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Phosphorylation of p53 in Normal and Simian Virus 40-Transformed NIH 3T3 Cells

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We observed six major tryptic phosphopeptides in p53 from simian virus 40-transformed and normal NIH 3T3 cells. Analyses of the phosphopeptides indicated that serines 37, 310 and/or 312, 389 and one or more of serines 7, 9, 12, 18, and 23 were phosphorylated. Phosphorylation of serines 310 and/or 312 was twofold higher in the simian virus 40-transformed cells as compared with that in normal NIH 3T3 cells.

p53 is a cellular protein of apparent molecular weight 53,000, which is present at low levels in normal cells and at high levels in a wide range of primary tumors and transformed cell lines (for reviews, see references 5, 26, and 31). The molecular function of p53 is unknown. However, it is a cell cycle-related nuclear protein which appears to be essential for the progression of cells from a quiescent to an actively growing state and therefore may be an important regulatory element in growth control (15, 20–23, 25, 30). p53 has also been classed as an oncogene based on its ability to rescue primary cells from senescence (12, 13); to cooperate, in place of *myc*, with an activated *ras* oncogene to transform primary rat embryo fibroblasts (7, 13, 28); and to transform previously immortalized cells to a tumorigenic phenotype without a significant change in cell morphology (6, 16, 35). The protein also forms stable complexes with the E1b tumor antigen of adenovirus (33) and the large tumor antigen (large T) of simian virus 40 (SV40; 17–19) and may have a direct involvement in SV40-mediated transformation (24).

p53 is a phosphoprotein (5, 26, 31) which lacks intrinsic protein kinase activity (34). Two phosphorylated amino acids, serines 312 and 389, have been identified in mouse p53 by using Edman sequencing (32); additional phosphorylation sites have been tentatively mapped to the amino terminus between amino acid residues 28 and 98. The functional significance of these phosphorylations is unclear. However, serine 389 may be important for transformation by SV40, since a reduction in phosphorylation of this residue at the nonpermissive temperature in SV40-*ts*A58-transformed cells correlates with a failure to form the large T/p53 complex (2, 8, 32).

To further characterize the phosphorylation sites of p53, we labeled SV40-transformed NIH 3T3 (SV3T3) cells for 16 h in phosphate-free Dulbecco-Vogt modified Eagle medium supplemented with 10% dialyzed calf serum and 2.5 mCi of $^{32}P_i$ per ml. Proteins were immunoprecipitated with anti-p53 monoclonal antibody PAb122 or PAb246 (36; kindly provided by E. Gurney, University of Utah, and J. Yewdell, Wistar Institute, respectively), resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and detected by autoradiography. p53 was eluted from the gel and oxidized as described previously (1) by using 20 μg of RNase A as a carrier. Digestion with tolylsulfonyl phenylalanyl chloromethyl ketone-treated trypsin was carried out by using a trypsin/carrier ratio of 1:50 (wt/wt) to minimize traces of chymotryptic activity. Phosphopeptides were separated in two dimensions by electrophoresis at pH 1.9 (1 kV for 40 min) or at pH 8.9 (1 kV for 15 min) and chromatography (11). p53 contained six major phosphopeptides, designated T1(a), T1(b), T2(a), T2(b), T3, and T4 (Fig. 1). Phosphoamino acid analysis (4) showed that each contained exclusively phosphoserine (data not shown).

