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Abelson Murine Leukemia Virus-Transformed Cells That Lack p53 Protein Synthesis Express Aberrant p53 mRNA Species

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Cells of the Abelson murine leukemia virus-transformed line L12 that lack the p53 protein also lack polyadenylated mRNA capable of directing the synthesis of p53 in a cell-free system. Direct analysis of stable polyadenylated mRNA from a variety of cell lines shows that all p53 producers shared a common mRNA species (2.0 kilobases) which hybridized with a p53-specific cDNA probe. This species, which appears to be the mature, normal-sized p53 mRNA, was totally undetectable in L12 cells, which did not produce p53 in vivo. However, L12 cells contained two major p53-specific mRNA species of a substantially larger size (3.5 and 6.5 kilobases) than the p53-specific mRNA in the p53-producing cells. Genomic DNA analysis uncovered an apparent alteration in the 5′ proximal part of only one p53 gene, which is unique to the L12 cell line. It is thus possible that the nonproducer phenotype of L12 cells is due at least in part to an alteration within a p53-specific DNA sequence. These findings define a system in which production of p53 appears to be efficiently regulated at the level of stable mRNA and which can be used to study the mechanisms controlling p53 expression in Abelson murine leukemia virus-transformed cells.

p53 is a cell-encoded protein that is synthesized in elevated amounts by many transformed cell lines (4, 7, 10–13, 23, 24, 26) and by primary tumor cells (20). It is a phosphoprotein (7, 13, 23) capable of complexing with several viral tumor antigens (10–12, 23). The fact that it is overproduced in a wide range of tumor cells suggests that accumulation of p53 may play a role in neoplastic transformation. To elucidate the molecular mechanism controlling p53 expression, we made a comparative study of an Abelson murine leukemia virus (Ab-MuLV)-transformed lymphoid cell line lacking the p53 protein (21, 23) and of cell lines that express the protein abundantly. L12 is an Ab-MuLV-transformed cell line that expresses the virus-encoded p120 Abelson *onc* gene product (abl) but lacks the cell-encoded p53 protein (21, 23). Injection of L12 cells into syngeneic mice induces the development of local tumors which are subsequently rejected, whereas other Ab-MuLV-transformed cells, overproducing both p120 and p53, develop into lethal tumors (21, 30).

The experiments described here were aimed at elucidating the molecular mechanism controlling p53 expression in the above-mentioned transformed cell lines. It was found that the inability of L12 cells to produce p53 is due to the total absence of mature p53-specific mRNA. This altered RNA pattern may result from a structural alteration in a p53-specific gene detected in the L12 cells.

MATERIALS AND METHODS

Cell lines. The Ab-MuLV-transformed lymphoid cell lines used were 2M3/M, also containing the Moloney helper virus; 2M3, a Moloney nonproducer cell line derived from 2M3/M (both of BALB/c origin); and L12 and 230-23-8 (a gift from N. Rosenberg, Tufts University, Medford, Mass.), of C57BL/J origin. Lymphoid cells were grown in RPMI-1640 medium enriched with 10% heat-inactivated fetal calf serum (Biolab, Israel) and 2×10^{-5} M beta-mercaptoethanol. 3T3-NIH fibroblasts and Meth A chemically transformed cells of BALB/c origin were grown in RPMI medium supplemented with 10% heat-inactivated calf serum. Hybridoma cell lines RA3-2C2 (3, 23) and PAb122 (6) 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 RA3-2C2 (3, 23) and PAb122 (6) established hybridoma cell lines. Antibodies were purified and concentrated by binding to Sepharose protein A columns (Sigma Chemical Co., St. Louis, Mo.). Goat anti-Moloney virus protein antibodies were obtained from the Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Md. The latter antibodies immunoprecipitate the p120 Ab-MuLV-encoded protein as well as the Moloney virus-related product.

In vitro and in vivo labeling of proteins and their immunoprecipitations. Total RNA was prepared essentially by the method of Auffray and Rougeon (1). Polyadenylated RNA was selected by oligodeoxythymidylic acid-cellulose chromatography (2) and translated in a cell-free reticulocyte lysate system (18). For the in vivo biosynthetic labeling, 10^7 cells were washed several times in phosphate-buffered saline and suspended in 1.5 ml of Dulbecco modified Eagle medium without methionine but enriched with 10% dialyzed heat-inactivated fetal calf serum and 250 μCi of [^{35}S]methionine (purchased from the Radiochemical Centre, Amersham, United Kingdom). Cells were incubated for 1 h at 37°C, washed in phosphate-buffered saline, and extracted into 5 ml of lysis buffer (10 mM Na_2HPO_4-NaH_2PO_4 [pH 7.5], 500 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate) at 0°C. In vivo-labeled cell lysates and the in vitro-translated products were precleared by repeated absorption onto *Staphylococcus aureus* and nonimmune serum. Equal amounts of radioactive protein were immunoprecipitated with various antibodies. Antigen-antibody complexes were collected by *S. aureus* (8). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed by the Laemmli method (9).

