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# Patterns of Proviral Insertion and Deletion in Avian Leukosis Virus-Induced Lymphomas

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**Sixty-eight lymphomas induced by eight different avian leukosis viruses have been analyzed on Southern blots for virus-induced mutations in the chicken c-*myc* gene. Sixty-six of the lymphomas exhibited a proviral insertion in c-*myc*, whereas one exhibited a new transduction of c-*myc*. Sixty-four of the proviral insertions were in the same transcriptional orientation as c-*myc*. Two were in the opposite transcriptional orientation. All of the insertions were upstream of the protein-coding sequences of c-*myc*, with most residing in the first exon or the first intron of c-*myc*. All of the lymphoma-inducing proviruses had deletions that included either sequences near the 5′ long terminal repeat (LTR) or an LTR. The most frequent lymphoma-inducing provirus appeared to have retained both of its LTRs, but had lost sequences near its 5′ LTR. The second and third most frequent lymphoma-inducing proviruses consisted of solo LTRs or of proviruses that had lost the 5′ LTR as well as some internal sequences. Twenty-four insertions were mapped in c-*myc*. Each of these mapped to within 150 base pairs of one of the five DNase I-hypersensitive sites that occur in a 3-kilobase region immediately 5′ to the protein-coding sequences of c-*myc*. One lymphoma contained a new c-*myc* transducing virus. This virus, MYC-3475, caused rapid-onset myelocytomatosis.**

The induction of B-cell lymphoma by avian leukosis viruses (ALVs) is associated with proviral insertions in the chicken c-*myc* gene (5, 9, 21). Virtually all of these insertions increase the expression of c-*myc* either by the initiation of transcripts in a proviral long terminal repeat (LTR) or by the enhancement of the expression of normal transcripts (5, 9, 14, 21). In no case has a lymphoma-inducing insertion truncated the c-*myc* protein product. Consequently, insertions are thought to cause lymphoma by up-regulating the transcription of c-*myc*.

In this manuscript we report data obtained from the analysis of 68 ALV-induced lymphomas for proviral insertions in c-*myc*. In agreement with the work of others, we find that the chicken c-*myc* is a frequent target for lymphoma-inducing insertions and that lymphoma-inducing proviruses have sustained deletions (4, 5, 9, 17, 21, 22, 29). However, in contrast to the implied results of others, we find that the deletions in lymphoma-inducing proviruses do not necessarily include all of one of the proviral LTRs. Rather, the most frequent form of lymphoma-inducing provirus has retained sequences in both of its LTRs but sustained an internal deletion. These internal deletions appear to include at least 500 base pairs (bp) near the 5′ LTR.

## MATERIALS AND METHODS

**Viruses.** Each of the eight ALVs used to induce lymphomas contained the *gag* (core proteins), *pol* (RNA-directed DNA polymerase), and *env* (envelope glycoproteins) genes and LTR sequences that are characteristic of replication competent ALVs. None contained transduced host sequences. Rous associated virus type (RAV-1) (28) was obtained from L. B. Crittenden of the Regional Poultry Research Laboratory, East Lansing, Mich. The RAV-60s were obtained from H. and T. Hanafusa, Rockefeller University, New York, N.Y. The RAV-60s represent four different recombinants between RAV-1 or RAV-2 and endogenous ALVs (26). Fujinami-associated virus was isolated from a stock of Fujinami sarcoma virus provided by H. Hanafusa. This isolate of Fujinami-associated virus has an *Eco*RI site in its LTR. Nontransforming subgroup E virus strain 2 is a recombinant of the Prague strain of Rous sarcoma virus type B and RAV-0 (35). It was obtained from J. Coffin, Tufts University School of Medicine, Boston, Mass. A molecularly cloned isolate of RAV-1 (pRAV-1) was obtained from J. M. Bishop and H. E. Varmus. Virus recovered from this clone is considered to be different from that obtained from L. B. Crittenden, since RAVs maintained in different laboratories frequently have different sequences in their enhancer regions (2, 10, 11, 40). Virus stocks were obtained by harvesting the culture medium of infected chicken or turkey cells.

**Lymphomas.** Lymphomas were harvested from K28 or (K28 × $15_1$)×K28 chickens that had been intravenously inoculated at 1 day of age with ~$10^6$ IU of an ALV (24–26). Tumor and control tissues were harvested from moribund or recently deceased chickens. A portion of these tissues was fixed in Formalin for subsequent histological analyses. The bulk of the tissue was quick-frozen and stored at −80°C to preserve the tumor DNA, RNA, and proteins for biochemical analyses.

**Analysis of tumor DNA.** DNA was harvested from tumor tissues, digested with restriction endonucleases, and analyzed on Southern blots as described by Miles and Robinson (15). DNA blots hybridized with the MYC, SMA, and IN2 probes were washed at higher stringencies than blots hybridized with other probes. These high-stringency washes (0.3× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 0.1% sodium pyrophosphate, 0.1% sodium dodecyl sulfate; 65°C) served to remove much of the nonspecific hybridization observed for probes with the G+C-rich regions of c-*myc*. Many of the Southern blots underwent sequential hybridizations with several probes. Probes were melted from blots by washing the blot for 15 min at 45°C in 0.3× SSC plus

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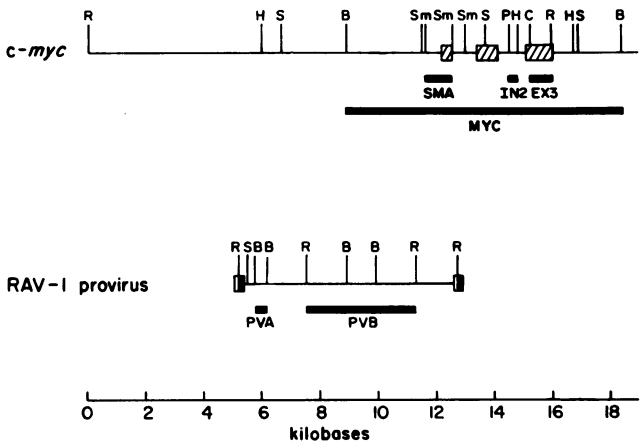


FIG. 1. Restriction endonuclease sites and probes for c-*myc* and RAV-1 sequences. Symbols: ▨, c-*myc* exons; ▬, probes; ◨, proviral LTR (the entire LTR was used as a probe for LTR sequences, the filled area 3′ to the *Eco*RI site represents sequences used for the 3′ LTR probe). Restriction endonucleases: B, *Bam*HI; C, *Cla*I; H, *Hin*dIII; P, *Pst*I; R, *Eco*RI; S, *Sac*I; Sm, *Sma*I. Sites for *Cla*I and *Pst*I have not been mapped throughout the c-*myc* sequence. Positions of exons and introns in c-*myc* are from Watson et al. (39), Shih et al. (32), and Linial and Groudine (14).

50% formamide followed by washing the blot for 5 min in 0.3× SSC–0.1% sodium dodecyl sulfate. Blots were then air dried and verified to be free of $^{32}$P-labeled DNA by autoradiography.

