of C-*abl* maps to human chromosome 9. It is also known that the genes for the murine and human forms of adenylate kinase (AK-1) map to mouse chromosome 2 and to human chromosome 9, respectively. This may prove significant since the human AK-1 gene has been mapped regionally to the end of the long arm of human chromosome 9, and therefore the C-*abl* gene may be located close by. If this proves to be so, then one must be prepared to inquire whether the human chromosome 9/22 translocation (Philadelphia chromosome) which frequently involves the end 9q and which correlates positively with chronic myeloid leukemia (CML) may alter the expression of the human C-*abl* gene and in turn influence tumor progression in CML.

STEPHEN P. GOFF*
Cancer Center, Massachusetts Institute of Technology, Cambridge 02139
PETER D'EUSTACHIO†
FRANK H. RUDDLE
Department of Biology, Yale University, New Haven, Connecticut 06511
DAVID BALTIMORE
Cancer Center, Massachusetts Institute of Technology

References and Notes

1. S. P. Goff, E. Gilboa, O. N. Witte, D. Baltimore, *Cell* **22**, 777 (1980).
2. N. Rosenberg and D. Baltimore, in *Viral Oncology*, G. Klein, Ed. (Raven, New York, 1980), p. 187.
3. N. Rosenberg, D. R. Clark, O. N. Witte, *J. Virol.* **36**, 766 (1980).
4. O. N. Witte, S. P. Goff, N. Rosenberg, D. Baltimore, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4993 (1980).
5. S. F. Ziegler, C. A. Whitlock, S. P. Goff, A. Gifford, O. N. Witte, *Cell* **27**, 477 (1981).
6. O. N. Witte, N. Rosenberg, D. Baltimore, *Nature (London)* **281**, 396 (1979).
7. J. Wang and D. Baltimore, unpublished observation.
8. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
9. P. D'Eustachio, A. L. M. Bothwell, T. K. Takaro, D. Baltimore, F. H. Ruddle, *J. Exp. Med.* **153**, 793 (1981).
10. D. Meruelo, M. Offer, N. Flieger, *ibid.* **154**, 1201 (1981).
11. J. Michaelson, *Immunogenetics* **13**, 167 (1981).
12. R. W. Ellis, D. DeFeo, T. Y. Shih, M. A. Gonda, H. A. Young, N. Tsuchida, D. R. Lowy, E. M. Scolnick, *Nature (London)* **292**, 506 (1981).
13. M. J. Murray, B.-Z. Shilo, C. Shih, D. Cowing, H. W. Hsu, R. A. Weinberg, *Cell* **25**, 355 (1981); M. Perucchio, M. Goldfarb, K. Shimizu, C. Lama, J. Fogh, M. Wigler, *ibid.* **27**, 467 (1981).
14. W. Bodmer and G. Todaro, personal communication.
15. C. A. Kozak, J. Lawrence, F. H. Ruddle, *Exp. Cell Res.* **105**, 109 (1977).
16. N. H. Ruddle, B. S. Conta, L. Leinwand, C. A. Kozak, F. H. Ruddle, P. Besmer, D. Baltimore, *J. Exp. Med.* **148**, 451 (1978).
17. E. Eicher, *Mouse News Lett.* **56**, 42 (1977).
18. This work was supported by grants GM-09966 (F.H.R.) and CA-14051 (D.B.) from the National Institutes of Health and by American Cancer Society grant VC-41 (D.B.).
* Present address: Columbia University College of Physicians and Surgeons, New York 10032.
† Present address: New York University Medical Center, 550 First Avenue, New York 10016.

