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Proviruses are adjacent to c-*myc* in some murine leukemia virus-induced lymphomas

(oncogenes)

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ABSTRACT **Sixty-one murine leukemia virus-induced lymphomas were examined for evidence of proviral insertions adjacent to known oncogenes. Five of these lymphomas were found to have alterations adjacent to c-*myc*. Two of the lymphomas with altered c-*myc* sequences were examined in detail. Evidence was obtained showing that the alterations in the c-*myc* sequences in these two lymphomas were the result of proviral integrations. This result suggests that lymphomagenesis by murine leukemia viruses can result from insertional mutagenesis of cellular oncogenes.**

The murine leukemia viruses (MLVs) are a family of retroviruses that induce lymphomas in mice and rats. Induction of lymphomas by MLVs occurs only after a long latent period, typically 4–12 months. No oncogenic sequences have been identified within the genomes of these viruses. Both the long latent period before onset of cancer and the absence of oncogenes are characteristics shared with three other families of retroviruses—the mouse mammary tumor viruses, the avian leukosis viruses (ALVs), and the reticulo-endotheliosis viruses. (For a review of this topic, see ref. 1.) ALV-induced B-cell lymphomas have been shown to contain proviral genomes integrated adjacent to the chicken c-*myc* gene (2–4) and ALV-induced erythroblastosis contain proviral genomes adjacent to the chicken c-*erb*-B gene (5). Reticulo-endotheliosis virus-induced lymphomas have also been shown to have proviral genomes integrated adjacent to c-*myc* (6). Mouse mammary tumor virus-induced carcinomas have been found to contain mouse mammary tumor proviruses integrated adjacent to specific sequences in the host genome (7, 8), and it seems likely that these common integration sites are adjacent to as yet unidentified cellular oncogenes. These integrations of proviral genomes adjacent to potentially oncogenic host genes are thought to induce tumor formation by perturbing either the expression of these genes or the activity of the resulting protein, a process termed insertional mutagenesis. The motivation for the work described here was to determine if the MLVs induce lymphomas by insertional mutagenesis, and, if so, to identify the target gene for such mutagenesis.

MATERIALS AND METHODS

DNAs were isolated, digested, fractionated by gel electrophoresis, transferred to nitrocellulose, and hybridized to [^{32}P]DNA as described (9). DNA of plasmid pv-*myc*3 was ^{32}P-labeled (to 4×10^8 dpm/μg) by nick-translation (10) and was used at 2 μCi/ml (1 Ci = 37 GBq) to detect the c-*myc* gene. This plasmid contains the v-*myc* gene of M-29 virus (11) inserted into the plasmid pBR322.

Sixty-one lymphomas were analyzed for altered c-*myc* sequences; 27 were induced in rats with Moloney MLV (MoMLV), 2 were induced in rats with AKR mouse-derived MLVs, 18 were induced in mice with MoMLV, and 14 occurred spontaneously in AKR mice. The thymomas with altered c-*myc* sequences analyzed here were induced as follows. Thymoma RT 1-3 was induced in a 1-day-old Fischer rat by intraperitoneal injection of 3×10^5 plaque-forming units of MoMLV. Virus was obtained from the clone 1 cell line (12). The thymoma developed at 145 days after injection. Thymoma RT 10-2 was induced in a 1-day-old Fischer rat by intraperitoneal injection of 3×10^7 plaque-forming units of MoMLV. Virus was obtained from the clone 2 cell line (13). The thymoma developed at 128 days after injection. Rats with labored breathing (a consequence of the thymoma filling the chest cavity) were sacrificed.

Diagnosis of tumors was done initially by the gross anatomical examination of dissected rats. Rats with lymphomas always had massively enlarged thymuses and sometimes had enlarged spleens, lymph nodes and/or mottled livers as well. DNAs from presumptive tumor samples were analyzed by digestion with the restriction endonuclease *Eco*RI, gel electrophoresis, and hybridization with [^{32}P]DNA from plasmid IA5, which detects MLV sequences. Because the enzyme *Eco*RI does not cleave within the viral genome and because proviruses can be integrated at many sites in the host cell genome, the appearance of a discrete pattern of viral fragments was taken as evidence for clonality of the material, which is expected for tumor tissue but not for normal tissue. All tumors exhibited this clonal pattern of proviruses.

