29. We would like to thank J. Fernandez for karyotyping of the hybrid clones; S. McDougal, J. K. Nicholson, B. Jones, and C. Dawson for their help with FACS SCAN analysis; L. Matlock and J. Sullivan for help in preparing the manuscript; F. Murphy and T. Folks for their helpful criticism and suggestions on the manuscript; and J. Curran for his continuing support.
30. Use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

24 May 1989; accepted 5 September 1989

p53: A Frequent Target for Genetic Abnormalities in Lung Cancer

TAKASHI TAKAHASHI, MARION M. NAU, ITSUO CHIBA, MICHAEL J. BIRRER, RICHARD K. ROSENBERG, MICHELLE VINOCOUR, MARK LEVITT, HARVEY PASS, ADI F. GAZDAR, JOHN D. MINNA*

Allele loss is a hallmark of chromosome regions harboring recessive oncogenes. Lung cancer frequently demonstrates loss of heterozygosity on 17p. Recent evidence suggests that the p53 gene located on 17p13 has many features of such an anti-oncogene. The p53 gene was frequently mutated or inactivated in all types of human lung cancer. The genetic abnormalities of p53 include gross changes such as homozygous deletions and abnormally sized messenger RNAs along with a variety of point or small mutations, which map to the p53 open reading frame and change amino acid sequence in a region highly conserved between mouse and man. In addition, very low or absent expression of p53 messenger RNA in lung cancer cell lines compared to normal lung was seen. These findings, coupled with the previous demonstration of 17p allele loss in lung cancer, strongly implicate p53 as an anti-oncogene whose disruption is involved in the pathogenesis of human lung cancer.

SPECIFIC CHROMOSOMAL DELETIONS have been reported in various human tumors, suggesting that anti-oncogenes ("tumor suppressor" genes) are important in the pathogenesis of these malignancies (*1*). Lung cancer cells appear to have many such abnormalities. Besides exhibiting many structural and numerical cytogenetic changes (*2*), comparison of tumor and normal tissue DNAs by means of restriction fragment length polymorphism (RFLP) probes revealed loss of heterozygosity in chromosome regions 3p, 13q, and 17p (*3–5*). Allele loss is highly suggestive of the presence of an anti-oncogene (*1*) and loss of the 13q allele provided a signpost leading to the discovery of inactivation of the retinoblastoma gene (*Rb*) in many, if not all, small cell lung cancers (SCLC) (*6*). Similarly, allele loss for chromosome regions 3p and 17p in both SCLC and non–small cell lung cancer (non-SCLC) suggested a search for anti-oncogenes in these chromosome areas. Since p53 is assigned to chromosome region 17p13 (*7*) and recent in vitro studies have suggested the possibility that p53 acts as an anti-oncogene (*8–10*), we explored the status of the p53 gene in lung cancer.

We examined 30 lung cancer cell lines (*11*) (13 SCLC, 14 non-SCLC, 2 extrapulmonary small cell carcinomas, and 1 pulmonary carcinoid) as well as samples from normal lung obtained at the time of surgical resection for lung cancer for p53 abnormalities. We used Southern (DNA) and Northern (RNA) blot analyses with a probe isolated from a normal human p53 cDNA clone (*12*) (Fig. 1 and Table 1). Two of the lung cancer cell lines (H358 and H660) showed homozygous deletions, one (H969) exhibited a genomic p53 DNA rearrangement, and the remainder showed no gross DNA structural abnormalities. H358 and H969 expressed no detectable mRNA even by the ribonuclease (RNase) protection assay (see below), whereas H660 exhibited very low level expression of a truncated 1.4-kb p53 mRNA. Four lines (H526, H82, H676, and H647) without gross structural DNA abnormalities expressed varying levels of abnormally sized mRNAs (2.6, 3.7, 2.3, and 3.7 kb, respectively), suggesting abnormal splicing, initiation, or termination. H676 also expressed a normally sized (2.8-kb) p53 mRNA as well as an abnormally sized one. Others expressed normally sized p53 mRNA at similar levels (for example, H23) or reduced levels (for example, H889) compared to normal lung.

