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Major deletions in the gene encoding the p53 tumor antigen cause lack of p53 expression in HL-60 cells

(human p53 cDNA/c-*myc* amplification)

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ABSTRACT **The tumor antigen p53 is overproduced in transformed cells of various species, including man. HL-60 is an exceptional human tumor cell line that does not express this protein. Hybridization of polyadenylylated mRNA of these cells with a human p53 cDNA probe (p53-H14), which we cloned, had indicated a total absence of the mature-size (3.0 kilobases) or any aberrant p53 mRNA species. Analysis of the genomic HL-60 DNA indicated that the p53 gene in these cells was significantly altered. Most of the gene was deleted, and the residual p53 sequences of these cells, which show weak homology, mapped to the corresponding 5′ region of the p53 gene. In agreement with previously documented results, we found that HL-60 cells have an amplified c-*myc* gene. We suggest that the deficiency of the p53 protein in HL-60 cells could have been overcome by using an alternative metabolic pathway. The c-*myc* product is a candidate for such an alternative protein.**

The cell-encoded tumor antigen p53 is found at elevated levels in tumor cells of different tissue types and species (1–5). Similar-sized proteins were detected in mouse, rat, hamster, and human cells (2, 6–8), suggesting that this protein is an essential conserved constituent of the cell.

Our approach in understanding the role of p53 in the process of malignant cell transformation consists of comparative studies between p53 producer cells and nonproducer variants. Previously we have described L12, a cell line transformed by Abelson murine leukemia virus (Ab-MuLV) that expresses a functional *abl* oncogene but lacks detectable levels of p53 (9–12). The p53 gene in L12 cells was inactivated by the integration of a Moloney murine leukemia virus (Mo-MuLV) into the first p53 intron (11). We have demonstrated that introduction of a functional p53 gene into L12 cells had changed their malignant phenotype from cells that develop into regressing tumors in syngeneic mice into cells that develop into lethal tumors (12). This suggested that acquisition of a fully transformed phenotype exhibited as development of lethal tumors in mice requires the expression of both p120, a product of the *abl* cytoplasmic oncogene, and p53, a transformation-related nuclear protein.

The complementation between the activity of a cytoplasmic oncogene and p53 was recently tested in a more direct assay. It was found that cotransfection of the p53 gene and the *ras* oncogene into rat primary embryonic fibroblasts caused the appearance of transformed foci, and thus p53 was classified as a member of the nuclear oncogene family (46, 47).

To further elucidate the molecular mechanism controlling p53 expression and function, it became important to expand our comparative study between p53 producer and nonproducer cells and thus explore for additional examples of p53 variants.

Analysis of human transformed cells had indicated the presence of appreciable p53 levels in a large number of cell lines (6). We found, however, that HL-60 is an exceptional human cell line that does not express detectable p53 protein and the specific mRNA. Analysis of genomic DNA sequences, using a human p53 cDNA probe, which we cloned, demonstrated that the p53 gene in the HL-60 underwent major deletions.

We suggest that the deficiency of p53 synthesis in HL-60 was overcome by using an alternative metabolic pathway for the cell cycle. One candidate is the product of c-*myc*, whose expression is amplified in the HL-60 cells (13, 14).

MATERIALS AND METHODS

Cell Lines. The Ab-MuLV-transformed cell line used was 230-23-8, of C57L/J mouse origin. The following human cell lines were tested: oat cells, a tissue-culture-established lung carcinoma, and HCT, a tissue-culture-established colon carcinoma (a gift of A. Fink at the Kaplan Hospital, Rehovot, Israel). SV-80 is a human transformed fibroblastic cell line expressing simian virus 40 (SV40), a DNA tumor virus (15). HL-60 is a human promyelocytic leukemic cell line (13, 14). Cells were grown in RPMI 1640 medium enriched with 10% heat-inactivated fetal calf serum (Biolab, Israel) and 20 μM 2-mercaptoethanol. 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 2 mM sodium pyruvate.

Antibodies. Monoclonal anti-p53 antibodies were obtained from the established hybridoma cell lines RA3-2C2 (16, 17), PAb122 (18), and PAb421 (19). Antibodies were purified and concentrated by binding to columns of staphylococcal protein A linked to Sepharose (Sigma). Monoclonal antibodies were obtained either from supernatants of the hybridoma cell lines or from ascitic fluid of syngeneic mice injected intraperitoneally with the hybridoma cell lines.

