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Analysis of recombinant DNA clones specific for the murine p53 cellular tumor antigen

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Three cDNA clones, corresponding to two non-overlapping regions of the mRNA coding for the mouse p53 cellular tumor antigen, were isolated and characterized. In hybridization-selection assays, these clones were capable of selectively binding p53 mRNA, as demonstrated by *in vitro* translation and immunoprecipitation with anti-p53 monoclonal antibodies. The p53 mRNA appeared to be the only messenger species specifically selected by these clones. The size of the p53 mRNA was found to be ~2 kb, and its levels to vary substantially among different types of transformed cells. Evidence was found for the existence of two distinct p53-specific genes in mouse genomic DNA. Two partially overlapping recombinant phage clones were obtained, both derived from the same p53-specific genomic DNA region. The orientation of the various cDNA clones relative to that of the p53 mRNA was established by S1 analysis and the relationship between the cDNA clones and the genomic ones was determined by comparative restriction enzyme mapping and nucleic acid hybridization.

Key words: cDNA clones/genomic clones/hybrid-selection/p53 tumor antigen

Introduction

Many types of cells transformed by a wide variety of agents, display elevated levels of a cellular phosphoprotein, p53 (reviewed in Klein, 1982). This protein is present in very low amounts in non-transformed cells (Linzer *et al.*, 1979; Simmons, 1980; Dippold *et al.*, 1981), where it has an extremely short half-life (Oren *et al.*, 1981). In at least two systems studied so far, a close correlation could be demonstrated between experimental modulation of the transformed phenotype and alterations in cellular p53 levels (Linzer *et al.*, 1979; Oren *et al.*, 1981, 1982). These findings are compatible with a model assuming that p53 is involved in regulating cell proliferation by controlling the entry from G1 into S phase, as suggested by Campisi *et al.* (1982). This model is supported by the recent observation that microinjection of anti-p53 monoclonal antibodies into quiescent 3T3 cells inhibits their serum-stimulated entry into S phase (Mercer *et al.*, 1982). The possibility therefore exists that overproduction of p53 may be directly involved in malignant transformation by continuously inducing cellular DNA replication. It should be noted, however, that p53 overproduction can in no way account for all types of transformation, since many transformed cells do not display markedly elevated levels of the protein and in a few cases it is not detectable at all (Crawford *et al.*, 1981; Rotter *et al.*, 1981).

In the light of the possibility that quantitative regulation of p53 expression is closely associated with transformation, it becomes obvious that this regulation and the parameters involved in it should be studied in more detail. Such a study is greatly dependent on the availability of p53-specific nucleic acid probes and on the isolation of the genes encoding this protein in various systems. Recently we described the construction of a short p53-specific cDNA clone from the mRNA of SV40-transformed mouse fibroblasts (Oren and Levine, 1983). We now report the isolation of two additional longer cDNA clones and of mouse genomic p53 clones and their use to study the basic features of the p53 mRNA and the corresponding genes.

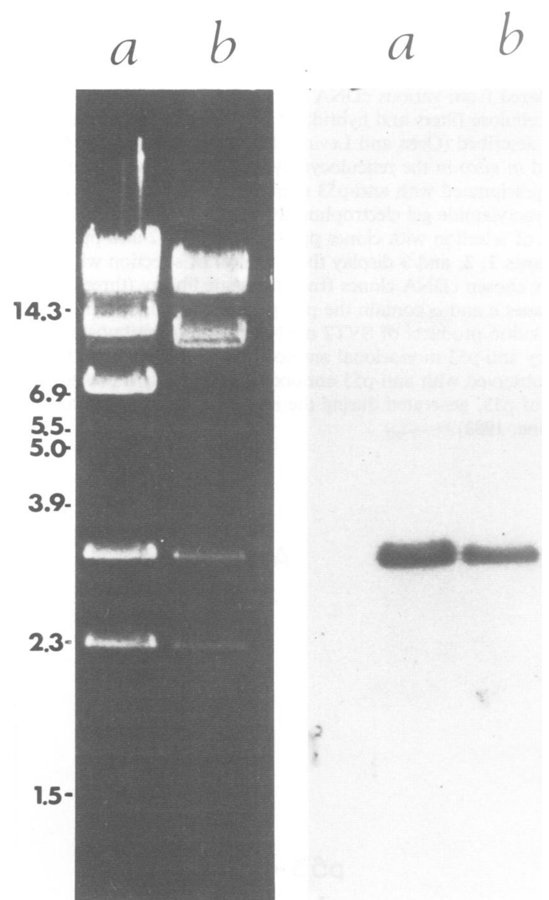

Fig. 1. Analysis of p53-specific genomic clones. Recombinant phages were isolated from a BALB/c mouse embryo genomic library by hybridization with radioactive pp53-208 cDNA as described in Results. DNA from phage Ch53-2 (**lane a**) and Ch53-11 (**lane b**) was digested with *Eco*RI and electrophoresed on a 0.7% agarose gel. The digestion products are depicted in the **left panel**. The 11.5-kb fragment generated from Ch53-11 can be better resolved from the short arm of the vector when the digest is electrophoresed for a longer period on a low-percentage agarose gel (data not shown). The gel was blotted and hybridized to radioactive cDNA clone pp53-208, as described in Materials and methods, and the autoradiogram is displayed in the **right panel**. The weak band seen at ~5.5 kb is probably a partial digestion product containing both the 3.3-kb and 2.3-kb fragments. Numbers on the left refer to the positions and sizes (in kb) of the fragments generated by digestion of phage Charon 4A DNA with *Bam*HI and *Eco*RI, serving as mol. wt. markers.

