Transactivation Ability of p53 Transcriptional Activation Domain Is Directly Related to the Binding Affinity to TATA-binding Protein*  

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J un Chang, Do-Hyung Kim, Seung Woo Lee, Kwan Yong Choi‡, and Young Chul Sung§§  

From the Department of Life Science and ‡Center for Biofunctional Molecules, Pohang University of Science and Technology, Pohang 790-784, Republic of Korea

Tumor suppressor protein p53 is a potent transcriptional activator and regulates cell growth negatively. To characterize the transcriptional activation domain (TAD) of p53, various point mutants were constructed in the context of Gal4 DNA binding domain and tested for their transactivation ability. Our results demonstrated that the positionally conserved hydrophobic residues shared with herpes simplex virus VP16 and other transcriptional activators are essential for transactivation. Also, the negatively charged residues and proline residues are necessary for full activity, but not essential for the activity of p53 TAD. Deletion analyses showed that p53 TAD can be divided into two subdomains, amino acids 1-40 and 43-73. An in vitro glutathione S-transferase pull-down assay establishes a linear correlation between p53 TAD-mediated transactivation in vivo and the binding activity of p53 TAD to TATA-binding protein (TBP) in vitro. Mutations that diminish the transactivation ability of Gal4-p53 TAD also impair the binding activity to TBP severely. Our results suggest that at least TBP is a direct target for p53 TAD and that the binding strength of TAD to TBP (TFIID) is an important parameter controlling activity of p53 TAD. In addition, circular dichroism spectroscopy has shown that p53 TAD peptide lacks any regular secondary structure in solution and that there is no significant difference between the spectra of the wild type TAD and that of the transactivation-deficient mutant type.

Transcriptional activators have been shown to stimulate in vitro the assembly of transcriptional preinitiation complexes (1, 2) as well as transcriptional elongation by RNA polymerase II (3). This stimulation is thought to depend on direct or indirect protein-protein interactions between transcriptional activators and the general transcriptional machinery and/or on relieving the inhibitory effects of chromatin (4, 5). Transcriptional activators can be divided into at least two discrete functional domains (6); a DNA binding/targeting domain is required to direct the activator to the appropriate DNA sequence element and then the transcriptional activation domain (TAD) can induce the enhanced transcription of target genes. TADs have been divided into three major classes according to a predominance of particular amino acid residues: acidic, proline-rich, or glutamine-rich (7). Of these classes, the acidic TADs appear to be unique in that they can apparently function universally in all eukaryotes tested from yeast to human (8).

Like other transcriptional activators, tumor suppressor protein p53 appears to have a modular domain structure; it contains an NH2-terminal region which functions as a TAD when coupled to a heterologous DNA binding domain (9, 10), a central site-specific DNA binding domain (11, 12), an oligomerization domain (13, 14), and a basic COOH-terminal nuclear localization domain (15). The NH2-terminal TAD of p53 is similar in size, net negative charge, and transactivating potency to the well defined TAD of herpes simplex virus virion protein 16 (HSV VP16) (16). This region is also rich in proline residues which are conserved through evolution (17). Like VP16 and a number of other transcriptional activators, p53 is thought to be a transactivator of the acidic type (9, 18).

Early studies suggested TFIID as the target for various activators (19, 20). Subsequently, the TATA-binding proteins (TBP) of yeast and human were shown to bind in vitro to the strong TADs of such viral and cellular activators as VP16 (21), E1A (22), Zta (23), and p53 (18, 24, 25). It has also been shown that another general transcription factor, TFIIH, interacts with various transactivators such as VP16 (26), Rel oncogene product (27), and CTF (28). Recent report showed that VP16 TAD and p53 TAD can also bind to TFIIH (29). In addition to general transcription factors, coactivators or adaptors are required for transactivation in the in vitro transcription system. The best characterized proteins among adaptors are the TBP-associated factors (TAFs) of the Drosophila melanogaster and humans (30–33). Recently, it was reported that p53 TAD can also interact with two subunits of the TFIID, TAFI140, and TAFI160 (34). Clearly, transcriptional activation appears to be more complicated than originally envisioned (6) and may involve multiple targets that make direct or indirect contacts in different spatial and temporal arrangements with TADs and the transcriptional machinery.

