RESEARCH ARTICLE

DNA Rearrangement and Altered RNA Expression of the c-*myb* Oncogene in Mouse Plasmacytoid Lymphosarcomas

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Abstract. *Three types of tumors termed plasmacytomas (ABPC's), lymphosarcomas (ABLS's), and plasmacytoid lymphosarcomas (ABPL's) arise in BALB/c mice treated with pristane and Abelson murine leukemia virus (A-MuLV). While most ABPC's and ABLS's contain integrated A-MuLV proviral genome and synthesize the v-*abl *RNA, most ABPL's do not. The ABPL tumors were examined for the expression of other oncogenes that may be associated with their transformed state, in the absence of transforming virus. These tumors expressed abundant c-*myb *RNA of unusually large size and showed DNA rearrangements of the c-*myb *locus.*

Proto-oncogenes, or c-oncogenes, are cellular DNA sequences homologous to the transforming genes (v-oncogenes) of acute transforming viruses (*1*). These genes are highly conserved through vertebrate evolution (*2*) and are thought to code for proteins that are associated with the control of cell growth, differentiation, and development (*3*). Mutations, deletions, or altered rates of expression of these genes are frequently associated with the development of neoplasia.

During studies with Abelson murine leukemia virus (A-MuLV), we and others observed that this virus induces in adult BALB/c mice a variety of lymphoid neoplasms predominantly of the pre–B cell series (ABLS tumors) (*4, 5*). However, when the mice are previously injected with pristane, which induces intraperitoneal granulomatous tissue (*6*), this virus also rapidly induces plasmacytomas (ABPC tumors) (*7*) and, occasionally, a morphological subset of lymphosarcomas characterized by plasmacytoid cytoplasm but with very little immunoglobulin production (ABPL tumors) (*8*).

A preliminary experiment indicated that ABLS's and ABPC's synthesized abundant A-MuLV RNA while most ABPL's, in striking contrast, did not. To understand the molecular mechanisms of the oncogenesis of these tumors, we undertook a study of the expression of *abl, myb*, and *myc* oncogenes (*9*) which have been associated with neoplasms of hematopoietic cells (*3*). In view of the finding that several murine plasmacytomas and Burkitt's lymphomas exhibit translocations involving the *myc* gene locus (*10–13*), we also looked for evidence of rearrangement of these three oncogenes in the genomes of these tumors. Five ABLS tumors, six ABPL tumors, and three ABPC tumors were used in the present study.

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Integration and Expression of A-MuLV

First, the three classes of neoplasms were studied for the integration and expression of the A-MuLV genome. As shown in Fig. 1a and Table 1, *abl* RNA is abundant in all ABLS and ABPC tumors. The bulk of the *abl* RNA in these tumors was a 6.7-kilobase (kb) polyadenylated [poly(A)] RNA, a size expected for the A-MuLV genomic RNA (*14*). In contrast, five of the six ABPL tumors (ABPL1, ABPL2, ABPL4, ABPL109, and ABPL133) did not show A-MuLV RNA in their cytoplasm. An exception was ABPL3, which contained large quantities of cytoplasmic A-MuLV RNA. Small amounts of 5.6-kb *abl* RNA also were detected in all three categories of tumors. This appears to be the transcriptional product of the endogenous c-*abl* proto-oncogene. Small amounts of 5.6-kb and 6.7-kb *abl* RNA's were detected in normal thymus, an organ known to express c-*abl* encoded protein NCP150 (*15*). Figure 1b shows Southern blot analysis (*16*) of the DNA's derived from the three classes of neoplasms after hybridization with v-*abl* probe. The DNA's from these tumors were digested with Kpn I, which cuts the proviral genome in the two flanking long terminal repeats (*14*), thereby releasing a 6.7-kilobase pair (kbp) fragment from the integrated proviral DNA. The c-*abl* gene, however, yielded two Kpn I fragments of 8.3 and 24.0 kbp. This distinction in fragment size between c-*abl* and v-*abl* allowed us to ascertain the presence or absence of an integrated proviral genome. As expected, the results show that all ABPC's, ABLS's, and ABPL3 contained at least one integrated proviral genome, while ABPL1, ABPL2, ABPL4, ABPL109, and ABPL133 did not show a 6.7-kbp proviral DNA fragment in their genomes.

