MOLECULAR AND CELLULAR BIOLOGY, Nov. 1995, p. 6474–6478 Vol. 15, No. 11
0270-7306/95/$04.00+0
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Reversal of In Vitro p53 Squelching by both TFIIB and TFIID

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Received 11 May 1995/Returned for modification 8 June 1995/Accepted 13 August 1995

p53, the protein encoded by one of the most significant human tumor suppressor genes, is a sequence-specific transcriptional activator. When activated by a double-stranded DNA break, p53 function arrests cells in G_1 and can induce apoptosis. Transcriptional activation function is critical for p53 tumor suppression, although transcriptional repressing and nontranscriptional functions of p53 may contribute. p53 activation requires that it bind to TFIID through interactions with TATA box-binding protein (TBP)-associated factors and potentially with TBP. Here, we studied the mechanism of p53 activation using in vitro transcription and a sufficiently high p53 concentration to squelch activated transcription. Squelching is thought to result when target molecules that interact with activation domains are titrated by binding to excess activator. Addition of either excess TFIIB or TFIID but not other proteins required for p53-activated transcription reversed squelching by high p53 concentrations, whereas neither stimulated transcription in reactions without excess p53. These results reveal that both TFIIB and TFIID are inhibited by high concentrations of p53 and suggest that p53 activation may work through direct or indirect interactions with both TFIIB and TFIID.

p53 is encoded by one of the most significant human tumor suppressor genes (20, 21, 36). It functions as a typical sequence-specific transcriptional activator (8, 17) that binds as a tetramer (15) to four tandem, alternatively inverted copies of a 5-bp consensus sequence (4, 13). When activated by a double-stranded DNA break (27), p53 arrests cells in G_1 and can induce apoptosis (reviewed in reference 14). Transcriptional activation function is critical for p53 tumor suppression (26, 29, 38), although the transcriptional repressing (3, 24, 33) and nontranscriptional functions (2, 5, 37) of p53 may contribute. p53 activation requires that it bind to TFIID (22, 34) through interactions with TATA box-binding protein (TBP)-associated factors (TAFs) (34) and potentially with TBP (22, 25, 32, 35).

Transcriptional activators stimulate the assembly and function of an approximately ribosome-sized (4-MDa) preinitiation complex composed of RNA polymerase II, general transcription factors, and, from recent experiments with *Saccharomyces cerevisiae*, a multisubunit SRB or mediator complex associated with the carboxy-terminal heptapeptide repeat of the largest polymerase II (Pol II) subunit (18, 19, 23). Functional interactions of activators with components of the preinitiation complex or intermediary coactivators have been detected through the process of squelching. Squelching is thought to result when target molecules that interact with activation domains are titrated by binding to excess activator (30). Here, we studied the mechanism of p53 activation using in vitro transcription and a sufficiently high p53 concentration to squelch activated transcription. We determined the component(s) of the in vitro reaction that is titrated at high p53 concentrations by assaying protein fractions and purified proteins for their ability to relieve the inhibition observed at high p53 concentrations. These experiments revealed that both TFIIB and TFIID can reverse p53 squelching in the in vitro reaction. These observations are consistent with the model that p53 stimulates transcription through direct or indirect interactions with both TFIIB and TFIID.

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MATERIALS AND METHODS

Expression and purification of p53 and p53 deletion mutants. HeLa cells were infected with recombinant vaccinia virus expressing an epitope-tagged (9) p53 (VV-ep53), and p53 was purified from the nuclear extract of infected cells by binding to a matrix of monoclonal antibody specific for the epitope tag followed by elution with the epitope peptide (9), all as described before (39). A recombinant vaccinia virus expressing epitope-tagged p53 with a deletion of residues 1 to 92 (VV-ΔN92) was constructed from pTM-eΔN92 as described before (7). pTM-eΔN92 was constructed by PCR amplification of the sequence encoding residues 93 to 160 of p53 from pcDNA-p53 (22) with primers that introduced an *Nco*I site and the sequence encoding the epitope MYPYDVPDYA at the 5′ end (GACCATGGACCCATACGATGTTCCAGATTACGCTTCATCTTCTGTC CCTTCCCAG) and an *Nco*I site at the 3′ end (GGCCATGGCGCGGACGC GGG). The amplified DNA was cloned between the *Nco*I sites in pTM-ep53 (39). ΔN92 was purified from HeLa cells infected with VV-eΔN92 by the procedure described above for the purification of p53. ΔN160 was produced by in vitro transcription translation in a rabbit reticulocyte extract with pTM-ΔN160 as the template. pTM-ΔN160 was constructed by cloning the 720-bp *Nco*I-*Bam*HI fragment from pcDNA-p53 between the *Nco*I and *Bam*HI sites of pTM.1 (7).