RNA and DNA blot analysis. Samples of 5 μg of polyadenylated RNA prepared from various cell lines were heated for 10 min at 60°C in 50% formamide–6% formaldehyde and

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running buffer (20 mM MOPS [morpholinepropanesulfonic acid, pH 7.0], 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 (28) and hybridized to nick-translated (19) whole plasmid pp53-271 (see below for description of the probe; specific activity, 10^8 cpm/μg). Hybridization was for 16 h at 43°C in 50% formamide–5× SSC (SSC is 0.15 M NaCl plus 0.015 sodium citrate)–5× Denhardt solution (5)–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 0.1× SSC–1% sodium dodecyl sulfate and were autoradiographed. Samples of restriction enzyme-digested high-molecular-weight DNA (5 μg) were electrophoresed on 0.8% agarose gels, blotted onto nitrocellulose filters (27), and hybridized to nick-translated pp53-271 DNA (19). Autoradiography was performed at −70°C with an intensifier screen for 1 week.

RESULTS

In vivo and in vitro analysis of p53 mRNA. In the first experiment, we tested whether L12 cells lacking the p53 protein also lacked the specific mRNA encoding it, or whether their mRNA transcripts were translated into short-lived, unstable p53. The latter situation was observed in BALB/c-3T3 cells, which express low p53 levels but transcribe efficiently the mRNA coding for this protein (16). In these experiments we compared L12 cells that did not synthesize p53 in vivo to 230-23-8, an independently established, Ab-MuLV-transformed p53 producer cell line. The 230-23-8 cell line is a p53 producer and is of the same C57BL/J cell origin. Cells were grown in culture to log phase, and RNA was prepared (1), selected for polyadenylated mRNA (2), and translated in a cell-free reticulocyte lysate system (18). The in vitro-translated products were immunoprecipitated with the following reagents: normal serum, RA3-2C2 monoclonal anti-p53 (3, 23), PAb122 monoclonal anti-p53 (6), and goat anti-Moloney containing antibodies against the p120 Abelson virus-encoded protein as well as against Moloney helper viral products. We also labeled whole cells in vivo with [^{35}S]methionine and immunoprecipitated the cell lysates with the same antibodies. As expected, the p120 virus-encoded protein in L12 was immunoprecipitated with specific antibodies, as were Moloney-related products (Fig. 1, goat anti-Moloney, lane a'). When polyadenylated L12 mRNA was translated in vitro, no p53 or any other specific protein could be detected in the translation products (Fig. 1, anti-p53, lane a). On the other hand, in vitro translation of mRNA from 230-23-8 clearly yielded a polypeptide of approximately 53 kilodaltons immunoprecipitating with anti-p53 monoclonal antibodies (Fig. 1, anti-p53, lane b). This result demonstrates that 230-23-8 cells, which display significant levels of p53 in vivo (Fig. 1, anti-p53, lane b'), indeed contain an mRNA species capable of directing the synthesis of p53 in a cell-free system. Similar results were also obtained with several other p53-producing transformed cell lines (data not shown).

All these findings support the conjecture that the lack of p53 in L12 cells results from the absence of specific polyadenylated mRNA coding for this protein. Previously analyzed systems displayed a quantitative regulation of cellular p53 levels owing to their modulation of the amount of translatable p53 mRNA (17) or to post-translational mechanisms (16). The L12 cells represent a unique system in which p53 synthesis is completely absent, apparently owing to an absolute lack of mature p53 mRNA.

Survey of p53-specific polyadenylated mRNA. Recently, p53-specific cDNA was cloned in plasmid pBR322 (15), permitting the direct study of p53 mRNA by nucleic acid hybridization. This clone, pp53-208 (15), was used to derive additional larger p53-specific cDNA clones from a similar cDNA library (14). One such clone, pp53-271, was analyzed by the hybrid selection assay and was clearly shown to be p53 specific (14). Radioactive clone pp53-271 DNA was next used as a probe to analyze polyadenylated RNA from the above-described cell lines. We tested mRNA of the following cell lines: L12 and 230-23-8, both of C57BL/J mouse origin; 2M3, an Ab-MuLV-transformed lymphoid cell line of BALB/c mouse origin, and 2M3/M superinfected with Moloney helper virus (both of these lines are high p53 producers); Meth A, a chemically transformed fibrosarcoma of BALB/c origin, synthesizing high levels of p53 (4, 22); and NIH-3T3 (16), a low in vivo producer of this protein. Polyadenylated mRNA of each cell line was separated through a formaldehyde agarose gel, transferred to nitrocellulose paper, and hybridized with radioactive p53 cDNA probe.

