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Human $TAF_{II}31$ protein is a transcriptional coactivator of the p53 protein

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ABSTRACT **The p53 protein activates transcription of a target gene by binding to a specific DNA response element and interacting with the transcriptional apparatus of RNA polymerase II. The amino-terminal domain of p53 interacts with a component of the TFIID basal transcription complex. The human TATA-binding-protein-associated factor $TAF_{II}31$, a component of TFIID, has been identified as a critical protein required for p53-mediated transcriptional activation. $TAF_{II}31$ and p53 proteins bind to each other via amino acid residues in the amino-terminal domain of p53 that are essential for transcription. Antibodies directed against $TAF_{II}31$ protein inhibit p53-activated but not basal transcription *in vitro*. These results demonstrate that $TAF_{II}31$ is a coactivator for the p53 protein.**

The p53 protein can be divided, both structurally and functionally, into three domains (1–3). The amino-terminal 42 residues of the p53 protein can activate transcription (4, 5) by interacting with one or more components of the RNA polymerase II (RNAPII) transcriptional apparatus. The p53 amino acid residues 22 and 23 are essential for this transcriptional activation (6). The sequence-specific DNA-binding domain of the p53 protein, residues 120–290 (2, 7), contains the mutations in p53 that are frequently associated with cancers (8). The carboxyl-terminal domain, residues 310–393, contains the major nuclear localization signals (9) and protein oligomerization sequences (2, 10). Both mutations in the p53 gene (8) and the interactions of viral and cellular oncogene products with the p53 protein (11–15) inactivate its ability to function as a transcription factor and a tumor suppressor (16, 17). Thus, understanding the mechanism by which p53 functions as a transcription factor regulating several different genes (18–20) will be an important part of deciphering its action as a tumor suppressor.

The RNAPII transcriptional machinery is composed of the enzyme and several basal transcription factors: TFIID, TFIIA, TFIIB, TFIIE, TFIIF, TFIIH, and TFIIJ (21). Among these, only TFIID is a sequence-specific DNA-binding protein; it is composed of the TATA-binding protein (TBP) and several TBP-associated factors (TAFs) (22). Several of the TAFs have been shown to play an essential role as coactivators of transcription through their interaction with specific transcriptional activators (22, 23). Different transcriptional activators target distinct TAFs in the TFIID complex for their functional activation of transcription (24, 25).

Drosophila $TAF_{II}40$ ($dTAF_{II}40$) has been shown to mediate transcriptional activation from an acidic activator, Gal4-VP16 (26), that resembles the acidic amino terminus of the p53 protein. The human homolog of $dTAF_{II}40$ was isolated and its cDNA was cloned by homology to the $dTAF_{II}40$ sequence.* This protein, $hTAF_{II}31$, was shown to be part of the TFIID complex and to bind to the wild-type p53 protein but not to the p53 double mutant defective at residues 22 and 23, which fails to activate transcription (6). Antibodies directed against $TAF_{II}31$ blocked p53-stimulated transcription *in vitro* but not basal transcription mediated by TFIID.

MATERIALS AND METHODS

Cloning and Expression of $hTAF_{II}31$. Two primers, 5′-ATGGAGTCTGGCAAGAC-3′ [the sequence beginning from the first ATG codon of a human EST fragment (expressed sequence tags; ref. 39) that was detected by screening the GenBank/EMBL database] and 5′-GACTCGAGTCGACATC-$GA(T)_{17}$-3′, were used to isolate the human cDNA by rapid amplification of cDNA ends (RACE), followed by PCR (27). The sequence encoding $hTAF_{II}31$ is shown in Fig. 1.

Preparation and Purification of Anti-$hTAF_{II}31$ Polyclonal Antibodies. Recombinant His_6-tagged $hTAF_{II}31$ produced in a pQE-9 expression vector (Qiagen) was purified as described in the Qiagen manual. The anti-$hTAF_{II}31$ antibodies were produced by rabbits and purified by affinity chromatography (28).

Purification of TFIID from HeLa Cells. Endogenous (e) TFIID from Hela S3/LTRα3 cells expressing hemagglutinin-tagged holo-eTFIID (provided by A. J. Berk) was purified as described (29). TFIID was partially purified from nuclear extracts through three columns: phosphocellulose, DEAE cellulose (Whatman DE-52), and single-stranded-DNA affinity column (30).

