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Immunologically Distinct p53 Molecules Generated by Alternative Splicing

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Transfection of a functional cloned p53 gene into an L12 p53 nonproducer cell line efficiently reconstituted p53 expression. The p53 protein synthesized in these clones was indistinguishable from that occurring naturally in tumor cells. When a p53 cDNA clone was used instead, we observed that the L12-derived clones exhibited a distinct immunological profile. In the present experiments we compared the immunological epitopes of p53 proteins encoded by several full-length cDNA clones. Immunoprecipitation of p53 proteins generated by in vitro transcription and translation of the various cDNA clones indicated variations in the content of immunological epitopes. Basically, two p53 protein species were detected. Both species contained the same antigenic determinants except the PAb421-PAb122 site, which was present in proteins encoded by p53-M11 and pcD-p53, but not in the p53 protein encoded by the p53-M8 cDNA clone. Sequence analysis of the various cDNA clones indicated the existence of a 96-base-pair (bp) insert in clone p53-M8 as compared with clone p53-M11 or pCD-p53. The 96-bp insert contained a termination signal which caused the premature termination of the protein, leading to the generation of a p53 product 9 amino acids shorter than usual. The existence of this insert also accounted for the lack of the PAb421-PAb122 epitope which was mapped to the 3′ end of the cDNA clone, following the 96-bp insert. This insert shared complete homology with the p53 intron 10 sequences mapping 96 bp upstream of the 5′ acceptor splicing site of p53 exon 11. It was therefore concluded that the different cDNA clones represented p53 mRNA species which were generated by an alternative splicing mechanism. Differential hybridization of the mRNA population of transformed fibroblastic or lymphoid cells with either the 96-bp synthetic oligonucleotide or the p53-M11 cDNA indicated that the various mRNA species are expressed in vivo.

The p53 tumor antigen has been shown to be a functional oncogene product (10, 14, 26, 35). By using different experimental approaches, it was shown that overproduction of p53 rendered cells malignant. Introduction of the p53 gene into primary embryonic fibroblasts by cotransfection with the *ras* oncogene induced the appearance of transformed cells, which developed into tumors in rats. This suggested that p53 belongs to the nuclear oncogene family and that like *myc*, *myb*, and E1A, p53 complemented the *ras* membrane-associated oncogene (10, 14, 26).

This was further supported by results obtained with Abelson murine leukemia virus (Ab-MuLV)-transformed variant p53 nonproducer L12 cells. In these cells we found that the endogenous p53 gene was inactivated by the integration of Moloney virus sequences into its first intron (37). Introduction of a functional genomic p53 clone into these nonproducer cells reconstituted the expression of p53 (35). This genetic manipulation changed the phenotype of these cells from cells that develop into regressing tumors into cells that develop into lethal tumors in syngeneic hosts (35). Our conclusion was that L12 cells, which express the *abl* oncogene but lack p53 expression, are only partially transformed. Expression of a completely transformed phenotype required the concomitant expression of *abl* and p53.

The p53 protein expressed in the L12-derived clones obtained by transfection of the p53 gene was indistinguishable from the p53 occurring naturally in tumor cells (35). Recently, however, we found that reconstitution of p53 expression in L12 cells by transfection of a full-length p53 cDNA clone (p53-M8) yielded L12-derived cell lines that expressed an immunologically discrete p53 form (34). The p53 proteins expressed by either the genomic or cDNA p53 clones exhibited similar peptide maps, but expressed different immunological epitopes (34). It was suggested that transfection of the p53 gene induces the expression of the entire group of the possible mRNA species transcribed off this gene, whereas the cloned p53 cDNA represents a single mRNA molecule that codes for a discrete species of p53 protein.

Results presented here suggest that the single functional p53 gene of the mouse is coding for at least two p53 mRNA species, generated by an alternative splicing mechanism. Each of these mRNA molecules can potentially be translated into a discrete p53 protein.

MATERIALS AND METHODS

Cells. 2M3, an Ab-MuLV-transformed lymphoid cell line expressing the *abl* oncogene product, and 2M3/M superinfected with the Moloney helper virus were grown in RPMI 1640 medium enriched with 10% heat-inactivated fetal calf serum (BioLab, Jerusalem, Israel). Hybridoma cell lines producing anti-p53 antibodies were grown in RPMI 1640 medium enriched with 20% heat-inactivated fetal calf serum supplemented with 20 mM L-glutamine and 20 mM sodium pyruvate.

Antibodies. Monoclonal anti-p53 antibodies were obtained from established hybridoma cell lines RA3-2C2 (7) and PAb421 (13). Monoclonal antibodies were obtained from supernatants of the hybridoma cell lines or ascitic fluid of syngeneic mice injected intraperitoneally with the hybrid-

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oma cell lines. Antibodies were purified and concentrated by binding to Sepharose-protein A columns (Sigma Chemical Co., St. Louis, Mo.).