The results of this experiment are illustrated in Fig. 2a. All cell lines except L12 contained a p53-specific mRNA of apparently identical size (2.0 kilobases [kb]). This species was virtually absent from the L12 cells. Instead, L12 cells displayed substantial levels of two larger polyadenylated RNA species, of approximately 6.5 and 3.5 kb, that hybridized the p53 cDNA. Very low amounts of a 6.5-kb mRNA were also detected in all p53 producers. This product may represent a precursor mRNA molecule, which is not unexpected since total (nuclear and cytoplasmic) RNA was used in these experiments. It is unclear whether this putative

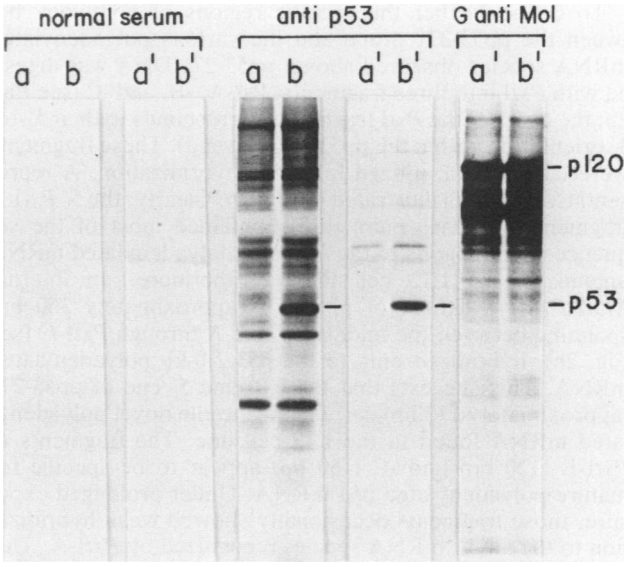

FIG. 1. The presence of polyadenylated p53 mRNA in Ab-MuLV-transformed lymphoid cells and its absence in L12 cells. Each sample of the in vivo-labeled (lanes a', b') or the in vitro-translated (lanes a, b) products was immunoprecipitated with the following antibodies: normal serum; anti-53 monoclonal antibodies containing a mixture of RA3-2C2 (3, 22) and PAb122 (6); or goat anti-Moloney (G anti Mol) containing anti-p120 antibodies as well as anti-Moloney-related products.

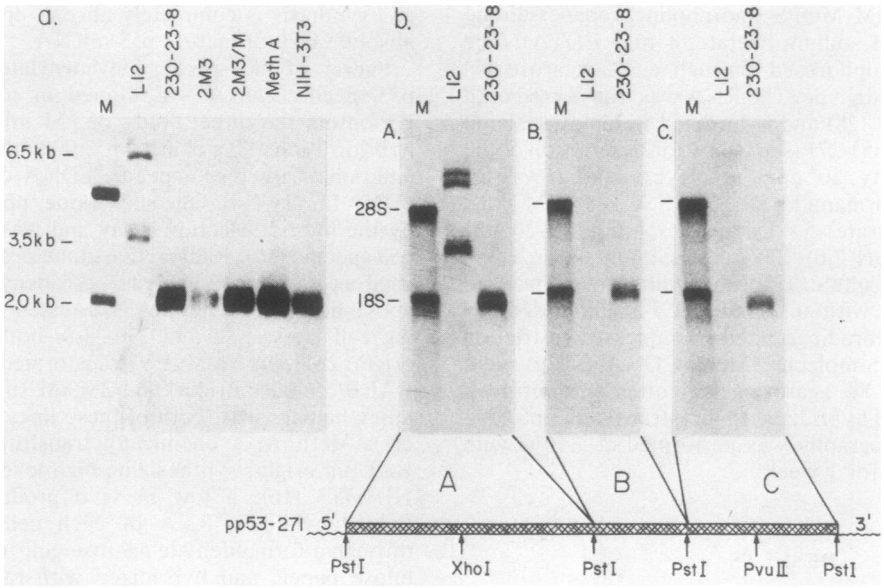

FIG. 2. Analysis of polyadenylated mRNA from various cellular sources for p53-specific sequences. Samples (5 μg) of polyadenylated RNA from the cell lines indicated in the figure were electrophoresed through a 1% agarose gel containing 6% formaldehyde. The RNA was transferred onto a nitrocellulose sheet (28) and hybridized to (a) nick-translated (19) whole plasmid pp53-271 or (b) a restriction enzyme-digested fragment nick translated to a specific activity of 10^8 cpm/μg. *Pst*I restriction enzyme fragments were isolated by agarose gel electrophoresis and purified by DEAE chromatography. Hybridization was for 16 h at 43°C in 50% formamide–5× SSC–5× Denhardt solution (5)–20 mM sodium phosphate (pH 7.0)–100 μg of salmon sperm DNA per ml–10% dextran sulfate. Hybridized filters were washed at 60°C with 0.1× SSC–0.1% sodium dodecyl sulfate and were autoradiographed. ^{32}P-labeled 18S and 28S ribosomal RNA were used as molecular weight markers.