Of the 35 tryptic peptides of murine p53, 18 contain serine residues (14, 29, 37). We compared these peptides with the observed phosphopeptides by determining the presence of particular amino acids within each phosphopeptide as follows. (i) The presence of cysteine or methionine was inferred by the comigration of ^{32}P-labeled peptides with peptides labeled with [^{35}S]cysteine or [^{35}S]methionine, respectively (16 h in cysteine-free or methionine-free Dulbecco-Vogt modified Eagle medium supplemented with 5% undialyzed calf serum and either 0.5 mCi of [^{35}S]cysteine per ml or 0.1 mCi of [^{35}S]methionine per ml, respectively). Detection of ^{32}P-labeled peptides in maps of mixed ^{35}S- and ^{32}P-labeled peptides was carried out by wrapping the thin-layer plates in four layers of aluminum foil to block ^{35}S counts. Autoradiography, using presensitized film with intensifying screens, was carried out at −70°C. To enhance ^{35}S detection, the plates were set aside for several weeks to allow the ^{32}P to decay and then immersed in 0.4% PPO (2,5-diphenyloxazole) in molten 2-methylnaphthalene and dried. Fluorography, using presensitized film, was carried out at −70°C. (ii) The presence of glutamic acid in a ^{32}P-labeled peptide was determined by its sensitivity to digestion by *Staphylococcus aureus* V8 protease under the appropriate conditions (9). (iii) Chymotrypsin digestion (3) revealed the presence of tryptophan, tyrosine, or phenylalanine. Sensitivity to cleavage by these enzymes was based on the disappearance of a phosphopeptide from its characteristic location, accompanied by the appearance of a phosphopeptide(s) in a new location(s). To illustrate this approach, Fig. 2 shows that when p53 tryptic phosphopeptides (Fig. 2A) were subjected to further cleavage by chymotrypsin, two phosphopeptides, T3 and T4, disappeared and were replaced by three new phosphopeptides, designated C1, C2a, and C2b (Fig. 2B). A mixture of the phosphopeptides generated by trypsin and chymotrypsin with those generated by trypsin alone showed that phosphopeptides C1, C2a, and C2b were different from phosphopeptides T3 and T4. Tryptic phosphopeptides T1a, T1b, T2a, and T2b were not further digested by chymotrypsin.

The results from these studies are summarized in Table 1;

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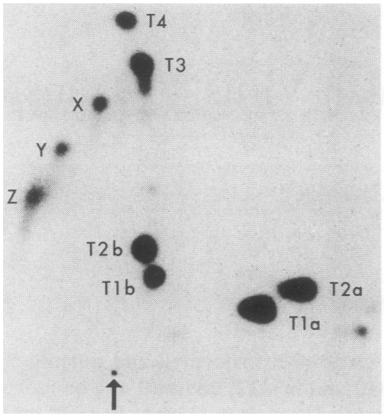

FIG. 1. Tryptic phosphopeptide map of metabolically labeled p53 from SV3T3 cells. ^{32}P-labeled proteins were extracted from SV3T3 cells, digested with trypsin, and resolved in the first dimension (horizontal direction) by electrophoresis at pH 1.9 and in the second dimension (vertical direction) by chromatography as described in the text. Approximately 5,000 Cerenkov cpm were loaded onto each plate, and presensitized film was exposed at −70°C for 24 h with an intensifying screen. The arrow indicates the origin. The major phosphopeptides are designated T1a, T1b, T2a, T2b, T3, and T4. Three minor phosphopeptides are designated X, Y, and Z.

the amino acid numbers used are based on the predicted amino acid sequence of murine p53 (14, 29, 37). The amino acid sequences of the candidate phosphopeptides are given in Table 2.

Tryptic phosphopeptides T1a and T1b had characteristics consistent with peptides 179–193 and/or 304–316. We eliminated peptide 179–193 as a candidate and showed that both phosphopeptides were derived from peptide 304–316, based on the following data. (i) The products of partial acid hydrolysis of T1a and T1b (in 6 N HCl at 110°C for 30 min) were identical (Fig. 3), indicating that the peptides contained the same phosphorylation site(s). Moreover, when T1a was redigested with trypsin, the product migrated to the position of T1b (data not shown). Of the two candidate peptides, only 304–316 can give rise to more than one tryptic product, because it has tandem basic residues at its N and C termini. (ii) We did not observe the release of phosphate during the second round of manual Edman degradation, as would be expected if peptide 179–193 were phosphorylated (data not shown), since the only serine residue in this peptide is at position 2. (iii) We calculated the two-dimensional mobilities of the serine-containing phosphopeptides of p53 as previously described (10). The theoretical mobilities of the tryptic products 303–316 and 304–317 are coincident and matched the actual migration of spot T1a under two different conditions of electrophoretic separation (i.e., at pH 1.9 and pH 8.9; data not shown), while the predicted migration of peptide 304–316 closely matched the actual migration of T1b (data not shown). The predicted migration of peptide 179–193 did not resemble the actual mobility of either T1a or T1b.

Peptide T1 contains two serine residues at positions 310 and 312. Our data did not distinguish which of these is the phosphorylated residue, but phosphorylation of this peptide is compatible with the observation of Samad et al. (32) that serine 312 is phosphorylated.