In vitro transcription-translation assay. The *Eco*RI insert of clones p53-M8 and p53-M11 and the *Bam*HI insert of the pCD-p53 clone were subcloned into the pSP65 transcription vector (Promega Biotech) in a 5′ orientation with regard to that of the SP6 bacteriophage polymerase promoter contained in this vector. The pSP65 plasmids containing the p53 inserts were linearized by *Hin*dIII digestion and extracted with phenol before precipitation. Approximately 1 μg of the linearized plasmid was transcribed with SP6 RNA polymerase (Anglian, Biotech). A fraction of the reaction mixture was used directly for translation in a rabbit reticulocyte lysate system (Anglian, Biotech).

Immunoprecipitation of p53 protein. 2M3 cells (10^7) at the logarithmic stage of growth were washed several times in phosphate-buffered saline and suspended in 1.5 ml of Dulbecco modified Eagle medium without methionine, enriched with 10% dialyzed heat-inactivated fetal calf serum and 250 μCi of [^{35}S]methionine (Amersham Corp., Arlington Heights, Ill.). Cells were incubated for 1 h at 37°C, washed in phosphate-buffered saline, and extracted into 2 ml of lysis buffer (10 mM Na_2HPO_4-NaH_2PO_4 [pH 7.5], 100 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate) at 4°C. Labeled-cell lysates were precleared by repeated absorption with *Staphylococcus aureus* and nonimmune serum. Equal amounts of in vivo-radiolabeled protein or in vitro-translated p53 were immunoprecipitated with specific antibodies. Antigen-antibody complexes were collected by S. aureus precipitation (16). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed by the method of Laemmli (17).

RNA blot analysis. Selected polyadenylated mRNA (1, 2) obtained from transformed cells or in vitro-transcribed RNA obtained from the various cDNA clones was heated for 10 min at 60°C in 50% formamide–6% formaldehyde (vol/vol)–running buffer (20 mM 4-morpholinepropanesulfonic acid [pH 7.5], 5 mM sodium acetate, 1 mM EDTA). The samples were electrophoresed through a 1% agarose gel containing 6% formaldehyde. The RNA was transferred onto a nitrocellulose sheet and hybridized to nick-translated DNA probes. Hybridization was for 16 h at 43°C in 50% formamide–750 mM NaCl–75 mM sodium citrate–5× Denhardt solution–20 mM sodium phosphate (pH 7.0)–100 μg of salmon sperm DNA per ml–10% dextran sulfate. Hybridized filters were washed extensively at 50°C with 15 mM NaCl–1.5 mM sodium citrate–0.1% sodium dodecyl sulfate and autoradiographed.

Isolation of various cDNA clones. Clones p53-M8 and p53-M11 were obtained by screening the original mouse Meth A cDNA library prepared in the *Eco*RI site of λgt10 (34) with a mouse-specific cDNA probe. Clone pCD-p53 was isolated from a concanavalin A-stimulated mouse T-cell line.

Nucleic acid sequencing. The M13 chain termination method (20, 32) was used for determining DNA sequences, using either the M13 universal primer or various synthetic oligonucleotide primers prepared according to the published p53 sequence (38).

RESULTS

Isolation of p53 cDNA clones coding for various immunological protein forms. We found that expression of p53 in the Ab-MuLV-transformed p53-nonproducer L12 cell line can be reconstituted by transfection by either the genomic p53 clone or the full-length p53 cDNA clones. Comparison of the immunological profile of the p53 proteins expressed in the L12-derived clones showed immunological variations. p53 protein expressed in L12 cells transfected by the cDNA clone p53-M8 bound the RA3-2C2 and PAb200.47 anti-p53 monoclonal antibodies as well as polyclonal anti-p53 serum but totally lacked the antigenic epitope for the PAb421-PAb122 monoclonal antibodies (34). The PAb421 and PAb122 anti-p53 monoclonal antibodies bind to the same antigenic domain (3). L12 clones derived by transfection of the genomic p53 clone bound the entire panel of anti-p53 antibodies tested, including PAb421-PAb122. On the basis of these results, we suggest that the p53 gene potentially codes for several types of p53 mRNA molecules and that the p53-M8 clone represents a unique mRNA species.

