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Evidence for a New Form of Retroviral *env* Transcript in Leukemic and Normal Mouse Lymphoid Cells

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Murine leukemia virus-related RNA species were examined in a set of radiation-induced T-cell leukemias from BALB/c mice. No evidence was found for linkage of viral long terminal repeat-derived (U5) sequences to information of host origin. A novel class of 2-kilobase (kb) *env*-related transcripts, about 1 kb shorter than normal viral *env* messenger, was found in all the leukemias. All of the 2-kb transcripts contained sequences homologous to the xenotropic virus-related *env* sequences in the Friend spleen focus-forming virus, representing the N-terminal portion of gp70. In two of the leukemias, these transcripts were found to contain both ecotropic p15E and U3 sequences in addition to the xenotropic gp70-related sequence. These two leukemias, but not others in which ecotropic sequences were absent from the 2-kb RNA, harbored several copies of a specific class of *env* recombinant proviruses. These proviruses possessed full-size *env* genes and were submethylated, as shown by *Sma*I and *Xma*I digests of proviral DNA. Low levels of 2-kb RNA were found in normal thymocytes from strains BALB/c, AKR, and 129 but not from congenic 129 G_{IX}^- mice. It is possible that the 2-kb RNA may originate by a novel splicing step that removes portions of the gp70 and p15E sequences from full-length *env* transcripts.

The expression of endogenous retroviruses in normal mice as well as in mouse lymphoid tumors has led many investigators to address the question of their role in leukemogenesis (6, 21, 34). T-cell lymphomas occurring spontaneously in AKR mice or after X-irradiation in C57BL and BALB/c are good models to study this problem. Because of the complex pattern of virus expression in the course of the life of AKR mice, leukemias induced by external agents such as radiation or chemicals in low-virus strains are potentially simpler systems. The mechanism of radiation leukemogenesis in BALB/c mice has been explored for the past several years in this laboratory (2, 3, 31). Irradiation of young BALB/c mice results in a high incidence of T-cell lymphoma (70%) by the age of 9 months. Radiation also results in early expression of ecotropic virus in these mice (3; P. O'Donnell and A. Silverstone, personal communication); the connection of this expression to leukemogenesis is as yet unclear.

If we suppose that the expression of the endogenous murine leukemia virus (MuLV) is in some way prerequisite for the development of radiation leukemia, some relevant experimental models must be considered. In B-cell leukemogenesis by avian leukosis viruses, an exogenous provirus is integrated in the tumor cells next to a cellular *onc* gene (c-*myc*); the promoter or enhancer (or both) sequences present in the long terminal repeat (LTR) of the provirus increases the expression of this gene (9, 20, 22). A second line of evidence connects T-cell leukemogenesis in inbred mice to recombinant MuLV *env* expression, e.g., in the case of spontaneous leukemia in AKR mice (6, 8). Although polytropic (mink cell focus-inducing [MCF] type) *env* recombinant MuLV has not been isolated from radiation leukemias of BALB/c mice, an ecotropic *env* recombinant virus with leukemogenic activity was isolated from one such leukemia (2; R. Ellis and E. Fleissner, unpublished data). An example of X ray-induced thymic lymphoma in outbred Swiss mice which lack germ-line ecotropic virus has been analyzed by Fischinger et al. (5). They found expression of an MuLV glycoprotein related to MCF-MuLV on the surface of thymoma cells in the absence of infectious virus expression.

To explore further the potential role of endogenous MuLV in radiation leukemogenesis, we analyzed the intracellular polyadenylated

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[poly(A)$^+$] RNAs of a set of BALB/c radiation leukemia cell lines (31). Specifically, we looked for new RNA species which could represent either cellular genetic information activated by a viral promoter or transcripts of proviruses with recombinant *env* genes. We have found a novel form of the latter type of transcript.

MATERIALS AND METHODS

Mouse strains. We used the following strains from the colony of E. A. Boyse at Memorial Sloan-Kettering Cancer Center: BALB/c, AKR, 129, and 129 G_{IX}^-. The mice were between 6 and 8 weeks of age.

Cells. Nine BALB/c radiation leukemias have been established in culture. Their origin and properties have been described previously (31).

