subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and about 50 μg of γ-IFN was recovered from gel slices in the 18,000 M_r region. Rabbits were injected with this dose of antigen every 2 weeks for 10 weeks. Six weeks after the last immunization, the animals received booster immunizations consisting of 200 μg of γ-IFN electrocluted from the SDS-PAGE slices. The antiserum contained 10^5 neutralizing units per milliliter.

9. P. W. Gray *et al.*, *Nature (London)* **295**, 503 (1982). Recombinant human γ-IFN (Genentech Inc.), specific activity 2×10^7 U/mg, assayed in human lung carcinoma A 549 infected with encephalomyelitis virus.
10. F. C. Ehrman *et al.*, *Science* **101**, 377 (1945).
11. H. Schellekens, A. de Reus, P. H. v.d. Meide, *J. Med. Primatol.* **13**, 235 (1984). The experiment with chimpanzees was performed at the Laboratory of Experimental Medicine and Surgery in Primates (LEMSIP), New York University Medical Center, under the veterinary supervision of C. J. Mahoney and E. Muchmore.
12. M. R. Hollingdale *et al.*, *Am. J. Trop. Med. Hyg.* **32** (No. 4), 685 (1983).
13. H. W. Murray *et al.*, *J. Immunol.* **134**, 1619 (1985).
14. C. F. Nathan, H. W. Murray, M. F. Wiebe, B. Y. Rubin, *J. Exp. Med.* **158**, 670 (1983); C. Nathan *et al.*, *ibid.* **160**, 600 (1984); R. D. Schreiber and A. Celada, *Lymphokines* **11**, 87 (1985).
15. E. R. Pfefferkorn and P. M. Geyre, *Infect. Immun.* **44**, 211 (1984); G. I. Byrne and D. A. Krueger, *ibid.* **42**, 1152 (1983); J. Turco and H. H. Winkler, *J. Exp. Med.* **158**, 2159 (1983); *Infect. Immun.* **45**, 781 (1984); L. M. de la Maza *et al.*, *J. Immunol.* **135**, 4198 (1985).
16. E. R. Pfefferkorn, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 908 (1984).
17. A. H. Cochrane *et al.*, in *Malaria*, J. Kreier, Ed. (Academic Press, New York, 1980), pp. 163–202; R. S. Nussenzweig and V. Nussenzweig. *Philos. Trans. R. Soc. London B* **307**, 117 (1984).
18. E. A. Ojo-Amaize, J. Vilcek, A. H. Cochrane, R. Nussenzweig, *J. Immunol.* **133**, 1005 (1984).
19. A. Rhodes-Feuillette *et al.*, *C. R. Acad. Sci.* **293**, 635 (1981); E. A. Ojo-Amaize *et al.*, *J. Immunol.* **127**, 2296 (1981).
20. W. A. Krotoski *et al.*, *Am. J. Trop. Med. Hyg.* **31**, 1291 (1982).
21. D. F. Clyde, *Bull. WHO* **59**, 391 (1981).
22. J. P. Vanderberg, R. S. Nussenzweig, H. Most, *J. Parasitol.* **54**, 1009 (1968).
23. Blood samples (from the ventral vein and artery of the tail) were centrifuged, the red blood cells were washed once with PBS, resuspended in 1 ml of PBS containing 0.015% (w/v) saponin (Sigma), incubated at 37°C for 5 minutes, and centrifuged. Normal rat liver DNA (50 μg) was added as a carrier to the pellet (parasites plus white cell nuclei). The cell pellet was homogenized with 150 m*M* NaCl, 10 m*M* EDTA, and 1% (w/v) SDS, and sodium perchlorate was added to 0.05*M*. A single extraction with an alcohol-phenol mixture was performed. The DNA in the aqueous phase was ethanol-precipitated and redissolved in water. The DNA in each blood sample was immobilized in 2.5-cm-diameter, 0.45-μm pore size nitrocellulose filters (Millipore) and hybridized with a genomic 2.2-kb repetitive *P. berghei* DNA probe, labeled with ^{32}P by nick translation. Background radioactivity was calculated from filters containing DNA from normal mouse blood and carrier rat DNA. A standard hybridization curve was constructed by probing triplicate tenfold dilutions of blood-stage *P. berghei* DNA. A linear relation between the radioactivity associated with the filters and the amount of parasite DNA immobilized was always obtained in the range of 100 pg to 1 μg of *P. berghei* DNA. The amounts of parasite DNA associated with the experimental filters were calculated by referring their specific radioactivity to the standard curve and expressing them as the means of the nanograms of *P. berghei* DNA detected in five animals.