To test this hypothesis, additional cDNA clones were isolated either from transformed Meth A fibroblasts, from which p53-M8 was previously isolated (34), or from a normal T-cell library (6), and the structures of their encoded proteins were compared. The following cDNAs were studied in detail: p53-M11 isolated from the Meth A cDNA library of BALB/c origin and the pCD-p53 cDNA clone isolated from a nontransformed helper T-cell clone, E.1 (6), which was reactive to hapten trinitrophenol on a BALB/c background. The latter cDNA library (5×10^5 PFU) (27) was made from poly(A)$^+$ RNA prepared 16 h after stimulation with concanavalin A, using the pCD vector of Okayama and Berg (24).

To identify and characterize the nature of the proteins encoded by the various p53 cDNA clones, we subcloned the inserts (all about the same size [1.85 kilobases]) into the pSP65 transcription vector (11). The cDNA clones obtained were linearized by digestion with *Hin*dIII, which is contained at the 3′-end polylinker site of the pSP65 transcription vector in relation to the cDNA insert. The authentic RNA transcripts generated by using the SP6 RNA polymerase were further translated in the in vitro reticulocyte lysate system (28). Figure 1 shows the p53 proteins synthesized in vitro by the various cDNA clones. In agreement with our previous in vivo studies, in which the p53-M8 was transfected into L12 cells, the p53-M8 cDNA dictated in this in vitro transcription-translation assay a p53 protein that efficiently immunoprecipitated with the RA3-2C2 and PAb200.47 anti-p53 monoclonal antibodies. However, this protein lacked the PAb421-PAb122 antigenic determinant(s) which was found in p53 encoded by p53-M11 and pCD-p53. p53-M11 and pCD-p53 clones dictated the synthesis of proteins identical in size that exhibited the same immunological epitopes. The latter proteins were indistinguishable from the p53 proteins detected in the in vivo-labeled 2M3 Ab-MuLV-transformed lymphoid cell line. In addition to variations in the content of antigenic determinants, we observed that the protein encoded by the p53-M8 clone is of smaller size than the p53 proteins encoded by the p53-M11 and pCD-p53 cDNA clones (Fig. 1).

Differences in the molecular weights of these p53 proteins encoded by the various cDNA clones and variation in their immunogenicity may point to the existence of alterations in the coding sequences. Comparison between the in vitro-synthesized proteins obtained from the individual cDNA-encoded p53 proteins and the in vivo-synthesized protein 2M3 (Fig. 1) revealed less significant size variations. It is conceivable that the in vivo-synthesized proteins represent all the p53 species which are not separable under these resolution conditions.

Mapping of variable region of cDNA clones. To localize the

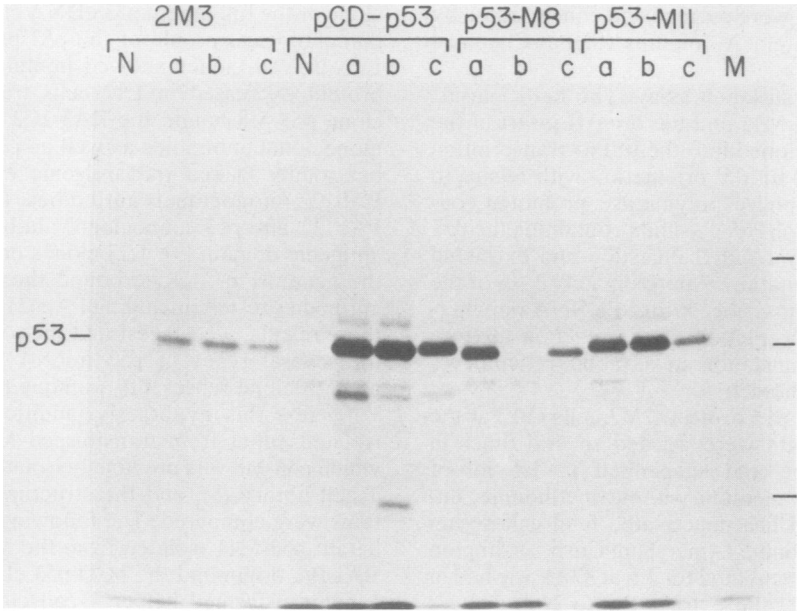

FIG. 1. p53 proteins generated by in vitro transcription and translation of various mouse p53 cDNA clones. Equal amounts of in vivo-labeled protein or in vitro-translated proteins were immunoprecipitated with either nonimmune serum (N) or each of the following anti-p53 monoclonal antibodies: (a) RA3-2C2 (7, 31); (b) PAb421 (13); (c) PAb200.47 (7); or PAb122 (data not shown). Lane M, Markers.