Here, we demonstrate that p53 TAD is a complex activation domain composed of two subdomains, in which positionally conserved hydrophobic residues are critical for activating function. The negatively charged residues and proline residues are also necessary for full activity, but not essential for the activity of p53 TAD. Mutations that severely impair the function of p53 TAD in vivo have been shown to diminish binding activity to TBP in vitro, indicating that the observed in vitro interaction is biologically relevant. Circular dichroism (CD) spectroscopy demonstrates that p53 TAD peptide does not have any detectable secondary structure at physiological condition.  

MATERIALS AND METHODS  

Plasmid Constructions and Mutagenesis—Gal4 DNA-binding domain expression plasmid, Gal4D, was constructed by inserting the 450-bp HindIII-Xmal fragment of pSG424 (8) into the HindIII-BamHI site of pcDNA (Invitrogen) following by flushing Xmal and BamHI overhangs. A DNA fragment encoding amino acids 1-73 of p53 was

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§ To whom correspondence should be addressed.

1 The abbreviations used are: TAD, transcriptional activation domain; TBP, TATA-binding protein; TAF, TBP-associated factor; bp, basepair(s); GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis; TFE, trifluoroethanol.
amplified from the human cDNA of p53 with two primers (5'-GGTCGG-GATCCAGAAGGCAGCGACATCA and 3'-GGTGAAGCTTACGCGGGAGCAGCCTC; BamHI and HindIII sites are underlined) and digested with BamHI and HindIII. The resulting DNA fragment was ligated into the BamHI-HindIII site of pSK(-) (Stratagene), yielding pSK-p53 TAD. Gal4D-p53 TAD was generated by inserting the 210-bp BamHI-HindIII fragment of pSK-p53 TAD into the BamHI-EcoRV site of Gal4D after flushing the HindIII overhang (Fig. 1). Oligonucleotide-directed mutagenesis was performed as described (35) using single-stranded DNA of pSK-p53 TAD. Mutations were identified by restriction endonuclease digestion and dyeoxy sequencing. The specific amino acid changes introduced by mutagenic primers are listed in Table I. The BamHI-HindIII DNA fragments of mutant derivatives were digested into the same site of Gal4D except for M41 and M241 in which HindIII site was used instead of HindIII site. The carboxy-terminal deletion mutant, Gal4D-p53 (1-40), was generated by ligating the 120-bp BamHI-HindIII DNA fragment of pSK-p53 TAD M41 into the same site of Gal4D. Gal4D-p53 (43-73) was obtained by inserting the 90-bp HindIII-HindIII DNA fragment of pSK-p53 TAD M41 into the EcoRI site of Gal4D after filling in cohesive ends with Klenow fragment of DNA polymerase I. Gal4D-p53 (1-40) M22 was generated by introducing M22 mutation into Gal4D-p53 (1-40).

The glutathione S-transferase (GST) fusion plasmids were made by using pGEX-KG which contains a GST gene under the control of a tac promoter and a flanked polymerizing site (36). pGEX-p53 TAD was constructed by inserting the 210-bp BamHI-HindIII DNA fragment of pSK-p53 TAD M41 and M241 into the same site of pGEX-KG, respectively. pGEX-p53 (1-40) was generated by inserting the 120-bp BamHI-HindIII DNA fragment of pSK-p53 TAD M41 into the same site of pGEX-KG. The reporter plasmid, G5E1 BCAT, was described previously (37).

Transfection and Chloramphenicol Acetyltransferase Assays— BHK-21 and COS-7 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Plasmid transfections were carried out by a DEAE-dextran method (38). Cells (10⁶) were seeded in a 100-mm dish 24 h before transfection and transfected with 1 μg each of the reporter and activator plasmids. At 48 h after transfection, cells were harvested and chloramphenicol acetyltransferase activity was measured as described previously (39). To determine expression levels of the Gal4 fusions, COS-7 cells were transfected in parallel with 2 μg of activator plasmids. Nuclear extracts were prepared as described previously (40) and electrophoretic mobility shift assays were performed as described (41) with DNA fragment containing five Gal4 binding sites. The amount of probes shifted by each derivative was quantitated using a Fuji BAS2000 photomager. The difference in transfection efficiency was normalized by using a second reporter plasmid, pG2L (Promega), containing a luciferase gene. Luciferase activity was assayed using the luciferase assay system (Promega) according to the supplier's recommendation. All chloramphenicol acetyltransferase assay data reported in this article were from points in the linear range of the assay.

