Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 130–134, January 1986
Genetics

The gene for human p53 cellular tumor antigen is located on chromosome 17 short arm (17p13)

(gene mapping/somatic cell hybrids/*in situ* hybridization/oncogenes)

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Communicated by Maxine F. Singer, August 15, 1985

ABSTRACT **A clone that cross-hybridizes with a mouse p53 probe has been isolated from a cDNA library of simian virus 40-transformed human fibroblasts. This cloned human p53 cDNA was used as a probe to examine DNAs obtained from human–rodent somatic cell hybrids that have segregated human chromosomes. The results show that the human p53 gene is located on chromosome 17. In addition, Southern analysis of hybrids prepared from human cells containing a chromosome 17 translocation allowed regional localization of the human p53 gene to the most distal band on the short arm of this chromosome (17p13). Localization of the p53 gene to 17p13 was confirmed by *in situ* hybridization of metaphase spreads with the human p53 probe.**

The p53 tumor antigen is found in elevated levels in a large variety of transformed cells (1, 2). Recently, we determined the sequence of the mouse p53-specific cDNA (3) and of the corresponding gene (4) and showed that it codes for a protein of 390 amino acids. We also have shown that the p53 sequences in the mouse genome are contained in a 16-kilobase (kb) *Eco*RI fragment comprising the functional gene and a 3.3-kb *Eco*RI fragment that carries an intronless processed pseudogene (3). In the mouse the pseudogene is located on chromosome 14, whereas the functional gene is on chromosome 11 (5, 6).

The cellular function of p53 is still unknown, but several experiments suggest its involvement in cell-cycle regulation. The synthesis of p53 is induced in lymphocytes by stimulation with concanavalin A (7) and in fibroblasts by serum stimulation of quiescent cells (8). This and the observation that injection of monoclonal anti-p53 antibodies into nuclei of fibroblasts at the time of serum stimulation inhibits DNA synthesis (9, 10) suggest that p53 may be a regulatory protein in the transition from G_0 to S phase. The *myc* gene product and p53 also have some similar properties (11). Furthermore, both have an overall structural resemblance and slightly common sequence features (4, 12). Recently it has been shown that under the regulatory control of strong promoters or enhancers, the gene for p53 can cooperate with the mutated *ras* oncogene in the transformation of embryonal cells (13–15), thus functioning in an analogous way to *myc* (16, 17). Based on these findings, p53 may be classified as a nuclear oncogene product.

To assess in more detail the possible participation of p53 in human tumors, it is essential to determine the chromosomal location of p53 in the human genome. Here we describe the cloning of a human p53 cDNA sequence and its use to probe DNA obtained from human–rodent somatic cell hybrid clones. The results show that the human p53 gene is present on chromosome 17 and that it is located on the most distal band of the short arm of this chromosome (17p13). This localization was confirmed by *in situ* hybridization.

MATERIALS AND METHODS

Cell Culture and Somatic Cell Hybridization. Human fibroblast lines used in cell fusions include diploid WI-38 (ATCC CCL75), a hypoxanthine phosphoribosyltransferase-negative ($HPRT^-$) simian virus 40 (SV40)-transformed WI-18 line (18), and four lines with balanced chromosome translocations (GM3196, GM0119, GM0073, and GM2658). The karyotype of both GM0119 and GM3196 is 46,XX,t(17;22)(p13;q11); GM3196 also contains a pericentric inversion of chromosome 5 (pter→p13::q13→p13::q13→qter). Both GM3196 fibroblasts and GM3197 lymphocytes originate from the same patient and have identical karyotypes. The karyotypes of GM0073 and GM2658 are 46X,t(X;14)(q13;q32) and 46,XX,t(2;6)(q11;q15), respectively. Rodent parental cell lines used were murine $HPRT^-$ L-A9 (19), mouse thymidine kinase-negative (TK^-) L-B82 (19) and LMTKCL1D (20), and an $HPRT^-$ derivative of the Chinese hamster V79 line (21). Human–rodent somatic cell hybrids were isolated and cloned in selective medium after cell fusion in polyethylene glycol as described (21). Hybrid cells were expanded, and the same preparation of cells was used for DNA isolation, isoenzyme analyses, and karyotyping.

Isoenzyme Analyses. Hybrid cell lines were analyzed for the presence of all human chromosomes (except Y) by standard isoenzyme analyses (22, 23) using the enzyme markers previously described (21). These analyses permit identification of the human chromosomes retained in hybrid cells.