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Deregulation of c-*myc* by Translocation of the α-Locus of the T-Cell Receptor in T-Cell Leukemias

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Two human T-cell leukemias carrying a t(8;14)(q24;q11) chromosome translocation were studied for rearrangements and expression of the c-*myc* oncogene. For one leukemia, rearrangement was detected in a region immediately distal (3′) to the c-*myc* locus; no rearrangements of c-*myc* were observed in the second case (DeF). However, studies with hybrids between human and mouse leukemic T cells indicated that in the leukemic cells of DeF, the breakpoint in chromosome 14 occurred between genes for the variable (V_α) and the constant (C_α) regions for the α chain of the T-cell receptor. The C_α locus had translocated to a region more than 38 kilobases 3′ to the involved c-*myc* oncogene. Since human c-*myc* transcripts were expressed only in hybrids carrying the 8q+ chromosome but not in hybrids containing the normal chromosome 8, it is concluded that the translocation of the C_α locus 3′ to the c-*myc* oncogene can result in its transcriptional deregulation.

SOME HUMAN T-CELL MALIGNANCIES carry specific chromosome rearrangements, predominantly translocations and inversions, that involve chromosome region 14q11.2 (*1*), the location of the locus for the α-chain of the T-cell receptor (*2*). One of the most common chromosome alterations in acute lymphocytic leukemia of the T-cell type is a t(11;14)(p13;q11) chromosome translocation (*3*). We have shown previously that the chromosome break at band 14q11 in these tumors directly involves the locus for the T-cell receptor between the genes for the variable (V_α) and for the constant (C_α) regions of the α-chain of the T-cell receptor and that the V_α genes are proximal and the C_α gene is distal to the 14q11 chromosome breakpoints (*4*). Thus the orientation of the α-locus of the T-cell receptor relative to the centromere is the opposite of that of the human immunoglobulin heavy-chain locus on chromosome 14 (*5*).

Recently a translocation between chromosome 8 and 14, with breakpoints at 8q24 (the locus of the c-*myc* oncogene) (*6*), and 14q11 (the locus for the α-chain of the T-cell receptor) (*2*), has been described in several T-cell neoplasms (*7–8*). These findings suggest that the locus for the α-chain may be involved in c-*myc* deregulation in some T-cell malignancies, similarly to the role of the human immunoglobulin loci in c-*myc* and *bcl*-2 deregulation in Burkitt lymphoma (*9*) and in other B-cell malignancies (*10*), respectively.

We have examined two cases of T-cell leukemias with a chromosome translocation t(8;14) involving band 14q11 and the distal long arm of chromosome 8. Figure 1A

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Table 1. Human genes in DeF-BW5147 cell hybrids.

