Stabilization and Activation of p53 by the Coactivator Protein TAF\textsubscript{II}31*

Received for publication, August 30, 2000, and in revised form, January 23, 2001
Published, JBC Papers in Press, February 1, 2001, DOI 10.1074/jbc.M007955200

Thomas Buschmann‡, Yahong Lin§, Nadia Aithmitti§, Serge Y. Fuchs‡, Hua Lu†, Lois Resnick-Silverman†, James J. Manfredi‡, Ze’ev Ronai‡, and Xiangwei Wu**

From the ‡Derald H. Ruttenberg Cancer Center, Mount Sinai School of Medicine, New York, New York 10029, the §Department of Biochemistry and Molecular Biology, Oregon Health Sciences University, Portland, Oregon 97201, and the ¶Huffington Center on Aging and Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas 77030

Regulation of the stability of p53 is key to its tumor-suppressing activities. mdm2 directly binds to the amino-terminal region of p53 and targets it for degradation through the ubiquitin-proteasome pathway. The coactivator protein TAF\textsubscript{II}31 binds to p53 at the amino-terminal region that is also required for interaction with mdm2. In this report, we demonstrate that expression of TAF\textsubscript{II}31 inhibits mdm2-mediated ubiquitination of p53 and increases p53 levels. TAF\textsubscript{II}31-mediated p53 stabilization results in activation of p53-mediated transcriptional activity and leads to p53-dependent growth arrest in fibroblasts. UV-induced stabilization of p53 coincides with an increase in p53-associated TAF\textsubscript{II}31 and a corresponding decrease in mdm2-p53 interaction. Non-p53 binding mutant of TAF\textsubscript{II}31 fails to stabilize p53. Our results suggest that direct interaction of TAF\textsubscript{II}31 and p53 not only mediates p53 transcriptional activation but also protects p53 from mdm2-mediated degradation, thereby resulting in activation of p53 functions.

Mutation of the p53 tumor suppressor gene is frequently associated with different forms of human cancers. The ability of p53 protein to suppress tumor growth is attributed to two major biological processes induced by p53: cell cycle arrest and apoptosis (reviewed in Refs. 1–3). Regulation of p53 protein stability plays a pivotal role in modulating p53 functions (4, 5). Under normal conditions, the half-life of p53 is limited to minutes. Cellular stress or DNA damage leads to a rapid stabilization of p53 protein and activation of p53-mediated checkpoint functions. Several proteins are known to affect p53 stability through protein-protein interactions, including HPV16 E6 (6), WT-1 (7), E1B 55K/E4orf6 (8), SV40 T antigen (9), JNK (11), HIF (12), p19ARF (13), p300 (14), and the cellular oncoprotein mdm2 (15, 16). Whereas mdm2, JNK, and E6 have been implicated in targeting p53 for degradation, WT1, E1B 55K/E4orf6, SV40T antigen stabilize p53 leading to increases in p53 levels. mdm2 functions as a pivotal p53 regulator and inhibits p53 functions by dual mechanisms. Interaction of mdm2 with p53 at its transactivation domain directly inhibits p53-mediated transcriptional activity (17, 18). Binding of mdm2 to p53 also targets p53 for degradation through ubiquitin-mediated pathways (19, 20). As one of p53 targets, expression of mdm2 is transcriptionally regulated by p53 (21), resulting in a feedback loop that regulates the level and activity of p53 (21). The biological implication of this regulatory loop is evident in that amplification of mdm2 gene has been found in about 30% of human soft tissue sarcomas and in other human cancers (22, 23), suggesting that negative regulation of p53 by mdm2 is important in the development of those tumors. Loss of mdm2, on the other hand, results in an up-regulation of p53 activity and subsequent aberration in cell cycle control and lethality in mice (24, 25).

As a transcription factor, the p53 protein activates transcription of target genes by binding RNA polymerase II complex. Two of the TATA-binding polypeptide (TBP)-associated factors (TAFs) within the general transcription factor TFIIID complex, TAF\textsubscript{II}31 and TAF\textsubscript{II}70, have been shown to interact with the amino-terminal activation domain of p53 directly in vitro (26, 27). It is generally believed that TBP mediates only basal transcription, whereas the TAF-TBP complex (TFIID) can mediate specific transcription factor-directed transactivation (28). Although the in vivo requirement for TAFs in p53-mediated transcription activation remains unclear, the fact that TAF\textsubscript{II}31 and TAF\textsubscript{II}70 support p53-mediated transcriptional activation in reconstituted transcription reactions suggests a critical role for TAFs in p53-mediated transactivation in vivo.