Purification of p53 and Its Mutants from a Baculovirus Expression System. Wild-type p53 protein and p53 mutants 22/23 and 14/19 were overexpressed in baculovirus-infected Sf9 insect cells and purified to apparent homogeneity by one step of immunoaffinity column chromatography (15, 18).

***In Vitro* Immunoprecipitation Assays.** Immunoprecipitation of p53 and associated proteins was carried out by methods described previously (6, 28).

Protein–Protein Interaction Assays Using Glutathione-Agarose Beads. Glutathione *S*-transferase (GST) fusion proteins were expressed from a pGEX-2T vector (Pharmacia). Assay mixtures contained fusion proteins (2 μg for $hTAF_{II}31$ and 3 μg for p53) on glutathione-agarose beads, ^{35}S-labeled proteins and buffer [20 mM Tris·HCl, pH 7.5/150 mM NaCl/2 mM dithiothreitol/0.2 mM phenylmethanesulfonyl fluoride/0.1 mM EDTA, 10% (vol/vol) glycerol/0.1% (vol/vol) Nonidet P-40]. The mixtures were incubated at 4°C for 1 hr and washed four times with the same buffer containing 500 mM NaCl. The washed beads were boiled in SDS sample buffer and the released proteins were electrophoresed in an SDS/polyacrylamide gel. The gels were dried and exposed to x-ray film.

***In Vitro* Specific Transcriptional Assays.** RNAPII, TFIIA, TFIIF, TFIIH, and TFIIJ were purified from HeLa cells

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Abbreviations: RNAPII, RNA polymerase II; TBP, TATA-binding protein; TAF, TBP-associated factor; e, endogenous; h, human; d, *Drosophila*; GST, glutathione *S*-transferase; AdMLP, adenovirus major late promoter; p53RE, p53 response element.

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. U25112).

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CATTAATGTGGGTGATTTAGCTCGAGAAGTCTGATCATCGGATATCATGGAGTCTGGCAA   60
                                              M  E  S  G  K    (5)

GACGGCTTCTCCCAAGAGCATGCCGAAAGATGCACAGATGATGGCACAAATCCTGAAGGA  120
 T  A  S  P  K  S  M  P  K  D  A  Q  M  M  A  Q  I  L  K  D   (25)

TATGGGGATTACAGAATATGAGCCAAGAGTTATAAATCAGATGTTGGAGTTTGCGTTCGG  180
 M  G  I  T  E  Y  E  P  R  V  I  N  Q  M  L  E  F  A  F  R   (45)

ATATGTGACCACAATTCTAGATGATGCAAAAATTTATTCAAGCCATGCTAAGAAAGCTAC  240
 Y  V  T  T  I  L  D  D  A  K  I  Y  S  S  H  A  K  K  A  T   (65)

TGTTGATGCAGATGATGTGCGATTGGCAATCCAGTGCCGCGCTGATCAGTCTTTTACCTC  300
 V  D  A  D  D  V  R  L  A  I  Q  C  R  A  D  Q  S  F  T  S   (85)

TCCTCCCCCAAGAGATTTTTTATTAGATATTGCAAGGCAAAGAAATCAAACCCCTTTGCC  360
 P  P  P  R  D  F  L  L  D  I  A  R  Q  R  N  Q  T  P  L  P   (105)

ATTGATCAAGCCATATTCAGGTCCTAGGTTGCCACCTGATAGATACTGCTTAACAGCTCC  420
 L  I  K  P  Y  S  G  P  R  L  P  P  D  R  Y  C  L  T  A  P   (125)

AAACTATAGGCTGAAATCTTTACAGAAAAAGGCATCAACTTCTGCGGGAAGAATAACAGT  480
 N  Y  R  L  K  S  L  Q  K  K  A  S  T  S  A  G  R  I  T  V   (145)

CCCGCGGTTAAGTGTTGGTTCAGTTACTAGCAGACCAAGTACTCCCACACTAGGCACACC  540
 P  R  L  S  V  G  S  V  T  S  R  P  S  T  P  T  L  G  T  P   (165)