precursor is identical to the major 6.5-kb RNA found in L12 cells. The 3.5-kb mRNA hybridizing with the p53 cDNA probe was unique to the L12 cell line and was not detected in other transformed cells studied here.

To define further the specific regions of homology between the pp53-271 probe and the various polyadenylated mRNA species observed above, pp53-271 DNA was digested with *Pst*I into three fragments, Pst-A, -B, and -C (see Fig. 2b; the order of the *Pst*I fragments corresponds to their 5′-to-3′ orientation within the pp53-271 plasmid). These fragments were labeled and utilized for RNA hybridization. A representative result is illustrated in Fig. 2b. Clearly, the 5′ *Pst*I A fragment (320 base pairs [bp]) contained most of the sequences homologous to the aberrant polyadenylated mRNA unique to the L12 cell line. Furthermore, an internal *Xho*I-*Pvu*II fragment of pp53-271 (approximately 350 bp) spanning between the middle of *Pst*I-A through *Pst*I-C (see Fig. 2b), hybridized only to the p53 2.0-kb polyadenylated mRNA. This suggests that the extreme 5′ end of pp53-271 (approximately 130 bp) corresponds to the novel polyadenylated mRNA found in the L12 cell line. The fragments of *Pst*I-B (120 bp) and -C (160 bp) appear to be specific for mature polyadenylated p53 mRNA. Under prolonged exposure, those fragments occasionally showed weak hybridization to the larger mRNA species recognized by *Pst*I-A. This is probably due to a contamination of fragments B and C with small amounts of fragment A.

Primer extension assay of mature p53 mRNA. The observation that clone pp53-271 contains at least two discrete regions of homology to the various p53 polyadenylated mRNA species necessitated the localization of this cDNA clone relative to the mature mRNA. This was performed by a primer extension assay. Fragments *Pst*I-B and -C, but not *Pst*I-A, were selected for primer extension because of the guanine-cytosine tails created by the particular cloning technique employed (14).

The selected fragments were labeled at their 3′ ends with the Klenow fragment of *Escherichia coli* polymerase I and were strand separated, and both strands were initially utilized independently for RNA hybridization followed by cDNA extension with unlabeled deoxynucleoside triphosphates by reverse transcriptase (29). Total polyadenylated mRNA used for the assay was obtained from Meth A, L12, and 2M3/M cells. Only one strand of each fragment was an effective primer in this assay, establishing the identity of the sense and antisense strands. The representative results of primer extension employing the antisense strands of fragments *Pst*I-B and -C are shown in Fig. 3. Primer extension products generated by both 3′-end-labeled antisense strands from fragments *Pst*I-B and -C indicated the same major 5′ stop in both Meth A and 2M3/M cell lines. Fragment *Pst*I-B yielded a final extension product of approximately 440 bp (Fig. 3b). Fragment *Pst*I-C in turn yielded a terminal extension product of approximately 600 bp (Fig. 3c). In accordance with our previous hybridization results (Fig. 2a, 2b), neither fragment produced primer extension products from L12 total polyadenylated mRNA. The products generated by L12 RNA were found to be similar to those obtained in the control, in which no external total polyadenylated mRNA was added (Fig. 3; compare lane L12 to control lane).

Taking into account a guanine-cytosine tail length of roughly 30 bp at the 5′ and 3′ ends of clone pp53-271 (14), it can be calculated that the final extension product extends to approximately 40 bp 5′ to the end of the *Pst*I A fragment. This suggests that clone pp53-271 is derived from the 5′-proximal region of the p53 mRNA. This is also in agreement

