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Sequences Outside of the Long Terminal Repeat Determine the Lymphomogenic Potential of Rous-Associated Virus Type 1

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Recombinant avian leukosis viruses have been constructed from the molecularly cloned DNAs of Rous-associated virus type 1 (RAV-1) and Rous-associated virus type 0 (RAV-0). Virus encoded by the cloned RAV-1 DNA induced a high incidence of B-cell lymphoma and a moderate incidence of a variety of other neoplasms. Virus encoded by the cloned RAV-0 DNA did not cause disease. Virus recovered from DNA constructions that encoded the *gag*, *pol*, and 5′ *env* sequences of RAV-0 and the 3′ *env* and long terminal repeat sequences of RAV-1 did not cause a high incidence of lymphoma. Rather, these constructed viruses induced a low incidence of a variety of neoplasms. Virus recovered from reconstructed pRAV-1 DNA had the same disease potential as did virus recovered from the parental pRAV-1 DNA. These results indicate that the long terminal repeat sequences of RAV-1 do not confer the potential to induce a high incidence of B-cell lymphoma.

Naturally occurring avian leukosis viruses (ALVs) as well as the helper viruses for the acute leukemia and sarcoma viruses of chickens cause cancer by insertion into or transduction of host proto-oncogene sequences (26, 27, 34, 35, 45, 49). To date, the type of malignancy induced by a helper virus has correlated with the host proto-oncogene that has been the target for an insertion or a transduction. The vast majority of ALV-induced lymphomas contain a provirus inserted in the proto-oncogene c-*myc* (26, 34, 49); the vast majority of ALV-induced erythroblastosis contain either a provirus inserted in the proto-oncogene c-*erb*B or a viral transduction of c-*erb*B (27, 45). In both ALV-induced lymphoma and erythroblastosis, most cancer-inducing insertions have resulted in a viral long terminal repeat (LTR) promoting unusually high levels of transcription of a chimeric RNA that contains truncated proto-oncogene sequences (a truncated RNA in the case of c-*myc* [62]; a truncated RNA and protein in the case of c-*erb*B [21, 32, 51, 71]).

Different ALVs have different oncogenic potentials. For example, helper viruses isolated from stocks of the Bryan high-titer strain of Rous sarcoma virus (Rous-associated virus types 1 and 2 [RAV-1 and RAV-2, respectively]) induce high incidences of B-cell lymphoma (52, 58) and erythroblastosis (3, 27), whereas helper viruses isolated from stocks of avian myeloblastosis (myeloblastosis-associated virus types 1 and 2) induce high incidences of B-cell lymphoma and nephroblastoma (64). Similarly, a transformation-defective (*src* gene deletion) mutant of the Prague strain of Rous sarcoma virus induces only a low incidence of a variety of cancers (54), whereas transformation-defective derivatives of Schmidt-Ruppin Rous sarcoma virus induce a high incidence of B-cell lymphoma (52). RAVs, myeloblastosis-associated viruses, and the transformation-defective derivatives of avian sarcoma viruses (those that have undergone complete deletion of transduced *src* sequences) have similar genetic organizations with the respective virion RNAs containing the *gag*, *pol*, and *env* genes as well as the terminal U5 and U3 regions that become the long terminal repeats (LTRs) of integrated proviral DNA.

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The genetic basis for the differences in the oncogenic potential of different ALVs is not understood. Experiments with naturally occurring as well as recombinant RAVs and myeloblastosis-associated viruses of different subgroups (ALVs are classified into subgroups according to their *env*-encoded host range) have indicated that the ability of a helper virus to cause B-cell lymphoma, erythroblastosis, or nephroblastoma is not determined by its subgroup (13, 52, 58, 64). Thus the ability of an ALV to undergo adsorption and penetration into target cells does not appear to determine whether it will induce a high incidence of B-cell lymphoma, erythroblastosis, or nephroblastoma. Little is known about the replication of oncogenic and nononcogenic ALVs in cells that are targets for the induction of erythroblastosis or nephroblastoma. However, it is known that ALVs with both high and low lymphomogenic potential undergo comparable levels of replication in the bursa of Fabricius (55). Since the vast majority of the cells in the bursa are B-cells, the ability of ALVs to replicate in B-cells does not appear to determine the ability of ALVs to induce B-cell lymphoma.