The observation that both TAF\textsubscript{II}31 and mdm2 bind to the same amino-terminal region of p53 suggests that TAF\textsubscript{II}31 may play an important role in regulating p53 protein stability and functions. To test this hypothesis, we have measured the effect of TAF\textsubscript{II}31 on ubiquitination and stability of p53 both in vivo and in vitro using either purified protein components or whole cell extracts (11). Our results showed that TAF\textsubscript{II}31 enhances p53 stability by inhibiting mdm2-mediated p53 ubiquitination and degradation. Expression of TAF\textsubscript{II}31 also induces p53-dependent transcriptional activation and cell cycle inhibition, suggesting that TAF\textsubscript{II}31 plays an active role in regulating p53 stability and activity in cells.

EXPERIMENTAL PROCEDURES

Cell Lines, Plasmids, and Transfection—All cells were maintained in Dulbecco’s modified Eagle’s medium, supplemented with 10% fetal bovine serum in 5% CO\textsubscript{2} incubators at 37 °C. The human TAF\textsubscript{II}31 expres-
sion vector was constructed by cloning human TAF\textsubscript{II31} cDNA into the pMTN expression vector under the control of SV40 promoter. The HA tag was engineered at the amino terminus. Mutant TAF\textsubscript{II31} was generated by polymerase chain reaction and constructed into pMTN with HA tag. The mdm2 and p53 expression plasmids have been described elsewhere (19). The mdm2 reporter plasmids were generated previously (39). Transfections were carried out using Fugene or N-[1-(2,3-diole- 

yloxy)propyl]-N,N,N-trimethyl ammonium methyl sulfate transfection reagent according to the manufacturer’s protocol (Roche Molecular Biochemicals). In transient transfection experiments, cells were harvested 48–72 h after transfection.

**Immunoprecipitation, Western, and Northern Blotting—**Cells were lysed in lysis buffer (20 mM HEPES, pH 7.5, 350 mM NaCl, 25% glycerol, 0.5% Nonidet P-40, 1 mM sodium vanadate, 0.5 mM phenylmethylsulfonyl fluoride, 1 \(\mu\)g/ml aprotinin, leupeptin, and leupeptin) by sonication on ice. Lysates were clarified by centrifugation at 14,000 \(\times\) g for 15 min at 4 °C. The protein concentrations were determined, and aliquots were stored at −80 °C.

For immunoprecipitation, equal amounts of lysates were immunoprecipitated using a monoclonal antibody against p53, pAb421, and protein A-agarose (Life Technologies, Inc.) for 16–24 h. The complex was washed three times with lysis buffer and subjected to SDS-polyacrylamide gel electrophoresis analysis. The proteins were transferred to a Hybond C membrane (Amersham Pharmacia Biotech), blotted by a rabbit polyclonal antibody against p53 (Santa Cruz), and developed using ECL (Amersham Pharmacia Biotech). Endogenous TAF\textsubscript{II31} was detected using a monoclonal antibody against TAF\textsubscript{II31}. mdm2 was detected by monoclonal antibody 2A10.

For Northern analysis, equal quantities of total RNA isolated by RNAzol (Life Technologies, Inc.) at 10–20 \(\mu\)g/sample were subjected to electrophoresis on a denaturing 1% formaldehyde agarose gel. The RNA was transferred to a nylon membrane and hybridized with \(^{32}\)PdCTP-labeled probes using the Rapid Hybridization System (Amersham Pharmacia Biotech).

**Pulse-Chase Labeling—**10.1 cells growing in 10-cm plates were transfected with p53, mdm2, and TAF\textsubscript{II31}. A protein labeling mixture containing \(^{35}\)Smethionine (50 \(\mu\)Ci; Amersham Pharmacia Biotech) was added to the cells 48 h after transfection and incubated for 15 min. The label was removed, and fresh media were added. The cells were harvested at 0, 1, and 2 h. The p53 protein was detected as described (11).