**DNAs used for hybridization probes.** The DNAs used for hybridization with c-*myc* were obtained from the recombinant plasmid pcMCBAM (38). Subclones of pcMCBAM were used to detect sequences containing the first exon, the second intron, and the third exon of c-*myc* (Fig. 1). The subclone of sequences in the third exon was a kind gift of M. Linial. On some blots, a *Sal*I fragment of cloned v-*myc* sequences (37) was used as a probe for third-exon sequences. This probe hybridizes with sequences in the 16-kilobase (kb) *Eco*RI fragment of c-*myc* that contains the first-, second-, and third-exon sequences as well as a ~20-kb fragment of c-*myc* that contains only third-exon sequences. The SMA and IN2 DNAs were prepared by isolating appropriate restriction endonuclease fragments of pcMCBAM from low-melting-point agarose gels and cloning them into pUC plasmids. The IN2 DNA was isolated from its cloning vector before nick translation. The PVA probe for proviral *gag* sequences is the fragment bounded by *Bam*HI sites at bp ~550 and ~1850 of proviral DNA. A pBR322 clone containing PVA sequences in the *Bam*HI site of pBR322 was kindly provided by workers in the laboratories of J. M. Bishop and H. E. Varmus. The PVB probe is the 3.8-kb *Eco*RI fragment of pRAV-1 DNA. This fragment is flanked by the *Eco*RI sites at bp ~2400 and ~6200 in proviral DNA. PVB DNA as well as sequences representing one complete LTR (internal *Eco*RI fragment from the tandem LTRs in pRAV-1) were isolated from *Eco*RI-digested pRAV-1 DNA. The 3′ LTR probe comprises sequences 3′ to the *Eco*RI site in the LTR. The isolation of this probe from p53, a cDNA clone of PrRSV-C, is described by Miles and Robinson (15).

**Sizing of DNA fragments.** Fragments detected on Southern blots were sized by comparing the mobility of the fragment with that of fragments of *Hin*dIII-digested lambda DNA. The positions of normal c-*myc* and proviral fragments (30, 39) were also used in the construction of the sizing curve.

**DNase I-treated DNA.** DNA isolated from DNase I-treated chromatin from the bursa of 2-week-old chickens was a kind gift of Mark Groudine and co-workers (29).

## RESULTS

**Screening of ALV-induced lymphomas for proviral insertions in c-*myc*.** Lymphomas were screened for proviral insertions in c-*myc* by sequentially hybridizing Southern blots of *Eco*RI-digested DNAs with probes for the third exon of c-*myc* (EX3 probe), for sequences 3′ to the *Eco*RI site in the LTR (3′ LTR probe), and for sequences within the most 5′ *Eco*RI fragment of the provirus (PVA probe) (Fig. 1). Hybridization of a lymphoma-specific fragment with the EX3 probe provided evidence for the involvement of c-*myc* in the generation of the lymphoma. Hybridization of the same fragment with the 3′ LTR probe suggested that the tumor was induced by an insertion in the same transcriptional orientation as c-*myc*. Failure of the tumor-specific band to hybridize with the PVA probe suggested that the 3′ LTR of a provirus was at the junction of proviral and c-*myc* sequences.

The initial screen detected novel *Eco*RI fragments of c-*myc* in 67 of the 68 lymphomas (Table 1); 80% of these were 2.9- to 4.0-kb *Eco*RI fragments that hybridized with the EX3 and 3′ LTR probes, but not with the PVA probe. Hereafter, such fragments are termed typical fragments, whereas fragments of other sizes or patterns of hybridization are termed atypical.

**Detailed mapping of selected lymphoma-inducing insertions.** All of the RAV-1-induced lymphomas as well as all of the lymphomas with atypical novel fragments were tested for 5′ as well as 3′ junctions of proviral and c-*myc* sequences, for deletions in proviral sequences, and for deletions or rearrangements in flanking host sequences. The goals of these studies were to define the patterns of lymphoma-inducing insertions associated with a virus that induces a high incidence of lymphoma and to obtain a number of examples of less frequent patterns of insertions.

**5′ and 3′ junctions of proviral and c-*myc* sequences.** Junction fragments of proviral and c-*myc* sequences were identified in *Eco*RI-digested DNA as well as in *Hin*dIII-*Eco*RI-digested DNAs (see Fig. 1 for relative positions of *Eco*RI and *Hin*dIII sites in c-*myc*). Southern blots of these digests were sequentially hybridized with the 3′ LTR, the LTR, the EX3 (*Eco*RI-digested DNAs), the IN2 (*Eco*RI-*Hin*dIII-digested DNAs), the SMA, and the MYC probes (Fig. 1). This series of hybridizations served to define both the 5′ and

TABLE 1. Lymphomas tested for novel c-*myc* fragments

| Inducing virus | No. with novel c-*myc* fragments[a] | | |
|---|---|---|---|
| | Typical | Atypical | None |
| RAV-1 | 26 | 6 | 1 |
| NY201RAV-60 | 3 | 1 | 0 |
| NY202RAV-60 | 1 | 0 | 0 |
| NY203RAV-60 | 10 | 1 | 0 |
| NY204RAV-60 | 0 | 1 | 0 |
| Fujinami-associated virus | 4 | 0 | 0 |
| Nontransforming subgroup E virus strain 2 | 3 | 1 | 0 |
| Recovered from pRAV-1 | 8 | 2 | 0 |

[a] A typical fragment is a 2.8- to 4.0-kb *Eco*RI fragment that hybridized with the EX3 and 3′ LTR but not the PVA probes. An atypical fragment exhibited a size or hybridization pattern that was not frequently observed. None indicates that the lymphoma did not exhibit a viral integration between the *Eco*RI site 5′ of c-*myc* and a *Bgl*I site ~4 kb 3′ of c-*myc* (Fig. 1).

TABLE 2. Orientations and patterns of deletion of lymphoma-inducing proviruses in a series of 32 RAV-1-induced lymphomas

| Orientation[a] | *Eco*RI fragment[b] | Proviral deletion | Host deletion, rearrangement | No. observed | Table[c] |
|---|---|---|---|---|---|
| Same | 2.9–3.9 | Sequences near 5′ LTR | Unlikely | 14 | 3 |
| Same | 2.9–3.5 | All except one LTR | Unlikely | 6 | 4 |
| Same | 3.0–7.3 | Including 5′ LTR | Likely | 5 | 5 |
| Same | 2.9–6.5 | Including 3′ LTR | Likely | 2 | 6 |
| Same | 3.2 | sequences near 5′ LTR, LTR (?) | Likely | 2 | 7 |
| Same | 10.5, 12.5 | ? | Likely | 2 | 8 |
| Opposite | 3.5, 7.5 | Including 5′ LTR | Likely | 1 | 9 |

[a] Transcriptional orientation of the provirus with respect to the transcriptional orientation of c-*myc*.
[b] Size in kilobases of novel fragment that hybridized with EX3 probe.
[c] Table in which each class is presented in detail.

3′ junctions of proviral and c-*myc* sequences, to map first-exon sequences in these junction fragments, and to identify LTR sequences that might be present in junction fragments (Fig. 2).

**Deletions or rearrangements in proviral or c-*myc* sequences.** Deletions or rearrangements were identified by hybridizing Southern blots of *Sac*I-digested DNAs with the MYC probe (Fig. 1). *Sac*I was chosen for these digests since a 7-kb *Sac*I fragment of c-*myc* encompassed the apparent acceptor sites for virtually all of the lymphoma-inducing insertions and since there is only one *Sac*I cleavage site in proviral DNA. If this site were present in a provirus, the insertion should result in two tumor-specific *Sac*I fragments. If this site is not present, the insertion should result in only one tumor-specific *Sac*I fragment. In either instance, the size of the novel *Sac*I fragment or fragments provides an estimate of the total amount of proviral (7.8-kb) and c-*myc* (7.0-kb) sequences retained in the locus which has sustained an insertion.

**Test for perturbations in first-exon sequences.** Since most of the lymphoma-inducing insertions appeared to reside in the first intron or exon of c-*myc*, the integrity of the first exon (a non-protein-coding exon) was examined by digesting lymphoma DNAs with *Sma*I, an enzyme that generates an ~1-kb fragment containing first-exon sequences (Fig. 1). The *Sma*I-digested DNAs were hybridized on Southern blots with the SMA probe. *Sma*I-digested DNAs displayed faint as well as intense novel fragments. The faint bands could be due to incomplete digests (*Sma*I is a methyl-sensitive enzyme) (Lym3095 and 1789; see Table 3) or limited sequence homology of a novel fragment with the SMA probe (Lym3029; see Table 5). Faint bands were not observed for other digests.