In this manuscript we report experiments designed to test the role of sequences at the ends of the genome of RAV-1 in lymphomogenic potential. The RAV-1 sequences tested included the COOH-terminus for the *env*-encoded gp85 protein, the entire coding sequence for the *env*-encoded gp37 protein, non-protein-coding sequences that lie between gp37 and U3; U3, R, U5, and 154 bases of the non-protein-coding sequence immediately downstream of U5 (see Fig. 1 and 2). gp37 is the *env*-encoded transmembrane protein that serves as an anchor in the viral membrane for the *env*-encoded gp85 glycoprotein (for a review see reference 17). The sequences between gp37 and U3 are a region of genetic diversity that contain signals for the plus-strand primer-binding site (for reviews see references 9 and 70). U3, R, and U5 make up the LTR sequences that flank integrated proviral DNA (for reviews see references 8 and 66). The LTRs of ALVs contain non-protein-coding sequences that play key roles in the reverse transcription of viral RNA, the integration of viral DNA, and the transcription of proviral DNA by RNA polymerase II. The transcriptional control elements in the LTR control both the rate of virus growth (10, 67) and the

steady-state levels of the expression of viral RNA in infected cells (73). In ALV-induced lymphomas, LTR sequences control c-*myc* expression (34, 49) and are the one region of the viral genome that appears to be essential to the maintenance of malignant growth (63, 74).

MATERIALS AND METHODS

Construction of recombinant DNAs. Plasmids containing the complete viral DNAs of RAV-1 and RAV-0 (pRAV-1, pRAV-0) were used for the construction of recombinant viruses. pRAV-1 is a derivative of pBR322 with RAV-1 DNA cloned at the *Sac*I site at base 255 of viral RNA. pRAV-1 was a gift of G. Payne, H. Varmus, and J. M. Bishop. pRAV-0 is pBR322 with RAV-0 DNA cloned at the *Sal*I site at ca. base 6050 of viral RNA. pRAV-0 was a gift of P. Tsichlis. Restriction endonuclease digestions and ligations were carried out with enzymes purchased from a number of suppliers. Fragments of restriction endonuclease-digested pRAV-1 and pRAV-0 were fractionated by electrophoresis on low-melting-point agarose gels. DNA fragments were recovered from gel slices (melted at 65°C) by phenol extraction followed by ethanol precipitation. In certain instances, ligations were carried out on fragments contained in plugs of low-melting-point agarose by melting the plugs at 65°C, cooling them to 37°C, adding ligase, and incubating them overnight at 14°C (14). Constructions were designated recALVs, with duplicates of each being designated by numbers that fell within blocks of 10 (i.e., duplicates of the 10s construction are designated 11, 12, 13, etc., whereas duplicates of the 20s construction are designated 21, 22, 23, etc.) Constructions made in the laboratory of H. L. Robinson are prefixed with WF (Worcester Foundation); constructions made in the laboratory of J. M. Coffin are prefixed with Bo (Boston).

Recovery of virus from plasmid DNAs. Viruses were recovered from plasmid DNA by cleaving viral sequences from the DNA, ligating the cleaved DNA, and transfecting the ligated product into turkey cells. Transfections were done with calcium phosphate precipitates by the method of Graham and Van der Eb (29) as modified by Loyter et al. (43). Transfected cultures were assayed for the development of interference to superinfection by an avian sarcoma virus of the same subgroup as the virus encoded in the transfected DNA (72). When interference was observed, the culture medium was harvested and used as a virus stock to infect 15_B *ev* 1 cells (15_B *ev* 1 cells are unusually permissive for ALV infections [53]). When the virus had grown to a maximum titer on 15_B *ev* 1 cells (assayed by testing for the amount of particulate RNA-directed DNA polymerase in culture medium [53]) the culture medium was harvested and used as a virus stock for oncogenicity tests. Turkey cells were used in the initial transfection, since they do not contain endogenous ALVs that might undergo recombination with the transfected virus. Virus titers were then boosted by a relatively short period of growth on 15_B *ev* 1 cells. In 15_B *ev* 1 cells the virus undergoes a low-frequency ($<1 \times 10^{-8}$ per infected cell per day of culture) of recombination with the endogenous virus that resides at *ev* 1.