AACCCCACAGACCATGTCTGTTTCAACTAAAGTAGGGACTCCCATGTCCCTCACAGGTCA  600
 T  P  Q  T  M  S  V  S  T  K  V  G  T  P  M  S  L  T  G  Q   (185)

AAGGTTTACAGTACAGATGCCTACTTCTCAGTCTCCAGCTGTAAAAGCTTCAATTCCTGC  660
 R  F  T  V  Q  M  P  T  S  Q  S  P  A  V  K  A  S  I  P  A   (205)

AACCTCAGCAGTTCAGAATGTTCTGATTAATCCATCATTAATCGGGTCCAAAAACATTCT  720
 T  S  A  V  Q  N  V  L  I  N  P  S  L  I  G  S  K  N  I  L   (225)

TATTACCACTAATATGATGTCATCACAAAATACTGCCAATGAATCATCAAATGCATTGAA  780
 I  T  T  T  N  M  M  S  S  Q  N  T  A  N  E  S  S  N  A  L  K   (245)

AAGAAAACGTGAAGATGATGATGATGACGATGATGATGATGATGACTATGATAATCTGTA  840
 R  K  R  E  D  D  D  D  D  D  D  D  D  D  D  Y  D  N  L  *    (264)

ATCTAGCCTTGCTGAATGTAACATGTATACTTGGTCTTGAATTCATTGTACTGATATTAA  900

ACATGCATGCTGGATGTTTTCAAGTTGTGTTTTAGAAAACTAATAATAATGAGTAAACAC  960

AGTTACCATACTTTTCAATTGAAATGAAGGTTTTTCATCAGCCTTAAAAGTGTAAGAAAA 1020

ATAAAGTGGTCATTCATTCGAAAAAAAAAAAAAAAAA                        1057
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FIG. 1. Nucleotide and amino acid sequences of the cloned hTAF$_{II}$31. The hTAF$_{II}$31 open reading frame spans nt 47–838. The star denotes the stop codon and the first methionine codon is in bold type.

(30–33). Recombinant (r) TBP, TFIIB, and TFIIE were purified by published methods (34–36). The reaction conditions for specific transcription assays were as described (30). Reaction mixtures (40 μl) were incubated at 30°C for 60 min and contained 0.6 mM ATP and CTP, 15 μM [α-^{32}P]UTP (10,000 cpm/pmol), and 0.5 μg of p53RE-AdMLP or PML-(C2AT)Δ50 DNA (37). The transcription factors added were TFIIA (DEAE-5PW fraction, 250 ng), rTFIIB (phosphocellulose fraction, 30 ng), yeast rTBP (S-Sepharose, 40 ng) or purified native TFIID (DE-52, 300 ng) or eTFIID (12CA5 immunoaffinity column, 60 ng), rTFIIE (Sephacryl 200, 30 ng), rTFIIF (gift of D. Reinberg, University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School; 40 ng), TFIIH (μMono Q, 40 ng), TFIIJ (phenyl-Superose, 200 ng), and RNAPII (DEAE-5PW, 50 ng). Purified baculovirus-expressed p53 proteins (15, 18) were used in the reaction. Products of the reactions were separated by urea/PAGE and were visualized by autoradiography.

RESULTS

Molecular Cloning of Human TAF$_{II}$31. The full-length dTAF$_{II}$40 amino acid sequence (26) was used with the Genetics Computer Group programs to screen the GenBank/EMBL database for homologies with human proteins or genes. An EST (39) sequence of 358 bp was located with an open reading frame of 312 bp (see the underlined portion in Fig. 1) by using BESTFIT. An EST DNA fragment of 314 bp was generated from HeLa mRNA by reverse transcription–PCR and used as a probe for screening. A cDNA clone encoding hTAF$_{II}$31 was isolated from a HeLa cDNA pool by a RACE–PCR technique (27). An identical clone was isolated independently by simultaneously screening a HeLa cDNA library. The complete coding region deduced from these cDNA sequences is shown in Fig. 1. This 1.1-kb cDNA appears to be nearly full length, as Northern blot analysis indicated a single mRNA species of $\approx$1.2 kb (unpublished data). The full open reading frame in this cDNA encodes a protein of 264 aa with a predicted molecular mass of 29.8 kDa and pI of 9.8.

