Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 8763–8767, November 1989
Cell Biology

Wild-type p53 can inhibit oncogene-mediated focus formation

(dominant negative mutations/tumor-suppressor genes/tumorigenesis)

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Communicated by I. Robert Lehman, July 25, 1989

ABSTRACT **Mutant forms of the p53 cellular tumor antigen elicit neoplastic transformation *in vitro*. Recent evidence indicated that loss of normal p53 expression is a frequent event in certain types of tumors, raising the possibility that such loss provides transformed cells with a selective growth advantage. Thus, it was conceivable that the mutants might contribute to transformation by abrogating normal p53 function. We therefore studied the effect of plasmids encoding wild-type (wt) p53 on the ability of primary rat embryo fibroblasts to be transformed by a combination of mutant p53 and *ras*. It was found that wt p53 plasmids indeed caused a marked reduction in the number of transformed foci. Furthermore, wt p53 plasmids also suppressed the induction of transformed foci by combinations of bona fide oncogenes, such as *myc* plus *ras* or adenovirus *E1A* plus *ras*. On the other hand, plasmids carrying mutations in the p53 coding region totally failed to inhibit oncogene-mediated focus induction and often even slightly stimulated it. Hence, such mutations completely abolished the activity of wt p53 that is responsible for the "suppressor" effect. The latter fact is of special interest, since similar mutations in p53 are often observed in human and rodent tumors. The inhibitory effect of wt p53 was most pronounced when early-passage cells were used as targets, whereas established cell lines were less sensitive. These data support the notions that wt p53 expression may be restrictive to neoplastic progression and that p53 inactivation may play a crucial role in tumorigenesis.**

A number of properties of the p53 cellular tumor antigen have led to the suggestion that it may be tightly associated with neoplastic transformation (1–4). Based on a variety of findings, the p53 gene was proposed to be an oncogene of the "nuclear," *myc*-like type (5–9).

While there are many instances of p53 overproduction in transformed cells, there are other cases in which p53 expression is altogether absent. The most striking case is that of mouse spleen tumors induced by the Friend erythroleukemia virus. The detailed analysis of those tumors, many of which have no detectable normal p53 at all, has led to the suggestion that, in some cases, loss of p53 function may be advantageous for tumorigenesis (10–13). Recent studies involving human tumors have lent strong support to this idea (14–17). A high proportion of human osteosarcomas exhibit no p53 synthesis; this is often correlated with the presence of gross rearrangements in the p53 genes (ref. 14; C. Miller and P. Koeffler, personal communication). The study of human colon cancer has been especially revealing (16, 17). One of the two p53 alleles is completely lost in at least 75% of cases. The other allele, in all cases studied so far, is invariably mutated. Most remarkably, the mutations fall within highly conserved protein domains; mutations in these regions have been shown to grossly alter the biological properties of mouse p53 (18–21).

The apparent paradox of p53 having both a positive and a negative effect on neoplastic processes seems now to be resolved, due to the finding that wild-type (wt) p53 is devoid of any demonstrable transforming activity, and the latter activity is exhibited only by plasmids encoding certain mutant forms of the protein (19, 20, 22). It was therefore conceivable that wt p53 may indeed interfere with transformation, and the mutant p53 could thus promote transformation by blocking normal p53 function, acting in a dominant negative fashion (23).

The idea that wt p53 may be restrictive to transformation was tested by studying the effect of plasmids encoding wt or mutant p53 on the ability of various oncogene combinations to elicit transformed foci *in vitro*. We report here that wt p53 plasmids exhibited a pronounced inhibitory effect in such transformation assays. The magnitude of the effect was directly correlated with the level of wt p53 expression and was totally abolished by point mutations in the protein. Our findings suggest that wt p53 expression may indeed have an adverse effect on transformed cells and that its inactivation may play a crucial role in tumorigenesis.