Synthesis of p53. For each individual cell line, 10^7 lymphoid cells or 1 plate of fibroblasts at a logarithmic stage of growth was washed several times in phosphate-buffered saline and resuspended in 1.5 ml of Dulbecco's modified Eagle's medium without methionine, enriched with 10% dialyzed heat-inactivated fetal calf serum and 250 μCi (1 Ci = 37 GBq) of [^{35}S]methionine (Amersham, England). Cells were incubated for 1–5 hr 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% $NaDodSO_4$) at 4°C. Labeled cell lysates were cleared by repeated absorption on *Staphylococcus aureus* and nonimmune serum. Equal amounts of radioactive protein were immunoprecipitated with specific antibodies. Antigen–antibody complexes were

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Abbreviations: Ab-MuLV and Mo-MuLV, Abelson and Moloney strains of murine leukemia virus; SV40, simian virus 40; bp, base pair(s); kb, kilobase(s).

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collected by *S. aureus* (20). $NaDodSO_4$/polyacrylamide gel electrophoresis was performed according to Laemmli (21).

Construction of a Human SV-80 λgT10 cDNA Library. Total polyadenylylated mRNA was denatured with 1 mM methylmercuric hydroxide prior to cDNA synthesis (22). The cDNA was synthesized essentially as described (23, 24). The first strand of cDNA was synthesized with 4 μg of denatured polyadenylylated mRNA as a template. The second cDNA strand was synthesized by a mixture of RNase H, *Escherichia coli* DNA ligase, and *E. coli* DNA polymerase. The double-stranded cDNA was treated with the Klenow fragment of *E. coli* DNA polymerase prior to methylation and *Eco*RI linker addition. Removal of excess linkers and size selection for cDNA molecules greater than 500 base pairs (bp) by agarose gel electrophoresis was performed as described (22). The cDNA was ligated to the *Eco*RI site of λgT10 and packaged by utilizing the Amersham λ *in vitro* packaging extracts (25). Recombinant phages were selected by plating onto the *E. coli* C600 *hflA* strain (26). The cloning efficiency averaged 4 × 10^5 plaques per μg of λgT10. The *Pst* I/*Kpn* I insert of p8R.4 mouse p53 genomic fragment containing the two 3′ most extreme exons was used for screening (11). Hybridizing plaques were purified and small-scale preparations were analyzed by restriction enzyme digestion. cDNA inserts were subcloned in the *Eco*RI site of puc13.

Southern Blot Analysis. Genomic hybridization DNA was prepared from individual cell lines. Cells were washed twice with phosphate-buffered saline, resuspended in lysis buffer [0.5% $NaDodSO_4$/50 mM Tris·HCl/5 mM EDTA, pH 7.5, containing proteinase K (Boehringer Mannheim) at 0.2 mg/ml] and incubated for 14 hr at 37°C. The solution was extracted twice with an equal volume of redistilled phenol and then extracted twice with an equal volume of chloroform/isoamyl alcohol (24:1, vol/vol). The DNA solution was adjusted to a final concentration of 0.3 M NaCl and 2 vol of ethanol was added. The DNA precipitate formed at room temperature was washed twice with 80% ethanol and suspended in 10 mM Tris·HCl/1 mM EDTA, pH 7.5. Aliquots of restriction enzyme-digested high molecular weight DNA (5 μg) were electrophoresed on a 0.8% agarose gel, blotted onto a nitrocellulose filter (27), and hybridized to nick-translated (28) DNA probes.

RNA Blot Analysis. RNA was prepared according to Auffray and Rougen (29) and selected for polyadenylylated molecules by oligo(dT)-cellulose chromatography (30). Aliquots (5 μg) of polyadenylylated RNA prepared from various cell lines were heated for 10 min at 60°C in 50% formamide/6% formaldehyde (vol/vol) and running buffer (20 mM 4-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 (31) and hybridized to nick-translated DNA probes (28). Hybridization was for 16 hr at 43°C in 50% formamide/750 mM NaCl/75 mM sodium citrate/5× Denhardt's solution (32)/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% $NaDodSO_4$ and autoradiographed.