In vitro transcription. Reaction mixes with HeLa cell nuclear extract contained 50 μg of protein in 50 μl of 60 mM KCl–12 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid)-KOH (pH 7.9)–6 mM $MgCl_2$–0.6 mM dithiothreitol–12% glycerol–0.5 mM nucleoside triphosphate mix–100 ng of p5RGCE4TCAT (19). Reaction mixes were incubated at 30°C for 60 min and assayed by primer extension as described before (1). In vitro transcription reaction mixes reconstituted with partially purified transcription factors were used at 50 μl and contained the same buffer and template concentrations plus 6 μg of fraction TFIIA, 75 ng of purified recombinant TFIIB (rTFIIB), 5 μg of fraction TFIIE/F Pol II, and 4 μg of fraction TFIID. Protein fractions TFIIA, TFIIE/F Pol II, and TFIID were prepared by chromatography on phosphocellulose and DEAE-Sepharose as described before (1). rTFIIB was prepared as described before (12). Epitope-tagged TFIID was purified as described before (1).

RESULTS

We established a p53-responsive in vitro transcription system with a synthetic target promoter containing five p53 binding sites (22) immediately upstream of the adenovirus E4 TATA box (p5RGCE4T) (see Fig. 2C). Epitope-tagged human p53 and an epitope-tagged deletion mutant of p53 lacking the N-terminal 92 amino acid residues, including the activation domain (10, 28, 31) (ΔN92), were purified from nuclear extracts of HeLa cells infected with recombinant vaccinia viruses expressing these proteins (Fig. 1A). The DNA-binding activities of the purified proteins were assayed by DNA coimmuno-

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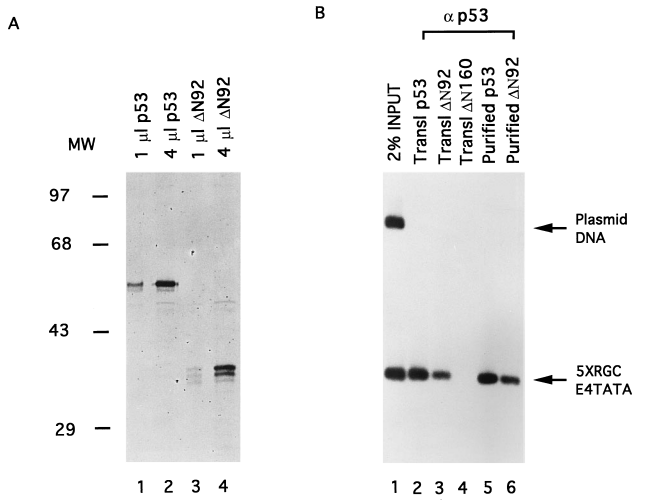

FIG. 1. Purified p53 proteins bind to specific DNA sites. (A) p53 (lanes 1 and 2) and ΔN92 (lanes 3 and 4) purified from nuclear extracts of HeLa cells infected with vaccinia virus vector VV-ep53 or VV-eΔN92, respectively, were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by silver staining. Sizes are shown in kilodaltons. (B) DNA immunoprecipitation assay. In vitro-translated p53 proteins (lanes 2 to 4) or purified p53 proteins (lanes 5 and 6) were incubated with an end-labeled DNA fragment containing five RGC p53 binding sites (16), the E4 TATA box (220 bp), and an end-labeled nonspecific DNA fragment (2.5 kb). p53 and its deleted forms were immunoprecipitated with monoclonal antibody pAb421 as described before (39), and the coprecipitating DNA was analyzed by agarose gel electrophoresis and autoradiography. A total of 2% of the DNA used in the binding reactions was analyzed in lane 1.

precipitation. pRGCE4T was cleaved and end labeled to produce (i) a DNA fragment containing five p53 binding sites and the E4 TATA box and (ii) a large fragment of the plasmid vector. These were incubated with either in vitro-translated (positive control) or purified p53 proteins and immunoprecipitated with an anti-p53 monoclonal antibody. Purified p53 and ΔN92 specifically immunoprecipitated the fragment containing p53 binding sites (Fig. 1B). As expected from earlier analyses of the p53 DNA-binding domain (4), further deletion of the p53 N terminus to amino acid 160 abolished DNA-binding activity (negative control [Fig. 1B, lane 4]). DNase I footprinting assays were also performed to establish the amount of purified protein required to completely occupy all five p53 DNA-binding sites in the p5RGCE4T template under transcription reaction conditions (data not shown). This amount of protein (~60 ng for p53 and ΔN92) is referred to as 1 footprinting unit.