**Seven different classes of lymphoma-inducing proviruses.** The above analyses revealed seven different classes of lymphoma-inducing proviruses (Table 2). Six of the classes contain proviruses in the same transcriptional orientation as c-*myc*, whereas the seventh contains proviruses in the opposite transcriptional orientation to c-*myc*. All of the classes contain proviruses inserted 5′ to the first coding exon (second exon) of c-*myc*. No proviruses were observed downstream of c-*myc*.

The next seven sections of the Results describe the different classes of lymphoma-inducing insertions. Tables 3 through 9 summarize the DNA fragments used to classify the insertions, whereas Fig. 2 through 4 present restriction

TABLE 3. Lymphoma-inducing proviruses[a]: same transcriptional orientation, internal deletions near 5′ LTR

| DNA | Junction fragments hybridizing with indicated probes (kb) | | | | | |
|---|---|---|---|---|---|---|
| | *Hin*dIII-*Eco*RI | | *Eco*RI | | *Sac*I (MYC) | *Sma*I (EX1) |
| | 5′ (LTR, MYC) | 3′ (LTR, 3′ LTR, MYC, IN2) | 5′ (LTR, MYC) | 3′ (LTR, 3′ LTR, MYC, EX3) | | |
| Lym1814 | 7.4 | 1.6 | $13^{NT}$ | $2.9^{NT}$ | $7.5^{NT}$ | NT |
| Lym4446 | 7.5 | 1.6 | $13^{NT}$ | $3.0^{NT}$ | $14^{NT}$ | NT |
| Lym3059 | 7.4 | 1.7 | $13.5^{+}$ | $3.0^{-}$ | $9^{-}$ | None |
| Lym2996 | 7.4 | 1.75 | $13.5^{+}$ | $3.1^{-}$ | $13.5^{-}$ | None |
| Lym1969 | 7.4 | 1.75 | $13.5^{+}$ | $3.0^{-}$ | $13.5^{-}$ | None |
| Lym3054 | 7.4 | 1.75 | $13.5^{+}$ | $3.1^{-}$ | $12.5^{-}$ | None |
| Lym2863 | 7.4 | 1.8 | $13.5^{+}$ | $3.0^{-}$ | $13.5^{-}$ | None |
| Lym2559 | 7.4 | 1.8 | $13.5^{+}$ | $3.0^{-}$ | $9.5^{-}$ | None |
| Lym3211 | 7.0 | 2.2 | $13^{+}$ | $3.5^{-}$ | $14^{-}$ | None |
| Lym1930 | 7.0 | 2.2 | $13^{+}$ | $3.3^{-}$ | $10.5^{-}$ | None |
| Lym1801 | 7.0 | 2.25 | $13^{+}$ | $3.4^{-}$ | $14.5^{+}$ | None |
| Lym4585 | 6.7 | 2.6 | NT | NT | $9.5^{NT}$ | NT |
| Lym3095 | 6.6 | 2.6 | $12.5^{+}$ | $3.9^{+}$ | $14^{-}$ | 1.7, (2.4), 6.2 |
| Lym1789 | 6.5 | 2.6 | $12.5^{+}$ | $3.9^{+}$ | $14^{?}$ | 1.6, (2.7), 6.5 |
| Lym1486[b] | 6.5 | 2.6 | $12.5^{?}$ | $3.9^{+}$ | $7.5^{-}$ | 1.4, (3.0) |

[a] All proviruses are RAV-1 except where otherwise indicated. + and − superscripts indicate hybridization and no hybridization, respectively, with the SMA probe for the *Eco*RI digest and with the PVA probe for the *Sac*I digest. NT indicates not tested. Parentheses indicate faint bands.
[b] NY201RAV-60 provirus.

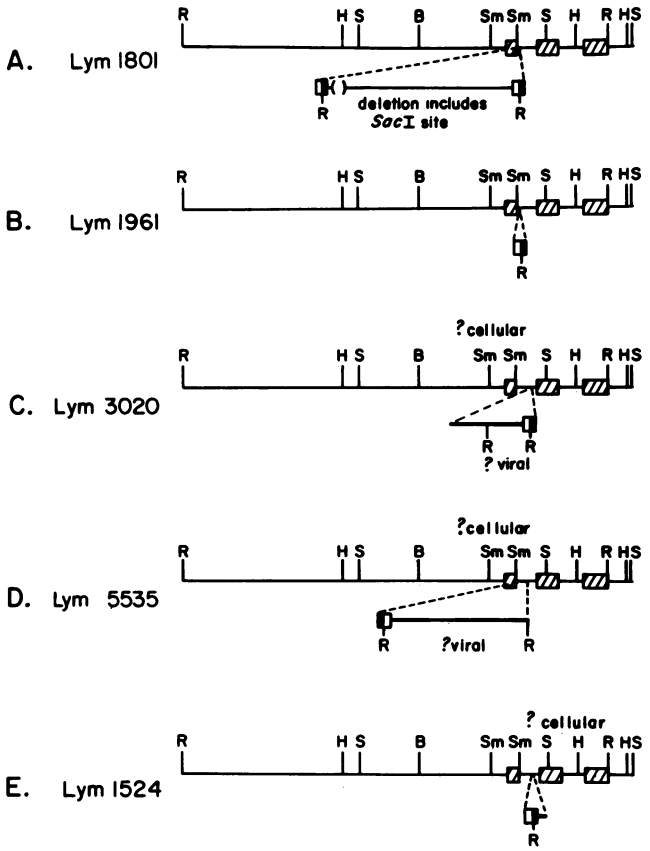


FIG. 2. Schematics of lymphoma-inducing proviruses. Each schematic represents a likely restriction map. A, Lym1801, a two-LTR provirus in the same transcriptional orientation as c-*myc* that has sustained an internal deletion (Table 3). B, Lym1961, a solo LTR in the same transcriptional orientation as c-*myc* (Table 4). C, Lym3020, a 5′ LTR-deleted provirus in the same transcriptional orientation as c-*myc* (Table 5). D, Lym5535; a 5′ LTR-deleted provirus in the opposite transcriptional orientation to c-*myc* (Table 9), E, Lym1524, a 3′ LTR-deleted provirus in the same transcriptional orientation as c-*myc* (Table 6). Parentheses indicate a deletion that includes the *Sac*I site. Other symbols and designations are as in Fig. 1.

endonuclease maps and fragments of representative insertions. The results section ends with the mapping of 24 of the insertions with respect to DNase-I hypersensitive sites in c-*myc* (see Fig. 5).

**Lymphoma-inducing proviruses with internal deletions.** The most frequently observed lymphoma-inducing provirus retained sequences in both of its LTRs but had deleted sequences near its 5′ LTR. This class of proviruses represented 44% of the RAV-1 insertions (Table 2). Southern blots of each of the 15 proviruses in this class exhibited two novel *Eco*RI-*Hin*dIII fragments (Table 3, Fig. 2A, Fig. 3). One of these, the 3′ junction of proviral and c-*myc* sequences, hybridized with the IN2 and 3′ LTR probes (Fig. 3, lanes 1 and 2). The other, the 5′ junction fragment, hybridized with the MYC and the LTR probes (Fig. 3, lanes 3 and 4). In individual lymphomas, the sum of the sizes of the 5′ and 3′ junction fragments ranged from 9.0 to 9.3 kb (Table 3). This sum is close to what would be expected for fragments with one proviral LTR (~0.3 kb) plus the sequences present in the 8.8-kb *Hin*dIII fragment of c-*myc* (Fig. 1).