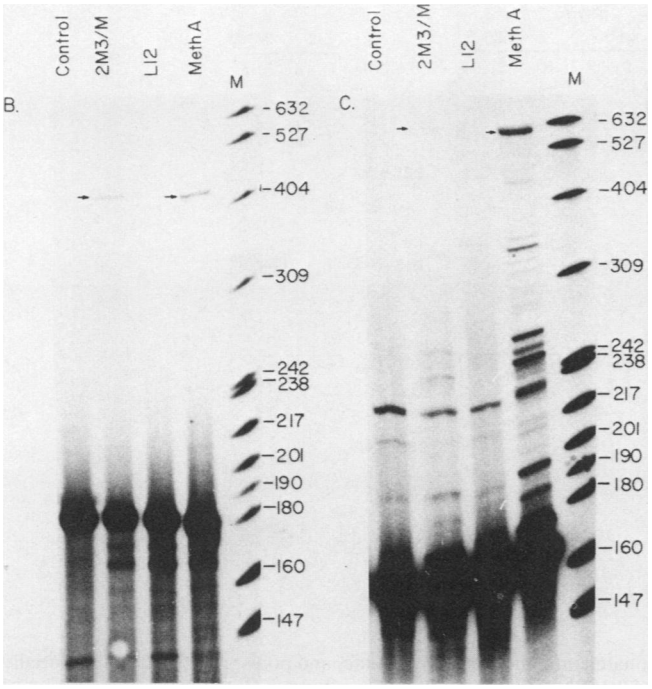

FIG. 3. Primer extension analysis of total polyadenylated RNA from p53 producer cell lines. The *Pst*I B and C fragments of clone pp53-271 were 3′ end labeled with the large fragment of DNA polymerase (Klenow) and were strand separated on a 5% polyacrylamide gel. In each experiment, 20 μg of polyadenylated mRNA was used. The cDNA extension products were fractionated on a 6% polyacrylamide–8 M urea gel. The 5′-end-labeled *Hpa*II fragments of plasmid pBR322 were used as size markers as indicated. The primer extension cDNA products generated by the antisense strands of *Pst*I-B (B) and *Pst*I-C (C) are shown. Hybridization conditions and reverse transcriptase cDNA primer extensions were performed essentially as described (29).

with the results obtained from the study of a series of cDNA clones covering a total of 1,700 bp of p53 mRNA (14).

Subcellular localization of p53 mRNA species. The results of the previously described experiments indicate a major difference in p53-specific mRNA populations between producer and nonproducer cells. To understand further the potential mechanisms governing the production and expression of p53 mRNA, we undertook a comparative cellular localization study of the p53 RNA.

Cells were fractionated into nuclear, cytoplasmic, and polysomal fractions (26), and polyadenylated and non-polyadenylated RNA was isolated and fractionated on formaldehyde agarose accordingly (1, 2). The fractionation conditions utilized were adjusted to minimize the possibility of leakage and contamination between the subcellular compartments. The p53-specific probes utilized were the *Pst*I-A (5′ specific), *Pst*I-C, and *Xho*I-*Pvu*II (3′ specific) fragments of clone pp53-271 (see Fig. 2b). Figure 4 illustrates results obtained when RNA was probed with *Pst*I-A (Fig. 4A.5′) and the *Xho*I-*Pvu*II fragment (Fig. 4C.3′). Similar results were obtained when *Pst*I-C replaced the *Xho*I-*Pvu*II fragment used as mature p53-specific probe.

The mature polyadenylated p53-specific mRNA, demonstrating a heterogeneous band ranging in size between 1.7 and 2.2 kb, was found predominantly in the polysomal fraction. The 3.5-kb polyadenylated mRNA of L12 was as prevalent in the polysomal fraction. Apparently, both polyadenylated RNA species are efficiently transported to the polysomes from the nucleus. The larger 6.5- and 7.5-kb polyadenylated species were most abundant in the nuclear fraction, but small amounts were also detected in the cytoplasmic and polysomal fractions. The cytoplasmic and polysomal non-polyadenylated RNAs demonstrated a discrete 1.7-kb p53-specific band. The non-polyadenylated 3.5-kb species of L12 cells was found predominantly in the cytoplasm. The nuclear non-polyadenylated fraction demonstrated similar heterogeneity in the two cell lines. The observation that the *Xho*I-*Pvu*II mature p53-specific mRNA probe hybridized to a heterogeneous non-polyadenylated nuclear RNA population in L12 cell line suggests that abnormal processing might play a role in the lack of mature polyadenylated p53 RNA. Furthermore, immunoprecipitation of polysomes from several cell lines (2M3, 2M3/M, Meth A, and 230-23-8) by anti-p53 monoclonal antibodies RA3-2C2 or PAb122 resulted in an enriched RNA population consisting of mature polyadenylated p53 mRNA and notably larger polyadenylated mRNAs, preferentially of 6.5 to 7.5 kb. Precipitation of polysomes from the p53 nonproducer L12 cell line only demonstrated the 6.5- and 7.5-kb species. Neither the 3.5-kb polyadenylated RNA species nor a normal 2.0-kb polyadenylated p53 mRNA of the L12 cell line was detected by these methods. A representative RNA hybridization of immunoprecipitated polysomes from L12 and 2M3/M cells is shown in Fig. 4A.5′ and C.3′ (lanes i.p.).