Karyotypic Analysis. Metaphase preparations of hybrid cells were examined by bright-field microscopy after Giemsa-11 staining (24) and a modification of the trypsin/Giemsa-banding technique (25).

Isolation of Human p53 cDNA Clone. An Okayama–Berg human fibroblast cDNA library (26) was plated, transferred to nitrocellulose filters, and hybridized as described (27) with a nick-translated 0.95-kb probe excised from the pp53-176 mouse cDNA clone (3). Hybridization was for 24 hr at 37°C in 50% formamide containing 5× NaCl/Cit (1× NaCl/Cit = 0.15 M NaCl/0.015 M sodium citrate), 1× Denhardt's solution (0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone), 20 mM sodium pyrophosphate (pH 7.0), and 0.5% $NaDodSO_4$ in the presence of denatured, sonicated, carrier *Escherichia coli* DNA at 100 μg/ml. This was followed by washing at room temperature in 2× NaCl/Cit and finally at 50°C in 2× NaCl/Cit for 30 min. A single positive colony (pp53H) was detected on screening 200,000 colonies.

Probes. The p176 clone of mouse p53 (3) was completely digested with *Xho* I and *Sac* II. The smaller *Xho* I–*Sac* II

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Abbreviations: kb, kilobase(s); TK, thymidine kinase; HPRT, hypoxanthine phosphoribosyltransferase; SV40, simian virus 40.

fragment that was isolated represents 950 bp of the 1170-bp coding sequence of the mouse p53 cDNA. The human probe was a 2.1-kb *Bam*HI fragment (Fig. 1) of the human pp53H cDNA. Each fragment was purified by 1% agarose gel electrophoresis, electroeluted, and radiolabeled by nick-translation with [^{32}P]dCTP for Southern hybridization or with ^{125}I-labeled dCTP (^{125}I-dCTP) for *in situ* hybridization of metaphase spreads.

DNA Isolation and Filter Hybridization. DNA was isolated from each hybrid cell line (28), digested with *Eco*RI or *Hin*dIII, size-fractionated by (0.7%) agarose gel electrophoresis, and transferred to positively charged Nylon membranes (Zetabind, AMF Cuno) in 20× NaCl/Cit. Membranes were hybridized for 24 hr at 42°C with the ^{32}P-labeled (nick-translated to about 10^8 cpm of DNA per μg) 2.1-kb *Bam*HI p53H fragment in 50% formamide containing 5× SPE (1× SPE = 0.15 M NaCl/0.01 M sodium phosphate/0.001 M EDTA, pH 7.4), 5× Denhardt's solution, 10% dextran sulfate, and sheared, denatured salmon sperm DNA at 200 μg/ml. Membranes were washed at room temperature in 2× NaCl/Cit containing 0.25% $NaDodSO_4$ and four times at 55°C in 0.1× NaCl/Cit containing 0.25% $NaDodSO_4$.

Human Metaphase Chromosome *in Situ* Hybridization. Metaphase spreads of normal human lymphocytes and GM3197 lymphocytes were prepared by standard procedures, and *in situ* hybridization was carried out by a modification of the technique of Harper and Saunders (29). Each slide was hybridized with 0.5×10^6 cpm of ^{125}I-dCTP-labeled p53H probe (10^8 cpm/μg) in 50% formamide containing 10% (wt/vol) dextran sulfate, 2× SCP (1× SCP = 0.12 M NaCl/0.015 M sodium citrate/0.02 M sodium phosphate, pH 6.0), 5× Denhardt's solution, 0.1 mM KI, 1 mM 5'-iododeoxycytidine, and sheared *E. coli* DNA at 100 μg/ml. Hybridization was at 37°C for 15 hr in a humid chamber. After hybridization, slides were washed three times for 20 min each in 50% formamide containing 2× NaCl/Cit and 0.1 mM KI at 39°C and rinsed five times for 2 min each in 2× NaCl/Cit at 39°C. The slides were further washed at 50°C for 15-min intervals three times in 2× NaCl/Cit containing 0.1 mM KI and then three times in 0.1× NaCl/Cit containing 0.1 mM KI and were dehydrated in an ethanol series. Autoradiography was performed with NTB2 emulsion, and the coated slides were stored at 4°C for 2–10 days before development of the emulsion.