Similar analyses of *Eco*RI digests of each of the internally deleted insertions confirmed the presence of LTR sequences at the 5′ and 3′ junctions of proviral and c-*myc* sequences (Table 3; Fig. 3, lanes 5 through 9). Again, the sum of these junction fragments approximated the size of one LTR plus the sequences present in the ~16-kb *Eco*RI fragment of c-*myc* (Table 3). Hybridization of blots of *Eco*RI-digested DNA with the SMA probe indicated that nine of the internally deleted proviruses resided 3′ to the first exon of c-*myc* (the SMA probe hybridized with the 5′ junction fragment), whereas two resided within the *Sma*I fragment that contains first-exon sequences (the SMA probe hybridized with both the 5′ and 3′ junction fragments).

Despite the presence of both LTRs and apparently unperturbed cellular sequences in each of these lymphomas, *Sac*I digests revealed only one tumor-specific fragment (Table 3, Fig. 2A, Fig. 3). In each case the tumor-specific *Sac*I fragment was larger than the 7.0-kb *Sac*I fragment of c-*myc* and smaller than the ~15-kb fragment expected for a complete provirus with a point mutation in its *Sac*I site. Twelve of the novel *Sac*I fragments were tested for hybridization with the PVA probe. Ten exhibited no discernible hybridization. Thus, most of the deletions appeared to include sequences from 155 bp (the *Sac*I site) to ~1,750 bp (the 3′ boundary of the PVA probe) immediately 3′ to the 5′ LTR. The largest deletions resulted in proviruses that appeared to retain little but two LTRs (lymphomas 1814 and 1486), whereas the smallest deletions appeared to encompass only 500 to 1,000 bp of proviral sequences (lymphomas 4446, 3211, 1801, 3095, and 1789). These striking results suggest that two LTR proviruses induce lymphomas when and only when they have deleted sequences near their 5′ LTR.

**Lymphoma-inducing proviruses with solo LTRs.** Nineteen percent of lymphoma-inducing RAV-1 proviruses (Table 2) appeared to have lost all of their sequences except for one LTR (Table 4, Fig. 2B). This class, referred to as the solo LTR class, was presumably generated by homologous recombination between the LTRs of an intact provirus (3, 36). *Hin*dIII-*Eco*RI as well as *Eco*RI digests of DNAs containing solo LTRs revealed LTR and apparently unperturbed host sequences at both the 5′ and 3′ junctions of proviral and c-*myc* sequences. The *Sac*I digests of these DNAs did not reveal a tumor-specific fragment. Presumably this was due to the similarity in size of the *Sac*I fragment of c-*myc* (7.0 kb) and the predicted *Sac*I fragment of c-*myc* with a solo LTR (7.3 kb).

**Lymphoma-inducing proviruses with 5′ LTR deletions.** Sixteen percent of lymphoma-inducing RAV-1 proviruses (Table 2) had sustained deletions of their 5′ LTR as well as some of their internal sequences (Table 5, Fig. 2C). *Hin*dIII-*Eco*RI as well as *Eco*RI fragments of these proviruses contained LTR sequences in their 3′ junction fragments but not their 5′ junction fragments. Since the 5′ junction fragments of these proviruses contained undefined amounts of viral sequences, the sizes of these fragments can not be used to estimate their content of c-*myc* sequences. However, four of the five 5′ LTR deleted proviruses had 5′ junction fragments that were either not detected or shorter than would be predicted from the restriction map of c-*myc*. In each of these cases, it is apparent that host sequences 5′ to the provirus have undergone major deletions or rearrangements. In two of the lymphomas (3018 and 4566) c-*myc* sequences 3′ as well as 5′ to the inducing provirus appear to have undergone deletions or rearrangements. Thus deletions that included a 5′ LTR as well as some internal sequences

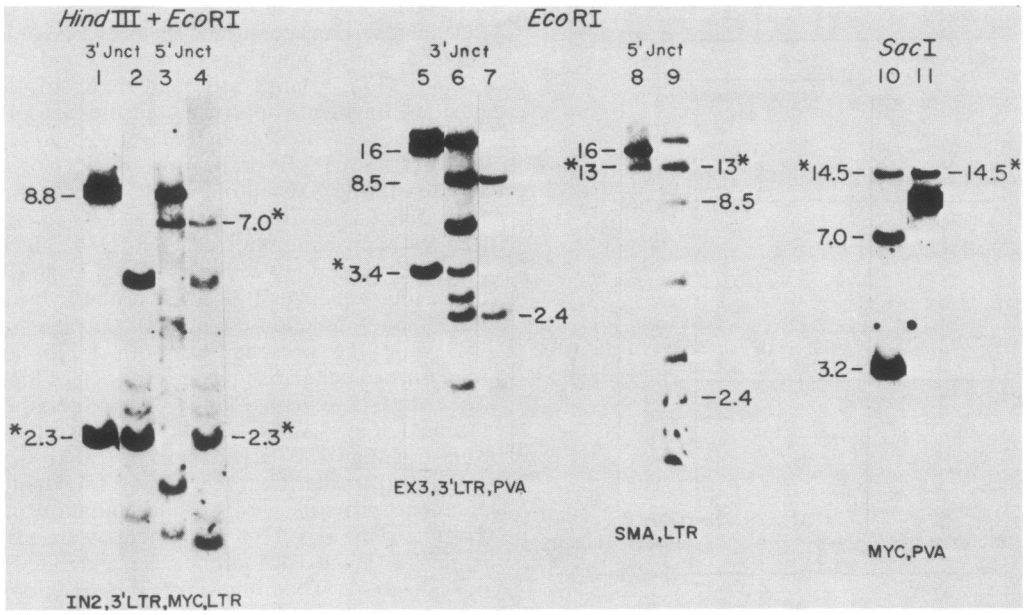


FIG. 3. Restriction endonuclease fragments used in the mapping of a lymphoma-inducing provirus with an internal deletion near its 5' LTR. Data are for the lymphoma-inducing provirus in Lym1801 (Table 3). For a schematic diagram of this provirus, see Fig. 2A. Each lane presents data from autoradiographs of Southern blots. Lanes obtained from sequential hybridizations of the same Southern blot are grouped. The restriction endonucleases used to digest Lym1801 DNA are indicted above the lanes, and DNA fragments used as hybridization probes are indicated below the lanes. Sizes of fragments are given in kilobases. Asterisks indicate tumor-associated fragments. Other fragments represent unperturbed c-*myc* sequences, the endogenous virus that resides at *ev*-1, or proviruses that were not integrated in c-*myc*. Hybridization of the 3' junction fragments with the MYC probe gave bands that were discernible in the original autoradiographs, but not in the reproductions. Clear evidence for these fragments is seen in the hybridization patterns of the same blot (*Hin*dIII-*Eco*RI digest) or of different blots (*Eco*RI digest) with the EX3 probe.

tended to be accompanied by deletions or rearrangements in host sequences (Table 5) (36).

**Lymphoma-inducing proviruses with 3' LTR deletions; a new transduction of c-*myc*.** Five of the proviruses in the same transcriptional orientation as c-*myc* had recombined c-*myc* sequences with sequences internal to the provirus. This was evidenced by the 3' junction fragments hybridizing with the PVA or PVB probes (Table 6, Fig. 2E, Fig. 4). Three proviruses appeared to have undergone recombination of proviral and c-*myc* sequences 5' to the *Eco*RI site at bp 2400 of proviral DNA (proviruses in lymphomas 3055, 1524, and 3475; Fig. 2E and 4A), and two to have undergone recombination of proviral and c-*myc* sequences 3' to this *Eco*RI site (proviruses in lymphomas 1463 and 1926; Fig. 4B).