Oncogenicity tests. All oncogenicity tests were performed by intravenous inoculation of 0.2 ml of a virus stock into 1-day-old K28 chickens. The K28 chickens used were the Robinson subline of K28. This random-bred subline has been selected for susceptibility to all known subgroups of ALVs (56) and only one endogenous virus, the one that resides at *ev* 1 (2). Groups of infected chickens were maintained in isolation. At 1 month postinfection, the growth of the test virus in the infected chickens was monitored by assaying sera for the amount of particulate RNA-directed DNA polymerase. Infected chickens were observed for disease for 1 year. Diseased birds were sacrificed when moribund. Diagnoses of different malignancies are based on the appearance of tumors at the time of autopsy as well as on the reading of histological sections of tumor tissue.

Recovery of virus from infected chickens. Virus was recovered from infected chickens by incubating cultured cells with serum harvested from an infected chicken at the time of its sacrifice. The RNAs of recovered viruses were analyzed for their genetic content by testing for RNase T1-resistant oligonucleotides as described by Coffin et al. (10).

RESULTS

Recombinant DNAs for the construction of test viruses. RAV-1, a virus that is known to cause a high incidence of B-cell lymphoma and a moderate incidence of a variety of other tumors in K28 chickens (58), and RAV-0, a virus that is known to cause no disease in K28 chickens (55), were chosen as parents for the construction of test viruses. RAV-1 is a subgroup A ALV that was isolated from a stock of the Bryan high-titer strain of Rous sarcoma virus (60). RAV-0 is a subgroup E ALV that is spontaneously expressed by the endogenous provirus that resides at *ev* 2 (1). RAV-1 has a higher growth potential than does RAV-0, with the LTR sequences of RAV-1 promoting the expression of ca. 10-times-higher levels of steady-state RNAs than those promoted by the LTR sequences of RAV-0 (10, 33, 67, 73).

Molecularly cloned DNAs for RAV-1 (pRAV-1) and RAV-0 (pRAV-0) were obtained and transfected into turkey cells for the recovery of infectious virus. The virus encoded by pRAV-1 caused a high incidence of lymphoma and a moderate incidence of a variety of other cancers, whereas the virus encoded by pRAV-0 did not cause disease (Table 1; see Fig. 2).

Figure 1 presents the restriction endonuclease maps and RNase T1-resistant oligonucleotide maps for the genomes of the viruses encoded by pRAV-1 and pRAV-0. The 23 mapped restriction endonuclease sites revealed only four

TABLE 1. Growth of parental and recombinant viruses[a]

Virus		Growth (10^3 cpm)[b]	
Origin	Subgroup	In culture	In chickens
pRAV-0	E	130	4 (1–10)
pRAV-1	A	34	2 (0.5–100)
Reconstructed pRAV-1	A	27	2 (0.5–10)
BO43	E	540	3 (1–20)
WF44	E	540	100 (40–500)
WF45	E	440	50 (10–200)
WF81	A	2	BG
WF82	A	3	BG
WF83	A	4	NT
WF84	A	2	NT

[a] The counts per minute of particulate reverse transcriptase per milliliter of culture medium cannot be used to compare titers of subgroup A and E viruses because subgroup A viruses have a higher ratio (ca. 10-fold) of infectious units to counts per minute of reverse transcriptase than do subgroup E viruses.

[b] Viremia is presented as the median value with the range given in parentheses. BG, Background; NT, not tested.