RESULTS

Lack of p53 Protein Synthesis in the HL-60 Cell Line. Structural analysis of p53 of various genetic origins has indicated that this protein is basically conserved (2, 6, 7), and thus p53 genes are expected to be homologous. Variation in the antigenic determinants bound by p53-specific monoclonal antibodies were, however, observed. For example, the determinant bound by the RA3-2C2 monoclonal antibody seems to be mouse specific and was not detected in other species tested (8).

Using a panel of specific monoclonal antibodies, we have screened a large number of human transformed cell lines for expression of p53. Fig. 1 illustrates several human cell lysates immunoprecipitated with specific anti-p53 monoclonal antibodies. Human cell lines express a p53 with an apparent molecular weight slightly larger than that of the protein expressed in transformed mouse cells. Apart from the SV-80 transformed fibroblasts, which exhibit two major p53 species, most of the cell lines tested expressed a single band of p53. In the SV-80 cells we have also detected the large T (tumor) antigen that is complexed with the p53 molecule. It appears that in this human cell line the complex T-p53 antigen is less stable than that observed in the mouse.

The HL-60 cells represent an exceptional category of human cells that seem to lack any p53 product immunoprecipitating with either the human-specific anti-p53 monoclonal antibodies (PAb421 and PAb122) (Fig. 1) or the polyclonal anti-p53 antibodies (data not shown). The fact that we could not detect p53 in these cells when labeled at different time periods, starting with a 5-min pulse up to a 24-hr pulse, strongly suggests that these cells totally lack the synthesis of p53 rather than produce this molecule with different kinetics.

Isolation of Human p53 cDNA Clones. To analyze the molecular mechanism responsible for the lack of p53 expression in HL-60 cells, it became important to use established human cDNA probes. The characterization of the p53 mRNA and the detailed analysis of the p53 gene in these cells required the availability of defined human cDNA probes.

Genomic hybridization of p53 sequences of various species, including rat, hamster, and human, indicated homology to mouse-specific probes. This was the case when either a p53 cDNA (33) or a cloned genomic fragment (p8R.4) (11) of mouse origin was tested (see Fig. 2 *A* and *B*, respectively). However, marked variations in the p53 gene structure and

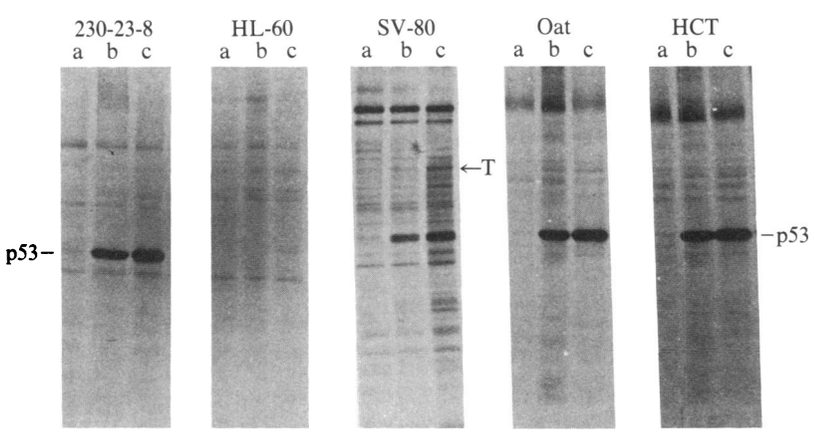

FIG. 1. Detection of p53 protein synthesis in human transformed cell lines. A kinetic study was performed to determine the optimal time period for metabolic p53 labeling for each individual cell line. Equal amounts of [^{35}S]methionine-labeled cell lysates were immunoprecipitated with nonimmune serum (lanes a), PAb122 (lanes b), or PAb421 (lanes c).