Comparison between dTAF$_{II}$40 (26) and hTAF$_{II}$31 amino acid sequences revealed very good homology in their amino-terminal regions (Fig. 2). Over the first 184 aa there is 44.6% identity and 60% similarity between the two proteins.

HTAF$_{II}$31 Is a Subunit of TFIID. The recombinant hTAF$_{II}$31 protein was expressed in *E. coli* using both the pGEX-2T and pQE-9 expression vectors. The polypeptide was either fused at the amino terminus with GST or tagged with six consecutive histidines for rapid purification by affinity chromatography. Polyclonal antibodies were raised in rabbits against the purified recombinant proteins and used to verify that the isolated cDNA clone encoded the authentic hTAF$_{II}$31 protein. eTFIID was purified from HeLa S3 cells to apparent homogeneity (Fig. 3*A*) by antibody affinity chromatography directed against an epitope fused to TBP which then was used to immunoselect the TFIID complex assembled *in vivo* (29). Consistent with published results (33), the purified TFIID is composed of TBP and 11 TAF polypeptides with molecular masses ranging from 17 to 250 kDa (Fig. 3*A*, lane 2). Western blot analysis (Fig. 3*B*) demonstrated that the native TFIID isolated in this fashion contained a 31-kDa polypeptide that reacted with the antibodies against hTAF$_{II}$31. As a control, TBP was also detected in the TFIID complex by Western blot analysis on the same membrane. Only the two expected polypeptides were detected in the analysis of either the crude protein fraction (Fig. 3*B*, lane 1) or the purified multisubunit TFIID (lane 2). As a second way to demonstrate that TBP and TAF$_{II}$31 are found in a complex, the phosphocellulose-purified 1.0 M salt fraction (contains TFIID) was incubated with anti-TAF$_{II}$31 antibodies. The immunoprecipitate was analyzed by SDS/PAGE and TBP was detected by Western blot with anti-TBP antibodies (Fig. 3*C*, lanes 1 and 2). These results demonstrate that the hTAF$_{II}$31 polypeptide encoded by the isolated cDNA clone is a subunit of TFIID.

p53 Interacts with hTAF$_{II}$31. Having established that the isolated clone encodes the hTAF$_{II}$31 of TFIID, the relationship between hTAF$_{II}$31 and p53 was explored. Wild-type p53 proteins, as well as mutant p53 proteins (22/23, 14/19), were purified to homogeneity from baculovirus-infected insect cells by one step of immunoaffinity chromatography (15, 18). The *in vitro* translated and labeled hTAF$_{II}$31, which migrated as a doublet in a denaturing polyacrylamide gel (Fig. 4, lane 2), was mixed with the unlabeled p53 protein and then incubated with

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hTAFII31 M   ESGKT...ASPKSMPKDAQMMAQILKDMGITEYEPRVINQMLEFAFRYVT 49
dTAFII40 MSAEKSDKAKISAQIKHVPKDAQVIMSILKELNVQEYEPRVVNQLLEFTFRYVT 58

hTAFII31 TILDDAKIYSSHAKKATVDADDVRLAIQCRADQSFTSPPPRDFLLDIARQRNQT 102
dTAFII40 CILDDAKVYANHARKKTIDLDDVRLATEVTLDKSFTGPLERHVLAKVADVRNSM 108

hTAFII31 PLPLIKPYSGPRLPPDRYCLTAPNYRLKSLQ..KKASTSAG.....RITVPRLS 149
dTAFII40 PLPPIKPHCGLRLPPDRYCLTGVNYKLRATNQPKKMTKSAVEGRPLKTVVKPVS 162

hTAFII31 VG.......SVTSRPSTPTLGTPTPQTMSVSTKVGTPMSLTGQRFTVQMPTSQS 196
dTAFII40 SANGPKRPHSVVAKQQVVTIPKPVIKFTTTTTTKTVGSSGGSGGGGGQEVKSES 216

hTAFII31 PAVKASIPATSAVQNVLINPSLIGSKNILITTNMMSSQNTANESSNALKRKRED 250
dTAFII40 TGAGGDLKMEVDSDAAAVGSIAGASGSGAGSASGGGGGGGSSGVGVAVKREREE 270

hTAFII31 DDDDDDDDDYDNL                                          264
dTAFII40 EEFEFVTN                                              278
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FIG. 2. Alignment of hTAF$_{II}$31 and dTAF$_{II}$40 amino acid sequences. Vertical bars denote identical residues. Double dots and single dots represent the similarity of the amino acids.