**In Vitro and in Vivo Ubiquitination Assays—**Baculovirus-expressed mdm2 and histidine-tagged p53 proteins were obtained as described (19). The TAF\textsubscript{II31} protein was produced by \textit{in vitro} transcription and translation (Promega). Ubiquitination assays were performed using lysis buffer and cytoplasmic lysates depleted of mdm2 and JNK that provide the necessary components. The mdm2 and/or TAF\textsubscript{II31} (in 1-ng quantities) were incubated on ice with bacterially expressed p53 (1–5 \(\mu\)g) bound to nickel-nitrotriacetic acid beads for 45 min. After extensive washes (four times with 1 mL of kinase buffer), the substrate-bound beads were equilibrated with 1× ubiquitination buffer (50 mM Tris-HCl, pH 8.0, 5 mM MgCl\(_2\), 0.5 mM dithiothreitol, 2 mM NaF, and 3 mM okadaic acid) with incubated in the same buffer supplemented with \(2 \times\) ATP, 10 mM creatine phosphate, 0.02 unit creatine phosphokinase, 2 \(\mu\)g of Ub HA, 1.5 mM ATP\(_{S}\) (Sigma), and 33% RL (w/v) in a total volume of 30 \(\mu\)l at 30 °C for 5 min. The reaction was stopped by adding 0.5 mL of 8 M urea in sodium phosphate buffer (pH 6.3) with 0.1% of Nonidet P-40. The beads were washed three times with the stop buffer and once with PBS supplemented with 0.5% Triton X-100, and the protein moiety was eluted with Laemmli sample buffer at 100 °C and subjected to Western blotting as described (11). For the \textit{in vivo} ubiquitination assay, the cells were transfected with his-p53 (1 \(\mu\)g), mdm2 (3 \(\mu\)g), TAF\textsubscript{II31} (3 \(\mu\)g), and HA-ubiquitin (3 \(\mu\)g). The cells were treated with MGL32 (10 \(\mu\)M, CalBiochem) for 12 h at 48 h after transfection and lysed in kinase buffer. The p53-ubiquitin complex was precipitated by nickel-nitrotriacetic acid beads and analyzed on SDS-polyacrylamide gel electrophoresis as described (19).

**Reporter Assay—**Saos-2 cells were transfected using the N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethyl ammonium methyl sulfate liposomal transfection reagent (Roche Molecular Biochemicals) as follows. Cells were plated in well of 6-well dishes and incubated overnight. Cells were fed with Dulbecco’s modified Eagle’s medium containing 10% FBS and incubated for an additional 3 h. N-[1-(2,3-dioleyloxy)propyl]-N,N-trimethyl ammonium methyl sulfate/DNA mixtures containing appropriate amounts of reporter constructs and expression plasmids were prepared according to the manufacturer’s instructions and incubated at room temperature for 15 min. Serum-free medium was then added to the mixtures and used to replace the medium in the wells. The dishes were incubated at 37 °C for 3 h, after which the transfection mix was removed and replaced with Dulbecco’s modified Eagle’s medium containing 10% FBS. After 48 h, the 6-well plates were placed on ice and washed once with PBS. The cells were then lysed by scraping into 120 \(\mu\)l of reporter buffer (Promega Luciferase Assay System), and supernatants were spun for 1 min at 14,000 rpm at 4 °C. The total protein concentration was determined using a commercially available assay (Bio-Rad). Of each sample, 40 \(\mu\)l were warmed to room temperature and mixed with Luciferase Assay Buffer (Promega). Light emission was determined in a TD-20e luminometer (Turner).

**Flow Cytometry Analysis—**The flow cytometry were performed as described (30). Briefly, cells were transfected with 2 \(\mu\)g of green fluorescence protein expression plasmid pEGFP-N (CLONTECH) and 10.0 \(\mu\)g of either control DNA (empty vector), or p53, or TAF\textsubscript{II31} expressing plasmid. Transfected cells were harvested 48–72 h later and washed twice in PBS. The cells were fixed in paraformaldehyde in PBS for 10 min and permeabilized in 70% ethanol for 16 h at 4 °C. Cells were washed two times with PBS containing 5 \(\mu\)g of propidium iodide and RNase A (5 \(\mu\)g/ml). Flow cytometric analysis was carried out in a fluorescence-activated cell sorting analyzer (Coulter). GFP positive cells were gated and analyzed for DNA content.