After discovering these gross abnormalities in the p53 gene, we wished to know if lung cancer cells expressing apparently normally sized transcripts contained subtle mutations and whether these occurred in tumor specimens taken directly from patients without intervening cell culture. We therefore performed RNase protection assays (*13*) using three overlapping probes isolated from a normal human p53 cDNA clone, which together covered nearly the entire coding region of the human p53 gene (*12*) (Fig. 2). RNAs from eight normal lung samples showed only full-length protection with the p53M and p53PA probes (*14*). In contrast, a SCLC (T1436) and a non-SCLC (T104) tumor sample showed abnormal RNase

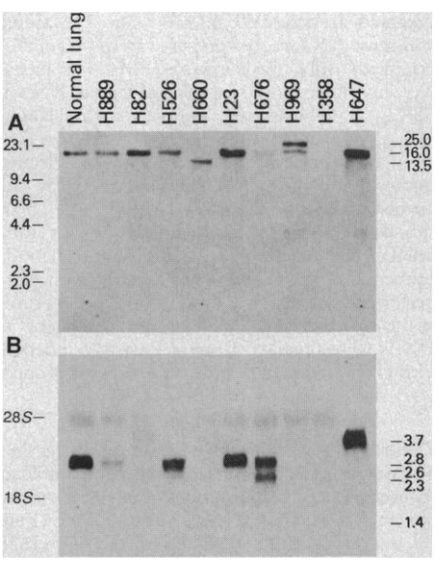

Fig. 1. The p53 DNA status and mRNA expression in lung cancer cell lines and normal human lung. (**A**) Southern blot analysis of p53 Eco RI–digested lung cancer cell line and normal lung DNAs (10 μg per lane). (**B**) Northern blot analysis of p53 in total cellular RNA (10 μg per lane) from lung cancer cell lines and a representative example of normal lung. In (A) numbers on the left indicate marker sizes in kilobases and numbers on the right indicate normal (16 kb) and abnormally sized p53 Eco RI fragments (in kilobases). Ethidium bromide staining showed amount of DNA loaded per lane is approximately equal except H676, which had less loaded than others. In (B) numbers on the right show both normal (2.8 kb) and abnormal p53 mRNA sizes (in kilobases), whereas the 28*S* ribosomal band serves as an internal marker for amount of RNA loaded. The probe used is a 1.8-kb Xba I–Xba I fragment prepared from a normal human p53 cDNA clone, php53c1, labeled with ^{32}P by the random primer technqiue (*26*). Methods for preparing DNA, RNA, and probe fragments along with analysis of Southern and Northern blots were as previously described (*27*). Additional restriction digests with Hind III and Bam HI confirmed the DNA abnormalities found in H358, H969, and H660 with Eco RI (*14*).

T. Takahashi, M. M. Nau, I. Chiba, M. J. Birrer, R. K. Rosenberg, M. Vinocour, A. F. Gazdar, J. D. Minna, National Cancer Institute–Navy Medical Oncology Branch, and Uniformed Services University of the Health Sciences, Bethesda, MD 20814.
M. Levitt, Division of Medical Oncology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15240.
H. Pass, Surgery Branch, National Cancer Institute, Bethesda, MD 20814.

*To whom correspondence should be addressed at the NCI–Navy Medical Oncology Branch, Bldg. 8, Room 5101, Naval Hospital, Bethesda, MD 20814.

cleavage patterns compared to normal lung sample N104 (Fig. 2). In each tumor sample, we identified two protected fragments that together equaled the length of the fully protected fragment, indicating that the abnormalities were point or very small mutations. In the case of T104, the site of cleavage mapped approximately to nucleotide (nt) 720; whereas in T1436 it mapped approximately to nt 540 of the sequence published by Zakut-Houri *et al.* (*12*).