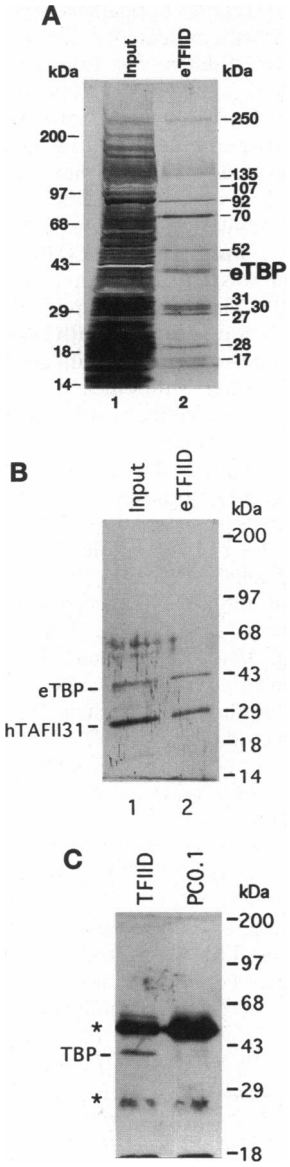

FIG. 3. $hTAF_{II}31$ is a subunit of human TFIID. (*A*) Purified human eTFIID was analyzed by SDS/PAGE followed by silver staining. Samples (4 μl) of input (DE-52 column, 0.5 M salt fraction, lane 1) and the eTFIID fraction (lane 2) of the 12CA5 immunoaffinity column were loaded onto a denaturing 5–15% polyacrylamide gradient gel. Molecular size markers are shown on the left and the subunits of TFIID are indicated on the right. (*B*) Western blot analysis of TFIID. The transfer membrane containing the same protein samples as in *A* was first incubated with polyclonal anti-$hTAF_{II}31$ antibodies (1:1000 dilution). After detection of $hTAF_{II}31$ by ECL (Amersham), the same membrane was incubated with monoclonal anti-TBP antibodies (1:2000 dilution). TBP was also visualized by ECL. (*C*) Coimmunoprecipitation of TBP by anti-$hTAF_{II}31$ antibodies. Samples (300 μl) of the 1.0 M salt fraction containing TFIID (lane 1) and the 0.1 M fraction (lane 2) from the phosphocellulose column were incubated with anti-$hTAF_{II}31$ antibodies (3 μg) and the complexes were analyzed by SDS/PAGE followed by Western blotting with anti-TBP antibodies. Stars denote the heavy and light chains of IgG.

monoclonal antibody PAb1801, directed against the human p53 protein. The resultant immunoprecipitate demonstrated that $hTAF_{II}31$ bound to the wild-type p53 protein and was coimmunoprecipitated by this antibody (Fig. 4, lanes 3 and 4). This interaction did not occur when p53 mutations made at residues 22/23 or 14/19 were employed (Fig. 4, lanes 5–8). $hTAF_{II}31$ protein was not immunoprecipitated in the absence of p53 protein (Fig. 4, lane 1) or when a nonspecific monoclonal antibody, PAb419, was used (lane 9). These results were readily repeated with a different p53-specific monoclonal antibody, PAb421.

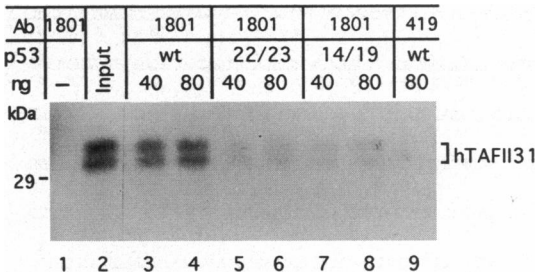

FIG. 4. Wild-type p53 binds to $hTAF_{II}31$. Samples (4 μl) of *in vitro* translated and radiolabeled $hTAF_{II}31$ were incubated with baculovirus-expressed wild-type (wt) or mutant (22/22 or 14/19) p53, together with anti-p53 (PAb1801; lanes 3–8) or irrelevant (PAb419; lane 9) monoclonal antibody. Lane 1, p53-free control; lane 2, labeled $hTAF_{II}31$ input (4 μl) directly loaded onto the SDS/10% polyacrylamide gel. Binding was detected by autoradiography.

