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On the mechanism of retrovirus-induced avian lymphoid leukosis: Deletion and integration of the proviruses

(RNA tumor virus/B-lymphocyte tumor/proviral DNA/MC-29 oncogene)

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ABSTRACT **There is considerable evidence that infection by avian lymphoid leukosis viruses can lead to tumor development in the target organ of the host. The mechanism by which virus-induced oncogenic transformation occurs, however, is not clearly understood. As a first step toward deciphering this process, we have characterized the proviruses of the lymphoid leukosis viruses in DNAs extracted from the leukotic and metastatic tumors by using restriction enzyme digestion and filter hybridization analysis with radioactive probes specific for the infecting genome. Our results indicate (*i*) that lymphoid leukosis tumors are clonal in origin; (*ii*) that there are multiple sites in the cellular genome of the target tissue where the virus DNA can integrate and that, in the majority of the tumors, at least one such site of each tumor is adjacent to a cellular sequence related to the oncogene of MC-29 virus; and (*iii*) that deletions and other structural alterations in the proviral DNA may facilitate tumorigenesis.**

The oncogenic retroviruses can be separated into at least two classes that appear to induce neoplasms by different molecular mechanisms. The more extensively characterized group includes viruses that induce rapid neoplasms, encode genes for cell transformation (probably of host origin), and are often defective, requiring a helper virus for infectivity or replication (1, 2). The second group induces neoplasms that have long latent periods, have no known genes coding directly for cell transformation, and are not defective in replication. Among these, some appear to have the potential for inducing several types of neoplasms (1, 2). The first class of viruses, although of basic interest in studies of *in vitro* cell transformation, are probably laboratory products, while the second class of viruses is likely to be responsible for the majority of naturally occurring retrovirus-induced neoplasms. Viral induction of avian lymphoid leukosis (LL) is an excellent model of neoplasm by a virus of the second group. The steps leading to mortality with LL include the infection of the target cell in the bursa of Fabricius, the transformation of the target cells not earlier than 3 to 4 weeks of age, the development of the grossly visible bursal tumor at 10–16 weeks of age, and the metastasis to visceral organs leading to massive lymphoid tumors and death, usually after 16 weeks of age (3).

The present studies are aimed at characterizing the newly integrated exogenous proviruses in LL tumor cell DNA to provide insight into the molecular events that lead to the development of LL.

MATERIALS AND METHODS

Cell Culture, Viruses, and Biochemicals. A RAV-1 virus stock, purified by three cycles of propagation at limiting dilutions, was used. Infection of chicken embryo fibroblast cultures was carried out at a multiplicity of 0.1, and the infected cells were passaged at least four times before DNA extraction. The media of such cultures contained a high level of reverse-transcriptase activity (4). For the synthesis of cDNA probes, concentrated Prague C virus, purified by repeated banding in sucrose gradients, was used (5). DNA polymerase I, DNase I, and restriction endonucleases were purchased from commercial sources, and [α-^{32}P]dCTP was from ICN.

Induction of Lymphoid Leukosis. Day-old chickens of a cross between RPRL (Regional Poultry Research Lab) lines $15I_5$ and 7_2 were inoculated by the intra-abdominal route with 10^5 infectious units of RAV-1. The chickens were free of common avian pathogens and reared in plastic canopy isolators to 12 weeks of age and then moved to semi-isolated cages. From 120 through 150 days, the birds were palpated for bursal enlargment twice weekly. Sixteen birds were killed; tumorous and representative nontumorous tissues were taken for DNA extraction. All tissue samples were immediately transferred to vessels containing liquid nitrogen and then stored at −70°C until use. For experiments to study the provirus in bursal tissue at preneoplastic stage, a portion of the bursa was surgically removed 4 weeks after virus inoculation.

DNA Extraction and Enzyme Digestions. Frozen tissues were homogenized in a glass barrel with a loose Teflon pestle in 40 vol of 10 mM Tris·HCl, pH 7.5/5 mM EDTA. Protease K (25 μg/ml) and $NaDodSO_4$ (1%) were added to the homogenate. After incubation at 37°C for 2 hr, the solution was adjusted to 0.1 M NaCl and extracted with phenol/chloroform. The DNA samples were concentrated by EtoH precipitation. Digestions of DNA with restriction endonucleases were conducted at 37°C for 2 hr. The digested DNAs were analyzed on 0.8% agarose gels and then transferred to nitrocellulose paper and hybridized with appropriate radioactive probes as described (6).

