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# p53 Binds to the TATA-binding Protein-TATA Complex*

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**Earlier reports show that p53, both wild type and mutants, may affect transcription. Wild-type p53 activates promoters with p53-binding sites while inhibiting promoters without binding sites. Mutant p53, on the other hand, has been shown to activate transcription from specific promoters. These observations suggest that both wild-type and mutant p53 may interact with a general transcription factor(s). In this report, we have shown that the cloned TATA-binding protein (TBP) from human and yeast interacts with human p53. TBP coimmunoprecipitates with wild-type or mutant human p53 when incubated with the p53-specific monoclonal antibody and Protein A-agarose. Wild-type murine p53 has also been found to interact with human TBP. Protein blot assays have demonstrated that the interaction between p53 and human TBP is direct. By gel retention analysis, we have shown that the complex of TBP and p53 (both wild type and mutant) can bind to the TATA box. The similar qualitative binding capability of wild-type and mutant p53 with human TBP and the similarity of the two complexes in binding to the TATA box suggest that the functional discrimination between wild-type and mutant p53 may not lie in their ability to bind TBP. The nature of the p53·TBP or p53·TBP·TATA complex may determine the success of transcription.**

The nuclear phosphoprotein p53 was first identified in association with simian virus 40 (SV40) large T antigen (1, 2). Expression of wild-type p53 has been demonstrated to negatively control cellular proliferation. Several lines of evidence indicate that the wild-type protein is a tumor suppressor. Wild-type p53 inhibits proliferation of transformed cells, suppresses oncogene-mediated cell transformation, and eliminates the tumorigenic potential of tumor-derived cell lines (3–13). On the other hand, tumor-derived mutant p53 cDNA clones were found to immortalize primary cells and cooperate with the *ras* oncogene in transformation of primary cells (9, 14, 15). p53 gene mutations are the most frequently reported genetic defects in human cancer (4, 16–20).

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p53 has been found to be associated with several viral-transforming proteins. SV40 T antigen (1, 2), adenovirus 5 E1B (21), and E6 of human Papillomavirus (22) bind to wild-type p53 presumably to inactivate p53's function some way leading to transformation or tumorigenesis (23). Wild-type p53 inhibits SV40 DNA replication *in vivo* and *in vitro*, presumably by binding to T antigen (24–27). E6 proteins of oncogenic human Papillomavirus can degrade p53 *in vitro* (28). Cellular proteins have also been found associated with p53. Among these are the heat shock protein hsc70 and two protein kinases, $p34^{cdc2}$ and casein kinase II (29–34). More recently, and of great significance, is the discovery that the product of the *mdm*-2 oncogene forms a tight complex with the p53 protein (35, 36). *mdm*-2 has been found to be amplified frequently in sarcomas, and it has been proposed that the amplification of *mdm*-2 in sarcomas leads to escape from p53-regulated cell growth (36).

In addition to interaction with viral and cellular proteins, several other interesting biochemical properties of p53 have also been identified. Wild-type (but not mutant) p53 binds to the SV40 early promoter (37), to the human ribosomal gene cluster (38), and to other genomic fragments (39, 40). p53 also binds to the murine muscle creatine kinase gene regulatory region (41), which was found to be p53-responsive (41, 42). The consensus sequence for its binding has been determined recently (39).

A growing body of experimental evidence indicates involvement of p53 in transcription. Initially, a p53-GAL4 fusion protein was shown to activate transcription from promoters containing GAL4-binding sites (43, 44). The transactivation domain was found to be contained within the N-terminal 42 amino acids (45). More recently, the wild-type protein (but not the mutant) has been demonstrated to be a sequence-specific transactivator for a promoter having synthetic upstream p53-binding sites *in vivo* and *in vitro* (40, 84, 85). Weintraub *et al.* (42) first demonstrated that the murine muscle creatine kinase enhancer could be activated by wild-type p53. Recently, Zambetti *et al.* (41) detailed binding of wild-type p53 to the murine muscle creatine kinase enhancer region and showed a relationship between p53-mediated activation and p53 binding. The *in vivo* transactivation of the murine muscle creatine kinase enhancer by wild-type p53 was inhibited by *mdm*-2 protein (35), presumably by forming a complex with p53. This suggests that *mdm*-2 may interfere with the normal function of the tumor suppressor.

Another group of experimental results suggests that overexpression of wild-type human p53 leads to the inhibition of gene expression *in vivo* for a number of cellular and viral promoters (46–51). Interestingly, wild-type p53 inhibited the human proliferating cell nuclear antigen and the multiple drug resistance gene (*MDR1*) promoter activities, while a few mutants activated the promoters *in vivo* (47, 51). Wild-type p53 also inhibited retinoblastoma gene promoter function (52). A mutational analysis of the retinoblastoma promoter showed a part of the basal promoter to be susceptible to p53