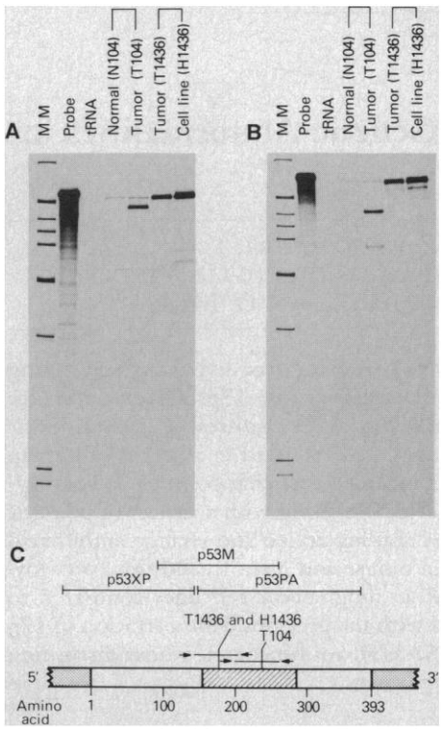

Fig. 2. Demonstration of p53 mutations by RNase protection assay and sequence analysis in normal lung and tumor RNAs of patient 104, (non-SCLC) and tumor and cell line RNAs derived from patient 1436 (SCLC). (**A**) RNase protection assay with probe p53M. (**B**) RNase protection assay with probe p53PA. (**C**) Schematic diagram of p53, including location of RNA probes [p53XP (Xba I–Pvu II, nt −132–433), p53M (Mbo II–Mbo II, nt 276–785) and p53PA (Pvu II–Acc I, nt 433–1127)], oligonucleotides (arrows) used in analysis, and mapped locations of p53 mutations. The nucleotide coordinates are from sequence of Zakut-Houri *et al.* (*12*). For RNase protection assays (A and B) M M (molecular marker) is Hinf I–digested pBR322; probe indicates ^{32}P-labeled RNA probe run alone; tRNA indicates reaction with tRNA in place of cellular RNA. RNAs from tissue samples were prepared as described in (*27*). The ^{32}P-labeled anti-sense RNA probes prepared from p53 cDNA fragments subcloned into pGEM4 (Promega) were hybridized to 10 μg of total cellular RNA at 58°C, the hybrids were digested with RNaseA (40 μg/ml), and protected fragments were analyzed by denaturing polyacrylamide gel electrophoresis followed by autoradiography as described (*13*). For sequence analysis (C), first-strand cDNA synthesis from 10 μg of total cellular RNA with p53-specific oligonucleotide primers and the subsequent PCR reaction were performed essentially as described (*15*). All primers had extraneous nucleotides comprising Eco RI sites at their 5′ ends (*28*). PCR-amplified fragments were subcloned in the Eco RI site of pGEM4; plasmid DNAs were prepared from at least two independent clones from each sample with the Miniprep Kit Plus (Pharmacia), and both strands were sequenced with the GemSeq K/RT System (Promega).

Table 1. Abnormalities of p53 in lung cancer lines. Terms and symbols for mRNA levels are as follows: +, easily detectable p53 transcripts comparable to levels found in normal lung; reduced or trace, greatly reduced amount of transcript compared to normal lung; undetectable, undetectable by both Northern blot analysis and the RNase protection assay. Full designation of the cell lines includes the prefix "NCI". All but H60, H69, H82, H187, H345, H378, and H510 were established from patients before treatment.