Expression of *myc*

We then examined all these tumors for the expression of *myc* RNA, which had earlier been shown to be present in high levels in mouse plasmacytomas (*17–19*), human Burkitt lymphomas (*19, 20*), and avian bursal lymphomas (*21*) (Fig. 2a and Table 1). All these tumors expressed a 2.4-kb *myc* RNA which is also a constituent of normal thymus and spleen RNA. The band intensities of *myc* RNA in the three classes of tumors varied considerably with the ABLS's generally containing the lowest amount of *myc* RNA (Fig. 2a and Table 1). Earlier studies demonstrated that most, but not all, BALB/c plasmacytomas expressed abundant 2.4-kb *myc* RNA. Some others express an abnormal 1.8-kb *myc* RNA (*12, 17–19*), which appears to result from DNA rearrangements (*12, 13, 17–19*) in the c-*myc* locus of these tumors. Some, but not all, ABPC's examined in the earlier studies, plus those shown in Fig. 2a, contained 1.8-kb *myc* RNA. Our studies show that none of the ABLS's or ABPL's contain such a small *myc* RNA. When the genomic DNA's of these tumors were examined for rearrangements in *myc* locus, none were found in these two classes of tumors with the exception of an additional Eco RI band very close to the germ line 21-kbp *myc* band in ABPL4 (Fig. 2b). Two of the ABPC's, ABPC24 and ABPC45, have *myc* rearrangements, but only ABPC24 produces an altered *myc* RNA (Fig. 2a and Table 1).

Rearrangement in *myb* Locus

As can be seen in Fig. 3a, both ABLS's and ABPL's contain a large quantity of 3.8-kb RNA and a trace amount of 4.2-kb RNA that hybridizes with a cloned probe from avian v-*myb* (*22*). Particularly dramatic elevations in the amount of *myb* RNA occur in ABPL-1 and ABPL2. In addition five of the six ABPL tumors contain a still larger form of *myb* RNA. The size of this ABPL-specific *myb* RNA varies among the tumors but is usually around 5.0 kb. The 3.8- and 4.2-kb *myb* RNA's can be seen in small amounts in ABPC's and in most normal cells, particularly thymus, but the 5.0-kb and larger *myb* RNA has no counterpart in any normal cell RNA that we have examined. The autoradiographic patterns of Southern blots of tumor DNA's probed with v-*myb* are shown in Fig. 3b. The Hind III digests of normal cells, as well as those of ABLS and ABPC tumors, showed the presence of three *myb*-hybridizing bands of 2.2, 2.8, and 3.7 kbp. In contrast, all six ABPL tumors examined (Table 1) contained an additional band of varying size, but usually larger than 3.7 kbp. The additional Hind III *myb* band in ABPL4 is very close to 3.7 kbp, but it is clearly visualized in Eco RI digests (data not shown). The larger band appeared to be a rearranged version of the 3.7-kbp germ line fragment, since this fragment generally hybridized with a distinctly diminished intensity in the tumors where a fourth fragment appeared. The appearance of a larger DNA fragment could be correlated with the appearance of a larger *myb* RNA in these tumors (except ABPL109), leading us to conclude that in these tumor cells the DNA rearrangement in the *myb* locus of one chromosome results in the synthesis of abnormal messenger RNA (mRNA) transcripts.