Tryptic phosphopeptides T2a and T2b had characteristics consistent with peptides 371–376 and/or 385–390. However, T2a and T2b gave identical fingerprints after partial acid hydrolysis (as described above), indicating that they contained the same phosphorylation site (Fig. 3). In confirmation, T2a migrated to the position of T2b after one cycle of manual Edman degradation (data not shown), indicating the loss of an N-terminal basic residue. The predicted two-dimensional mobilities of the peptides indicated that T2(a,b) was the C-terminal peptide 385–390 (data not shown). The single serine residue in this peptide, at position 389, has been reported to be phosphorylated (32).

Analysis of T3 indicated that it comprised amino acids 1 to

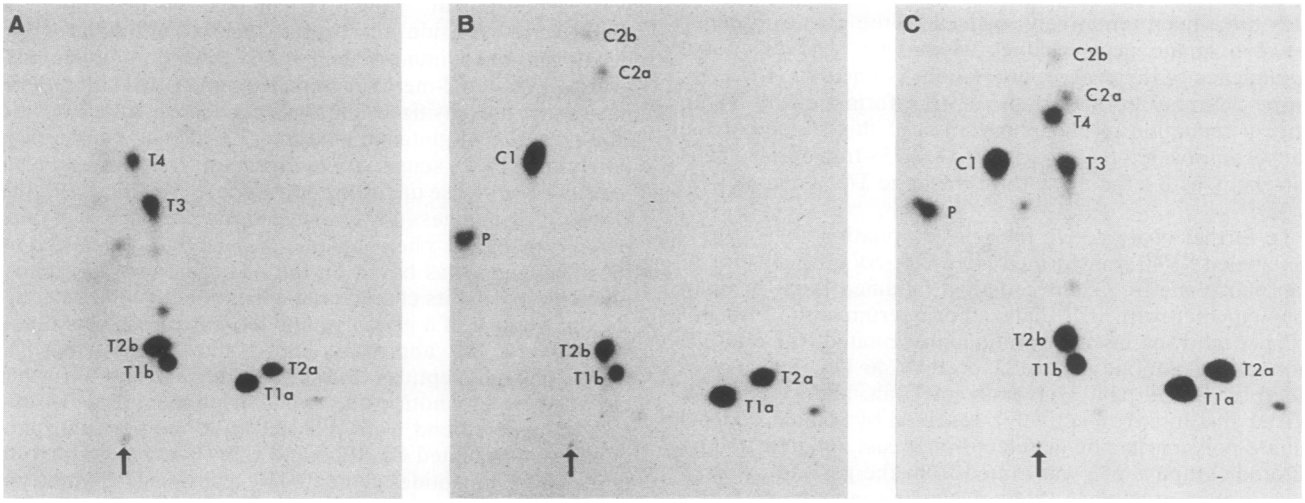

FIG. 2. Chymotryptic digestion of tryptic phosphopeptides of metabolically labeled p53 from SV3T3 cells. ^{32}P-labeled proteins were extracted from SV3T3 cells and digested with trypsin. An aliquot of the trypsin-digested material was then further digested with chymotrypsin. The peptides were separated in two dimensions on cellulose thin-layer plates by electrophoresis at pH 1.9 (horizontal direction) and chromatography (vertical direction). (A) p53 digested with trypsin alone. (B) p53 digested with trypsin and then with chymotrypsin. (C) A mixture of the peptides from panels A and B. P, position of free P_i. The arrows indicate the origins. Approximately 1,400 Cerenkov cpm were loaded onto each map. In each case, presensitized film was exposed at −70°C for 3 days with an intensifying screen.

TABLE 1. Identification of serine phosphate-containing peptides of p53

Tryptic spot	Cuts with:		Methionine present	Cysteine present	Possible peptide(s)
	V8	Chymotrypsin			
T1(a)	−	−	−	+	179–193 304–316
T1(b)	−	−	−	+	179–193 304–316
T2(a)	−	−	−	−	371–376 385–390
T2(b)	−	−	−	−	371–376 385–390
T3	+	+	+	−	1–27
T4	+	+	+	+	28–62