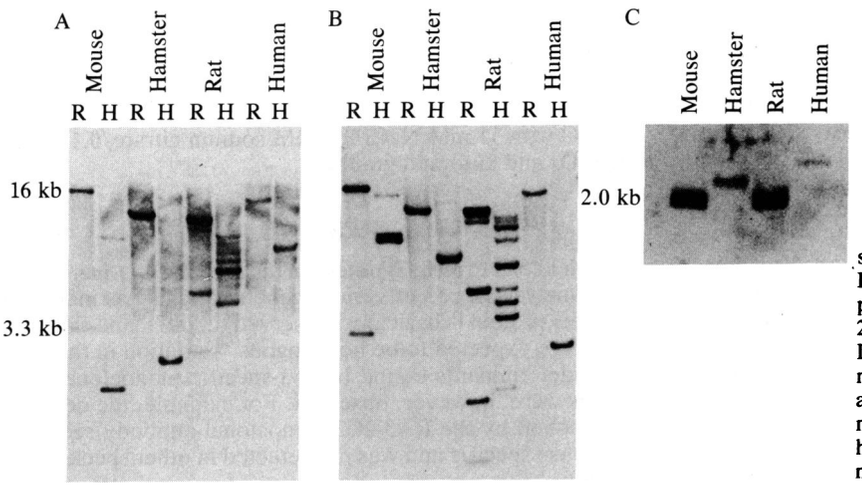

FIG. 2. Conservation of the p53 gene in various species. Equal amounts of DNA digested with *Eco*RI (R) or *Hin*dIII (H) were separated by electrophoresis and hybridized with either the pp53-271pstB fragment (11, 33) (*A*) or the p8R.4 *Kpn* I/*Pst* I genomic fragment containing the 3′-proximal region of the p53 gene (11) (*B*). For RNA blot analysis, equal amounts of polyadenylylated mRNA of the various species were separated and hybridized with the *Kpn* I/*Pst* I insert of the genomic p8R.4 clone (*C*). kb, Kilobases.

copy number of the various species were observed. Fig. 2 *A* and *B* illustrate the results obtained with the following cell lines: 230-23-8 Ab-MuLV-transformed murine cells, Co60 SV40-transformed hamster fibroblasts, B77 avian sarcoma virus-transformed rat cells, and SV-80 human transformed fibroblasts (Fig. 2 *A* and *B*). In addition, RNA blot analysis using the mouse genomic probe (p8R.4) showed that all species tested had a single distinct homologous p53-specific mRNA. The estimated sizes of the p53 polyadenylylated mRNAs were as follows: mouse, 2.0 kb; hamster, 2.4 kb; rat, 2.0 kb; and human, 3.0 kb (Fig. 2*C*). In our hands the p8R.4 *Pst* I/*Kpn* I p53 genomic fragment (containing two p53 principal exons closest to the 3′ end) (11) consistently showed a higher efficiency of hybridization with both genomic DNA and mRNA of all the species tested.

On that basis, our strategy for establishing a human cDNA probe was to prepare a human cDNA library of SV-80 transformed cells in the gt10 λ phage and use the mouse p8R.4 genomic fragment as a probe for screening and identifying human p53 cDNA clones. We screened 100,000 independent plaques and found p53-positive clones at a frequency of 1 out of 12,000. Hybridizing plaques were purified and analyzed with restriction enzymes. Another round of screening using the 3′ p8R.4 probe revealed a maximal cDNA size of 2.3 kb. A range of cDNA inserts were subcloned in the *Eco*RI site of puc 13. One representative 2.3-kb cDNA insert, p53-H14, spanning 75% of the mature human mRNA and lacking about 700 bp at the 5′ end, was used as the principal p53-specific human probe for further analysis.

Lack of Expression of the Mature p53 mRNA in HL-60 Cells. In the L12 murine Ab-MuLV-transformed p53 nonproducer cells we have detected aberrant p53 mRNA species larger than the mature 2.0-kb p53 mRNA (10). This mRNA molecule was homologous with the mature p53 mRNA at the 5′-proximal region only (10). These aberrant p53 mRNA species are most likely the product of a fusion transcriptional event initiating in the first p53 exon and terminating within the Mo-MuLV sequences that have integrated into the first p53 intron of these cells (10, 11).

Prompted by these observations, we decided to study whether the human HL-60 nonproducer cells also express aberrant p53 transcripts or were devoid of any p53 mRNA molecules.

RNA blot analysis of polyadenylylated mRNA of the various human transformed cells of lymphoid or fibroblastic origin, employing the human p53-H14 cDNA probe, indicated that all p53 producer cells expressed a characteristic 3.0-kb mRNA population identical in size to that detected with the p8R.4 mouse genomic probe (compare Fig. 3*A* to Fig. 2*C*).

