Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 5146–5150, July 1988
Genetics

Human p53 oncogene contains one promoter upstream of exon 1 and a second, stronger promoter within intron 1

(gene regulation/oncogene expression/chloramphenicol acetyltransferase assay/Epstein–Barr virus-based vector)

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Communicated by Christian B. Anfinsen, March 7, 1988

ABSTRACT **To gain insight into how transcription of the human p53 oncogene is controlled, we characterized the regulatory regions of the gene. A 3.8-kilobase-pair (kbp) *Eco*RI restriction fragment encompassing the 5′ end of the human p53 gene, as well as subfragments generated by restriction digests, was cloned upstream of the *Escherichia coli* chloramphenicol acetyltransferase (CAT) gene and CAT activity was assayed in extracts of transfected cells. Two types of CAT vectors were used: Epstein–Barr virus *oriP*-derived constructs that were stably introduced into the human cell lines K562, Raji, and HL-60, and pSV0-CAT-derived constructs that were transiently introduced into the monkey cell line COS. By this approach we have identified two promoters for the human p53 gene. One promoter, p53*P*1, is located 100–250 bp upstream of the 218-bp noncoding first exon; a second, stronger promoter, p53*P*2, maps within the first intron. CAT activity and expression of CAT RNA indicate that p53*P*2 functions up to 50-fold more efficiently than p53*P*1. We conclude that the expression of the human p53 gene may be controlled by two promoters and that differential regulation of these promoters may play an important role in the altered expression of the gene in both normal and transformed cells.**

The p53 oncogene product, a 53-kDa protein, has been detected at elevated levels in transformed cells of various species, including cells derived from tumor biopsy samples (1) and cells transformed in culture by DNA viruses (2), retroviruses (3), chemical carcinogens (4), and x-irradiation (4). In addition, increased expression of p53 can lead to cell immortalization (5), to increased tumorigenicity of cells (6, 7), and to transformation of normal primary embryonic rat fibroblasts through cooperation with the activated c-Ha-*ras* oncogene (5, 8, 9). p53 may also play a role in the regulation of cell proliferation. p53 expression increases prior to DNA synthesis when resting cells are stimulated to undergo cell division (10, 11), and inhibition of p53 expression, either by microinjection of anti-p53 monoclonal antibodies (12) or by p53 antisense RNA, inhibits DNA synthesis (13).

Regulation of p53 expression has been shown to occur at the level of p53 protein stability. In cells transformed by simian virus 40 (SV40), p53 is found in a complex with the SV40 large tumor (T) antigen (2). This association results in an increase in the stability and steady-state level of p53 (14). Regulation of p53 expression also occurs at the level of mRNA abundance. After mitogenic stimulation of normal human lymphocytes or murine 3T3 cells, p53 mRNA levels increase 5- and 20-fold, respectively, within 6–14 hr (11, 15).

Unlike transcription of some other cellular protooncogenes, transcription of the p53 gene has not been shown to be activated in transformed cells by chromosomal translocations, gene amplifications, or retroviral insertions. The possibility exists, therefore, that altered expression of p53 in some transformed cells results from mutations within regulatory regions of the gene or from altered expression of trans-acting factors that are required for appropriate expression of the p53 gene.

To understand how transcription of the p53 gene is regulated, we first located the DNA sequences that mediate expression of the gene, with the aim of understanding their function(s) and of eventually identifying cellular factors that they may bind. Our results indicate that expression of the human gene for p53 may be controlled by two promoters having different strengths. One promoter, designated p53*P*1, is located 100–250 base pairs (bp) upstream of the 218-bp noncoding first exon (16). A second promoter, designated p53*P*2, functions more efficiently and maps within the 5′ 1.2 kbp of intron 1.

MATERIALS AND METHODS

Cell Lines. K562 is a human leukemic cell line derived from a patient with chronic myelogenous leukemia (17). HL-60 is a human cell line derived from a patient with acute promyelocytic leukemia (18). Raji is an Epstein–Barr virus (EBV)-genome-positive cell line derived from a Burkitt lymphoma biopsy sample (19). K562, HL-60, and Raji were grown in RPMI 1640 culture medium containing 10% (vol/vol) fetal bovine serum. COS cells are SV40-transformed monkey fibroblasts (20) and were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum.