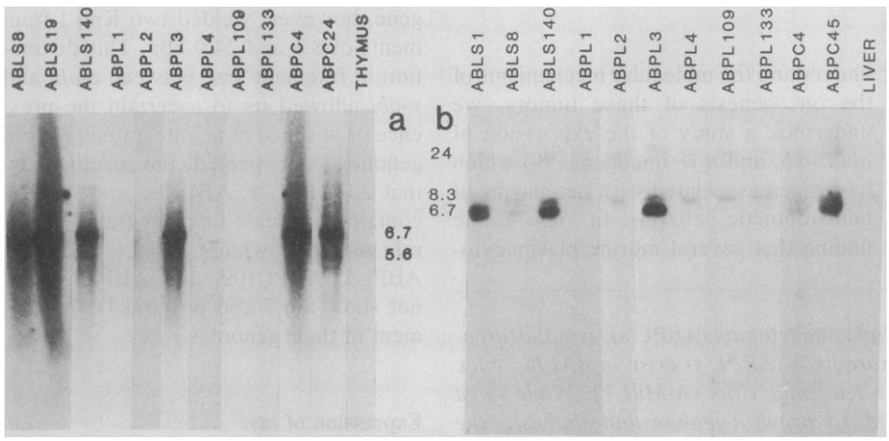

Fig. 1. Hybridization with *abl* probe. Solid tumors were excised from subcutaneous or mesenteric sites after transplantation of ascites tumor cells into syngeneic mice. Spleens and tumors were frozen in liquid nitrogen and pulverized with mortar and pestle. (a) Total RNA was prepared as described (*17, 32*) and twice enriched for poly(A) containing molecules on oligodeoxythymidylate (dT) cellulose. This RNA (5 μg) was subjected to electrophoretic separation on 1 percent agarose containing 5 m*M* methylmercury hydroxide, blotted onto diazotized phenylthioether paper (Schleicher and Schuell), and hybridized under stringent conditions (*17*) with a 1.2-kbp Bgl II fragment that had been isolated from cloned A-MuLV (*14*), subcloned into pBR322 with the use of Eco RI linkers, and labeled with ^{32}P by nick translation (*33*). Tissue and tumor sources of RNA's are indicated at the top, and sizes (in kilobases) of hybridizing bands are indicated in the margins. These sizes were determined from ethidium bromide–stained plant virus standards [tobacco mosaic virus, 6.34 kb (*34*), brome mosaic virus, 3.4, 3.1, 2.3, and 0.87 kb (*35*)]. (b) *abl* Hybridization of blots of Kpn I digests of genomic DNA (25 μg) from the tumors and liver as indicated. High molecular weight DNA was prepared (*17, 36*) and digested with Kpn I. The digested genomic DNA (25 μg) was subjected to electrophoresis in 0.7 percent agarose gels, blotted onto nitrocellulose (*16*), and hybridized with the same mouse *abl* probe used in (a). Sizes of hybridizing fragments are indicated in the margins. These sizes were determined from ethidium bromide–stained Hind III fragments of phage λ DNA.

Cytoplasmic μ-Chain RNA

We have made some studies of the surface antigens on the lymphosarcomas in order to determine how best to classify them in terms of lymphocyte differentiation pathways (*23*); we have also characterized four of the ABLS's and five of the ABPL's for immunoglobulin M (IgM) μ-chain expression. In comparison to splenocytes, which make large amounts of μ-chain mRNA, both types of tumors express small amounts of cytoplasmic μ-chain RNA (Fig. 4). The tumors contain varying amounts of several forms of μ-chain mRNA. The 2.4-kb form presumably encodes μ chains of secreted IgM (μ_s), and the 2.7-kb form probably encodes μ chains of IgM that remain mem-