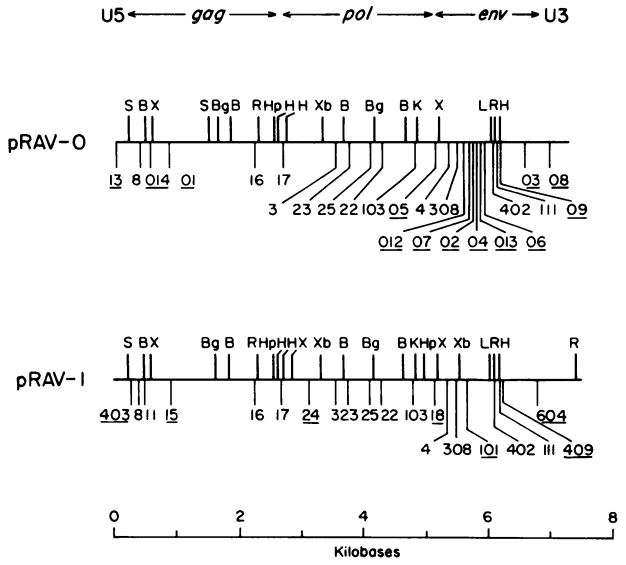

FIG. 1. Restriction endonuclease maps and RNase T1-resistant oligonucleotide maps of viral sequences in pRAV-0 and pRAV-1. The maps show viral sequences as the RNA genome would appear. Restriction endonuclease sites (B, *Bam*HI; Bg, *Bgl*II; H, *Hin*dIII; Hp, *Hpa*I; S, *Sac*I; L, *Sal*I; Xb, *Xba*I; X, *Xho*I; K, *Kpn*I; R, *Eco*RI) are indicated above the genome, and oligonucleotide markers (numbers) are indicated below the genome. Oligonucleotides that are unique to RAV-0 or RAV-1 are underlined.

differences between the genomes of RAV-1 and RAV-0. The oligonucleotide maps revealed more differences, with the most striking differences mapping to *env* and U3. Of 14 oligonucleotides in the *env* and U3 sequences of RAV-0, 9 were unique to RAV-0.

Construction of DNAs for the recovery of recombinant viruses. To test for a role of gp37 and LTR sequences in lymphomogenic potential, the recALV40s and the recALV80s were constructed by using the *Sac*I site at base 255 and the *Sal*I site at ca. base 6050 in pRAV-1 and pRAV-0 (Fig. 2). *Sal*I and *Sac*I were used to generate ca. 5.8-kilobase (kb) *Sac*I-*Sal*I and ca. 1.8-kb *Sal*I-*Sac*I restriction endonuclease fragments from pRAV-1 and pRAV-0. The ca. 5.8-kb fragments contained all of the coding sequences in *gag* and *pol* as well as *env* sequences that encode the subgroup determinants of gp85 (20). The ca. 1.8-kb fragments contained sequences encoding the COOH terminus of gp85, all of the sequences encoding gp37, non-protein-coding sequences between gp37 and U3, all of the LTR, and 154 bases of non-protein-coding sequences immediately 3′ of U5. The ca. 5.8-kb fragments of pRAV-0 were then ligated to the ca. 1.8-kb fragments of pRAV-1 to form the recALV40s (pBo43, pWF44, and pWF45). Similarly, the ca. 5.8-kb fragments of pRAV-1 were ligated to the ca. 1.8-kb fragments of pRAV-0 to form the recALV80s (pWF81, pWF82, pWF83, and pWF84). The ca. 5.8-kb and ca. 1.8-kb fragments of pRAV-1 were also ligated to reconstruct pRAV-1 (Fig. 2).

Each of the constructed DNAs was tested for the *Sac*I and *Eco*RI restriction endonuclease sites that are diagnostic for the ca. 5.8- and ca. 1.8-kb fragments of pRAV-0 and pRAV-1. As expected, the recALV40s contained the *Sac*I site that is diagnostic for the ca. 5.8-kb fragment of pRAV-0 and *Eco*RI site that is diagnostic for the ca. 1.8-kb fragment of pRAV-1, whereas the recALV80s contained neither the *Sac*I site that is diagnostic for the ca. 5.8-kb fragment of pRAV-0 nor the *Eco*RI site that is diagnostic for the ca. 1.8-kb fragment of pRAV-1 (Fig. 3). The *Sac*I and *Eco*RI sites in the reconstructed pRAV-1 were also consistent with the reconstructed plasmid's having been obtained by ligation of the appropriate fragments of pRAV-1 (Fig. 3).