MATERIALS AND METHODS

Plasmids. Most of the plasmids employed in this study have been described before, some of them under different names. pLTRp53wt encodes wt mouse p53 under the transcriptional control of a Harvey sarcoma virus long terminal repeat (LTR) and is identical to pLTRNc9 (20). pLTRp53m is essentially similar, except that the p53 cDNA is derived from Meth A fibrosarcoma cells (20, 24) and carries point mutations at positions corresponding to residues 168 and 234 of the protein; it is identical to pLTRc5 (20). pLTRp53cG9 (9, 19, 25) contains a chimera of mouse p53 cDNA and genomic DNA; it encodes a protein with a mutation at residue 135 (19, 20). pLTRp53dl is identical to the previously described pLTRcGS, a deleted derivative of pLTRp53cG missing the bulk of the protein-coding region (25). The construction of pCMVp53wt and pCMVp53m will be described in detail elsewhere. These plasmids contain p53 cDNA segments identical to those present in pLTRp53wt and pLTRp53m, respectively. Transcription is driven by the immediate-early enhancer–promoter of the human cytomegalovirus (CMV) (26). In addition, each plasmid contains a chimeric intron, generated by fusing the 5′ part of the first CMV intron to the 3′ part of the first p53 intron. pCMVp53dl was prepared from pCMVp53wt by cleaving this plasmid DNA with *Xho* I and *Kpn* I, blunting the ends, and religating. This results in a deletion of about 600 base pairs, removing approximately the N-terminal half of the protein. In addition, the region encoding the C-terminal half, 3′ to the *Kpn* I site, is now positioned out of phase (data not shown). Consequently, the polypeptide

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Abbreviations: CMV, cytomegalovirus; LTR, long terminal repeat; REF, rat embryo fibroblast; wt, wild type.

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encoded by this plasmid contains only the first 15 amino acids of authentic mouse p53.

Cells and Transfections. Primary rat embryo fibroblasts (REFs) were prepared from 16-day-old Fischer rat embryos and maintained as described (5). Cells were usually passaged two or three times before being transfected. Approximately 8×10^5 cells growing in a 90-mm dish were transfected by the calcium phosphate coprecipitation method (5). Following transfection and glycerol shock, cells were maintained for 2–3 days in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, and then the serum concentration was reduced to 5%. Rat-1 cells, an established fibroblastic cell line, were maintained, transfected, and subjected to mycophenolic acid selection as described (9).

Protein Analysis. Proteins were labeled with [^{35}S]methionine, extracted, immunoprecipitated, and analyzed by SDS/polyacrylamide gel electrophoresis as described (5, 20).

RESULTS

Effect of wt p53 on Transformation of REFs by Mutant p53 Plus *ras*. Primary REFs can become neoplastically transformed through the combined action of plasmids overproducing mutant p53 and activated Ha-*ras* (5–7, 19, 20). If the transforming, mutant p53 plasmids were indeed working by interfering with the activity of wt p53, then one should be able to antagonize their transforming effects by increasing the amount of wt p53. We therefore studied the effect of various p53 plasmids on the transformation of REFs by a combination of *ras* and pLTRp53cG9, which directs the abundant synthesis of mutant p53 (20). In addition to previously described plasmids driven by a retroviral LTR (20), we used a new set of plasmids employing the strong CMV immediate-early enhancer–promoter (26). In each case, the effects of adding a wt p53 plasmid to the transfection mixture were compared to those elicited by similar plasmids encoding either a heavily deleted (dl) or point-mutated (m) p53 (see *Materials and Methods* for plasmid descriptions). It should be noted that while the latter mutant plasmid, pLTRp53m, possessed an inherent transforming activity, it was much lower than that of pLTRp53cG9 (ref. 20; unpublished results), most likely because the presence of introns in pLTRp53cG9 causes it to express much higher levels of mutant protein (ref. 22; see also Fig. 2).

The results of such an analysis are summarized in Table 1. Inclusion of wt p53 plasmids in the assay led to a major reduction in the number of transformed foci induced by *ras* and pLTRp53cG9. The effect was particularly pronounced when plasmid pCMVp53wt was employed. Further, there was a consistent correlation between the extent of inhibition and the amount of wt p53 plasmid used (Fig. 1). Inclusion of the control deletion plasmid (pLTRp53dl; Table 1) also led to a reduction in focus numbers. This may have been due, at least in part, to competition with pLTRp53cG9 for common transcription factors. Nevertheless, this inhibitory effect was much milder than that exerted by the wt p53 plasmids. Furthermore, the results shown in Fig. 1 have already been normalized for this apparent inhibition by pLTRp53dl.