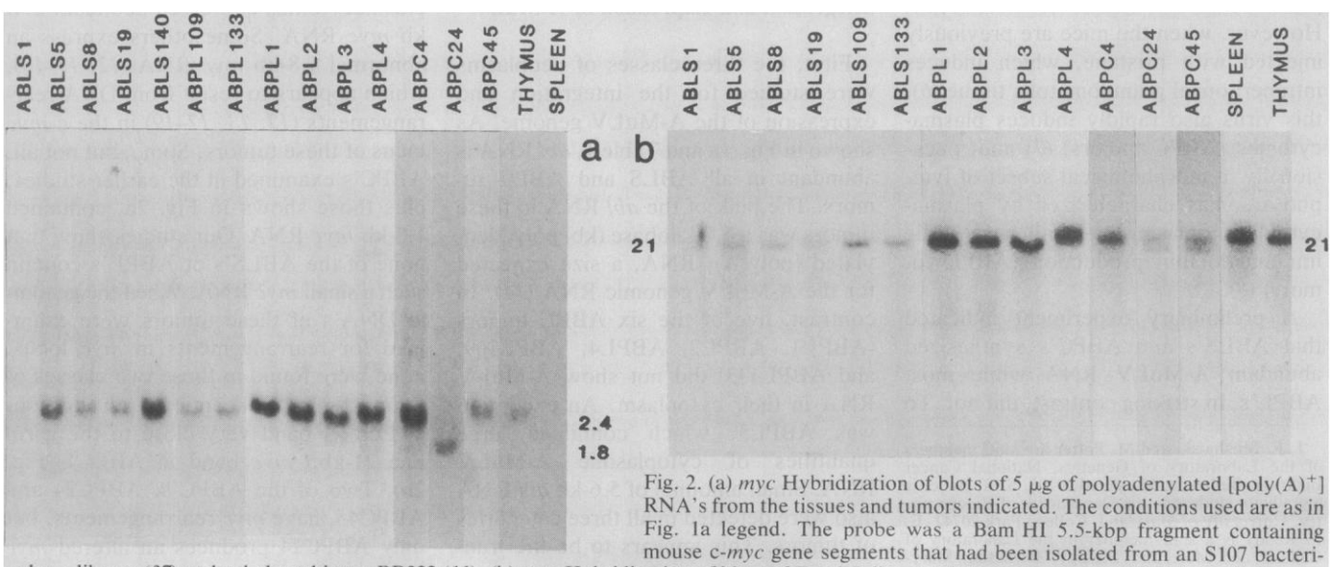

Fig. 2. (a) *myc* Hybridization of blots of 5 μg of polyadenylated [poly(A)$^+$] RNA's from the tissues and tumors indicated. The conditions used are as in Fig. 1a legend. The probe was a Bam HI 5.5-kbp fragment containing mouse c-*myc* gene segments that had been isolated from an S107 bacteriophage library (*37*) and subcloned into pBR322 (*11*). (b) *myc* Hybridization of blots of Eco RI digests of 25 μg of genomic DNA from the tumors and tissues indicated. Conditions are given in Fig. 1b and the probe was that used in Fig. 2a.

brane bound (μ_m) (*24*). Many of the tumors also contain smaller forms of μ-chain RNA, probably analogous to those reported in other transformed B lymphocytes (*25*). We conclude the ABLS and ABPL tumors are committed to the B lymphocyte differentiation pathway.

Proto-oncogenes and Hit-and-Run Mechanisms

The mechanism of A-MuLV induced transformation is not known; this process may involve more than one step. Most studies indicate that A-MuLV integrates in the form of a provirus which is then actively transcribed into v-*abl* RNA which encodes a *gag-abl* fusion protein (*gag*, gene for a structural protein in retroviruses), thought to be responsible for cell transformation (*26*). A second possible mechanism of A-MuLV induced transformation involves secondary genetic changes incurred during a transient virus infection. This mechanism can be inferred from the demonstration that inserted A-MuLV genomes can be eliminated from cloned, transformed lymphoid lines without the loss of the transformed phenotype (*27*). Elimination of A-MuLV appears to be a relatively common occurrence in some lymphoid cells (*28*). The ABPL cells, which lack A-MuLV proviral genome, provide a model system to study secondary changes that lead to maintenance of the malignant state in the absence of transforming virus. "Hit-and-run" phenomena have been demonstrated for various virus systems, such as herpes simplex virus (*29*). Our data suggest that hit-and-run mechanisms might involve rearrangements of cellular proto-oncogenes leading to their activation. We have shown that the ABPL tumors, although lacking A-MuLV proviral genome, have undergone rearrangements in the c-*myb* locus, which nonetheless usually remains transcriptionally active. The ABPL109 tumor appears to be an exception in that it has a *myb* rearrangement but no evidence of an altered RNA transcript.