Cell lines	*mos*	*myc*	IgH	C_α	V_α	NP	Human chromosomes*				Human c-*myc* transcripts
							8	8q+	14	14q−	
DeF	+	+	+	+	+	+	++	++	++	++	+++
BW5147	−	−	−	−	−	−	−	−	−	−	−
563 BC5	+	+	+	+	+	+	+	−	++	++	−
563 BB4	+	+	+	+	+	+	±	−	++	+	ND
563 BD3	+	+	−	−	+	+	+	−	−	++	−
563 AA1	+	+	−	−	+	+	+	−	−	+	ND
563 BB2	+	+	+	+	−	−	−	+	−	−	ND
563 BD2	−	−	−	−	+	+	−	−	−	++	ND
563 BA5-BC10	+	+	+	+	−	−	−	++	−	−	++
563 BA5-BB3	+	+	+	+	−	−	ND†	ND	ND	ND	ND
563 BA5-DE7	+	+	+	+	+	+	±	+	+	++	ND
563 AC3	+	+	+	+	+	+	−	+	−	++	+

*Percentages of metaphases containing the relevant human chromosome. A minimum of 18 metaphases of each hybrid were examined. −, none; ±, 1 to 10%; +, 10 to 30%; ++, >30%. †ND, not done. For human c-*myc* transcripts +'s and −'s were determined on the basis of analysis of the gel in Fig. 4.

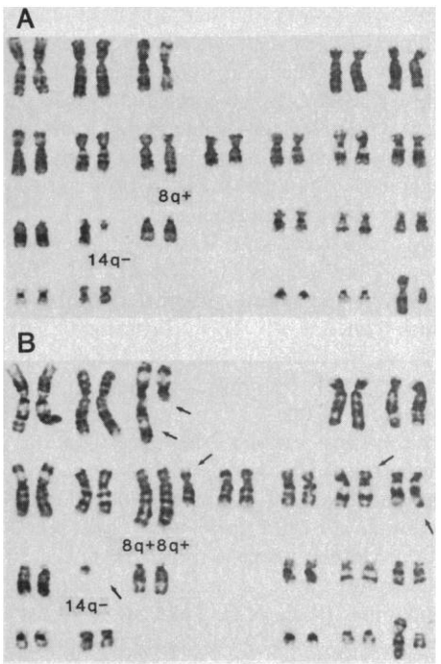


Fig. 1. (A) Representative karyotype of leukemic cells from patient DeF with t(8;14) translocation and pericentric inversion of chromosome 9 as the only abnormalities, the latter was shown to be a constitutional variant in normal T cells during clinical remission. The breakpoint on chromosome 14 is at band q11, and the segment translocated to chromosome 8 reflects loss, and possible rearrangement, of chromosome 14 material. (B) Representative karyotype of T-cell leukemia cell line SKW-3 with t(8;14)(q24;q11). In addition to two copies of the 8q+ chromosome and a 14q−, resulting from the translocation, other abnormalities (arrows) include a t(3q;3q), a t(8p;11p), a 12q+, and absence of the other chromosome 14. These results are consistent with the previous cytogenetic analysis of SKW-3 cells (*8*).

shows the karyotype of leukemic cells from the bone marrow of a case (DeF), with the t(8;14) and a constitutional inversion of chromosome 9 as the only alterations. This patient is a boy with an acute lymphocytic leukemia (ALL) of the T-cell type. The karyotype of a cell line, SKW-3, derived from a 55-year-old male with T-cell chronic lymphocytic leukemia (CLL) is shown in Fig. 1B. The SKW-3 leukemia cells carried two copies of the 8q+ chromosome derived from a t(8;14)(q24;q11) and had additional chromosomal abnormalities.

By cleavage of DeF DNA with Bam HI, Eco RI, and Hind III and hybridization of the fragments with a human c-*myc* cDNA clone (Ryc 7.4) (*9, 11*), we determined that the c-*myc* gene was in its germline configuration in DeF cells. Since the Bam HI site 5′ to the c-*myc* gene is 14.5 kilobases (kb) from the first exon of c-*myc* (*12*), these results indicate that the breakpoint was either more than 14.5 kb 5′ to the first exon or was 3′ to the c-*myc* gene. We then used three probes (pCA1.7S, pPA1.3SB, and pPA0.2S) specific for the flanking region 3′ to the c-*myc* oncogene (Fig. 2A) to determine whether the rearrangements occurred within 38 kb 3′ to the c-*myc* oncogene. We did not detect rearrangements within this region of DNA in DeF cells.