region responsible for the variations among these cDNA clones, we linearized the pSP65 cDNA constructs at different restriction sites within the coding and noncoding sequences. The various linearized constructs were further exposed to transcription-translation in the in vitro assay. Linearizing the cDNA clones with *Kpn*I or *Stu*I generated mRNAs that encode for identical p53 proteins by the various clones, respectively (Fig. 2). As expected, the p53 proteins encoded by the plasmids linearized with *Kpn*I were smaller than those obtained when *Stu*I was used. The difference in size was retained, however, when the pSP65-cDNA plasmids were digested with *Apa*I before transcription and translation. These analyses suggest that p53-M8 and p53-M11 vary at the 3′ end of their coding sequences bordering at the *Stu*I restriction site. This was further confirmed by the observation that a pSP65 hybrid molecule generated by connecting the 5′ half segment of p53-M11 (*Eco*RI to *Kpn*I) and the 3′ half segment of p53-M8 (*Kpn*I to *Eco*RI) clone p53-N103 dictated the synthesis of a p53 protein identical in size to that of the p53-M8 parental clone (Fig. 2E).

Comparison of the in vitro-transcribed RNA products by direct Northern analysis indicated that the RNA molecule transcribed off clone p53-M8, although coding for a smaller-size protein, was larger than that transcribed off clone p53-M11 (Fig. 3). This suggests that the protein size reduction cannot be accounted for by a simple DNA deletion in p53-M8.

Sequence analysis of the various p53 cDNA clones. To characterize in detail these structural variations, we analyzed the coding-region sequences of the different cDNA clones. Four differences were found between p53-M8 and p53-M11. Three of the four were base substitutions at nucleotide positions 395, 503, and 702 (Fig. 3). Each of these substitutions predicted an amino acid difference between the p53 protein synthesized by p53-M8 and that synthesized by p53-M11. All three changes were nonconservative. Those changes in p53-M8 and p53-M11 were: from cysteine to phenylalanine (G→T, nucleotide 395), from glycine to glutamine (A→G, nucleotide 503), and from isoleucine to methionine (G→T, nucleotide 702). However, only one base substitution was found between p53-M8 and pCD-p53 at nucleotide position 395. It should be noted that more base substitutions were found between clones p53-M8 and p53-M11, which were derived from the same library, compared with the number of substitutions found between p53-M8 and pCD-p53, which originated from different libraries.

Two alterations at positions 503 and 702 were also found between the genomic and the cDNA clones derived from different strains of mice (4). These observations imply that the polymorphism observed here is neither a reflection of strain differences nor merely a result of a cloning artifact, although the biological significance of this phenomenon remained unclear.

A major structural difference between the cDNA clones was an insertion of 96 base pairs (bp) into clone p53-M8 compared with p53-M11 and pCD-p53. This insert was located between nucleotide positions 1091 and 1092 (Fig. 4). The 96-bp insertion contained a TGA stop codon, and therefore the p53 protein encoded by p53-M8 was terminated at amino acid position 381, whereas the p53-M11 and pCD-p53 clones encoded 390 amino acids. Thus, p53 protein from p53-M8 was 9 amino acids shorter than p53 from p53-M11 and pCD-p53. This DNA modification could explain the existence of a larger-size mRNA product transcribed off clone p53-M8 which codes for a smaller-size p53 protein.

The origin of the 96 bp of inserted sequence was clarified by comparing the inserted sequence with the p53 genomic sequence. To that end we sequenced the p8R.H4 mouse p53 genomic subclone (37) which contains the 3′-end coding sequences of the mouse p53 gene. The inserted sequence found in the p53-M8 cDNA clone showed complete homol-

