Direct Interaction Between the Transcriptional Activation Domain of Human p53 and the TATA Box-binding Protein*

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The human p53 tumor suppressor gene product can activate transcription by RNA polymerase II in the yeast, Saccharomyces cerevisiae, as well as in human cells. Several viral transcriptional activator proteins have been shown to directly contact TBP, the TATA box-binding subunit of the general initiation factor, TFIIID. In this report, we use protein affinity chromatography to show that the cellular transcription factor, p53, interacts directly and specifically with yeast TBP. The TBP binding domain of p53 was localized to its N-terminal 73 amino acids. This highly acidic portion of p53 functions as a transcriptional activation domain and is deleted in some tumors induced by the Friend leukemia virus. A human tumor-derived oncogenic point mutation of p53, which lies outside the activation domain of p53, but reduces its ability to activate transcription, greatly reduced the ability of p53 to bind yeast TBP in vitro. This mutation probably affects the overall conformation of the protein and indirectly interferes with the ability of p53 to contact TBP and activate transcription. In contrast, a mutated oncogenic form of p53 that is unaffected in its ability to activate transcription bound yeast TBP as well as wild type p53. The human TBP activity in a HeLa extract also bound to the activation domain of p53. Our data support a general model in which DNA-bound activator proteins activate transcription by interacting with TBP.

The human p53 tumor suppressor gene encodes a potent transcriptional activator that can function in both yeast and mammalian cells (1). p53 is thought to regulate cell growth negatively by regulating transcription during the cell cycle (for review, see Ref. 2). Wild type p53, and not mutant forms of p53 derived from tumors, also can inhibit the growth of the yeast Saccharomyces cerevisiae (3). Like other transcriptional activators (4), p53 therefore appears to have a modular domain structure; it contains a site-specific DNA binding domain near its C terminus (5) and a transcriptional activation domain within its N-terminal 73 amino acids (1). Insertions by the Friend leukemia virus that confer a tumorigenic phenotype in murine erythroleukemia cells often result in the expression of a truncated form of mouse p53, known as p44, which lacks its N-terminal activation domain (6). Mammalian derived point mutations in both human and murine p53, however, lie outside this defined activation domain (for review, see Ref. 7). Such mutations impair the ability of p53 to activate transcription in transfected cells (8) and usually affect the ability of p53 to bind to DNA (9). At least some of these mutations, however, also affect the ability of the p53 activation domain to function in both yeast and mammalian cells, probably because these point mutations change the overall conformation of p53 (8).

The N-terminal activation domain of p53 is similar in size, net negative charge, and transactivating potency to the well defined acidic activation domain of the herpes simplex virus virus protein 16 (VP16) (10). Like VP16, and a number of other transactivators (4), p53 therefore appears to be a transactivator of the acidic type. The acidic activation domain of VP16 has been shown to interact directly with the TATA box-binding protein, TBP, a subunit of the RNA polymerase II general initiation factor TFIIID (11). Mutations in VP16 that compromise its ability to activate transcription (12) also compromise its ability to bind TBP (13). Two other transcriptional activators, adenovirus E1A (14, 15) and the Epstein-Barr virus transcription factor Zta (16), also directly contact TBP. An interaction with TBP may be a general feature of transcriptional activation mechanisms (17).

In this report we have used protein affinity chromatography to show that wild type human p53 binds specifically and directly to yeast TBP. The TBP-binding region of p53 was localized to its N-terminal transcriptional activation domain. The human TFIIID activity in a HeLa cell extract was also detected binding to this activation domain of p53. By using two human tumor-derived point mutations in p53, we found that the binding of p53 to yeast TBP correlates with the ability of its activation domain to function in vivo. These data emphasize the importance of interactions between transcriptional activators and TBP in regulating the initiation of transcription. They also lend additional support to the notion that p53 functions to suppress tumor growth by activating transcription.

Experimental Procedures

Construction of p53 Expression Plasmids—pGST-p53 1-73, a plasmid encoding a fusion protein of glutathione S-transferase (GST) and the first 73 amino acids of wild type p53, was made by ligating a blunt-ended 219-bp Ncol-HaeII fragment of the human p53 cDNA in plasmid pSPprop53 (18) into the Smal site of expression vector pGEX2T (Pharmacia LKB Biotechnology Inc.). pGST-p53 140-393, a fusion of GST and the C-terminal 253 amino acids of p53, was constructed by cloning a blunted 760-bp Ncol-BamHI fragment of the p53 cDNA from plasmid pSPprop53 into the Smal site of pGEX2T. pGST-p53, a plasmid encoding a fusion protein of GST and full-length human wild type p53, was constructed by cloning the 420-bp Ncol fragment of plasmid pSPprop53 into the NotI site of plasmid pGST-p53 140-393. pGST-p53 R273H, a plasmid encoding R273H.