RESULTS

Some MLV-Induced Lymphomas Contain Altered c-*myc* Genes. Restriction endonuclease digestion, gel electrophoresis, and nucleic acid hybridization were used to determine whether MLV proviruses were present adjacent to potentially oncogenic genes in lymphoma DNAs. Such proviruses would perturb the pattern of restriction endonuclease cleavage sites in the vicinity of the gene. DNAs from 61 MLV-induced lymphomas were analyzed for alterations adjacent to c-*myc*—27 induced in Fischer rats by MoMLV, 2 induced in Wistar/Furth rats with AKR mouse-derived MLVs, 18 induced in mice by MoMLV, and 14 occurring spontaneously in AKR mice. Five of the lymphomas were found to have alterations in the sequences adjacent to c-*myc*—4 induced in Fischer rats by MoMLV and 1 induced in a Wistar/Furth rat by AKR-derived MLVs. Two of the MoMLV-induced lymphomas were selected for detailed analysis.

The evidence that these lymphomas have alterations in the DNA sequences adjacent to c-*myc* is shown in Fig. 1. DNAs from thymoma and control tissues were digested with *Eco*RI, fractionated by gel electrophoresis, and hybridized to ^{32}P-labeled *myc* DNA. DNA extracted from the liver of a normal, uninfected rat produced a single hybridizing fragment of about 20 kilobases (kb) in length (lane A). DNA from

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Abbreviations: MLV, murine leukemia virus; MoMLV, Moloney MLV; ALV, avian leukosis virus; kb, kilobase(s); MCF, mink cell focus-forming.

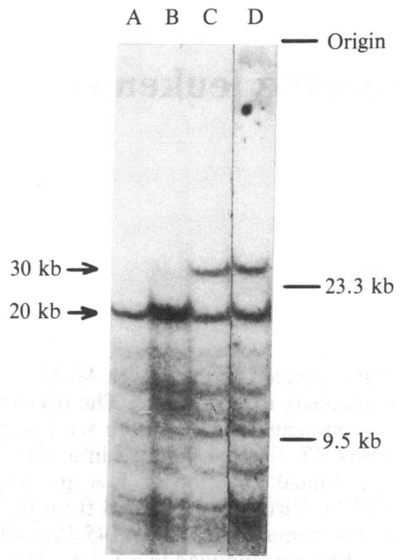

FIG. 1. Altered c-*myc* sequences and MLV proviral sequences in *Eco*RI-digested lymphoma DNAs. The arrows to the left indicate the sizes of major c-*myc* hybridizing fragments. Lines to the right indicate the mobility of *Hin*dIII-digested λ DNA size standards. The large number of fainter hybridizing DNA fragments seen in the figure (and which are seen reproducibly with the pv-*myc*3 probe) are not observed after hybridization with mouse c-*myc* sequences (data not shown) and thus appear not to derive from the rat c-*myc* gene. The DNAs were derived from the following sources: lane A, the liver of an uninfected Fischer rat; lane B, the brain of rat 1-3; lane C, the thymoma of rat 1-3 (RT 1-3); lane D, the thymoma of rat 10-2 (RT 10-2).

thymoma RT 1-3 (induced by infection of a rat with MoMLV) produced an altered pattern of c-*myc* fragments. In addition to the ≈20-kb fragment observed in the normal rat DNA, a fragment of about 30 kb was produced (lane C). DNA from the brain of the same rat that produced RT 1-3 was used as a control nontarget tissue. This DNA produced only the normal 20-kb fragment (lane B), demonstrating that the altered band observed in the thymoma does not represent a genetic polymorphism, but rather a tumor-specific change. A second thymoma, designated RT 10-2, was also induced by infection of a rat with MoMLV. DNA extracted from this thymoma displays the same 20- and 30-kb c-*myc* fragments observed in RT 1-3.