On the other hand, we detected rearrangements distal (3′) to the c-*myc* gene in SKW-3 cells (Fig. 2B). The breakpoint in these cells was between the first Eco RI and the first Hind III site 3′ to the c-*myc* oncogene (Fig. 2A). Since the orientation of the λ and κ immunoglobulin loci (*13, 14*) and of the locus for the α-chain of the T-cell receptor is the same (*4*), the present result is consistent with the "variant" t(8;22) and the t(2;8) chromosome translocations in Burkitt lymphomas, where the breakpoints are 3′ to the involved c-*myc* oncogene (*13, 15*). Thus there appears to be heterogeneity in breakpoints on chromosome 8 in T-cell leukemias with a t(8;14) and in Burkitt lymphoma (*16*).

To determine whether the breakpoint in DeF leukemic cells, as predicted on the basis of the orientation of the α-chain locus of the T-cell receptor (*4*), is 3′ to the c-*myc* oncogene and whether the breakpoint in T-cell leukemias carrying the t(8;14) chromosome translocation involves the locus for the α-chain directly, we fused DeF leukemic cells with BW5147 mouse T-cell leukemia cells that are deficient in hypoxanthine phosphoribosyltransferase (*4*). The hybrids were assayed for the presence of human chromosomes; for the c-*mos* oncogene, which is located at band 8q11 (*17*); for the c-*myc* oncogene; for the V_α and C_α regions; and for the expression of nucleoside phosphorylase (NP), the human constitutive isozyme that has been localized to a region of chromosome 14 proximal to the V_α genes at 14q11 (*4*).

Hybrids 563 BB2 and 563 BA5-BC10 (that have retained the 8q+ chromosome) contained the human c-*myc* oncogene and the C_α gene, but had lost the V_α gene and NP expression (Table 1 and Fig. 3). On the contrary, hybrids 563 BD3, 563 AA1, and 563 BD2 (that have retained the 14q− chromosome) contained V_α genes and expressed human NP but had lost the human c-*myc* and the C_α gene. Since hybrid cells carrying the 8q+ chromosome in the absence of both the normal chromosome 8 and the 14q− chromosome contain the germline c-*myc*, while hybrids with only the 14q− chromosome have lost c-*myc*, the chromosome 8 breakpoint in the DeF leukemic cells must be 3′ (distal) to the involved c-*myc*. This is similar to the variant chromosome translocations in Burkitt lymphomas (*13, 14*). We have previously shown that the 5′ end of the c-*myc* oncogene is more proximal than its 3′ end at band q24 of chromosome 8 (*6*). In addition, since hybrid cells containing the 8q+ chromosome in the absence of the other relevant human chromosomes also retained all three DNA segments (pCA1.7S, pPA1.7SB, and pPA0.2S) 3′ to the c-*myc* oncogene (data not shown), we conclude that the chromosome breakpoint in DeF cells is more than 38 kb 3′ to the involved c-*myc* oncogene. Finally, these results indicate that the chromosome breakpoint on chromosome 14 in DeF cells is between the V_α and the C_α genes.

