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Repression of the basal c-fos promoter by wild-type p53

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ABSTRACT

Mutations in the p53 gene are the most common genetic alterations observed in many inherited and sporadic forms of human cancer. Recent studies indicate that wild-type p53 may be involved in the regulation of gene expression. In the present report we examined the effect of p53 on the human c-fos promoter. Using a transient co-transfection assay we show that wild-type human p53, but not a transforming mutant of p53, negatively regulates the activity of the c-fos promoter in a dose-dependent manner. Promoter deletion analysis maps a sequence confering p53 repression to the basal promoter region between nucleotides −53 and +42 relative to the cap site. In contrast, p53 strongly stimulates transcription when a sequence previously reported to bind p53 (TGCCT repeat) was inserted in front of the HSV-TK promoter driving CAT. These findings raise the question as to whether p53 may mediate its inhibitory effect on c-fos gene expression by interfering, directly or indirectly, with components of the basal transcriptional machinery.

INTRODUCTION

The nuclear phosphoprotein p53 is a frequent target for mutations in a wide variety of human cancers. Previously classified as a tumor antigen and oncoprotein it has emerged as a tumor suppressor negatively regulating the cell cycle and requiring loss of function mutations for tumor formation (for review see 1). A large number of studies have shown that the wild-type p53 can suppress or inhibit transformation of cells in culture by either viral or cellular oncogenes (2,3,4), growth of transformed cells in culture (4−7) and formation of tumors in animals (8). Two hypotheses have been suggested to explain how p53 normally regulates passage through the cell cycle: a) it may act to promote or inhibit the assembly of a DNA replication initiation complex, and/or b) p53 could act as a transcriptional regulator promoting or repressing cellular promoters regulating the expression of growth control genes.

Several properties of p53 have been described yielding insights into possible functions of p53 as a transcriptional regulator in the cell: a) it is frequently found in the cell nucleus (9,10); b) it has been shown to possess DNA binding activities (4, 11−13); c) studies using hybrid proteins in which the DNA binding domain of yeast Gal4 was fused to the amino-terminal region of p53 have provided evidence for a strong transactivating domain in the p53 protein (15−17); and d) recent studies showed that wild-type (wt) p53 may act to down-regulate the activity of various cellular promoters of growth control genes such as the c-fos and c-jun protooncogenes (18,19).

In the present study we analyzed in greater detail the effects of p53 on the human c-fos promoter. We report here that wt p53 can inhibit c-fos gene expression in a dose-dependent manner, and that a sequence confering p53 sensitivity maps to the basal promoter region. No evidence was obtained for direct binding of p53 to this sequence. In contrast, p53 strongly activates transcription when a sequence previously reported to bind p53 (13) was inserted in front of the HSV-TK promoter driving CAT. These findings suggest that the effects of p53 on the c-fos promoter may be mediated by its ability to interfere, directly or indirectly, with components of its basal transcriptional machinery.

MATERIALS AND METHODS

Cells and Transfection Procedure

Glioblastoma cell lines G102 (kind gift of Dr. Jenkins, Mayo Clinic) and T98G (ATCC, Rockville,MD) were passaged in DMEM supplemented with 10% fetal calf serum and plated at a density of 10^5 per 2.5 cm dish in a 6-well Corning culture plate. Cells were serum-starved 24h before transfection. Cells at 70% confluency were transfected using a method employing lipopolyamine-coated DNA (20,21). The DNA/lipid complex was freshly obtained by mixing DNA solution with lipopolyamine solution, complex formation allowed to occur, and added to the cells in serum free medium. 2μg of DNA/threefold excess of lipid charge was routinely used. The transfection step lasted 2−4h. Cells were placed routinely in low serum (0.1%) for 16−20h and subsequently stimulated with 8-bromo-cAMP, the phorbol ester PMA (12-o-tetra-decanoyl phorbol-13-acetate) or 10% serum for 24h. Cells were collected, protein extracted, extract normalized after determining total protein (Biorad), and assayed for CAT activity (22). Chloramphenicol and its acetylated derivatives were extracted with ethyl acetate, separated by TLC, and autoradiographed. Degree of conversion of chloramphenicol into its acetylated derivatives was determined by liquid scintillation counting. Maximum levels of expression in the absence of p53 were arbitrarily set to 1, and% repression or stimulation by p53 calculated.