Addition of increasing amounts of p53 to transcription reaction mixes with HeLa cell nuclear extract resulted in 10-fold stimulation of transcription over that observed in the absence of p53, whereas ΔN92 repressed transcription from the low level observed without added p53 (Fig. 2A). Purified p53 did not stimulate transcription from templates lacking p53 binding sites (data not shown). We conclude that the p53 activation domain is required to stimulate transcription in this in vitro system. Farmer et al. (8) also reported that purified p53 activates transcription in vitro and showed that p53 mutants defective in specific DNA binding fail to activate transcription.

Overexpression of activators in vivo leads to inhibition of transcription, a phenomenon known as squelching (30). Squelching is thought to result when target molecules required for activated transcription are titrated by binding to excess activator. To determine whether excess p53 could squelch transcription in this in vitro system, we added increasing amounts of p53 to the in vitro reaction mix (Fig. 2B). Maximum transcription was

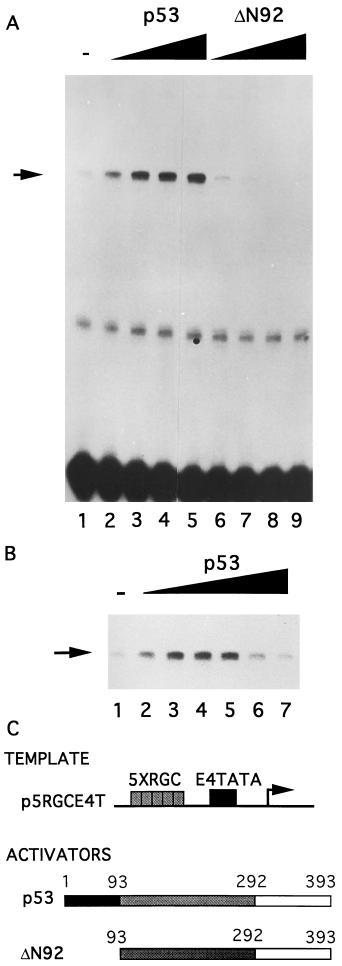

FIG. 2. Purified p53 squelches transcription at high concentrations. (A) In vitro transcription reactions in a HeLa cell nuclear extract with p5RGCE4T as the template. Lane 1, no added p53; lanes 2 to 5, 0.25, 0.5, 1, and 2 footprinting units (fpu), respectively, of purified p53 (1 fpu [60 ng of purified p53 or ΔN92] is just sufficient to fill the five p53 binding sites on the p5RGCE4T plasmid under transcription conditions, as assayed by DNase I footprinting); lanes 6 to 9, 0.25, 0.5, 1, and 2 fpu, respectively, of ΔN92. In this and subsequent in vitro transcription assays, specifically initiated RNA was analyzed by primer extension (arrow). (B) In vitro transcription reactions in HeLa cell nuclear extract. Lane 1, no added p53; lanes 2 to 7, 0.25, 0.5, 1, 2, 4, and 8 fpu, respectively, of p53. (C) Schematic diagrams of the p5RGCE4T template and the activators used in the in vitro transcription reactions. The solid, shaded, and open rectangles represent the p53 activation (10, 28, 31), DNA-binding, and tetramerization domains (reviewed in reference 4), respectively.

observed when sufficient p53 was added to saturate the p53 binding sites on the template (1 footprinting unit). However, when 4 or more footprinting units of p53 were added to the in vitro reaction mix, transcription was inhibited, suggesting that targets of the p53 activation domain were titrated at high p53 concentration (Fig. 3).