Type of mutations	mRNA level	Tumor cell type	Cell line
Homozygous deletion	Undetectable	Bronchioloalveolar	H358
Homozygous deletion with truncated mRNA	Reduced	Extrapulmonary small cell	H660
DNA rearrangement	Undetectable	Adenocarcinoma	H969
Abnormal size mRNA	+	Small cell	H526
	+	Adenocarcinoma	H676
	+	Adenosquamous	H647
	Trace	Small cell	H82
Point or small mutation	+	Small cell	H1436, H1450
	+	Pulmonary carcinoid	H727
	+	Adenocarcinoma	H23
	+	Bronchioloalveolar	H820
	+	Adenosquamous	H125
	+	Large cell	H661
	Reduced	Small cell	H889, H1092
	Reduced	Adenocarcinoma	H920
None detected	Trace	Small cell	H60, H69, H209, N417
	Reduced	Squamous	H520
None detected	+	Small cell	H187, H345, H378
	+	Extrapulmonary small cell	H510
	+	Adenosquamous	H596
	+	Squamous	H226
	+	Large cell	H460, H1385

To confirm the results obtained by the RNase protection assay, we used cDNA synthesis from total cellular RNA coupled with polymerase chain reaction (PCR) (*15*) (Fig. 2). First-strand cDNA was primed with p53-specific oligonucleotides located 3′ of the mutations. Using additional p53-specific oligonucleotides flanking 5′ of the mutations and the original 3′ oligonucleotides as a primer set, we amplified the cDNAs by PCR. The PCR products were cloned into pGEM4, and the inserts were sequenced. In T104 a 3-bp in-frame internal deletion was identified that resulted in the deletion of asparagine at amino acid (aa) 239. When we sequenced the same region of p53 mRNA prepared from the patient's normal lung (N104), no deletion was found and our sequence was in complete agreement with the published normal p53 sequence (*12, 16*). In T1436, a point mutation (T to G at nt 537) was detected that resulted in substitution of a glutamine for a histidine at aa 179. Since we had established both a tumor cell line (H1436) and a normal B-lymphocyte line (BL1436) from this patient, the same RNase protection and sequencing analysis was applied to both cell lines. The identical mutation found in the tumor specimen was detected in the tumor cell line H1436, whereas no mutation was found in the B-lymphocyte line BL1436. In both cases the mutations found by sequencing corresponded to the sites of the lesions detected by the RNase protection assays. In studies of other primary lung cancer samples, we have found over ten additional tumors with mutations in the open reading frame by the RNase protection assay (*14*). These data indicate that the p53 gene abnormalities have occurred in tumors harvested directly from patients, appear to represent somatic events, and are retained in the cultured tumor cells.

To expand these observations, we applied the RNase protection assay to the lung cancer cell lines in order to identify subtle changes similar to those seen in the primary tumor samples. Many examples of such mutations were found (Fig. 3). In four SCLC lines (H889, H1092, H1436, and H1450), five non-SCLC lines (H23, H125, H661, H820, and H920), and one pulmonary carcinoid line (H727), we identified two protected fragments that together equaled the length of the normally protected fragment, indicating that the abnormalities were point or very small mutations. Using the three RNase protection probes, we were able to unambiguously map the approximate sites of all of these mutations. To confirm these

abnormalities, we sequenced three samples with the cDNA and PCR techniques described above. A single base pair mutation was identified in H23, which resulted in the substitution of isoleucine for methionine at aa 246 (transition from ATG to ATC). A similar point mutation in H889, a G to C transition (TGC to TCC), resulted in the substitution of a serine for cysteine at aa 242. In the case of H1450, we also sequenced this region of p53 cDNA from the normal B-lymphocyte line (BL-7) established from the same patient. A point mutation (CTT to CGT, leucine to arginine at aa 194) was identified only in the tumor cell line, H1450, but not in BL-7, again indicating that the mutations occurred as a somatic event. The three RNase protection probes were also applied to the five lung cancer cell lines (H82, H526, H660, H676, and H647) exhibiting abnormal p53 mRNAs (Fig. 1). Abnormal cleavage patterns were easily seen in all except H82 where the very low level of expression required 10 μg of polyadenylated RNA for documentation of a mutant mRNA (*14*).