Fig. 3*A* clearly shows that HL-60 cells did not express any detectable mature-size p53 mRNA molecules. Also, no defined aberrant mRNA species larger or smaller in size were observed in these cells by employing the p53-H14 human p53 cDNA probe.

These observations support the conclusion that the lack of p53 synthesis in the HL-60 cells was due to the absence of a mature p53 3.0-kb transcript that can be translated *in vivo* into a mature p53 molecule.

Deletions in the p53 Gene of HL-60 Cells. Prompted by the observation that HL-60 cells lack the mature p53 mRNA, we speculated that the p53 gene in these cells was altered. For that purpose we analyzed the genomic DNA sequences hybridizing with the p53 human-specific cDNA probes (p53-H14). A unique pattern of p53 genomic fragments was found in all p53 producer human cell lines when probed either with the human p53-H14 cDNA or with the mouse clones. Comparison between the p53 specific genomic DNA fragments obtained from p53 producer human cell lines and the HL-60 nonproducer cell line clearly showed that the p53 gene of the latter underwent major alterations (Fig. 4*A*). The genomic single fragment of HL-60 generated by various restriction enzymes hybridized weakly with the entire 2.3-kb *Eco*RI insert of p53-H14 and exhibited significant deletions and rearrangements. Judging on the basis of hybridization intensity, we have concluded that the homology of the remaining genomic p53 DNA sequence in the HL-60 cells is very low. Utilizing defined segments of the p53-H14 cDNA clone probing either the 5′ or the 3′ region of the p53 gene indicated that most of the p53 exons in HL-60 cells were deleted. The residual p53 sequences of these cells, showing weak homology with the present available human p53 cDNA probes, were mapped to the corresponding 5′ region of the gene. Results summarized in Fig. 5 show that *Eco*RI/*Hin*dIII digestion of SV-80 genomic DNA gave rise to a typical pattern of

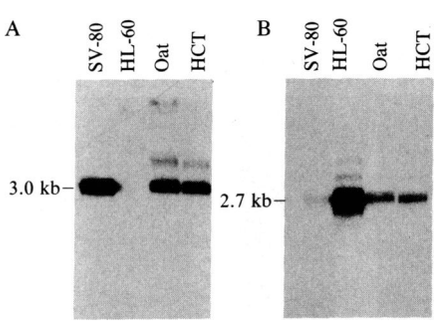

FIG. 3. RNA blot analysis of the various human cell lines. Equal amounts of polyadenylylated mRNA were separated and hybridized to the human p53-H14 *Eco*RI insert (*A*) or the *Cla* I/*Eco*RI insert of PMC41-3RC genomic 3′ probe of the c-*myc* gene (34). Ribosomal RNA was used as a molecular size reference.

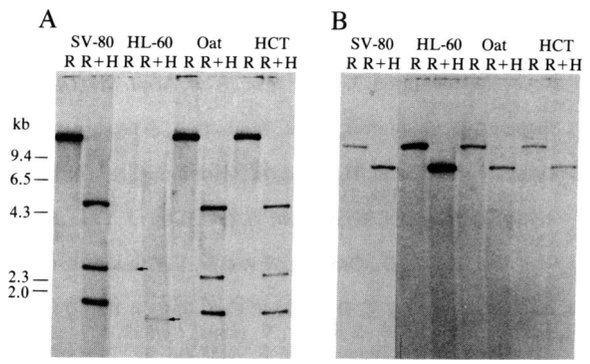

FIG. 4. Southern blot analysis of various human cell lines. Equal amounts of DNA of the various cell lines were digested with *Eco*RI (R) or double-digested with *Eco*RI and *Hin*dIII (R + H). (*A*) DNA blots were hybridized with the human p53-H14 cDNA *Eco*RI insert. (*B*) The same filters were extensively washed with 0.5 M NaOH for 15 min at room temperature to remove all the radioactive signals and subsequently hybridize with the c-*myc* probe, the *Cla* I/*Eco*RI insert of the PMC41-3RC 3′ probe of the c-*myc* gene (34).