GST Pull-down Experiment— GST fusion proteins were expressed in Escherichia coli DH5α and were purified by using glutathione-Sepharose beads (Pharmacia Biotech Inc.) in accordance with the supplier's recommendation. 35S-Labeled human TBP was generated by using a coupled transcription-translation reticulocyte lysate (NTT system, Promega) with linearized pETH11D plasmid (42) as a template. 200 ng of GST-p53 TAD and mutant derivatives coupled to 20 μl of glutathione-Sepharose beads was incubated at 4°C with 35S-labeled TBP in 600 μl of a buffer solution containing 40 mM HEPES-KOH, pH 7.5, 150 mM KCl, 0.5 mM EDTA, 5 mM MgCl2, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and 0.1% Nonidet P-40 for 1 h. To minimize potential bead losses during subsequent washes, the buffer was mixed with glutathione beads to adjust a total bead volume of 20 μl/reaction. Following this incubation, the beads were washed five times with the same buffer solution and bound proteins eluted with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) loading buffer. The proteins were separated by 10% SDS-PAGE and visualized by autoradiography. Signals were quantitated on a Fuji BAS2000 photomager and plotted to obtain a graphical representation of the results.

Purification of p53 TAD Peptides— For large scale production of p53 TAD, E. coli DH5α cells containing pGEX-p53 TAD were induced with 0.2 mM isopropyl-β-D-thiogalactopyranoside and harvested 4 h after induction. The fusion protein was purified from the soluble extract by use of binding affinity to glutathione-Sepharose beads. The p53 TAD peptide was released from the GST moiety in a buffer containing 100 mM NaCl and 2.5 mM CaCl2 using 1 μg of thrombin (Sigma)/1 mg of fusion protein. The peptide was further purified by gel filtration chromatography using Superose 12 (Pharmacia). The peptide after the gel filtration step was found to be homogeneous as judged by Coomassie Blue staining of the gel after SDS-PAGE. The identity of the peptide was determined by amino acid composition analysis. The M22 mutant derivative was also purified by the same method.

CD Spectroscopy—CD experiments were performed with a spectropolarimeter Jasco J-720. A cuvette with 0.1-cm path length was used for all spectral measurements. Measurements were made at room temperature in 5 mM phosphate buffer. The concentrations of peptides were determined by absorbance at 280 nm in the phosphate buffer. The used peptide concentrations were 17 μM for wild type p53 TAD and 15 μM for the M22 mutant. All spectra were corrected for background using the phosphate buffer and averaged from the spectra of at least four scans. The pH values were measured with a microelectrode calibrated at two reference pH values.

RESULTS

Mutational Analysis of p53 TAD—The preponderance of acidic amino acids within p53 TAD suggests that negative charge is a critical component of the activation domain structure. To test whether activation function is simply related to the net negative charge, we constructed Gal4D-p53 TAD and replaced, in combination, the acidic amino acids within the activation domain with uncharged or positively charged residues (Fig. 1). From the relative activities of such mutants (Table I), we infer that negative charge is necessary for the optimal activity of p53 TAD. The M41 mutant was less active than the M2 mutant, indicating that mutations of negatively charged residues, Glu-2 and Glu-3, had a less effect on the activity than mutations on Asp-41 and Asp-42 residues. The M241 mutant was less active than the M41 mutant, showing that replacement of increasing numbers of acidic residues with other residues led to a progressive decrease in transcriptional activation. It was reported previously that the acidic residues
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The p53 TAD is also rich in proline residues (19.2%), which is a characteristic of another class of TAD, such as CTF/NF-1 (44). When M12 and M34 mutants were tested, there were about 39 and 24% reduction in p53 TAD-mediated transactivation, respectively. As expected, the M1234 mutant containing mutations in four Pro residues was shown to be about 71% reduction in the transactivation (Table I), indicating that there was additive effect with these mutations and that proline residues are also required for the optimal activity of p53 TAD.