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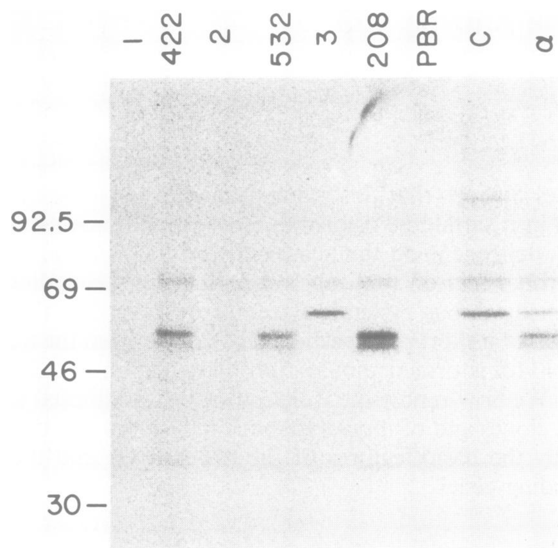

Fig. 2. Hybridization-selection analysis of p53-specific cDNA clones. DNA was prepared from various cDNA clones and from pBR322, immobilized on nitrocellulose filters and hybridized to 200 μg total cytoplasmic SVT2 RNA as described (Oren and Levine, 1983). The selected RNA was translated *in vitro* in the reticulocyte lysate system, and products were immunoprecipitated with anti-p53 monoclonal antibody and analysed by SDS-polyacrylamide gel electrophoresis. **Lanes 422, 532** and **208** display the products of selection with clones pp53-422, pp53-532 and pp53-208, respectively. **Lanes 1, 2,** and **3** display the products of selection with pools of randomly chosen cDNA clones from the same library (three clones in each pool). **Lanes c** and α contain the polypeptides immunoprecipitated from the translation products of SVT2 mRNA by control monoclonal antibody (c) and by anti-p53 monoclonal antibody (α). The lower band in the doublet observed with anti-p53 antibodies is probably a proteolytic cleavage product of p53, generated during the immunoprecipitation process (Oren and Levine, 1983).

Results

Isolation of p53 genomic clones

The previously described p53 cDNA clone pp53-208 (Oren and Levine, 1983) was used as a probe to screen a library of partial *Eco*RI restricted BALB/c mouse embryo DNA cloned in bacteriophage Charon 4A (Zakut *et al.*, 1980). 4 x 10^5 plaques were analysed and two hybridizing phages were isolated. The DNA of these phages was digested with *Eco*RI followed by hybridization with the cDNA-containing plasmid pp53-208. As shown in Figure 1, both phages share two common *Eco*RI fragments of 3.3 kb and 2.3 kb in addition to one unique fragment of 7 kb in Ch53-2 and 11.5 kb in Ch53-11. Of these, only the 3.3-kb fragment hybridized to the plasmid pp53-208 DNA (Figure 1). These findings are in agreement with the fact that pp53-208 hybridizes to a 3.3-kb fragment from *Eco*RI-digested mouse DNA (Oren and Levine, 1983), and indicate that both genomic clones are derived from the same region of the mouse genome.

Isolation and identification of p53-cDNA clones

A cDNA library was constructed from enriched poly(A)$^+$ RNA of mouse SVT2 cells, as described in Materials and methods, and screened by hybridization with the cDNA insert of plasmid pp53-208. Out of ~15 000 recombinant plasmid-containing colonies screened, three hybridized specifically with the pp53-208 probe. Two of these, pp53-422 and pp53-532, containing inserts of ~510 and 280 bp, respectively, were taken for further analysis. The DNA of these plasmids was analysed by hybridization selection assays, employing anti-p53 monoclonal antibodies (Oren and Levine, 1983). The results of this analysis are shown in Figure 2. It is obvious that both pp53-422 and pp53-532, as well as the previously isolated pp53-208, efficiently bound the mRNA coding for p53. A variety of other clones from the same cDNA library,