Another interesting exception to this general picture is ABPL3, which contains transcriptionally active A-MuLV proviral genomes and has a rearranged c-*myb*. Although we have no direct evidence that A-MuLV integrated initially and then was lost from cells of the ABPL type, we have adopted this as our working hypothesis. In fact, ABPL3 may be a clue that this was the actual operative mechanism since it has elements of ABPL's, namely, rearranged *myb* DNA and a 5.0-kb *myb* RNA, and it also has v-*abl* RNA and at least one copy of A-MuLV proviral genome integrated in its DNA. The excision process may have occurred at another, particularly unstable site of A-MuLV integration, for ex-

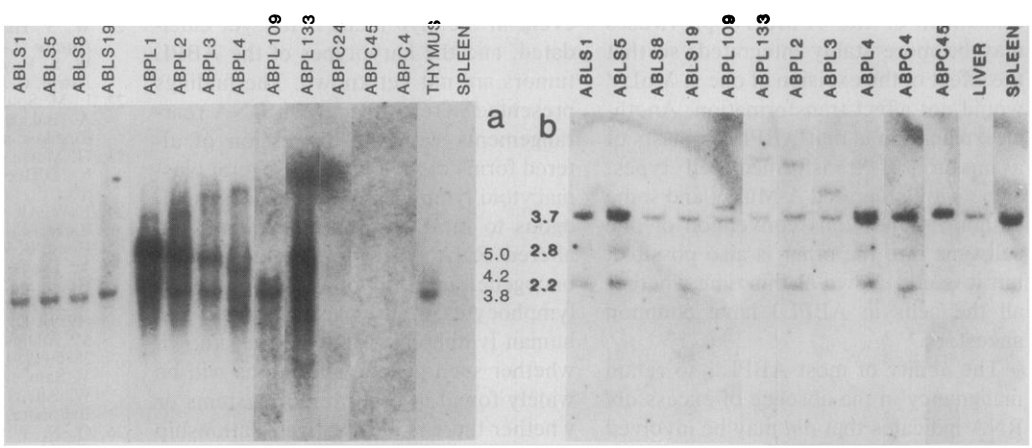

Fig. 3. (a) *myb* Hybridization of blots of 5 μg of poly(A)$^+$ RNA's from tissues and tumors indicated. The *myb* probe was a Kpn I–Xba I 1.3-kbp fragment isolated from cloned avian myeloblastosis virus (*22*), subcloned in pBR322. Conditions for RNA electrophoresis are given in the legend for Fig. 1a. Conditions for hybridization are the relaxed conditions described (*17*). Intensity of hybridization and background levels varied considerably in the different blots assembled. (b) *myb* Hybridization of blots of Hind III digests of 25 μg of genomic DNA from the tumors and tissues indicated. The conditions for DNA electrophoresis are as in Fig. 1b. The probe and hybridization conditions are as in (a).

Table 1. Summary of *onc* gene hybridizations.

Tumor	Transplant generation	*abl*		*myc*			*myb*		
		Viral RNA*	Proviral DNA†	2.4-kb RNA‡	1.8-kb RNA§	Rearranged DNA‖	3.8-kb RNA¶	> 4.5-kb RNA#	Rearranged DNA**
ABLS1	13	++++	++	+	–	–	++	–	–
5	12	++++	+	++	–	–	++	–	–
8	13	++++	+	++	–	–	++	–	–
19	11	++++	+	++	–	–	++	–	–
140	6	++++	++	+++	–	–	++	–	–
ABPL1	9	–	–	+++	–	–	++	++	+
2	7	–	–	+++	–	–	++	++	+
3	11	++++	++	+++	–	–	++	+	+
4	16	–	–	+++	–	+	++	+	+
109	2	–	–	+++	–	–	++	–	+
133	8	–	–	+++	–	–	++	++	+
ABPC4	14	++++	+	+++	–	–	–	–	–
24	9	++++	+	+	+++	+	–	–	–
45	23	++++	++	+++	–	+	–	–	–
Spleen		–	–	+	–	–	+	–	–
Thymus		–	–	++	–	–	++	–	–