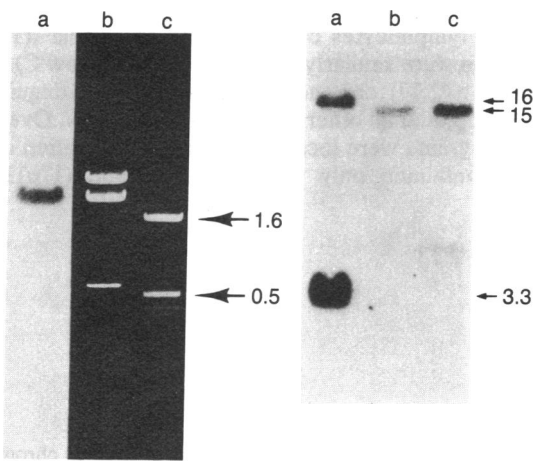

FIG. 1. (*Left*) Southern hybridization of p53H with the mouse p53 probe. The p53H plasmid was digested with *Bam*HI, electrophoresed in a 1% agarose gel, and transferred to nitrocellulose. Lanes: a, hybridization with the mouse p53 probe; b, ethidium bromide stain before transfer; c, *Hinf* I-digested pBR322 size markers after ethidium bromide staining. The 2.1-kb hybridizing band was eluted from low-melting-point agarose and used as a probe. (*Right*) Hybridization of mouse and human genomic DNA with mouse and human p53 probes. Each DNA (10 μg) was digested with *Eco*RI, electrophoresed in 0.7% agarose, and transferred to nitrocellulose. Lanes: a, mouse DNA hybridized with mouse probe; b, human DNA hybridized with the mouse probe; c, human DNA hybridized with the human probe. Size is shown in kilobase pairs.

RESULTS

Human p53 cDNA Clone. By screening a cDNA library of SV40-transformed human fibroblasts (26) with a mouse p53 probe (*Xho* I–*Sac* II fragment of plasmid p176; ref. 3), we isolated a cDNA clone that cross-hybridized to the mouse probe. Digestion of this cloned DNA with *Bam*HI yielded two cDNA-containing fragments of 2.1 and 0.6 kb, only the first of which hybridized to the mouse probe (Fig. 1 *Left*). While this paper was in preparation we received a manuscript describing a similar p53 cDNA clone (30). Restriction enzyme mapping and partial DNA sequencing of our clone demonstrates its identity to that described by Matlashewski *et al.* (30). Hence the 0.6-kb fragment (Fig. 1 *Left*) contains the 3' untranslated region of the human p53 cDNA, including an *Alu*-like sequence, whereas the 2.1-kb fragment contains predominantly coding sequences and includes at its 5' end approximately 100 bp derived from the vector used for cloning. This 2.1-kb fragment was isolated from an agarose gel and used as a probe for further experiments. The sequence of this fragment was determined recently, and p53 protein expression was observed in COS cells after transfection with the p53 gene in an expression vector (31).

The results of Southern blot hybridization of mouse and human DNA with either the mouse or the human p53 probes is shown in Fig. 1 *Right*. It is clear that both probes detected the same 15-kb band in human DNA, and the signal produced by the human probe was significantly stronger than that by the mouse probe. The results in Fig. 1 *Right* also suggest that the human genome does not contain a processed pseudogene corresponding to the one carried on the 3.3-kb band in the mouse (see lane a). As suggested by the hybridization pattern of hamster DNA, the latter also appears to contain only a single p53 gene and no pseudogenes (5).

Chromosomal Mapping of the Human p53 Gene. A major 15-kb human p53 band was clearly resolved from a 12- to 13-kb homologous hamster band in *Eco*RI digests of human–Chinese hamster hybrid DNAs (Fig. 2). The presence of the human band correlated only with the presence of human chromosome 17 (Fig. 3), indicating that the functional human p53 gene (p53H) is located on that chromosome. Southern analysis of two groups of human–hamster hybrids (Table 1, series A and B) disclosed only one discordancy between the segregation of the p53H gene and chromosome 17. The single discordancy may represent a difference in sensitivity between detection of the p53 gene and the isoenzyme marker or a partial deletion of chromosome 17 in that hybrid.

A faint 3.9-kb band hybridizing with the p53H probe was sometimes observed in the human and hybrid DNAs (Fig. 2). Hybridization with this fragment was quite weak and frequently not detectable. It was only found in hybrids that showed intense hybridization with the 15-kb human band, and it probably does not represent a processed pseudogene. The 3.9-kb band likely represents a 5' fragment of the functional p53 gene, since a band of similar size cross-hybridizes with a mouse genomic 5' probe (unpublished results).