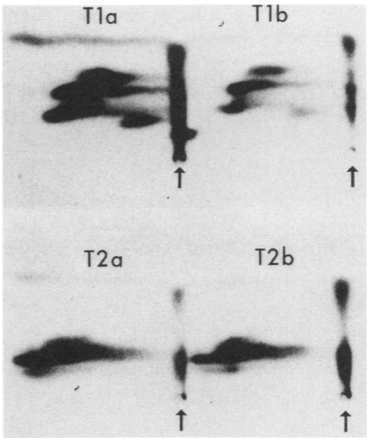

FIG. 3. Fingerprinting of p53 phosphopeptides T1a, T1b, T2a, and T2b. The peptides were eluted from a map similar to the one shown in FIG. 1 and then partially hydrolyzed in 6 N HCl for 30 min at 110°C. The products were resolved in two dimensions by electrophoresis at pH 8.9 (horizontal direction) and chromatography (vertical direction). Approximately 200 to 600 Cerenkov cpm were loaded in each sample, and presensitized film was exposed at −70°C for 7 days with an intensifying screen. The arrows indicate the origins.

27. No other serine-containing peptide in the predicted p53 sequence was compatible with these results. Similarly, the data indicate that T4 was peptide 28–62. The theoretical mobilities of peptides 1–27 and 28–62 are very similar to the experimentally observed migrations of phosphopeptides T3 and T4, respectively, and confirmed our identification of these peptides (data not shown). The T4 data are consistent with previous observations that phosphorylation sites lie between amino acids 28 and 98 (32).

Peptide T4 contains two serine residues, at positions 37 and 58. We favor serine 37 as the site of phosphorylation because phosphopeptides C2a and C2b migrated to the predicted locations of two related serine 37-containing peptides generated by chymotryptic cleavage of T4 at tandem sites (Fig. 2). We were unable to detect a corresponding serine 58-containing phosphopeptide at its predicted location. Analysis of the V8 products of T4 supported this conclusion (data not shown).

Peptide T3 contains five serine residues, at positions 7, 9, 12, 18, and 23. Several of these serine residues may be phosphorylated, because three other minor phosphopeptides, designated X, Y, and Z (Fig. 1), lie on a diagonal with T3, as would be expected if they were multiply phosphorylated species of T3. (We have been unable to recover enough radioactivity in X, Y, or Z to test their relatedness to T3.)

We have not characterized the phosphorylation sites further for the following reasons. (i) The distance between the N-terminal amino acid in each phosphopeptide and the closest serine residue is in every case too great to be able to detect the release of phosphate (during β-elimination) by manual Edman degradation. (ii) We have been unable to recover enough radioactivity in each peptide for automated

TABLE 2. Tentatively identified phosphopeptides of p53[a]

Tryptic spot	Corresponding peptide
T1(a,b)	179 193 CSDGDGLAPPQHLIR 304 316 K/R/ALPTCTSASPPQK/K/KP
T2(a,b)	371 376 K/K/GQSTSR/H 385 390 K/K/VGPDSD (C terminal)
T3	1 27 (N terminal)MTAMEESQSDISLELPLSQETFSGLWK/L
T4	28 62 K/LLPPEDILPSPHCMDDLLLPQDVEEFFEGPSEALR/V

[a] The N- and C-terminal amino acids in each peptide are numbered according to their positions in the p53 sequence. The trypsin cleavage sites are indicated by slashes. The N- and C-terminal amino acids of p53, which are present in peptides 1–27 (T3) and 385–390 (T2a,b), respectively, are indicated.