three major fragments (1.8, 3.0, and 5.0 kb) hybridizing with the 5′ 1.2-kb *Eco*RI/*Ava* I fragment of p53-H14. In addition, the genomic 3′ 1.8-kb *Eco*RI/*Hin*dIII fragment specifically hybridized with the two other p53 cDNA fragments tested (0.7- and 0.4-kb probes II and III, respectively, in Fig. 5). In HL-60 cells the only hybridization event was with the 5′ 1.2-kb *Eco*RI/*Ava* I fragment of p53-H14, reflected as a single band, smaller in size and reduced in homology. On the basis of results obtained by using a human cDNA probe that spans 75% of the p53 gene, we concluded that most of the p53 gene was deleted. HL-60 cells retained a 5′ part of the p53 gene that was probably rearranged.

c-*myc* Amplification in the HL-60 Cell Line Tested Here. Genomic analysis of HL-60 cells have previously shown the presence of amplified c-*myc* sequences (13) that efficiently are transcribed into a 2.7-kb c-*myc* mRNA product (14). This was detected in HL-60 cells passaged *in vitro* or in primary

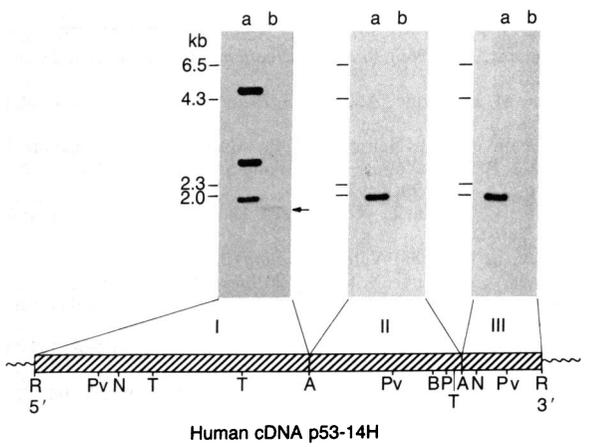

FIG. 5. Comparison of the p53 gene in HL-60 cells to that of SV-80 transformed cells. Equal amounts of DNA obtained from SV-80 cells (lanes a) or from HL-60 cells (lanes b) were double digested with *Eco*RI and *Hin*dIII, separated by gel electrophoresis, and hybridized with *Eco*RI/*Ava* I-digested segments generated from the human p53-H14 cDNA probe. The probes used in their 5′ to 3′ orientation were as follows: I, *Eco*RI/*Ava* I (1.2 kb); II, *Ava* I/*Ava* I (0.7 kb); and III, *Ava* I/*Eco*RI (0.4 kb). The last probe showed consistently higher background because of the presence of repetitive sequences. The p53-H14 fragments were isolated by agarose gel electrophoresis, electroeluted, and concentrated by Elutip filtration (Schleicher & Schuell). The restriction enzymes used for analysis of human cDNA clone p53-H14 were R, *Eco*RI; Pv, *Pvu* II; N, *Nco* I; T, *Taq* I; A, *Ava* I; B, *Bam*HI; and P, *Pst* I.

tumor cells obtained from patients with acute promyelocytic leukemia (13, 14). Since the HL-60 cells in which we have observed the alteration in p53 sequences were passaged for several months in our laboratory, reevaluation of the nature of c-*myc* was essential.

Polyadenylylated mRNA of the various human cell lines tested for p53 expression were probed in turn with the *Cla* I/*Eco*RI insert of clone PMC41-3RC, derived from the 3′-proximal region of the human c-*myc* gene (34). Fig. 3*B* shows that all the transformed lines exhibit, though at various amounts, the expected 2.7-kb c-*myc* mRNA (14). In contrast to the total lack of p53 mRNA observed in the HL-60 cells (Fig. 3*A*), a significantly augmented level of c-*myc* mRNA was evident in these cells (Fig. 3*B*).

In agreement with previously documented results, we found that HL-60 cells tested here demonstrated the amplified nonrearranged c-*myc* gene (Fig. 4*B*), thus confirming the authenticity of the HL-60 cells tested here. This is in marked contrast to the major deletion observed in the p53 gene of these cells. All other transformed cell lines tested did not show overt amplification of c-*myc*.