Regional Localization of p53 on Chromosome 17 Short Arm. Although the human (15 kb) and mouse (16 kb) p53 bands were not adequately separated in *Eco*RI digests of hybrid DNAs, the human p53 gene could be chromosomally mapped in human–mouse hybrids by using *Hin*dIII digests. The

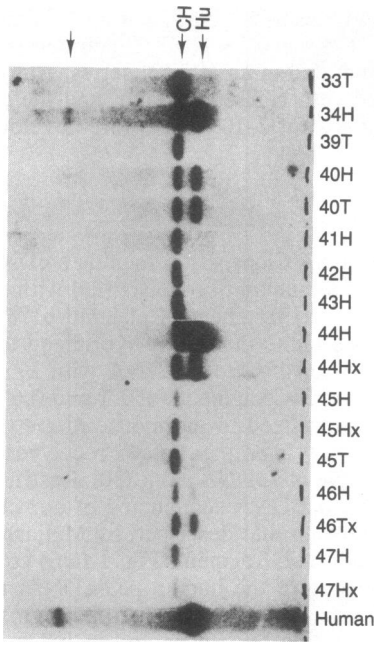

FIG. 2. Southern hybridization of ^{32}P-labeled p53H probe with *Eco*RI-digested, size-fractionated DNA isolated from representative human–hamster somatic cell hybrids of series A (Table 1) and controls. The 15-kb human (Hu) and 12-kb hamster (CH) bands hybridizing with the p53H probe are shown. A 3.9-kb human band also hybridized very weakly with the probe (lower arrow). The 15-kb human p53 band is present in hybrids 34H, 40H, 40T, 44H, 44HX, 46H, and 46TX.

human p53 probe hybridized strongly with 2.7- and 7-kb fragments in *Hin*dIII digests of human DNA (Fig. 4), whereas the same probe reacted only with a doublet of about 7 kb in mouse DNA. Using the 2.7-kb p53-specific band for analysis of human–mouse hybrids, we confirmed the assignment of the p53 gene to human chromosome 17 (Table 1, series C).

The p53 gene was regionally localized to human chromosome band 17p13 by analysis of another group of nine human–mouse hybrids (Table 1, series D). These hybrids were isolated after fusing human fibroblasts containing a reciprocal 17;22 chromosome translocation with TK$^-$ mouse fibroblasts. Hence, survival of these hybrid cells in HAT selective medium (100 μM hypoxanthine/1 μM aminopterin/16 μM thymidine/100 μM glycine) was dependent on the presence of human TK encoded by the normal human chromosome 17 or the 17;22 translocation chromosome. After human chromosome segregation during prolonged propagation of the cells, it could be anticipated that many hybrid clones would retain one, but not both, of these chromosomes bearing the gene encoding TK. The galactokinase and TK loci are closely linked (32) on the long arm of human chromosome 17 (17q21–q22). As expected, all of these hybrids that were cultured in selective HAT medium expressed human galactokinase. In contrast, only four of these hybrids (56H, 57H, 63H, and 64H) retained the p53H gene, while three others (53H, 54H, and 155) had lost this sequence. The human p53 gene was also not detected in two other clones that lost human galactokinase during back selection in 5-bromodeoxyuridine (53B) or prolonged growth under nonselective conditions (166B).

The simplest explanation for these results is that the p53 locus is distal to the translocation breakpoint on the short arm of human chromosome 17. Hence, those hybrids retaining only the 17;22 translocation chromosome do not contain p53H, although retention of the human gene for TK permits their survival in HAT medium. The fact that all three of these hybrids also express a chromosome 22 locus (mitochondrial aconitase) that would be retained on the 17;22 translocation chromosome is consistent with this interpretation. Direct evidence for retention of the 17;22 translocation chromosome was obtained by karyotypic analysis (Figs. 5 and 6) of one of these clones. The translocation chromosome was found in metaphases of this hybrid, whereas no normal chromosome 17 could be detected. The combined results of our karyotypic analysis and Southern analyses clearly indicate that the p53 gene is located on the most distal short arm band (p13) of human chromosome 17.

