er, our TEM data and crystallization mechanisms of hematite nanospheres provide strong evidence for the existence of ferric iron hydroxide particles having a colloidal origin presumably by about 2.5 billion years ago. Such particles raise the question of whether the iron was transported from its source areas in a colloidal state or whether the colloidal particles formed during oxidation in the depositional basins and thus facilitated deposition. Although they are features from a small part of an iron formation, they are widespread in our samples and provide strong direct evidence that colloidal processes were involved in the development of Precambrian iron formations. Other workers should be alert for such potential indicators of the presence and extent of colloidal activity.

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33. We thank R. C. Morris for providing samples and data, C. M. Lesher and S. Guggenheim for helping us acquire the samples, R. A. Eggleton for valuable suggestions, T. M. Han, H. D. Holland, C. Klein, and G. L. LaBerge for suggestions and reprints, and D. Burt and P. Knauth for helpful reviews. Electron microscopy was performed at the Facility for HREM at Arizona State University (ASU), which is supported by the National Science Foundation (NSF) and ASU. This study was supported by NSF grant EAR-870529.

4 June 1990; accepted 9 July 1990

Different Tumor-Derived p53 Mutants Exhibit Distinct Biological Activities

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In its wild-type form, the protein p53 can interfere with neoplastic processes. Tumor-derived cells often express mutant p53. Full-length mutant forms of p53 isolated so far from transformed mouse cells exhibit three common properties in vitro: loss of transformation-suppressing activity, gain of pronounced transforming potential, and ability to bind the heat shock protein cognate hsc70. A tumor-derived mouse p53 variant is now described, whose site of mutation corresponds to a hot spot for p53 in human tumors. While absolutely nonsuppressing, it is only weakly transforming and exhibits no detectable hsc70 binding. The data suggest that the ability of a p53 mutant to bind endogenous p53 is not the sole determinant of its oncogenic potential. The data also support the existence of gain-of-function p53 mutants.

THE CELLULAR PHOSPHOPROTEIN p53 is expressed aberrantly in a variety of neoplastic cells (*1*). Plasmids encoding various mutant forms of p53 possess a distinct oncogenic activity both in vitro (*2–6*) and in vivo (*7*), although wild-type (wt) p53 plasmids actually abolish oncogene-mediated focus formation (*8, 9*). Thus, p53 mutants may facilitate cell immortalization and transformation by interfering with a normal function of wt p53 (*5, 8–11*). Indeed, studies involving mouse (*12*) and human (*13–16*) tumors suggest that wt p53 may possess a tumor-suppressor activity, whose elimination may cause the development of many types of neoplasia.

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Most transforming variants of p53 studied exhibit a specific interaction with the major heat shock protein cognate, hsc70 (*17*), which may be important in endowing these variants with oncogenic properties (*4, 6*). There are, however, at least two deletion mutants of p53 that can immortalize primary rodent cells in the absence of any detectable complex with hsc70 (*2, 5*).

The formation of a complex between p53 and other proteins, such as the SV40 large T antigen, the adenovirus type 5 58-kD E1b protein, and hsc70, is correlated with an increased intracellular stability of p53 (*4, 18, 19*). Nevertheless, such interactions are not a prerequisite for p53 stabilization (*2, 11, 18, 20, 21*), and the common denominator in all these cases may, in fact, be the inability of mutant p53 molecules to perform their normal function efficiently.

In cell line IP3, which originated from an ultraviolet light–induced mouse fibrosarcoma, p53 is greatly stabilized in the absence of any detectable complex with hsc70 (*21*). It was suggested that IP3 cells carry a mutant p53 gene whose protein product can be stabilized without the involvement of a p53-hsc70 complex.