Hybridization Reagents. The radiolabeled nucleotides in all of the following probes were derived from [α-^{32}P]dCTP. (*i*) $cDNA_{3'}$, which carries the 3′-terminal sequences (≤200 nucleotides) of the viral genome, was synthesized by using the avian myeloblastosis virus polymerase on ≤8S poly(A) containing RNA and $oligo(dT)_{12-18}$ (P-L Biochemicals) as primer. Oligo(dT)-primed $cDNA_{3'}$ was then purified by chromatography twice on oligo(dT)-cellulose after hybridizing to poly(A) (6). (*ii*) $cDNA_{5'}$, which represents the 5′-terminal 101 nucleotides of the viral genome, was synthesized by using detergent-activated virion as described (7) and purified by isolation of the 101-

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Abbreviations: LL, lymphoid leukosis; LLV, lymphoid leukosis virus; MDal, megadalton(s); LTR, long terminal repeat; ev, endogenous viral; TS, tumor specific; CSV, chicken syncitial virus.

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mer from a 10% polyacrylamide gel (7). (*iii*) $cDNA_{rep*}$ was synthesized in the same way as $cDNA_{5'}$ except that the gel-purification step was omitted. This probe, enriched in $cDNA_{5'}$, carries ≥80% sequences of the entire genome. It is capable of detecting all three *Sac* I-derived endogenous virus fragments corresponding to the major loci as described by Astrin *et al.* (8). In addition, $cDNA_{rep*}$ also detects a 2.5-megadalton (MDal) end fragment (see Fig. 1*B*, lanes 1 and 2), which preferentially hybridizes to $cDNA_{5'}$. (*iv*) DNA probes specific for the oncogene of MC-29 (avian myelocytomatosis virus strain 29) (1, 9) were prepared by nick translation (10) of a plasmid clone, pMyc-Pst, supplied to us by D. Sheiness and J. M. Bishop (University of California, San Francisco). pMyc-Pst, which carries principally the putative oncogene, was derived by subcloning a *Pst* fragment of a DNA clone carrying the entire MC-29 genomic sequence.

RESULTS AND DISCUSSION

Viral Etiology and Development of Lymphoid Leukosis. Twenty-nine day-old ($15I_5 \times 7_2$) chickens were inoculated with avian lymphoid leukosis virus (LLV), RAV-1. All birds either died of or were killed bearing lymphomas by 253 days of age. Tissues were taken from 16 of the birds for DNA extractions and histopathological examinations. Among these 16, all except 1 contained lesions in the bursa of Fabricus; 5 also developed secondary spleen or liver tumors. Thus, in our experimental system, a near 100% incidence of bursal lymphoma was obtained after virus inoculation. Such a high lymphoma incidence, together with the presence of RAV-1 proviruses in all the tumor samples (see below), is consistent with a viral etiology for this disease.

Strategies for the Identification of Exogenous Provirus. The studies described here are principally based on digestion analyses with *Sac* I and *Eco*RI and hybridization with the sequence-specific probes $cDNA_{rep*}$, $cDNA_{3'}$, and $cDNA_{5'}$. $cDNA_{rep*}$ carries sequences representing the entire RAV-1 viral genome. $cDNA_{3'}$ and $cDNA_{5'}$, on the other hand, are specific for the 3′ and 5′ terminal sequences of the viral RNA genome (see *Materials and Methods*). The sequences contained in $cDNA_{3'}$ and $cDNA_{5'}$ (shown in Fig. 1*A* as boxed 3 and 5) together comprise the long terminal repeat (LTR) present at both ends of the provirus. As the 3′-terminal region (≈200 nucleotides) of the RAV-1 genome does not share much homology with any endogenous viral (ev) sequence in chicken chromosome (11, 12), we have used $cDNA_{3'}$ extensively to distinguish the infecting RAV-1 DNA from ev sequences.