Recombinant Plasmids. A 3.8-kbp *Eco*RI restriction fragment containing ≈2.2 kbp of DNA sequence upstream of the human p53 gene, the 218-bp noncoding first exon, and 1.3 kbp from the 10-kbp first intron was obtained from a λ Charon 4A library of genomic DNA isolated from the SV40-transformed human cell line SV80 (21). The plasmid pSV0-CAT consists of pBR322, the chloramphenicol acetyltransferase (CAT) coding sequences (22) upstream of the SV40 small tumor (t) antigen intron and polyadenylylation sequences, and a polylinker immediately upstream of the CAT coding sequences. p220-CAT is derived from the plasmid p220.2, provided by Bill Sugden and John Yates (McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI). p220.2 was derived from the plasmid p201 by insertion of a polylinker from pUC12 downstream of the polyadenylylation site of the herpes simplex virus 1 thymidine kinase gene. The construction of p201 has been described (23). The plasmid p220-CAT, shown in Fig. 1*A*, confers resistance to hygromycin B, carries the EBV nuclear antigen 1 (EBNA-1) coding sequences and the EBV origin of replication (*oriP*) and replicates as a plasmid both in *Escherichia coli* and in human cells (23, 24). The CAT coding sequences were isolated from pSV0-CAT on a 1.6-kbp

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Abbreviations: SV40, simian virus 40; EBV, Epstein–Barr virus; CAT, chloramphenicol acetyltransferase.

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*Hin*dIII–*Bam*HI restriction fragment and cloned into the *Hin*dIII and *Bam*HI restriction sites of p220.2

Gene Transfer. Ten micrograms of plasmid DNA was introduced into 2×10^7 exponentially growing cells by electroporation (25). After 48–72 hr, the cells were transferred to complete medium plus hygromycin B (Calbiochem) at 150 μg/ml. DNA transfections were performed by the calcium phosphate coprecipitation method (26). COS cells at 50–75% confluence in 100-mm culture dishes were transfected with 10 μg of plasmid DNA. Cells were harvested for CAT assays after 72 hr. CAT assays were performed as described (22). Control experiments indicated that all assays were in the linear range with respect to time and concentration of cell extract.

RNA Analysis. Total cellular RNA was isolated (27) and further purified by sedimentation through 5.7 M CsCl. RNA (20 μg) was electrophoresed in 1% agarose/2.2 M formaldehyde gels, transferred to nitrocellulose (28), and hybridized to nick-translated DNA. For nuclease S1 mapping, total RNA (20 μg) was precipitated with the heat-denatured DNA probe (25,000 cpm) labeled at its 5′ ends by reaction with [γ-^{32}P]-ATP and bacteriophage T4 polynucleotide kinase. The precipitate was resuspended in 10 μl of hybridization buffer [40 mM Pipes, pH 6.5/400 mM NaCl/1 mM EDTA/80% (vol/vol) formamide], heated at 75°C for 15 min, and incubated at 45°C. After 18 hr at 45°C, 200 μl of cold S1 buffer (30 mM sodium acetate, pH 4.5/250 mM NaCl/2.5 mM zinc acetate) containing 600 units of nuclease S1 (Boehringer Mannheim) was added. The mixture was incubated at 37°C for 90 min and the reaction was stopped by extraction with phenol. The S1-resistant products were separated by electrophoresis in 5% acrylamide/8 M urea gels.