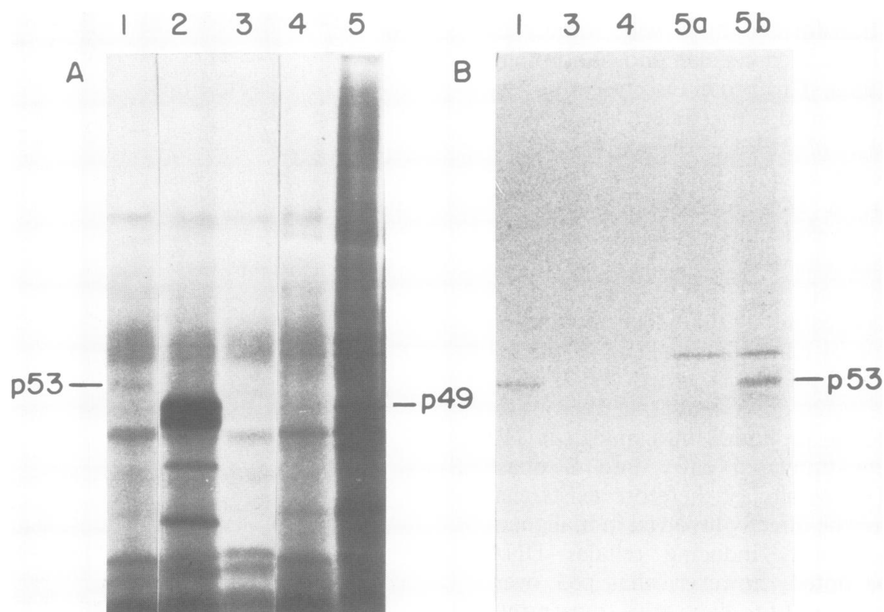

Fig. 3. Hybridization selection analysis of cDNA clones. DNA of clone pp53-271 **(lane 1)**, of a cDNA clone specific for a 49-kd major cellular polypeptide **(lane 2)**, and of pBR322 **(lane 4)** was analysed essentially as described in Figure 2. **Panel A** shows the direct analysis of the translation products, without any immunoprecipitation. **Lane 3** displays the endogenous background of the reticulocyte lysate used in this experiment, while **lane 5** shows the polypeptides generated by translation of non-selected SVT2 mRNA. The reactions analysed in **panel A** were further immunoprecipitated with either control antibody **(panel B,** 5a) or with anti-p53 monoclonal antibody **(panel B,** all other lanes). The positions of p53 and of the abundant 49-kb polypeptide (p49) are also indicated.

serving as randomly-chosen controls, failed to select any detectable p53 mRNA (Figure 2 and unpublished data). Thus, both additional cDNA clones must also be derived from the p53 mRNA.

To obtain cDNA clones complementary to other parts of the mRNA, use was made of the genomic p53 clones described above. The 3.3-kb *Eco*RI fragment was prepared from clone Ch53-2, nick-translated, and hybridized to the same cDNA library from which clones 422 and 532 had been isolated. Two additional clones hybridizing with the genomic probe but not with clone pp53-208, were isolated. The larger of the two, termed pp53-271 (insert size 600 bp), was further characterized.

Since pp53-271 did not show cross hybridization with pp53-208 it was necessary to establish its relationship to the p53 mRNA. DNA from clone pp53-271 was isolated and assayed by hybridization selection (Figure 3). When the translation products of the selected RNA were analysed directly, without further immunoprecipitation, only a single polypeptide could be detected which was not made with RNA selected by pBR-322 DNA (panel A, lanes 1 and 4). Immunoprecipitation of the translation products with anti-p53 monoclonal antibody established the identity of this band as p53 (panel B, lane 1). This result further confirms that pp53-271 is indeed a p53-specific cDNA clone. Furthermore, it shows that p53 mRNA is the only translatable cellular messenger capable of efficiently hybridizing to this clone. When a control cDNA clone derived from an abundant mRNA species was reacted with the same amount of RNA, much more protein was made by the selected mRNA (panel A, lane 2). The fact that mRNA selected by clone pp53-271 directs the synthesis of such minute amounts of protein demonstrates that the corresponding messenger is either extremely rare or very inefficiently translated in the reticulocyte lysate.

Analysis of p53-specific mRNA

The cloned p53 cDNA was employed to study the size and relative levels of p53 mRNA in different transformed mouse cells. Polyadenylated RNA, either cytoplasmic or total cellular, was prepared from various sources, and equal