*6.7-kb *abl* RNA. †6.7-kbp Kpn I band of *abl* DNA. ‡*myc* RNA transcript from c-*myc* unrearranged proto-oncogene. §*myc* RNA transcript from rc-*myc* rearranged proto-oncogene. ‖Eco RI restriction fragment different from germ line c-*myc* fragment of 21 kbp. ¶*myb* RNA transcript from c-*myb* unrearranged proto-oncogene. #*myb* RNA transcript from rc-*myb* rearranged proto-oncogene, usually > 4.5 kb in size. **Hind III restriction fragment different from those of germ line c-*myb* fragments of 2.2, 2.8, and 3.7 kbp.

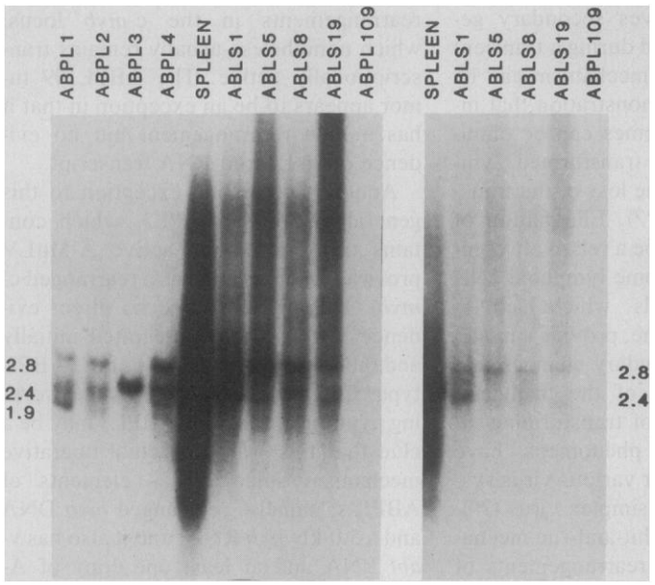

Fig. 4. Cμ (constant portion of μ chain) hybridization of blots of 5 μg of poly(A)$^+$ RNA's from spleen and the tumors indicated. The conditions for RNA electrophoresis and blotting are given in the legend to Fig. 1a. The probe was the Pst I insert containing mouse IgM Cμ sequences isolated from a complementary DNA clone pMK1 (*38*). The bands of hybridization of RNA from the ABLS tumors and spleen were so intense that a shorter exposure of these lanes is included in the right panel. Degradation of spleen RNA is probably responsible for the hybridization in the area containing RNA smaller than 2.4 kb.

ample, adjacent to c-*myb*, leading to the *myb* RNA and DNA abnormalities. The remainder of the A-MuLV proviruses may be more stably integrated, so that the effect of the excision of one A-MuLV would not affect transformation. Another explanation is that ABPL3 consists of a mixture of transformed cell types, some with integrated A-MuLV and some without. Continuous conversion of one cell type into the other is also possible, but it is not known at this time whether all the cells in ABPL3 have common ancestors.

The ability of most ABPL's to retain malignancy in the absence of excess *abl* RNA indicates that *abl* may be involved in only one stage in a complex, multistage process that finally yields a fully neoplastic cell (*30*). The other *onc* genes seen in abundance in the tumors discussed here, *myb* and *myc*, may play key roles in other stages of this process.

Burkitt's lymphoma cells and some other undifferentiated B-lymphomas in man and plasmacytoma cells in mouse (*10*) are associated with a high frequency of nonrandom chromosome translocations involving immunoglobulin and *myc* gene loci. These translocations may be related in some way to a high transcriptional activity (*31*) as well as to DNA rearrangements involving these genes during certain stages of B-lymphocyte development. The associated enhancement of *myc* transcription may confer a growth advantage to these cells.