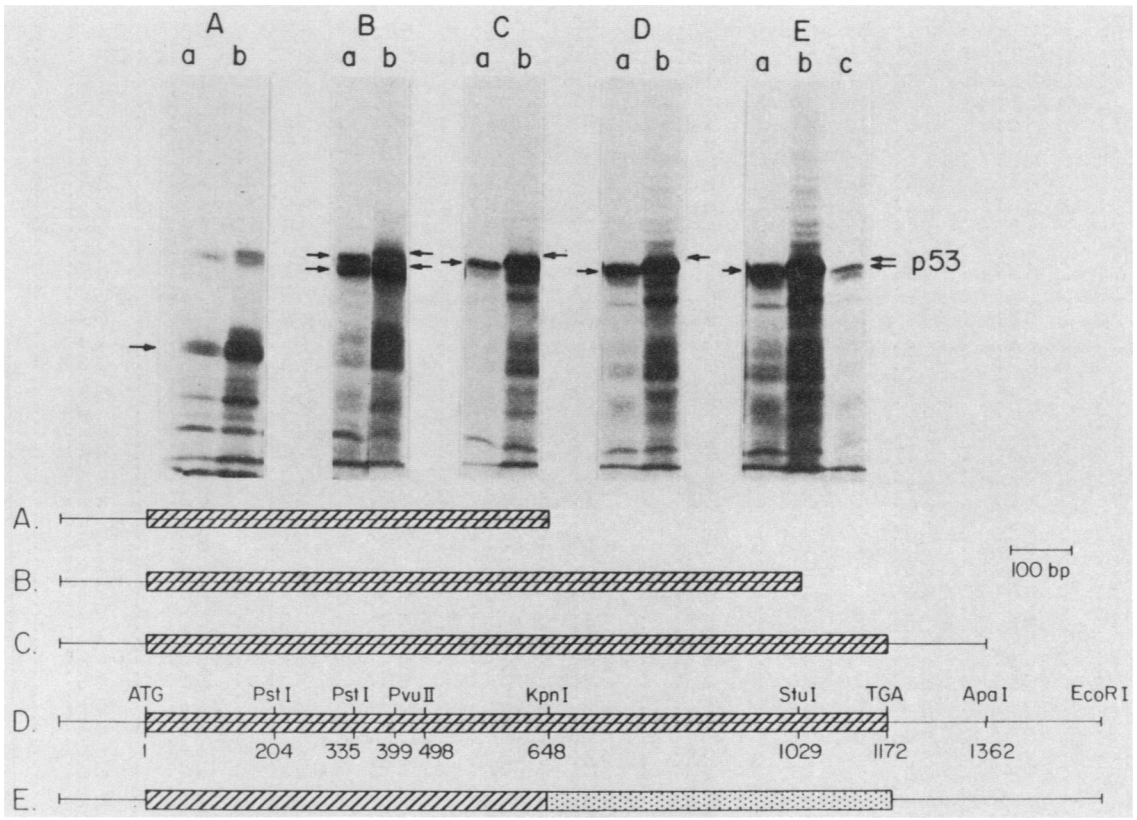

FIG. 2. p53 proteins encoded by pSP65-p53 cDNA clones linearized with different restriction enzymes. p53-M8 (a) and p53-M11 (b) cDNA plasmids were linearized with the following restriction enzymes: *Kpn*I (A); *Stu*I (B); *Apa*I (C); and *Hin*dIII (D). Specific proteins were generated by an in vitro transcription-translation assay (as described in Materials and Methods). Please note that digestion with *Stu*I and *Kpn*I was partial, and some of the unlinearized plasmid dictated the synthesis of an intact p53 protein. E represents the proteins obtained from *Hin*dIII-linearized p53-M8 (a), p53-M11 (b), and the chimeric molecule p53-N103 (c) which was constructed by ligating the 5′ *Eco*RI-*Kpn*I fragment of p53-M11 with the 3′ *Kpn*I-*Eco*RI fragment of p53-M8. Hatched boxes represent the coding sequences of the p53-M11 cDNA clone, and the dotted boxes represent the coding sequences of p53-M8.

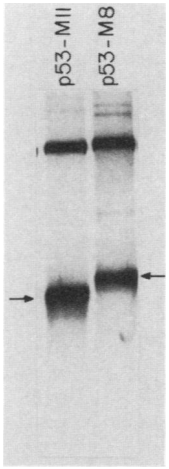

FIG. 3. Northern analysis of RNA molecules transcribed under in vitro conditions. The various cDNA clones were linearized with *Hin*dIII to generate a full-length transcript. RNA was transcribed by the specific SP6 polymerase. Equal amounts of RNA were separated through agarose gels and hybridized to the nick-translated (30) *Eco*RI insert of the mouse p53-M11 or p53-M8 cDNA clone. The arrows indicate the different RNA products obtained. The large bands represent the linearized plasmids.

ogy to 96 bp mapping at the 3′ end of intron 10 located upstream of the acceptor splicing site of exon 11 (Fig. 5).

In vivo expression of the various mRNA species. The above results strongly suggest that the mRNA molecule represented by the p53-M8 cDNA clone was generated by an alternative splicing mechanism, giving rise to a p53 protein which is of slightly reduced molecular size. In our next experiment we tested the frequency of the expression of the various p53 mRNA species in vivo. Polyadenylated selected mRNA obtained from either Meth A fibroblastic transformed cells, from which the cDNA library was established, or from 2M3/M, pre-B leukemic transformed cells, was hybridized with either the full-length cDNA insert of clone p53-H-11 or the 96-bp synthetic primer. Results (Fig. 6B) indicated that the mRNA species represented by clone p53-M8 detected the 96-bp synthetic oligonucleotide and is expressed by Meth A cells as well as by the 2M3/M cells. This indicated that the p53-M8 cDNA clone represents an authentic mRNA p53 species which is expressed in both cell lines tested, rather than reflecting a possible cloning artifact. All other in vivo-expressed mRNA species, including that represented by p53-M8, were detected with the *Eco*RI insert of clone p53-M11.

