38802–38812
- Hess, J., Porte, D., Munz, C., and Angel, P. (2001) *J. Biol. Chem.* **276**, 20029–20038
- Lee, J. S., See, R. H., Deng, T., and Shi, Y. (1996) *Mol. Cell. Biol.* **16**, 4312–4326
- Lively, T., Ferguson, H., Galasinski, S., Seto, A., and Goodrich, J. (2001). *J. Biol. Chem.* **276**, 25582–25588
- Ito, T., Yamauchi, M., Nishina, M., Yamamichi, N., Mizutani, T., Ui, M., Murakami, M., and Iba, H. (2001) *J. Biol. Chem.* **276**, 2852–2857
- Franklin, C. C., McCulloch, A. V., and Kraft, A. S. (1995) *Biochem. J.* **305**, 967–974
- Albright, S., and Tjian, R. (2000) *Gene (Amst.)* **242**, 1–131
- Bell, B., and Tora, L. (1999) *Exp. Cell Res.* **246**, 11–19
- Tora, L. (2002) *Genes Dev.* **16**, 673–675
- Brou, C., Chaudhary, S., Davidson, I., Lutz, Y., Wu, J., Egly, J. M., Tora, L., and Chambon, P. (1993) *EMBO J.* **12**, 489–499
- Jacq, X., Brou, C., Lutz, Y., Davidson, I., Chambon, P., and Tora, L. (1994) *Cell* **79**, 107–117
- Mengus, G., May, M., Carre, L., Chambon, P., and Davidson, I. (1997) *Genes Dev.* **11**, 1381–1395
- Pham, A. D., Muller, S., and Sauer, F. (1999) *Mech. Dev.* **84**, 3–16
- Walker, A. K., Rothman, J. H., Shi, Y., and Blackwell, T. K. (2001) *EMBO J.* **20**, 5269–5279
- Freiman, R. N., Albright, S., Zheng, S., Sha, W. C., Hammer, R. E., and Tjian, R. (2001) *Science* **293**, 2084–2087
- Bell, B., Scheer, E., and Tora, L. (2001) *Mol. Cell* **8**, 591–600
- Dunphy, E., Johnson, T., Auerbach, S. S., and Wang, E. H. (2000) *Mol. Cell. Biol.* **20**, 1134–1139
- Green, M. (2000) *Trends Biochem. Sci.* **25**, 59–63
- Martin, J., Halenbeck, R., and Kaufmann, J. (1999) *Mol. Cell. Biol.* **19**, 5548–5556
- Metzger, D., Scheer, E., Soldatov, A., and Tora, L. (1999) *EMBO J.* **18**, 4823–4834
- Yamit-Hezi, A., and Dikstein, R. (1998) *EMBO J.* **17**, 5161–5169
- Dubrovskaya, V., Lavigne, A.-C., Davidson, I., Acker, J., Staub, A., and Tora, L. (1996) *EMBO J.* **15**, 3702–3712
- Oehler, T., and Angel, P. (1992) *Mol. Cell. Biol.*, **12**, 5508–5515
- Hagmeier, B. M., Konig, H., Herr, I., Offringa, R., Zantema, A., van der Eb, A.,

<sup>3</sup> C. Munz and P. Angel, unpublished observation.