RESULTS

Identification of Two p53 Promoters That Control Expression of the CAT Gene. To localize the sequences involved in regulating expression of the p53 gene, the 3.8-kbp fragment (Fig. 1*B*, fragment a), containing the 5′ portion of the p53 gene was cloned upstream of the CAT gene on the plasmid p220-CAT (Fig. 1*A*). In addition, this 3.8-kbp DNA fragment was digested with *Xba* I, which cuts this fragment into two parts (Fig. 1*B*, fragments b and c) that were also cloned in p220-CAT. The 2.4 kbp upstream of the *Xba* I site consist of noncoding sequence and ≈106 bp from the first exon; the 1.4 kbp downstream of the *Xba* I site consist of ≈112 bp from the first exon and 1.3 kbp from intron 1. These constructs were introduced into K562 cells by electroporation, cells were selected for stable resistance to hygromycin B, and CAT activity was assayed. Cells carrying the "empty" CAT vector (p220-CAT) expressed no detectable CAT activity, whereas the vectors carrying the p53 upstream sequences directed expression of the CAT gene (Fig. 1*C*). The 3.8-kbp DNA fragment and the 2.4 kbp upstream of the *Xba* I site, as shown previously in HeLa cells (16), directed expression of CAT in K562 cells. In addition, the sequence downstream of the *Xba* I site directed CAT expression reproducibly at 3- to 10-fold higher levels than the sequence upstream of the *Xba* I site (Fig. 1*C*, Table 1). These results indicate that at least two promoters map near the 5′ end of the human p53 gene. One of these promoters, p53*P*1, is located upstream of the single *Xba* I site within the first exon, and a second promoter, p53*P*2, is located downstream of the *Xba* I site. As demonstrated below, p53*P*2 maps within intron 1 of the human p53 gene.

We also tested the promoter activities of p53*P*1 and p53*P*2 in two other human cell lines, HL-60 and Raji. In parallel, we compared the activities of p53*P*1 and p53*P*2, the SV40 early promoter, and the Moloney murine leukemia virus long terminal repeat (Table 1). p53*P*1 expressed about the same level of CAT activity as the SV40 promoter in both K562 and HL-60, whereas p53*P*2 expressed up to 20-fold more CAT activity than either p53*P*1 or the SV40 promoter. Extracts of