To determine whether any of these 3' LTR-deleted proviruses represented a new transduction of c-*myc*, tumor-specific *Eco*RI fragments were analyzed for sequences in the second intron of c-*myc*. The IN2 probe hybridized with all of the 3' junction fragments except for that in lymphoma 3475 (Fig. 4C). Thus the novel provirus in lymphoma 3475 was a candidate for a new transduction of c-*myc*. Proof that this provirus represented a transducing virus was obtained by intravenous inoculation of a filtered homogenate of lymphoma 3475 into 1-week-old chickens. Three of the four inoculated chickens succumbed to myelocytomatosis between 12 and 14 weeks after inoculation. DNAs prepared from these tumors exhibited the same novel 2.9-kb *Eco*RI fragment as lymphoma 3475 (data not shown). Thus the

TABLE 4. Lymphoma-inducing proviruses[a]: same transcriptional orientation, solo LTRs

| DNA | Junction fragments hybridizing with indicated probes (kb) | | | | | |
|---|---|---|---|---|---|---|
| | *Hin*dIII-*Eco*RI | | *Eco*RI | | *Sac*I (LTR, MYC) | *Sma*I (SMA) |
| | 5' (LTR, MYC) | 3' (LTR, 3' LTR, MYC, IN2) | 5' (LTR, MYC, SMA) | 3' (LTR, 3' LTR, MYC, EX3) | | |
| Lym1804 | 7.5 | 1.6 | 13.5 | 2.9 | 7.0 | 1.0 |
| Lym1359 | 7.3 | 1.75 | 13.5 | 3.0 | 7.0 | 1.0 |
| Lym1971 | 7.3 | 1.7 | 13.5 | 3.1 | 7.0 | 1.0 |
| Lym2545 | 7.2 | 1.8 | 13.5 | 3.1 | 7.0 | 1.0 |
| Lym1961 | 7.0 | 2.0 | 13.0 | 3.3 | 7.0 | 1.0 |
| Lym3011 | 7.2 | 2.25 | 13.0 | 3.5 | 7.0 | 1.0 |

[a] All of the proviruses are RAV-1 proviruses.

TABLE 5. Lymphoma-inducing proviruses[a]: same transcriptional orientation, deletion includes the 5' LTR

| DNA | Junction fragments hybridizing with indicated probes (kb) | | | | | |
|---|---|---|---|---|---|---|
| | *Hin*dIII-*Eco*RI | | *Eco*RI | | *Sac*I (MYC) | *Sma*I (SMA) |
| | 5' (MYC) | 3' (LTR, 3' LTR, MYC, IN2) | 5' (MYC) | 3' (LTR, 3' LTR, MYC, EX3) | | |
| LymR326 | 10 | 1.7 | None | $3.0^-$ | 10 | None |
| Lym3020 | 7.6 | 1.62 | $13.5^+$ | $3.0^-$ | 9 | None |
| Lym3018 | None | 2.55 | None | $3.7^-$ | 6.8 | 1.7 |
| Lym3029 | None | 2.5 | $15.5^+$ | $3.7^+$ | 11 | (1.4) |
| Lym1549[b] | 5.8 | 3.8 | NT | $4.6^+$ | 11.5 | None |
| Lym4566 | NT | NT | $5.0^-$ | $7.3^-$ | 7.2 | None |

[a] All of the proviruses are RAV-1, except where otherwise indicated. Superscripts, NT, and parentheses are explained in footnote *a* of Table 3.
[b] NY204RAV-60 provirus.

TABLE 6. Lymphoma-inducing proviruses[a]: same transcriptional orientation, deletion includes the 3′ LTR

| DNA | 5′ *Eco*RI junction fragment | | | | 3′ *Eco*RI junction fragment | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | MYC | LTR | SMA | EX3 | IN2 | 3′ LTR | PVA | PVB | SMA |
| Lym1463[b] | 9.5 | + | − | 6.5 | + | − | − | + | + |
| Lym1926 | 12.5 | + | + | 5.4 | + | − | − | + | − |
| Lym3055 | NT[c] | | | 4.5 | + | + | + | NT | + |
| Lym1524[d] | 12 | + | + | 3.1 | + | + | + | NT | − |
| Lym3475[e] | NT | | | 2.9 | − | + | + | NT | NT |

[a] Lym1926 and Lym3055 contain RAV-1 proviruses.
[b] NY202RAV-60 provirus.
[c] NT, Not tested.
[d] NY203RAV-60 provirus.
[e] Nontransforming subgroup E virus strain provirus.

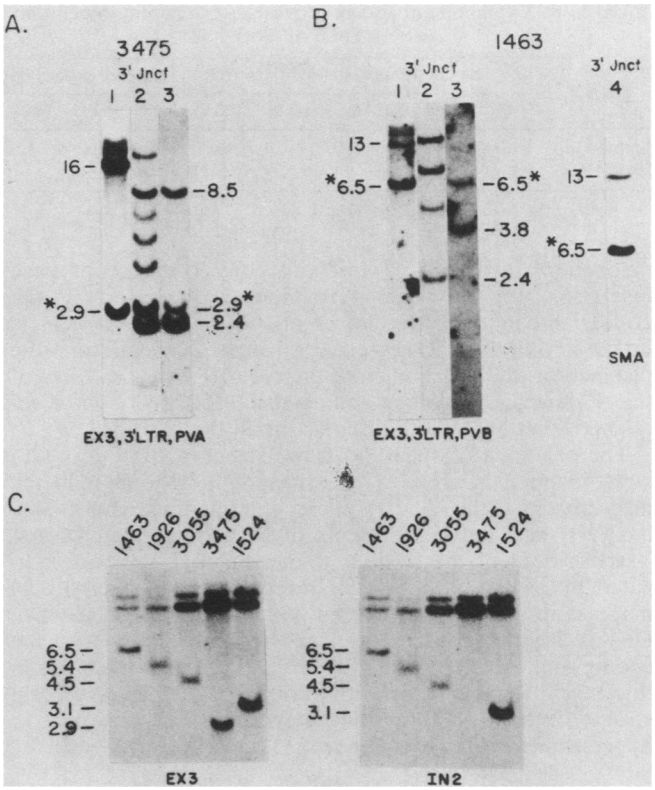


FIG. 4. Restriction endonuclease fragments used in the mapping of lymphoma-inducing proviruses with 3′ LTR deletions. A, Data used in the mapping of the lymphoma-inducing provirus in Lym3475 (Table 6). B, Data used in the mapping of the lymphoma-inducing provirus in Lym1463 (Table 6). C, Data for the mapping of intron 2 sequences in lymphoma-inducing proviruses with 3′ LTR deletions (Table 6). All of the data are for *Eco*RI-digested DNAs. The DNA being analyzed is indicated above the lanes. Other designations are as in Fig. 2. The data in C come from three sequential hybridizations of a Southern blot without removal of the probe between hybridizations. The first hybridization was with a *Pst*I-*Hin*dIII fragment of MYC that is 3′ to the coding sequences for c-*myc*. This probe hybridized with the ~20-kb fragment. The second hybridization was with the IN2 probe. The third hybridization was with the EX3 probe. The IN2 and EX3 probes hybridize with the 16-kb c-*myc* fragment as well as with tumor-associated fragments.

novel c-*myc* fragment in lymphoma 3475 represents a new c-*myc* transducing virus. This virus has been named MYC-3475.

**Lymphoma-inducing proviruses with internal deletions and deletions or rearrangements of flanking host sequences.** Two of the lymphoma-inducing proviruses appeared to have internal deletions as well as deletions or rearrangements in flanking host sequences (Table 7). One of these (in lymphoma 4591) had a novel *Sac*I fragment that was smaller than the acceptor *Sac*I fragment of c-*myc*. The other (in lymphoma 2963) had *Eco*RI-*Hin*dIII fragments that were larger than would have been expected for one LTR plus the acceptor c-*myc* fragment.