***In Situ* Hybridization of Metaphase Spreads with p53H Probe.** Metaphase spreads of normal human lymphocytes were hybridized with ^{125}I-labeled p53H probe to localize the p53 gene *in situ* (Table 2, columns A and B). The grain distribution exceeded background only on the short arm of chromosome 17, and all of these grains were specifically localized over the telomeric band 17p13. Metaphase spreads of GM3197 lymphocytes containing the reciprocal t(17;22) translocation were similarly examined (Table 2, row C). The very short t(22;17) chromosome was easily distinguished from G group, and all other, human chromosomes. Over 7% of the total grains were localized on the translocation chromosome containing only the telomeric band (17p13) of

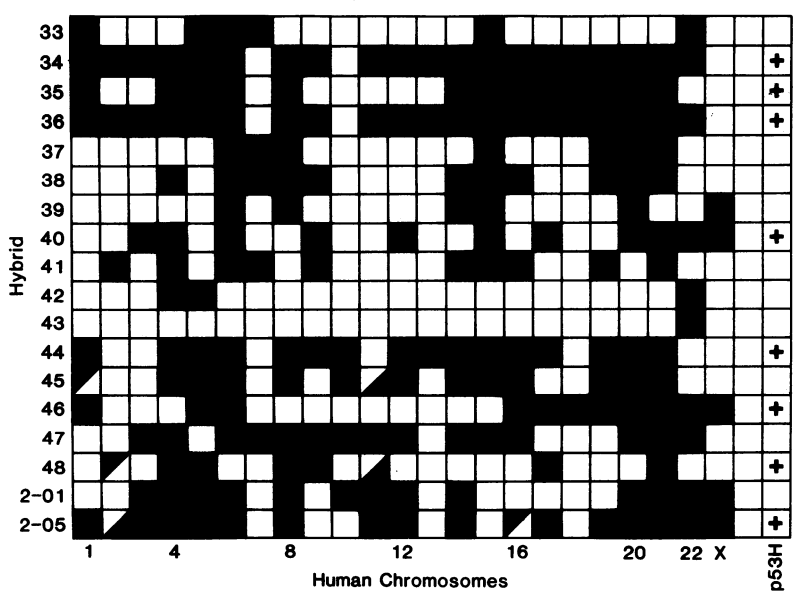

FIG. 3. Distribution of specific human chromosomes in human–hamster hybrid cell lines. Individual hybrid cell lines are represented on the ordinate, and specific human chromosomes are represented on the abscissa. Solid boxes indicate the presence of a specific human chromosome in a hybrid line, and open boxes indicate absence of the chromosome. When only the upper left corner of a box is darkened, only the short arm of that chromosome was retained, whereas the right lower corner is darkened when only the long arm is retained. The presence or absence of the human p53 gene is indicated in the far right column, and it correlates only with the presence or absence of human chromosome 17 in these hybrids.

Table 1. Segregation of p53 gene with specific human chromosomes

Human chromosome	% discordancy				Total
	A	B	C	D	
1	28	25	59	22	33
2	34, 41	55, 35	59	22, 33	43
3	41	20	94	44	48
4	45	11	65	ND	40
5	38	40	76	33	47
6	45	45, 40	59	22	44
7	62	70	18	11	48
8	48	30	63	ND	38
9	28	45	53	44	40
10	55	65	76	44	61
11	48, 52	20	65	56	45
12	41	30	59, 24	11, 44	35
13	28	36	0	22	22
14	48	20	12	33	31
15	55	60	29	33	48
16	52, 24	15, 50	53	44	40
17	0	5	0	(33)	|<2|*
18	24	50	35	56	37
19	38	15	76	33	40
20	38	15	59	33	36
21	31	25	41	56	35
22	45	40	65	77	51
X	52	25	35	22	37

Detection of the human p53 gene correlated with the presence or absence of human chromosome 17 in four series of human–rodent hybrids. Discordancy represents either the presence of a specific human chromosome in the absence of the p53 (human) gene or the presence of the gene despite the absence of the chromosome in that hybrid. When two numbers are given, they represent the percentage of discordancy with chromosomal short arm and long arm markers, respectively. Series A was human (GM0073)–hamster hybrids 33-48H&T (33, 34); 14 of 29 hybrids contained the p53 gene. B was a series of eight human (GM2658)–hamster hybrids and 12 subclones, and 12 were positive for the p53H gene. C was a series of human–mouse hybrids and subclones, and 10 of 17 were positive for p53. D was a series of nine hybrids (four positive for p53) isolated after fusing human fibroblasts (GM3196 or GM0119) containing a reciprocal translocation of chromosomes 17 and 22 with LMTK$^-$ cells; four hybrids retaining the normal chromosome 17 exhibited the p53 gene, whereas three hybrids retaining only the 17;22 translocation chromosome did not retain the p53 gene. Discordant segregation of chromosome 17 and p53H in series D actually represents discordancy between p53H and the 17;22 translocation chromosome. ND, not done.