To further explore this possibility, the sequence of IP3 p53 mRNA was investigated by means of the polymerase chain reaction (PCR) (*22*). Three separate IP3 mRNA preparations were each used for the synthesis of cDNA. From the three ensuing PCR reactions, seven clones were isolated and sequenced (*22*). All exhibited a change from C to T at nucleotide position 808 [amino acid 270; numbering as in (*23*)], resulting in replacement of an arginine with a cysteine. None of the cDNA clones corresponded to wt p53. Hence, if IP3 cells contain a wild-type allele (or another allele encoding a different mutant p53), it is either silent or expressed in small amounts.

The mutation in p53cys270 falls within a highly conserved region of the protein (homology box V) (*24*) that is essential for T antigen binding (*25*). Therefore, cDNA encoding the mutant p53cys270 was placed under the Harvey sarcoma virus long terminal repeat. The plasmid thus generated (pLTRIP3) was cotransfected into primary rat embryo fibroblasts (REF) together with plasmids encoding SV40 (strain 776 cloned into the Bam HI site of pBR322; pSVBam) and activated *ras* (pEJ6.6). In a parallel transfection, pCMVNc9, encoding mouse wt p53 (*9*), was substituted for pLTRIP3. Cell lines were established and analyzed by immunoprecipitation with either a monoclonal antibody (MAb) PAb421, specific for p53, or MAb PAb419, directed against SV40 large T antigen. When an extract from pLTRIP3-transfected cells (SVRLTRIP3) was immunoprecipitated with PAb419, only a very minor fraction of the exogenous mouse p53cys270 was found associated with T antigen (Fig. 1). By contrast, in the cell line expressing mouse wt p53 (SVRCMVNc9), the ratio between mouse and endogenous rat p53 in the pellet immunoprecipitated with PAb419 was similar to their ratio in the total population. Thus, the mutation at position 270 greatly reduces the binding of p53 to T antigen.

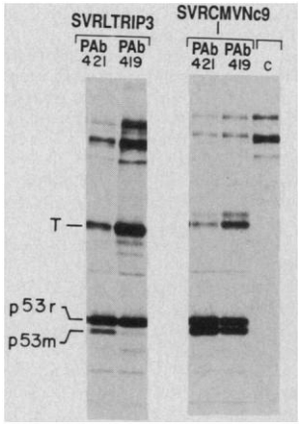

Fig. 1. Analysis of proteins in SV40 plus *ras* cotransformants. Cell lines were established from foci derived by cotransfection of REF with pSVBam plus pEJ6.6 and either pLTRIP3 (cell line SVRLTRIP3) or pCMVNc9 (cell line SVRCMVNc9). The cells were labeled with [^{35}S]methionine, and extracts were immunoprecipitated with PAb421 or PAb419; C, control hybridoma culture medium; p53r, rat p53; p53m, mouse p53; and T, T antigen.

The IP3 cell line was derived from a tumor, and we therefore wished to assess its transforming potential. Mutant p53 plasmids containing genomic DNA transform more efficiently than those comprised solely of cDNA (*6*). Therefore, the Cys^{270} mutation was introduced by site-directed mutagenesis into a plasmid containing a chimera of cDNA and genomic DNA (*26, 27*). The resultant plasmid was used to transfect REF together with activated *ras*. When compared to two other plasmids, identical in design but encoding different p53 mutants (p53val135 and p53phe132), p53cys270 elicited a reduced number of foci (Table 1, Fig. 2). Not only were the foci fewer, but they also were often fainter, slower growing, and less easily expandable into stable cell lines (*28*). Thus, at least under the experimental conditions of the standard cotransformation assay, p53cys270 is significantly less transforming than the other two mutants. Yet, when tested for its ability to inhibit focus formation by *myc* and *ras*, p53cys270 exhibited no suppressor activity (Table 2); neither did p53cys270 have any significant effect on transformation by *ras* plus either p53val135 or p53phe132 (*28*). Moreover, p53cys270 reproducibly caused a slight enhancement of focus formation by *myc* plus *ras*, as previously reported for other p53 mutants (*8, 9*).