Immunoprecipitation

Cells from each cell line were grown to near confluency and labeled by incubation for 2h in methionine-free medium containing 50 μCi/ml 35S-Translabel (Amersham). Following incubation, the monolayers were washed with phosphate-buffered saline (PBS), cells lysed in situ in 2ml ice-cold 10mM Tris, 0.14M NaCl, 0.5% NP40 (pH 8.0). The recovered lysate was centrifuged for 10 min at 4°C and preabsorbed with protein A-Sepharose . Preabsorbed lysate was centrifuged and supernatant immediately used for immunoprecipitation using the following anti-p53 monoclonal antibodies (Oncogene Science): PAb421 (36); PAb240 (37); and PAb1620 (38,39β). PAb421 reacts with both wild-type and mutant p53. PAb 240 recognizes mutant but not wild-type p53.

PAb1620 reacts preferentially with wild type p53. Immunoprecipitated proteins were resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using 12% resolving minigel with a 4% stacking gel. ^{35}S-labeled p53 was visualized by autoradiography, with exposure at −70°C.

Plasmids

pFC700 contains the human c-fos promoter extending ca. 700 nucleotides upstream of the cap site (−700/+42) linked to the the chloramphenicol acetyl transferase reporter gene (23). All deletion mutants are derived from this parent plasmid: plasmid −225: −225/+42, plasmid 99: −99/+42, plasmid 72: −72/+42, plasmid 53: −53/+42, plasmid 225−99/53: −225/−99 and −53/+42, plasmid 225/99: −700/−99 and −73/+42, plasmid 63/53: −700/−63 and −53/+42.

pBLCAT2: contains the herpes simplex virus thymidine kinase promoter (−105/+51) linked to CAT (24).

pBL64CAT: the DNA element CTTGCCTGGACTTGCCTGG (13) was first inserted into the SmaI site of pbluescript SK and recombinants verified by sequence analysis. A BamH1/SalI fragment bearing the DNA element and portions of the pBSK polylinker was then inserted in sense orientation into the BamH1/SalI site of pBLCAT2., generating pBL64CAT. pBL87CAT carries a mutation in the second TGCCT box of the upstream DNA element (CTTGCCTGGACTTTTTTGG) cloned into pBLCAT2.

pJ7CAT: contains the SiCMV-IE94 promoter coupled to CAT (25).

pC53SN3: contains 1.8kb of full length p53 cDNA cloned into pCMV-neo-Bam under the transcriptional control of the immediate early enhancer/promoter of the human cytomegalovirus (CMV) (6). pC53SCX3 encodes a mutant form of p53 and differs from pC53SN3 by a single point mutation causing a change from val to ala at residue 143 (6).

RESULTS

p53 inhibits c-fos gene expression

In order to determine whether p53 is capable of regulating c-fos promoter activity, cells were co-transfected with a plasmid expressing wt p53 (pC53SN3) under the control of the CMV promoter/enhancer together with plasmids containing the c-fos promoter (−700/+42), and deletions thereof, linked to the CAT reporter gene. The response of the promoters was monitored by assaying for stable CAT enzyme. Cells were transfected by a novel, highly efficient and consistent transfection procedure using lipopolyamine-coated DNA as previously described (20,21). The high sensitivity and reproducibility of this method allowed us to study reliably dose-response effects of p53 (test experiments on the cells used in this study showed transfection effeciencies varying by < 5%).