The *myb* locus also appears to be highly active in cells at the stage of differentiation exhibited by the ABLS and ABPL tumors. The transcriptionally activated state of this locus may play an important role in processes such as virus integration (*31*). The recombination event in the *myb* locus is not yet elucidated, and the karyotypes of the ABPL tumors are not yet known. The findings presented here connect *myb* DNA rearrangements with the expression of altered forms of *myb* RNA in several plasmacytoid lymphosarcomas. This is analogous to the DNA rearrangements and altered RNA's seen in another proto-oncogene, *myc*, in other tumors of B lymphocytes, mouse plasmacytomas and human lymphomas. It is not yet known whether such genetic alterations will be widely found in other tumor systems or whether there is a causative relationship between these alterations and transformation. Molecular cloning and detailed structural and biological analyses of such aberrantly rearranged genes should give us a better understanding of the various mechanisms that contribute to a complex series of changes that are responsible for the induction and maintenance of the malignant state.

References and Notes

1. K. Bister and P. H. Duesberg, *Adv. Viral Oncol.* **1**, 3 (1982).
2. J. M. Bishop *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **44**, 919 (1980).
3. T. Graf and D. Stehelin, *Biochim. Biophys. Acta* **651**, 245 (1982).
4. H. T. Abelson and L. S. Rabstein, *Cancer Res.* **30**, 2213 (1970); R. Risser, M. Potter, W. P. Rowe, *J. Exp. Med.* **148**, 714 (1978); N. Rosenberg and D. Baltimore, *ibid.* **143**, 1453 (1976).
5. M. D. Sklar, E. M. Shevach, I. Green, M. Potter, *Nature (London)* **253**, 550 (1975); E. J. Siden, D. Baltimore, D. Clark, N. Rosenberg, *Cell* **16**, 389 (1979).
6. P. N. Anderson and M. Potter, *Nature (London)* **222**, 994 (1969).
7. M. Potter, M. D. Sklar, W. P. Rowe, *Science* **182**, 592 (1973).
8. E. Premkumar, M. Potter, P. A. Singer, M. D. Sklar, *Cell* **6**, 149 (1975); M. Potter, E. P. Reddy, N. A. Wivel, *Natl. Cancer Inst. Monogr.* **48**, 311 (1978).
9. *abl* is the gene encoding the transforming element in the transforming virus, A-MuLV; *myb* is, analogously, derived from avian myeloblastosis virus and *myc* is derived from MC29, an isolate of avian myelocytomatosis virus [see (*3*) for a review].
10. G. Manolov and Y. Manolova, *Nature (London)* **237**, 33 (1972); D. Benjamin, I. T. Magrath, R. Maguire, C. Janus, H. D. Todd, R. G. Parsons, *J. Immunol.* **129**, 1336 (1982); S. Ohno *et al.*, *Cell* **18**, 1001 (1979).
11. R. Taub *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7837 (1982).
12. G. L. C. Shen-Ong, E. J. Keath, S. P. Piccoli, M. D. Cole, *Cell* **31**, 443 (1982).
13. S. Crews, R. Barth, L. Hood, J. Prehn, K. Calame, *Science* **218**, 1319 (1982); L. J. Harris, R. B. Lang, K. B. Marcu, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4175 (1982).
14. S. P. Goff, E. Gilboa, O. N. Witte, D. Baltimore, *Cell* **22**, 777 (1980); A. Srinivasan, E. P. Reddy, S. A. Aaronson, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2077 (1981).