Most chickens of a ($15I_5 \times 7_2$) cross have three ev loci, *ev* 6, *ev* 1, and *ev* 2.§ We have used *Sac* I digestion to document the presence of exogenous proviruses in tumor DNAs and to identify their integration patterns. This is based on the following considerations: First, *Sac* I has a single cleavage site in RAV-1 proviral DNA, and the fragment sizes are determined not only by the location of this site in the viral genome but also by the nearest enzyme cleavage site in the flanking cellular sequence (Fig. 1*A*). Therefore, *Sac* I digestion can provide information concerning the integration site of exogenous proviral DNA. Second, as shown by Astrin and coworkers (8, 13), *Sac* I digestion of normal chicken DNAs gives a relatively simple fragmentation pattern of the ev sequences; additional bands corresponding to the newly integrated exogenous provirus in the tumor DNA can be readily identified. On cleavage of the genomic DNA with *Sac* I and hybridization with $cDNA_{rep*}$, the ev sequences are shown as four bands of M_r 13, 5.9, 3.7, and 2.5 MDal. In the example shown in Fig. 1*B*, both nontumor (lane 1) and tumor tissue (lane 2) DNA display these four bands. DNA from the tumor displays two additional bands (M_r 8 and 4.0 MDal), which we refer to as tumor specific or TS bands. The exogenous origin of the TS bands was established by hybridization with $cDNA_{3'}$ which detects only RAV-1 DNA. The specificity of this probe is shown by the complete absence of *ev*-related fragments in the DNA from nontumor tissue (lane 3). Hybridization of the tumor DNA with $cDNA_{3'}$ (lane 4) shows two distinct bands with size identical to the TS bands detected by $cDNA_{rep*}$.

In contrast to *Sac* I, there are several cleavage sites for *Eco*RI in the viral genome, which therefore allows us to analyze the internal structural arrangement of the exogenous proviral DNA (Fig. 1*A*). More important, ev sequences lack the two outer *Eco*RI sites (indicated by open triangles), which are found only in the exogenous proviral DNA. Consequently, either the 1.4- or the 0.7-MDal fragment specifically indicates the presence of ev sequences in cellular DNA, as seen by comparing the DNA pattern of a RAV-1 infected culture of chicken embryo fibroblasts with that of an uninfected culture (lanes 5 and 6). The 1.4-MDal fragment (indicated by triangle) is present only in the

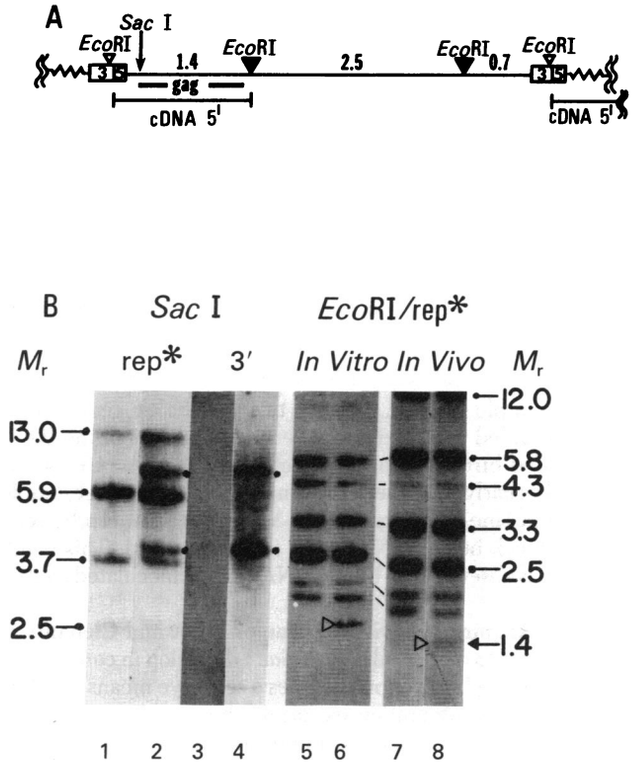

FIG. 1. Restriction enzyme cleavage maps of a colinearly integrated RAV-1 provirus DNA and identification of tumor-specific (TS) proviral DNA. M_r in MDal. (*A*) Cleavage maps of *Eco*RI and *Sac* I. Open triangles indicate *Eco* RI sites not present in the *ev* sequences. 3'5' represents the LTR, which is located at both termini of the viral DNA and carries the 3′- and 5′-terminal sequences of the RNA genome. The wavy line denotes the flanking cellular sequences. The bars indicate the *Eco*RI fragments detectable by $CDNA_{5'}$. (*B*) Restriction enzyme digestion analysis of proviral DNA. The DNA samples were extracted from bursa tumor 10 (lanes 2 and 4), from the nontumorous thymus (lanes 1 and 3) of the same bird, from the *in vitro* RAV-1-infected (lane 6) or uninfected (lane 5) chicken embryo fibroblasts of line ($15I_5 \times 7_2$), and from the bursal tissues of a bird inoculated with RAV-1 4 weeks earlier (lane 8) and of an uninoculated bird (lane 7). They were digested with *Sac* I or *Eco*RI and analyzed on 0.8% agarose gels and by Southern blotting hybridizations with $cDNA_{rep*}$ and $cDNA_{3'}$.