Altered c-*myc* Sequences in MoMLV-Induced Thymomas Result from Proviral Insertion Adjacent to the c-*myc* Gene. The alterations of the rat c-*myc* gene observed in the MLV-induced thymomas RT 1-3 and RT 10-2 could result from rearrangement of host sequences, as has been described in mouse plasmacytomas (14–18), or could result from proviral insertions. If the observed polymorphisms were due to proviral insertions adjacent to the rat c-*myc* gene, the altered c-*myc* fragments should contain both *myc* and viral fragments resulting in comigration of viral and *myc* fragments on gels. Unfortunately, most lymphomas induced in rats by MoMLV have 10 or more proviruses, making coincidental comigration of viral and c-*myc* sequences highly likely. The most favorable case is RT 10-2, which has only 5 MLV proviruses. Hybridization of *Eco*RI, *Eco*RI+*Sal* I, *Eco*RI+*Cla* I, and *Eco*RI+*Nru* I digestions of RT 10-2 DNA with both *myc* and MLV probes always reveals comigration of the novel c-*myc* and viral fragment, but usually reveals coincidental comigration of the normal c-*myc* and a viral fragment as well (data not shown). Clearly, comigration of viral and *myc* sequences cannot be used to determine if the altered c-*myc* fragments observed here are due to proviral insertion.

An alternative approach to showing that the perturbations

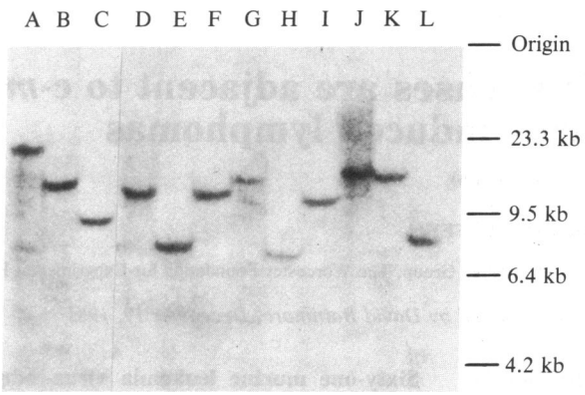

FIG. 2. Restriction endonuclease cleavage sites adjacent to c-*myc* in normal rat DNA. DNA from the liver of an uninfected Wistar/Furth rat was digested with a series of restriction endonucleases, fractionated by gel electrophoresis, and hybridized to a c-*myc* sequence probe. To the right are indicated *Hin*dIII λ size markers, as in Fig. 1. The DNA was digested with the following enzymes: lane A, *Eco*RI; lane B, *Hin*dIII; lane C, *Hin*dIII + *Eco*RI; lane D, *Bam*HI; lane E, *Bam*HI + *Eco*RI; lane F, *Bam*HI + *Hin*dIII; lane G, *Sac* I; lane H, *Sac* I + *Eco*RI; lane I, *Sac* I + *Hin*dIII; lane J, *Kpn* I; lane K, *Kpn* I + *Eco*RI; lane L, *Kpn* I + *Hin*dIII.

of the c-*myc* genes are due to proviral insertion is to show that the restriction map of the tumor-specific c-*myc* fragment contains the spectrum of restriction sites characteristic of an MLV provirus. Fig. 2 displays some of the data used to locate restriction sites adjacent to the normal c-*myc* sequences and Fig. 3 shows some of the data used to characterize the alterations in RT 1-3 and RT 10-2.

A summary of restriction endonuclease cleavage data from 14 gels is summarized in Table 1. These data were used to construct a preliminary restriction endonuclease cleavage map of the normal c-*myc* fragment and the tumor-specific c-*myc* fragments (Fig. 4).