15. O. N. Witte, N. E. Rosenberg, D. Baltimore, *Nature (London)* **281**, 396 (1979).
16. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
17. J. F. Mushinski, S. R. Bauer, M. Potter, E. P. Reddy, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1073 (1983).
18. J. M. Adams, S. Gerondakis, E. Webb, J. Mitchell, O. Bernard, S. Cory, *ibid.* **79**, 6966 (1982).
19. K. B. Marcu *et al.*, *ibid.* **80**, 519 (1983).
20. R. Dalla-Favera, S. Martinotti, R. C. Gallo, J. Erikson, C. M. Croce, *Science* **219**, 963 (1983); J. Erikson, A. ar-Rushdi, H. L. Dwenga, P. C. Nowell, C. M. Croce, *Proc. Natl. Acad. Sci. U.S.A.*, in press; J. M. Adams, S. Gerondakis, E. Webb, L. M. Corcoran, S. Cory, *ibid.* **80**, 1982 (1983); R. Dalla-Favera, F. Wong-Staal, R. C. Gallo, *Nature (London)* **299**, 61 (1982).
21. W. S. Hayward, B. G. Neel, S. M. Astrin, *Nature (London)* **290**, 475 (1981); B. G. Neel, W. S. Hayward, H. Robinson, J. Fang, S. M. Astrin, *Cell* **23**, 323 (1981).
22. L. M. Souza, M. J. Briskin, R. L. Hillyard, M. A. Baluda, *J. Virol.* **36**, 325 (1980); K. E. Rushlow *et al.*, *Science* **216**, 1421 (1982).
23. H. Morse, F. Mushinski, M. Potter, S. Bauer, L. D'Hoostelaere, K. Holmes, unpublished data.
24. F. W. Alt *et al.*, *Cell* **20**, 293 (1980); J. Rogers, P. Early, C. Carter, K. Calame, M. Bond, L. Hood, R. Wall, *ibid.*, p. 303; P. Early, J. Rogers, M. Davis, K. Calame, M. Bond, R. Wall, L. Hood, *ibid.*, p. 313.
25. R. P. Perry and D. E. Kelley, *ibid.* **18**, 1333 (1979); D. J. Kemp, A. W. Harris, S. Cory, J. M. Adams, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2876 (1980); E. Siden, F. W. Alt, L. Shinefeld, V. Sato, D. Baltimore, *ibid.* **78**, 1823 (1981); F. W. Alt, N. Rosenberg, V. Enea, E. Siden, D. Baltimore, *Mol. Cell. Biol.* **2**, 386 (1982).
26. O. N. Witte, N. Rosenberg, M. Paskind, A. Shields, D. Baltimore, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2488 (1978); O. N. Witte, N. Rosenberg, D. Baltimore, *J. Virol.* **31**, 776 (1979); A. Srinivasan, C. Y. Dunn, Y. Yuasa, S. G. Devare, E. P. Reddy, S. A. Aaronson, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5508 (1982).
27. N. Rosenberg, *Curr. Top. Microbiol. Immunol.* **101**, 95 (1982).
28. D. J. Grunwald *et al.*, *J. Virol.* **43**, 92 (1982).
29. D. A. Galloway and J. K. McDougall, *Nature (London)* **302**, 21 (1983).
30. M.-A. Lane, A. Sainten, G. M. Cooper, *Cell* **28**, 873 (1982); *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5185 (1981).
31. M. Breindl, L. Bacheler, H. Fan, R. Jaenisch, *J. Virol.* **34**, 373 (1980).
32. C. Auffray and F. Rougeon, *Eur. J. Biochem.* **107**, 303 (1980).
33. P. J. W. Rigby, M. Dieckmann, G. Rhodes, P. Berg, *J. Mol. Biol.* **113**, 237 (1977).
34. D. L. D. Caspar, *Adv. Protein Chem.* **18**, 37 (1963).
35. L. C. Lane and P. Kaesberg, *Nature (London) New Biol.* **232**, 40 (1971).
36. F. Polsky, M. H. Edgell, J. G. Seidman, P. Leder, *Anal. Biochem.* **87**, 397 (1978).
37. I. Kirsch, J. V. Ravetsch, S.-P. Kwan, E. E. Max, R. L. Ney, P. Leder, *Nature (London)* **293**, 585 (1981).
38. M. R. Knapp *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2996 (1982).

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