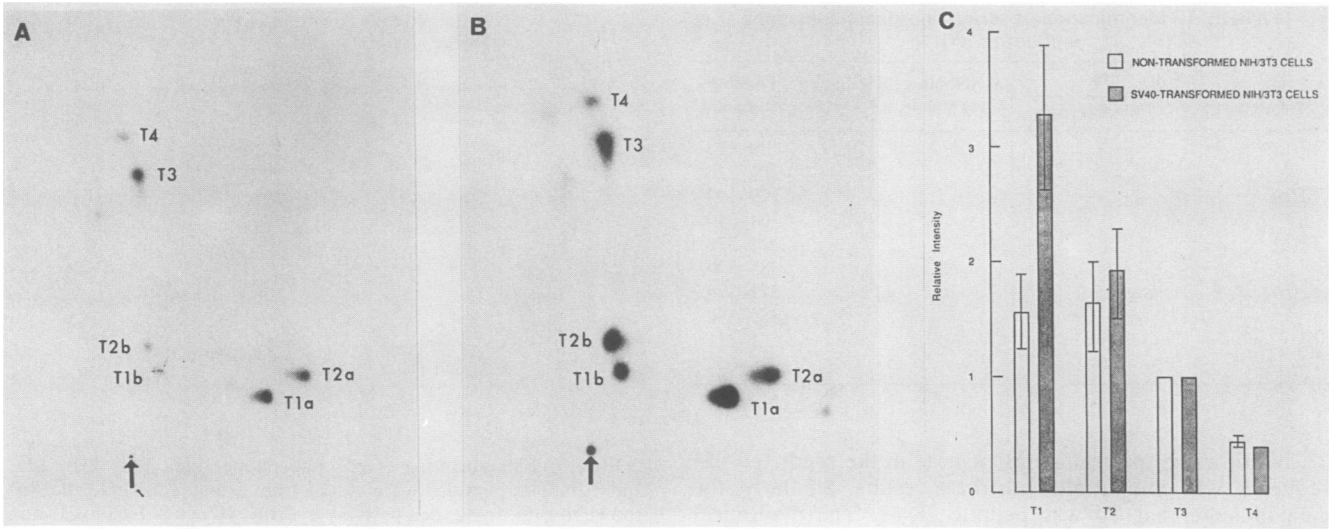

FIG. 4. Comparison of phosphopeptides of metabolically labeled p53 from nontransformed and SV40-transformed NIH 3T3 cells. Phosphopeptide maps of p53 from nontransformed and SV40-transformed NIH 3T3 cells were prepared as described in the text (with electrophoresis at pH 1.9). In each case, presensitized film was exposed at −70°C with an intensifying screen. Cerenkov counts per minute in each sample and the exposure times were: (A) p53 from NIH 3T3 cells, 400 cpm, 7 days; and (B) p53 from SV40-transformed NIH 3T3 cells, 5,000 cpm, 24 h. The arrows indicate the origins. The relative amounts of the phosphopeptides in each cell line are shown in panel C. The intensities of the spots on the autoradiograms were estimated by scanning. The values for peptide T1 were calculated by summing the intensities of spots T1a and T1b. Similarly, the T2 values were summed from T2a and T2b. For each autoradiograph, an arbitrary value of 1.0 was assigned to T3, and the values for the other phosphopeptides were normalized to this. The data presented in the bar chart were averaged from four autoradiographs of p53 peptides from the nontransformed cells and from three autoradiographs of the SV40-transformed cells (all from different experiments). The standard errors are shown (where appropriate) in the form of error bars.

sequencing. (iii) Even if we could identify the phosphorylation sites by sequencing, we would expect to have difficulty in interpreting whether the release of phosphate occurs at residue 9 or 12 in peptide T3 (amino acids 1 to 27), because it is not known whether the initiation codon of mouse p53 is at codon 1 or 4 of the predicted protein sequence (14, 29, 37).

Differences in the half-lives of p53 from the nontransformed cells ($t_{1/2} < 1.5$ h) and from the SV40-transformed cells ($t_{1/2} > 24$ h) (26) made it difficult to obtain steady-state labeling of p53 from the transformed cells with [^{35}S]methionine. Therefore, we were unable to accurately compare the ^{32}P/^{35}S ratios of p53 from the two cell lines, and thus, we were unable to determine the levels of phosphorylation. However, a comparison of the phosphopeptide maps of p53 from nontransformed and SV40-transformed NIH 3T3 cells shows that the same phosphopeptides were present in p53 from the two cell lines (Fig. 4A and B). We quantified the peptides by scanning the autoradiographs to determine whether there were any transformation-related differences in the relative levels of phosphorylation at individual sites (Fig. 4C). We normalized the data such that for each map, the value of each peptide was expressed relative to T3. Our results consistently indicate that phosphorylation of peptide 304–316 (T1a and T1b, containing serines 310 and 312) was 1.5- to 2-fold higher in the SV40-transformed cells as compared with that in the nontransformed cells (Fig. 4C). (We reached the same conclusion when the values were expressed relative to T2 or T4.) There were no significant transformation-related differences in any of the other phosphopeptides. Studies of p53 phosphorylation in a range of other cell lines, which we are presently carrying out in collaboration with others, support these conclusions and suggest that the twofold increase we observed with the NIH cells is dependent on association with T antigen rather than on transformation per se (unpublished observations). Samad et al. (32) also found a transformation-related difference of similar magnitude in p53 phosphorylation, but at serine 389. It is possible that differences in the labeling conditions, extraction conditions, or cells used (i.e., we compared normal and SV40-transformed NIH 3T3 cells, whereas Samad et al. [32] compared 3T3 cells transformed by an SV40 *ts*A mutant at permissive and nonpermissive temperatures) would highlight one subtle change in phosphorylation state in preference to another. The changes in p53 phosphorylation, although small, may be important for transformation, particularly if they are characteristic of a subclass of p53 molecules. Changes in pp60$^{c\text{-}src}$ phosphorylation caused by the polyomavirus middle T antigen are confined to a small fraction of the pp60$^{c\text{-}src}$ population but are important for polyomavirus transformation (3).