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a fusion protein of GST and full-length p53 containing an oncogenic arginine to histidine point mutation at amino acid 273, was similarly constructed in two steps, using DNA from plasmid pHR4-2 (19). pGST-p53 R175H, a plasmid encoding a GST-p53 fusion protein with an oncogenic point mutation, arginine to histidine at position 175 (20), was created by oligonucleotide-directed mutagenesis (21) of plasmid pGST-p53.

Protein Purification—All of the plasmids described above expressed fusion proteins having the predicted mobility during electrophoresis on polyacrylamide gels containing SDS when expressed in E. coli.

Fusion proteins used for affinity chromatography were covalently purified to homogeneity by cation exchange chromatography on Bio-Rex 70 resin (Bio-Rad) and tested for identity by Western blot analysis (23) with the p53 monoclonal antibody PAB421 (24). All fusion proteins used for affinity chromatography were covalently coupled at a fixed concentration of 25 μM to Affi-Gel 10 active ester agarose beads (Bio-Rad) as described elsewhere (25). Recombinant yeast TBP (26) produced in E. coli was purified as described elsewhere (11).

Affinity Chromatography—All affinity chromatography experiments were performed essentially as described previously (11, 13, 25). Extracts were made from an E. coli strain which produces yeast TBP under the control of a bacteriophage T7 RNA polymerase promoter (26) and from the control strain BL21 (DE3) (27). The extracts were chromatographed on DE52 (Whatman), and the flow-through fractions were dialyzed into ACB (10 mM HEPES, 1 mM EDTA, 1 mM dithiothreitol, 10% glycerol) containing 0.1 M NaCl, prior to affinity chromatography. 300-μl aliquots of this extract were passed over 20-μl dried volume micro-affinity columns (28). All columns were subsequently washed with 12 column volumes (240 μl) of ACB containing 0.1 M NaCl and were then eluted with three column volumes (60 μl) of ACB containing 0.5 M NaCl. In some affinity chromatography experiments, 2 μg of purified yeast TBP was mixed with 2 μg each of protein low molecular weight standards (Bio-Rad) in 100 μl of ACB containing 0.1 M NaCl, and this mixture was chromatographed on 20-μl micro-affinity columns of GST or GST-p53. Columns were then washed with ACB containing 0.1 M NaCl, and fractions of three

![Silver-stained SDS-polyacrylamide gel of high salt eluates from affinity columns that were loaded with E. coli extracts containing recombinant yeast TBP.](image1)

**FIG. 3.** The transcriptional activation domain of p53 binds yeast TBP. Silver-stained SDS-polyacrylamide gel of high salt eluates from affinity columns that were loaded with E. coli extracts containing recombinant yeast TBP. The affinity column ligands contained various portions of p53. Lane 1, eluate from a GST-p53 1-73 column; lane 2, eluate from a GST-p53 140-393 column; lane 3, eluate from a column containing full-length p53; lane 4, eluate from a GST control column.

![ Autoradiogram of RNA transcripts initiated at the Ad2MLP.](image2)

**Fig. 4.** p53 also binds human TBP activity. Autoradiogram of RNA transcripts initiated at the Ad2MLP. Transcription reactions contained RNA polymerase II and human general initiation factors. Lane 1, complete reaction including recombinant human TBP; lane 2, reaction lacking human TBP; lane 3, reaction lacking human TBP and supplemented with eluate from a GST column over which HeLa extract was passed; lane 4, reaction lacking human TBP supplemented with eluate from a GST-p53 column over which HeLa extract was passed. The position of the Ad2MLP transcript is indicated by the arrow.
column volumes each (60 µl) were collected. The columns were finally eluted with ACB containing 0.5 M NaCl. In all of the experiments presented, one-half of each eluted fraction was analyzed by SDS-PAGE on a gel containing 11% polyacrylamide and was visualized by silver staining.

In Vitro Transcription—Transcripts were initiated at the Ad2MLP in the plasmid pMLC2AT-50 (29) in reactions containing purified calf thymus RNA polymerase II (25), TFIIA/J (30) and TFIH (31) fractions purified from HeLa nuclear extract, and six bacterially expressed recombinant human proteins: TFII B (32), TBP (33), TFII E (34), TFIIE (34), RAP30 (65), and RAP74 (36). Transcription in vitro and RNA analysis were done essentially as described elsewhere.8 Reactions lacking human TBP were supplemented with dialyzed high salt eluate from either a GST or a GST-p53 column. To prepare these eluates, these columns were loaded with 300 µl of HeLa whole cell extract (25), subsequently washed with 10 column volumes of ACB containing 0.1 M NaCl, and eluted with three volumes of ACB containing 0.5 M NaCl.