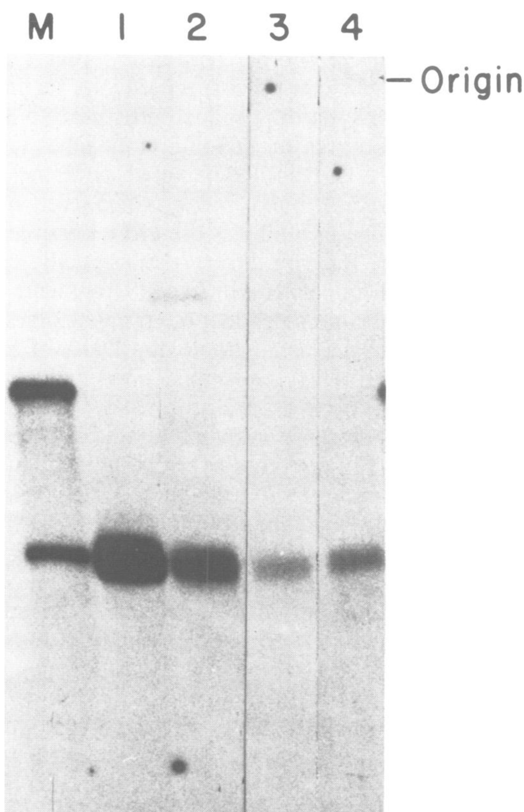

Fig. 4. Analysis of p53-specific mRNA. Poly(A)$^+$ RNA was prepared from a variety of sources, twice chromatographed over oligo(dT)-cellulose and fractionated over a 1% formaldehyde agarose gel, blotted to nitrocellulose and hybridized to the radiolabeled *Xho*I-*Pvu*II fragment of clone pp53-271 (sp. act. 10^8 c.p.m./μg). **Lane 1**, 5 μg cytoplasmic IB-9 RNA; **lane 2**, 5 μg total cellular RNA from Friend erythroleukemia cells; **lane 3**, 5 μg total cellular RNA from an XRPC-24 tumor; **lane 4**, RNA extracted from SVT2 polysomes immunoprecipitated with anti-p53 monoclonal antibody (Oren and Levine, 1983; ~5 ng). **Lane M** displays the positions of radioactive 28S and 18S rRNA.

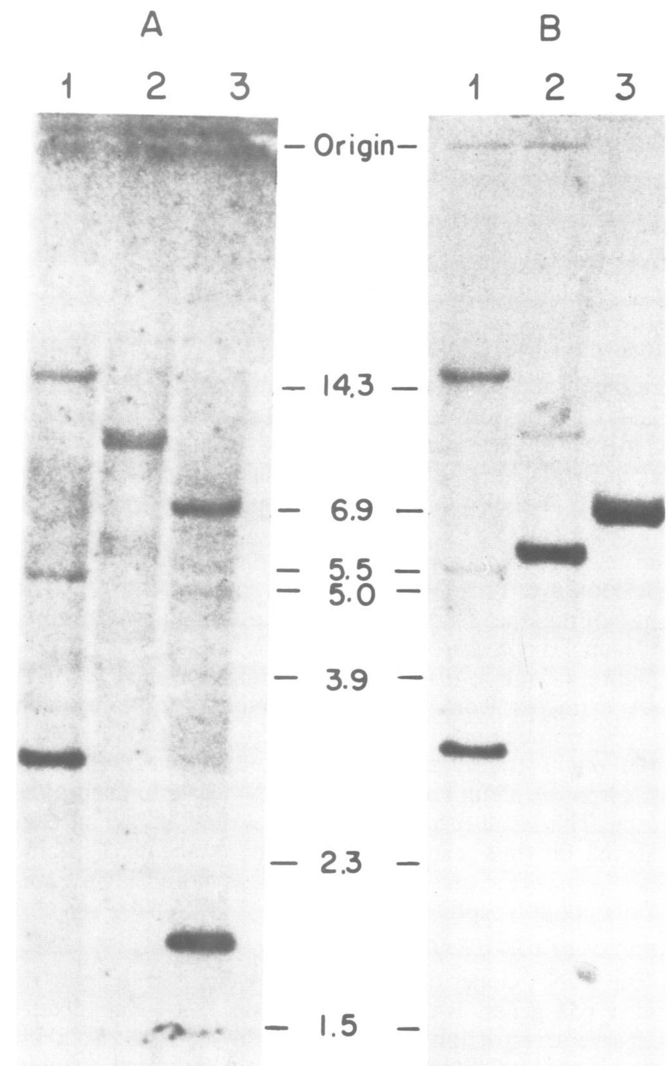

Fig. 5. Southern analysis of p53-specific mouse DNA. BALB/c mouse liver DNA (20 μg/sample) was digested with different restriction enzymes, electrophoresed and blotted to nitrocellulose as described in Materials and methods. Duplicate samples were hybridized with the following radioactive probes: **panel A**, the whole cDNA insert of clone pp53-208, excised with *Pst*I; **panel B**, the internal *Xho*I-*Pvu*II fragment of clone pp53-271. The enzymes used were: **lane 1**, *Eco*RI; **lane 2**, *Bam*HI; **lane 3**, *Hind*III. The numbers in the middle of the figure denote the positions and sizes (in kilobase pairs) of the fragments generated by digestion of Charon 4A DNA with *Bam*HI and *Eco*RI.