DNA and RNA extraction and hybridization. DNA was extracted by the method of Gross-Bellard et al. (7). Hybridization analysis of DNA cut with restriction enzymes, by gel electrophoresis and transfer to nitrocellulose, was as described by Southern (27). Total RNA was extracted from exponentially growing cells (10^6 cells per ml) or from cell suspensions of thymocytes. Cells were pelleted and, after washing in cold phosphate-buffered saline, lysed at 37° for 5 min in STE buffer (0.02 M Tris, pH 7.4, 0.1 M NaCl, 0.001 M EDTA) containing 200 μg of proteinase K per ml with 1% sodium dodecyl sulfate. RNAs were extracted twice with phenol saturated in sodium acetate (0.1 M), pH 4.5, and ethanol precipitated. Poly(A)$^+$ RNA was selected on oligodeoxythymidylate [oligo(dT)]-cellulose and then denatured with glyoxal as described by McMaster and Carmichael (17). Size separations (15 μg of leukemia-derived RNAs, 30 μg of thymocyte RNA) were carried out by electrophoresis on 1% agarose gels in 10 mM phosphate buffer, pH 6.8. The RNAs were blotted on 2-aminophenylthioether paper (26) and then hybridized with ^{32}P-labeled DNA probes. Conditions of hybridization and washing have been described by Saule et al. (25).

Derivation and labeling of DNA probes. (cf. Fig. 4 for schematic representation of probe sequences.) pBR322 clones of the AKV viral genome (pAKV) and the AKV LTR (pLTR) were obtained from D. Lowy and S. Chattopadhyay at the National Institutes of Health, Bethesda, Md. A U3-specific probe (pAKV U3) was prepared from pLTR by cutting with *Kpn*I and treatment with *Bal*31 to remove about 100 nucleotides on both sides of the *Kpn*I site. The fragment obtained after cutting by *Pst*I was cloned in pBR322 (restriction enzymes and *Bal*31 were purchased from Boehringer Mannheim and from Bethesda Research Laboratories). A probe specific for the U5 region of AKV was prepared by cutting pLTR with *Kpn*I and *Eco*RI, releasing a DNA fragment containing about 20 nucleotides of the repeat sequence (R) of the LTR, the entire U5 sequence, and about 600 nucleotides from the mouse genomic DNA sequence contiguous to U5 in the pLTR plasmid. Two clones of *env* sequences from the spleen focus-forming virus (SFFV) of the Friend virus complex were also used in these studies. One sequence was bounded by the *Bam*HI and *Eco*RI sites in the N-terminal part of the SFFV *env* region [pSFFV(BE)]. The second probe was bounded by the *Ava*I site near the middle of the BE fragment and by the *Eco*RI site [pSFFV(AE)]. pSFFV(BE) was obtained from E. Athan and D. Dina at the Albert Einstein College of Medicine, Bronx, N.Y.; pSFFV(AE) was from A. Oliff of this institute. Finally, we used a probe specific for part of the p15E region of AKV, designated pAKV5, which was from W. Herr, Harvard University, Cambridge, Mass. (10). Plasmids were nick translated in the presence of [^{32}P]dCTP (600 Ci/mM) and [^{32}P]dATP (600 Ci/mM), both from New England Nuclear, using 10 U of DNA polymerase (grade I; Boehringer Mannheim) according to the method developed by Rigby et al. (23). Probes were separated from triphosphate by spin dialysis through Sepharose 6B (18). The nick translated probes had a specific activity of about 2×10^8 cpm/μg.

RESULTS

Hybridization of poly(A)$^+$ RNA from radioinduced leukemias with the LTR and genomic probes from AKV. Ten micrograms of poly(A)$^+$ RNA from each leukemia was size separated, blotted on APT paper, and hybridized with ^{32}P-labeled pLTR. The patterns of hybridization of the RNAs from four leukemias are shown in Fig. 1. Although some variation was observed in the virus-related RNAs from the individual leukemias, an RNA of genomic size (8.5 kilobases [kb]) and species of about 3 kb, which corresponded to viral *env* mRNA, were seen in most preparations. In addition, we found in these four leukemias, and in all the radiation leukemias tested, a new RNA 2 kb in size, which hybridized with the LTR probe.