Recovery of virus from the constructed DNAs. Viruses were recovered from the DNA in plasmid vectors by transfection of excised and ligated viral DNAs into avian cells. Culture medium from infected cells was saved as a virus stock once the titers of virus (counts per minute per milliliter of particulate RNA-directed DNA polymerase) had reached a maximum (Table 1). Since the ratio of infectious units to counts per minute of reverse transcriptase is higher for subgroup A viruses than subgroup E viruses, the levels of reverse transcriptase cannot be used to compare the titers of infectious virus in stocks of subgroup A and subgroup E ALVs. However, comparison of the titers of particulate reverse

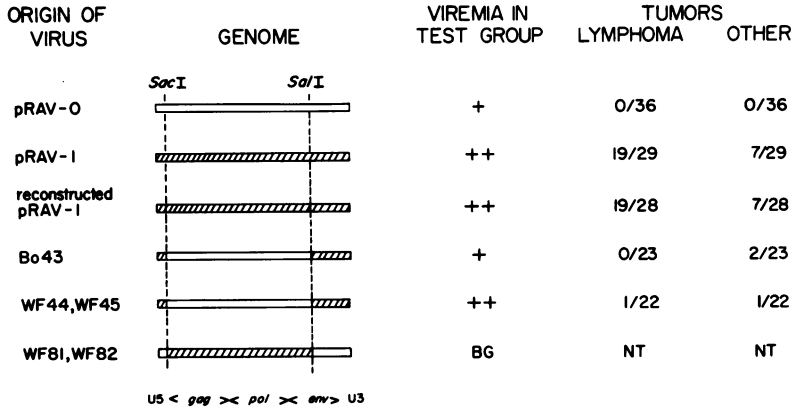

FIG. 2. Recombinant viruses: genomes and lymphomogenic potential. The viral genomes are shown as the RNA genome would appear. Parental clones (pRAV-1 and pRAV-0) are shown in the upper half of the figure, and recombinant clones (Bo43, WF45, WF81, WF82, and reconstructed pRAV-1) are shown in the lower half. The levels of viremia in test groups are taken from Table 1, with the + or ++ estimates of infectious virus taking into account the fact that subgroup A ALVs have ca. 10-fold-higher ratios of infectious units to counts per minute of reverse transcriptase than do subgroup E viruses. Data for the oncogenic potential are for chickens that were observed for 1 year after infection. See Table 2 for a listing of the different types of tumors that occurred during the test.

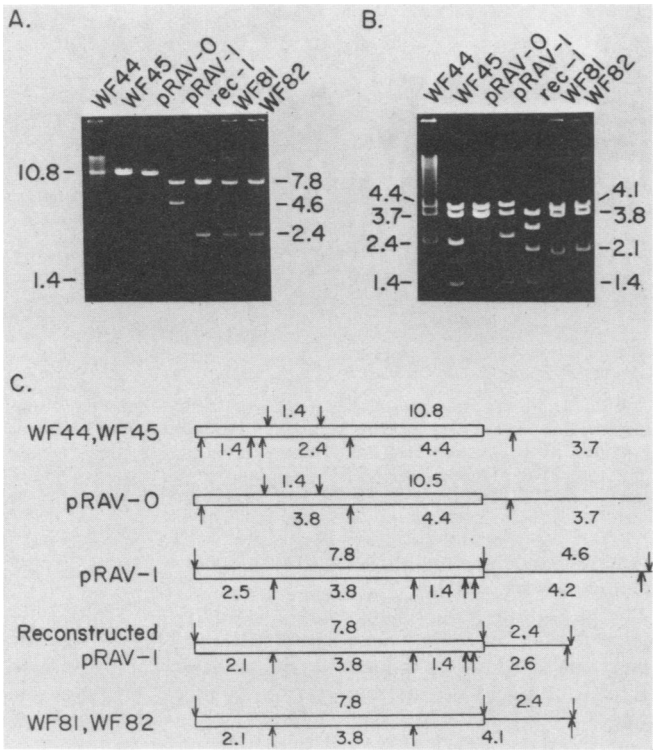

FIG. 3. Recombinant viruses: diagnostic restriction endonuclease fragments. *Eco*RI and *Sac*I fragments of the parental and recombinant viruses are displayed in panels A and B; schematics of the *Eco*RI and *Sac*I sites in these viruses are presented in panel C. In the schematics, the cloned DNA has been linearized around the 5′ junction of vector and viral sequences. In WF44, WF45, and pRAV-0, this junction is a *Sal*I site. These DNAs are cloned in pBR322. In pRAV-1, reconstructed pRAV-1, WF81, and WF82, this junction is a *Sac*I site. pRAV-1 is cloned in a derivative of pBR322. Reconstructed pRAV-1, WF81, and WF82 are cloned in pUC12. In each case *Sac*I sites are indicated above the line and *Eco*RI sites are indicated below the line. Symbols: ▭, viral sequences; ———, vector sequences.