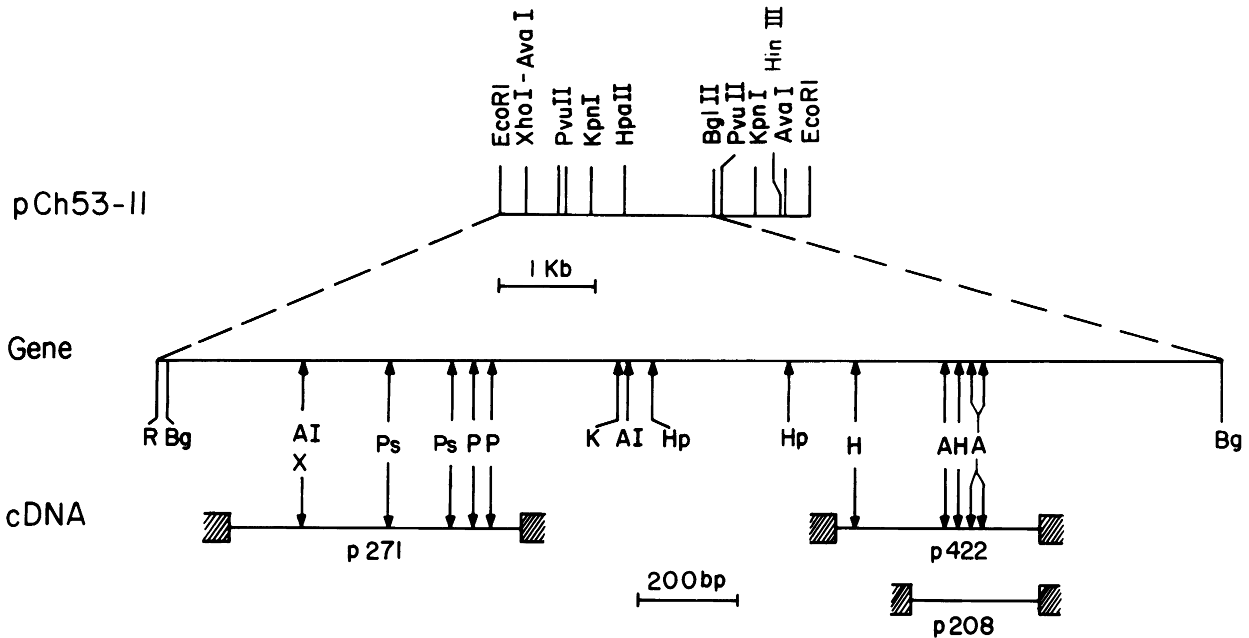

Fig. 6. Partial restriction enzyme maps of the cDNA clones pp53-271, pp53-422 and pp53-208, and the subcloned genomic 3.3-kb fragment (pCh53-11). The maps indicate the identical restriction enzyme sites and the relative locations of homologous sequences between the cDNA and the genomic clones. A, *Ava*II; AI, *Ava*I; Bg, *Bgl*II; H, *Hin*fI; Hp, *Hpa*II; K, *Kpn*I; P, *Pvu*II; Ps, *Pst*I; R, *Eco*RI; X, *Xho*I. The *Hin*fI and *Ava*II sites in the genomic DNA were determined only for the region homologous to pp53-422.

amounts of each preparation were analysed as described in Figure 4. As seen in the Figure, the p53-specific probe detects a rather broad RNA band, possessing an average size of ~2 kb. It is obvious that there are substantial differences in p53 mRNA levels among different transformed cells. In addition, p53 mRNA can easily be detected not only in tissue-culture cells but also in solid tumors, such as the one induced by injection of XRPC-24 plasmacytoma cells. Interestingly, total cellular RNA from Friend leukemia cells also displays a high mol. wt. (7.5 kb) p53-specific species (lane 2). The nature of this species is still unknown, although it may represent a nuclear precursor of p53 mRNA.

Figure 4, lane 4, displays the hybridization detected with RNA extracted from polysomes immunoprecipitated with anti-p53 monoclonal antibodies (Oren and Levine, 1983). Based on its transcriptional activity, the total amount of mRNA in the aliquot analysed in lane 4 was estimated to be ~5 ng. This result further demonstrates that the 2-kb mRNA observed in this experiment is indeed the p53-specific messenger species, present on polysomes synthesizing the corresponding polypeptide.

Analysis of p53-specific mouse DNA

The cDNA clones described above were used to study the mouse p53 genes. BALB/c mouse liver DNA was digested with several restriction enzymes and analysed by the Southern transfer method (Southern, 1975), employing two different cDNA clones as labeled probes. The probes used were derived from clones pp53-208 and pp53-271, and the results are shown in Figure 5. Three bands are seen in the *Eco*RI lanes, whereas two bands are present in each of the other lanes. It should be noted that each of the broad bands seen in lanes A2 and B3 constitutes a true doublet; the individual fragments can clearly be resolved upon longer electrophoretic separation on a low percentage agarose gel (data not shown). The weak 5.5-kb fragment was not reproducibly observed in different experiments, especially those employing DNA of several sources other than liver. We therefore believe that it does not represent a distinct, different p53-specific DNA segment, but is rather a partial digestion product, identical to the one observed upon digestion of the genomic clone DNA (compare with legend to Figure 1). It should be noted, nevertheless, that in liver DNA this weak band was retained even after exhaustive *Eco*RI digestion, and it is therefore possible that its presence or absence may be due to tissue-specific chemical modifications. The upper 16-kb *Eco*RI fragment, on the other hand, is unique and a product of complete digestion, as observed in many experiments employing DNA of a variety of mouse strains and cell types (unpublished data).