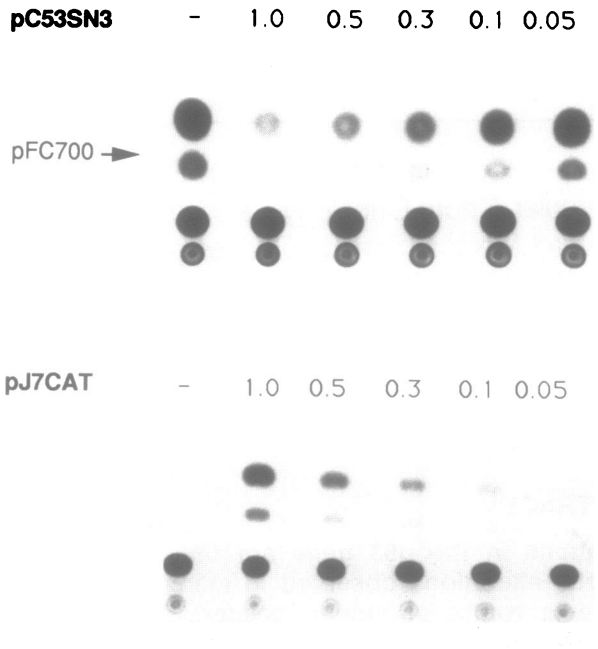

Figure 1. Dose-dependent repression of the human c-fos promoter by wt p53. Glial G102 cells were transfected with 2μg pFC700 (contains 700 nucleotides of the 5'-flanking region of the c-fos gene) and wt p53 expression plasmid (pC53SN3) at varying amounts (1−0.05μg, the amount added is shown above each lane, in μg). Total amounts of DNA was kept constant using pCMV-neo-Bam as a control. Alternatively, cells were transfected with varying amounts of pJ7-CAT (1−0.05μg) bearing the SCMVIE94 promoter, together with pUC19 (2μg) and pCMV-neo-Bam accordingly to keep total amounts of DNA to 3μg. Experiments shown are representatives of several independent experiments (n > 5).

Glial G102 cells, a cell line derived from a human glioblastoma (a kind gift of Dr R. Jenkins, Mayo Clinic, Rochester MN) were transfected with each CAT plasmid (2μg) together with varying amounts of pC53SN3 and pCMV-neo-Bam (parent plasmid of pC53SN3 lacking the p53 coding region), which served as a control to keep the total amount of DNA transfected constant and to account for any potential promoter competition. As shown in Fig1., expression of p53 strongly down-modulates the c-fos promoter extending ca.700 nucleotides upstream of the cap site (pFC700). The c-fos promoter was induced by cAMP to obtain high levels of expression. The degree of repression by p53 depended on the amount of p53 plasmid transfected and thus presumably p53 protein synthesized. Strong inhibition (average 90% inhibition) was reached at 1μg of pC53SN3. The dose-dependent response nicely matched the degree of expression observed from a CAT-construct driven by a CMV-promoter/enhancer (pJ7CAT,25) transfected under identical conditions in the range of 1−0.05μg of DNA (pUC19 was used to keep total amounts of DNA constant). This indicates that the effects on c-fos promoter occur within a linear range of p53 expression. p53 similarly down-modulated serum, PMA-induced, and resting c-fos promoter activity (data not shown).

Fig.2 shows a study using different deletion mutants of the c-fos promoter, in an attempt to map a region confering p53