**RESULTS**

**Stabilization of p53 in Vivo by TAF\textsubscript{II31}—**As a first step to address the possible role of TAFs in regulating p53 stability, mammalian expression vectors encoding human TAF\textsubscript{II31} and p53 were transfected into 10.1 mouse Balb/c 3T3 fibroblast cells that lack endogenous p53 (31). Co-transfection of mdm2 and p53 decreased expression levels of p53 as a result of its destabilization, as previously reported (15, 16) (Fig. 1A). Expression of TAF\textsubscript{II31} in 10.1 cells increased the levels of p53 protein by 2-fold (Fig. 1A). Furthermore, forced expression of TAF\textsubscript{II31} attenuated mdm2-mediated p53 degradation in a dose-dependent manner (Fig. 1A). TAF\textsubscript{II31} did not change mRNA levels of p53 (Fig. 1A), suggesting that the elevation of p53 levels by TAF\textsubscript{II31} was not due to increased transcription of the p53 gene. In a duplicate experiment, expression levels of TAF\textsubscript{II31} protein in the transfection experiment were confirmed by Western analysis (Fig. 1A). Pulse-chase labeling with \(^{35}\)Smethionine revealed that the half-life of p53 was extended in cells expressing TAF\textsubscript{II31} (Fig. 1B), supporting the notion that induction of p53 levels by TAF\textsubscript{II31} is due to enhanced p53 stability. To determine whether TAF\textsubscript{II31} mediates its effect on p53 through mdm2 or through an independent mechanism, p53 and TAF\textsubscript{II31} were cotransfected into a p53/mdm2 double null cell line. The results demonstrated that p53 levels are not affected by the expression of TAF\textsubscript{II31} in p53/mdm2 null cells, indicating that stabilization of p53 by TAF\textsubscript{II31} is mdm2 dependent (Fig. 1C). Because binding of p53 by mdm2 is required for mdm2-mediated p53 degradation (15, 16), these results suggest that TAF\textsubscript{II31} either out-competes mdm2 for binding to p53 or excludes mdm2 from the p53 complex, thus preventing mdm2-mediated p53 degradation.

**TAF\textsubscript{II31} Blocks mdm2-mediated Ubiquitination of p53—**It has been shown that mdm2 targets p53 for ubiquitination, and we demonstrated that TAF\textsubscript{II31} inhibits mdm2-mediated degradation of p53. To extend our finding that the effect of TAF\textsubscript{II31} on p53 is at the level of ubiquitination, an \textit{in vitro} ubiquitination assay was used to determine the degree of p53 ubiquitination after expression of TAF\textsubscript{II31} (19). In this assay nickel bead-bound histidine-tagged p53 (his-p53) was incubated with TAF\textsubscript{II31} and the targeting protein mdm2. Subsequently, the ubiquitination reaction was carried out using reticulocyte lysates depleted of mdm2 and JNK (Fig. 2A). The addition of
Vivo cells were pulse-labeled with [35S]methionine for 10 min and chased at TAFII31. 10.1 cells were transfected with p53, TAFII31, and mdm2. The Bclonal antibody against HA epitope. attenuation of mdm2 on p53 ubiquitination. and coexpression of TAFII31 blocked mdm2-mediated ubiquitination of p53 in vitro (Fig. 2B). These data indicate that TAFII31 attenuates the effect of mdm2 on p53 ubiquitination.

To determine the effect of TAFII31 on p53 ubiquitination in vitro, mdm2, TAFII31, and his-p53 expression plasmids were cotransfected into Balb/c 3T3 fibroblasts in various combinations together with the HA-tagged ubiquitin expression vector. After treatment with proteasome inhibitor MG132, the transfected p53 was purified on nickel beads and detected by immunoblotting with pAb421 antibody to p53. The degree of polyubiquitinated p53 was measured using polyclonal anti-HA antibody. The p53 ubiquitination was measured using immunoblot probed with anti-HA antibody. The p53-ubiquitin is represented as smears of more slowly migrating proteins in the upper part of the membrane. Consistent with the p53 ubiquitination by TAFII31 in vitro, 10.1 cells were transfected his-p53, p53, and TAFII31 (3 μg) in various combinations. The p53 complex was captured on nickel beads, and p53-ubiquitin was detected by Western blot against HA. Levels of p53 were determined using antibody against p53 on a Western blot.