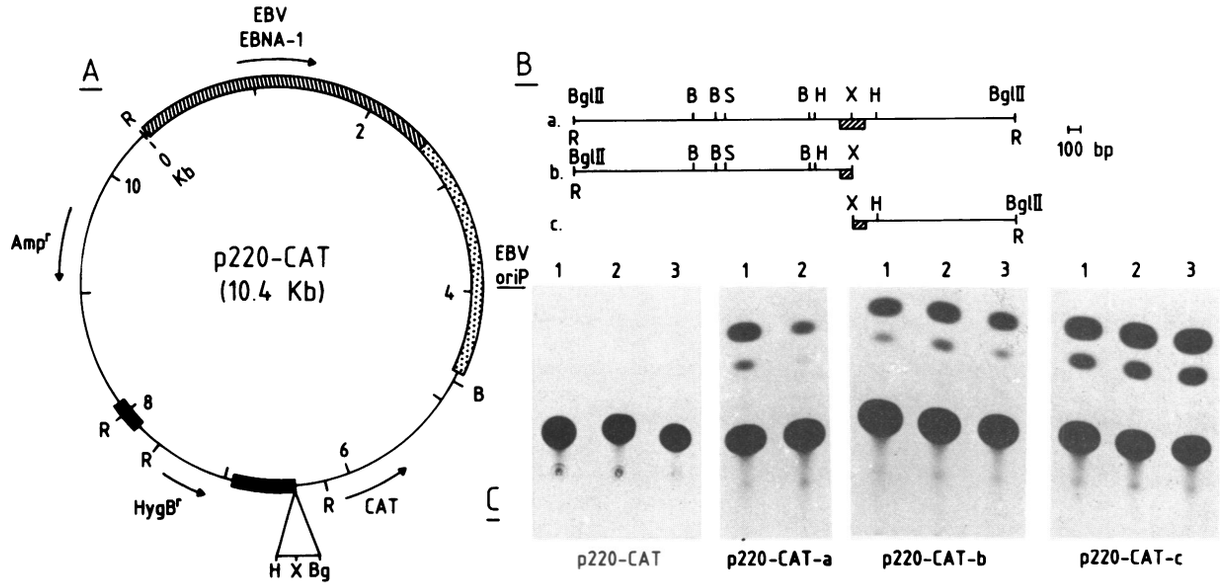

FIG. 1. Expression of CAT in K562 cells. (*A*) CAT expression vector p220-CAT. The gene for resistance to hygromycin B (HygB^r) is expressed by using the promoter and polyadenylylation sequence of the herpes simplex virus thymidine kinase gene (black boxes). The putative origin of replication of EBV, *oriP*, is represented by the stippled box. The EBV nuclear antigen 1 (EBNA-1) coding sequence is represented by the hatched box. Sequences from pBR322 that express resistance to ampicillin (Amp^r) and allow replication in *E. coli* are shown by a thin line. The CAT coding sequence is upstream of the SV40 small tumor (t) antigen intron and polyadenylylation sequence. Arrows indicate direction of transcription. Kb, kbp. (*B*) Restriction maps of DNA inserted into p220-CAT. The 3.8-kbp DNA fragment encompasses the 5′ region of the human p53 gene. Hatched box represents the 218-bp noncoding first exon. Region to the right of the hatched box represents the 5′ portion of the 10-kbp first intron. Recognition sites are shown for restriction endonucleases *Bgl* II, *Eco*RI (R), *Bam*HI (B), *Sma* I (S), *Hin*dIII (H), and *Xba* I (X). (*C*) CAT activity in K562-derived cells. The following constructs were introduced by electroporation into K562 cells: p220-CAT (the "empty" CAT vector), p220-CAT-a (the vector containing insert a), p220-CAT-b (the vector containing insert b), and p220-CAT-c (the vector containing insert c). Numbers at the top of each autoradiogram represent different populations of cells tested. One hundred micrograms (protein) of cell extract was assayed for CAT activity in a 60-min reaction. Lower spots are [^{14}C]chloramphenicol; upper two spots are its acetylated derivatives.

Table 1. CAT activity in cells expressing CAT from the two p53 promoters

Promoter(s)	% chloramphenicol acetylated		
	K562	HL-60	Raji
p53*P*1 + p53*P*2	1.8, 3.6	4.5, 4.6	6.8, 5.2
p53*P*1	1.9, 2.3, 2.0	2.7, 1.2	NT
p53*P*2	17.8, 10.1, 6.1	32.6, 68.5	NT
SV40	2.5, 2.8	1.4, 3.0	NT
Mo-MuLV LTR	93, 88	9.8, 6.3	NT
None	0.2	0.1	0.2

The cells listed all stably carry p220-CAT constructs, either with or without inserts. p53*P*2 + p53*P*1, entire 3.8 kbp; p53*P*1 and p53*P*2, 2.4-kbp and 1.4-kbp fragments, respectively; Mo-MuLV LTR, Moloney murine leukemia virus long terminal repeat. Thirty micrograms (protein) of cell extract was assayed for CAT activity. Each value represents the percent chloramphenicol acetylated in a 60-min reaction and is the average of at least two assays of individual populations. NT, not tested.

cells expressing CAT from p53*P*2 consistently contained more CAT activity than cells expressing CAT from p53*P*1 (Fig. 1, Table 1).

One explanation for these observations is that cells carrying the p220-CAT construct with p53*P*2 maintain more copies of the transfected plasmid DNA and that the observed relative increase in CAT activity is a result of gene dosage. To address this question, we measured the number of stably maintained copies of the respective promoters by Southern analysis (29). Total DNA from K562 cells expressing CAT, either from p53*P*1 or from p53*P*2, was digested with restriction enzymes so that the promoter would be released from p220-CAT. The restriction digests released DNA fragments of the expected sizes (Fig. 2), 2.4 kbp (containing p53*P*1) and 1.4 kbp (containing p53*P*2), that hybridized to the ^{32}P-labeled