To confirm the p53–$hTAF_{II}31$ interaction by a different technique, GST affinity beads were employed. *In vitro* translated and labeled $hTAF_{II}31$ protein was incubated with wild-type or mutant GST-p53 fusion proteins or GST beads. The results of $hTAF_{II}31$ binding to p53 are presented in Fig. 5 with the percentage of p53–$hTAF_{II}31$ binding (after subtraction for the GST-bead background) indicated in the bar graph. The amino-terminal domain of p53 (GST plus aa 1–92 of p53; G1-92 in Fig. 5) retained 80% of the p53-$hTAF_{II}31$ binding when compared with the intact GST-p53 protein (the wild-type p53–$hTAF_{II}31$ binding is given as 100%). By comparison, the carboxyl-terminal domain of p53 (aa 320–393) retained only 5% of the wild-type binding to $hTAF_{II}31$. Moreover, when the GST-1–92 fusion proteins of p53 mutated at 22/23 or 14/19 were used, the binding dropped to less than 2% or 8%, respectively. Thus, the domain of p53 interacting with

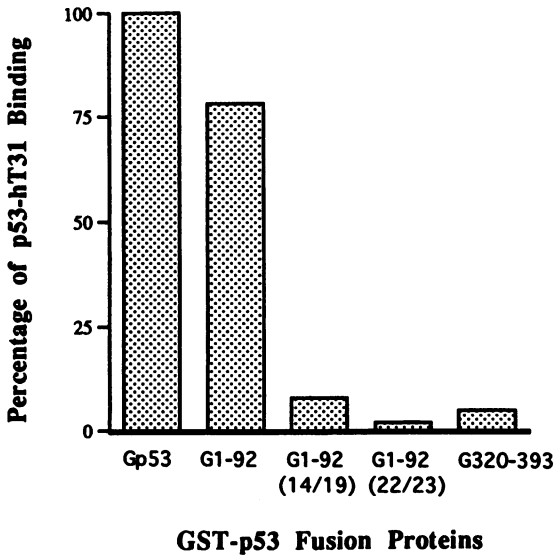

FIG. 5. Amino terminus of p53 binds to $hTAF_{II}31$. *In vitro* translated and ^{35}S-labeled $hTAF_{II}31$ protein was incubated with GST-p53 beads and washed. The washed beads were boiled in SDS sample buffer before SDS/10% PAGE. The level of $hTAF_{II}31$ bound was determined for GST fusion proteins with wild-type p53 (Gp53), p53 amino terminus (G1-92), p53 amino terminus with double mutations [G1-92(22/23) or G1-92(14/19)], and p53 carboxyl terminus (G320-393). Bound $hTAF_{II}31$ signals were quantitated by phosphor imager. Results are represented as the percentage of p53–$hTAF_{II}31$ binding (with Gp53 as 100%) for each p53 fusion protein.

hTAF$_{II}$31 is contained in aa 1–92. Furthermore, the amino acids at 22/23 or 14/19 of this domain appear to be critical in contacts between p53 with hTAF$_{II}$31 in the transcriptional machinery. Similar results were obtained when GST-hTAF$_{II}$31 was used to bind free p53 protein or its mutant forms (data not shown).