**Lymphoma-inducing proviruses with unusually large 3′ junction fragments.** Two lymphoma-inducing proviruses had unusually large 3′ junction fragments (Table 2). These 12.5- and 10.5-kb *Eco*RI fragments hybridized with the EX3 and 3′ LTR probes, but did not hybridize with the PVA probe (Table 8). Since the novel fragments did not hybridize with the PVA probe, they appeared to represent 3′ LTR sequences linked to c-*myc* sequences. If this configuration is correct, then one would expect no perturbation in the size of the 7.0-kb *Sac*I fragment of c-*myc* (Fig. 1). However, *Sac*I digests of these tumors displayed novel fragments that were 4 to 6 kb larger than the normal *Sac*I fragment of c-*myc*. The position and sequence content of these insertions are unclear.

**Lymphoma-inducing proviruses with opposite transcriptional orientation.** The least frequent class of lymphoma-inducing proviruses (Table 2) contained insertions in the opposite transcriptional orientation to c-*myc* (Table 9, Fig. 2D). Interestingly, the two proviruses in this class had undergone deletions which included their 5′ LTRs. One of these deletions (in lymphoma 4586) appears to have resulted in the recombination of sequences 5′ to the *Eco*RI site at bp ~2400 in proviral DNA with c-*myc*, whereas the other appears to have resulted in the recombination of sequences 3′ to this site with c-*myc*.

**Mapping of proviral insertions with respect to DNase I-hypersensitive sites.** The 24 lymphoma-inducing insertions with no discernible deletions or rearrangements in their 3′ junction fragments (lymphomas in Tables 3 and 4; lymphomas R326, 3020, 3029 and 1549 in Table 5) could be

TABLE 7. Lymphoma-inducing proviruses[a]: same transcriptional orientation, poorly defined deletions of viral and host sequences

| DNA | Junction fragments hybridizing with indicated probes (kb) | | | | | |
|---|---|---|---|---|---|---|
| | *Hin*dIII-*Eco*RI | | *Eco*RI | | *Sac*I (MYC) | *Sma*I (SMA) |
| | 5′ (LTR, MYC) | 3′ (LTR, 3′ LTR, MYC, IN2) | 5′ (LTR, MYC) | 3′ (LTR, 3′ LTR, MYC, EX3) | | |
| Lym4591 | 7.4 | 1.6 | NT | NT | 4.8 | NT |
| Lym2963 | 8.2 | 2.6 | None | 3.2+ | 10 | (2.6), (2.1) |

[a] Both of the proviruses are RAV-1 proviruses. Superscripts, NT, and parentheses are explained in footnote *a* of Table 3.

TABLE 8. Lymphoma-inducing proviruses[a]: apparent integration site ~6 kb 5′ of c-*myc*

| DNA | Junction fragments hybridizing with indicated probes (kb) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Eco*RI | EX3 | SMA | 3′ LTR | PVA | *Sac*I | MYC | PVA |
| Lym4438 | 10.5 | + | + | + | − | 11 | + | + |
| Lym2563 | 12.5 | + | + | + | − | 12.5 | + | ? |

[a] Both of the proviruses are RAV-1.

mapped in c-*myc* (Fig. 5). In *Hin*dIII-*Eco*RI digests of these insertions, the 3′ junction fragment is bounded by the *Hin*dIII site in the second intron of c-*myc* and the *Eco*RI site in the proviral LTR (Fig. 2A through C). Therefore the distance of the insertion from the *Hin*dIII site is the size of the 3′ junction fragment minus the ~150 bp of viral sequences that lie 3′ to the *Eco*RI site in the LTR.

The positions of open chromatin structures in c-*myc* (29) were mapped relative to the sites of lymphoma-inducing insertions by using *Hin*dIII-*Eco*RI junction fragments as size markers for *Hin*dIII fragments of DNase I-treated DNAs. The positions of the DNase I-hypersensitive sites are indicated by asterisks in Fig. 5. Interestingly, each of the 24 mapped insertions was within 150 bp of one of the five DNAse I-hypersensitive sites that lie immediately 5′ to the coding sequences for c-*myc*. Since only 1,350 of the bases in this 2,750-base region are within 150 bp of an open chromatin structure, the probability that integrations occurred next to hypersensitive sites by chance is $(1{,}350/2{,}750)^{24}$ or $<10^{-6}$.

## DISCUSSION

Sixty-eight ALV-induced lymphomas have been analyzed for proviral insertions into or new transductions of c-*myc* (Table 1). The most frequent form of lymphoma-inducing provirus had retained sequences in both of its LTRs, but lost sequences near its 5′ LTR (Table 2). Thus, contrary to accepted belief, ALV proviruses do not have to lose an LTR to activate the transcription of downstream host sequences.

**Patterns of deletion in lymphoma-inducing proviruses.** More than 75 lymphoma-inducing proviruses that are upstream of and in the same transcriptional orientation, 7 that are upstream of but in the opposite transcriptional orientation, and 1 that is downstream of c-*myc* have been analyzed for deletions (Tables 3 through 9) (22, 27, 29). Each of these has lost sequences that include an LTR or sequences near the 5′ LTR. Among insertions in the same transcriptional orientation, the most frequent deletion is the deletion of sequences near the 5′ LTR (Table 2). Among insertions in the opposite transcriptional orientation, four have lost sequences that include the 5′ LTR (Table 9) (22), two have lost sequences near the 5′ LTR (21, 22, 40), and one has lost the 3′ LTR (29). The one downstream provirus has lost sequences that include the 5′ LTR (21). Before our study, all upstream proviruses in the same transcriptional orientation as c-*myc* had been assumed to have lost one LTR. This erroneous assumption was based on the absence of the *Sac*I site near the 5′ LTR (27).

The loss of sequences near the 5′ LTR in all two LTR lymphoma-inducing proviruses suggests that this deletion is required for two LTR proviruses to up-regulate the transcription of adjacent host sequences. In normal two-LTR proviruses, the 5′ and 3′ LTRs have distinct activities, with the 5′ LTR initiating and the 3′ LTR providing the polyadenylation signal for transcripts (J. Coffin and S. Herman, personal communication). This specialization of the LTRs favors transcription of viral rather than flanking host sequences (8). Known functions of the selectively deleted sequence include a splice donor site (30), signals for the packaging of RNA into virions (31), and the 5′ sequences of all of the viral proteins (30). The selectively deleted sequence also appears to be a region with high secondary structure as evidenced by the presence of two hypersensitive sites in nuclease S1-treated chromatin (7). Which, if any, of these features may be relevant to the selection for 5′ deletions in two LTR lymphoma-inducing proviruses is not known.

All of the mapping of lymphoma-inducing insertions is based on Southern blot analyses which do not rigorously define fragment sizes or sequence contents. Proviruses that are classified as solo LTRs may have some internal sequences, and proviruses that are classified as two LTR proviruses with internal deletions may have deletions that extend into the 5′ LTR. However, we feel that most of the classifications will be correct, since lymphoma-inducing ALVs that have been sequenced include a true solo LTR and a true two LTR provirus with a deletion near its 5′ LTR (40).

**Deletions and rearrangements in flanking host sequences.** The vast majority of solo and two LTR proviruses were not associated with deletions or rearrangements in flanking host sequences (Tables 3 and 4). Interestingly, these proviruses were inserted downstream of the major hypersensitive site that lies immediately 5′ to the first exon of c-*myc* (hypersensitive site II) (Fig. 5) (29). In contrast, the six insertions upstream of hypersensitive site II were associated with easily detected deletions or rearrangements in host sequences. These six insertions included three 5′ LTR-deleted proviruses (lymphomas 1549 and 4566, Table 5; lymphoma 4586, Table 9), one 3′ LTR-deleted provirus (lymphoma 1463, Table 6), and both of the apparently far upstream proviruses (Table 8). Hence, deletions in host sequences may be necessary for insertions that are upstream of hypersensitive site II to activate c-*myc*.