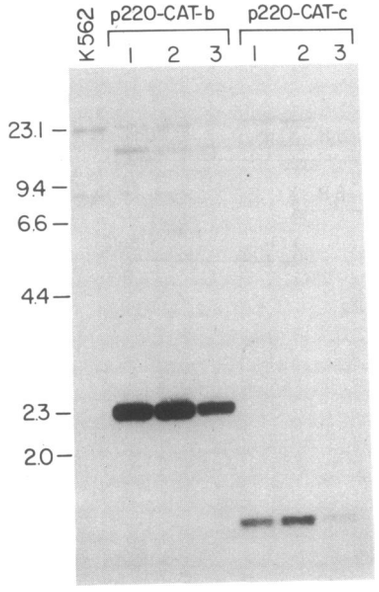

FIG. 2. Southern analysis of p53 promoter sequences introduced into K562 cells on the vector p220-CAT. Five micrograms of total DNA from cells designated p220-CAT-b, carrying the 2.4-kbp DNA fragment containing p53*P*1, was digested with *Xba* I. DNA from cells designated p220-CAT-c, carrying the 1.4-kbp DNA fragment containing p53*P*2, was digested with *Xba* I and *Bgl* II. Lane numbers 1–3 represent DNA from the same populations assayed for CAT activity in Fig. 1*C*. Hybridization was to the complete ^{32}P-labeled 3.8-kbp DNA fragment. The large DNA species present only in the CAT-positive populations are vector sequences that have hybridized to contaminating vector sequences in the radiolabeled hybridization probe. Size markers (in kbp) were derived from λ DNA digested with *Hin*dIII.

3.8-kbp DNA fragment. By measuring the intensities of the hybridization signals obtained from the introduced DNA relative to that obtained from the high molecular weight endogenous DNA, we estimated that the K562-derived cells carrying p53*P*1-bearing plasmids contained 10–20 plasmid copies per cell, whereas the K562 cells carrying p53*P*2-bearing plasmids contained 5–10 plasmid copies per cell. Thus, the increase in CAT activity observed in cells carrying p53*P*2 cannot be explained by gene dosage and is therefore likely to result from p53*P*2 being a stronger promoter.

p53*P*2 Maps Within Intron 1. To localize the two p53 promoters, we introduced deletions into the 3.8-kbp DNA fragment with various restriction endonucleases. These derivatives were inserted into the CAT vector pSV0-CAT, and the resulting constructs (Fig. 3) were introduced into COS cells. Cell extracts were prepared and assayed for CAT activity. As shown in Fig. 3, two promoter activities were also detected in transfected COS cells. Digestion of the 2.4-kbp fragment contained p53*P*1 activity with *Hin*dIII localized p53*P*1 to within the 350 bp upstream of the *Xba* I site (Fig. 3*e*). Reversing the orientation of either the entire 3.8-kbp fragment (Fig. 3*b*) or of the sequence containing only p53*P*1 (Fig. 3*d*) resulted in no detectable CAT activity. Furthermore, constructs that contained the entire 2.4 kbp upstream of the *Xba* I site consistently yielded one-half to one-quarter the activity of constructs that contained only the 350 bp containing p53*P*1 (compare Fig. 3 *c* and *e*). These results indicate that an element that negatively regulates p53*P*1 may be present upstream of the promoter. As was the case in K562, a second promoter, p53*P*2, located within the 1.4 kbp downstream of the *Xba* I restriction site within exon 1, expressed a higher level of CAT activity (Fig. 3*f*). To ensure that the observed CAT activity was not somehow due to pBR322 sequences being brought next to p53 sequences, we separated these sequences by reintroducing the 2.4-kbp upstream fragment, which was deleted from this construct, in the inverted orientation. This construct remained competent to express CAT activity (Fig. 3*g*). Deletion of the remaining exon 1 sequence by digestion with *Hin*dIII did not affect the ability of the remaining 1.2 kbp of intron 1 to promote CAT activity (Fig. 3*h*). These results indicate that a promoter is present within the 5′ 1.2 kbp of intron 1 of the human p53 gene.