*For chromosome 17, series D was not included in the analysis of total hybrids.

chromosome 17. These results unambiguously confirmed the localization of the p53 gene to 17p13.

DISCUSSION

Our work describes the assignment of the p53 gene to human chromosome 17. This result is based upon isolation of a human p53 cDNA clone, which was used to probe DNA from human–rodent somatic cell hybrids after human chromosome segregation. Further analysis of hybrids retaining a translocated chromosome 17 showed that the human p53 gene is located on the most distal band of the short arm of this chromosome (i.e., 17p13) and this localization was confirmed unequivocally by *in situ* hybridization.

The localization of the human p53 gene is of interest because nonrandom chromosome aberrations have been described in association with many human neoplasms, and cellular protooncogenes have been localized to some of these regions (35–37). Another protooncogene (c-*erb*A) was recently assigned to human chromosome 17, but it was localized to the long arm (q21–q22) of this chromosome (38, 39). A specific 15;17 chromosome translocation has been reported in acute promyelocytic leukemia, but the breakpoint is also on the long arm of chromosome 17 (q11). A similar chromosome 17 breakpoint was described in a human leukemia with both 17;21 and 9;22 reciprocal translocations (40). It is interesting that a fragile site on chromosome 17 (p12) is situated near the p53 locus, but we are unaware of any reported tumor-specific chromosome aberrations involving the short arm of chromosome 17. Our results suggest that such aberrations may be detected in the future.

The functional p53 gene has previously been assigned to

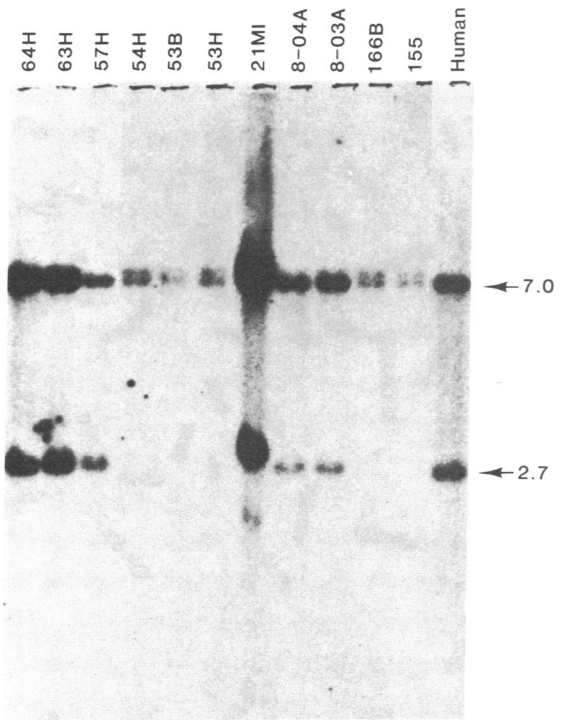

FIG. 4. Hybridization of a ^{32}P-labeled p53H probe with *Hin*dIII-digested, electrophoretically separated DNA isolated from human–mouse hybrids and human HeLa DNA. A strongly hybridizing 2.7-kb p53-specific band in human DNA was used to identify cell hybrids containing the human p53 gene. In contrast, the 7-kb p53-specific human band is not resolved from a 7-kb doublet of homologous mouse sequences, and the 7-kb band cannot be used in this analysis. The human p53 gene can be regionally localized to chromosome 17p13, since it was not detected in hybrids retaining the 17;22 human translocation chromosome (53H, 54H, and 155), whereas the gene was present in hybrids (57H, 63H, 64H, 8-03A, 8-04A, and 21M1) retaining a normal human chromosome 17.

Table 2. *In situ* hybridization of p53H probe with metaphase chromosomes

	No. of grains at chromosome location			Total grains	No. of metaphases
	t17;22	17p	17q		
A	—	12 (6.3)	2 (1.0)	192	10
B	—	5 (4.9)	1 (1.0)	102	17
C	25 (7.2)	11 (3.2)	4 (1.4)	346	42

Metaphase spreads of normal human lymphocytes (rows A and B) and those containing a reciprocal translocation t17;22(p13;q11) (row C) were hybridized with an ^{125}I-dCTP-labeled p53H probe. Results show the number of grains (percent of total in parentheses) located on the short arm (17p) and long arm (17q) of chromosome 17 as well as grains on the translocation chromosome t17;22, which contains only band 17p13 from chromosome 17.