**Sites of integration of lymphoma-inducing proviruses.** The

TABLE 9. Lymphoma-inducing proviruses[a]: opposite transcriptional orientation, deletion includes the 5′ LTR

| DNA | Junction fragments hybridizing with indicated probes (kb) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Hin*dIII-*Eco*RI | | *Eco*RI | | | | | |
| | | | | 3′ | | | | |
| | 5′ (3′ LTR, MYC) | 3′ (MYC, IN2) | 5′ (3′ LTR, MYC) | MYC, EX3 | LTR | PVA | PVB | SMA |
| Lym5535[b] | 7.4 | 1.8 | 12.5 | 3.5 | − | − | + | − |
| Lym4586 | 4.5 | 5.8 | None | 7.5 | − | + | NT | + |

[a] Lym4586 contains an RAV-1 provirus. Symbols are as in Table 3.
[b] Provirus encoded by the virus recovered from pRAV-1.

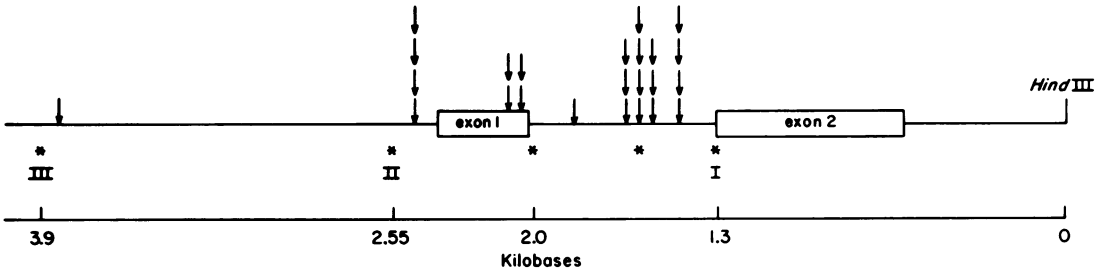


FIG. 5. Position of DNase I-hypersensitive sites and lymphoma-inducing RAV-1 proviruses in c-*myc*. Symbols: ↓, proviral insertion; *, DNAse I-hypersensitive site. The mapping of the DNase I-hypersensitive sites against our sized tumor fragments resulted in positions of hypersensitive sites that were within 100 bp of those reported by Schubach and Groudine (29).

sites of 24 of the lymphoma-inducing insertions could be mapped in c-*myc* (Fig. 5). The mapping of these insertions was in good agreement with prior studies (32), with most of the insertions mapping in the 3′ end of the first intron. Since it had been proposed that retroviral DNAs integrate near regions of open chromatin structure (1, 29), the sites of insertions were also mapped with respect to hypersensitive sites in DNase I-treated bursal chromatin. Remarkably, the sites of each of the 24 insertions mapped within 150 bp of one of the five DNase I-hypersensitive sites that lie in a 2,600-bp region immediately 5′ to the first coding exon of c-*myc* (29). None of the integrations occurred in the 1,400 bp in this region that lie more than 150 bp from a DNase I-hypersensitive site.

**Frequency of generation of new c-*myc*-transducing viruses.** One lymphoma was found to have a new c-*myc*-transducing virus, whereas four were found to have proviruses with 3′ LTR deletions that were upstream of and in the same transcriptional orientation as c-*myc* (Table 6). Since new transductions can be initiated by 3′ LTR-deleted proviruses (6), we anticipated that several of these proviruses might have generated c-*myc*-transducing viruses. To our surprise, most such proviruses were not associated with new transductions. Thus, the low incidence of new *myc*-transducing viruses may reflect the generation of transducing viruses by only a minority of 3′ LTR-deleted proviruses (Table 6) as well as the infrequent occurrence of such proviruses in lymphomas (Table 2).

**Frequency of induction of lymphoma by proviruses in the opposite transcriptional orientation to c-*myc*.** Only one of the 32 RAV-1-induced lymphomas contained a provirus in the opposite transcriptional orientation to c-*myc* (Table 2). This is in contrast to the occurrence of four proviruses in the opposite transcriptional orientation to c-*myc* in a series of eight RAV-2-incuded lymphomas (22). The probability that this difference in frequency was due to chance is <0.01. Since lymphoma induction in such cases is due to the enhancement of c-*myc* transcription (21), it seems possible that the enhancer sequences in a virus determine the frequency with which a stock causes lymphoma by insertions in the opposite transcriptional orientation to c-*myc*.

**Other lymphoma-inducing proviruses.** Reticuloendotheliosis viruses, murine leukemia viruses, and feline leukemia viruses also induce lymphomas by insertions in c-*myc* (13, 18, 19, 33). Lymphoma-inducing reticuloendotheliosis virus insertions appear to be very similar to lymphoma-inducing ALV insertions. The vast majority of these are deleted proviruses in the same transcriptional orientation as c-*myc*. The most frequent pattern of proviral deletion results in two LTR proviruses that have lost sequences near the 5′ LTR, the second most frequent pattern is proviruses that have deleted sequences including the 5′ LTR, and the third most frequent pattern is solo LTRs (23, 34; H.-J. Kung, personal communication). The similarities in position, orientation, and patterns of deletion of lymphoma-inducing insertions in these two families of viruses suggest that ALV and reticuloendotheliosis virus activate the expression of the chicken c-*myc* gene by similar mechanisms. In contrast, lymphoma-inducing murine leukemia virus proviruses tend to be integrated in the opposite transcriptional orientation to c-*myc*, reside almost exclusively upstream of the first exon of c-*myc*, and have typically not sustained deletions (13, 33). At present little is known about lymphoma-inducing insertions of feline leukemia viruses. However, in contrast to ALV, reticuloendotheliosis virus, and murine leukemia virus insertions, feline leukemia virus insertions may transduce c-*myc* at a high frequency as evidenced by the isolation of *myc*-containing viruses from a number of T-cell lymphomas in pet cats (12, 16, 18).

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## LITERATURE CITED

1. **Breindl, M., K. Harbers, and R. Jaenisch.** 1984. Retrovirus-induced lethal mutation in collagen I gene of mice is associated with an altered chromatin structure. Cell **38**:9–16.
2. **Coffin, J. M.** 1985. Genome structure, p. 17–73. *In* R. Weiss, N. Teich, H. Varmus, and J. Coffin (ed.), Molecular biology of RNA tumor viruses II (supplement). Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
3. **Copeland, N. G., K. W. Hutchison, and N. A. Jenkins.** 1983. Excision of the DBA ecotropic provirus in dilute coat-color revertants of mice occurs by homologous recombination involving the viral LTRs. Cell **33**:379–387.
4. **Fung, Y.-K. T., L. B. Crittenden, and H.-J. Kung.** 1982. Orientation and position of avian leukosis virus DNA relative to the cellular oncogene c-*myc* in B-lymphoma tumors of highly susceptible $15I_5 \times 7_2$ chickens. J. Virol. **44**:742–746.
5. **Fung, Y.-K. T., A. M. Fadly, L. B. Crittenden, and H.-J. Kung.** 1981. On the mechanism of retrovirus-induced avian lymphoid leukosis: deletion and integration of the provirus. Proc. Natl. Acad. Sci. USA **78**:3418–3422.