The two cDNA clones used as probes – pp53-208 and pp53-271 – are totally non-overlapping, as determined directly by their inability to cross-hybridize and confirmed by their structural analysis (Figure 6). Since both these clones hybridize to the same *Eco*RI bands of 16 kb and 3.3 kb, these two bands must represent two distinct p53-specific genes. Although it is still unclear whether both or only one of these p53-specific DNA segments are functional in encoding the protein, we shall refer to both as murine p53 genes (with the reservation that one may actually be a pseudogene).

Comparison of the hybridization of *Bam*HI and *Hind*III-digested mouse DNA with clones pp53-208 and pp53-271, reveals that the patterns obtained with the two probes are not identical. In fact, in each case the probes detect one common band and one which differs between pp53-208 and pp53-271. Since the 3.3-kb *Eco*RI fragment contains neither a *Bam*HI nor a *Hind*III site within the region homologous to the cDNA clones (Figure 6), these data imply that the second p53 gene (represented by the 16-kb *Eco*RI fragment) must contain cleavage sites for both enzymes. At least one site for each enzyme must be located between the regions complementary to pp53-208 and to pp53-271.

Structural analysis of the genomic and cDNA clones

To determine the relationship between the cDNA clones and

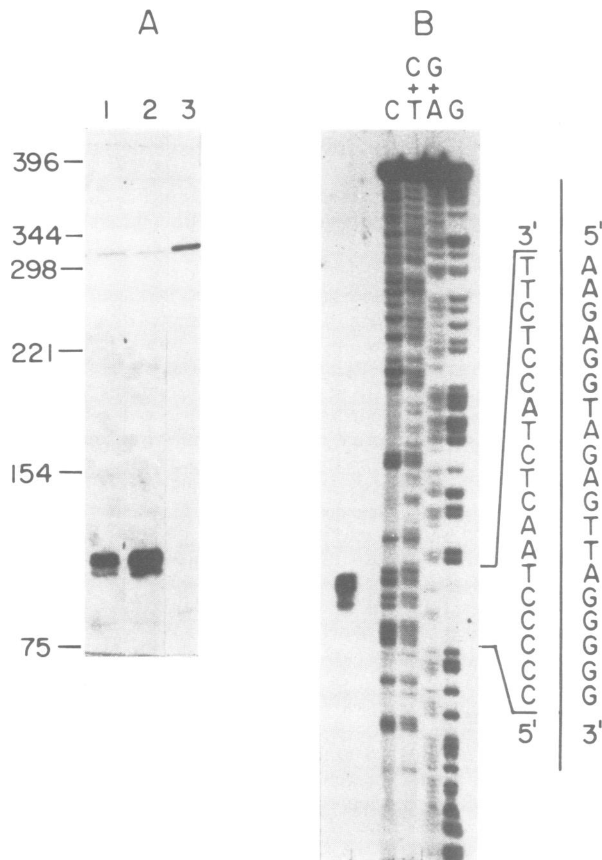

Fig. 7. S1 analysis of p53-specific DNA. The end-labeled 320-bp *Xho*I-*Eco*RI fragment was prepared as described in Materials and methods, re-annealed with cytoplasmic poly(A)$^+$ RNA from IB-9 cells and subjected to S1 analysis (Berk and Sharp, 1977). The products were separated on a 4% polyacrylamide gel containing 8 M urea **(panel A)**. **Lane 1**, 25 000 ^{32}P c.p.m. of DNA and 1 μg mRNA; **lane 2**, 25 000 c.p.m. of DNA and 3 μg mRNA; **lane 3**, 5000 c.p.m. of DNA, incubated in the absence of RNA and loaded without any S1 treatment. The numbers on the left side denote the positions and sizes (in base pairs) of denatured DNA fragments derived by *Hinf*I digestion of pBR322 DNA. **Panel B**: the 110 bp bands seen in **panel A** were cut out, eluted and reelectrophoresed on a 6% polyacrylamide-urea sequencing gel, in parallel with a Maxam Gilbert sequencing ladder of the initial 320 bp *Xho*I-*Eco*RI fragment.

the genomic ones, the 3.3-kb genomic fragment hybridizing to pp53-208 (Figure 1) was subcloned in pBR322 and denoted pCh53-11. Clones pp53-208, pp53-422 and pp53-271, as well as pCh53-11, were analysed and compared by restriction enzyme mapping. Comparison of the maps of these clones (Figure 6) suggests several conclusions. Clones pp53-208 and pp53-422 possess overlapping sequences reflecting the fact that pp53-208 was used as a probe to isolate the latter. Furthermore, pp53-208 seems to be entirely contained within the larger pp53-422. On the other hand, there is no overlap between clone pp53-271 and pp53-422 and the region of the genomic clone homologous to pp53-271 is located ~650 bp upstream to the sequences homologous to pp53-422.