We have previously shown that transloca-

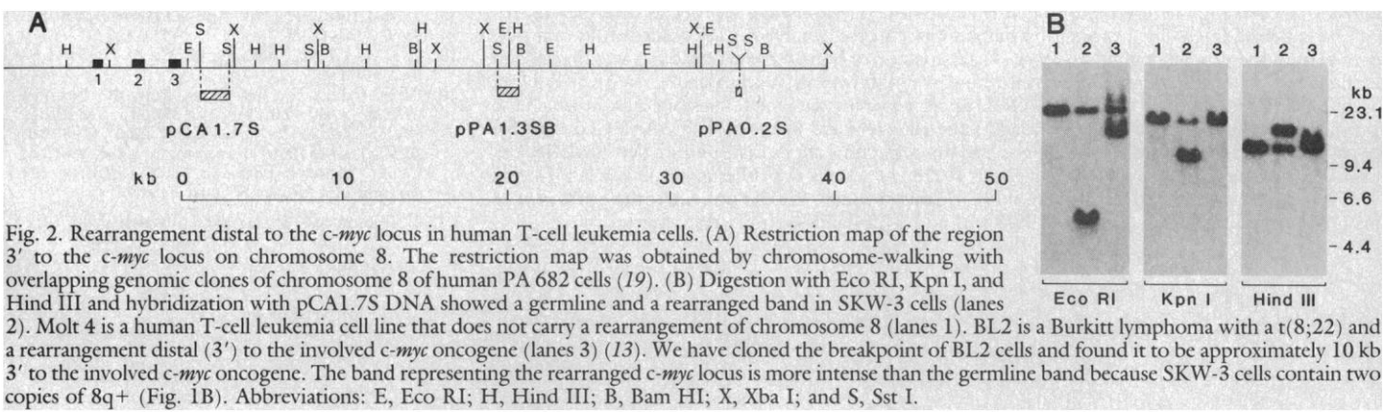
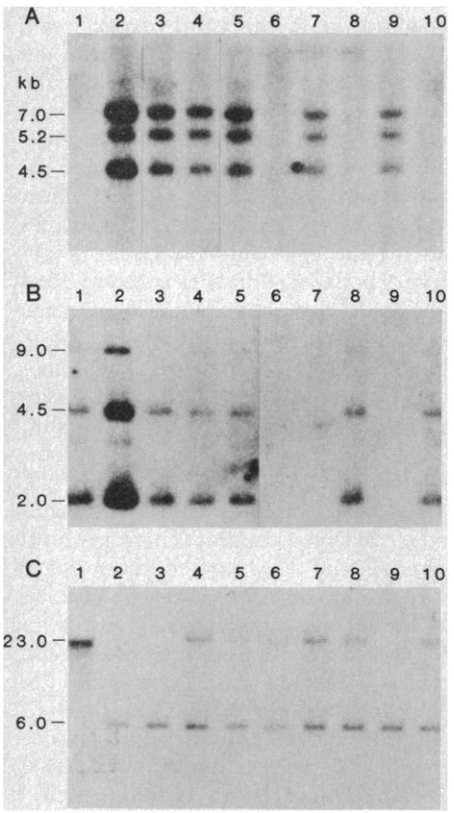


Fig. 2. Rearrangement distal to the c-*myc* locus in human T-cell leukemia cells. (A) Restriction map of the region 3′ to the c-*myc* locus on chromosome 8. The restriction map was obtained by chromosome-walking with overlapping genomic clones of chromosome 8 of human PA 682 cells (*19*). (B) Digestion with Eco RI, Kpn I, and Hind III and hybridization with pCA1.7S DNA showed a germline and a rearranged band in SKW-3 cells (lanes 2). Molt 4 is a human T-cell leukemia cell line that does not carry a rearrangement of chromosome 8 (lanes 1). BL2 is a Burkitt lymphoma with a t(8;22) and a rearrangement distal (3′) to the involved c-*myc* oncogene (lanes 3) (*13*). We have cloned the breakpoint of BL2 cells and found it to be approximately 10 kb 3′ to the involved c-*myc* oncogene. The band representing the rearranged c-*myc* locus is more intense than the germline band because SKW-3 cells contain two copies of 8q+ (Fig. 1B). Abbreviations: E, Eco RI; H, Hind III; B, Bam HI; X, Xba I; and S, Sst I.