14 June 1982; revised 26 August 1982

Mouse c-myc Oncogene Is Located on Chromosome 15 and Translocated to Chromosome 12 in Plasmacytomas

Abstract. *Hybridization studies with viral oncogene probes indicate that c-myc, the cellular gene homologous to the transforming gene of avian myelocytomatosis virus, resides on mouse chromosome 15 and in many plasmacytomas is translocated to the antibody heavy chain gene locus on chromosome 12. The transcriptional orientation of the translocated c-myc sequence is opposite the orientation of the adjacent C_α gene that codes for the heavy chain of immunoglobulin A. The translocated c-myc sequence is not the same oncogene detected in murine plasmacytomas by the NIH-3T3 cell transformation assay.*

Characteristic chromosomal translocations are often associated with lymphoid tumors in both mice and humans (*1*). It has been postulated that these abnormalities may contribute to the generation of tumors by altering the expression of certain cellular genes, referred to as oncogenes (*1*). To further understand the role of translocations in tumorigenesis, it is important to clone the region of DNA containing the translocation. This has been accomplished with the use of murine plasmacytomas, which characteristically have a translocation of the distal part of chromosome 15 to chromosome 12 [t(12;15)] or 6 [t(6;15)] (*1*).

Plasmacytomas are tumors of antibody-producing plasma cells. In diploid antibody-producing cells, there are two chromosomal copies of each antibody gene family. Plasmacytomas, like normal antibody-producing cells, have one chromosome that is rearranged and expresses an antibody polypeptide (productive re-

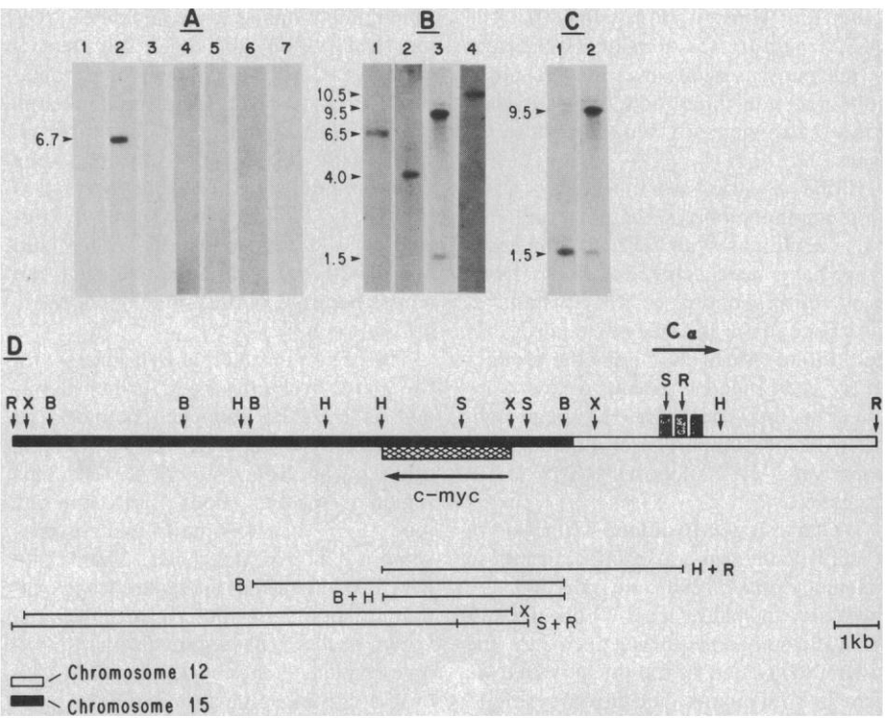

Fig. 1. Hybridization of the v-myc probe to Ch603α30 DNA. (A) Ch603α30 DNA cleaved with Bam HI (lanes 1 to 3) or Hind III (lanes 4 to 7) was subjected to electrophoresis, hybridized on nitrocellulose with the following viral oncogene probes: lane 1, v-fes; lane 2, v-myc; lane 3, v-yes; lane 4, v-src; lane 5, v-erb; lane 6, v-myb; and lane 7, v-abl. (B) Hybridization of Ch603α30 DNA digested with Hind III (lane 1), Bam HI plus Hind III (lane 2), Sst I plus Eco RI (lane 3), and Xba I (lane 4) with the v-myc probe. (C) Ch603α30 DNA digested with Sst I plus Eco RI in both lanes; lane 1 was hybridized with the 3.6-kb Sal I fragment from the v-myc clone that contains sequences from the 5′ part of the gene; lane 2 was hybridized with the 2.2-kb Sal I fragment from the v-myc gene that contains the 3′ part of the gene. [The v-myc probe contains a 1.5-kb Pst I fragment from MCV38 inserted in the Pst I site of pBR322 (*8*).] (D) Map of Ch603α30 DNA. Arrows indicate the 5′ to 3′ direction of transcription for the C_α gene segment and the c-myc sequences. Hatched area denoted "c-myc" shows the region which hybridizes with the v-myc probe, as indicated by the lines below the map showing the fragments from each digest which hybridize to the probe. R indicates Eco RI; B indicates Bam HI; H indicates Hind III, S indicates Sst I, and X indicates Xba I.