To further explore the quantitative aspects of the system, a transient-expression experiment was performed (Fig. 2). It is evident that under these assay conditions, pCMVp53wt is 8- to 10-fold more efficient than pLTRp53wt in directing the synthesis of p53 protein. Interestingly, both wt plasmids make far less p53 than pLTRp53cG9, and yet they very effectively inhibit focus induction by the latter mutant plasmid. This quantitative relationship argues against the notion that wt p53 merely competes with a positive transforming activity of the mutant. Rather, it indicates that wt p53 provides a distinct, intrinsic activity in this system.

Table 1. Effect of p53 plasmids on transformation of REFs by mutant p53 plus *ras*

Additional cotransfected DNA	Foci per dish (no. of dishes)
None	28 (6)
pSP6	23 (1)
pLTRp53dl	9 (2)
pLTRp53m	20 (2)
pLTRp53wt	3 (2)
pCMVp53dl	10 (2)
pCMVp53m	14 (6)
pCMVp53wt	0.4 (10)

Each transfection mixture contained 2 μg of pLTRp53cG9, encoding mutant p53 (19, 20), and 2 μg of pEJ6.6, carrying an activated Ha-*ras* gene (27), as well as 5 μg of additional plasmid DNA as indicated. Foci were scored 3 weeks after transfection. Results are given as average number of foci per transfected dish and were compiled from several independent transfections. Numbers in parentheses represent the number of transfection dishes scored to obtain the average value. pSP6 refers to pSP64 (28), which served as a vector control for pCMV plasmids. pLTRp53m and pLTRp53wt are identical to the previously described pLTRc5 and pLTRNc-9, respectively (20). pCMVp53m and pCMVp53wt encode mutant and wt p53, respectively, under the control of the CMV immediate-early promoter (26). In addition, they contain the first noncoding CMV exon and a chimeric intron. pCMVp53dl is a deletion derivative of pCMVp53wt.

Effect of wt p53 on Transformation of REFs by *myc* Plus *ras*. Nonestablished REFs can be very efficiently transformed by a combination of pEJ6.6 and plasmids encoding the Myc protein (30). To determine whether *myc* plus *ras*-mediated transformation was also affected by the cellular levels of wt p53, the transfection experiments summarized in Table 2 were performed. Since transformation efficiencies varied severalfold among individual experiments, we chose to display the results of each group of transfections separately. As seen from Table 2, *myc* plus *ras*-mediated transformation was also very sensitive to the presence of wt p53 expression plasmids. Inclusion of pCMVp53wt in the transformation mixture reduced the number of foci by a factor of at least 20. Most remarkably, no such reduction was seen when a plasmid encoding mutant p53 (pCMVp53m) was used. The protein encoded by pCMVp53m carries two point mutations, at residues 168 and 234 (refs. 20 and 33; see also *Materials and*

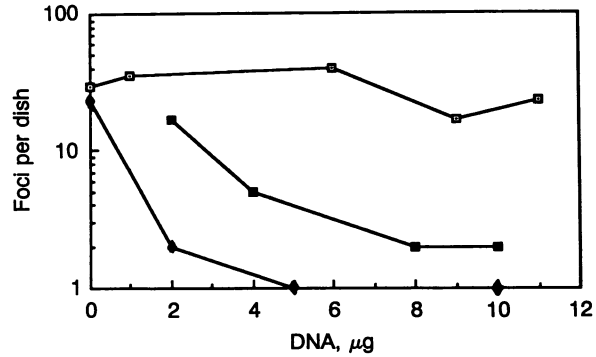

FIG. 1. Effect of wt p53 expression plasmids on transformation by mutant p53 plus *ras*. REFs were transfected as described in Table 1. Identical cultures were each transfected with 2 μg of pLTRp53-cG9, 2 μg of pEJ6.6, and various amounts of pSP6 (open squares), pLTRp53wt (solid squares), or pCMVp53wt (diamonds). In the cases of pLTRp53wt and pCMVp53wt, total DNA in each transfection mixture was kept constant by adding the appropriate amount of pLTRp53dl or pSP6, respectively. Numbers on the horizontal axis refer to the net amount of "inhibitor" DNA present in any given transfection mixture. Foci-per-dish values are the average of duplicate transfection dishes.