6. **Goldfarb, M., and R. A. Weinberg.** 1981. Generation of novel, biologically active Harvey sarcoma virus via apparent illegitimate recombination. J. Virol. **38**:136–150.
7. **Groudine, M., and K. F. Conklin.** 1985. Chromatin structure and *de novo* methylation of sperm DNA: implications for activation of the paternal genome. Science **228**:1061–1068.
8. **Hayward, W. S.** 1977. Size and genetic content of viral RNAs in avian oncovirus-infected cells. J. Virol. **24**:47–63.
9. **Hayward, W. S., B. G. Neel, and S. M. Astrin.** 1981. ALV-induced lymphoid leukosis: activation of a cellular *onc* gene by promoter insertion. Nature (London) **290**:475–480.
10. **Ju, G., L. Boone, and A. M. Skalka.** 1980. Isolation and characterization of recombinant DNA clones of avian retroviruses: size heterogeneity and instability of the direct repeat. J. Virol. **33**:1026–1033.
11. **Ju, G., and A. M. Skalka.** 1980. Nucleotide sequence analysis of the long terminal repeat (LTR) of avian retroviruses: Structural similarities with transposable elements. Cell **22**:379–386.
12. **Levy, L. S., M. B. Gardner, and J. W. Casey.** 1984. Isolation of a feline leukaemia provirus containing the oncogene *myc* from a feline lymphosarcoma. Nature (London) **308**:853–855.
13. **Li, Y., C. A. Holland, J. W. Hartley, and N. Hopkins.** 1984. Viral integrations near c-*myc* in 10–20% of MCF247 induced AKR lymphomas. Proc. Natl. Acad. Sci. USA **81**:6801–6811.
14. **Linial, M., and M. Groudine.** 1985. Transcription of three c-*myc* clones is enhanced in chicken bursal lymphoma cell lines. Proc. Natl. Acad. Sci. USA **82**:53–57.
15. **Miles, B. D., and H. L. Robinson.** 1985. High-frequency transduction of c-*erb*B in avian leukosis virus-induced erythroblastosis. J. Virol. **54**:295–303.
16. **Mullins, J. I., D. S. Brody, R. C. Binari, Jr., and S. M. Cotter.** 1984. Viral transduction of c-*myc* gene in naturally occurring feline leukaemias. Nature (London) **308**:856–858.
17. **Neel, B. G., W. S. Hayward, H. L. Robinson, J. Fang, and S. Astrin.** 1981. Avian leukosis virus-induced tumors have common proviral integration sites and synthesize discrete new RNAs: oncogenesis by promoter insertion. Cell **23**:323–334.
18. **Neil, J. C., D. Hughes, R. McFarlane, N. M. Wilkie, D. E. Onions, G. Lees, and O. Jarrett.** 1984. Transduction and rearrangement of the *myc* gene by feline leukaemia virus in naturally occurring T-cell leukaemias. Nature (London) **308**:814–820.
19. **Noori-Daloii, M. R., R. A. Swift, H. J. Kung, L. B. Crittendon, and R. L. Witter.** 1981. Specific integration of REV proviruses in avian bursal lymphomas. Nature (London) **294**:574–576.
20. **Pachl, C., W. Schubach, R. Eisenman, and M. Linial.** 1983. Expression of c-*myc* RNA in bursal lymphoma cell lines: identification of c-*myc* encoded proteins by hybrid-selected translation. Cell **33**:335–344.
21. **Payne, G. S., J. M. Bishop, and H. E. Varmus.** 1982. Multiple arrangements of viral DNA and an activated host oncogene (c-*myc*) in bursal lymphomas. Nature (London) **295**:209–214.
22. **Payne, G. S., S. A. Courtneidge, L. B. Crittenden, A. M. Fadly, J. M. Bishop, and H. E. Varmus.** 1981. Analysis of avian leukosis virus DNA and RNA in bursal tumors: viral gene expression is not required for maintenance of the tumor state. Cell **23**:311–322.
23. **Ridgway, A. A., R. A. Swift, H. J. Kung, and D. J. Fujita.** 1985. *In vitro* transcription analysis of the viral promoter involved in c-*myc* activation in chicken B lymphomas: detection and mapping of two RNA initiation sites within the reticuloendotheliosis virus long terminal repeat. J. Virol. **54**:161–170.
24. **Robinson, H. L., B. P. Blais, P. N. Tsichlis, and J. M. Coffin.** 1982. At least two regions of the viral genome determine the oncogenic potential of avian leukosis viruses. Proc. Natl. Acad. Sci. USA **79**:1225–1229.
25. **Robinson, H. L., L. Jensen, and J. M. Coffin.** 1985. Sequences outside of the long terminal repeat determine the lymphomogenic potential of Rous-associated virus type 1. J. Virol. **55**:752–759.
26. **Robinson, H. L., M. N. Pearson, D. W. DeSimone, P. N. Tsichlis, and J. M. Coffin.** 1979. Subgroup E avian leukosis virus associated disease in chickens. Cold Spring Harbor Symp. Quant. Biol. **44**:1133–1142.
27. **Rovigatti, V., C. Rogler, B. Neel, W. Hayward, and S. Astrin.** 1982. Expression of endogenous oncogenes in tumor cells. Results, p. 319–330. *In* A. H. Owens, D. S. Caffey, and S. B. Baylin (ed.), Tumor cell heterogeneity. Academic Press, Inc., New York.
28. **Rubin, H., and P. K. Vogt.** 1962. An avian leukosis virus associated with stocks of Rous sarcoma virus. Virology **17**:184–194.
29. **Schubach, W., and M. Groudine.** 1984. Alteration of c-*myc* chromatin structure by avian leukosis virus integration. Nature (London) **307**:702–708.
30. **Schwartz, D. E., R. Tizard, and W. Gilbert.** 1983. Nucleotide sequence of Rous sarcoma virus. Cell **32**:853–869.
31. **Shank, P. R., and M. Linial.** 1980. Avian oncovirus mutant (SE21Q1b) deficient in genomic RNA: characterization of a deleted provirus. J. Virol. **36**:450–456.
32. **Shih, C.-K., M. Linial, M. M. Goodenow, and W. S. Hayward.** 1984. Nucleotide sequence 5′ of the chicken c-*myc* coding region: localization of a non-coding exon that is absent from *myc* transcripts in most ALV-induced lymphomas. Proc. Natl. Acad. Sci. USA **81**:4697–4701.
33. **Steffen, D.** 1984. Proviruses are adjacent to c-*myc* in some murine leukemia virus-induced lymphomas. Proc. Natl. Acad. Sci. USA **81**:2097–2101.
34. **Swift, R. A., E. Shaller, R. L. Witter, and H.-J. Kung.** 1985. Insertional activation of c-*myc* by reticuloendotheliosis virus in chicken B lymphoma: nonrandom distribution and orientation of the provirus. J. Virol. **54**:869–872.
35. **Tsichlis, P. N., and J. M. Coffin.** 1980. Recombinants between endogenous and exogenous avian tumor viruses: role of the c region and other portions of the genome in the control of replication and transformation. J. Virol. **33**:238–249.
36. **Varmus, H. E., N. Quintrell, and S. Ortiz.** 1981. Retroviruses as mutagens: Insertion and excision of a nontransforming provirus alter expression of a resident transforming provirus. Cell **25**:23–26.
37. **Vennstrom, B., C. Moscovici, H. M. Goodman, and J. M. Bishop.** 1981. Molecular cloning of the avian myeloblastosis virus genome and recovery of infectious virus by transfection of chicken cells. J. Virol. **39**:625–631.
38. **Vennstrom, B., D. Sheiness, J. Zabielski, and J. M. Bishop.** 1982. Isolation and characterization of c-*myc*, a cellular homolog of the oncogene (v-*myc*) of avian myelocytomatosis virus strain 29. J. Virol. **42**:773–779.
39. **Watson, D. K., E. P. Reddy, P. H. Duesberg, and T. S. Papas.** 1983. Nucleotide sequence analysis of the chicken c-*myc* gene reveals homologous and unique coding regions by comparison with the transforming gene of avian myelocytomatosis virus MC29, *gag-myc*. Proc. Natl. Acad. Sci. USA **80**:2146–2150.
40. **Westaway, D., G. Payne, and H. E. Varmus.** 1984. Proviral deletions and oncogene base-substitutions in insertionally mutagenized c-*myc* alleles may contribute to the progression of avian bursal tumors. Proc. Natl. Acad. Sci. USA **81**:843–847.