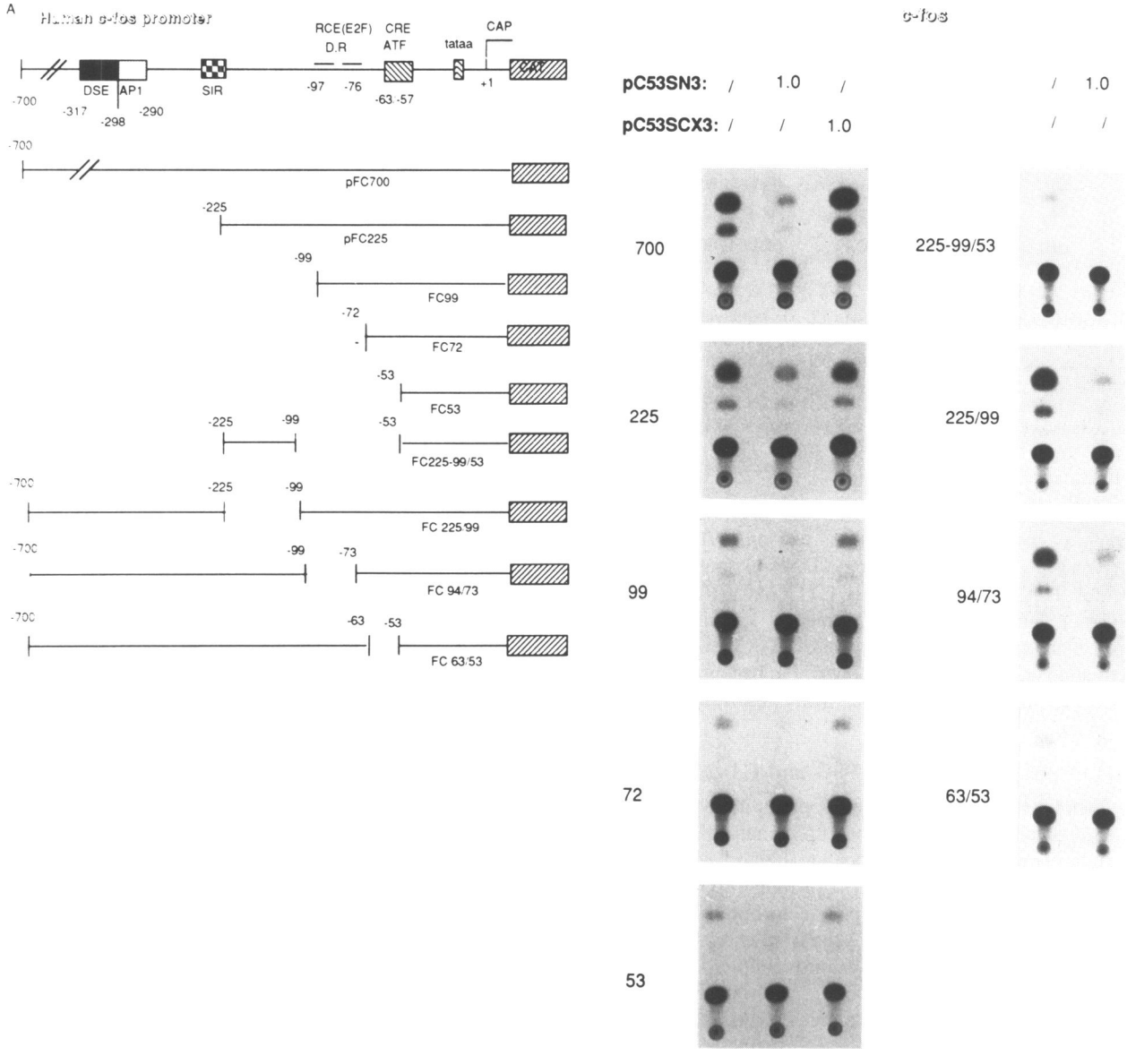

Figure 2. Deletion analysis and assay of regulatory elements of the c-fos promoter for response to wt p53. The structure of the parent plasmid pFC700 and the mutants derived thereof (kind gift of Drs. R. Prywes,T. Fisch,R. Roeder) are shown in Fig.2A. Elements indicated constitute: DSE: dyad symmetry element (27), SIR: serum inducible repeat (28), CRE: cAMP response element (29,30), RCE: retinoblastoma control element (31), and the TATA box. Fig2B: effects of pC53SN3 (1μg) and its 143-mutant counterpart pC53SCX3 (1μg) on expression of CAT plasmids (2μg) are shown as indicated above each lane. Plasmids were co-transfected in G102 cells with pCMV-neo-Bam as a control to keep total DNA constant. Results shown are representatives of n > 3 independent experiments.