DISCUSSION

On the basis of structural and functional criteria, the oncogene family members were categorized into two major complementation groups (35, 36). Results recently emerging from different experimental systems have suggested that p53 tumor antigen is most probably a member of the nuclear oncogene family and like myc, myb, and E1A proteins, p53 complements the function of oncogenes of the second category. In the case of the L12 Ab-MuLV-transformed p53 nonproducer cells, which changed their malignant phenotype, it was suggested that p53 probably complemented the p120 *abl* oncogene (12). Expression of these two products in the L12 variant cells yielded the appearance of fully transformed lethal tumors (12). Furthermore, it was shown that cotransfection of p53 with the *ras* oncogene induces the appearance of transformed foci in primary embryonic rat fibroblastic monolayers (46, 47).

Human HL-60 cells studied here are the second example of a cell line that does not express p53. In the HL-60 case, shut-off of p53 expression was induced by major deletions and rearrangement in the p53 genomic structure. In Ab-MuLV-transformed cell line L12, the example reported earlier (9–11), we found that the functional p53 gene was inactivated by the integration of a Mo-MuLV into the first p53 intron (11). Southern blot analysis of the L12 cells indicated that both p53 chromosomal alleles in these cells contained the same altered structure (11). Likewise, the HL-60 cells described here showed the loss of the normal p53 allele while retaining the altered counterpart. The fact that these two independently established p53 nonproducer cell lines exhibit a similar pattern of alteration of one p53 allele and concomitant loss of the intact p53 allele suggest that homozygotization of the altered inactive p53 allele in these leukemic cells is probably advantageous.

The L12 murine p53 nonproducer cells previously studied were suggested to be partially transformed and the acquisition of a complete transformed phenotype required the introduction of a functional p53 gene (12).

Conversely, the HL-60 p53 nonproducer cells are most probably fully transformed, because these cells have been cloned from a human promyelocytic leukemia (13, 14). In these tumor cells, we assume that the deficiency in p53 expression was overcome by accentuated production of an alternative protein.

In addition to being associated with the malignant process, it was suggested by several investigators that p53 plays a role in the normal cell cycle. Studies by Milner and Milner (37)

showed that p53 is induced in cells treated with mitogens and suggested that it starts functioning early during the transition from G_0 to G_m. Using quiescent nontransformed NIH-3T3 cells, stimulated with serum to start cell division, Mercer *et al.* (38) showed that microinjection of anti-p53 monoclonal antibodies into the cells inhibited DNA synthesis. They suggested that p53 is synthesized as a late G_1 protein. Reich and Levine (39) reached a similar conclusion after analyzing 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.

We would like to suggest that the deficiency of p53 synthesis in HL-60 cells was overcome by using an alternative metabolic pathway for the cell cycle. One such potential candidate is the c-*myc* product, whose expression is accentuated in HL-60 cells (13, 14).

The notion that p53 and myc are analogous proteins in the cell was previously suggested (40). The basis for that assumption was the observation that these two proteins accumulate in the nuclear compartment (40–42), both are in correlation with the cell cycle (39, 43, 44), and mitogenic stimulation of resting cells yielded the synthesis of these two proteins (37, 38, 43). Recently Bienz *et al.* (45) suggested that although p53 and myc genes do not share significant sequence homology they have similarities in the general structure of their molecules. The fact that p53 gene, like the myc gene, can complement the *ras* oncogene in inducing transformation of primary embryonic rat fibroblasts (46, 47) suggests also analogy in function, which is in agreement with the phenomenon described here.

Unlike in the HL-60 cells, c-*myc* in the L12 p53 nonproducer was not amplified (data not shown). In that case, cells became fully transformed only when cloned p53 gene was introduced (12). If indeed p53 and c-myc are functional interchangeable proteins, it is expected that introduction of c-*myc* at a high copy number into L12 cells instead of p53 gene will also change the malignant phenotype of these cells.

It was suggested that the c-*myc* amplification has occurred in the germ line, and freshly cultured HL-60 cells or cells passaged in culture for a long period exhibit a similar pattern of c-*myc* amplification (13, 14). At this stage of analysis it is not clear whether p53 gene deletion and c-*myc* amplification occurred in these cells in a dependent or independent manner. It is possible that p53 deletion and loss of the intact allele led to the selection of cells overproducing c-myc or, alternatively, the c-*myc* amplification caused selection of cells with no p53 expression. Any of the pathways used gave rise to cells that cannot support the concomitant overproduction of these two proteins.

We hypothesize that cell survival and expression of a malignant phenotype require a physiological balance between the expression of these two proteins.

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