Addition of the factor(s) titrated by high concentrations of p53 to the squelched transcription reaction mix should reverse the inhibition by resupplying the titrated factor (Fig. 3). Consequently, we tested the ability of protein fractions that can support p53-activated transcription (see below) to reverse the inhibition caused by high p53 concentrations (Fig. 4). As before, addition of 0.5 or 1 footprinting unit of p53 resulted in stimulation of transcription, while addition of 4 footprinting units inhibited activated transcription (Fig. 4A, lanes 1 to 4). Addition of partially purified TFIIA and a TFIIE/F Pol II

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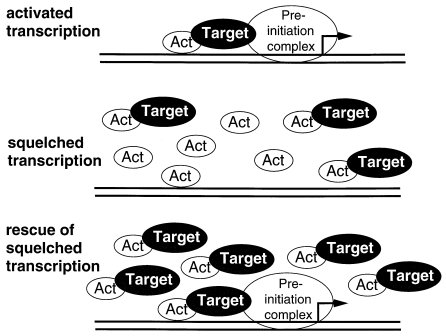

FIG. 3. Model for transcriptional squelching in vitro by excess activator and rescue of squelching by addition of the functional target of the activator. (Top) At the optimal activator (Act) concentration, activator bound to the test promoter interacts with a critical target molecule required for activated transcription. (Middle) At a high activator concentration, target molecules are sequestered by excess activators, and few target molecules are available to interact with the activator bound to the test promoter. (Bottom) Addition of sufficient target molecules to interact with all the activators restores activated transcription.

fraction (which also contains TFIIH [11]) failed to reverse p53 squelching. However, addition of either purified rTFIIB (12) or partially purified TFIID did reverse the inhibition, arguing that both TFIIB and a component of the partially purified

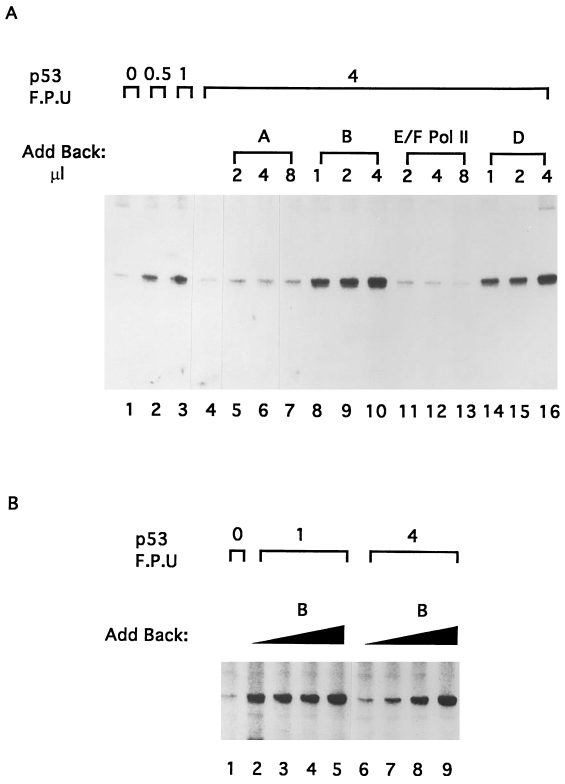

FIG. 4. Both purified rTFIIB and purified TFIID rescue p53-squelched transcription. (A) In vitro transcription reactions in a HeLa cell nuclear extract. Reaction mixes contained the indicated footprinting units (F.P.U.) of purified p53 (1 F.P.U. is ~60 ng). The reaction mixes in lanes 5 to 16 were supplemented with partially purified TFIIA (2 μg/μl), purified rTFIIB (75 ng/μl), TFIIE/F Pol II fraction (2.5 μg/μl), or partially purified TFIID (3 μg/μl). (B) TFIIB is not limiting before the addition of excess p53. Reactions were done with HeLa cell nuclear extract and the indicated amounts of purified p53. The reaction mixes in lanes 3 to 5 and 7 to 9 were supplemented with additional TFIIB as follows: 1 μl (75 ng) (lanes 3 and 7), 2 μl (lanes 4 and 8), or 4 μl (lanes 5 and 9).

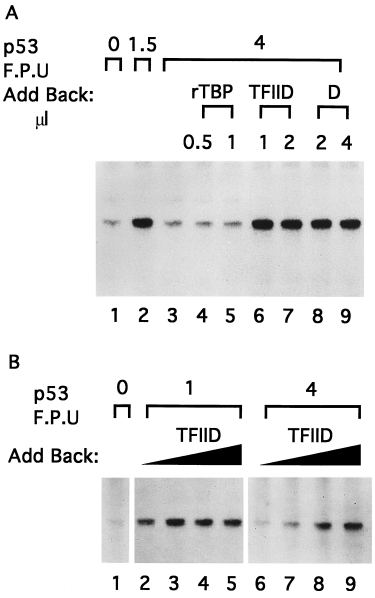

FIG. 5. Purified TFIID rescues p53 squelching. (A) In vitro transcription was performed with partially purified general transcription factors and rTFIIB. Reaction mixes contained the indicated amounts of purified recombinant TBP, purified epitope-tagged TFIID, or partially purified TFIID fraction (3 μg/μl). (B) TFIID is not limiting before the addition of excess p53. Transcription reactions were performed with partially purified factors and rTFIIB as in part A, with the indicated footprinting units (F.P.U.) of purified p53. The reaction mixes in lanes 2 and 6 contained no additional TFIID. Purified TFIID was added to the reaction mixes in lanes 3 and 7 (1 μl), 4 and 8 (2 μl), and 5 and 9 (4 μl). Different preparations of purified epitope-tagged TFIID were used in the experiments in parts A and B.