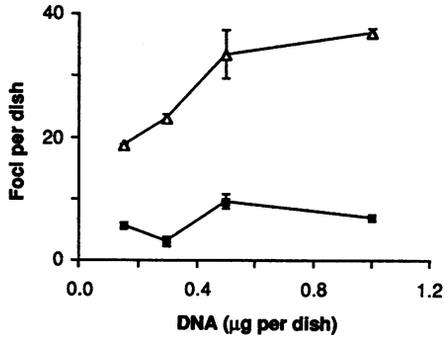

Fig. 2. Dose-response curve of transformation by p53cys270 (■) and p53val135 (△). Transfections were as described in Table 1. Foci were scored 16 days after transfection.

Cell lines established from *ras* plus p53cys270 foci were next subjected to protein analysis. The endogenous p53 in IP3 cells, which naturally express p53cys270, is very stable (*21*). To determine whether this increased stability was due to the mutation, we performed a pulse-chase analysis (Fig. 3). Densitometric scanning of the autoradiogram revealed a $t_{1/2}$ of 5.6 hours for p53 in this p53cys270 plus *ras*–transformed line (*28*). Hence, the mutation at position 270 does appear to be a major determinant of the

Table 1. Transformation of REF by various p53 mutants in combination with *ras*. REF were transfected and analyzed as described (*9*). In each experiment, 4 μg of pEJ6.6, encoding mutant human *ras* (*32*) was transfected along with 4 μg of pLTRcGXK (p53dl), encoding a highly deleted p53 polypeptide (*9*); pLTRp53cG (p53val135) (*27*); pLTRp53cGcys270; or pLTRp53cGphe-132, except for experiment 5, where 1.5 μg of each plasmid DNA was used. Plasmid pLTRp53cGphe132, encoding phenylalanine at position 132, was constructed by site-directed mutagenesis as described for pLTRp53cGcys270. Transfections were usually done in duplicate, and the numerical value in each column represents an average. Foci were scored about 12 days after transfection. ND, not done. Standard error was calculated on the basis of all individual dishes.

Plasmid	Foci per dish for experiments					
	1	2	3	4	5	Average
p53dl	ND	ND	0	0.5	1	0.6 ± 0.2
p53cys270	0	1	8	3	7	4.2 ± 1.2
p53val135	ND	26	23	19	25	23.1 ± 2.6
p53phe132	18	29	51	ND	86	43.6 ± 11.0

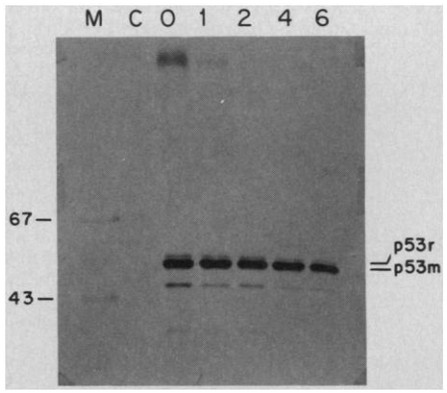

Fig. 3. Pulse-chase analysis of p53cys270. A focus derived by transfection of REF with p53cys270 plus *ras* was expanded into a cell line. Cells were labeled with [^{35}S]methionine for 30 min and then chased for the periods (in hours) indicated on top, in presence of nonradioactive methionine. Cell extracts were subjected to protein analysis by immunoprecipitation and SDS–polyacrylamide gel electrophoresis (SDS-PAGE) as described (*3*). Numbers of the left refer to the molecular weights (in kilodaltons) of marker (M) polypeptides. C, aliquot of non-chased cell extract, identical to the one in lane O, but reacted with control hybridoma culture medium.

increased half-life of this p53 variant. Nevertheless, p53cys270 in IP3 cells is more stable [$t_{1/2}$ of >8 hours; (*21*)], confirming the idea that the half-life of p53 is determined both by its primary structure and by its intracellular environment (*21, 29*). In these p53cys270-overexpressing cells, the endogenous, presumably wild-type, rat p53 was also stabilized to a $t_{1/2}$ well above 1 hour.