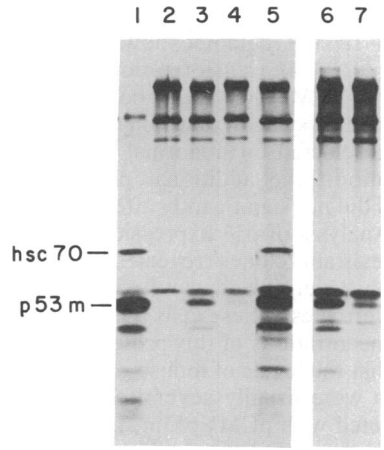

FIG. 2. Transient expression of p53 in cells transfected with various plasmids. REFs were transfected with plasmid DNA (15 μg per dish) by the calcium phosphate method (5), in the presence of chloroquine (100 μg/ml). A glycerol shock (2 min) was performed 8 hr later. Forty-eight hours after transfection, each culture was labeled for 45 min with 100 μCi (3.7 MBq) of [^{35}S]methionine. Extracts were prepared and processed by immunoprecipitation and SDS/polyacrylamide gel electrophoresis as described (5, 20). All lanes represent reactions with the p53-specific monoclonal antibody PAb421, except lane 2, which represents a negative control reaction in which hybridoma culture medium was used. The extracts were derived from the following sources: lane 1, clone 6 cells, transformed by mutant p53 plus *ras* (29); lanes 3–5, REFs transfected with pCMVp53wt, pLTRp53wt, and pLTRp53cG9, respectively; lane 2 contained a mixture of the three extracts used for lanes 3–5. Lanes 6 and 7 represent a longer autoradiographic exposure of the samples shown in lanes 3 and 4, respectively. p53m indicates the position of the transfected mouse p53; hsc70 is the 70-kDa heat shock protein cognate (29).

Methods). Similarly, another mutant plasmid, carrying a p53 cDNA with a single point mutation at amino acid 135 of mouse p53 (19, 20), also failed to inhibit focus induction in this system (data not shown). Hence, the point mutations present in these plasmids completely abolish the "suppressor" activity of wt p53. Interestingly, there often was a slight enhancement of the transforming activity of *myc* plus *ras* in the presence of pCMVp53m. This could indicate a synergism between elevated *myc* expression and abrogated wt p53 activity in the transformation process.

Table 2. Effect of p53 plasmids on transformation of REFs by various oncogenes

Additional cotransfected DNA	Foci per dish (no. of dishes)				
	myc plus *ras*			*E1A* plus *ras*	*E1A* plus *E1B*
	Exp. I	Exp. II	Exp. III		
None	105 (2)	50 (2)	16 (2)	123 (5)	105 (2)
pSP6		39 (2)			
pSPCMV		22 (4)			
pLTRp53dl			18 (2)	54 (3)	
pLTRp53wt		22 (4)			
pCMVp53dl		29 (4)		46 (2)	
pCMVp53m	115 (4)	60 (7)	26 (4)	88 (7)	100 (2)
pCMVp53wt	5 (4)	2 (10)	0 (4)	8 (11)	16 (2)

REFs were transfected as in Table 1. The *myc* gene was provided by plasmid pLTRmyc (31), and *ras* by pEJ6.6 (27); both were used at 2 μg per transfection. Plasmid pLA8, carrying the adenovirus *E1A* region as well as part of the *E1B* region (32), was also employed at 2 μg per transfection. Transfections under *E1A* plus *ras* included pLA8 and pEJ6.6 and were scored after 2 weeks; only typical large colonies were counted. Transfections under *E1A* plus *E1B* included only pLA8 and were scored after 3 weeks. In most cases, each column represents data compiled from several independent experiments. pSPCMV contains only the CMV enhancer–promoter inserted into pSP64; all other plasmids are as described in Table 1, except that in Exp. III, pLTRp53dl stands for the deletion plasmid pLTRcGXK (25).