TFIID fraction are potential targets of the p53 activation domain.

To determine if TFIID was the component of the partially purified TFIID fraction that rescued p53 squelching, we performed further studies in a reconstituted transcription system. p53-activated transcription was observed with partially purified TFIIA, rTFIIB, a partially purified TFIIE/F Pol II fraction, and partially purified TFIID (Fig. 5A, lanes 1 and 2). The concentrations of protein fractions used in this and subsequent reconstituted in vitro transcription reactions were established in a preliminary series of transcription reactions. Each protein fraction (TFIIA, rTFIIB, TFIIE/F Pol II, and TFIID) was titrated in reactions with 1 footprinting unit of p53 and used at the concentration that was just sufficient to achieve maximal transcription. Under these conditions, addition of 4 footprinting units of p53 inhibited activated transcription (Fig. 5A, lane 3). Both partially purified (Fig. 5A, lanes 8 and 9) and extensively purified (Fig. 5A, lanes 6 and 7, and Fig. 6) TFIID rescued the squelched reaction. As expected, an equivalent number of purified recombinant TBP molecules (Fig. 6) could not rescue the squelched reaction because TFIID TAF subunits are required for p53 activation (34).

We considered the possibility that TFIIB or TFIID might be a limiting factor in the in vitro transcription reactions even before addition of excess p53. If this were the case, addition of these factors would increase both the activated (1 footprinting unit of p53) and squelched (4 footprinting units of p53) transcription reactions. However, addition of excess TFIIB (Fig. 4B) or TFIID (Fig. 5B) did not significantly stimulate the activated level of transcription observed with 1 footprinting unit of p53. This was the expected result for the reconstituted transcription reactions, since the conditions of the in vitro

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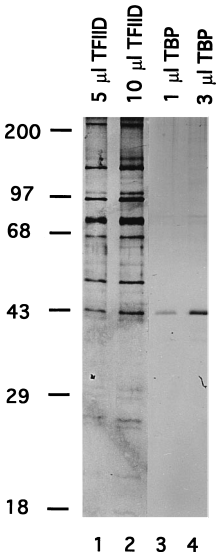

FIG. 6. Silver-stained gel of the purified TFIID and recombinant TBP preparations used in Fig. 5A. Molecular size markers are shown at the left (in kilodaltons).

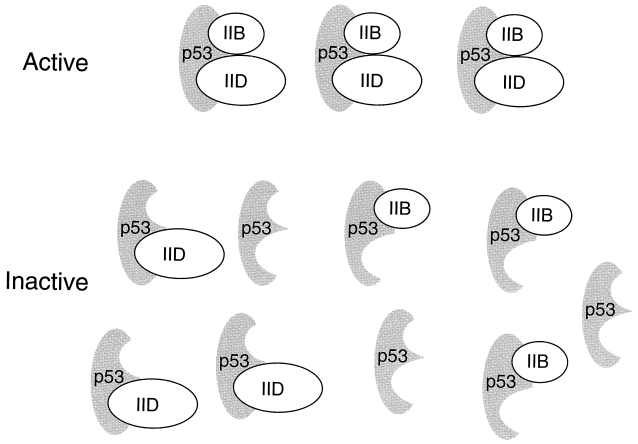

FIG. 7. Model for the rescue of p53 squelching by addition of either TFIIB or TFIID. A p53-TFIIB-TFIID complex, shown in the upper portion of the figure, is proposed to be required for p53 activation. At excess p53 concentrations, shown in the lower portion of the figure, inactive complexes in which p53 has bound to either TFIIB or TFIID but not to both are formed. The addition of TFIIB can produce active p53-TFIIB-TFIID complexes by the incorporation of TFIIB into the p53-TFIID complexes. Similarly, the addition of TFIID to p53-TFIIB complexes can form active p53-TFIIB-TFIID complexes.

reaction were established through a series of titrations so that none of the required protein fractions were limiting. While addition of excess TFIIB and TFIID did not significantly stimulate transcription in reactions with 1 footprinting unit of p53, as before, it did significantly stimulate the squelched transcription reactions with 4 footprinting units of p53 (Fig. 4B and 5B). Similar results were observed for addition of rTFIIB to squelched reactions with either nuclear extract (Fig. 4) or the reconstituted transcription system (data not shown). These results indicate that TFIIB and TFIID did not become limiting in these transcription reactions until transcription was squelched by excess p53. As diagrammed in Fig. 3, these results are consistent with a model for p53 activation in which p53 interacts directly or indirectly with both TFIIB and TFIID.