Two Species of CAT RNA Are Present in Cells Expressing CAT from Both p53*P*1 and p53*P*2. If both p53 promoters are functional when present together on the 3.8-kbp DNA fragment, then two CAT mRNAs initiating at different sites should be present in the cell. To determine whether this was the case, RNA was extracted from K562-derived cells that carried p220-CAT containing the entire 3.8-kbp fragment. The RNA was subjected to electrophoresis followed by blot hybridization to the ^{32}P-labeled CAT coding sequences. Two CAT-specific RNAs, approximately 1.3 and 2.9 kilobases (kb) long, were detected (Fig. 4*A*). The presence of two CAT mRNAs is consistent with there being two promoters as indicated by the CAT assays described above. In addition, the difference in the steady-state levels of the two CAT RNAs is consistent with there being two promoters of different strengths. The difference in size of the two CAT mRNAs (≈1.6 kb) indicates that p53*P*2 may lie ≈1.6 kbp downstream of p53*P*1, thus locating p53*P*2 near the 3′ end of the 3.8-kbp genomic DNA fragment. To test this, we performed S1 nuclease mapping of the 5′ end of mRNA initiating at p53*P*2. RNA from K562 cells and from K562 cells expressing the CAT gene from the entire 3.8-kbp genomic fragment was hybridized in solution to a ^{32}P-labeled DNA fragment spanning the intron 1 sequences from the end of the 3.8-kbp clone and extending 1.2 kbp upstream (Fig. 4*B*). An S1-resistant species of ≈75 bases was observed only with RNA from K562 cells expressing CAT, indicating that transcripts expressed from p53*P*2 initiate ≈75 bp from the end of the DNA fragment (≈1.2 kbp downstream of exon 1). These

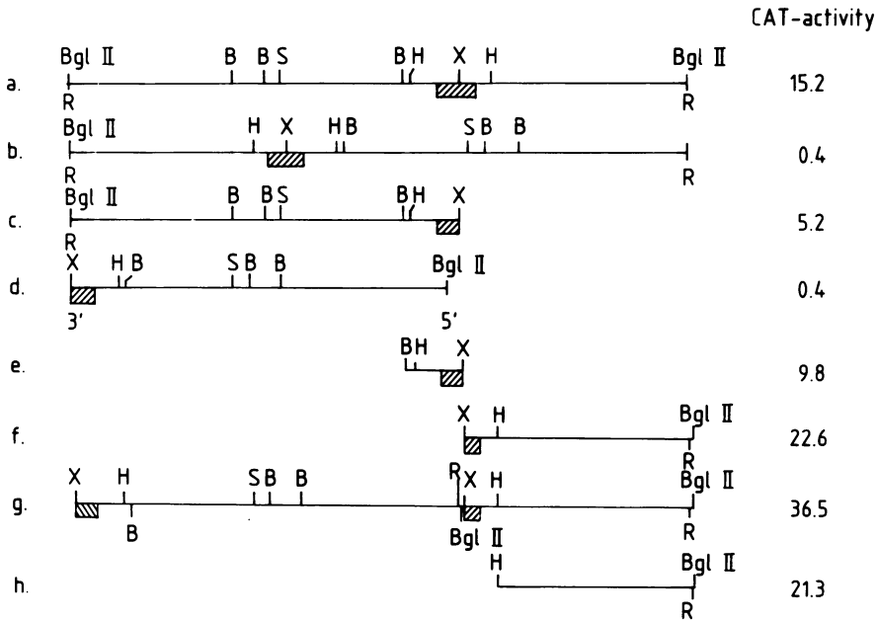

FIG. 3. Localization of the sequences required for activity of p53*P*1 and p53*P*2. Restriction maps are of DNAs introduced into pSV0-CAT and tested for promoter activity in COS cells. Restriction endonuclease sites abbreviated as in Fig. 1. (*a*) The entire 3.8-kbp DNA fragment encompassing both p53*P*1 and p53*P*2. (*b*) The 3.8-kbp DNA fragment cloned in the opposite orientation. (*c*) The 2.4-kbp fragment upstream of the *Xba* I site within exon 1 (hatched box). (*d*) The 2.4-kbp fragment cloned in the opposite orientation. (*e*) The 350-bp *Bam*HI–*Xba* I fragment consisting of the 5′ half of exon 1 plus 250 bp upstream of exon 1. (*f*) The 1.4-kbp fragment downstream of the *Xba* I site within exon 1. (*g*) The 1.4-kbp fragment (from *f*) was separated from upstream pBR322 sequence by insertion of the upstream 2.4-kbp fragment into the *Xba* I site in the opposite orientation. (*h*) Deletion of the remaining exon 1 sequence by digestion of *f* with *Hin*dIII and *Xba* I. The constructs were transfected onto COS cells, and cell extracts were prepared 48–72 hr later. Cell extracts containing 20 μg of protein were assayed for CAT activity. CAT activity is expressed as percent chloramphenicol acetylated in a 60-min reaction and is the average of two independent determinations.