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LITERATURE CITED

1. **Beemon, K., and T. Hunter.** 1978. Characterization of Rous sarcoma virus *src* gene products synthesized in vitro. J. Virol. **28**:551–566.
2. **Carroll, R. B., and E. G. Gurney.** 1982. Time-dependent maturation of the simian virus 40 large T antigen-p53 complex studied by using monoclonal antibodies. J. Virol. **44**:565–573.
3. **Cartwright, C. A., P. L. Kaplan, J. A. Cooper, T. Hunter, and W. Eckhart.** 1986. Altered sites of tyrosine phosphorylation in pp60$^{c\text{-}src}$ associated with polyomavirus middle tumor antigen. Mol. Cell. Biol. **6**:1562–1570.
4. **Cooper, J. A., B. M. Sefton, and T. Hunter.** 1983. Detection and quantification of phosphotyrosine in proteins. Methods En-

zymol. **99**:387–402.
5. **Crawford, L. V.** 1983. The 53,000-dalton cellular protein and its role in transformation. Int. Rev. Exp. Pathol. **25**:1–50.
6. **Eliyahu, D., D. Michalovitz, and M. Oren.** 1985. Overproduction of p53 antigen makes established cells highly tumorigenic. Nature (London) **316**:158–160.
7. **Eliyahu, D., A. Raz, P. Gruss, D. Givol, and M. Oren.** 1984. Participation of p53 cellular tumor antigen in transformation of normal embryonic cells. Nature (London) **312**:646–649.
8. **Greenspan, D. S., and R. B. Carroll.** 1981. Complex of simian virus 40 large tumor antigen and 48,000-dalton host tumor antigen. Proc. Natl. Acad. Sci. USA **78**:105–109.
9. **Houmard, J., and G. R. Drapeau.** 1972. Staphylococcal protease: a proteolytic enzyme specific for glutamoyl bonds. Proc. Natl. Acad. Sci. USA **69**:3506–3509.
10. **Hunter, T., M. A. Hutchinson, W. Eckhart, T. Friedmann, A. Esty, P. La Porte, and P. Deininger.** 1979. Organization of T antigens in the polyoma virus genome. Cold Spring Harbor Symp. Quant. Biol. **44**:131–139.
11. **Hunter, T., and B. M. Sefton.** 1980. Transforming gene product of Rous sarcoma virus phosphorylates tyrosine. Proc. Natl. Acad. Sci. USA **77**:1311–1315.
12. **Jenkins, J. R., K. Rudge, P. Chumakov, and G. A. Currie.** 1985. The cellular oncogene p53 can be activated by mutagenesis. Nature (London) **317**:816–818.
13. **Jenkins, J. R., K. Rudge, and G. A. Currie.** 1984. Cellular immortalization by a cDNA clone encoding the transformation-associated phosphoprotein p53. Nature (London) **312**:651–654.
14. **Jenkins, J. R., K. Rudge, S. Redmond, and A. Wade-Evans.** 1984. Cloning and expression analysis of full length mouse cDNA sequences encoding the transformation associated protein p53. Nucleic Acids Res. **12**:5609–5626.
15. **Kaczmarek, L., M. Oren, and R. Baserga.** 1986. Co-operation between the p53 protein tumor antigen and platelet-poor plasma in the induction of cellular DNA synthesis. Exp. Cell Res. **162**:268–272.
16. **Keleakar, A., and M. D. Cole.** 1986. Tumorigenicity of fibroblast lines expressing the adenovirus E1a, cellular p53, or normal c-*myc* genes. Mol. Cell. Biol. **6**:7–14.
17. **Kress, M., E. May, R. Cassingena, and P. May.** 1979. Simian virus 40-transformed cells express new species of proteins precipitable by anti-simian virus 40 tumor serum. J. Virol. **31**:472–483.
18. **Lane, D. P., and L. V. Crawford.** 1979. T antigen is bound to a host protein in SV40-transformed cells. Nature (London) **278**: 261–263.
19. **Linzer, D. I. H., and A. J. Levine.** 1979. Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. Cell **17**:43–52.