TAFII31 and p53 Interaction Is Required for p53 Stabilization—To test whether TAFII31-p53 interaction is required for the ability of TAFII31 to stabilize p53, we generated mutant TAFII31 truncated at either the amino or carboxyl terminus. The TAF-C expresses amino acids 130–264 of TAF II31 and is mdm2-dependent. Immortalized fibroblast cells from p53/mdm2 double knockout mice were cotransfected with p53 expressing plasmid (1 μg) and TAFII31 (2–10 μg). The levels of p53 were detected by immunoprecipitation-Western blotting.
by TAFII31, we first analyzed p53 transcriptional activity using a luciferase reporter containing p53 binding sites from the human p21/WAF-1 gene (29). Transfection of TAF II31 did not alter the basal transcriptional activity of the reporter containing p53 binding sites from the human p21/WAF-1 gene (29). Transfection of TAF II31 did not alter the basal transcriptional activity of the reporter containing p53 binding sites (Fig. 4 A), whereas cotransfection of p53 and TAFII31 super-activated the reporter activities in a dose-dependent manner (Fig. 4A). The synergetic activation of the p53 reporter gene by TAFII31 may be attributed to its dual effects on p53 stability and its function as a p53 coactivator. The effect of TAFII31 mutants on p53-mediated transcription activation was also tested in similar assays. In contrast to the wild type protein, both mutants inhibited p53-activated transcription (Fig. 4B). Furthermore, these mutants inhibit wild type TAFII31-mediated p53 transcription activation (Fig. 4B). Because TAFII31 functions as a bridge to link p53 to the transcriptional machinery (26, 27), our data suggest that expression of TAF mutants disrupts the formation of p53 transcription complex and thus blocks p53-mediated transcriptional activity.

**Activation of p53-mediated Transcriptional Activation by TAFII31**—Stabilization of p53 in cells is expected to result in activation of p53 downstream pathways, including activation of p53-mediated transcription activity, inhibition of cell cycle progression, and/or activation of p53-mediated apoptosis. To elucidate the biological implications of p53 stabilization mediated by TAFII31, we first analyzed p53 transcriptional activity using a luciferase reporter containing p53 binding sites from the human p21/WAF-1 gene (29). Transfection of TAFII31 did not alter the basal transcriptional activity of the reporter containing p53 binding sites (Fig. 4 A), whereas cotransfection of p53 and TAFII31 super-activated the reporter activities in a dose-dependent manner (Fig. 4A). The synergetic activation of the p53 reporter gene by TAFII31 may be attributed to its dual effects on p53 stability and its function as a p53 coactivator. The effect of TAFII31 mutants on p53-mediated transcription activation was also tested in similar assays. In contrast to the wild type protein, both mutants inhibited p53-activated transcription (Fig. 4B). Furthermore, these mutants inhibit wild type TAFII31-mediated p53 transcription activation (Fig. 4B). Because TAFII31 functions as a bridge to link p53 to the transcriptional machinery (26, 27), our data suggest that expression of TAF mutants disrupts the formation of p53 transcription complex and thus blocks p53-mediated transcriptional activity.

**Forced Expression of TAFII31 Induces p53-dependent Growth Suppression**—To further explore the consequences of activation of p53-mediated transcription by TAFII31, we used colony formation assay to monitor whether expression of TAFII31 could affect cell growth (Fig. 4C and Table I). Transfection of p53 into either 12.1 cells (p53 wild type) or 10.1 cells (p53-null) resulted in a 8-fold reduction in plating efficiency, as a result of p53-mediated growth arrest in both cell types. Transfection of TAFII31 into 12.1 cells resulted in a 7-fold decrease in colony formation. This effect was not seen in 10.1 cells. These data suggest that the ability of TAFII31 to reduce the number of colonies formed is mediated by p53. Fluorescence-activated cell sorter analysis of cell transiently transfected with TAFII31 further confirmed this finding. Expression of TAFII31 causes accumulation of cells in G1 and reduction of cells in S phase of the cell cycle in a p53-dependent manner (Fig. 4D). These results demonstrate that expression of TAFII31 leads to activation of p53-mediated growth arrest.