In tumor line H23 only the abnormal RNase cleavage pattern was seen, indicating expression of only a mutant p53 allele (Fig. 3). However, in H889 the presence of a fully protected band in addition to the smaller bands suggested either the presence of transcripts from normal and mutant alleles or incomplete digestion of the RNA hybrids protected by the mutant allele (*13, 17*). Conveniently, the mutation in H889 created a new Nco I site (GCATGG to CCATGG) (Fig. 3), allowing us to distinguish between these two possibilities. Southern blot analysis with Nco I revealed that only the mutant allele was retained in the genome, whereas analysis of the cDNA and PCR products containing codon 242 of H889 RNA, when digested with Nco I, produced only two subfragments consistent with the expression of only the mutant allele (*14*). In fact, T1436, H1436, H1450, and T104 also showed full-length protection in addition to abnormal subfragments by the RNase protection assay. However, in the cDNA and PCR-generated clones so far examined we found only mutant sequences.

From this study, the p53 gene appears to be frequently affected in all types of lung cancer. We have found gross DNA abnormalities, including homozygous deletions and rearrangements, and major mRNA abnormalities, including absent or trace levels of expression and abnormally sized mRNAs. In addition, a number of different point mutations and a small internal deletion were found in tumor specimens as well as cell lines. The region between aa 156 and aa 288 in human p53 is very homologous (92%) to the comparable region in mouse p53, suggesting the functional importance of this region of the protein (*12*). Thus, it was of interest to find that all of the point or small mutations mapped in this region (Fig. 3). These observations are reminiscent of the *Rb* abnormalities reported in several different tumor types including SCLC (*6, 18*).

Many findings suggest that normal p53 may function as an anti-oncogene. Inactivation of the p53 gene is frequently associated with progression of mouse erythroleukemia induced by Friend leukemia virus (*8*). The ability of an altered p53 gene to cooperate with an activated *ras* gene to transform primary rodent cells can be observed with a number of different p53 mutations, whereas normal p53 from both mouse and man fails to cooperate with *ras* to transform primary rodent cells (*9, 19, 20*). Transgenic mice carrying mutant p53 genes have been shown to develop lung cancers (*21*). Together these observations raise the possibility that mutant p53 may act in a dominant-negative fashion by forming inactive oligomers and sequestering the normal p53 (*9, 20, 22*). In fact, normal p53 acts to suppress cotransformation of *ras* with either mutant p53, E1a, or c-*myc* in primary rodent cells (*10*). It seems likely, therefore, that normal p53 is an important negative growth regulator, and that mutations of the p53 gene may result in the loss of suppression or cooperate with other oncogenes to stimulate neoplastic growth, or both. Two lesions are required to inactivate *Rb* in SCLC. Two lesions in p53 also must have occurred in some lung cancers (those that only have mutant p53). We have not yet resolved whether some lung cancers could express both normal and mutant forms of p53. If both mutant and normal p53 proteins coexist in the cell, the subsequent loss of the normal p53 gene would favor a transformation event.

To determine how common p53 mutations are in the genesis of human tumors, Masuda *et al.* sampled 134 human carcinomas and found DNA rearrangements of the p53 gene only in osteogenic sarcomas (*23*). However, 17p allele loss was reported in over 75% of colon cancers (*24*) and p53 point mutations were found in two colon cancer xenografts selected for such 17p allele loss (*25*). If the 17p allele loss is a surrogate for p53 mutation, the incidence of 17p allele loss (47%) in lung cancer reported by Yokota *et al.* (*5*) indicates this is a frequent event. In the present work we have identified p53 abnormalities in 17 of 30 (57%) lung cancer cell lines, representing all histological types of lung cancer. An additional 17% (5/30) expressed very low levels of p53 mRNA. To date we have studied a panel of 50 lung cancer tumor samples and have