Altered p53 genomic DNA pattern in L12 nonproducer cells. In an attempt to elucidate further the complex molecular mechanisms responsible for the altered p53 mRNA patterns observed, an analysis of the genomic DNA fragments hybridizing to the p53-specific probes was performed. DNA from a variety of cell lines was digested with several restriction enzymes, electrophoresed on agarose gels, transferred to nitrocellulose, and hybridized to nick-translated clone pp53-271 DNA. These results are summarized in Fig. 5I and Table 1. Examination of the *Eco*RI digests reveals that all p53-producing cell lines (of both BALB/c and C57BL/J genetic origin) displayed the same 3.3- and 16-kb *Eco*RI fragments; this is in agreement with the previous observation (14). However, nonproducer L12 cells contain an apparently identical 3.3-kb band and a larger fragment (approximately 23.0 kb) hybridizing with the p53-specific probes. The fact that *Eco*RI, *Hin*dIII, and *Bam*HI do not cut within clone pp53-271 (14) implies that the genomic region related to the cDNA is duplicated or represents two independent p53 related genes. The different size of the *Eco*RI fragment is not due to strain-specific restriction site polymorphism since 230-23-8 cells of the same C57BL/J genetic background display the usual 16-kb band. To characterize in more detail the alteration in L12 DNA, extensive restriction enzyme digestion and double digestion of genomic DNA from p53 producer and nonproducer cell lines was performed with fragments *Pst*I-A, *Xho*I-*Pvu*II, and *Pst*I-C of pp53-271 as probes. The results indicate the existence of two noncontiguous p53 genes, one contained within the 3.3-kb *Eco*RI fragment and the second within the 16-kb *Eco*RI fragment. We mapped approximately 20 kb around the 3.3-kb *Eco*RI fragment, which does not overlap the 16-kb fragment, and found no variations between the p53 producer and nonproducer cells. Employing fragment *Pst*I-A, derived from the 5′ end of the mRNA (see above), we found an altered restriction enzyme pattern in the 5′ region of the p53 *Eco*RI 23.0-kb genomic DNA segment in L12 cells (Fig. 5II A.5′). Howev-

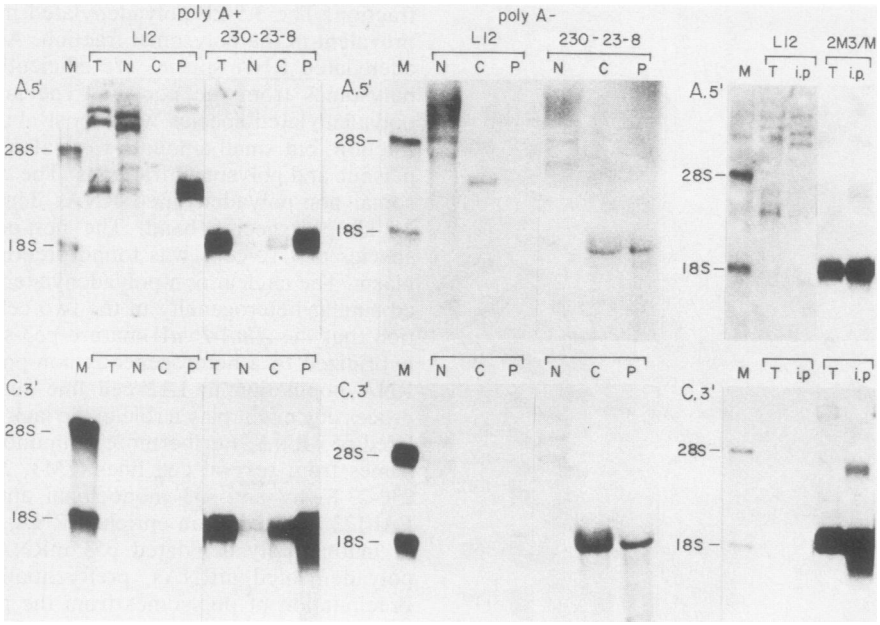

FIG. 4. Subcellular localization of p53 RNA species. Cells were fractionated into nuclear, cytoplasmic, and polysomal fractions essentially as described (25). Cells were lysed by hypotonic shock in the presence of 0.1% Nonidet P-40. Polysomes were isolated through a cushion of 65% sucrose. RNA was isolated from washed nuclei by LiCl-urea (1). The cytoplasmic and washed polysomal RNA was prepared by phenol extraction. Polyadenylated (poly A^+) RNA was isolated by repetitive oligodeoxythymidylic acid column chromatography (2). Immunoprecipitation of polysomes was performed as previously described (25). The washed polysomal anti-p53 RA3-2C2 monoclonal antibody complex was isolated by protein A-Sepharose. RNA was phenol extracted and purified by oligodeoxythymidylic acid chromatography. Five micrograms of polyadenylated RNA, 40 μg of non-polyadenylated (poly A^-) RNA, and an estimated 5 ng of immunoprecipitated RNA were fractionated on 1.2% formaldehyde agarose gels. The probes utilized for hybridization were *Pst*I-A and *Pst*I-C nick translated to a specific activity of 10^8 cpm/μg as described in the legend to Fig. 2. The following RNA fractions were included: T, total; N, nuclear; C, cytoplasmic; P, polysomal; i.p., immunoprecipitated polysomes.