- Herrlich, P., and Angel, P. (1993) *EMBO J.* **12**, 3559–3572
35. Hughes, M., Sehgal, A., Hadman, M., and Bos, T. (1992) *Cell Growth Differ.* **3**, 889–897
36. van Dam, H., Wilhelm, D., Herr, I., Steffen, A., Herrlich, P., and Angel, P. (1995) *EMBO J.* **18**, 1798–1811
37. Hibi, M., Lin, A., Smeal, T., Minden, A., and Karin, M. (1993) *Genes Dev.* **7**, 2135–2148
38. Lavigne, A.-C., Mengus, G., May, M., Dubrovskaya, V., Tora, L., Chambon, P., and Davidson, I. (1996) *J. Biol. Chem.* **271**, 19774–19780
39. Wilhelm, D., Bender, K., Knebel, A., and Angel, P. (1997) *Mol. Cell. Biol.* **17**, 4792–4800
40. Zoumpourlis, V., Papassava, P., Linardopoulos, S., Gillespie, D., Balmain, A., and Pintzas, A. (2000) *Oncogene* **19**, 4011–4021
41. Bannister, A. J., Oehler, T., Wilhelm, D., Angel, P., and Kouzarides, T. (1995) *Oncogene* **11**, 2509–2514
42. van Dam, H., Huguier, S., Kooistra, K., Baguet, J., Vial, E., van der Eb, A. J., Herrlich, P., Angel, P., and Castellazi, M. (1998) *Genes Dev.* **12**, 1227–1239
43. Vries, R. G., Prudenziatti, M., Zwartjes, C., Verlaan, M., Kalkhoven, E., and Zantema, A. (2001) *EMBO J.* **20**, 6095–6103
44. Goodrich, J. A., Hoey, T., Thut, C. J., Admon, A., and Tjian, R. (1993) *Cell* **75**, 519–530
45. May, M., Mengus, G., Lavigne, A. C., Chambon, P., and Davidson, I. (1996) *EMBO J.* **15**, 3093–3104
46. Benkoussa, M., Brand, C., Delmotte, M. H., Formstecher, P., and Lefebvre, P. (2002) *Mol. Cell. Biol.* **22**, 4522–4534
47. Fronsdal, K., Engedal, N., Slagsvold, T., and Saatcioglu, F. (1998) *J. Biol. Chem.* **273**, 31853–31859
48. Jung, D. J., Sung, H. S., Goo, Y. W., Lee, H. M., Park, O. K., Jung, S. Y., Lim, J., Kim, H. J., Lee, S. K., Kim, T. S., Lee, J. W., and Lee, Y. C. (2002) *Mol. Cell. Biol.* **22**, 5203–5211
49. Kouzarides, T., and Ziff, E. (1988) *Nature* **336**, 646–651
50. Chinenov, Y., and Kerppola, T. K. (2001) *Oncogene* **20**, 2438–2452
51. Sano, Y., Harada, J., Tashiro, S., Gotoh-Mandeville, R., Maekawa, T., and Ishii, S. (1999) *J. Biol. Chem.* **274**, 8949–8957
52. Baichwal, V. R., Park, A., and Tjian, R. (1992) *Genes Dev.* **6**, 1493–1502
53. Papavassiliou, A. G., Chavrier, C., and Bohmann, D. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 11562–11565
54. Greenall, A., Willingham, N., Cheung, E., Boam, D., and Sharrocks, A. (2001) *J. Biol. Chem.* **276**, 16207–16215
55. Lim, F., Kraut, N., Frampton, J., and Graf, T. (1992) *EMBO J.* **11**, 643–652
56. Sano, Y., Tokitou, F., Dai, P., Maekawa, T., Yamamoto, T., and Ishii, S. (1998) *J. Biol. Chem.* **273**, 29098–29105
57. Oehler, T., Pintzas, A., Stumm, S., Darling, A., Gillespie, D., and Angel, P. (1993) *Oncogene* **8**, 1141–1147
58. Wulf, G. M., Ryo, A., Wulf, G. G., Lee, S. W., Niu, T., Petkova, V., and Lu, K. P. (2001) *EMBO J.* **20**, 3459–3472
59. Arias, J., Alberts, A. S., Brindle, P., Claret, F. X., Smeal, T., Karin, M., Feramisco, J., and Montminy, M. (1994) *Nature* **370**, 226–229
60. Chiang, C.-M., and Roeder, R. (1995) *Science* **267**, 531–536



**TAF7 (TAF<sub>II</sub>55) Plays a Role in the Transcription Activation by c-Jun**  
Christine Munz, Eleni Psichari, Dimitris Mandilis, Anne-Claire Lavigne, Maria Spiliotaki,  
Thomas Oehler, Irwin Davidson, Laszlo Tora, Peter Angel and Alexander Pintzas

*J. Biol. Chem.* 2003, 278:21510-21516.

doi: 10.1074/jbc.M212764200 originally published online April 3, 2003

---

Access the most updated version of this article at doi: [10.1074/jbc.M212764200](https://doi.org/10.1074/jbc.M212764200)

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

This article cites 60 references, 32 of which can be accessed free at  
<http://www.jbc.org/content/278/24/21510.full.html#ref-list-1>