sensitivity. The structure of the c-fos parent plasmid and 5'-deletion mutants derived thereof are shown in Fig.2A. A mutant form of p53 (p53SCX3, cloned into pCMV-neo-Bam) differing from its cellular counterpart by a single point mutation causing a change from valine to alanine at residue 143 (originally isolated from a colorectal carcinoma, 6) was used to determine wt-specific effects of p53. Both have previously been characterized for their translational efficiency (26) and effects on cell growth (6). Expression of exogenous wt and 143-mutant p53 proteins in transfected cells was monitored by Western blot analysis (using antibody PAb421, Oncogene Science) and revealed comparable, with expression of mutant protein 1.3 fold of that of wt p53 protein (data not shown). As shown in Fig.2B wt p53, but not mutant p53, was able to repress 8-bromo-cAMP-induced c-fos promoter activity of all deletion constructs described. The average degree of repression was somewhat higher from reporter constructs bearing sequences upstream of nucleotide −225 (e.g. 90% inhibition for the −700 deletion construct as compared to 70% inhibition for the −53 deletion construct), indicating that upstream sequences may in part further promote the transcriptional repression mediated by p53. Further studies will be necessary to clarify this issue. Both pFC225−99/53 and pFC63/53 expectedly exhibited a low level of activity as the cAMP response element is deleted in these constructs.

p53 as a transcriptional activator

As opposed to its inhibitory effects on the c-fos promoter, p53 (1μg) had no effect on HSV-TK promoter activity in T98G cells (pBLCAT2, Fig.3). Introducing a previously characterized p53 DNA binding sequence (CTTGCCTGGACTTGCCTGG) (13)

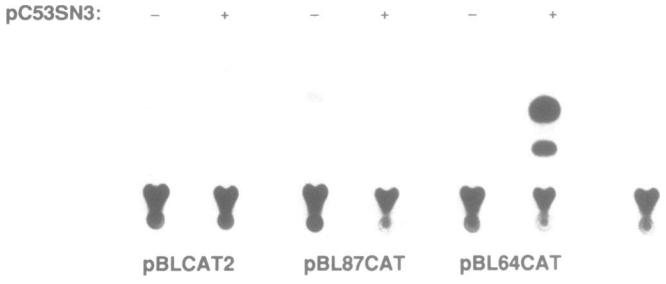

Figure 3. Activation of gene expression by wt p53. p53 expression plasmids were co-transfected with pBLCAT2 (HSV-TK promoter) and derivatives thereof. pBL64CAT bears the TGCCT repeat sequence in sense orientation upstream of the TK promoter. In pBL87CAT the second TGCCT box is mutated to TTTTT. Panel on the right shows background activity from non-transfected cells. T98G cells were co-transfected as described above and assayed for CAT activity 48h later.

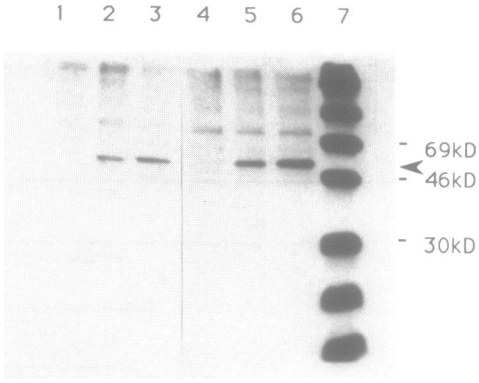

Figure 4. Expression of cellular p53 protein. Immunoprecipitation with PAb1620 (lanes 1,4), PAb240 (lanes 2,5), and PAb421 (lanes 3,6) of metabolically labeled protein extracts from G102 cells (lanes 1–3), and T98G cells (lanes 4–6). Molecular weight standard is shown in lane 7.

in sense-orientation upstream of the TK promoter, however promoted a strong transactivation by p53 (Fig.3). This responsiveness was not confered by a DNA element mutated in the second TGCCT box (CT**TGCCT**GGACT**TTTTT**GG in pBL87CAT), emphasizing the sequence specificity of the response element. Mutant p53 (pC53SCX3) displayed no transactivating activity (data not shown). No background activity was observed in extracts obtained from non-transfected cells (Fig.3, panel on far right).