results are consistent with the observed sizes of the CAT mRNA seen by blot hybridization analysis.

DISCUSSION

To gain insight into the regulation of the p53 gene, we have begun to locate DNA sequences that control expression of the gene. We constructed recombinant plasmids that contained all or portions of a 3.8-kbp DNA fragment composed of noncoding sequence encompassing the 5′ end of the human p53 gene upstream of the CAT gene. These constructs were introduced into cells and assayed for their ability to express the CAT gene. Our results indicate that the human p53 gene contains at least two promoters that are capable of initiating transcription of the CAT gene. p53*P*1 was described previously (16) and maps to the 250 bp upstream of the first exon. Constructs that contain, in addition to p53*P*1, sequence upstream of p53*P*1 yield one-half to one-quarter the CAT activity of constructs containing only p53*P*1. These results indicate that the sequence upstream of the promoter may contain elements that negatively regulate p53*P*1. A second promoter, p53*P*2, is located within intron 1 and is up to 50-fold more efficient than p53*P*1 in expressing the CAT gene (Table 1, Fig. 4*A*). Transcripts originating from p53*P*2 were mapped by S1 analysis and found to initiate ≈1.2 kbp downstream of exon 1 (Fig. 4*B*). Sequence upstream of p53*P*1 may also suppress the expression of p53*P*2. The CAT activity expressed from constructs containing the entire 3.8-kbp DNA fragment, containing both p53*P*1 and p53*P*2, was consistently lower than the CAT activity expressed from constructs containing only p53*P*2 (Table 1).

Consistent with the findings presented here, a study by Bienz-Tadmor *et al.* (30), comparing the structures of the murine and human p53 regulatory regions, showed that these sequences are highly conserved and that the murine p53 promoter also contains an upstream negative regulatory element. They also identified a highly conserved sequence containing extensive dyad symmetry within exon 1 and suggested that this sequence may play a role in the translation efficiency of the mRNA (30). Initiation of transcription downstream of this sequence, at p53*P*2 in the case of the human p53 gene, would eliminate this region of dyad symmetry and possibly, as may be the case in c-*myc*, increase the translational efficiency of the mRNA (31).

The control of gene expression by multiple promoters having different strengths has been observed for other genes. Expression of the mouse α-amylase gene, *Amy-A*^{1} (32), and the yeast invertase gene, *SUC2* (33), is controlled by dual promoters. The c-*myc* gene is controlled by at least four promoters (34–36). The multiple promoters of c-*myc* appear to be involved in altered regulation of the gene in transformed cells as well as during the cell cycle of cells (37).

At present, no biological role for the presence of two p53 promoters can be assigned. One possibility is that p53*P*2 is the promoter for a gene located within the 10-kbp first intron of the p53 gene. Alternatively, differential usage of two promoters may be involved in regulation of p53 expression during the cell cycle or in its overexpression in transformed cells. Bendori *et al.* (38), studying expression of p53 during terminal differentiation of murine erythroleukemia cells, demonstrated the induction of expression of a larger species of p53 mRNA. The structure of this induced RNA is consistent with transcription initiating at a site ≈200 bp upstream. Further, it is possible that, like activation of c-*myc*, activation of expression of the p53 gene in transformed cells results from alterations in the regulatory regions of the gene. The regulatory regions of the p53 gene used for this study were isolated from a transformed human cell line, SV80. One hypothesis to explain the overexpression of p53 in SV80 cells is that alterations have occurred in the p53 gene, thus activating transcription. Mutations occurring within intron 1 may have been responsible for the activation of p53*P*2, which

