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Common Proviral Integration Region on Mouse Chromosome 7 in Lymphomas and Myelogenous Leukemias Induced by Friend Murine Leukemia Virus

JONATHAN SILVER* AND CHRISTINE KOZAK

Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20205

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Friend murine leukemia virus (F-MuLV) induces a variety of hematopoietic neoplasms 2 to 12 months after inoculation into newborn mice. These neoplasms are clonal or oligoclonal and contain a small number of F-MuLV insertions in high-molecular-weight DNA. To investigate whether different tumors have proviral insertions in the same region, a provirus-cellular DNA junction fragment from an F-MuLV-induced myelogenous leukemia was cloned in λgtWES, and a portion of the flanking cellular DNA sequence was used in blot-hybridization studies of 34 additional F-MuLV-induced neoplasms. Three of these additional neoplasms (one myelogenous leukemia and two lymphomas) were found to have altered copies of the flanking cellular sequence. Restriction enzyme analysis of genomic DNA from these tumors revealed that in each case a proviral copy of F-MuLV had inserted into the same 1.5-kilobase region; all proviruses had the same orientation. Using mouse-Chinese hamster somatic cell hybrids, we mapped this common integration region, designated *Fis-1*, to mouse chromosome 7. *Fis-1* is distinct from three oncogenes on mouse chromosome 7, Ha-*ras*, *fes*, and *Int-2*, based on restriction enzyme analysis and blot hybridization. Therefore, *Fis-1* appears to be a novel sequence implicated in both lymphoid and myeloid leukemias induced by F-MuLV.

Studies on the mechanism of tumorigenicity by long-latency retroviruses have shown that independent tumors may have proviruses inserted in the same region of chromosomal DNA. This observation was initially made in chicken DNA in which the provirus of avian leukosis virus was found to have integrated near the oncogene *myc* in a majority of B-cell lymphomas (9, 21, 24) and near *erbB* in many cases of erythroblastosis (7, 19). Subsequent studies in mice identified retroviral insertions near *myc* in thymic lymphomas (4, 18, 29, 35) and integrations near *myb* in Abelson virus-induced plasmacytoid lymphosarcomas (20). In addition, several common integration regions not previously identified as oncogenes have been found in mouse mammary tumor virus-induced mammary carcinomas (23, 25), in Moloney virus-induced thymic lymphomas in rats (16, 37), and in lymphomas induced by a variety of murine leukemia viruses (MuLVs) in mice (5). It has been hypothesized that virus insertions in these regions play a causal role in tumorigenesis (23). Consistent with this hypothesis, proviral integration in these regions is seen early in the natural history of tumors (27) and is often associated with transcription of adjacent cellular genes (4, 6, 7, 9, 18–22, 24, 29, 35). Many of the common integration regions identified to date appear to be specific for a particular virus, host strain, or tumor type.

Friend MuLV (F-MuLV) is somewhat unusual in that it can induce a variety of hematopoietic neoplasms including T- and B-cell lymphoma, myelogenous leukemia, and erythroblastosis (32). The type of neoplasm induced depends in large part on the strain of mouse inoculated (33). It was of interest to attempt to identify common integration regions in F-MuLV-induced neoplasms, both to look for new oncogenes and to investigate whether such common integration regions are specific for certain mouse strains or tumor types.

The approach adopted was the same as that used by other investigators (23), namely, to identify a tumor with a small number of F-MuLV proviral insertions, clone virus-cell junction fragments from this tumor, and then use flanking cellular DNA probes from such clones to screen additional tumors for alterations in this region of cellular DNA. In the F-MuLV system, this strategy is complicated by the fact that induction of neoplasms by F-MuLV is frequently associated with the appearance of recombinant mink cell focus-forming (MCF) viruses. For the purpose of cloning provirus-cell junction fragments we wanted to avoid tumors with MCF proviruses. We reasoned that in such tumors, MCF proviruses might be integrated in common integration regions, and we could not easily clone MCF provirus-cell DNA junction fragments because of the high degree of homology between Friend MCF and multicopy endogenous retroviral sequences. Since F-MuLV does not induce MCF viruses in C57BL/6 mice (30), we selected a tumor induced by F-MuLV in a C57BL/6 mouse to clone provirus-cell DNA junction fragments.

With a flanking-sequence probe from this C57BL/6 tumor, we screened 35 neoplasms induced by F-MuLV in a variety of mouse strains. We report here that four of these neoplasms have a copy of F-MuLV inserted in the same orientation in a 1.5-kilobase (kb) region of cellular DNA designated *Fis-1* for Friend integration site. Two of the neoplasms with proviral insertions in *Fis-1* were myelogenous leukemias and two were lymphomas. *Fis-1* is located on mouse chromosome 7 and appears to be distinct from previously identified oncogenes and common integration sites.