Individual foci induced by *myc* plus *ras* in the presence of different p53 plasmids were next expanded into cell lines and subjected to protein analysis. Of a total of 11 lines established from foci generated in the presence of pCMVp53wt, 10 failed to express any detectable mouse p53, and a single line had very low levels (Fig. 3). On the other hand, mouse p53 could easily be detected in almost all lines originating in cultures transfected with *ras* plus *myc* plus pCMVp53m. It therefore seems likely that the few *myc* plus *ras* transformants seen in the presence of pCMVp53wt represent cells in which this plasmid was either not present at all or inefficiently expressed.

Effect of wt p53 on E1A-Dependent Transformation of REFs. The effect of wt p53 was next investigated in a system involving a viral oncogene, the adenovirus *E1A* gene. Like *myc*, *E1A* can efficiently transform REFs in concert with *ras* (32). As seen in Table 2, pCMVp53wt had a pronounced inhibitory effect also in this system. This particular plasmid

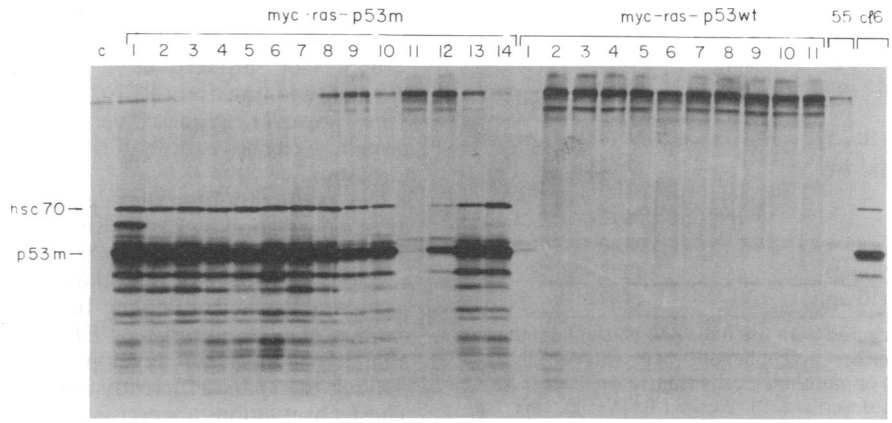

FIG. 3. Analysis of p53 expression in transformed cell lines. Foci generated following REF transformation by *myc* plus *ras* (see Table 2) were isolated and expanded into cell lines. Cultures of individual lines were labeled with [^{35}S]methionine (40 μCi per 60-mm dish) and processed as described in Fig. 2, using the anti-p53 monoclonal antibody PAb421. For lane c, an aliquot identical to the one represented in the adjacent lane 1 was incubated with control hybridoma culture medium. The groups marked as p53m and p53wt were derived from transfections in which the additional plasmid was pCMVp53m or pCMVp53wt, respectively. Clone 55 is a line transformed by *myc* plus *ras* alone (D.E., unpublished work), and cl6 refers to clone 6 (see Fig. 2). Other symbols are as in Fig. 2.

combination also provided another advantage: plasmid pLA8, in addition to containing the *E1A* region, also includes most of the *E1B* region. Consequently, it can also transform REFs in the absence of *ras* (32). The resultant foci, however, develop much more slowly and differ in morphology from the *E1A* plus *ras* ones (32). This permitted us to study the effect of wt p53 on *E1A* plus *E1B*-mediated transformation, in the absence of *ras*, by scoring pertinent typical foci at late times after transfection. As seen in Table 2, pCMVp53wt also greatly reduced the number of foci elicited by pLA8 alone. Thus, excess wt p53 is inhibitory for *E1A* plus *E1B*-mediated transformation, too. As in the case of *myc* plus *ras*, mutant p53 failed to exhibit any detectable inhibitory activity.

Effect of wt p53 on Established Cell Lines. In all the above experiments, wt p53 was found to inhibit the neoplastic transformation of REFs. One major concern could be that this merely represented a nonspecific "toxic" effect of the wt p53 plasmids. We therefore determined the effects of pCMVp53wt on a transfection system that does not depend on neoplastic transformation as the selected feature. To that end, we employed established cells of the Rat-1 cell line. In the first set of experiments cells were transfected with the plasmid ptkgpt (9) and selected for drug resistance. As seen in Table 3 (top three lines), cotransfection with pCMVp53wt had only a slight effect on the number of mycophenolic acid-resistant colonies. The same plasmid combination was next tested in the added presence of activated *ras*. Again, the numbers obtained with pCMVp53wt were only slightly below those seen in the presence of the pSP6 control or pCMV-p53m.