Previous studies on the VP16 TAD have suggested that the acidic residues contribute to its activity, but intervening hydrophobic residues are more important than other residues (45). TADs of a number of transactivators exhibit a conserved pattern of hydrophobic residues (45). Since p53 TAD also shows the similar pattern of positionally conserved hydrophobic residues (Fig. 2), we generated various mutants in which conserved hydrophobic amino acids were replaced with hydrophilic ones. When these mutants were tested for transactivation activity in BHK-21 and COS-7 cells, the activities of several mutants were significantly impaired (Table I), indicating that the positionally conserved hydrophobic residues are more important than other residues (45). Underlined letters of p53 TAD indicate identity in all TADs. The effect of mutations on Leu-22 and Trp-23 reduced p53 TAD-mediated transactivation by about 85%. In contrast, mutations on both Val-31 and Leu-26 resulted in approximately 88% loss of the activity. Also, single amino acid change on Phe-19 reduced the activity by about 85%. In contrast, mutations on both Val-31 and Leu-32, which are not positionally conserved, did not impair the transactivation function but rather enhance the activity. Therefore, we concluded that the positionally conserved hydrophobic residues, Phe-19, Leu-22, Trp-23, Leu-25, and Leu-26 are critical for transactivation function of p53 TAD. These residues are identical in all sequences of p53 protein from several species (17). The effect of mutations on Leu-22 and Trp-23 is consistent with a previous report (43), but those of mutations on Phe-19 and on Leu-25 and Leu-26 do not exactly coincide with their results in which human p53 mutant protein containing the double mutation on Leu-14 and Phe-19 was observed to have a 50% reduction in chloramphenicol acetyltransferase activity compared with wild type p53. In addition, the Leu-25 and Leu-26 double mutant showed either enhanced or reduced activity in Saos-2 cells, depending on p53-responsive elements either from the creatine phosphokinase gene or from the mdm-2 gene (43).

To compare the expression level among different Gal4 fusion proteins, electrophoretic mobility shift assay was performed using a labeled DNA fragment containing five Gal4 binding sites and showed that there was no significant difference among them (data not shown). The difference in the chloramphenicol acetyltransferase activity is, therefore, due to the intrinsic biological activity of different Gal4 fusion proteins, but not by the different level of Gal4 fusion proteins in the transfected cells. Although the transactivating abilities of mutants constructed in the foregoing studies were severely impaired, residual activity still remained, suggesting that p53 TAD is composed of separable subdomains just like VP16 (46) and Epstein-Barr virus Rta transactivator (47). It was previously shown that the minimal activation domain of p53 lies within the first 42 amino acids of the protein (48). Since Gal4D-p53(1–40) consistently showed about 30–38% activity of Gal4D-p53 TAD, which contains the residues 1–73, residues 43–73 appear to be necessary for the full p53 TAD-mediated transactivation. To be certain that residues of p53 from 43 to 73 also contain an autonomous TAD, Gal4D-p53(43–73) was constructed and tested for the transactivating ability. The resulting plasmid showed about 6% activity of Gal4D-p53 TAD (Table I), indicating that there is an autonomous TAD in this subregion. In the case of VP16, the truncated activation domain possesses approximately 50% of wild type intrinsic activity, whereas the addition of COOH-terminal subdomain restored the full activity (46). Gal4D-p53(1–40) M22, which deletes the COOH-terminal subregion from M22 mutant, completely lost the residual activity of M22 mutant (Table I), demonstrating that the residual activity comes from the separable COOH-terminal subdomain, and that Leu-22 and Trp-23 are absolutely required for the function of minimal activating region (residues 1–40) of p53.