DISCUSSION

To identify the targets of p53 in its function as a transcriptional activator, we identified the activities that are titrated at high p53 concentrations in an in vitro transcription reaction. High concentrations of p53 inhibited p53-activated transcription (Fig. 2B). This phenomenon, which has been observed for a number of activators, is known as squelching (30). It is thought to occur when excess activators stably bind target molecules required for activation, titrating the target molecules so that none are available to interact with activator bound to the promoter being assayed (Fig. 3). In this model, the titrated target molecule can be determined by identifying a component of the transcription reaction that relieves the inhibition when its concentration is increased (Fig. 3, rescue of squelched transcription). When this strategy was applied to a p53-squelched reaction, either purified TFIIB (Fig. 4) or purified TFIID (Fig. 5) relieved the inhibition. It is significant that additional TFIIB or TFIID did not stimulate transcription at lower concentrations of p53. Consequently, TFIIB and TFIID did not become limiting until excess p53 was added. These results indicate that both TFIIB and TFIID are inhibited at high p53 concentration.

The observation that the transcriptional inhibition caused by excess p53 could be overcome by adding additional TFIIB or TFIID suggests that the inactivation is stoichiometric. This would occur if p53 formed a stable complex with TFIIB and TFIID, as diagrammed in Fig. 3. Earlier results from in vitro studies demonstrated that recombinant p53 can form a complex with extensively purified TFIID (22), probably through interactions with TBP (22, 25, 32, 35) and TAFs (34). However, no significant interaction was observed between purified recombinant p53 and TFIIB (22). A significant difference between this earlier study and the current analysis is that the inhibition of TFIIB activity observed in the current analysis was observed in an in vitro transcription reaction requiring multiple factors. It is possible that the inactivation of TFIIB activity observed in the current studies requires another factor in the in vitro transcription reaction that is present in excess so that it is not titrated at high p53 concentration. This could be another one of the characterized general transcription factors or an uncharacterized p53 coactivator. Consequently, the postulated interaction between p53 and TFIIB may be indirect, potentially requiring a bridging factor.

The results observed in these studies are strikingly different from those observed in similar studies with a Gal4-E1A activator (1). In the experiments with Gal4-E1A, only TFIID could rescue the squelched reaction. Addition of TFIIB had no effect. This difference raises the possibility that Gal4-E1A and p53 activate transcription by different mechanisms. It is significant that either TFIIB or TFIID can rescue p53 squelching. This result can be explained by the model shown in Fig. 7. In this model, p53 activation requires the formation of a p53-TFIID-TFIIB complex. As discussed above, this postulated complex may include other factors that are not titrated in our in vitro transcription reactions at high p53 concentrations. At high p53 concentrations, p53 molecules would be bound to complexes containing either TFIIB or TFIID, but few would bind to complexes containing both TFIIB and TFIID. In this situation, addition of TFIIB could fill the empty TFIIB site in complexes with TFIID, and addition of TFIID could fill the empty TFIID site in complexes with TFIIB. Consequently, addition of either TFIIB or TFIID would produce functional complexes containing both TFIIB and TFIID.

We tested whether purified p53, TFIID, and TFIIB would

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form a complex containing all three proteins in vitro under transcription reaction conditions and failed to observe such a complex (unpublished results). However, the formation of a stable complex containing the three proteins might require other factors present in the transcription reactions. Another possibility consistent with these results is that p53 targets both TFIIB and TFIID but cannot interact with these factors simultaneously. More complex models are also consistent with the data, including models in which a high concentration of TFIIB or TFIID drives the formation of an activated complex by mass action. However, the observation that both TFIIB and TFIID can rescue p53 squelching in these in vitro reactions suggests that TFIIB and TFIID are in some manner, direct or indirect, targets of the p53 activation mechanism. It is also possible that the inhibition of TFIIB activity by excess p53 may be unrelated to the mechanism of p53 activation. In some situations, p53 can inhibit transcription from promoters without a linked p53 binding site (3, 24, 33). This raises the possibility that the inhibition of TFIIB and TFIID observed in these studies is actually due to a p53 repressing function which is independent of p53's activation function.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant CA-25235 from the National Cancer Institute. X.L. was supported by a postdoctoral fellowship from the Damon Runyon-Walter Winchell Cancer Research Fund.