In agreement with the above-described in vitro data showing size differences between the p53 transcripts (Fig. 3), the p53-M8 corresponding mRNA expressed in vivo seemed to

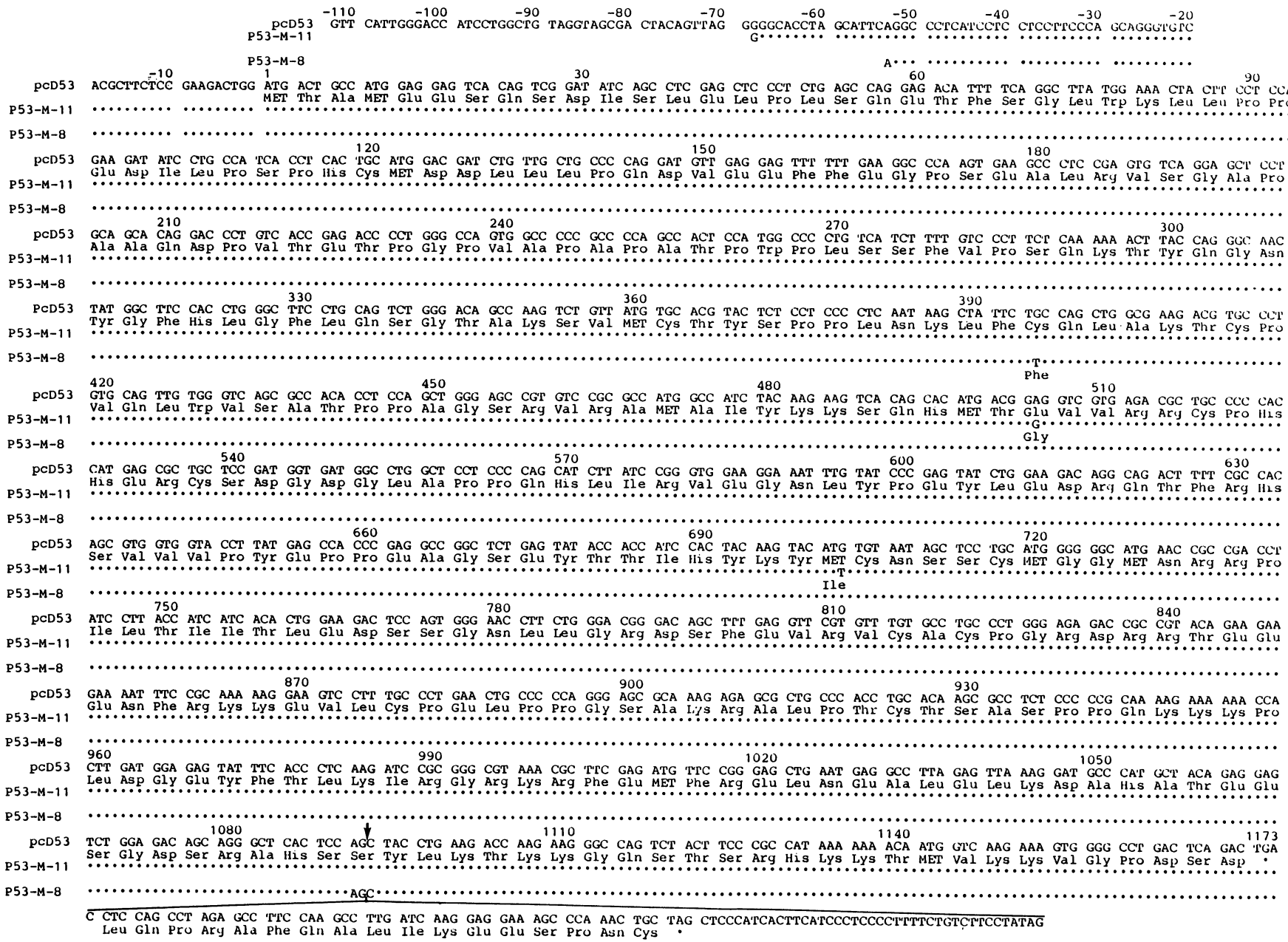

FIG. 4. Nucleotide sequence comparison of p53 cDNA clones pCD-p53, p53-M11, and p53-M8. Nucleotide 1 marks the first base of the presumed start codon ATG. Nucleotides identical to the pCD-p53 sequence are marked as dots. The site of the 96-bp insertion is indicated by a small arrow.