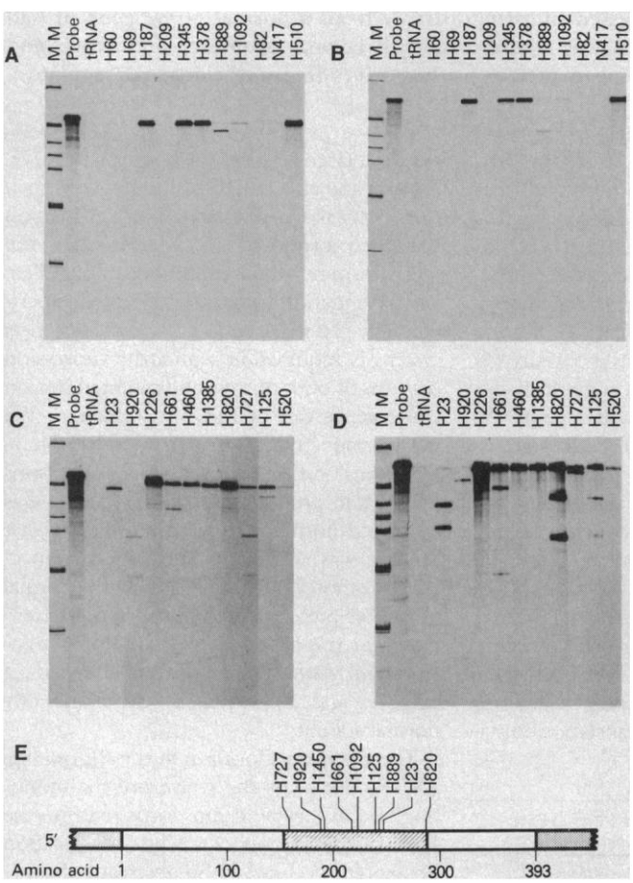

Fig. 3. Demonstration of p53 mutations by the RNase protection assay in lung cancer cell line RNAs, including the location of the mapped mutations within the open reading frame of the p53 gene. RNase protection assay of SCLC (**A** and **B**) and non-SCLC (**C** and **D**) RNAs with probes p53M (A and C) and p53PA (B and D). (**E**) Schematic diagram of p53 (Fig. 2) with mapped locations of respective mutations in various cell lines determined by patterns with the various p53 RNA probes. Methods were as described in the Fig. 2 legend. The cDNA synthesis primer and the PCR primer set were the same as that used in Fig. 2 (*28*).

identified p53 abnormalities in about 20% by the RNase protection assay. In fact, we have probably underestimated the frequency of p53 abnormalities both in tumor samples and cell lines, since the RNase protection assay has a detection rate of <50% for single base pair mutations (*13, 17*).

From recent studies, including this report, it is clear that lung cancers have suffered many alterations to known or suspected anti-oncogenes. In fact, the alterations of the *Rb* and p53 genes, as well as one or more putative anti-oncogenes in chromosome region 3p, appear to coexist in ten SCLC and nine non-SCLC cell lines that we have studied for all three abnormalities (*2, 3, 6, 14*). Using cytogenetic or RFLP analysis, or both, all of the SCLCs and six of the non-SCLCs have 3p deletions or loss of heterozygosity. Probably all of the SCLC cell and at least two of the non-SCLC cell lines have abnormalities of the *Rb* gene. Thus, seven of the SCLC cell lines have all three abnormalities. Although none of the non-SCLC lines have all three abnormalities, four have a p53 and a 3p abnormality and one has a p53 and an *Rb* lesion. Future studies addressing when these lesions develop and whether their correction reverses the malignant phenotype should help us not only to understand the molecular mechanism of initiation and progression of lung cancer but provide new approaches to prevention, diagnosis, prognosis, and possibly therapy.