As is illustrated in Fig. 4, the tumor-specific fragments of RT 1-3 and RT 10-2 can be explained as insertions into the normal c-*myc* fragment. Below the insertions into RT 1-3 and RT 10-2 is shown the restriction endonuclease cleavage map

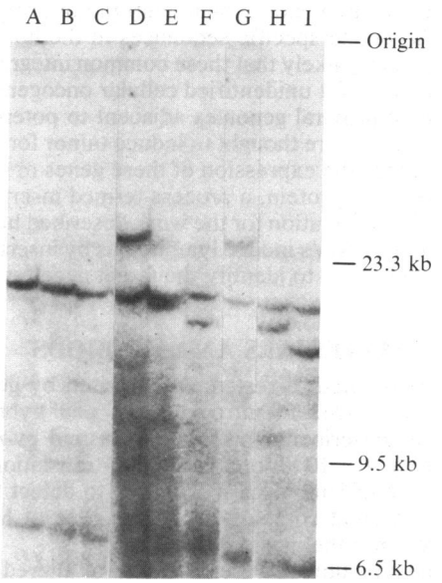

FIG. 3. Restriction endonuclease cleavage sites in the altered c-*myc* fragments in RT 1-3 and RT 10-2. Lanes A–C contain DNA from normal rat liver, lanes D–F from RT 10-2, and lanes G–I from RT 1-3. Lanes A, D, and G were digested with *Eco*RI, lanes B, E, and H with *Eco*RI + *Cla* I, and lanes C, F, and I with *Eco*RI + *Sal* I. To the right are indicated *Hin*dIII λ size markers, as in Fig. 1.

Table 1. *myc*-hybridizing fragments observed in lymphoma DNAs

Enzyme	Size of fragments, kb		
	Normal	RT 1-3	RT 10-2
*Eco*RI	18.6 ± 1.8 (15)	26.4 ± 2.4 (7)	28.5 ± 1.4 (6)
*Hin*dIII	12.1 ± 1.3 (7)		
*Hin*dIII + *Eco*RI	8.6 ± 0.4 (4)		
*Bam*HI	10.9 ± 1.5 (4)		
*Bam*HI + *Eco*RI	7.6 ± 0.4 (4)	7.3 (1)	
*Bam*HI + *Hin*dIII	10.5 ± 2.1 (3)		
Cla I + *Eco*RI	18.7 ± 0.8 (4)	16.3 ± 0.7 (4)	18.4 ± 2.1 (4)
Kpn I	14.2 ± 1.0 (5)	9.3 (1)	9.8 (1)
Kpn I + *Eco*RI	13.8 ± 1.6 (4)		
Kpn I + *Hin*dIII	7.7 ± 0.5 (2)		
Nru I + *Eco*RI	18.1 ± 3.1 (2)	12.3 ± 1.8 (2)	13.5 ± 1.4 (2)
Pst I	2.3 ± 0.2 (2)		
Pst I + *Eco*RI	2.4 ± 0.1 (4)	2.4 (1)	
Pst I + *Hin*dIII	2.5 ± 0.1 (2)		
Sac I	12.3 ± 1.6 (5)		
Sac I + *Eco*RI	7.2 ± 0.6 (5)	6.8 (1)	
Sac I + *Bam*HI	10.5 ± 0.8 (3)		
Sac I + *Eco*RI	18.5 ± 0.9 (7)	15.0 ± 0.9 (6)	16.3 ± 1.5 (7)
Xba I	9.3 ± 0.2 (2)		
Xba I + *Eco*RI	4.4 ± 0.5 (5)		
Xba I + *Hin*dIII	7.4 ± 0.5 (3)		

Results presented are the average of the number of measurements shown in parentheses (n). For $n \geq 3$, size is presented ± standard deviation. For $n = 2$, size is presented ± range. Each measurement is derived from a separate gel. The sizes of the fragments were calculated by using size standards run in an adjacent lane, by the formula of Southern (19). The standard deviation is presented to provide an indication of the reproducibility of the measurements. In addition to experiment-to-experiment variability, there are systematic errors in these measurements (differences in the salt concentration between the sample lanes and marker lanes, lane-to-lane mobility variation). Thus, the accuracy of these measurements is somewhat lower than the standard deviation would indicate.

of the MoMLV genome. The insertions adjacent to c-*myc* in RT 1-3 and RT 10-2 contain the restriction endonuclease cleavage sites of the MoMLV genome, in the same order as they are present in the MoMLV genome. Particularly striking are the presence of cleavage sites for the restriction endonucleases *Cla* I, *Nru* I, and *Sal* I, because these enzymes cleave rat DNA extremely infrequently.