The presence of a virus-related RNA in the 2-kb size range in all the leukemias was reminiscent of findings in the case of virus-induced avian leukemias (9, 20, 22). In the avian model, only viral U5 sequences are present in the same RNA which contains c-*myc* sequences. We

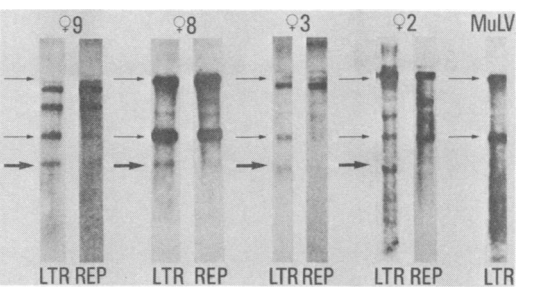

FIG. 1. RNA blots of poly(A)$^+$ RNA from four leukemias (first eight tracks) and from NIH 3T3 cells infected by Moloney MuLV (last track). The pairs of tracks corresponding to each leukemia show hybridization of the same blot with probes representing either the LTR region of AKR ecotropic virus (LTR) or the complete genome of this virus (REP). The upper two arrows designate positions of genomic-size (8.5 kb) and *env* (3 kb) transcripts; the bold arrows designate 2-kb transcripts detected in all the leukemias with the LTR probe.

therefore tested these RNAs for the presence of viral structural gene sequences by hybridization with a probe, pAKV, representing the complete genome of ecotropic virus from the AKR mouse. (The AKR ecotropic virus is virtually identical to the BALB/c endogenous ecotropic virus.) This probe produced only a very faint signal with the 2-kb RNA, which indicated that only a small fraction of the pAKV sequence hybridized with this RNA species (Fig. 1). Since the AKV probe contained the entire LTR sequence, it was likely that pLTR and pAKV detected the same sequences in the 2-kb RNAs, suggesting that the latter contained little, if any, ecotropic virus structural gene information.

Because these results were reminiscent of the avian lymphoma model, they prompted us to analyze the contributions of U5 and U3 in the hybridization of the 2-kb RNA with pLTR. Figure 2 shows the results of sequential tests on the same Northern blot of poly(A)$^+$ RNA from the leukemia RL♂1 with U3 and U5 probes. The 2-kb RNA hybridized with U3 as well as with U5. This result indicated that the RNA probably did not derive from downstream transcription of a cellular gene, originating in a viral LTR sequence, since such transcripts are not expected to be terminated by viral U3 sequences.

Hybridization of leukemia-derived RNAs with *env*-specific probes. We therefore tested other viral structural gene probes for their ability to hybridize with the 2-kb species. In the left panel of Fig. 3 are shown the results with a xenotropic virus-specific probe (BE) representing a 0.6-kb segment of the *env* region of the Friend SFFV (cf. Fig. 4). Poly(A)$^+$ RNAs from four leukemias are shown. The 2-kb RNA in all four hybridized with the BE probe. This result showed that at least partial gp70 coding sequences of xenotropic specificity were present in the 2-kb species. As was the case with the LTR probe (Fig. 1), we also detected both genome-size (8.5 kb) and *env* (3 kb) mRNA transcripts with the BE probe in all four leukemias. A 7.5-kb RNA with xenotropic specificity was also prominent. In some cases (RL♀2 and ♀6), viral RNAs of about 6 kb and also of supergenomic (ca. 9.5 kb) size were seen. Since these latter species were not regularly detected, we did not investigate them further.

When the same blot was tested with the U3 probe (right panel of Fig. 3), we observed that the 2-kb RNA in one leukemia (RL♀2) hybridized strongly, whereas others hybridized only weakly (RL♂6 and RL♀6) or undetectably (RL♀3). Thus, with respect to the relative reactivity of their 2-kb RNA with the BE and U3 probes, RL♀2 and the other three leukemias behave in a reciprocal fashion. In probing blots of RNA from fibroblasts infected with either ecotropic or xenotropic BALB/c-derived viruses, we found that the U3 probe displayed a strong specificity for ecotropic virus RNAs (data not shown). Therefore, the results in Fig. 3

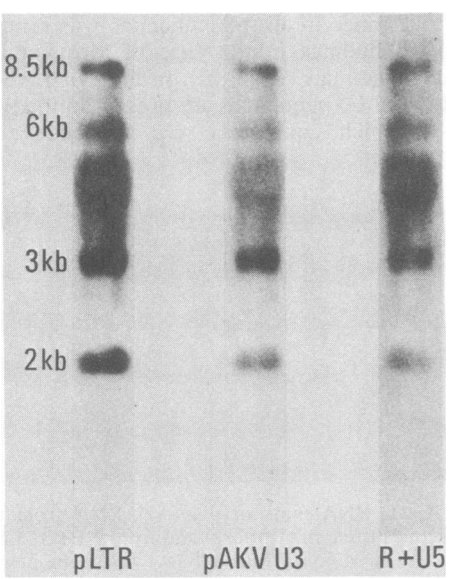

FIG. 2. Hybridization of the same blot of poly(A)$^+$ RNA from the leukemia RL♂1 with three probes representing the complete LTR sequence of AKR ecotropic virus (pLTR) and the U3 or U5 portions of the LTR. The U5 probe contains about 20 base pairs of the R segment located between U3 and U5. R sequences were removed from the U3 probe by digestion with *Bal*31.