**Association of TAFII31 with p53 in Cells after UV Treatment**—Because forced expression of TAFII31 leads to stabilization of p53 and subsequent growth suppression, the association of p53 and TAFII31 in cells was then explored. Analysis of the p53-TAFII31 complex was carried out in Swiss3T3 cells before and after DNA damage (UV irradiation). Immunoprecipitation using monoclonal antibodies against p53 and immunoblot with antibodies to TAFII31 identified TAFII31 bound to p53 after UV exposure. The amount of TAFII31 protein associated with p53 was proportional to the levels of p53, whereas the levels of mdm2 associated with p53 were inversely correlated with those of TAFII31 (Fig. 5). TAFII31 was in a complex with p53 in untreated cells, because TAFII31 was immunoprecipitated by anti-p53 antibody at time 0 where low levels of p53 is expressed. This band is specific, because it is not present in 10.1 cells which lack p53 (data not shown). The increasing TAFII31 associated with p53 after UV was not due to an increase in TAFII31 levels as a result of UV exposure, because the total TAFII31 protein did not change before and after UV treatment. These results suggest that TAFII31 contributes to the stabilization of p53 after DNA damage.
We have demonstrated in this study that expression of the coactivator protein TAFII31 contributes to the regulation of p53 stability through mdm2-mediated p53 ubiquitination pathway. Stabilization of p53 by TAFII31 leads to functional activation of p53-mediated transcriptional activation and induction of cell cycle inhibition, suggesting that TAFII31 contributes positively to p53 functions in cells. Although most of the experiments in this study are performed in transient transfection assays which result in overexpression of proteins, the fact that there is a positive correlation between endogenous p53 stabilization and p53-TAFII31 association after DNA damage (Fig. 5) suggests that TAFII31 plays an important role in the regulation of p53 activity in vivo. This is consistent with the observation that expression of TAFII31 induces growth arrest mediated by endogenous p53 (Fig. 4, C and D, and Table I).

The mdm2 protein regulates both the activity and levels of p53 through a negative feedback loop. The TAFII31-p53 interaction, on the other hand, seems to form a positive loop to activate p53 and its downstream pathways. It appears that TAFII31 can directly inhibit mdm2-p53 interaction and stabilize p53 as suggested in the in vitro ubiquitination assay (Fig. 2A). It is further supported by the fact that p53-TAFII31 interaction is required for TAFII31-mediated p53 stabilization (Fig. 3). As a transcription coactivator, TAFII31 may recruit p53 to the transcription complex (26, 27), which leads to transcriptional activation of p53 as seen in Fig. 4A. The recruitment of p53 to the transcription complex by TAFII31 may also protect p53 from the mdm2-mediated degradation. These properties of TAFII31 reinforce its function as coactivator of p53. Although the mechanistic basis of regulation of p53 function by endogenous TAFII31 requires additional investigation, the finding that the effect of TAFII31 on p53 requires protein-protein interactions suggests that TAFII31 may directly compete with mdm2 for p53 binding, and a delicate balance between mdm2 and TAFII31 determines the levels and stability of p53.

TAFII31 is part of the TFIID complex, containing TBP and many other TAFs. It has been shown that TAF-TAF interactions are highly conserved to allow interchange between homologues of TAFs from different species in transcription reactions in vitro (26, 32). Sequence comparison of Drosophila dTAFII40 and human TAFII31 as well as dTAFII60 and TAFII70 showed that the amino-terminal region is highly conserved (data not shown), suggesting that this region may be used for TAF-TAF interactions. Consistent with this notion, it has been shown that the conserved amino-terminal domain of TAFII70 interacts with TBP, TAFII250, and TAFII31 (33). The fact that the carboxyl half of TAFII31 contains the p53 binding domain further supports this possibility (Fig. 3). Thus, TAFII31 functions as a bridge between p53 and the general transcription machinery using its carboxyl terminus to interact with p53 while binding to TFIID using its amino-terminal domain.