tion of the c-*myc* locus to the heavy-chain locus on chromosome 14 (*6*), or the translocation of either the λ or the κ locus to the c-*myc* oncogene on chromosome 8 (*13, 14*), results in the transcriptional deregulation of the c-*myc* oncogene involved in the translocation (*9, 13, 14*). In Burkitt lymphoma, the c-*myc* oncogene involved in the translocation fails to respond to the normal transcriptional control and is expressed constitutively at elevated levels (*9, 13, 14*) while the c-*myc* oncogene on the normal chromosome 8 in Burkitt lymphoma is transcriptionally silent (*9, 13, 14*). Therefore, we have examined the expression of the c-*myc* oncogene involved in the t(8;14) chromosome translocation versus that of the c-*myc* oncogene on the normal chromosome 8 in somatic cell hybrids between BW5147 mouse leukemic and DeF human leukemic cells.

We have previously shown that the transcripts of the mouse and the human c-*myc* oncogene can be distinguished by S_1 nuclease protection experiments (*9*). Hybrid 563 BA5-BC10 (containing the 8q+ chromosome) expressed human c-*myc* transcripts while hybrids 563 BC5 and 563 BD3 (containing the normal 8) did not (Fig. 4, lanes 5, 6, and 7). The lower c-*myc* expression in hybrid 563 BA5-BC10 compared to that of the parental DeF cells is due to the presence of the 8q+ chromosome in only 16 of 28 metaphases examined in the hybrid and to the fact that the parental mouse BW5147 cell is near tetraploid. Thus, the involved human c-*myc* gene is diluted at least four- to sixfold in the hybrid. Hybrid 563 AC3, which contains the 8q+ chromosome in 10% of its cells but has lost the normal chromosome 8, also expressed human c-*myc* transcripts (Table 1). The results indicate that the translocation of the C_α locus of the T-cell receptor to a region 3′ to the c-*myc* oncogene results in its transcriptional deregulation in a T-cell background. This observation closely parallels previous findings concerning c-*myc* deregulation in Burkitt lymphomas (*9, 13, 14*). Thus the locus for the α-chain of the T-cell receptor seems to contain genetic elements capable of activating gene transcription *in cis* over considerable chromosomal distances.