Because the data used to construct Fig. 4 are the average of data from 14 different gels, gel-to-gel variability reduces the precision of location of the cleavage sites. Comparisons within individual gels can be used to localize the *Cla* I, *Sal* I, and *Nru* I cleavage sites relative to each other to within 0.5–1 kb. The sites are localized precisely as predicted by insertion of a MoMLV genome. In conclusion, the sum of all of the above data provides strong evidence that the altered c-*myc* fragments observed in the lymphomas RT 1-3 and RT 10-2 are due to proviral insertion.

DISCUSSION

The data presented here show that some MLV-induced lymphomas have altered c-*myc* sequences and that in at least

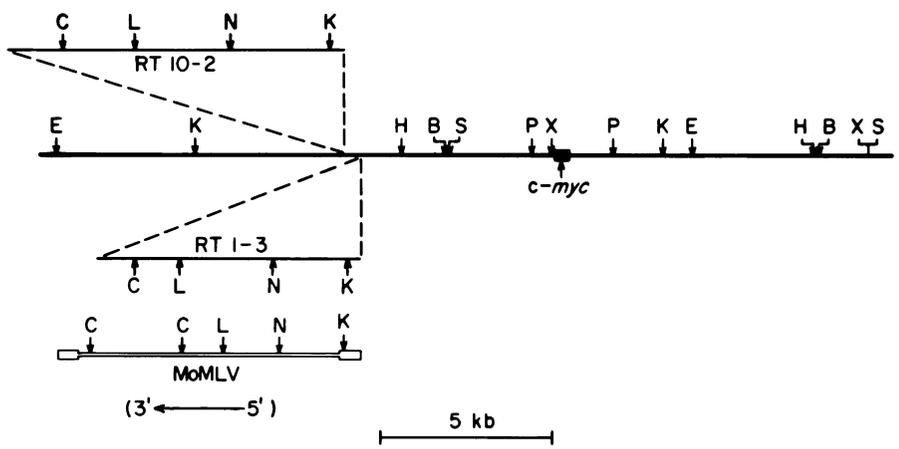

FIG. 4. Map of the restriction endonuclease cleavage sites adjacent to c-*myc* in normal rat, RT 1-3, and RT 10-2 DNAs. The endonucleases are abbreviated as follows: B = *Bam*HI, C = *Cla* I, E = *Eco*RI, H = *Hin*dIII, K = *Kpn* I, L = *Sal* I, N = *Nru* I, P = *Pst* I, S = *Sac* I, and X = *Xba* I. Data from Table 1 were used to locate restriction endonuclease cleavage sites on this map. One limitation to this map is that only the first cleavage sites on either side of the c-*myc* sequences are located on this map. (Fragments produced by more distant cleavage will not be detected with the c-*myc* probe.) Positioning of the sites is derived from subtraction of the sizes of large DNA fragments. Thus, the precision with which these sites are located is limited. To construct an unbiased map, data from Table 1 were used without correction. For example, in every case that *Eco*RI-digested RT 1-3 and RT 10-2 DNA are run in adjacent lanes, they exactly comigrate. Because of gel to gel variation, however, the average sizes of these fragments differ.

two lymphomas, these alterations are the result of proviruses integrated adjacent to the c-*myc* gene.

Two alternative explanations for the observed novel c-*myc* fragments can be excluded. The first alternative explanation is that these alterations represent genetic polymorphisms in individual animals rather than tumor-specific changes. Such polymorphisms would not be expected, because Fischer rats are inbred. The most direct evidence against this explanation, however, is that in the case of RT 1-3, the altered c-*myc* fragment is present only in the tumor tissue, not in normal tissue from the same animal (Fig. 1). Because the alterations in RT 1-3 and RT 10-2 are clearly related, genetic polymorphism in this latter case is also extremely unlikely.