§ Among the 16 characterized birds, numbers 1–13 carry all three *ev* loci. Numbers 14–16 lack *ev* 2.

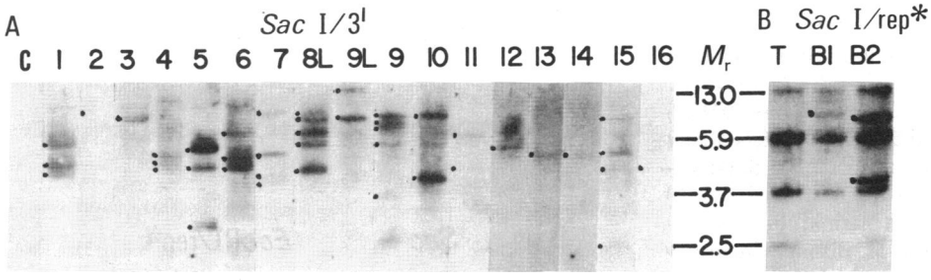

FIG. 2. TS proviral DNA as identified by *Sac* I digestion. (*A*) $cDNA_{3'}$ hybridization with the DNA samples isolated from bursal or liver (L) tumors. Lane C (control) represents the normal thymus DNA of bird 1. (*B*) $cDNA_{rep}$ hybridization with the DNA samples from bursal nodules 1 (B1) and 2 (B2) and normal thymus (T) of bird 10. Dots indicate the TS bands—i.e., fragments detected in the tumor tissue but not in the normal tissue of the same bird. M_r in MDal.

infected sample (lane 6). Indeed, this specific exogenous viral marker enabled us to demonstrate that, in >90% of the RAV-1 inoculated birds, extensive infection of the bursa tissue had occurred as early as 4 weeks after inoculation; a typical example is shown in lane 8, where the 1.4-MDal fragment can be seen in the 4-week bursal DNA of the inoculated bird. This band, however, is absent in the bursal DNA of an uninoculated control (lane 7).

Newly Acquired Provirus in Tumor DNA and Clonality of the Tumors. As discussed above, *Sac* I digestion in conjunction with $cDNA_{3'}$ hybridization provides a sensitive means for identification of the integration pattern of the newly acquired proviruses. A survey of DNA of all bursal tumors by this analysis shows that each tumor DNA displays at least one TS band (Fig. 2*A*), providing strong evidence that all tumors acquired at least all or a portion of one exogenous provirus.

It is noteworthy that DNA samples taken from bursal tissues of birds at preneoplastic stages, when assayed by the same method, do not have any TS band, although extensive infection of the target tissue by exogenous viruses can be documented (Fig. 1*B*; unpublished results). These data suggest that the initial infection of the target tissue by RAV-1 results in the integration of proviral DNA at many sites in the cellular genome of a large number of cells. The fact that TS bands can be identified in all tumors at the terminal stage indicates that each tumor results from selective growth of a homogeneous population of cells (which are characterized by a common proviral DNA structure). The origin of the tumors, therefore, is probably clonal. This conclusion is further supported by the observation that DNAs isolated from multiple tumor nodules located on the same bursa display TS bands distinct from one another, indicating that these different tumor nodules are derived from independently infected and transformed cells. An example is given in Fig. 2*B*; the two bursal tumor nodules (B1 and B2) of bird 10 have entirely different *Sac* I-TS band (indicated by dots) patterns when compared with each other or with the normal thymus tissue control (lane T). These observations are consistent with the results of others (14–16), which also indicated that LL tumors are consequences of clonal growths of transformed cells.

The data in Fig. 2 also show the size variation of TS bands in different tumors, suggesting that integration in a number of sites can lead to the development of a tumor. However, another equally plausible, but not mutually exclusive, possibility is that deletion within the proviral DNA contributes to size variation.