Another feature apparent from Fig. 3 is that unlike cell lines transformed by a combination of *ras* and other p53 mutants, this one did not exhibit any demonstrable coprecipitation of hsc70 with p53cys270. Similar results were obtained in a transient transfection experiment (*28*). Thus, p53cys270 is stabilized without forming a detectable complex with hsc70. We are currently testing whether the presence of cysteine at position 270 is specifically required for this effect.

Transfected mutant forms of p53 tend to form hetero-oligomers with the endogenous, presumably wild-type, p53 of the recipient cells (*3, 5*). It has been suggested that the transfected mutant p53 thereby neutralizes a putative tumor-suppressor activity of the wt p53 (*8–10*). To see whether p53cys270 could also interact with the endogenous REF p53, we performed a serial immunoprecipitation experiment (Fig. 4). ^{35}S-labeled extracts from cell lines transformed by *ras* and different p53 mutants were first reacted with MAb RA3-2C2, which binds only with mouse p53 (Fig. 4, lanes 2) (*30*). An identical aliquot of each extract was reacted, in parallel, with MAb PAb421, which can react with both mouse and rat p53 (lanes 1). As is evident from the comparison between the two MAbs (Fig. 4, lanes 1 and 2) and from the subsequent reprecipitations (lanes 3 to 5), MAb RA3-2C2 brought down similar amounts of coprecipitated endogenous rat p53 in lines overexpressing either p53val135 or p53cys270. Surprisingly, overproduced p53phe132, which is very transforming (Table 1), exhibited less binding to rat p53. It is evident from the data that p53cys270 binds the endogenous p53 as efficiently as do other, more potently transforming p53 mutants. Furthermore, this interaction is not a consequence of the selective pressure applied while establishing the transformed line, since a similar behavior was also exhibited by p53cys270 when expressed transiently in REF (*28*). Finally, the proportion of endogenous p53 found in complex with a given p53 mutant also depends on the ratio between the latter and the former; a larger fraction of endogenous p53 appeared to be uncomplexed in lines expressing relatively small amounts of mutant p53 (*28*).

Table 2. Effect of various p53 plasmids on transformation of REF by *myc* plus *ras*. Transfection conditions and plasmids are as described in Table 1, except that foci were typically scored about 10 days after transfection. In each experiment, *ras* (pEJ6.6, 1.5 μg per dish) and *myc* [pLTRmyc, 1 μg per dish; (*9*)] were cotransfected along with 1.5 μg of plasmid encoding a p53 variant. Wild-type p53 was encoded by pLTRp53cGwt (*26*). All other p53 plasmids were as in Table 1. Each value represents the average of four transfection dishes from three separate experiments.

Plasmids	Foci per dish (Average ± SE)
p53dl	93 ± 40
p53val135	145 ± 27
p53cys270	167 ± 31
p53phe132	109 ± 14
p53wt	0.25 ± 0.25

Observations of aberrant expression of p53 in tumors (*12–16*) have suggested that p53, in its wild-type context, may be a tumor suppressor. If this is indeed the case, and if the in vitro suppression assay is relevant to the in vivo situation, one may expect that all tumor-derived p53 mutants will be devoid of in vitro suppressor activity, although not all mutants may necessarily exhibit efficient transforming capacity. The behavior of p53cys270 supports this idea and indicates that loss of suppressor activity and acquisition of transforming potential may be separable and may involve two distinct functions of p53.