transcriptase among the subgroup E viruses indicates that virus recovered from the recALV40s gave titers up to 4 times higher than those of virus recovered from pRAV-0. This was expected, since the recALV40s contain the highly efficient transcriptional control elements found in the LTR of RAV-1 (10, 67). Comparison of the titers of the subgroup A viruses indicates that viruses recovered from the recALV80s grew ca. 10 times less well than virus recovered from pRAV-1. This again was anticipated, because the recALV80s contained the only moderately efficient transcriptional control elements found in the LTR sequences of pRAV-0.

Oncogenic potential of recALV40s, recALV80s, and recovered pRAV-1. Viruses recovered from the recALV40s, the recALV80s, and reconstructed pRAV-1 were inoculated intravenously into 1-day-old K28 chickens. At 1 month after inoculation, the growth of the viruses was monitored by testing sera for the amount of particulate RNA-directed DNA polymerase (Table 2). The growth of virus recovered from the reconstructed pRAV-1 was similar to that recovered from pRAV-1. Virus recovered from each of the recALV40s grew in chickens. However, virus recovered from WF44 and WF45 established higher levels of viremia (>10 times higher) than did virus recovered from Bo43. Since independent test groups inoculated with the same virus had never shown such a difference in viremias at 1 month postinoculation, the oncogenicity test data for Bo43 have not been pooled with those for WF44 and WF45. Virus recovered from the recALV80s could not be detected in sera of the inoculated chickens. A second group of chickens was inoculated with virus recovered from the recALV80s. These chickens received both intravenous (0.2 ml) and intraperitoneal (0.8 ml) inoculations. At 1 month postinoculation, sera were harvested from this group and tested for particulate RNA-directed DNA polymerase. Again, the level of viremia was below that which could be detected by tests for particulate reverse transcriptase. Since we could not obtain an easily detected viremia, these chickens were sacrificed.

By 3 months after infection, the chickens infected with virus recovered from the reconstructed pRAV-1 had begun to develop malignancies. By 1 year after infection, 19 of 28 had developed lymphoma and 7 of 28 had developed other malignancies (Fig. 2; Table 2). In contrast, during the same period only one lymphoma and one kidney tumor were observed in the 22 chickens inoculated with virus recovered from WF44 and WF45. These unexpected results clearly indicate that the 3′ *env* LTR sequences of RAV-1 do not determine the ability of RAV-1 to induce a high incidence of B-cell lymphoma.

Analysis of virus in chickens infected with virus recovered from Bo43. To verify that the virus growing in chickens inoculated with virus recovered from Bo43 was indeed encoded by Bo43, virus was isolated from the sera of two test chickens. The RNAs of the isolated viruses were analyzed for RNase T1-resistant oligonucleotides. Fingerprints of the oligonucleotides revealed only those characteristic of viruses encoded by the recALV40s (Fig. 4). Thus the virus in this test group had an oligonucleotide map that was indistin-

TABLE 2. Fate of chickens in oncogenicity tests

Origin of virus	No. of chickens at risk	No. of survivors	No. of deaths from cancer[a]				No. of non-cancer deaths[b]
			Lymphoma	Kidney	Erythroblastosis	Other	
pRAV-0	36	33	0	0	0	0	3
pRAV-1	29	0	19	4	2	1	3
Reconstructed pRAV-1	28	0	19	5	1	1	2
Bo43	23	20	0	1	0	1	1
WF44	11	8	(1)	0	0	0	2
WF45	11	9	0	1	0	0	1

[a] Parentheses indicate a diagnosis for which we do not have histology. Other deaths from cancer include tumors of whose diagnosis we were not sure.
[b] Most non-cancer deaths were due to trauma.

guishable from that of viruses recovered from WF44 and WF45.