A second alternative explanation for the observed alterations of c-*myc* is that they result from a genetic rearrangement other than proviral insertion, such as recombination or chromosomal translocation. The evidence against this possibility is that in both lymphomas the configuration of altered restriction endonuclease cleavage sites observed is precisely what would be predicted by proviral insertion. Introduction of exactly this configuration of sites, in a specific order and at specific positions relative one to another, would be extremely unlikely to occur by any mechanism other than proviral insertion.

The data presented here do not provide direct evidence that the observed proviral insertions were involved in lymphoma induction. However, the following facts argue that these proviral insertions do play an important role in lymphomagenesis. (*i*) MLVs integrate at many sites in the host cell genome (20). As a result, it would not be expected that 4 of 61 lymphomas would have a provirus within the same 20-kb region by chance. (*ii*) The c-*myc* gene is potentially oncogenic. (*iii*) MLVs are biologically very similar to ALVs, which have been shown to induce lymphomas by insertional mutagenesis of chicken c-*myc* (2–4). Thus, the identification of MLV proviruses adjacent to c-*myc* provides the strongest evidence to date that MLVs can induce tumors by insertional mutagenesis.

Restriction endonuclease cleavage analysis of the MLV proviruses adjacent to c-*myc* in the two MoMLV-induced lymphomas (Fig. 4) leads to two additional conclusions. (*i*) The orientation of the MoMLV proviruses in these two lymphomas is such that simple read-through transcription of c-*myc* would not occur. If the effect of these proviruses is to alter the level of c-*myc* transcription, this effect must be mediated by a more subtle mechanism, perhaps involving enhancer sequences (21). A similar conformation of proviral and c-*myc* sequences has been observed in ALV-induced lymphomas (3). (*ii*) The mink cell focus-forming (MCF) class of recombinant MLVs has been proposed as intermediates in the induction of lymphomas by MLVs (22, 23). The restriction endonuclease cleavage map of the proviruses found adjacent to c-*myc* is inconsistent with their being MCF recombinants of MoMLV, but rather is consistent with their being intact, unaltered MLV proviruses (24, 25). In fact, none of the five proviruses present in the RT 10-2 lymphoma is an MCF-type provirus (unpublished data). This fact indicates that MCF viruses *per se* may have an obligatory role in induction of leukemias by MLVs.

In mice, substantial evidence has been presented that stimulation of the target T lymphocytes by viral antigens, either directly or indirectly (26, 27), is responsible for induction of lymphomas by MLVs. How can these experiments be reconciled with the results in support of insertional mutagenesis as the mechanism of lymphoma induction by MLVs? There are a number of possible explanations, two of which are as follows. (*i*) MLVs play two roles in the induction of lymphomas. First, they produce an expansion of the target population by immune stimulation and then produce malignant transformation by insertional mutagenesis. (*ii*) The role of MLVs is different in different animals, immune stimulation being responsible for lymphomagenesis in some, and insertional mutagenesis in others. In considering this second possibility, it is interesting to note that all of the experiments implicating immune stimulation have been done in mice, whereas almost all of the results implicating insertional mutagenesis have been obtained in rats.

Among the 61 MLV-induced lymphomas examined, only 5 had apparent alteration in the restriction sites adjacent to c-*myc*. The restriction endonuclease digestions used in these experiments would have revealed integrations within 17 kb of one side of the rat c-*myc* sequences or 8 kb of the other side or within about 10 kb of either side of the mouse c-*myc* sequences. Although it is possible that the remaining tumors have proviruses in the region of c-*myc*, but more than 8–17 kb away, or that some mechanism other than insertional mutagenesis is responsible for these lymphomas, I favor the hypothesis that in these other lymphomas, insertional mutagenesis has affected cellular oncogenes other than c-*myc*. In support of this hypothesis, Tsichlis and co-workers have shown two integration sites that are common to a number of MLV-induced lymphomas (ref. 28; P. N. Tsichlis, personal communication); both of these are at least 10 kb away from c-*myc* and represent good candidates for as yet unidentified oncogenes. Interestingly, both of the integration sites identified by Tsichlis *et al.*, as well as the c-*myc* gene, reside on mouse chromosome 15. The significance of this last observation is not apparent. If indeed MLVs induce lymphomas by insertional mutagenesis of genes other than c-*myc*, analysis of such lymphomas could represent a way of identifying heretofore unknown oncogenes.