It is commonly believed that the transforming potential of p53 mutants stems from their ability to form complexes with the endogenous wt p53, thus relieving its putative suppressor activity (*8–10*). However, the data presented above indicate that these two properties, transforming capacity and endogenous p53 binding, are not directly correlated. Hence, although binding of endogenous p53 by a given p53 mutant may be a prerequisite for transformation, it may not be sufficient for generating a high transforming potential. It is therefore conceivable that, in addition to their ability to sequester wt p53, mutants such as p53val135 or p53phe132, have some additional feature that is responsible for their increased oncogenicity. Such mutants may then exhibit also a gain of function, whereas p53cys270 may represent a pure loss-of-function mutant. Nevertheless, p53cys270 can enhance oncogene-mediated focus-formation at least as efficiently as the other mutants (Table 2). Admittedly, the extent of the enhancement is very modest, although reproducible. Yet, it is tempting to speculate that this assay, in which transformation is driven by two potent dominant oncogenes, scores solely for the ability of a p53 mutant to abrogate wt p53–mediated suppression. Such abrogation may, in fact, be strictly determined by the capacity of the mutant to complex its endogenous counterpart. Thus, a nontransforming p53 mutant may still be manifest as dominant negative in vivo, as long as it occurs on a background of activated proto-oncogenes.

The residue in human p53, Arg^{273}, that corresponds to the mutation in mouse p53cys270, was found to be mutated in several human tumors and tumor-derived lines (*16, 31*), and may constitute a hot spot for mutations in tumors (*16*). Furthermore, p53 genes from many additional human tumors contain mutations between amino acid positions 239 and 281 of human p53 (*15, 16*). Because such mutations may have a similar phenotype to mouse p53cys270, exhibiting low transforming capacity, a transformation assay may not always be the best choice for analysis of p53 mutants encountered in human tumors.

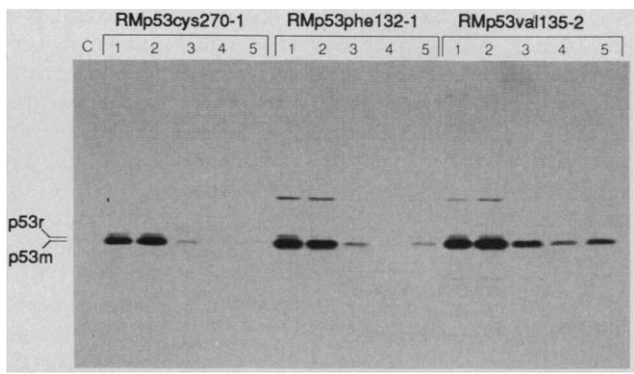

Fig. 4. Binding of endogenous rat p53 to transfected mutant mouse p53 in various lines. REF were .co-transfected with pEJ6.6 plus pLTRmyc and either pLTRcGcys270, pLTRcGphe132, or pLTRcGval135, as described in Table 2. Cells were labeled for 4 hours with [^{35}S]methionine. Extract aliquots containing equal amounts of acid-insoluble radioactivity were either reacted with PAb421 (lane 1) or subjected to two consecutive rounds of incubation with RA3-2C2 (lanes 2 and 3). The supernatant of the second round was divided in two equal parts, which were reacted with RA3-2C2 (lane 4) and PAb421 (lane 5), respectively. The polypeptides precipitated in each round were analyzed by SDS-PAGE.