***In Vitro* Transcriptional Activity of p53.** To address the functional significance of the p53–hTAF$_{II}$31 interaction, a cell-free reconstituted system for transcriptional analysis was prepared. Two copies of a p53RE DNA sequence derived from either the *WAF-1* (20) or *mdm-2* (15, 18) gene were placed 5′ to the adenovirus major late promoter (AdMLP) (37). The AdMLP promoter was linked to a G-less cassette of 400 bases located 3′ to the transcriptional initiation site (37). These constructs were then employed as templates for *in vitro* transcription assays (30). Human RNAPII and the basal transcription factors TFIID, TFIIA, TFIIB, TFIIE, TFIIF, TFIIH and TFIIJ were purified as described in *Materials and Methods*. Typically, an *in vitro* transcription reaction mixture contained, in addition to the aforementioned transcription factors and RNAPII, DNA templates with or without a p53 response element (p53RE), wild-type or mutant p53 protein, and ribonucleotides as described. RNA transcripts of 400 nt were produced from the reaction (Fig. 6*A*). The addition of baculovirus expressed wild-type p53 protein (100 ng) increased RNA production about 19-fold (Fig. 6*A*, compare lanes 1 and 2), whereas the same amount of a codon 175 mutant p53 protein was without effect (lane 5). As anticipated, the transcriptional activities of the p53 mutant 22/23 or 14/19 significantly decreased the level of RNA produced, to about 3-fold above basal levels (Fig. 6*A*, lanes 3 and 4).

The stimulation of RNA synthesis by wild-type p53 was dose dependent; RNA transcripts increased from 5-fold to 10-fold when p53 was increased from 30 ng to 60 ng (Fig. 6*B*, compare lanes 2 and 3 with lane 5). The p53RE sequence was required for transcription activation and stimulation by the p53 protein (Fig. 6*B*). Transcription activation by p53 required all the basal transcriptional factors, including RNAPII.

hTAF$_{II}$31 Mediates Transcriptional Activation by p53. To test whether hTAF$_{II}$31 could in fact mediate transcriptional activation by p53, the effect of purified anti-hTAF$_{II}$31 polyclonal antibodies (28) on p53-activated *in vitro* transcription was investigated in the reconstituted system described above. p53 (60 ng) stimulated the transcription directed by the DNA template containing p53RE from the *WAF-1* promoter up to 12-fold (Fig. 6*C*, compare lanes 2 and 3). This transcriptional activation required TFIID, as there was no transcription at all in the absence of TFIID (Fig. 6*C*, lane 1). The affinity-purified anti-hTAF$_{II}$31 antibodies inhibited specifically the p53-activated transcription, as the RNA production dropped to the basal level (Fig. 6*C*, compare lanes 2 and 5) in the presence of the antibodies but not the preimmune serum (compare lanes 4 and 5). The basal transcription directed by the AdMLP promoter was not affected by the anti-hTAF$_{II}$31 antibodies (Fig. 6*C*, lanes 2 and 5), even though purified TFIID was used in the experiment. Furthermore, addition of twice the amount of purified TFIID to the reaction mixture overcame some of the inhibitory effect of the anti-hTAF$_{II}$31 antibodies on the p53-activated transcription (Fig. 6*C*, compare lane 6 with lanes 3 and 5). α-Amanitin (0.02 μg/ml), an RNAPII-specific inhibitor, inhibited this transcription (Fig. 6*C*, lane 7). These results demonstrate that hTAF$_{II}$31 coordinates p53-mediated transcriptional activation.