The identical positions of restriction sites in the p53 cDNA clones and Ch53-11 confirm that the latter recombinant clone does contain sequences derived from murine genomic p53-specific DNA. These data also support the conclusion that the three cDNA clones (pp53-208, pp53-422 and pp53-271) are derived from a continuous region in the genome and hence probably from the same mRNA species.

S1 analysis of genomic p53-specific DNA

To determine the transcriptional orientation of the p53 mRNA and to compare the structure of the genomic clone with that of the mRNA, S1 analysis was performed, as described in Materials and methods. The results are shown in Figure 7. It is obvious that the mRNA hybridizes to and protects a region extending ~110 bp from the *Xho*I in the direction of the left-hand *Eco*RI site (cf., Figure 6). The exact nucleotide sequence of the region comprising the end of the protected fragment was established by eluting this fragment from the gel and re-electrophoresing it on a sequencing gel in parallel with partial degradation products (Maxam and Gilbert, 1977) of the 5′ end-labeled *Xho*I-*Eco*RI fragment used for this analysis (Figure 7, panel B). The ability to be protected from S1 establishes the fact that the labeled probe was derived from the strand of the DNA complementary to p53 mRNA. The orientation of the transcript is therefore opposite to that of the labeled probe, namely from left to right on the map displayed in Figure 6. Thus, pp53-271 is derived from a region of the p53 mRNA upstream (5′) to that represented in clones pp53-208 and pp53-422.

This S1 analysis suggests that there must exist a significant difference between the sequence of Ch53-11 and that of the p53 mRNA, starting at a point ~110 bp 5′ to the *Xho*I site. The possible implications of these data are considered in the Discussion.

Discussion

Several recombinant DNA clones, specific to the mouse p53 cellular tumor antigen, were isolated and characterized. Two cDNA clones, pp53-208 and pp53-271, are clearly derived from different, non-overlapping regions of the p53 mRNA. Using these clones as probes, data were obtained suggesting the existence of two distinct p53-specific genes in the mouse genome. This conclusion differs from that suggested by our previous results (Oren and Levine, 1983), where a single gene was implicated due to the failure to detect the upper, 16-kb *Eco*RI band. Since this band can be detected by a similar probe (Figure 5, panel A), the previous result must have been due to an experimental problem. At this stage, it is still unclear whether both genes are transcriptionally active or whether one is a pseudogene. Work is now in progress to distinguish between these possibilities.

The S1 analysis data suggest that while the sequences of the genomic clone Ch53-11 and that of the corresponding mRNA are identical throughout the region extending ~110 bp from the *Xho*I site in the 5′ direction of the mRNA, they diverge from each other upstream to that point. The most likely explanation is that the divergence point constitutes the 3′ end of an intron. However, one may also consider the possibility that the mRNA used in this experiment was transcribed off the second p53 gene, and that the sequences of the two genes differ markedly in a particular region, leading to an S1-sensitive site. Direct sequence comparison of the two genes should enable a definitive distinction between these possibilities.

Additional work employing p53-specific DNA probes will be needed to clarify the expression patterns of the pertinent genes in normal *versus* transformed cells and, eventually, to elucidate the relationship between p53 and cellular transformation.

Materials and methods

Cells and tumors

SV40-transformed mouse SVT2 cells were the gift of A.J. Levine. IB-9 cells, derived from a fibrosarcoma induced by methylcholanthrene (De Baetselier et al., 1980) were the gift of S. Katzav. Exponentially growing Friend erythroleukemia cells were the gift of R. Bendori and A. Kimchi. XRPC-24 mouse plasmacytoma tumors were propagated in BALB/c mice by s.c. injection of tumor-derived cells.

Extraction of nucleic acids

Total cellular RNA from tumors and tissue-culture cells was extracted by the guanidine-thiocyanate procedure (Chirgwin et al., 1979). Cytoplasmic RNA was prepared from cultured cells as previously described (Oren et al., 1981). RNA samples to be analysed were twice chromatographed over an oligo(dT)-cellullose column and ethanol precipitated prior to further manipulation. Total mouse genomic DNA was prepared according to Blin and Stafford (1976).

For the preparation of plasmid DNA, bacteria containing the desired plasmid were grown in enriched M9 medium to an OD_{550} of 0.15. Cytidine was then added to a final concentration of 1 mg/ml (Norgard et al., 1979) and the culture allowed to reach an OD_{550} of 0.4. Chloramphenicol was then added to 100 mg/liter and, after 20 h at 37°C, the cells were lysed and plasmid DNA extracted by standard procedures (Katz et al., 1973). Following isopycnic centrifugation in CsCl/ethidium bromide and removal of the dye, plasmid DNA was diluted 3-fold with water and immediately precipitated with ethanol. Phage DNA was prepared according to the procedure of Blattner et al. (1977).