20. **Malzman, W., and L. Czyzyk.** 1984. UV irradiation stimulates levels of p53 cellular tumor antigen in nontransformed mouse cells. Mol. Cell. Biol. **4**:1689–1694.
21. **Mercer, W. E., C. Avignolo, and R. Baserga.** 1984. Role of the p53 protein in cell proliferation as studied by microinjection of monoclonal antibodies. Mol. Cell. Biol. **4**:276–281.
22. **Mercer, W. E., C. Avignolo, H. Liu, and R. Baserga.** 1984. The p53 protein and cell proliferation, p. 377–382. *In* G. F. Vande Woude, A. J. Levine, W. C. Topp, and J. D. Watson (ed.), Cancer cells. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **Mercer, W. E., D. Nelson, A. B. DeLeo, L. J. Old, and R. Baserga.** 1982. Microinjection of monoclonal antibody to protein p53 inhibits serum-induced DNA synthesis in 3T3 cells. Proc. Natl. Acad. Sci. USA **79**:6309–6312.
24. **Michalovitz, D., D. Eliyahu, and M. Oren.** 1986. Overproduction of protein p53 contributes to simian virus 40-mediated transformation. Mol. Cell. Biol. **6**:3531–3536.
25. **Milner, J.** 1984. Different forms of p53 detected by monoclonal antibodies in non-dividing and dividing lymphocytes. Nature (London) **310**:143–145.
26. **Oren, M.** 1985. The p53 cellular tumor antigen: gene structure, expression and protein properties. Biochim. Biophys. Acta **823**:67–78.
27. **Oren, M., W. Maltzman, and A. J. Levine.** 1981. Post-translational regulation of the 54K cellular tumor antigen in normal and transformed cells. Mol. Cell. Biol. **1**:101–110.
28. **Parada, L. F., H. Land, R. A. Weinberg, D. Wolf, and V. Rotter.** 1984. Cooperation between gene encoding p53 tumor antigen and *ras* in cellular transformation. Nature (London) **312**:649–651.
29. **Pennica, D., D. V. Goeddel, J. S. Hayflick, N. C. Reich, C. W. Anderson, and A. J. Levine.** 1984. The amino acid sequence of murine p53 determined from a cDNA clone. Virology **134**:477–482.
30. **Reich, N. C., and A. J. Levine.** 1984. Growth regulation of a cellular tumor antigen, p53, in non-transformed cells. Nature (London) **308**:199–201.
31. **Rotter, V., and D. Wolf.** 1984. Biological and molecular analysis of p53 cellular-encoded tumor antigen. Adv. Cancer Res. **43**:113–141.
32. **Samad, A., C. W. Anderson, and R. B. Carroll.** 1986. Mapping of phosphomonoester and apparent phosphodiester bonds of the oncogene product p53 from simian virus 40-transformed 3T3 cells. Proc. Natl. Acad. Sci. USA **83**:897–901.
33. **Sarnow, P., Y. S. Ho, J. Williams, and A. Levine.** 1982. Adenovirus E1b-58kd tumor antigen and SV40 large T antigen are physically associated with the same 54 kd cellular protein in transformed cells. Cell **28**:387–394.
34. **Van Roy, F., L. Fransen, and W. Fiers.** 1984. Protein kinase activities in immune complexes of simian virus 40 large T-antigen and transformation-associated cellular p53 protein. Mol. Cell. Biol. **4**:232–239.
35. **Wolf, D., N. Harris, and V. Rotter.** 1984. Reconstitution of p53 expression in a nonproducer Ab-MuLV-transformed cell line by transfection of a functional p53 gene. Cell **38**:119–126.
36. **Yewdell, J. W., J. V. Gannon, and D. P. Lane.** 1986. Monoclonal antibody analysis of p53 expression in normal and transformed cells. J. Virol. **59**:444–452.
37. **Zakut-Houri, R., M. Oren, B. Bienz, V. Lavie, S. Hazum, and D. Givol.** 1983. A single gene and a pseudogene for the cellular tumor antigen p53. Nature (London) **306**:594–597.