DISCUSSION

ALV-induced lymphomas result from rare events that occur in infected cells (46). Consequently, viral genes can influence lymphomogenic potential by determining the frequency with which a virus initiates a lymphoma-inducing event and by determining the ability of a virus to maintain a lymphoma-inducing event. In lymphomogenesis by ALVs the most clearly defined event is mutation of c-*myc* to an oncogene by a proviral insertion (34, 49). Many of these insertions are deleted proviruses. Analyses of these deletions indicate that the LTR is the only region of the provirus that is necessary for the maintenance of a lymphoma-inducing insertion (50, 74). In this paper we report that the LTR of a highly lymphomogenic virus (when recombined into a benign virus) does not confer the potential to induce a high incidence of lymphoma (Fig. 2; Table 2). These results suggest that viral proteins play key roles in determining the frequency of the establishment of lymphoma-inducing insertions.

LTR sequences and lymphomogenic potential. Most, if not all, cancer-associated proviral insertions either deregulate (to constitutive synthesis) or up-regulate the expression of the target proto-oncogene (18, 26, 34, 47, 49). Thus the role of LTR sequences in the conversion of proto-oncogenes to oncogenes appears to be inappropriate expression of the target proto-oncogene by the transcriptional control elements in the LTR. The LTR sequences of Rous sarcoma virus (and its associated helper viruses and transformation-defective derivatives) contain transcriptional control elements that are highly active in a variety of cell types (28). Since these LTRs are active in a variety of cell types, it is not surprising that they confer the potential to induce a variety of malignancies (55). In contrast, the LTRs of thymoma-inducing murine leukemia viruses (MuLVs) contain enhancer sequences that are relatively T-cell specific (5, 39). In most instances these LTRs confer the potential to induce T-cell lymphoma but do not confer the potential to induce a broad spectrum of malignancies (6, 7, 16, 37, 39).

***env*-encoded host range determinants and lymphomogenic potential.** In ALV-infected chickens neither *env*-encoded host range determinants nor the level of expression of *env* determinants influences the incidence of lymphoma (13, 50, 52, 58). In contrast, in MuLV-infected mice and in feline-leukemia-virus-infected cats, specific host range determinants, as well as the level of expression of these determinants, appears to influence the time of onset and incidence of thymoma (37; for reviews see references 25 and 38). These host range determinants (designated MCF for MuLVs and subgroup B for feline leukemia viruses [24, 31]) have been hypothesized to possibly accelerate the progression of lymphoma by serving as mitogens for preleukemic or leukemic cells (23, 42, 44).

***env*-encoded anchor proteins and lymphomogenic potential.** gp37 is a transmembrane protein with substantial sequence diversity in its intracellular COOH-terminal domain (4, 40; for a review see reference 9). The results in Fig. 2 clearly indicate that the gp37 protein of RAV-1 does not determine the ability of RAV-1 to induce a high incidence of B-cell lymphoma. Studies on the disease potential of thymoma-inducing MuLVs have indicated that sequences encoding the analogous protein of MuLVs (p15E) can augment the lymphomogenic potential of MuLVs with thymotropic LTR sequences (37). In this instance, p15E may affect disease potential by influencing the maturation and display of *env*-encoded MCF antigens (25).

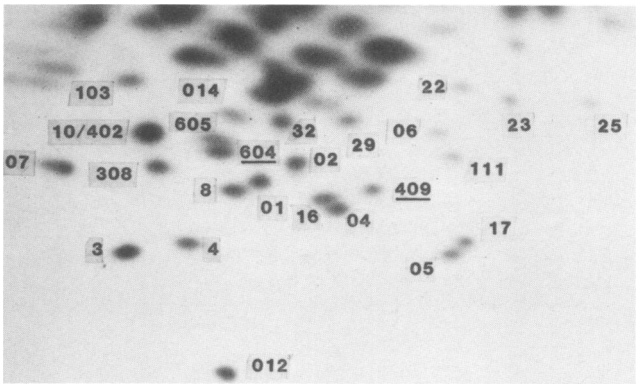

FIG. 4. Fingerprint of the RNase T1-resistant oligonucleotides observed in virus cultured from a chicken inoculated with the virus encoded by Bo43. See Fig. 1 for map of oligonucleotides that are diagnostic for pRAV-0 and pRAV-1. Sequences that are unique to the 3′ *env* LTR fragment of pRAV-1 are underlined.