Since Rat-1 cells can be efficiently transformed by *ras*, we next assessed the consequences of wt p53 expression on this process using a focus assay. As seen in Table 3, the ability of *ras* to transform Rat-1 cells was only marginally sensitive to wt p53, as was also transformation by v-*src*. Essentially similar results were obtained with another established cell line, mouse NIH 3T3 (data not shown). With plasmid pSSV-11, encoding v-*sis* (34), transformation was also not markedly inhibited by pCMVp53wt (data not shown). It therefore appears that in this system, in which the transfected cells had already been selected for abnormal control of proliferation (immortalization), the additional phenotypic changes induced by *ras* did not significantly affect the cellular response to wt p53. Analysis of p53 expression in several mycophenolic acid-resistant clones revealed that Rat-1 cells can tolerate a low level of added wt p53 expression (data not shown). Nevertheless, these cells are probably still sensitive to higher concentrations of this protein. This was suggested by the fact that the levels of mouse p53 seen in pCMVp53wt transfectants were usually severalfold lower than those in lines transfected with pCMVp53m. In addition, there was a higher proportion of mouse p53 expressors among the pCMVp53m transfectants (4 out of 6 lines) than among the pCMVp53wt transfectants (6 out of 15). Thus, very high wt p53 levels are probably deleterious to Rat-1 cells. Nevertheless, both the conversion to drug resistance and the transformation of established cells by oncogenes were much less sensitive to wt p53 than the previously described systems, involving REF transformation. Hence, while excess wt p53 may well exert a general growth-inhibitory effect on at least some established cell lines, it preferentially suppresses focus formation in the REF system.

Table 3. Effect of p53 plasmids on transformation of established Rat-1 cells

Transfected DNA	Transformants per dish (no. of dishes)
	MPA-resistant
ptkgpt + pSP6	77 (3)
ptkgpt + pCMVp53m	50 (3)
ptkgpt + pCMVp53wt	34 (3)
ptkgpt + *ras* + pSP6	79 (2)
ptkgpt + *ras* + pCMVp53m	58 (3)
ptkgpt + *ras* + pCMVp53wt	43 (3)
	Dense foci
ras + pCMVp53m	111 (4)
ras + pCMVp53m (10 μg)	143 (4)
ras + pCMVp53m (15 μg)	89 (1)
ras + pCMVp53wt	107 (4)
ras + pCMVp53wt (10 μg)	54 (4)
ras + pCMVp53wt (15 μg)	55 (2)
ras + pLTRp53dl	134 (2)
ras + pLTRp53wt	106 (2)
src + pSPCMV (10 μg)	20 (2)
src + pCMVp53m (10 μg)	33 (2)
src + pCMVp53wt (10 μg)	24 (2)

Rat-1 cells were transfected with the indicated plasmid combinations and scored for either mycophenolic acid (MPA, 25 μg/ml)-resistant colonies (9) or morphologically transformed dense foci. Plasmid ptkgpt has been described (9). Plasmid fpGV-100, carrying the v-*src* oncogene, was a generous gift of M. Weber and T. Parsons (University of Virginia, Charlottesville). Transfection mixtures contained the following amounts of DNA per dish: ptkgpt, 0.5 μg; pEJ6.6 (*ras*), 1 μg; fpGV-100, 5 μg; and one of the various p53 plasmids, 5 μg unless otherwise indicated. Mycophenolic acid-resistant colonies and *ras*-transformed foci were scored ≈2 weeks after transfection, whereas *src* transformants were scored after 3 weeks.