er, no difference was observed between the p53 gene contained in the 23-kb *Eco*RI fragment of the L12 cell line and that in the 16-kb *Eco*RI fragment of the p53 producer cell line hybridizing with the *Xho*I-*Pvu*II or *Pst*I-C probes (Fig. 5II C.3').

These data suggest that the inability of L12 cells to produce mature mRNA may be related to a change at the 5'-proximal part of the p53-specific gene contained in the 23-kb *Eco*RI fragment. No variations were detected in the p53-specific gene contained in the 3.3-kb *Eco*RI fragment.

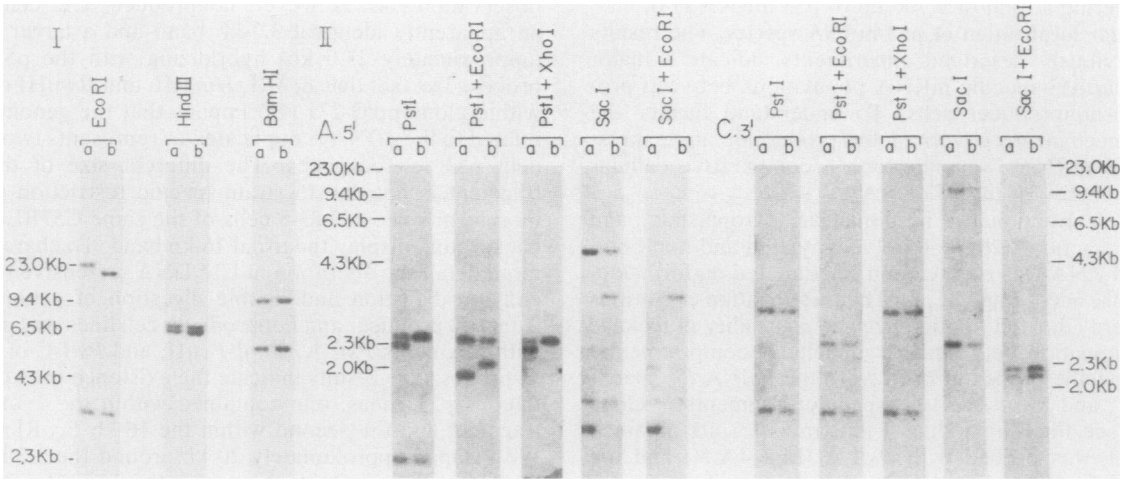

FIG. 5. Southern blot analysis of p53-specific sequences in Ab-MuLV-transformed mouse lymphoid cells. High-molecular-weight DNA from Ab-MuLV-transformed mouse lymphoid cell lines L12 (lanes a) and 230-23-8 (lanes b) was digested and double digested with several restriction enzymes described in Table 1. Samples (5 μg) were electrophoresed on 0.8% agarose gels, blotted onto nitrocellulose filters, and hybridized with (I) the whole clone pp53-271 DNA and (II) *Pst*I A or C fragments. The *Hin*dIII fragments of lambda DNA were used as molecular weight markers.

TABLE 1. Size distribution in various transformed cell lines of restriction fragments hybridized with pp53-271 DNA[a]

Cell line	p53 producer	Genetic origin	Sizes of restriction fragments (kb)				
			*Eco*RI	*Bam*HI	*Hin*dIII	*Sac*I	*Pst*I
2M3	+	BALB/c	16.0, 3.3	10.5, 6.5	7.5, 7.25		
2M3/M	+	BALB/c	16.0, 3.3	10.5, 6.5	7.5, 7.25	14, 5.5, 4.0, 2.5, 1.8, 0.6	3.5, 3.0, 2.8, 1.5, 1.0, 0.35
Meth A	+	BALB/c	16.0, 3.3	10.5, 6.5	7.5, 7.25	14, 5.5, 4.0, 2.5, 1.8, 0.6	3.5, 3.0, 2.8, 1.5, 1.0, 0.35
230-23-8	+	C57BL/J	16.0, 3.3	10.5, 6.5	7.5, 7.25	14, 5.5, 4.0, 2.5, 1.8, 0.6	3.5, 3.0, 2.8, 1.5, 1.0, 0.35
L12	−	C57BL/J	23.0, 3.3	10.5, 6.5	7.5, 7.25	14, 5.5, 2.5, 1.8, 1.5, 0.6	3.5, 3.0, 2.3, 1.5, 1.0, 0.35