Frequent Deletion of the Provirus in Tumor DNA. Evidence for the deletion of viral sequences from some of these proviruses was provided by experiments in which *Eco*RI-cleaved tumor DNA was hybridized with $cDNA_{5'}$ probe. Fig. 1*A* shows that $cDNA_{5'}$ can specifically detect the 1.4-MDal *Eco*RI fragment near the left end, which carries the entire *gag* (group-specific-antigen) sequence. As discussed above (Fig. 1*B*), the 1.4-MDal gag-containing fragment can be readily detected in the undeleted RAV-1 provirus found both in *in vitro* infected cells and in the bursal tissue of inoculated birds at preleukosis stages. By contrast, in many tumor DNAs (e.g., 2, 3, 5, 9L, and 12 in Fig. 3*A*), the 1.4-MDal fragment (triangle) is completely absent. A similar conclusion was reached from hybridizations with $cDNA_{rep}$ or probes specific for the gag sequences and from *Sac* I digestion analysis (data not shown). These data thus demonstrate that some of the RAV-1 provirus in the LL tumors have undergone extensive structural alteration.

Multiple Integration Sites of the Proviruses in Tumor DNA. Hybridization of *Eco*RI-cleaved tumor DNA with $cDNA_{5'}$ also detects the right-end viral-cell junction fragment and provides reliable information concerning the integration site of proviral DNA (Fig. 1*A*), because the M_r of such fragments cannot be influenced by the potentially extensive deletion(s) in the viral genome. To identify the junction fragments, individual tumor DNAs were compared with DNAs from normal tissues (e.g., thymus or muscle) of the same animals. The representative samples of normal tissue DNAs shown in lanes C1 and C2 of Fig. 3*A* serve as controls for tumor DNA samples in lanes 1–13 and 14–16, respectively. In both controls, only the fragments corresponding to the endogenous viruses were detected: there are seven *Eco*RI-*ev* fragments in C1 DNA, including the very faint

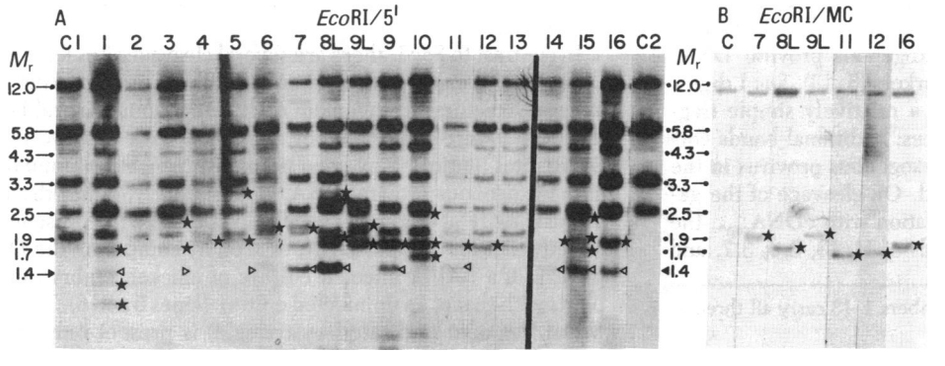

FIG. 3. Deletion and integration of the proviruses as analyzed by *Eco*RI digestion. (*A*) $cDNA_{5'}$ hybridization with DNA samples of bursal or liver (L) tumors developed in birds 1–16. Normal thymus controls, C1 and C2, are from birds 9 and 16. (*B*) pMyc-Pst hybridization with representative tumor DNA samples. Triangles indicate the 1.4-MDal *Eco*RI fragments and stars represent the right-end viral-cell junction fragments. M_r in MDal.

Table 1. Identification of fragments

Bird	Sample	M_r of right-end cell-viral junction, MDal	*Eco*RI 1.4-MDal fragment
1.	Bursa*	1.8,† 1.3, 0.9	+, Δ
2.	Bursa‡		Δ
3.	Bursa	2.3, 1.7†	Δ
4.	Bursa	1.8†	+
5.	Bursa	2.8, 1.8†	Δ
6.	Bursa	2.0†	+
7.	Bursa	2.0†	+
8.	Liver	2.8, 2.6, 1.85†	+
9.	Bursa	1.7†	+
	Liver 1	2.0,† 1.7	Δ
	Liver 2	2.0,† 1.7	Δ
	Liver 3	2.0,† 1.7	Δ
	Liver 4	2.0,† 1.7	Δ
10.	Bursa 1	ND	ND
	Bursa 2	2.4,† 1.7, 1.5	Δ
11.	Bursa	1.7†	+
	Liver	1.7†	Δ
	Spleen	1.7†	Δ
12.	Bursa	1.75†	Δ
13.	Bursa‡		+
14.	Bursa	1.8†	+
15.	Bursa	2.4,† 1.8,† 1.7†	+
16.	Bursa	1.8†	+

Right-end cell-viral junction fragments were identified by $cDNA_{5'}$.