DISCUSSION

The findings reported here demonstrate that plasmids encoding wt mouse p53 efficiently interfere with the ability of different oncogene combinations to elicit neoplastically transformed foci upon transfection of nonestablished REFs. These findings do not necessarily argue that wt p53 is directly interfering with the transformation process. In fact, they may well be consistent with a general adverse effect of excess wt p53 on cell proliferation. It is quite likely that excess wt p53 activity may also restrict the growth of various nontransformed cells. The data suggest, however, that different cell types are differentially sensitive to this inhibitory effect of wt p53. It is thus conceivable that the levels of the protein that are present and tolerated in normal cells may become restrictive once those cells undergo certain types of neoplastic processes. In this case, inactivation of p53 may confer a strong selective advantage on such transformed cells. Our data clearly show that mutations in at least certain sites in the p53 molecule abolish the inhibitory effects of this protein.

The same mutations that were shown here to extinguish the "suppressor" property of wt p53, can also confer upon p53 the ability to transform REFs in concert with *ras* (5, 19, 20). This, however, requires very high levels of the mutant protein, well above those of wt p53 sufficient to inhibit focus formation.

One prediction from these experiments is that in the case of transformation by *ras* and overproduced mutant p53, the latter may act by blocking the inhibitory activity of the endogenous wt p53 rather than by providing a dominant, positive oncogenic stimulus. In fact, relative to the combinations of *myc* plus *ras* or *E1A* plus *ras*, foci elicited by mutant p53 plus *ras* develop more slowly and are often difficult to expand efficiently into transformed cell lines (5). These observations are consistent with the notion that stable transformation in this system, although facilitated by the presence of overproduced mutant p53, may require additional events (oncogene activation?) to occur after transfection. Our findings do not exclude the possibility that other p53 mutants than those employed here, or even the same ones in a different cellular context, may contribute to transformation

directly rather than through the inactivation of the endogenous wt p53.

How can mutant p53 interfere with the activity of the endogenous wt molecules? Clearly, this process may require a great excess of the former over the latter. This is indicated by the fact that all lines generated from foci induced by *ras* plus mutant p53 (at least those mutants tested so far) exhibit very high levels of the introduced p53 (refs. 18–20 and 22; unpublished results). The large excess of mutant p53 could interfere with the function of the endogenous counterpart simply by competition for common molecular targets. Interestingly, in such mutant p53-transformed cells, practically all the endogenous p53 is tightly complexed with the transfected mutant protein (20, 35). Such a complex could be directly responsible for the functional inactivation of the endogenous wt p53.

The basis for the inhibitory effect of wt p53 plasmids on focus induction by *myc* plus *ras* or *E1A* plus *ras* is even less clear. It is clear, however, that the inhibitory effect of p53 in these systems can be abolished by mutations in the protein. It may thus be very significant that mutations in similar parts of the p53 molecule have been found both in human tumors (17) and in mouse tumors (refs. 20, 32, and 36; O. Halevy and M.O., unpublished data).

The work presented here seemingly conflicts with earlier studies, which demonstrated that p53 expression is actually essential for ongoing cell proliferation, and that downregulating p53 levels by either anti-p53 antibodies (37) or antisense transcripts (38) results in growth arrest. This conflict may be resolved by assuming that in normal cells, p53 acts within a tightly controlled chain of growth-regulatory events. Blocking any of these steps in normal cells may abort the entire process and inhibit cell proliferation. On the other hand, when this pathway becomes grossly perturbed by events such as deregulated *myc* overexpression, p53 may become dispensable and may in fact hinder uncontrolled cell proliferation. Although the precise mode of action of p53 is still unknown, our findings predict that loss of wt p53 function could provide a positive selective advantage in certain neoplastic processes. The recent evidence from various tumor systems is highly consistent with this notion.

Note Added in Proof. Our findings and conclusions are highly consistent with a recent report by Levine and coworkers (39).

M.O. is grateful to Dr. I. Robert Lehman for generous hospitality. We wish to thank all the members of our lab for many stimulating discussions throughout the course of this work. We thank Drs. M. Horowitz, M. Weber, K. Robbins, and B. Fleckenstein for the generous gifts of plasmids pLTRmyc, fpGV-100, pSSV-11, and pRR23, respectively, and M. Fagan for dedicated secretarial assistance. This work was supported by Grant RO1 CA40099 from the National Cancer Institute and by grants from the US–Israel Binational Science Foundation, the Fund for Basic Research, Israel Academy of Sciences and Humanities, and the Laub Foundation.

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