[a] Southern blot analysis was performed as described in the legend to Fig. 5. Cell lines 2M3, 2M3/M, 230-23-8, and L12 are of pre-B lymphoblastoid origin. Meth A is of a methylcholanthrene-transformed fibroblastoid origin. Values represent the approximate size (in kb) of the hybridizing DNA fragments generated with respective restriction enzymes. The fragment sizes were calculated by comparing their relative migrations with that of *Hin*dIII-digested lambda phage DNA coelectrophoresed on the same gel.

DISCUSSION

The present results define a unique system in which p53 production is controlled by the presence or absolute absence of the corresponding mature 2.0-kb mRNA. All p53 producer cell lines, including the nontransformed NIH-3T3 fibroblasts, contain a 2.0-kb mRNA consisting of the mature p53 coding species. L12 cells, which are devoid of p53 protein, fail to produce this 2.0-kb mRNA but exhibit two major p53-specific mRNA species, of 3.5 and 6.5 kb. The aberrant polyadenylated p53 mRNA species produced in the L12 cell line is homologous only in part to the normal mature polyadenylated p53 mRNA. Primer extension assay has shown that the pp53-271 cDNA used in the present experiments maps to the 5′-proximal part of the mRNA. By means of the cDNA clone pp53-271, the homologous region was located at the 5′ end of the normal p53 mRNA.

Our conclusions are complicated by the presence of at least two potential noncontiguous p53 genes, one of which is contained in a 3.3-kb *Eco*RI fragment and appears to be a processed pseudogene (14; Zakut et al., in press) and the second of which is contained in a 16-kb *Eco*RI fragment and is most likely the principal active gene (14). Analysis of p53 genomic sequences in L12 cells implies an apparent reorganization in only the p53-related gene contained in the 23-kb *Eco*RI fragment. Detailed genomic analysis with defined fragments of pp53-271 indicates that the rearrangement of a p53 gene in the nonproducer L12 cell line maps to its 5′ region (see Fig. 5II). At present, however, we cannot conclude that this significant rearrangement at the 5′ end of one of the p53 genes can solely account for the origin of the additional 7 kb found in the L12 *Eco*RI 23-kb fragment (Fig. 5I).

The lack of the 16.0-kb p53-specific *Eco*RI fragment in L12 cells inplies that the normal homolog is not present in these cells. Therefore, in addition to the rearrangement in one of the p53 genes, those cells must have undergone a chromosomal event resulting in the deletion of normal p53-specific sequences. Alternatively, it is possible that an identical rearrangement has taken place in the two homologous chromosomes in this cell line, which was derived from an inbred mouse strain. We recently mapped the p53 gene to chromosome 11 (V. Rotter, D. Wolf, D. Pravtcheva, and F. H. Ruddle, Cell. Mol. Biol, in press), and we are presently testing the possibility that either L12 cells have lost one homolog of chromosome 11 or that these chromosomes have undergone structural changes.

Overall, our present working hypothesis is that the 16-kb *Eco*RI fragment represents the principal active p53 gene. Since this gene is structurally altered in L12 cells, it is conceivable that this alteration is the principal cause of the aberrant nonproductive p53 mRNA in those cells. The DNA alterations may result in the synthesis of a markedly different primary transcript from the p53 gene. Alternatively, the primary transcript may be relatively similar to the normal one, with novel processing patterns primarily accounting for the final aberrant polyadenylated mRNAs of L12. The initial site of novel processing lies most likely in the 5′ coding region of the p53 mRNA near the *Xho*I site of clone pp53-271, probably constituting an exon-intron junction. The rearrangement found to be in the 23-kb *Eco*RI fragment of L12 cells in relation to the unaltered 16.0-kb *Eco*RI fragment of the p53 producer cell line 230-23-8 has prompted their cloning for closer scrutiny. We have isolated the normal 16.0-kb *Eco*RI fragment of the 230-23-8 cell line and are presently mapping the various mRNA species to the relevant genomic fragments.

In summary, we have analyzed the Ab-Mulv-transformed variant cell line L12, which expresses a functional oncogene product p120 but lacks detectable concentrations of the cell-encoded p53. It is conceivable that the apparent absence of detectable mature p53 mRNA in the L12 cells is because the principal active p53 gene is altered. We are presently trying to introduce an active p53 gene into L12 cells and to test whether expression of p53 will change the phenotype of these cells, which develop rejectable tumors in C57BL/J mice to cells which are lethal for syngeneic hosts.

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