In Vitro TBP Binding Activity of p53 TAD and Mutants—Previous studies showed that p53 TAD interacts directly and specifically with yeast and human TBP (18, 24). The binding activities of wild type p53, mutant p53R175H (18, 25), and Gal4-p53 fusion proteins (24) to TBP were reported to correlate with their transactivation abilities in vivo, suggesting that p53 TAD activates transcription by directly interacting with TBP. In contrast, Lin et al. (43) reported that wild type p53 and transactivation-deficient mutants, including R175H mutation, could bind equally well to human TBP when tested with immunoprecipitation and far-Western analysis. Thus, it remains controversial whether TBP is the target molecule of p53 TAD, and binding activity of p53 TAD to TBP is directly related to p53 TAD-mediated transactivation. To clarify this discrepancy,
The residues of wild type p53 TAD from 1 to 73 and its derivatives were placed under the GST gene to generate pGEX-p53 TAD fusion constructs (Fig. 1). The GST-p53 TAD fusion protein and its derivatives were expressed in E. coli and purified by affinity chromatography (Fig. 3A). The purified fusion proteins were assayed for the activity to bind in vitro translated human TBP in a GST pull-down experiment. As shown in Fig. 3B and Table I, the levels of TBP precipitated by GST-p53 TAD and mutant derivatives are linearly correlated with the ability of transactivation in vivo. Binding reactions were performed under nonsaturating condition, where GST-p53 TAD and mutant derivatives were a limiting factor. Under this condition, about 20% of input TBP bound to the GST-p53 TAD. We have repeated these binding assays at several times with different batches of fusion proteins and in vitro translated TBP. Relative binding activities were reproducible and resulted in the same relative order for TBP binding. This establishes a direct relationship between transactivation ability in vivo and the binding activity of the p53 TAD to TBP in vitro (Fig. 3C). The TBP binding activities of M22, M25, and M19 mutants lacking critical hydrophobic residues but bearing identical net negative charge were significantly decreased when compared with that of wild type p53 TAD, indicating that the binding of p53 TAD to TBP is not due to nonspecific ionic interaction between the positively charged region of TBP and negatively charged p53 TAD. These results do not agree well with previous report in which the binding ability of p53 protein to TBP was not affected by the mutations at residues 22 and 23 (43). This inconsistency may result from the use of different proteins in which full-length p53 protein was used for interaction in the previous report and assay conditions (43). It was recently reported that COOH-terminal region of p53 protein (amino acid residues 318–393) can interact with TBP independently (49), indicating that the previous result is due to the interaction between TBP and COOH-terminal region of nonfunctional p53 mutant proteins. Taken together, we suggest that TBP is one of direct target molecules for the p53 TAD and that the binding strength of p53 TAD to TBP (TFIID) is an important parameter controlling the rate of transcription initiation in eukaryotes.

**CD Spectroscopy of a Wild Type and a Mutant p53 TAD—**

Because of a linear correlation between transactivation ability of p53 TAD and the binding activity of p53 TAD to TBP in vitro, it is very likely that purified p53 TAD peptide and mutant derivatives are biologically relevant species. To determine the structural difference between a wild type p53 TAD and a nonfunctional mutant, M22, the solution structures of purified p53 TAD and M22 were analyzed by CD spectroscopy. To obtain the CD spectra, p53 TAD peptide and M22 peptide were further purified to near homogeneity by gel filtration chromatography after thrombin cleavage as described under "Materials and Methods." The CD spectrum of p53 TAD peptide at neutral pH showed no apparent α-helical structure when analyzed by Yang’s method (Fig. 4A; Ref. 50). Spectra were also measured in the presence of trifluoroethanol (TFE), a dehydrating solvent which promotes helical formation, to stimulate a more hydrophobic milieu. In the presence of up to 50% TFE, p53 TAD peptide had little content of helical structure (Fig. 4A). When the effect of pH was investigated to determine the local interactions, there was no dramatic structural transition during the change of pH from 2.9 to 9.0 (data not shown). The structural study of nonfunctional p53 TAD mutant, M22, was also investigated in the same way. The mutant peptide showed no significant secondary structure when analyzed with the change of TFE (Fig. 4B). In addition, there was no difference between the spectra of the wild type TAD and that of M22, indicating that the mutation, which makes p53 TAD nonfunctional, does not change the overall structure of the peptide.

**DISCUSSION**

The determination of the critical amino acid residues and protein structures involved in mediating the biological activity of TADs would represent an important step toward understanding the mechanism of transcriptional activation. Since...
The requirement of COOH-terminal subdomain in residues and proline residues (acidic: 25.8%, proline: 22.6%). The COOH-terminal subdomain contains a similar level of acidic cells when linked to Gal4 DNA-binding domain (Table I). This activating region (amino acids 1–40) in BHK-21 and COS-7 ids 43–73) has weaker transactivation ability than minimal subdomains. The COOH-terminal subdomain (amino acids Phe-19, Leu-22, Trp-23, Leu-25, and Leu-26) are critical since our results revealed that the conserved hydrophobic residues are unstructured negative noodles which become structured upon interaction with some part of the transcription apparatus (59). This “induced fit” model seems to be further supported by our results and previous data on VP16 TAD (55, 56) showing that transcriptionally compromised mutants of p53 TAD have reduced binding ability to TBP (54). In addition, there are accumulative evidences that several nonfunctional TADs have reduced binding abilities to TBP, suggesting that there is the biological relevance of these interactions (22, 57, 58).