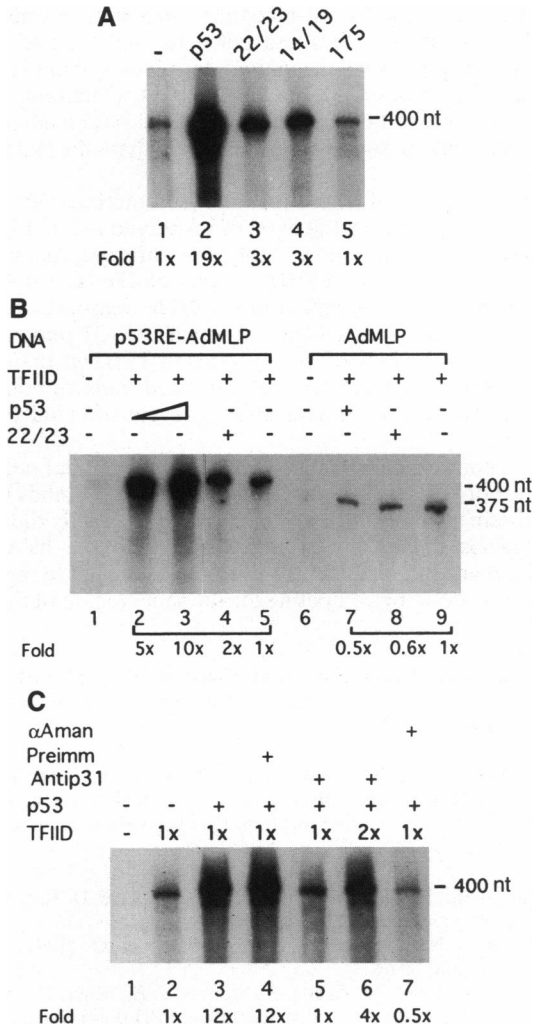

FIG. 6. hTAF$_{II}$31 mediates transcriptional activation by p53. (*A*) The *in vitro* transcription assay was performed as described in *Materials and Methods*. In addition to basal transcription factors and RNAPII, 100 ng of the indicated p53 protein, and 0.5 μg of p53REΔ-50 DNA were used in the reaction. The synthesized RNA of 400 nt was detected by autoradiography. Fold, increases in RNA synthesis are compared with the control (lane 1), to which no p53 was added. (*B*) The reaction was carried out with two different DNA templates: p53RE-containing (lanes 1–5) and p53RE-free (lanes 6–9) AdMLP promoters. Where indicated, 30 ng (lane 2) or 60 ng (lanes 3 and 7) of wild-type p53 or 60 ng of the 22/23 mutant (lanes 4 and 8) were present. For lanes 2–5 and 7–9, 50 ng of purified eTFIID as shown in Fig. 3*A* was employed. Quantitation was done by phosphor imager. (*C*) Purified anti-hTAF$_{II}$31 antibodies (60 ng), pre-immune IgGs (Preimm; equivalent amounts) were preincubated with eTFIID (1× and 2× are 60 ng and 120 ng). The mixtures were then added to transcription mixtures. The reaction was started by addition of nucleotides. For lane 7, α-amanitin (αAman, 0.02 μg/ml) was present during the incubation.

DISCUSSION

hTAF$_{II}$31 cDNA, the human homolog of dTAF$_{II}$40 cDNA (26), has been identified and the protein it encodes has been shown to be a subunit of TFIID. By immunoselecting an epitope-tagged TBP expressed in HeLa cells (29), the associated factors were purified and hTAF$_{II}$31 was shown to copurify with other TAFs in this complex. Several lines of evidence demonstrate that TAF$_{II}$31 interacts with the p53 transcription factor. p53 and TAF$_{II}$31 in solution associate and are coimmunoprecipitated by anti-p53 monoclonal antibodies. Mutations in residues 14/19 or 22/23 of the amino terminus of p53 disrupt the association of p53 with TAF$_{II}$31 in solution. These same double mutations reduce or eliminate the p53 protein's ability to act as a transcription factor *in vitro* and *in vivo* (6). These mutations have no effect upon the ability of p53 to bind to its sequence-specific DNA recognition site and they do not alter the conformation of p53 as measured by conformation-sensitive monoclonal antibodies (6). Similarly, GST-hTAF$_{II}$31

protein columns specifically recognized the amino terminus of the p53 protein (aa 1–92) and failed to bind (or had much reduced binding) with the amino terminus containing the 14/19 or 22/23 mutations. The 22/23 and 14/19 mutants of the p53 protein, which fail to bind to $hTAF_{II}31$, and mediated *in vitro* transcription significantly less well than the wild-type protein.

The relevance of these protein interactions for p53-mediated transcriptional activation was demonstrated by the observation that polyclonal antibodies directed against the $hTAF_{II}31$ protein in the TFIID complex blocked the ability of p53 to stimulate transcription *in vitro*. The reason to believe that the antibodies acted directly on $hTAF_{II}31$ and not by general steric blocking of other factors in TFIID is that these antibodies inhibited only the p53-simulated transcription. The TFIID basal level of transcription was not affected by the antibodies.

It is of considerable interest that the major cellular negative regulator of p53 activity, the Mdm-2 protein (15), binds to the p53 protein at residues 14/19 and 22/23 (6), exactly the same amino acids important for interaction with the $hTAF_{II}31$ protein. It appears then that two proteins designed to regulate p53 activity do so by competing for the same region of the p53 protein.

Note. After this paper was completed, Thut *et al.* (38) published similar results of experiments utilizing the TAFs from *Drosophila* or fusion proteins with $hTAF_{II}31$.

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