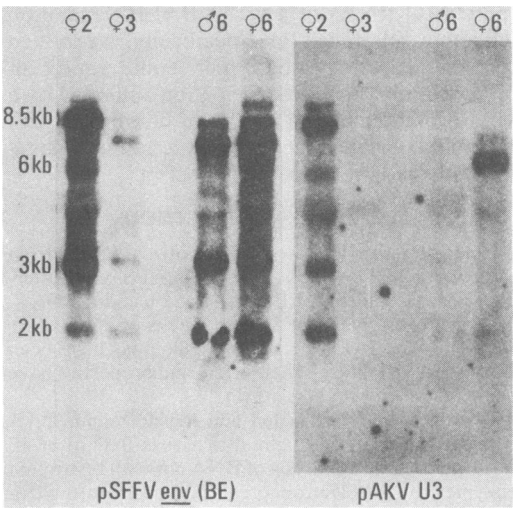

FIG. 3. Poly(A)$^+$ RNAs from four leukemias hybridized with either the xenotropic *env* BE probe from SFFV or the ecotropic U3 probe from AKR ecotropic virus. The same blot was used for hybridizations in the left and right panels.

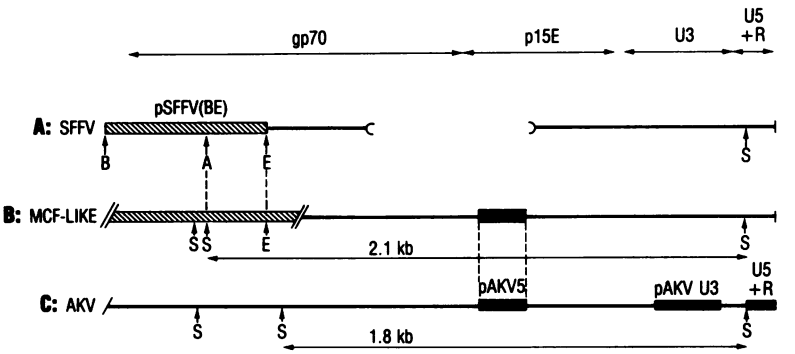

FIG. 4. Schematic representation of restriction sites and location of probe sequences in the genomes of SFFV, an MCF-like recombinant virus, and AKR ecotropic virus (AKV). Restriction sites: B, *Bam*HI; S, *Sma*I; E, *Eco*RI; A, *Ava*I. Viral genomes: A, SFFV; B, MCF; C, AKV.

indicated that the 2-kb RNAs of different radiation leukemias were composed of different ratios of ecotropic and xenotropic virus-related information. The 2-kb RNA from RL♂1 was also found, like that from RL♀2, to exhibit a relatively strong reactivity with the U3 probe (cf. Fig. 2) as compared to its reactivity with the BE probe (not shown). The 2-kb RNAs from several other radiation leukemias tested were unlike the 2-kb species in RL♂1 and RL♀2 and more closely resembled the species in RL♀3, RL♂6, and RL♀6. We also observed size differences among the RNA species in the 3-kb size range detected with the BE probe. The 3-kb species detected in RL♂1 and RL♀2 was somewhat smaller than those found in the other leukemias (compare ♀2 and ♀3 in Fig. 3).

To examine further the nature of *env*-related information in the 2-kb transcript, RNA blots were probed with a cloned DNA representing about 170 base pairs of the N-terminal region of ecotropic p15E (see Fig. 4). This probe, pAKV5, detected the 2-kb species in RNA from RL♂1 and RL♀2, but not in RL♂6 or RL♀6 (Fig. 5). These results parallel those for the U3 probe and show that in two leukemias the 2-kb species contains an ecotropic p15E sequence as well as xenotropic gp70 information. The relatively weak signal observed with the pAKV5 probe for the 2-kb RNA as compared with the 3-kb ecotropic *env* RNA (compare Fig. 5 to Fig. 2 and 3) suggests that only a part of the sequence represented by pAKV5 is present in 2-kb RNA, i.e., that a deletion of *env* information in the RNA may start in the probed sequence and extend in the 5′ direction into gp70 coding sequences.