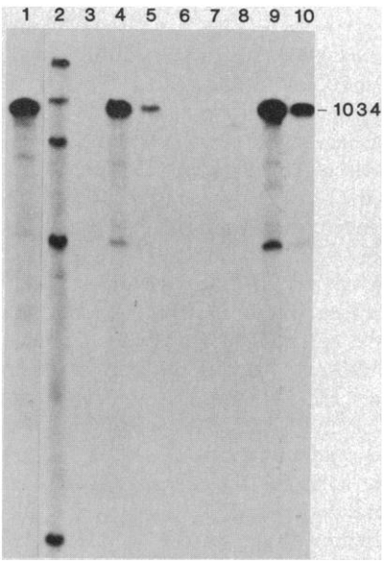


Fig. 3 (left). Southern blotting analysis of hybrid clones. (A) Hybridization of Hind III–digested cellular DNA with the V_α probe (*4*). Lane 1, BW5147 DNA; lane 2, DeF DNA; lanes 3–10, hybrid DNA's (lane 3, 563 BC5; lane 4, 563 BD3; lane 5, 563 BD2; lane 6, 563 BA5 BC10; lane 7, 563 AA1; lane 8, 563 BB2; lane 9, 563 BA5 DE7; lane 10, 563 BA5-BB3). (B) Hybridization of Hind III–digested cellular DNA with the C_α probe (*4*). DeF DNA is in lane 2 and BW 5147 DNA is in lane 6 and the hybrid DNA's are: lane 1, 563 BA5-BC10; lane 3, 563 BA5-BB3; lane 4, 563 BA5-DE7; lane 5, 563 BB4; lane 7, 563 BD3; lane 8, 563 BC5; lane 9, 563 AA1; lane 10, 563 BD2. (C) Hybridization of Bam HI–digested cellular DNA hybridized to the c-*myc* cDNA (Ryc 7.4) probe (*6*). Lane 1, DeF DNA; lane 2, BW5147 DNA; hybrid DNA's are in lanes 3–10 (lane 4, 563 BA5-BC10; lane 5, 563 BB4; lane 6, 563 BB2; lane 7, 563 BD3; lane 8, 563 BC5; lane 9, 563 BD2; lane 10, 563 BA5 BB3). Fig. 4 (right). S1 nuclease protection analysis of RNA expressed in parental and hybrid cells. The S1 probe was pRyc 7.4, a human c-*myc* cDNA clone that carries a 1.2-kb insert of the human c-*myc* cDNA in pBR322 (*9*). The pRyc 7.4 plasmid DNA was digested by Bcl I, 5′ end-labeled with ^{32}P and used as a probe as described (*9, 13, 14*). The expected fragment protected by human c-*myc* messenger RNA (1034 nucleotides) encompasses most of the protein-coding sequence (*9*). Expression of human c-*myc* transcripts was detected in DeF human leukemia cells (lane 1); in Jurkat human T-cell lymphoma cells (lane 9); in SKW-3 T-cell leukemia cells (lane 4); in GM1500-6TG-OUB tumorigenic human lymphoblastoid cells, in which the expression of c-*myc* transcripts is very elevated (lane 10) (*18*); and in hybrid 563 BA5-BC10 (lane 5). The mouse BW5147 leukemic cells express mouse c-*myc* transcripts that are not detectable by the human c-*myc* probe (lane 8) as shown previously (*9, 13, 14*). The size marker in lane 2 is 5′ ^{32}P-labeled 0 × 174 digested with Hae III. In lane 3, no RNA was included. We did not detect the expression of human c-*myc* transcripts in hybrids 563 BD3 and 563 BC5 (lanes 6 and 7, respectively).

REFERENCES AND NOTES

1. L. Zech *et al.*, *Int. J. Cancer* **32**, 43 (1981); F. Hecht, R. Morgan, B. K.-M. Hecht, S. D. Smith, *Science* **226**, 1445 (1984).
2. C. M. Croce *et al.*, *Science* **227**, 1044 (1985).
3. D. Williams *et al. Cell* **36**, 101 (1984).
4. J. Erikson, D. Williams, J. Finan, P. C. Nowell, C. M. Croce, *Science* **229**, 784 (1985).
5. J. Erikson, D. L. Williams, J. Finan, P. C. Nowell, C. M. Croce, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5611 (1982).
6. R. Dalla-Favera *et al.*, *ibid.*, p. 7824.
7. C. J. F. Coubet *et al.*, *C. R. Acad. Sci. Ser. B.* **300**, 171 (1985).
8. E. A. Shima *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
9. K. Nishikura *et al.*, *ibid.* **80**, 4822 (1983); A. ar-Rushdi *et al.*, *Science* **222**, 390 (1983).
10. Y. Tsujimoto, J. Cossman, E. Jaffe, C. M. Croce, *Science* **228**, 1440 (1985).
11. J. Erikson, A. ar-Rushdi, H. Drwinga, P. C. Nowell, C. M. Croce, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 820 (1983).
12. L. C. Showe *et al.*, *Mol. Cell. Biol.* **5**, 501 (1985).
13. C. M. Croce *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 6922 (1983).
14. J. Erikson *et al.*, *ibid.*, *p.* 7581.
15. B. S. Emanuel *et al.*, *Nucleic Acids Res.* **13**, 381 (1985).
16. C. M. Croce and P. C. Nowell, *Blood* **65**, 1 (1985).
17. J. F. Coubet *et al.*, *EMBO J.* **4**, 2245 (1985).
18. C. M. Croce *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3170 (1984).
19. L. K. Sun, L. C. Showe, C. M. Croce, *Nucleic Acids Res.*, in press.

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