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22. Two synthetic oligonucleotides, containing an Eco RI site, flanking the 5′ end (primer 1) or 3′ end (primer 2) of the p53 mRNA were used. The sequences of the oligonucleotides were 5′-GGAATTCAGGCCCTCATCCT-3′, beginning 57 bp upstream of the initiator ATG, and 5′-GGAATTCAGCCCTGAAGTCATAAGA-3′, 167 bp downstream of the translation termination codon. The first strand of cDNA was generated from cytoplasmic RNA with primer 2 and reverse transcriptase as described by C. Marcelle *et al.* [*Genes Chromosomes Cancer* **1**, 172 (1989)], except that the reaction was incubated at 42°C for 1 hour. The RNA was then degraded by adding NaOH to 0.3 M and incubating for 20 min at 70°C, followed by neutralization. The two primers were then used to amplify the resultant 1.3-kb p53 cDNA segment, including the entire protein coding region. Reaction conditions were as described [N. Firon, N. Eyal, E. H. Kolodny, M. Horowitz, *Am. J. Hum. Genet.* **46**, 527 (1990)]. After 35 cycles, the PCR products were cleaved with Eco RI and ligated directly into the Eco RI site of plasmid Bluescript SK (Stratagene). Inserts were excised with Eco RI, cleaved with Pst I and Sac II, and the resultant fragments were subcloned again in Bluescript SK. Plasmids were subjected to DNA sequencing with the use of the Sequenase kit (U.S. Biochemical).
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33. We thank M. Horowitz and N. Eyal for use of the PCR machine and for many helpful discussions, J. Traub for skillful technical assistance, A. Raz for the IP3 cell line, and M. Fagan for manuscript preparation. Supported in part by PHS grant R01 CA 40099 from the National Cancer Institute, by the U.S.-Israel Binational Science Foundation, and by grants from the Israel Cancer Research Fund, the Fund for Basic Research administered by the Israel Academy of Sciences and Humanities, and the Laub Foundation. M.O. is a scholar of the Leukemia Society of America, Inc., and O.H. is a fellow of the Israel Cancer Research Fund.

14 February 1990; accepted 18 July 1990

Impact of Mass Treatment of Onchocerciasis with Ivermectin on the Transmission of Infection

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Onchocerciasis is a major blinding disease that, until recently, has been essentially untreatable. Ivermectin is a safe and effective drug for the mass treatment of onchocerciasis and when used on an individual basis, it reduces the ability of the treated person to transmit *Onchocerca volvulus* infection. In the present study, the effect of community-based ivermectin treatment on the degree of transmission within the community was assessed by determining the incidence of new infection in children. Ivermectin was distributed annually on three occasions to the eligible members of a population of approximately 14,000 people living on a rubber plantation in a forest area endemic for onchocerciasis. After 2 years, the prevalence of infection in 5-year-old children decreased by 21%. The annual incidence in an uninfected cohort of children decreased by 35% and, after age-specific adjustment, the reduction in incidence in 7- to 12-year-old children was 45%. Thus, community-based distribution of ivermectin led to a significant reduction in incidence of new infection. These findings suggest that ivermectin can be important in reducing the transmission of onchocerciasis.

APPROXIMATELY 85 MILLION PEOple live in areas endemic for onchocerciasis, and 18 million people are infected with *Onchocerca volvulus* (*1*). Onchocerciasis causes blindness or visual loss in 1 to 2 million of these infected people. More than half of the inhabitants of hyperendemic areas will become blind before death, and life expectancy of those who are blind is one-third that of their sighted peers (*2*).

Onchocerciasis is caused by the filarial parasite *O. volvulus*. In nature, humans are the only known reservoir. Adult worms, found in tissue nodules, produce millions of microfilariae that migrate throughout the body, concentrating particularly in the skin and eye. This infection is transmitted by black flies of the simulium species and has a prepatent period of 9 to 15 months (*1*). Adult worms may live up to 18 years in the human host.

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Ivermectin is effective against a wide range of parasites (*3*) and is an effective and well-tolerated drug for individual treatment of onchocerciasis (*4–7*). It promises to revolutionize treatment of onchocerciasis by making feasible large-scale community-based treatment (*8, 9*). Ivermectin also may decrease the ability of infected individuals to contribute to transmission (*10*) and lead to a decrease in vectorial transmission (*11, 12*). However, given the efficiency of the simulium vectors and the high transmission capacity in endemic areas, it is unclear whether the effect of ivermectin seen in individuals would be seen with community-based treatment or whether it would be of programmatic importance. The following study tested whether treating a large population with ivermectin would decrease transmission sufficiently to reduce the incidence of new infection.

This study was conducted between September 1987 and November 1989 at the Liberian Agricultural Company (LAC) rubber plantation where approximately 14,000 people live (*8*). Prevalence of infection there increases with age, ranging from 26% at age 5 to 86% at 20 years (*8*). All persons over 12 years of age were eligible for treatment with