Analysis of p53 phenotype in T98G and G102 cells

Figure 4 shows the results of an imunoprecipitation experiment aimed at determining the phenotype of p53 in the glial cells used in this study. Three different monoclonal antibodies which differ in their ability to recognize wild-type or mutant p53 were used. As shown in Fig.4, endogenous p53 from both cell lines was recovered by PAb421, recognizing both wild-type and mutant p53, and pAb240 that specifically immunoprecipitates mutant p53. In contrast, the wild-type specific antibody PAb1620 did not immunoprecipitate p53 from either cell line, suggesting a mutant conformation for p53 in these cell lines.

DISCUSSION

The results presented in this report demonstrate that wt human p53 can down-modulate the transcriptional activity of induced and non-induced human c-fos promoter in glial cells and that this repression is mediated through the basal promoter region extending from nucleotide −53 to +42 relative to the cap site.

Expression of wt p53, but not a transforming mutant, is associated with a strong repression of cAMP induced c-fos promoter activity. The findings that repression is also observed upon induction of c-fos by serum and the phorbol ester PMA, which is consistent with previous reports (18,19), indicate that the effects of p53 are not strictly dependent on the type of stimulus. In fact the same pattern of response in observed in the absence of any stimulus (N.Kley and R.Chung, unpublished observation). Several functional elements in the human c-fos promoter have been identified. These include the dyad symmetry element (DSE,27), the serum-inducible repeat (SIR,28), an octanucleotide direct repeat (23), a cAMP response element (CRE,29,30), a retinoblastoma control element (RCE,31), and the TATA box. None of these characterized elements upstream of the TATA box, were absolutely required for p53 mediating its inhibitory effects, as all deletion mutants described in Fig.2 were efficiently repressed by p53. Thus, repression mediated by p53 can largely be accounted for by an ability of p53 to interfere with c-fos basal promoter activity. However we cannot exclude that the presence of upstream sequences further enhance the ability of p53 to repress c-fos promoter activity.

p53 repressed c-fos promoter activity similarly in other glial cell lines, including T98G cells, and Hela cells (data not shown), indicating that the effects are not restricted to one particular cell type. As indicated by the immunoprecipitation experiments (Fig.4), the effects of transfected p53 occurred in cells expressing p53 of mutant phenotype (the exact nature of the mutation is as yet unclear). It is conceivable that the effects of transfected p53 may vary in their potency, depending on whether the cellular p53 phenotype is wild-type or mutant, and on the nature of the mutation.

The high sensitivity of our transfection protocol furthermore allowed us to show that the effects of p53 were titratable and occur in a linear range of p53 expression. This is an important issue concerning the specificity of the p53 mediated inhibition of cellular promoters, as already remarked by Ginsberg et al (19). Indeed, we have observed consistent, marked differences in the sensitivity of cellular promoters to p53 (N.Kley, R.Chung, unpublished observation). Such future studies may yield more insights into the physiological targets of p53 under conditions where it is not overexpressed.

The molecular mechanisms underlying the inhibitory effects of wt p53 on promoter activity are not understood. It has recently been described that p53 can bind, directly or indirectly, to a region in the SV40 early promoter and a cellular DNA sequence (TGCCT repeat) (13,14). We have not found such sequences in the −53/+42 region of the c-fos promoter. Furthermore, DNA binding studies in which we observed clear binding of p53 to the TGCCT repeat sequence, but not a mutated sequence, failed to detect any p53 binding to oligonucleotides spanning the −53/+42 region (data not shown), indicating that p53 may exert its effect on the c-fos promoter indirectly. It is unlikely that the inhibitory effects are due to an unspecific repression of transcriptional activity, as activity of the HSV-TK promoter is not down regulated by p53 (Fig. 3). Furthermore, introducing the TGCCT repeat sequence upstream of the TK promoter