Although TAFII31 enhances p53-mediated transcription activation, the mutant TAFII31 lacking either amino or carboxyl p53 transciptional activity. All activities were normalized to reporter alone. C, TAFII31 suppresses colony formation in a p53-dependent manner. 10.1 (no p53) and 12.1 (wild type p53) cells were transfected with neo vector, p53, TAFII31 (2 μg each). The Geneticin-resistant colonies were counted 2–3 weeks after transfection and stained with the Giemsa stain. D, effect of p53 and TAFII31 expression on cell cycle progression. 10.1 and 12.1 cells were transiently transfected with control vector, p53, and TAFII31 DNA, as indicated. A GFP expressing plasmid was cotransfected to mark the transfected cells. The cells were subjected to fluorescence-activated cell sorter analysis, and GFP positive cells were analyzed for DNA content. Cell cycle distribution for each fluorescence-activated cell sorter was presented. The experiments were repeated, and data represent one set of the experiments.
TABLE I

| Transfected DNA             | 12.1 cells (p53 wild type) |          | 10.1 cells (p53-null) |          |
|----------------------------|----------------------------|----------|-----------------------|----------|
| neo vector                 | Expt 1                     | 41       | Expt 2                | 102      | Expt 3                | 93       | Mean    | 77       | Expt 1 | 32       | 100      | Expt 2 | 11        | 9        | Expt 3 | 12       | 28       | Mean    | 70       |         | 70       |         |
| neo vector + p53           | 3                          | 12       | 12                    | 9         | 3                     | 11       | 17           | 10       |
| neo vector + TAFp31        | 6                          | 15       | 14                    | 12        | 28                    | 72       | 57           | 52       |

**FIG. 5.** Association of TAFp31 with p53 in Swiss 3T3. Swiss 3T3 cells were treated with UV at 50J/m². The cells were harvested at the indicated time points. Lysates were immunoprecipitated by pAB421, and the membrane was blotted with anti-p53, anti-mdm2, or anti-TAFp31 antibodies. Purified TAFp31 served as a positive control (pos. cont.). Amount of TAFp31 in untreated and UV-treated cells were analyzed using whole cell extracts (WCE), and 100 μg quantities of lysates were subjected to Western blotting analysis using the anti-TAFp31 antibody. IP, immunoprecipitation; IB, immunoblot.

*Acknowledgments*—We thank Drs. Stuart Aaronson, David Sassoon, Z. Q. Pan, and J. Licht for helpful discussions, Dr. S. Jones at University of Massachusetts for the gift of p53/mdm2 double-null fibroblasts, and Dr. Z. Luo at Boston University for providing the pMTN plasmid.