We thank Carol Eng for excellent technical assistance.

REFERENCES

1. **Boyer, T. G., and A. J. Berk.** 1993. Functional interaction of adenovirus E1A with holo-TFIID. Genes Dev. **7:**1810–1823.
2. **Caelles, C., A. Helmberg, and M. Karin.** 1994. p53-dependent apoptosis in the absence of transcriptional activation of p53-target genes. Nature (London) **370:**220–223.
3. **Chin, K. V., K. Ueda, I. Pastan, and M. M. Gottesman.** 1992. Modulation of activity of the promoter of the human MDR1 gene by ras and p53. Science **255:**459–462.
4. **Cho, Y., S. Gorina, P. D. Jeffrey, and N. P. Pavletich.** 1994. Crystal structure of p53 tumor suppressor-DNA complex: understanding tumorigenic mutations. Science **265:**346–355.
5. **Crook, T., N. J. Marston, E. A. Sara, and K. H. Vousden.** 1994. Transcription activation by p53 correlates with suppression of growth but not transformation. Cell **79:**817–827.
6. **Davison, B. D., J.-M. Egly, E. R. Mulvihill, and P. Chambon.** 1983. Formation of stable preinitiation complexes between eukaryotic class B transcription factors and promoter sequences. Nature (London) **301:**680–686.
7. **Elroy-Stein, O., T. R. Fuerst, and B. Moss.** 1989. Cap-independent translation of mRNA conferred by encephalomyocarditis virus 5′ sequence improves the performance of the vaccinia virus/bacteriophage T7 hybrid expression system. Proc. Natl. Acad. Sci. USA **86:**6126–6130.
8. **Farmer, G., J. Bargonetti, H. Zhu, P. Friedman, R. Prywes, and C. Prives.** 1992. Wild-type p53 activates transcription in vitro. Nature (London) **358:** 83–85.
9. **Field, J., J.-I. Nikawa, D. Broek, B. MacDonald, L. Rodgers, I. A. Wilson, R. A. Lerner, and M. Wigler.** 1988. Purification of a *ras*-responsive adenyl cyclase complex from *Saccharomyces cerevisiae* by use of an epitope addition method. Mol. Cell. Biol. **8:**2159–2165.
10. **Fields, S., and S. K. Jang.** 1990. Presence of a potent transcription activating sequence in the p53 protein. Science **249:**1046–1049.
11. **Flores, O., H. Lu, and D. Reinberg.** 1992. Factors involved in specific transcription initiation by mammalian RNA polymerase II: identification and characterization of factor IIH. J. Biol. Chem. **267:**2786–2793.
12. **Ha, I., W. S. Lane, and D. Reinberg.** 1991. Cloning of a human gene encoding the general transcription initiation factor IIB. Nature (London) **352:**689–695.
13. **Halazonetis, T. D., and A. N. Kandil.** 1993. Conformational shifts propagate from the oligomerization domain of p53 to its tetrameric DNA binding domain and restore DNA binding to select p53 mutants. EMBO J. **12:**5057–5064.
14. **Hartwell, L. H., and M. B. Kastan.** 1994. Cell cycle control and cancer. Science **266:**1821–1828.
15. **Jeffrey, P. D., S. Gorina, and N. P. Pavletich.** 1995. Crystal structure of the tetramerization domain of the p53 tumor suppressor at 1.7 angstroms. Science **267:**1498–1502.
16. **Kern, S. E., K. W. Kinzler, A. Bruskin, D. Jarosa, P. Friedman, C. Prives, and B. Vogelstein.** 1991. Identification of p53 as a sequence-specific DNA-binding protein. Science **252:**1708–1711.
17. **Kern, S. E., J. A. Pietenpol, S. Thiagalingam, A. Seymour, K. W. Kinzler, and B. Vogelstein.** 1992. Oncogenic forms of p53 inhibit p53-regulated gene expression. Science **256:**827–830.
18. **Kim, Y. J., S. Bjorklund, Y. Li, M. H. Sayre, and R. D. Kornberg.** 1994. A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. Cell **77:**599–608.
19. **Koleske, A. J., and R. A. Young.** 1994. An RNA polymerase II holoenzyme responsive to activators. Nature (London) **368:**466–469.