0036-8075/82/1224-1319$01.00/0

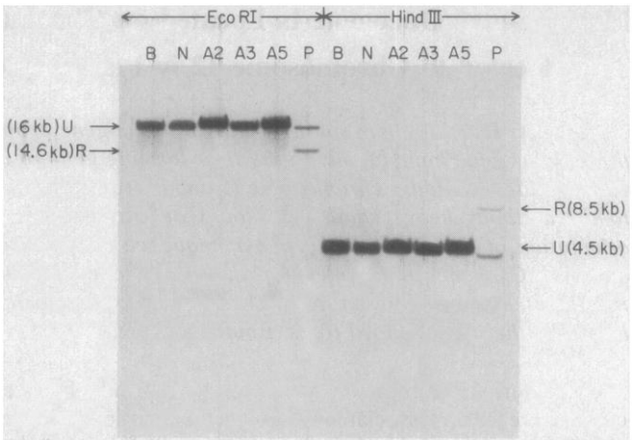

Fig. 2. Restriction enzyme cut DNA from NIH-3T3 cells transformed by S107 plasmacytoma DNA hybridized to the ^{32}P-labeled Msp I fragment. In (*4*) this probe is referred to as nonimmunoglobulin–rearranging DNA (NIRD). Genomic DNA was cut with Eco RI or Hind III, subjected to electrophoresis on 0.55 percent agarose gels, transferred to nitrocellulose filters, and hybridized to the probe. The DNA samples are BALB/c liver DNA (B), NIH-3T3 cell DNA (N), three different primary transformants of NIH-3T3 cells transformed by S107 DNA (A2, A3, A5).

arrangement). The other chromosome is either unrearranged or undergoes a rearrangement that does not result in the expression of an antibody polypeptide (nonproductive rearrangement). Chromosome 12 contains the immunoglobulin heavy chain gene locus and chromosome 6 contains the kappa light chain gene locus (*2*). Thus, it is possible that the t(12;15) and t(6;15) translocations found in murine plasmacytomas may result in some of the nonproductive antibody gene rearrangements found in these tumors.

Clone libraries composed of DNA from immunoglobulin A (IgA)–producing plasmacytomas with t(12;15) translocations have been screened with a DNA probe corresponding to the constant region gene of the IgA heavy chain (C_α). In addition to clones containing the productively rearranged C_α gene, others were obtained that contain the C_α gene with the translocated part of chromosome 15 approximately 2 kilobases (kb) 5′ to the C_α gene (*3–5*).

We have recently cloned a junction of t(12;15) from two IgA-producing mouse plasmacytomas, M603 and M167 (*4*). We have shown that at least 10 kb of DNA from chromosome 15 is joined into the switch (S) region of the unexpressed C_α gene in both clones. The chromosome 15 sequence is identical in the two clones by heteroduplex and restriction enzyme analysis.