Isolation of mouse p53 genomic clones

A partial *Eco*RI mouse embryo DNA library prepared in our laboratory (Zakut et al., 1980) was screened by *in situ* plaque hybridization to nick-translated mouse pp53-208 plasmid DNA, according to published procedures (Benton and Davis, 1977).

Construction and screening of cDNA library

A cDNA library from sucrose-fractionated SVT2 mRNA was prepared essentially as described (Oren et al., 1983). Transformed bacteria were plated to yield an average density of 600 – 1000 colonies per 90 mm dish. Colonies were grown to a diameter of 1 – 2 mm, transferred to nitrocellulose filters (Thayer, 1979) and hybridized to the appropriate probe (see below). Positive colonies were picked, re-isolated and confirmed by growing them to a small scale, extracting their DNA (Birnboim and Doly, 1979), and hybridizing it to the same radioactive probe as before. Final identification of p53-specific clones was by the hybrid-selection method (Ricciardi et al., 1979; Oren and Levine, 1983).

Preparation of hybridization probes

Clone pp53-208 DNA was digested to completion with *Pst*I. The insert was separated on a 5% polyacrylamide gel, electroeluted and nick- translated with *Escherichia coli* DNA polymerase I to an approximate specific activity of 10^8 c.p.m./μg (Rigby et al., 1977). For the screening of genomic libraries, whole plasmid pp53-208 DNA was nick-translated.

Gel analysis and nucleic acid hybridization

DNA from various plasmids and bacteriophages was digested with restriction enzymes, electrophoresed on agarose gels and blotted onto nitrocellulose (Southern, 1975). The blots were hybridized with radiolabeled probe at 67°C for 16 h in 5 x SSC, 5 x Denhardt's solution (Denhardt, 1966), 20 mM sodium pyrophosphate pH 7.0, 0.5% SDS, in the presence of 100 μg/ml denatured sonicated *E. coli* DNA. Following several repeated washes at 67°C with 1 x - SSC, 20 mM sodium pyrophosphate and 0.2% SDS, blots were exposed to X-ray film with an intensifying screen at −70°C. For genomic blots, BALB/c high mol. wt. DNA (20 μg/sample) was digested to completion with the appropriate restriction enzymes, and fractionated over a 0.6% agarose gel in Tris-acetate-EDTA buffer (TAE). Blotting and hybridization were as described (Rechavi et al., 1982). Polyadenylated RNA was fractionated over a 1% formaldehyde gel (Rave et al., 1979) and blotted onto nitrocellulose (Thomas, 1980) in 10 x SSC. Hybridization was at 42°C for 48 h in 50% formamide, 5 x SSC, 1 x Denhardt's solution, 50 mM sodium phosphate pH 6.8, and 100 μg/ml denatured salmon sperm DNA. Hybridized filters were washed repeatedly at room temperature with 2 x SSC, 0.5% SDS and 20 mM sodium pyrophosphate pH 7.0, followed by 30 min at 50°C with 0.1 x SSC, 0.5% SDS, 20 mM sodium pyrophosphate.

Hybridization selection

DNA of the plasmid to be tested was immobilized on nitrocellulose filters and hybridized with 200 μg of total cytoplasmic RNA from either SVT2 or IB-9 cells. Hybridization, isolation of bound RNA and *in vitro* translation were as described (Oren and Levine, 1983). The translation products were either analysed directly or after immunoprecipitation with anti-p53 monoclonal antibody L421 (Harlow et al., 1981).

Restriction enzyme mapping

Restriction endonuclease mapping of purified phage and plasmid DNA was performed with the indicated enzymes employing the conditions specified by the supplier. Restricted phage DNA was further analysed by agarose gel electrophoresis, blotting and hybridization to p53-specific cDNA plasmid clones and to specific restriction fragments derived from these cDNA clones.

S1 analysis

S1 analysis was performed essentially according to Berk and Sharp (1977). The DNA of the genomic subclone pCh53-11 was purified over a Biogel A-50 (Biorad) column, digested with *Xho*I, treated with bacterial alkaline phosphatase (Boehringer-Mannheim), 5′ end-labeled with polynucleotide kinase (P/L Biochemicals) and recut with *Eco*RI. The resultant 320 nucleotide fragment was labeled to an approximate specific activity of 8 x 10^6 c.p.m./μg single-stranded DNA. The products of the kinase reaction were heat-denatured in the presence of dimethyl sulphoxide, separated on a 5% polyacrylamide gel, and the single-stranded 320 nucleotide fragment was eluted and subjected to S1 analysis. Reannealing was at 52°C for 3 h in 20 μl of 80% formamide, 400 mM NaCl, 40 mM pipes pH 6.4, 1 mM EDTA, and was terminated by dilution into 200 μl of S1 buffer (250 mM NaCl, 30 mM NaAc pH 4.5 1 mM $ZnSO_4$, 25 μg/ml *E.coli* DNA) and incubation for 45 min with 500 units S1 nuclease (Miles) at 37°C. The digestion products were analysed as described in the legend to Figure 7.

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