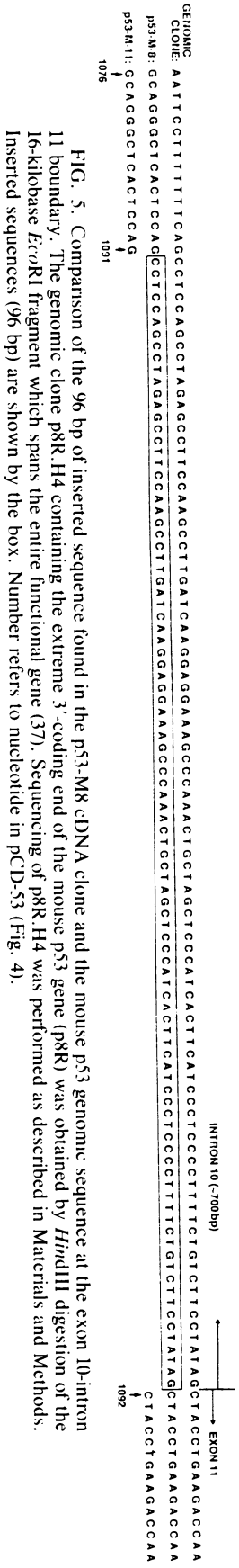

FIG. 5. Comparison of the 96 bp of inserted sequence found in the p53-M8 cDNA clone and the mouse p53 genomic sequence at the exon 10-intron 11 boundary. The genomic clone p8R.H4 containing the extreme 3'-coding end of the mouse p53 gene (p8R) was obtained by *Hin*dIII digestion of the 16-kilobase *Eco*RI fragment which spans the entire functional gene (37). Sequencing of p8R.H4 was performed as described in Materials and Methods. Inserted sequences (96 bp) are shown by the box. Number refers to nucleotide in pCD-53 (Fig. 4).

represent the larger-size molecules of the mRNA population. Indeed, the 96-bp synthetic oligonucleotide seemed to hybridize to the slower-migrating p53 mRNA molecules of the total population detected by the p53-M11 cDNA insert (Fig. 6, compare A with B).

DISCUSSION

The existence of a single functional p53 gene in mice and humans was shown by several groups (12, 18, 36–38). However, more than one biological activity was associated with the p53 protein. p53 was proven to be a functional oncogene which is involved in the induction of the malignant process (9, 10, 14, 26, 34, 35). Introduction of cloned p53 gene into nontransformed primary embryonic fibroblasts complemented the *ras* oncogenes in transforming such cells into tumor cells (10, 14, 26). Similarly, we found that cells partially transformed with Ab-MuLV acquired a completely transformed phenotype upon transfection with either genomic or cDNA p53 plasmids (34, 35).

At the same time, however, p53 was found to play a role in the cell cycle of the normal cell (19, 22, 29). This assumption was mostly based on the observations that p53 was expressed in a number of nontransformed cell types, e.g., normal mouse thymocytes (31), cells of 12 to 14-day-old embryos (23), and NIH 3T3 fibroblasts (25). Moreover, it was shown that p53 was induced in cells treated with mitogen, which is thought to start functioning early during the transition from G0 to G1. Mercer et al. (19) showed that injecting anti-p53 monoclonal antibodies into the cells inhibited DNA synthesis in quiescent nontransformed NIH 3T3

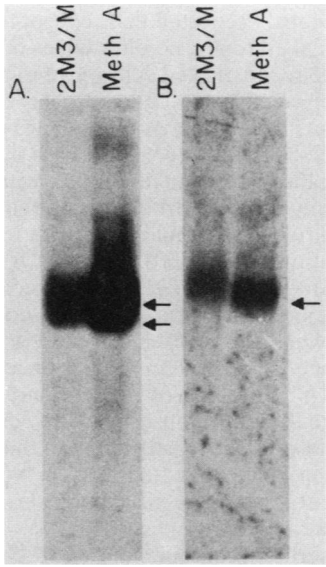

FIG. 6. In vivo expression of the various p53 mRNA species. Equal amounts of poly(A)$^+$ selected mRNA of Meth A chemically transformed fibroblasts or Ab-MuLV-transformed 2M3/M cells were separated through agarose gel electrophoresis and hybridized with the nick-translated (30) *Eco*RI fragment of p53-H-11 (A) or the 96-bp labeled synthetic oligonucleotide (B). The last probe was prepared by annealing the following synthetic single-strand DNA at 55°C for 30 min. The 5'-end primer was: CTCCAGCCTAGAGCCTTCCAA GCCTTGATCAAGGAGGAAAGCCCAAACTGCAGC, and the 3'-end primer was GCTATAGGAAGACAGAAAAGGGGAGGGAT GAAGTGATGGGAGCTAGAGGTT. The primers were labeled with the Klenow fragment and radioactive dCTP (15). The arrows indicate the different-size p53 mRNA species.

cells stimulated with serum, suggesting that p53 is synthesized as a late G0 protein (19). A similar conclusion was reached by Reich and Levine (29) examining the steady-state levels of p53 mRNA and p53 protein synthesis in a synchronous population of NIH 3T3 fibroblasts, obtained by releasing a culture from density-dependent growth inhibition.