Correlation of somatically acquired *env* recombinant proviruses with the presence of a 2-kb transcript having both xenotropic and ecotropic *env* sequences. In a previous report it was noted that the *env* protein species produced in RL♂1 and RL♀2 appeared to be serologically related to *env* protein found in a BALB/c leukemia induced by an *env* recombinant MuLV (31). We therefore inquired whether the differing ratios of reactivities to xenotropic (BE) and ecotropic (p15E and U3) probes found in 2-kb RNAs might reflect a selective occurrence of *env*-recombinant viral genomes in these leukemias. To test this proposition, we hybridized Southern blots of leukemia DNAs with the ecotropic pAKV5 probe, since this p15E region is frequently con-

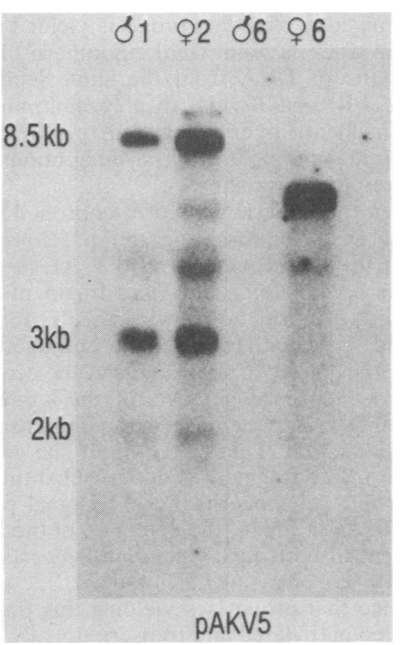

FIG. 5. Poly(A)$^+$ RNAs from four leukemias hybridized with the pAKV5 probe representing a 170-base pair sequence in the N-terminal region of ecotropic p15E (cf. Fig. 4).

served in leukemogenic recombinant MuLVs of the MCF type (10). We used a restriction enzyme, *Sma*I, which cuts in the middle of the BE sequence and at a similarly located site in ecotropic *env* sequences (near the beginning of the *env* gene) as well as in the viral LTR (see Fig. 4). The distance between these *Sma*I sites is 2.1 kb. In contrast, ecotropic proviruses are the source of a 1.8-kb *Sma*I fragment. This is because ecotropic, but not xenotropic, viruses possess an additional *Sma*I site just beyond the region equivalent to the BE sequence. Recombinant viruses which have substituted the N-terminal portion of gp70 with xenotropic virus-related sequences might be expected to have lost this *Sma*I site. If such recombinants retained appropriate ecotropic p15E sequences, the 2.1-kb *Sma*I fragment produced would hybridize with the pAKV5 probe, unlike analogous fragments generated from endogenous viral genomes of the xenotropic class.

To visualize all fragments of interest and to assess their level of methylation, we analyzed DNAs digested by either *Xma*I or *Sma*I. These enzymes are isoschizomers but *Xma*I, unlike *Sma*I, is insensitive to cytosine methylation. Results for four leukemias are shown in Fig. 6. DNAs from RL♂1 and RL♀2 do in fact display the 2.1-kb pAKV5-reactive bands indicative of *env* recombinant proviruses. The intensity of the bands reflects multiple proviruses. (The dark 1.8-kb bands appear to represent multiple integrations of ecotropic proviruses; the single germline copy of such a provirus yielded a light band in track N with *Xma*I.) Southern blots of digestions of DNA from the same leukemias with *Eco*RI and *Bam*HI display multiple junction fragments hybridizing with pAKV5, confirming that multiple proviral integrations have occurred (data not shown).