* Bird 1 carries three proviruses; two of them carry deletion in the *gag* gene, and the other appears to carry an intact *Eco*RI 1.4-MDal fragment.

† Also detectable by pMyc-Pst.

‡ Although the detections of the right-end junction fragments by $cDNA_{5'}$ in birds 2 and 13 are not obvious, TS fragments hybridizable to pMyc-Pst are present in these tissues. Birds 2 and 13 carry c-myc containing TS fragments of 1.8 and 2.4 MDal, respectively. +, The left-end internal *Eco*RI 1.4-MDal fragment is present; Δ, the *Eco*RI 1.4-MDal fragment is absent; ND, not determined.

1.7-MDal band, which is weakly detectable by $cDNA_{5'}$. C2 DNA has a similar *Eco*RI cleavage pattern, except that the two small fragments (1.9 and 1.7 MDal) of ev 2 are missing. When the tumor DNAs were compared with these controls, new fragments of different sizes appeared. Those fragments, indicated by stars, were identified as right-end cell-viral junction fragments¶ and their sizes are given in Table 1. (Identification of some of the new fragments that migrate at positions close to the ev fragments—e.g., the 1.7-MDal band—was aided by the significantly higher intensity of that band seen in tumor tissue over the corresponding ev fragment observed in normal tissue DNA of the same bird.) The size heterogeneity of the end fragments indicates multiple integration sites. However, it appears that the right-end junction fragments in the size range 1.7–2.5 MDal are more common than others. It is also noteworthy that, in several cases, the tumor DNA carries more than one TS end fragment and, hence, more than one provirus. These multiple RAV-1 proviruses possibly resulted from multiple virus infections of the progenitor cell of a monoclonal tumor. Alternatively, these samples may represent semiclonal tumors in which several tumor clones coalesced together, as has been suggested for certain terminal LL tumors, based on histopathological evidence (17).

¶ For those samples which carried deletions in the 1.4-MDal fragment, it is important to rule out the possibility that these new bands of novel sizes are derived from the gag-containing 1.4 MDal internal fragment by structural alterations. This was accomplished by further hybridization of these bands with DNA probes specific for gag region. All of the right-end fragments assigned above failed to hybridize to such a probe.

Linkage of the RAV-1 Provirus with the MC-29 Related Endogenous Sequences. Recent studies by Hayward *et al.* (18) strongly implicate a cellular sequence related to the oncogene of the acute leukemia virus, MC-29, in LL virus leukemogenesis. The progenitor sequence of MC-29 oncogene (designated as c-myc) has been shown to be highly conserved and present in the genomes of all vertebrates (9). We wished to determine whether the infecting RAV-1 DNA is physically linked to the c-myc in the LL tumors characterized in this study. To examine this possibility, a cloned DNA pMyc-Pst that specifically carries the MC-29 oncogene sequence was used as a molecular hybridization probe. Representative samples for pMyc-Pst hybridizations to *Eco*RI-cleaved tumor DNAs are shown in Fig. 3*B*. In normal tissue (lane C), only one high M_r band corresponding to the *c-myc* locus is detected; in the tumor tissues (lanes 7, 8, etc.), additional bands (indicated by stars) are also observed. The sizes of these additional bands are primarily in the 1.7–2.5 MDal range and match well with the corresponding viral-cell junction fragments assigned by hybridization with $cDNA_{5'}$ in Fig. 3*A*. These results indicate that, in these LL tumor DNAs, the *c-myc* gene (on one of the two chromosomes) is joined with the RAV-1 provirus. Based on this analysis, we could demonstrate that, in all tumors in which the right-end junction fragment can be clearly detected by $cDNA_{5'}$, linkage between the RAV-1 provirus and the c-myc sequence exist (see Table 1). In most of the samples in which multiple RAV-1 proviruses are present, a single one is linked to the c-myc sequence. In one case (i.e., bird 15, Table 1), all three proviruses are linked to the c-myc. We take the most straightforward interpretation and suggest that bird 15 bursal tumor consists of three coalescing tumor clones and each carries a RAV-1 provirus integrating next to the *c-myc* gene, but at a slightly different position.