The question raised, therefore, is whether these diverse activities of p53 are mediated by more than one p53 protein species. The existence of a heterogeneous population of p53 protein species in tumor cells was occasionally observed on two-dimensional gels. Moreover, in normal thymocytes two immunologically distinct forms of p53 which potentially represent functional molecules were observed (21). The results presented here suggest that the single mouse p53 gene is coding for several p53 mRNA transcripts each of which may be involved in a different biological activity.

The major structural difference between the cDNA coding for a larger p53 protein species, clones p53-M11 and pcD-p53, and the cDNA coding for a smaller protein, clone p53-M8, was the existence of a 96-bp insert at the 3′ end of the p53-M8 clone. This insert, which contains a premature protein termination signal, accounts for the transcription of a 96-bp-longer transcript which, nevertheless, is translated into a shorter protein.

The molecular weights of the p53 proteins encoded by p53-M8, p53-M11, and pCD-p53 are calculated to be 42,387, 43,400, and 43,347, respectively. This size difference is consistent with our observation in the pSP65 system. Indeed, the difference in migration of p53 protein encoded by p53-M8 compared with those encoded by p53-M11 and pCD-p53 on sodium dodecyl sulfate acrylamide gels could account for the calculated 9-amino acid difference representing about a 1-kilodalton difference (Fig. 1).

We have previously reported that, except for variations in the immunological epitopes, no differences in the size of p53 expressed in L12 reconstituted cells by genomic (pul8R-6) or cDNA (p53-M8) constructs could be observed (34, 35). In those experiments we compared a single p53 protein species encoded by a cDNA clone (p53-M8) with a heterogeneous p53 protein population encoded by p53 genomic reconstituted or naturally in vivo-produced protein. Under such resolution conditions, we could not make any conclusions about size variations. In the present analysis, we found that the p53-M8 encoded for a protein 9 amino acids shorter than other p53 proteins. This size difference could have been observed only when the shorter p53 protein was compared with a homogeneous p53 protein species encoded by another individual cDNA clone coding for the larger p53 protein species. The size difference observed between the various in vitro mRNA transcripts is consistent with the size difference detected in the in vivo-expressed mRNA molecules. In all, p53-M8 represents the larger-size molecules which contain the 96-bp insert.

The 96-bp insertion found in clone p53-M8 also generated a p53 protein which lacks the PAb421-PAb122 antigenic determinant(s). By preparing a series of BAL 31-deleted p53 constructs, Wade-Evans and Jenkins (33) have recently mapped the various antigenic epitopes of the murine p53 protein. They found that PAb421-PAb122 maps between nucleotides 1110 and 1155 (Fig. 5). The 96-bp insert of clone p53-M8 which is between nucleotides 1091 and 1092 gave rise, therefore, to a p53-M8-specific mRNA transcript containing a stop codon before the PAb421-PAb122-coding sequences. Therefore, the specific mRNA transcribed from the p53-M8 did not translate the PAb421-PAb122-coding sequence.

The 96-bp insert of clone p53-M8 shared complete homology with intron-specific sequences mapping 96 bp within the extreme 3′ end of intron 10 and bordering the acceptor splicing site of exon 11. This strongly suggests that the insertion was created by using an altered splicing site. The p53-M8 clone most probably used a different acceptor site which is 96 bp upstream of the acceptor site used by p53-M11 or pCD-p53. However, all three mRNA products used the common donor site. The acceptor splicing site used by p53-M8 showed agreement with the consensus sequence (5).

p53 cDNA clones which had a 96-bp insertion were found at a high frequency (40% of p53 cDNA clone isolated) in a Meth A fibroblast library, whereas none of the cDNA clones from a nontransformed helper-T-cell clone (eight cDNA clones analyzed; data not shown) hybridized with the synthetic 96-bp probe. This result suggests that some alteration was introduced in one of the p53 alleles of Meth A fibroblasts which produces a new acceptor splice site and leads to formation of a p53 RNA with an insertion of 96 nucleotides. Several examples of defective RNA splicing owing to introduction of mutations have been reported in studies of β-thalassemia (8). In this case the mutations are at IVS-2 positions, all of which create sequences that look much like the consensus sequence for a donor splicing junction.

The observation that the various p53 transcript species described here are expressed in vivo in transformed lymphoid and fibroblastic cells indicates that the various p53 cDNA clones described here represent authentic mRNA molecules that are transcribed and translated in the cells. The availability of these cloned p53 cDNAs will therefore facilitate our further experiments regarding the existence of possible variations in the biological functions of these defined p53 protein species.

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