20. **Lane, D. P.** 1994. p53 and human cancers. Br. Med. Bull. **50:**582–599.
21. **Levine, A. J., A. Chang, D. Dittmer, D. A. Notterman, A. Silver, K. Thorn, D. Welsh, and M. Wu.** 1994. The p53 tumor suppressor gene. J. Lab. Clin. Med. **123:**817–823.
22. **Liu, X., C. W. Miller, P. H. Koeffler, and A. J. Berk.** 1993. The p53 activation domain binds the TATA box-binding polypeptide in holo-TFIID, and a neighboring p53 domain inhibits transcription. Mol. Cell. Biol. **13:**3291–3300.
23. **Lodish, H., D. Baltimore, A. J. Berk, S. L. Zipursky, P. Matsudaira, and J. Darnell.** 1995. Molecular cell biology, 3rd ed., p. 453–456. Scientific American Books, Inc., New York.
24. **Mack, D. H., J. Vartikar, J. M. Pipas, and L. A. Laimins.** 1993. Specific repression of TATA-mediated but not initiator-mediated transcription by wild-type p53. Nature (London) **363:**281–283.
25. **Martin, D. W., R. M. Munoz, M. A. Subler, and S. Deb.** 1993. p53 binds to the TATA-binding protein-TATA complex. J. Biol. Chem. **268:**13062–13067.
26. **Momand, J., G. P. Zambetti, D. C. Olson, D. George, and A. J. Levine.** 1992. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. Cell **69:**1237–1245.
27. **Nelson, W. G., and M. B. Kastan.** 1994. DNA strand breaks: the DNA template alterations that trigger p53-dependent DNA damage response pathways. Mol. Cell. Biol. **14:**1815–1823.
28. **O'Rourke, R. W., C. W. Miller, G. J. Kato, K. J. Simon, D. Chen, C. V. Dang, and H. P. Koeffler.** 1990. A potential transcriptional activation element in the p53 protein. Oncogene **5:**1829–1832.
29. **Pietenpol, J. A., T. Tokino, S. Thiagalingam, W. S. El-Deiry, K. W. Kinzler, and B. Vogelstein.** 1994. Sequence-specific transcriptional activation is essential for growth suppression by p53. Proc. Natl. Acad. Sci. USA **91:**1998–2002.
30. **Ptashne, M.** 1988. How eukaryotic transcriptional activators work. Nature (London) **335:**683–689.
31. **Raycroft, L., H. Wu, and G. Lozano.** 1990. Transcriptional activation by wild-type but not transforming mutants of the p53 anti-oncogene. Science **249:**1049–1051.
32. **Seto, E., A. Usheva, G. P. Zambetti, J. Momand, N. Horikoshi, R. Weimann, A. J. Levine, and T. Shenk.** 1992. Wild-type p53 binds to the TATA-binding protein and represses transcription. Proc. Natl. Acad. Sci. USA **89:**12028–12032.
33. **Shen, Y., and T. Shenk.** 1994. Relief of p53-mediated transcriptional repression by the adenovirus E1B 19-kDa protein or the cellular Bcl-2 protein. Proc. Natl. Acad. Sci. USA **91:**8940–8944.
34. **Thut, C. J., J. L. Chen, R. Klemm, and R. Tjian.** 1995. p53 transcriptional activation mediated by coactivators TAFII40 and TAFII60. Science **267:**100–104.
35. **Truant, R., H. Xiao, C. J. Ingles, and J. Greenblatt.** 1993. Direct interaction between the transcriptional activator domain of human p53 and the TATA box-binding protein. J. Biol. Chem. **268:**2284–2287.
36. **Vogelstein, B., and K. W. Kinzler.** 1992. p53 function and dysfunction. Cell **70:**523–526.
37. **Wagner, A. J., J. M. Kokontis, and N. Hay.** 1994. Myc-mediated apoptosis requires wild-type p53 in a manner independent of cell cycle arrest and the ability of p53 to induce p21waf1/cip1. Genes Dev. **8:**2817–2830.
38. **Yew, P. R., and A. J. Berk.** 1992. Inhibition of p53 transactivation required for transformation by adenovirus early 1B protein. Nature (London) **357:** 82–85.
39. **Yew, P. R., X. Liu, and A. J. Berk.** 1994. Adenovirus E1B oncoprotein tethers a transcriptional repression domain to p53. Genes Dev. **8:**190–202.

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