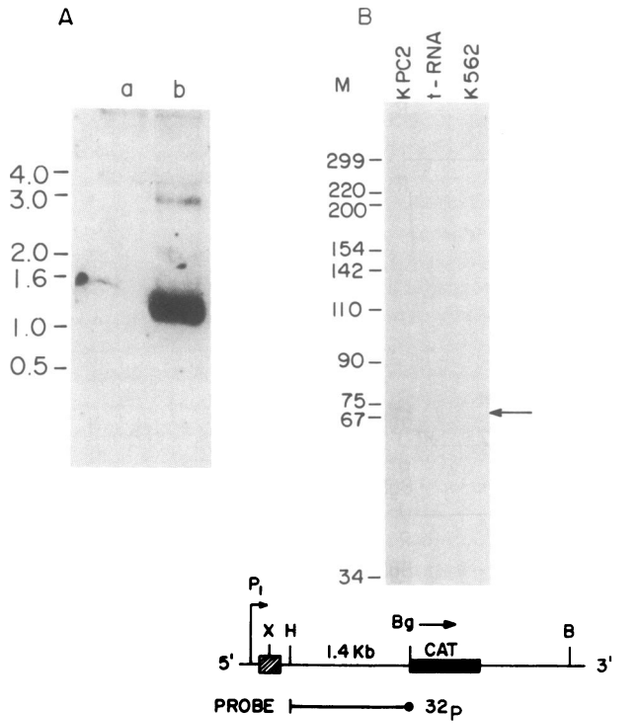

FIG. 4. Analysis of CAT RNA from K562 cells and cells carrying the complete 3.8-kbp DNA fragment encompassing p53*P*1 and p53*P*2. (*A*) Twenty micrograms of total cellular RNA from CAT-negative K562 cells (lane a) and from a clone of K562 cells expressing CAT (lane b) were electrophoresed in 1% agarose/2.2 M formaldehyde gels, transferred to nitrocellulose, and hybridized to ^{32}P-labeled CAT coding sequence (Fig. 1*A*). Size markers (in kb) were derived from known restriction fragments of DNA that were denatured and electrophoresed as described above. A 3.5-kb species of RNA seen in lane b (from CAT-positive cells) was also detected in lane a (K562) and was more evident on an overexposed autoradiogram (data not shown). (*B*) Nuclease S1 analysis of CAT RNA initiated from p53*P*2. The end-labeled DNA probe illustrated below the autoradiogram was hybridized to total RNA (20 μg) from K562 cells, to total RNA (20 μg) from K562 cells expressing CAT (KPC2), and to 10 μg of tRNA. Hybrids were digested with nuclease S1 and analyzed by electrophoresis in 5% acrylamide/8 M urea gels. A protected species of $\approx$75 bases was seen only in K562 cells expressing CAT from p53*P*2. Size markers were taken from ^{32}P-labeled *Msp* I and *Hin*fI restriction fragments of pBR322. Below the autoradiogram is an illustration of the sequence containing p53*P*1 (indicated by arrow), exon 1, and the intron 1 sequence that contain p53*P*2 activity cloned upstream of the CAT gene. Bg, *Bgl* II; other restriction sites are abbreviated as in Fig. 1.

may ordinarily be silent. Since the first exon of p53 is not translated, initiation of transcription within intron 1 would not lead to an altered protein. Consistent with this hypothesis, we have observed by blot analysis an mRNA that hybridizes to intron 1 sequences in SV80 cells but not in Raji cells (data not shown). In addition, we have isolated a cDNA clone of mRNA of SV80 cells that contains intron 1 sequence.

This work was supported in part by a grant from the Cancer Research Institute, by Public Health Service Grant IR01 CA4002 from the National Institutes of Health, and by a grant from the Leo and Julia Forcheimer Center for Molecular Genetics, the Weizmann Institute of Science. V.R. is the incumbent of the Norman and Helen Asher Professorial Chair in Cancer Research. D.R. is a Fellow of the Leukemia Society of America.

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