REFERENCES AND NOTES

1. B. Ponder, *Nature* **335**, 400 (1988).
2. J. Whang-Peng *et al.*, *Science* **215**, 181 (1982).
3. S. Naylor, B. Johnson, J. Minna, A. Sakaguchi, *Nature* **329**, 451 (1987); H. Brauch *et al.*, *N. Engl. J. Med.* **317**, 1109 (1987); B. E. Johnson *et al.*, *J. Clin. Invest.* **82**, 502 (1988).
4. K. Kok *et al. Nature* **330**, 578 (1987).
5. J. Yokota, M. Wada, Y. Shimosato, M. Terada, T. Sugimura, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 9252 (1987); 17p allele loss was found in ten of ten informative cases of SCLC (H. Brauch, J. Minna, B. Zbar, unpublished data).
6. J. W. Harbour *et al.*, *Science* **241**, 353 (1988).
7. O. W. McBride, D. Merry, D. Givol, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 130 (1986); M. Isobe, B. S. Emanuel, D. Givol, M. Oren, C. M. Croce, *Nature* **320**, 84 (1986).
8. M. Mowat, A. Cheng, N. Kimura, A. Bernstein, S. Benchimol, *ibid.* **314**, 633 (1985).
9. P. Hinds, C. Finlay, A. Levine, *J. Virol.* **63**, 739 (1989); M. Green, *Cell* **56**, 1 (1989).
10. C. Finlay, P. Hinds, A. J. Levine, *ibid.* **57**, 1083 (1989); M. Oren, personal communication.
11. M. Brower, D. N. Carney, H. K. Oie, A. F. Gazdar, J. D. Minna, *Cancer Res.* **46**, 798 (1986); D. N. Carney *et al.*, *ibid.* **45**, 2913 (1985).
12. R. Zakut-Houri, B. Bienz-Tadmaor, D. Givol, M. Oren, *EMBO J.* **4**, 1251 (1985).
13. E. Winter, F. Yamamoto, C. Almoguera, M. Perucho, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7575 (1985).
14. T. Takahashi *et al.*, data not shown.
15. P. Doherty, M. Huesca-Contreras, H. Dosch, S. Pan, *Anal. Biochem.* **177**, 7 (1989).
16. P. Lamb and L. Crawford, *Mol. Cell. Biol.* **6**, 1379 (1986).
17. R. M. Myers, Z. Larin, T. Maniatis, *Science* **230**, 1242 (1985).
18. S. Friend *et al.*, *Nature* **323**, 643 (1986); W.-H. Lee *et al.*, *Science* **235**, 1394 (1987); J. M. Dunn, R. A. Phillips, A. J. Becker, B. L. Gallie, *ibid.* **241**, 1797 (1988); E. Y.-HP. Lee *et al.*, *ibid.* p. 218; J. H. Horowitz *et al.*, *ibid.* **243**, 937 (1989).
19. J. R. Jenkins, K. Rudge, P. Chumakov, G. A. Currie, *Nature* **317**, 816 (1985); C. A. Finlay *et al.*, *Mol. Cell. Biol.* **8**, 531 (1988); S. P. Tuck and L. Crawford, *Oncogene Res.* **4**, 81 (1989).
20. D. Eliyahu *et al.*, *Oncogene* **3**, 313 (1988).
21. A. Lavigueur *et al.*, *Mol. Cell. Biol.* **9**, 3982 (1989).
22. B. Rovinski and S. Benchimol, *Oncogene* **2**, 445 (1988).
23. H. Masuda, C. Miller, H. Koeffler, H. Battifora, M. Cline, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 7716 (1987).
24. B. Vogelstein *et al.*, *Science* **244**, 207 (1989).
25. S. Baker *et al.*, *ibid.*, p. 217.
26. A. P. Feinberg and B. Vogelstein, *Anal. Biochem.* **132**, 6 (1983).
27. M. Nau *et al.*, *Nature* **318**, 69 (1985).
28. Primers for cDNA synthesis were prepared with a 380B DNA synthesizer (Applied Biosystems) according to vendor instructions. They included 5′-AATTCAAAGCTGTTCCGTCCCAGTAGATT TACTC for T1436 and H1436; and 5′-ATGCG-AATTCAAAGCTGTTCCGTCCCAGTAGAT for N104 and T104. The PCR primer set for T1436 and H1436 was 5′-ATGCGAATTCCAACTGGC-CAAGACCTGCCCTGTG and 5′-ATGCGAATT-CAGTGTTTCTGTCATCCAAATACTC; the primer set for N104 and T104 was 5′ATGCGAATT-CCATCTTATCCGAGTGGAAGGAAAT and 5-ATGCGAATTCAAAGCTGTTCCGTCCCAGT-AGATT.