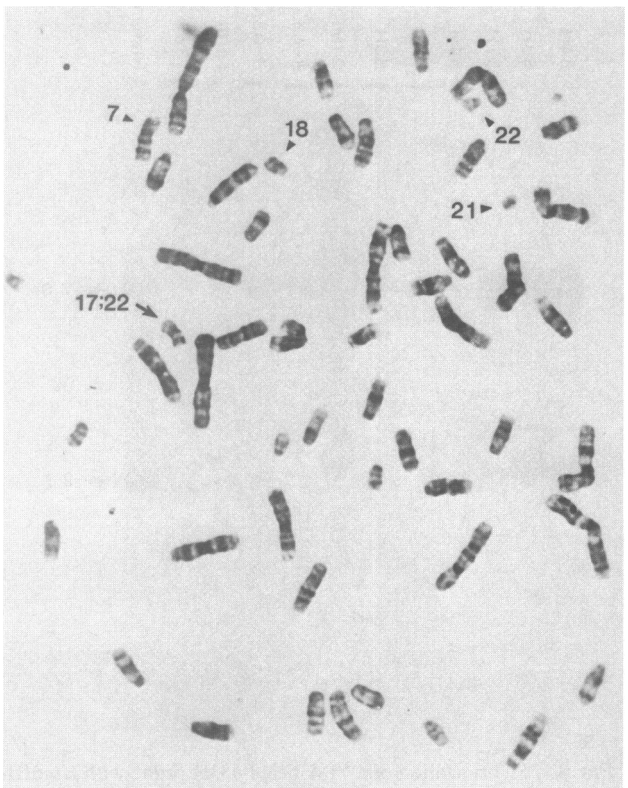

FIG. 5. Typical G-banded metaphase of hybrid 155 showing the 17;22 human translocation chromosome as well as other human chromosomes that are present. A normal chromosome 17 was not detected in metaphases from this line.

mouse chromosome 11, whereas a murine p53-processed pseudogene is located on chromosome 14. Since sequences homologous to genes located on both the short and long arms of human chromosome 17 are all present on mouse chromosome 11 (41), the localization of p53 to human chromosome 17 is not surprising. The p53 gene also has been mapped to an analogue of human chromosome 17 in African green monkey kidney cells (M. Oren and M. Singer, personal communication).

There have been previous efforts to chromosomally map human antigens expressed in SV40-transformed cells that were immunoprecipitable from extracts with anti-SV40 tumor antibody (42). It was concluded that the expression of a 55-kDa human protein coprecipitated by the anti-SV40 tumor antibody from SV40-transformed human–mouse somatic cell hybrids was dependent on the retention of human chromosome 7. Our results, which map the functional p53 gene to chromosome 17, indicate that the 55-kDa protein in the earlier study was probably not the p53 tumor antigen. It is unlikely that the results of Stitt and Mangel are explained by the presence of a regulatory gene for expression of p53 on chromosome 7 because one of their two hybrids expressing the human 55-kDa protein was reported to have lost human chromosome 17.

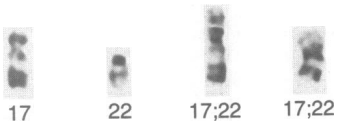

FIG. 6. Comparison of normal human chromosomes 17 and 22 with the 17;22 translocation chromosome shown in Fig. 5. Chromosomes 17 and 22 from a normal human metaphase preparation are shown at the left and the 17;22 translocation chromosome from hybrid 155 is displayed on the far right. The adjacent 17;22 chromosome represents an artificial construction of this translocation chromosome using parts of normal chromosome 17 (17p13→17qter) and chromosome 22 (22q11→22qter). The arrow indicates the breakpoint.

We thank Beverly Miller for preparation of this manuscript and Holly Fuhrer for photography. Human GM0119, GM3196, GM0073, and GM2658 cells were provided by the Institute for Medical Research. D.G. thanks Dr. S. Aaronson (National Institutes of Health) for providing support and hospitality during this research and Drs. M. Krause, I. M. Chiu, and F. Mushinski for their help at several stages of this work.

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