When the same DNA blot was probed with a cloned DNA representing the p15E-proximal half of the BE sequence (pSFFV-AE [see Fig. 4]), the 2.1-kb *Xma*I and *Sma*I fragments were also found to hybridize with this xenotropic-specific sequence (Fig. 7). The conjunction of the xenotropic gp70 sequence and the ecotropic p15E sequence of pAKV5 in the same *env*-derived DNA fragment is a direct demonstration that elimination of the *Xma*I/*Sma*I site generating the 1.8-kb fragment is due to substitution of xenotropic sequence in the N-terminal portion of gp70. As in Fig. 6, the detection of the 2.1-kb fragment in both *Sma*I and *Xma*I digests from the two leukemias, RL♂1 and RL♀2, is also evidence that proviruses yielding this fragment are hypomethylated and transcriptionally active (11, 19). In Fig. 7, the prominent 2.3-kb band in *Xma*I, but not *Sma*I, digests presumably represents germline copies of xenotropic *env* sequences (24, 28). (This band is less prominent in the track [N] showing the digest of normal BALB/c liver DNA, because somewhat less DNA was loaded in this sample, as was apparent in the ethidium bromide staining of the original gel.)

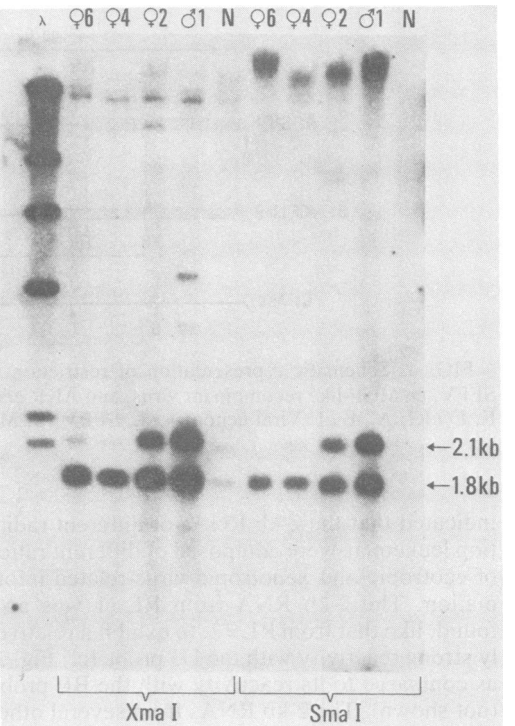

FIG. 6. Southern blot of DNAs from four BALB/c radiation leukemias, RL♀6, RL♀4, RL♀2, and RL♂1, cut with either *Xma*I or *Sma*I. Tracks (N) representing normal BALB/c liver DNA cut with either enzyme were included as controls. The first track on the left shows *Hin*dIII-cut λ marker DNA. The probe used was a cloned DNA fragment from the N-terminal half of the p15(E) part of the AKR ecotropic virus *env* gene (pAKV5). The 1.8-kb restriction fragments contain *env* and U3 sequences and represent predominantly somatically acquired proviruses; the 1.8-kb fragment from the single copy of ecotropic germline provirus can be seen in the N track for the *Xma*I digest (not in the *Sma*I digest due to methylation of this provirus). The 2.1-kb fragments from RL♀2 and RL♂1 DNA represent *env* recombinant (MCF-like) proviruses. These fragments on the same blot also hybridized with a xenotropic-specific gp70 probe (see Fig. 7).

It should be emphasized that a 2.1-kb *Sma*I fragment containing both ecotropic and xenotropic *env* sequences was found in only two out of the nine BALB/c radiation leukemias which we examined. It was therefore striking that the same two leukemias (RL♂1 and RL♀2) ex-

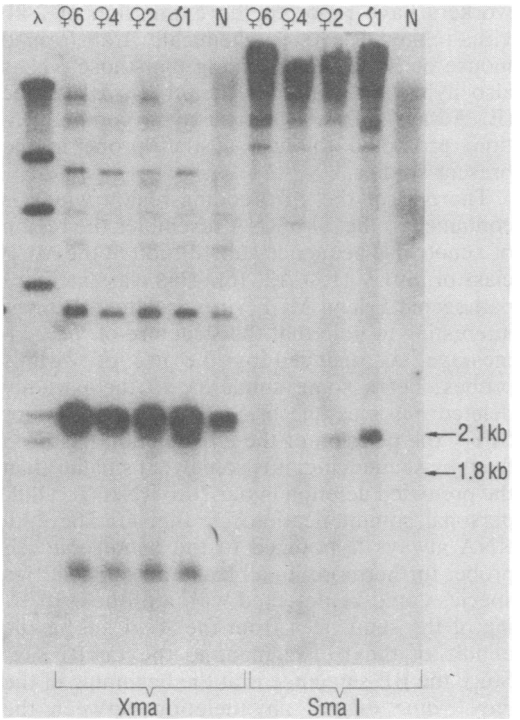

FIG. 7. Hybridization of the same Southern blot shown in Fig. 6 with the xenotropic *env* probe BE. The 2.1- but not the 1.8-kb *Sma*I and *Xma*I fragments were detected by this probe. Also detected were 2.3-kb *Xma*I fragments representing multiple germline viral genomes with *env* sequences of the xenotropic class. These fragments were not reactive with the ecotropic pAKV5 probe (cf. Fig. 6). They appeared to be highly methylated as shown by their absence in the *Sma*I digest.

pressed 2-kb transcripts with recombinant characteristics.