Non-protein-coding sequences between gp37 and LTR have also been hypothesized to affect the lymphomogenic potential of ALVs by influencing the ability of the proviral 3′ LTR to act as a downstream promoter (68). The results in Fig. 2 clearly indicate that such sequences do not alone determine the ability of RAV-1 to induce a high incidence of B-cell lymphoma.

***gag-pol* sequences and lymphomogenic potential.** If 3′ *env*-LTR sequences are necessary but not sufficient for the induction of a high incidence of lymphoma (Fig. 2; Table 2) and if the 5′ *env* sequences of ALVs do not affect lymphomogenic potential (52, 58), then *gag-pol* sequences must play an important role in lymphomogenic potential. Neither *gag* nor *pol* gene products appear to restrict the replication of ALVs in B-cells (55). Therefore, we think that *gag-pol* sequences affect lymphomogenic potential either by determining the frequency with which RAV-1 proviruses become inserted into c-*myc* or by determining the frequency with which inserted proviruses convert c-*myc* from a proto-oncogene to an oncogene. For example, the frequency with which insertions occur in c-*myc* may be determined by integration functions encoded in *pol* (19, 22, 30, 36, 61).

A striking influence (10-fold) of *gag-pol* sequences on lymphomogenic potential may be relatively unique to ALVs. *gag-pol* sequences have been found to play a role in the induction of lymphoma by the MuLV MCF247. However, this role is minor, increasing the disease incidence from 60 to 80% of infected mice (37). The remarkable influence of *gag-pol* sequences in lymphomogenesis by ALVs may reflect the central role of promoter insertions in c-*myc* in the genesis of ALV-induced lymphomas. In MuLV-induced lymphomas the less specific regional localization of lymphoma-associated insertions (12, 15, 41, 65, 69), the possible later timing of lymphoma-associated insertions (in leukemic as opposed to pre-leukemic tissue [11, 41, 46, 48]), and the frequent occurrence of complete proviruses in the target proto-oncogene (12, 15, 41, 65, 69) may result in a more peripheral role for *gag-pol* sequences in lymphoma induction.

ALV *gag-pol* sequences have also been found to play a key role in determining the ability of these viruses to induce osteopetrosis (P. R. Shank, P. J. Schatz, L. M. Jensen, P. N. Tsichlis, J. M. Coffin, and H. L. Robinson, Virology,

in press). The role of *gag-pol* sequences in the induction of osteopetrosis, however, is most likely different from its role in the induction of lymphoma, since osteopetrosis is a nonclonal outgrowth of osteoblasts in which the inducing virus is undergoing the persistent synthesis of viral and proviral DNA (57).

RAV-1 genes in the induction of other cancers. Virus recovered from pRAV-1 and reconstructed pRAV-1 induced a moderate incidence of a variety of malignancies (Fig. 2; Table 2). In contrast, the 3′ env LTR sequences of pRAV-1 conferred the potential to induce a low incidence of a variety of tumors. Since different ALV-induced tumors involve different genetic events (27, 34, 45, 49), RAV-1 sequences that determine the incidence of non-B-cell tumors may be different from those that determine the incidence of lymphoma.

Low growth potential of the recALV80s. Although we have not rigorously quantitated the growth potential of the recALV80s, our impression is that these viruses grow more poorly (i.e., require more passages to grow to maximum titers) than would have been predicted from their having the LTR sequences of RAV-0 and the envelope antigens of RAV-1. If this is so, the unusually low growth potential of these viruses could be due to the fact that the gp85 sequences of RAV-1 do not work effectively with the gp37 sequences of RAV-0. Alternatively, the low titers of particulate reverse transcriptase could reflect the compounding in the recALV80s of the intermediate titers of reverse transcriptase observed for viruses with the LTR sequences of RAV-0 with the intermediate titers of reverse transcriptase observed for viruses with the subgroup A antigens of RAV-1.

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