Note Added in Proof. Since submission of this manuscript, a recombinant DNA clone containing both MoMLV sequences and c-*myc* sequences was isolated from RT 10-2 DNA. The structure of this clone is consistent with all of the results presented in this paper. An additional datum obtained from analysis of this clone is that the MoMLV provirus and the adjacent c-*myc* gene in RT 10-2 are in opposite transcriptional orientations.

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1. Weiss, R., Teich, N., Varmus, H. & Coffin, J. (1982) *RNA Tumor Viruses* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
2. Hayward, W. S., Neel, B. G. & Astrin, S. M. (1981) *Nature (London)* **290,** 475–480.
3. Payne, G. S., Bishop, J. M. & Varmus, H. E. (1982) *Nature (London)* **295,** 209–214.
4. Neel, B. G., Hayward, W. S., Robinson, H. L., Fang, J. & Astrin, S. M. (1981) *Cell* **23,** 323–334.
5. Fung, Y.-K. T., Lewis, M. G., Kung, H.-J. & Crittenden, L. (1983) *Cell* **33,** 357–368.
6. Noori-Daloii, M. R., Swift, R. A., Kung, H.-J., Crittenden, L. B. & Witter, R. L. (1981) *Nature (London)* **294,** 574–576.
7. Nusse, R. & Varmus, H. E. (1982) *Cell* **31,** 99–109.
8. Peters, G., Brookes, S., Smith, R. & Dickson, C. (1983) *Cell* **33,** 369–377.
9. Steffen, D., Bird, S., Rowe, W. P. & Weinberg, R. A. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4554–4558.
10. Rigby, P. W., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113,** 237–241.
11. Vennstrom, B., Moscovici, C., Goodman, H. M. & Bishop, J. M. (1981) *J. Virol.* **39,** 625–631.
12. Fan, H. & Paskind, M. (1974) *J. Virol.* **14,** 421–429.

13. Shields, A., Witte, O. N., Rothenberg, E. & Baltimore, D. (1978) *Cell* **14,** 601–609.
14. Adams, J. M., Gerondakis, S., Webb, E., Corcoran, L. M. & Cory, S. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1982–1986.
15. Taub, R., Kirsch, I., Morton, C., Lenoir, G., Swan, D., Tronick, S., Aaronson, S. & Leder, P. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7837–7841.
16. Marcu, K. B., Harris, L. J., Stanton, L. W., Erikson, J., Watt, R. & Croce, C. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 519–523.
17. Mushinski, F. J., Bauer, S. R., Potter, M. & Reddy, E. P. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1073–1077.
18. Shen-Ong, G. L. C., Keath, E. J., Piccoli, S. P. & Cole, M. D. (1982) *Cell* **31,** 443–452.
19. Southern, E. M. (1979) *Anal. Biochem.* **100,** 319–323.
20. Steffen, D. & Weinberg, R. A. (1978) *Cell* **15,** 1003–1010.
21. Levinson, B., Khoury, G., Vande Woude, G. & Cruss, P. *Nature (London)* **295,** 568–572.
22. Hartley, J. W., Wolford, N. K., Old, J. L. & Rowe, W. P. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 789–792.
23. Fischinger, P. J., Nomura, S. & Bolognesi, D. P. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 5150–5155.
24. van der Putten, H., Quint, W., van Raaij, J., Maandag, E. R., Verma, I. M. & Berns, A. (1981) *Cell* **24,** 729–739.
25. Chattopadhyay, S. K., Lander, M. R., Gupta, S., Rands, E. & Lowy, D. R. (1981) *Virology* **113,** 465–483.
26. Weissman, I. L. & McGrath, M. S. (1982) *Curr. Top. Microbiol. Immunol.* **98,** 103–112.
27. Ihle, J. N. & Lee, J. C. (1982) *Curr. Top. Microbiol. Immunol.* **98,** 85–102.
28. Tsichlis, P. N., Straus, P. G. & Hu, L. F. (1983) *Nature (London)* **302,** 445–449.