Two hypotheses for the role of critical hydrophobic residues are that these residues are necessary for either maintaining the structure of the activation domain or the direct interaction with TBP. Based on the results of CD spectroscopy, there is no significant structural difference between wild type p53 TAD and M22 mutant. This suggests that Leu-22 and Trp-23 may be directly interacting residues with TBP and may not be involved in structural determination. In contrast to highly ordered DNA binding modules, activation domains may be not so highly ordered on their own, but appear to become structured only upon interactions with target molecules. This hypothesis is supported by the finding that the biological function of TADs does not require a well defined amino acid sequence. Based on this sequence flexibility, it was suggested that acidic regions are unstructured negative noodles which become structured upon interaction with yeast and human TBP. The CD spectra of purified p53 TAD (A) and the M22 mutant (B) obtained at pH 7.0 with several different concentrations of TFE. a, 0%; b, 10%; c, 20%; d, 30%; e, 50%.

### p53 TAD

p53 is an important tumor suppressor protein and contains a distinct TAD, including acidic residues (23.3%) and proline residues (19.2%), we have chosen p53 TAD to study the molecular mechanism of transcriptional activation. Due to its high content of acidic and proline residues, p53 TAD may fall into the category of a combination of acidic and proline-rich domain as in the cases of Jun and Fos (51, 52). Our mutational analyses showed that the negatively charged residues and proline residues of p53 TAD are necessary for full activity but not essential for the transactivation ability. Several reports recently suggested that acidic residues are not essential for transactivation function, but hydrophobic and bulky aromatic residues may be more important in defining the transactivation domain. Importance of hydrophobic residues was also observed in p53 TAD, since our results revealed that the conserved hydrophobic residues (Phe-19, Leu-22, Trp-23, Leu-25, and Leu-26) are critical for transactivation ability (Fig. 2 and Table I).

Interestingly, p53 TAD is composed of two separate functional subdomains. The COOH-terminal subdomain (amino acids 43–73) has weaker transactivation ability than minimal activating region (amino acids 1–40) in BHK-21 and COS-7 cells when linked to Gal4 DNA-binding domain (Table I). This COOH-terminal subdomain contains a similar level of acidic residues and proline residues (acidic: 25.8%, proline: 22.6%). The requirement of COOH-terminal subdomain in cis for optimal transactivation ability of p53 TAD suggests the possibility that the subdomain may be required for stabilizing the interaction between p53 TAD and the target molecules. Alternatively, the subdomain may directly contact with different cellular factor(s). It has been shown that the full-length VP16 TAD, but not NH2-terminal subdomain, interacts with TAFI140 (53), whereas NH2-terminal subdomain can interact with TBP (54) and TFII B (26). Many characteristics of p53 TAD are shared with those of VP16 TAD, including (i) essential bulky hydrophobic residues, (ii) an overall negative charge, (iii) the lack of secondary structure in solution (55, 56), (iv) separable two subdomains, and (v) in vitro interaction with yeast and human TBP. These findings suggest that p53 TAD has the same mechanism of action as does the VP16 TAD. We have observed that overexpression of p53 TAD can efficiently inhibit the function of VP16 TAD and vice versa in an in vivo squelching experiment. Also apparently shared with VP16 TAD is that transcriptionally compromised mutants of p53 TAD have reduced binding ability to TBP (54). In addition, there are accumulative evidences that several nonfunctional TADs have reduced binding abilities to TBP, suggesting that there is the biological relevance of these interactions (22, 57, 58).

Two hypotheses for the role of critical hydrophobic residues are that these residues are necessary for either maintaining the structure of the activation domain or the direct interaction with TBP. Based on the results of CD spectroscopy, there is no significant structural difference between wild type p53 TAD and M22 mutant. This suggests that Leu-22 and Trp-23 may be directly interacting residues with TBP and may not be involved in structural determination. In contrast to highly ordered DNA binding modules, activation domains may be not so highly ordered on their own, but appear to become structured only upon interactions with target molecules. This hypothesis is supported by the finding that the biological function of TADs does not require a well defined amino acid sequence. Based on this sequence flexibility, it was suggested that acidic regions are unstructured negative noodles which become structured upon interaction with some part of the transcription apparatus (59). This “induced fit” model seems to be further supported by our results and previous data on VP16 TAD (55, 56) showing the apparent lack of any detectable α-helical or other structure. Tight interactions between TADs and target molecules seem to be dependent on hydrophobic interactions that are formed by the induced fit. However, it is necessary to perform the structural analysis in a complex form of p53 TAD and TBP for a complete definition of the induced structure.

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