2-kb RNAs detected in normal mouse thymocytes. When we analyzed total poly(A)$^+$ RNAs from thymuses of 6- to 8-week-old BALB/c mice by hybridization with BE probe, we detected 7.5-, 3-, and 2-kb RNAs (Fig. 8). These three species were not detected when hybridization was carried out with the AKV-derived U3 probe. This is probably a reflection of a xenotropic specificity in the U3 sequences of these RNAs, but the low concentration of these viral transcripts in normal BALB/c tissue makes a definite conclusion difficult. When the same amount of RNA from spleen and bone marrow was analyzed as for thymocytes, only very faint bands corresponding to the three thymocyte species were observed (data not shown). We also analyzed the poly(A)$^+$ RNA from BALB/c liver and found no expression of MuLV-related mRNA in this organ. As in the case of BALB/c, normal thymocytes from AKR mice at 2 months of age expressed 7.5-, 3-, and 2-kb RNA species detectable with the BE probe (Fig. 8). When the same RNAs were hybridized with pAKV U3 (not shown), we observed full-size 8.5-kb genomic and 3-kb *env* mRNAs, a result which can be attributed to the chronic expression of the ecotropic endogenous virus in AKR.

We were interested in comparing the expression of viral RNAs in thymocytes of the congenic strains 129 and 129-G_{IX}^- (29, 33) with the pattern observed with normal BALB/c and AKR thymocytes after hybridization with the BE probe. We observed the usual three bands at 7.5, 3, and 2 kb in strain 129, but not in 129 G_{IX}^- (Fig. 8). This result suggests that in strain 129 mice expression of all three RNAs is controlled by the same genetic locus or by very closely linked loci.

DISCUSSION

The compositions of poly(A)$^+$ RNAs from nine radioinduced BALB/c cultured leukemias were analyzed by Northern blotting with the use of various cloned viral DNA probes. Viral DNAs in the size range of genomic and *env* mRNAs were generally present in the leukemias. In addition, a 2-kb RNA was found in all of the leukemias, which hybridized to a probe representing the LTR of the AKR ecotropic virus (Fig. 1). This RNA species hybridized with a probe containing only the U5 region of the

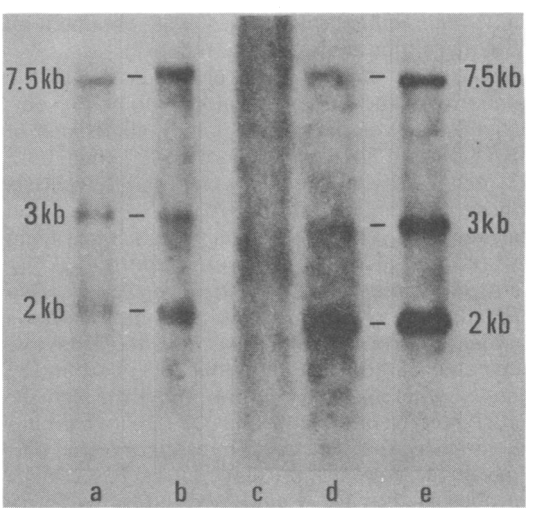

FIG. 8. Poly(A)$^+$ RNAs from normal mouse thymocytes of strains BALB/c (a), AKR (b), 129 G_{IX}^- (c), and 129 (d). Track (e) shows RNA from the radiation leukemia RL♀4. The BE probe was used.