actually promotes transactivation by p53 under identical conditions (Fig. 3). In contrast, insertion of a sequence mutated in the second TGCCT box (TGCCT-TTTTT), previously shown to be incapable of p53 binding (13), does not promote transactivation. Thus binding of p53 to this DNA recognition sequence correlates with its ability to activate transcription. This ability of p53 to transactivate genes is consistent with the recent findings by Weintraub et al. (32) showing that p53 enhances MCK-promoter activity. It is likely that this effect of p53 is mediated by a TGCCT-repeat-like sequence residing within the p53 response element characterized by these authors (32).

The structure of the p53 protein resembles that of known transcription factors consisting of two functional domains; a carboxy-terminal basic region, possibly involved in DNA binding, and an acidic and proline rich amino-terminal region. Studies using hybrid proteins in which the DNA-binding domain of yeast GAL4 was fused to the amino-terminal region of p53 have already provided evidence for a strong transactivating domain in the p53 protein (15,16,33). The relative contribution of the acidic and proline rich region in mediating transactivation by wt p53 has yet to be determined. The failure of the ala143 mutation, which lies outside this region of p53 to transactivate gene expression, indicates that subtle alterations in conformation are critical to p53 function. This is consistent with the recent findings by Raycroft et al. (34) that the human 143-mutant p53/GAL4 fusion protein is unable to activate transcription.

In addition to its apparent ability to bind DNA (13,14), the wt form of p53 has been reported to interact with various viral proteins such as the simian virus large tumor antigen, the human papilloma virus 16 E6 protein and the adenovirus E1B 55-kDa protein (35). It is tempting to speculate that p53 may interact in a similar way directly or indirectly with components of the basal transcriptional machinery and thereby mediate repression. On the other hand, p53 may mediate its effects via regulating the expression of genes whose long-lived products interact in a sequence specific manner with the basal promoter region, with potential regulatory interactions with the basal transcriptional machinery. Clearly, the identification of cellular components mediating transcriptional effects of p53 will be a major step towards a better understanding of the role of p53 in regulating cellular proliferation.