To investigate the possibility that an oncogene originally located on mouse chromosome 15 might be present on our clones containing the t(12;15) junction, we utilized the Southern blot method (*6*). Restriction enzyme cut DNA from one of the t(12;15) junction clones, Ch603α30 (α30), was hybridized with various viral oncogene probes. Viral oncogenes (v-onc genes) are contained in the genomes of acutely transforming retroviruses and are derived from homologous cellular genes (c-onc genes). Therefore v-onc probes can be used to test whether the α30 clone contains the corresponding c-onc gene. Figure 1A shows Southern blots of Bam HI or Hind III digests of α30 DNA hybridized with cloned probes containing v-onc genes from feline sarcoma virus (v-fes), Y73 avian sarcoma virus (v-yes), MC29, avian myelocytomatosis virus (v-myc), Abelson leukemia virus (v-abl), avian erythroblastosis virus (v-erb), SR-A avian sarcoma virus (v-src), and avian myeloblastosis virus (v-myb) (*7, 8*). The v-myc probe hybridized to α30 sequences; the others did not. Therefore, we conclude that the mouse homolog of the c-myc oncogene is present on α30.

The region of α30 that hybridizes with the v-myc probe has been further identified as a 2.7-kb sequence bounded on one side by a Hind III site and on the other by an Xba I site (Fig. 1B). This region is located about 1 kb from the junction of chromosome 15 and chromosome 12 in α30 (Fig. 1D). The 1.7-kb Xba I fragment that spans the translocation junction does not hybridize with the v-myc probe, thus demonstrating that v-myc sequences do not occur within 1 kb on the chromosome 15 side of the junction. The Ch167α7 (α7) clone from plasmacytoma M167 has been shown to be homologous to α30 in this region (*4*); therefore α7 also contains the c-myc gene. Furthermore, inspection of the published restriction maps of rearranged C_α clones from plasmacytomas J558 and S107 reveals that these clones appear to be homologous to α30 in this region and also contain the sequences homologous to the v-myc probe (*3–5*). Identification of the c-myc gene on these clones, in conjunction with our previous demonstration that this portion of these clones is located on chromosome 15 in normal cells, now establishes that the mouse c-myc gene is normally located on chromosome 15. In plasma cell tumors it is translocated to a position within 3.5 kb of the C_α gene on chromosome 12 in at least four different plasmacytomas.

Figure 1C shows that v-myc hybridizes to two Sst I plus Eco RI fragments of 1.5 and 9.5 kb. We were able to determine the 5′ to 3′ orientation of the mouse c-myc sequences by probing Sst I digests of α30 with 5′ and 3′ fragments from the v-myc probe generated by restricting the cloned DNA with Sal I (*8*). The 3′ fragment of v-myc hybridizes to both Sst I fragments, while the 5′ fragment of v-myc hybridizes only to the 1.5 kb Sst I fragment (Fig. 1C). Thus the orientation of the c-myc sequences in α30 is opposite to that of the immunoglobulin C_α gene (Fig. 1D). This finding is in agreement with Adams *et al.* (*5*) who demonstrated on their J558 clone that there was a transcription unit in this region which is transcribed from the DNA strand complementary to that which encodes the C_α gene.

Several investigators have detected the presence of activated oncogenes in tumor cell DNA's by utilizing the NIH-3T3 cell transformation assay (*9*). In these experiments, DNA from tumor cells is introduced into NIH-3T3 fibroblast cells by calcium phosphate–mediated transfection. The DNA is transferred in pieces of more than 30 kb and a given recipient cell may integrate from 0.1 to 1 percent of the mass of the donor DNA. Many nonlymphoid tumors and most lymphoid tumors cause morphological transformation of NIH-3T3 cells at a high frequency when their DNA's are transfected into the cells. The appearance of transformation is due to the transfer of an activated oncogene from the tumor cell genome to the NIH-3T3 cell genome.