29. We thank D. Givol and M. Oren for the php53c1 cDNA clone and helpful discussions; S. Stephenson, H. Oie, E. Russell, and L. Preis for assistance; J. Viallet, T. Winton, D. Ihde, J. Mulshine, and I. Linnoila for help in obtaining tumor and normal lung RNAs and materials for cell culture; J. Battey, F. Kaye, S. Segal, and D. Buchhagen for critical review of the manuscript. Generous support was provided in part by the G. Harold and Leila Y. Mathers Charitable Foundation.

30 June 1989; accepted 11 September 1989

Normal Expression of a Rearranged and Mutated c-*myc* Oncogene After Transfection into Fibroblasts

ADAM RICHMAN AND ADRIAN HAYDAY*

Expression of the c-*myc* oncogene is deregulated in a variety of malignancies. Rearrangement and mutation of the c-*myc* locus is a characteristic feature of human Burkitt's lymphoma. Whether deregulation is solely a result of mutation of c-*myc* or whether it is influenced by the transformed B cell context has not been determined. A translocated and mutated allele of c-*myc* was stably transfected into fibroblasts. The rearranged allele was expressed indistinguishably from a normal c-*myc* gene: it had serum-regulated expression, was transcribed with normal promoter preference, and was strongly attenuated. Thus mutations by themselves are insufficient to deregulate c-*myc* transcription.

IN HUMAN BURKITT'S LYMPHOMA (BL) cells, the region of chromosome 8 that contains c-*myc* is often translocated to the immunoglobulin heavy chain (IgH) locus (*1*). The translocated allele is expressed abnormally, whereas the unrearranged copy is usually transcriptionally silent (*2*). Studies with human peripheral B cells in culture (*3*) and with transgenic mice (*4*) suggest that c-*myc* gene deregulation represents one step in the multistep generation of a fully transformed BL phenotype. All translocated c-*myc* alleles have sequence alterations upstream of exon II (*5, 6*), frequently in the vicinity of the dual promoters P1 and P2 and near the exon I–intron I boundary. In normal cells the latter region contains sites for both attenuation of ongoing transcription (manifested by greater RNA polymerase density on exon I sequences relative to intron I and exon II) and translation initiation of a large form of the c-*myc* protein (*7*). A cause and effect relationship between such mutations and deregulated c-*myc* expression has been proposed (*5, 6*); however the mechanism by which mutations might affect c-*myc* regulation has not been tested experimentally. To make such a test, a transfection system is required in which the expression patterns of both normal and mutated human c-*myc* genes can be directly compared. We now demonstrate that a BL-derived c-*myc*-IgH translocation locus that bore mutations within and proximal to the c-*myc* gene was expressed normally in transfected fibroblast cells: it was accurately transcribed from c-*myc* promoters P1 and P2 with normal promoter preference, transcription was regulated in response to serum, ongoing transcription was attenuated near the end of exon I, and the mRNA had apparently normal stability.

The c-*myc* translocation in the BL cell line AW-Ramos joins the c-*myc* and the immunoglobulin heavy chain locus within the IgM switch region (*8*). The translocation breakpoint occurs 340 bp upstream of c-*myc*

Biology Department, Yale University, New Haven, CT 06511.

*To whom correspondence should be addressed.