REFERENCES

1. Oren, M., and Rotter, V. (1999) Cell Mol. Life Sci. 55, 9–11
2. May, P., and May, E. (1999) Oncogene 18, 7621–7636
3. Levine, A. J. (1997) Cell 88, 325–331
4. Ashcroft, M., and Vousden, K. H. (1999) Oncogene 18, 7637–7643
5. Oren, M. (1999) J. Biol. Chem. 274, 36031–36034
6. Huijbregtse, J. M., Scheffner, M., and Howley, P. M. (1991) EMBO J. 10, 4129–4135
7. Maheswaran, S., Engert, C., Bennett, P., Heinrich, G., and Haber, D. A. (1995) Genes Dev. 9, 2143–2156
8. Querido, E., Marcellus, R. C., Lai, A., Charbonneau, R., Teodoro, J. G., Ketner, G., and Brantley, P. E. (1997) J. Virol. 71, 3788–3788
9. Tiemann, F., Zerrahn, J., and Deppert, W. (1995) J. Virol. 69, 6115–6121
10. Reindolin, E., Kohler, M., Kraiss, S., Oren, M., and Montenarh, M. (1990) Oncogene 5, 137–145
11. Fuchs, S. Y., Adler, V., Buschmann, T., Yin, Z., Wu, X., Jones, S. N., and Ronai, Z. (1998) Genes Dev. 12, 2658–2663
12. An, W. G., Kanekal, M., Simon, M. C., Maltepe, E., Blagusicikov, M. V., and Neckers, L. M. (1998) Nature 392, 405–408
13. Zhang, Y., Xiong, Y., and Yarborough, W. G. (1998) Cell 92, 725–734
14. Grossman, S. R., Perez, M., Kung, A. L., Joseph, M., Mansur, C., Xiao, Z. X., Kumar, S., Howley, P. M., and Livingston, D. M. (1998) Mol. Cell 2, 405–415
15. Hupert, Y., Maya, B., Kazaz, A., and Oren, M. (1997) Nature 387, 296–299
16. Kubbutat, M. H., Jones, S. N., and Vousden, K. H. (1997) Nature 387, 299–303
17. Momand, J., Zambetti, G. P., Olsen, D. C., George, D., and Levine, A. J. (1992) Cell 69, 1237–1245
18. Oliner, J. D., Pietenpol, J. A., Thiagalingam, S., Gyrus, J., Kinzler, K. W., and Vogelstein, B. (1993) Nature 362, 857–860
19. Fuchs, S. Y., Adler, V., Buschmann, T., Wu, X., Jones, S. N., and Ronai, Z. (1998) Oncogene 17, 2543–2547
20. Honda, R., Tanaka, H., and Yasuda, H. (1997) FERS Lett. 420, 25–27
21. Wu, X., Bayle, J. H., Olsen, D., and Levine, A. J. (1998) Genes Dev. 7, 1126–1132
22. Oliner, J. D., Kinzler, K. W., Meltzer, P. S., George, D. L., and Vogelstein, B. (1992) Nature 358, 80–83
23. Cordon-Cardo, C., Llutes, E., Drobnjak, M., Oliva, M. R., Pollack, D., Woodruff, J. M., Marechal, V., Chen, J., Brennan, M. F., and Levine, A. J. (1994) Cancer Res. 54, 784–789
24. Jones, S. N., Roe, A. E., Donohue, L. A., and Bradley, A. (1995) Nature 378, 206–208
25. Montes de Oca, Luna, R., Wagner, D. S., and Lozano, G. (1995) Nature 378, 203–206
26. Thut, C. J., Chen, J. L., Klemm, E., and Tjian, R. (1995) Science 267, 100–104
27. Lu, H., and Levine, A. J. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 5154–5158
28. Berk, A. J. (1999) Curr. Opin. Cell Biol. 11, 330–335
29. Reznik-Silverman, L., St Clair, S., Maurer, M., Zhao, K., and Manfredi, J. J. (1998) Genes Dev. 12, 2102–2107
30. Relax, F., Wei, X., Li, W., Pan, J., Lin, Y., Bowtell, D. D., Sassoon, D. A., and Wu, C. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 2105–2110
31. Harvey, D. M., and Levine, A. J. (1995) Genes Dev. 5, 2375–2385
32. Chen, J. L., Attardi, L. D., Verrizier, C. P., Yokomori, K., and Tjian, R. (1994) Cell 79, 93–105
33. Hisatake, K., Ohta, T., Takada, Guermah, M., Horikoshi, M., Nakatani, Y., and Roeder, R. G. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 8195–8199
34. Martinez, E., Kundu, T. K., Fu, J., and Roeder, R. G. (1998) J. Biol. Chem. 273, 25781–25785
35. Wieczorek, E., Brand, M., Jaeg, X., and Tora, L. (1998) Nature 393, 187–191
36. Amrolia, P. J., Ramamurthy, L., Saluja, D., Tanese, N. J., and Mancini, D. E. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 10051–10056
37. Grant, P. A., Schietz, D., Pray-Grant, M. G., Steger, D. J., Reese, C. E., Yates, J. R., III, and Workman, J. L. (1998) Cell 94, 45–53
38. Mizzen, C. A., Yang, X. J., Kokubo, T., Brownell, J. E., Bannister, A. J., Owen-Hughes, T., Workman, J., Wang, L., Berger, S. L., Kouzarides, T., Nakatani, Y., and Allis, C. D. (1996) Cell 87, 1261–1270
39. Fuchs, S. Y., Adler, V., Pincus, M. R., and Ronai, Z. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 10541–10546

*p53-dependent suppression of cell plating efficiency by TAFp31*

Each transfection mixture contained 2 μg of neo vector, p53, TAFp31, and 5 μg of salmon sperm DNA as carrier. The geneticin-resistant colonies were counted 2–3 weeks after transfection. Expt, experiment.