Utilizing this assay, Lane *et al.* (*10*) have detected an activated oncogene in two murine plasmacytomas, S107 and MOPC315. We wanted to determine whether the oncogene they detected was related to the t(12;15) translocation seen in the IgA-producing plasmacytomas. If the DNA surrounding the translocation point, presumably including the c-myc gene, were responsible for the transformation of the NIH-3T3 cells then we should detect it by hybridizing probes derived from DNA around the translocation point to restriction enzyme cut DNA from the primary NIH-3T3 cell transformants. The results (Fig. 2, lanes P) of

an Eco RI and Hind III cut plasmacytoma DNA that was hybridized to a ^{32}P-labeled Msp I fragment derived from chromosome 15 and within 100 base pairs of the translocation point (*4*) show a rearranged band that corresponds to the translocation as well as an unrearranged band. Both of the rearranged Hind III and Eco RI fragments contain the c-myc gene and sequences from both chromosomes 15 and 12. If the translocated c-myc gene is present in the NIH-3T3 cell transformants, then hybridization of Eco RI or Hind III cut DNA with the Msp I fragment or v-myc probes should show the occurrence of a rearranged band in addition to the unrearranged, NIH-3T3 cell, band. This rearranged band would usually be the same size as the plasmacytoma rearranged band but occasionally might differ since the donor DNA is randomly sheared. Figure 2 shows the result of three different primary transformant DNA's cut with Eco RI and Hind III and hybridized to the Msp I fragment probe (the v-myc probe shows the same result). There is no evidence of a rearranged band in either of the three transformants, only a band corresponding to the unrearranged band that is seen in both the BALB/c and NIH-3T3 cell genomes. The conclusion is that there must be another gene, not the translocated c-myc gene, that is altered in murine plasmacytomas and causes transformation of NIH-3T3 cells in transfection experiments. This situation is similar to the leukemia virus-induced chicken bursal lymphomas in that those tumors have a c-myc gene activated by insertion of retroviral sequences but a different cellular oncogene, unrelated to c-myc, is detected in the NIH-3T3 cell transformation assay (*11*). These findings indicate there are at least two oncogenes that are altered in both murine plasmacytomas and avian bursal lymphomas and are consistent with the current concept of oncogenesis as a multistep process, although their roles in the generation of lymphoid tumors are unknown.

Thus we have demonstrated a specific chromosomal translocation involving an oncogene in transformed cells (*12*). The accumulated evidence from karyotypic analysis and molecular cloning suggests that translocation of the c-myc gene is a consistent feature of murine plasmacytomas and seems likely to play a role in the genesis of these tumors. Two other types of alterations of the c-myc gene have been discovered in other tumors. In chicken bursal lymphomas, the c-myc gene is altered by insertion of avian leukosis virus either 5′ or 3′ to the gene. The result is an increase in the number of c-myc transcripts with respect to that seen in various untransformed cells (*13*). Amplification of the c-myc gene has been demonstrated in the human promyelocytic leukemia line HL-60 (*14*). Amplification of the gene also is accompanied by an increase in the number of transcripts. In plasmacytomas, the precise effect of the translocation on the c-myc gene is unknown; however, this rearrangement could potentially alter transcriptional regulatory sequences, promoter sequences, splice sequences, or coding sequences. In fact, Adams *et al.* (*5*) report data indicating that the translocated c-myc gene synthesizes transcripts that differ in size from those produced by the untranslocated gene. The availability of clones containing the translocated c-myc gene should allow us to examine the nature and concentration of c-myc transcripts from the rearranged chromosome and determine whether other important alterations in the c-myc sequence accompany the translocation.

STEPHEN CREWS
RICHARD BARTH
LEROY HOOD

Division of Biology,
California Institute of Technology,
Pasadena 91125

JOHN PREHN
KATHRYN CALAME

Department of Biological Chemistry,
University of California, Los Angeles,
Medical School, Los Angeles 90025