RESULTS AND DISCUSSION

Protein affinity chromatography can often be used to identify protein-protein interactions with greater sensitivity than methods involving co-immunoprecipitation (28). In the experiments described here, we have used affinity chromatography to test whether the human p53 protein is able to selectively bind to the TATA box-binding factor, TBP. For use as affinity column ligands, mutant and wild type forms of human p53 were produced in E. coli as fusion proteins with glutathione S-transferase (GST) (22) (Fig. 1).

Since the human p53 protein can function as a transactivator in the yeast S. cerevisiae, we first asked if the yeast TBP present in an E. coli extract was found in the flow-through (lane 1) and used as ligands for affinity chromatography. As before, the yeast TBP present in an E. coli extract bound to a GST-p53 column (Fig. 3, lane 3), but not to a GST control column (lane 4). Affinity columns containing the N-terminal amino acids 1–73 of p53 bound yeast TBP (Fig. 3, lane 1), while a C-terminal fusion containing amino acids 140–393 of p53 did not (lane 2). Therefore, yeast TBP binds to the same N-terminal region of p53 that functions as an acidic activation domain (1). We also tested whether the activation domain of p53 could bind mammalian TBP. The eluates of GST and GST-p53 1–73 columns over which human HeLa cell extracts had been passed were assayed for human TBP activity using a reconstituted in vitro transcription system. No Ad2MLP transcript was seen when human TBP was omitted from the reaction (see Fig. 4, lane 2). Addition of the eluate from a control GST column to this reaction did not restore transcription (lane 3). When the eluate of a GST-p53 1–73 column was added, however, transcription of the Ad2MLP was restored (lane 4). These data indicate that the human TBP activity in a HeLa extract can bind to the activation domain of p53.

Oncogenic versions of p53 often cannot activate transcription (8, 38). Transactivation could be reduced if such mutant forms of p53 failed either to bind DNA or to communicate with the RNA polymerase II initiation complex. To test whether such mutant forms of p53 could bind TBP, wild type p53 was directly compared with the oncogenic mutants R273H (19) and R175H (20). The R175H point mutant has been shown to be unable to activate transcription in vitro (38) when expressed as a chimera with the DNA binding domain of GAL4. In contrast, the point mutant R273H is able to activate transcription in vivo in both yeast (1) and human cells (8) when assayed as a chimera with the DNA binding domain of GAL4. However, the R273H mutant form of p53 cannot activate transcription on its own because it is unable to bind to the p53 consensus DNA sequence (39).

Purified yeast TBP was mixed with molecular weight marker proteins and applied to columns containing GST, GST-p53 WT, GST-p53 R175H, and GST-p53 R273H. As shown in Fig. 5, almost all of the yeast TBP was found in the high salt eluate (E) of the wild type GST-p53 and GST-p53 R273H columns, while most was found in the flow-through (lane 1) and wash fractions (lanes 2 and 3) from the GST-p53 R175H column. This R175H mutant form of p53 is therefore compromised in its ability to bind yeast TBP, while the R273H mutant form, which can still activate transcription as a chimera with GAL4, binds to yeast TBP in a manner similar to wild type p53. Our data therefore suggest that an
interaction between the acidic activation domain of p53 and TBP is important for transcriptional activation by p53. Another less well characterized oncogenic mutant form of p53, C135S (40), also failed to bind yeast TBP (data not shown). We expect that this mutant form, when assayed in vivo as a GAL4 chimera will also fail to activate transcription. p44, the truncated form of p53 observed in some Friend virus-induced tumors (6), lacks its N-terminal activation domain and therefore would be unable to interact with TBP and activate transcription. Our observations support the concept that tumor suppression by p53 is directly related to its ability to activate transcription by interacting with TBP bound to the TATA box of the adjacent promoter.

Several viral oncoproteins are thought to function by inactivating the growth suppressing activities of the recessive cellular oncoproteins p53 and retinoblastoma (for review, see Ref. 2). Human adenoviruses have two oncoproteins, E1A and E1B (41). The products of the E1A gene interact with the retinoblastoma protein (42), while one of the products of the E1B gene interacts with the acidic N-terminal region of p53 (43). E1B may contribute to the oncogenicity of human adenoviruses (44) because it represses the activation potential of wild type p53 in vivo (37). This repression of transcription by E1B may occur because the interaction between E1B and p53 excludes the interaction of p53 with TBP in adenovirus-infected cells.

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