On the Mechanisms of Oncogenic Transformation. The mechanism by which LLV induces oncogenic transformation is especially intriguing because there is no evidence indicating that LLV codes for an oncogenic product. It has been postulated that specific integration of the LLV DNA into a site near a host oncogene might promote the expression of the oncogene (19). This possibility is particularly attractive in view of the fact that the two LTRs flanking the viral genome contain characteristics of promoters for eukaryotic transcription (20, 21) and that the sequence in the left-end LTR participates in the genesis of viral mRNAs (22, 23). Similarly, the right-end LTR may promote the transcription of downstream cellular sequences (24). The recent identification of novel mRNA species in LLV induced tumors, which carry both LTR-related sequences and sequences possibly of host origin supports this hypothesis (15, 16, 18).

The relationship of specific integrations to oncogenic transformation. Hayward *et al.* (18) have recently reported that, in the LL tumors, LLV proviruses are integrated next to the *c-myc* genes and that enhanced expression of MC-29 sequences are observed (18). These authors have suggested that insertion of the LLV provirus promotes the expression of the *c-myc* gene, thereby triggering the oncogenic transformation. Our data confirm some of their observations. We find that, in most of the LL tumors described here, at least one RAV-1 provirus of each tumor is covalently joined to the endogenous *myc* locus; however, as seen by the various sizes of the RAV-1-onc_{MCV} joining fragments, the exact integration sites of RAV-1 proviruses are not always identical in individual tumors. These results suggest that integration of RAV-1 at one of several sites near the c-myc gene is conducive to transformation. Recently, we have extended this analysis to the LL-like tumors induced by chicken syncitial vi-

ruses (CSV). We have previously shown that CSV, a member of the reticuloendotheliosis virus that bears no genetic relationship to LLV, is capable of inducing LL with similar latency and pathology (25). In this case too, we have been able to demonstrate linkage between the c-myc the CSV provirus in all tumors characterized (unpublished results). As CSV DNA and RAV-1 DNA, including their LTRs, share very little sequence homology with each other (26, 27), the finding that they are both integrated at positions next to the *c-myc* gene in LL tumors strongly implicates this gene and, possibly, adjacent sequences in the transformation of lymphocytes. The detailed mechanisms whereby the integration of either RAV-1 or CSV promotes the expression of the *c-myc* gene have yet to be elucidated.

The significance of the viral deletions to oncogenic transformation. One striking finding is the detection of extensive deletions of proviral DNA in at least 40% of the tumors analyzed. It is possible that deletions of the viral genome that disrupt the transcriptional program of viral RNA facilitate the transcription of the downstream cellular sequences. Perhaps the transcription of viral RNA from the left LTR extending into the right LTR may affect the initiation at the right LTR. A disruption of the transcriptional program caused by a deletion in the proviral DNA may expose the right LTR and allow efficient transcription of the downstream putative oncogene. The following observations are consistent with the importance of the LTR in the transformation process: (*i*) all tumor tissues analyzed in this study contain at least one LTR sequence (identified by $cDNA_{3'}$ and $cDNA_{5'}$ probes) and (*ii*) one tumor (5) harbors extensively deleted proviruses which possess very little, if any, viral sequences other than the LTRs (unpublished data).

Alternatively, the deletion of viral sequences may play a role in the selective growth of the tumor clones. Those cells in which the expression of viral antigens is eliminated by deletion may therefore be rendered less immunogenic and able to escape the host immune response. Histopathological examination shows that, at the onset of the disease, there are many microscopically observed enlarged bursal follicles (considered to be the transformed cell clones) (28, 29). Immune selection may account for the finding that only a limited number develop into tumors.

Irrespective of the role of deletion of provirus in the tumorigenic process, our data show that the presence of a complete provirus is not required at the terminal stage of the tumor. This finding lends further support to the hypothesis that the oncogene(s) involved in the maintenance of cells in the transformed and tumorous state is of cellular rather than of viral origin.

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