LTR and very poorly to a probe representative of the whole ecotropic virus genome (Fig. 1 and 2). These are properties predicted by the promoter insertion model of retroviral oncogenesis (9). In preliminary experiments, we tested several molecularly cloned oncogenes (*myc*, Ki-*ras*, Ha-*ras*, *mos*, and *fes*) and found no hybridization with the 2-kb RNA and no abnormal expression of the mRNAs corresponding to these oncogenes. We did not pursue this analysis further with probes for other oncogenes because we could show that the 2-kb RNA also hybridized to the U3 region of the LTR, a result which is not predicted by the viral promoter insertion model, and that the RNA hybridized to a xenotropic *env*-specific probe (a *Bam*HI-*Eco*RI fragment [BE] from the *env* gene of SFFV) (Fig. 2 and 3). With the latter probe we also observed in all the leukemias 3-kb xenotropic *env* mRNAs, which correlates well with the expression of *env* glycoprotein with xenotropic specificity as reported by Tress et al. (31).

Some insight into the variable composition of 2-kb RNA species emerged from comparisons of the intensity of hybridization signals obtained with a xenotropic probe (BE) and the ecotropic probes U3 and pAKV5. The 2-kb RNAs from most leukemias gave a much stronger signal with the xenotropic probe than with the ecotropic probes. In two leukemias, RL♂1 and RL♀2, the 2-kb RNA reactivity with the ecotropic probes was greatly enhanced with respect to the signal with the xenotropic probe. These results are consistent with the hypothesis that, at least in these two leukemias, 2-kb RNA is expressed from a recombinant viral genome. Evidence that these two leukemias do in fact harbor amplified *env* recombinant proviruses was obtained by Southern blot analysis (Fig. 6 and 7).

The form of 2-kb RNA characteristic of the majority of the leukemias appears to be present, at a low level, in normal lymphoid tissues of BALB/c and AKR mice. In strain 129 mice, both 3- and 2-kb *env* species were found, whereas these species of viral mRNA were not detected in congenic 129 G_{IX}^- mice, which differ from strain 129 at the *Gv-1* locus controlling G_{IX} antigen expression (29, 30). Levy et al. also found that expression of multiple viral RNAs is under control of *Gv-1* (14). The fact that we detected a 2-kb RNA in thymocytes of strain 129 mice, whereas Levy et al. did not, can be attributed to our use of a probe (BE) which has xenotropic specificity and hybridizes to the partial gp70 sequence present in this RNA. (The probes used by Levy et al. derived from ecotropic Moloney MuLV, which is only very weakly homologous to xenotropic *env* sequences in the region represented by the BE probe [I. Verma, personal communication].) Kirschmeier and coworkers have reported the expression of 2-kb virus-related RNAs in chemically transformed mouse cells (13). The finding that those RNAs also hybridized with the xenotropic BE probe (E. Athan and D. Dina, personal communication) prompted the use of that probe in the present studies.

The part of the gp70 coding region which is contained in the 2-kb RNA resembles the region of xenotropic sequence substitution in the MCF class of MuLV (1, 4, 12, 16). This class includes pathogenic Friend MCF virus isolates, and it is interesting to note that the structure of the 2-kb message, as deduced by the use of various probes, bears some similarity to the partially deleted *env* message of SFFV (4, 15, 32). For SFFV the position of the *env* deletion has been fixed by sequencing; it is somewhat smaller than the predicted deletion in our 2-kb RNA (A. Oliff, personal communication; cf. Fig. 4). The 2-kb RNA always hybridized to the xenotropic BE probe; furthermore, in all leukemias tested, this species could be detected with a probe consisting of the sequences from the *Ava*I site in the middle of the BE segment to the *Eco*RI site. Since the BE sequence is at the beginning of the *env* coding region, any deletion between the *Ava*I site and the U5 sequence would have to be quite small (<0.5 kb), assuming a normal splice junction between the latter sequence and *env*. A deletion of about 1 kb of normal *env* mRNA sequences is predicted from the (highly conserved) size of the 2-kb species. The simplest model (in which a single deletion is responsible for the size difference) would locate this deletion between the *Eco*RI site in the gp70 sequence of MCF viruses and a point in the p15E region defined by the pAKV5 probe.

An interesting possibility is that the 2-kb RNA results from a specific splicing event, occurring at a low level, perhaps preferentially in certain cell types, e.g., lymphoid cells. The coordinate control of 3- and 2-kb *env* mRNAs by the *Gv-1* locus in 129 mice fits this hypothesis. Such a model is also consistent with the finding of *env* recombinant proviruses having no apparent *env* deletion in the two leukemias which express a recombinant form of 2-kb RNA. This model can be tested by the isolation and sequencing of cDNA clones of 2-kb RNA and comparison of these sequences with data on full-length viral *env* genes.

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