References and Notes

1. G. Klein, *Nature (London)* **294**, 313 (1981).
2. P. D'Eustachio, D. Pravtcheva, K. Marcu, F. H. Ruddle, *J. Exp. Med.* **151**, 1545 (1980); T. Meo, J. Johnson, C. V. Beechey, S. J. Andrews, J. Peters, A. G. Searle, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 550 (1980); D. Swan, P. D'Eustachio, L. Leinwand, J. Seidman, D. Keithley, F. H. Ruddle, *ibid.* **76**, 2735 (1979).
3. I. R. Kirsch, J. V. Ravetch, S.-P. Kwan, E. E. Max, R. L. Ney, P. Leder, *Nature (London)* **293**, 585 (1981); L. Harris, R. B. Lang, K. B. Marcu, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4175 (1982).
4. K. Calame, S. Kim, P. Lalley, R. Hill, M. Davis, L. Hood, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
5. J. Adams, S. Gerondakis, E. Webb, J. Mitchell, O. Bernard, S. Cory, *ibid.*, in press.
6. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
7. C. J. Sherr, L. A. Fedele, M. Oskarsson, J. Maizel, G. Vande Woude, *J. Virol.* **34**, 200 (1980); N. Kitamura, A. Kitamura, K. Toyoshima, Y. Hirayama, M. Yoshida, *Nature (London)* **297**, 205 (1982); A. Srinivasan, E. P. Reddy, S. A. Aaronson, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2077 (1981); B. Vennström, L. Fanshier, C. Moscovici, J. M. Bishop, *J. Virol.* **36**, 575 (1980); W. J. DeLorbe, P. A. Luciw, H. M. Goodman, H. E. Varmus, J. M. Bishop, *ibid.* **36**, 50 (1980); L. M. Souza *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5177 (1980).
8. B. Vennström, C. Moscovici, H. M. Goodman, J. M. Bishop, *J. Virol.* **39**, 625 (1981).
9. For review see G. M. Cooper, *Science* **217**, 801 (1982).
10. M. A. Lane, A. Sainten, G. M. Cooper, *Cell* **28**, 873 (1982).
11. G. M. Cooper and P. E. Neiman, *Nature (London)* **292**, 857 (1981).
12. G. L. C. Shen-Ong, E. J. Keath, S. P. E. Piccoli, and M. D. Cole (*Cell*, in press) have also shown that c-myc is translocated in murine plasmacytomas.
13. W. S. Hayward, B. G. Neel, S. M. Astrin, *Nature (London)* **290**, 475 (1981); G. S. Payne, J. M. Bishop, H. E. Varmus, *ibid.* **295**, 209 (1982).
14. S. Collins and M. Groudine, *ibid.* **298**, 679 (1982); R. D. Favera, F. Wong-Staal, R. C. Gallo, *ibid.* **299**, 61 (1982).
15. We thank M. Lane and G. Cooper for providing the S107/NIH-3T3 transformed cell lines and S. Aaronson, J. M. Bishop, M. Cline, and D. Slamon for providing cloned oncogenes. Supported by NIH grants (L.H. and K.C.) and by a postdoctoral fellowship from the Anna Fuller Fund (R.B.).

21 October 1982

Variable Ultrasound Echogenicity in Flowing Blood

Abstract. *Real-time ultrasound imaging of large abdominal veins revealed bloodstream echogenicity of variable intensity. This variability is largely due to the entrance and persistance of tributary blood currents that show different echogenicity. Red cell aggregation is probably an important cause of bloodstream echoes and their variable intensity.*

Ultrasound reflective imaging of flowing bloodstreams has revealed low-amplitude echoes (*1–3*). In real-time scanning, such echoes have been observed to move with the flow. In this report we describe what we believe to be a previously unreported finding: ultrasonic echoes from the bloodstream of large veins show zonal variability in intensity related to inflow from tributaries. By scanning along veins we found that the echogenicity of the blood in a large vein is significantly affected by the number and intensity of echoes from blood entering from tributaries. Thus tributary blood that is hypoechogenic relative to blood in the collecting vein produces a hypoechogenic current that moves downstream in the larger vein and may be traced directly to the tributary. Similar flows have been seen in tributary blood hyperechogenic with respect to the prevailing echogenicity of the main venous channel.

An ultrasonic scanner (*4*) was used to scan directly the surfaces of the inferior vena cava and portal veins surgically exposed in eight dogs anesthetized with nitrous oxide and halothane. Figure 1A shows two distinct hypoechogenic zones in an ultrasound cross section of the

0036-8075/82/1224-1321$01.00/0