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REFERENCES

1. Levine, A.J., Momand, J., and Finlay, C.A. (1991) Nature 351, 453–456 .
2. Finlay, C.A. Hinds, P.W., and Levine, A.J. (1989) Cell 57, 1083–1089 .
3. Eliyahu, D., Michalovitz, D., Eliyahu, S., Pinhasi-Kimhi, O., and Oren, M. (1989) Proc.Natl.Acad.Sci.USA 86, 8763–8767.
4. Michalovitz, D., Halevy, O., Oren, M. (1990) Cell 62, 671–680 .
5. Mercer, W.E., Shields, M.T., Amin, M., Sause, G., Apella, E., Romano, J.W., and Ullrich, S.J. (1990) Proc.Natl.Acad.Sci 87, 6166–6170 .
6. Baker, S.J., Markowitz, S., Fearon, E.R., Wilson, J.K.W., and Vogelstein, B. (1990) Science 249, 912–915 .
7. Martinez, J., Georgoff, I., Martinez, J., and Levine, A.J. (1991) Genes Dev. 5, 151–159 .
8. Chen, P.L., Chen, Y., Bookstein, R., and Lee, W.-H Science (1990) 250, 1576–1580 .
9. Lane, D.P., and Benchimol, S. (1990) Genes Dev. 4, 1–8 .
10. Ginsberg, D., Michael-Michalovitz, D., Ginsberg, D., and Oren, M. (1990) Mol.Cell.Biol. 11, 582–585 .
11. Steinmeyer, K., and Deppert, W. (1988) Oncogene 3, 501–507 .
12. Kern, S.E., Kinzler, K.W., Baker, S.J., Nigro, J.M., Rotter, V., Levine, A.J., Friedman, P., Prives, C., and Vogelstein, B. (1991) Oncogene 6, 131–136 .
13. Kern, S.E., Kinzler, K.W., Bruskin, A., Friedman. P.N., Prives, C., and Vogelstein, B. (1991) Science 252, 1708–1711 .
14. Bargonetti, J., Friedman, P.N., Kern, S.E., Vogelstein, B., and Prives.C. (1991) Cell 65, 1083–1091.
15. Fields, S., and Jang, S.K. (1990) Science 249, 1046–1049 .
16. Raycroft, L., Wu, H., and Lozano, G. (1990) Science 249, 1049–1051 .
17. O'Rourke, R.W., Miller, C.W., Kato, G.J., Simon, K.J., Chen, D.l., Dang, C.V., and Koeffler, H.P. (1990) Oncogene 5, 1829–1832 .
18. Santhanam, U., Ray, A., and Seghal, P.B., (1991) Proc.Natl.Acad.Sci. USA, 88, 7605–7609
19. Ginsberg, D., Mechta, F., Yaniv, M., and Oren, M. (1991) Proc.Natl.Acad.Sci. USA, 88, 9979–9983
20. Behr, J.P., Demeneix, B., Loeffler, J.P., and Perez-Motul.J. (1989) Proc.Natl.Acad.Sci. USA 86, 6982–6986 .
21. Demeneix, B., Kley, N., and Loeffler, J.P. (1990) DNA and Cell Biol., 9, 335–345 .
22. Gorman, C.M., Moffat, L.M., and Howard, B.H., (1982) Mol.Cell.Biol. 2, 1044–1051
23. Fisch, T.M., Prywes, R., and Roeder, R.G. (1987) Mol.Cell.Biol. 7, 3490–3502 .
24. Luckow, B., and Schuetz, G. (1988) Nucl. Acid Res., , 5490
25. Morgenstern, J.P., and Land, H. (1990) Nucleic Acid Res. 18, 1068 .
26. Diller, L., Kassel, J., Nelson, C.E., Gryka, M.A., Litwak, G., Gebhardt, M., Bresac, B., Ozturk, M., Baker, S.J., Vogelstein, B., and Friend, S.H. (1990) Mol.Cell.Biol. 10, 5772–5782 .
27. Treisman, R. (1986) Cell 46, 567–574 .
28. Visvader, J., Sassone-Corsi, P., and Verma, I.M. (1988) Proc.Natl.Acad.Sci. USA 85, 9474–9478 .
29. Sassone-Corsi, P., Visvader, J., Ferland, L., Mellon, P.L., and Verma, I.M. (1988) Genes Dev. 2, 1529–1538 .
30. Fisch, T.M., Prywes, R., Simon, M.C., and Roeder, R.G. (1989) Genes. Dev. 3, 198–211 .
31. Robbins, P.D., Horovitz, J.M., and Mulligan, R.C. (1990) Nature 346, 668–670 .
32. Weintraub, H., Hauschka, S., and Tapscott, S.J. (1991) Proc.Natl.Acad.Sci. USA, 88, 4570–4571
33. O'Rourke, R.W.Miller, C.W., Kato, G.J., Simon, K.J., Chen, D.I., Dang, C.V., and Koeffler, H.P. (1991) Oncogene, 5, 1829–1832
34. Raycroft, L., Schmidt, J.R., Yoas, K., Hao, M., and Lozano, G. (1991) Mol.Cell.Biol., 11, 6067–6074
35. Levine, A.J. (1990) Virology, 177, 419–426
36. Harlow, E., Crawford, L.V., Pim, D.C., and Williamson, N.M. (1981) J.Virol., 39, 861–869
37. Gannon, J.V., Greaves, R., Iggo, R., and Lane, D.P. (1990) EMBO J., 9, 1595–1602
38. Ball, R.K., Siegl, B., Quellhorst, S., Brandner, G., and Braun, D.G. (1984) EMBO J., 3, 1485–1491
39. Milner, J., Cook, A., and Sheldon, M. (1987) Oncogene, 1, 453–455