MATERIALS AND METHODS

Virus, mice, and hematopathology. Biologically cloned F-MuLV was a gift from Janet Hartley (National Institute of Allergy and Infectious Diseases [NIAID]). Mice were obtained from the Small Animal Section, National Institutes of Health, inoculated as neonates, and sacrified when they developed splenomegaly, lymphadenopathy, or respiratory

* Corresponding author.

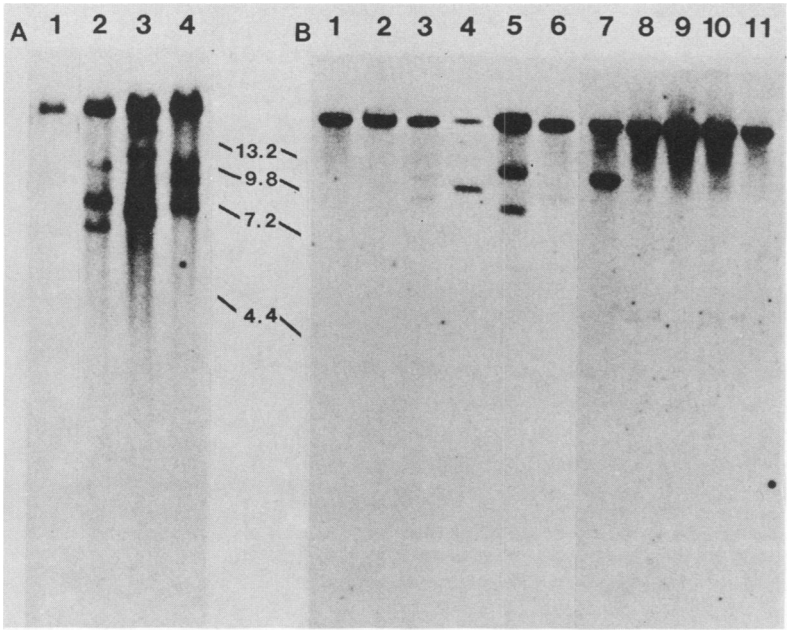

FIG. 1. Blot hybridization of tumor DNA with an endogenous ecotropic envelope probe and a probe consisting of unique sequence cellular DNA from the region flanking a proviral insertion. (A) DNA (10 μg) from normal C57BL/6 liver (lane 1) or different F-MuLV-induced myelogenous leukemias in C57BL/6 mice (lanes 2 through 4) was cut with *Eco*RI, electrophoresed through 0.6% agarose, transferred to nitrocellulose (34), and hybridized to a ^{32}P-labeled ecotropic *env*-specific probe (1). The nitrocellulose membrane was washed in 0.1× SSC at 55°C. Lanes: 2, tumor 577-3; 3, tumor 580-1; 4, tumor 580-3. (B) DNA (15 μg) was cut with *Eco*RV, electrophoresed and transferred as in panel A, and hybridized to the flanking-sequence probe. Lane 1, Myeloid leukemia from a BXD-2 mouse; lane 2, control NFS/N liver; lanes 3 through 11, F-MuLV induced leukemias: lane 3, tumor 580-1; lane 4, tumor 580-3; lane 5, lymph node DNA from tumor 875-3; lane 7, tumor 906-8. DNA from normal C57BL/6, AKR/N, and C3H mice gave the same single 17-kb band as in NFS/N mice. Numbers in middle show kilobases.

distress. Diagnoses were based on gross pathology, Wright-Giemsa, myeloperoxidase, and nonspecific esterase stains of blood and tumor tissues, and microscopic examination of fixed tissues according to described criteria (30).

Molecular cloning. Genomic DNA was digested to completion with *Eco*RI and ligated to *Eco*RI arms of bacteriophage λgtWES. Phage plaques were screened with a ^{32}P-labeled ecotropic virus envelope (*env*) probe (1) generously provided by Malcolm Martin (NIAID). Phage inserts were subcloned in pBR322, and portions of the inserts were transferred to pUC8. Blot hybridizations were done in the presence of dextran sulfate (36) at 42°C, and nitrocellulose membranes were washed in 0.1× SSC (15 mM sodium chloride plus 1.5 mM sodium citrate) at 55 or 65°C as indicated.

Tumor DNAs. DNA was prepared from the greatly enlarged spleens, lymph nodes, or thymuses of tumor-bearing mice. Tumor DNAs from 20 AKR lymphomas were kindly provided by Charles E. Buckler (NIAID), and DNAs from nine BXD-2 myelogenous leukemias were a gift from Richard Bedigian (Jackson Laboratory, Bar Harbor, Maine).

Somatic cell hybrids. Mouse-Chinese hamster somatic cell hybrids (12) were scored for the presence of mouse chromosomes by karyology and typing for mouse markers located on 15 mouse chromosomes (13).

RESULTS

Cloning of an F-MuLV-flanking cellular DNA junction fragment. A series of F-MuLV-induced myelogenous leukemias in C57BL/6 mice were analyzed by blot hybridization with an ecotropic virus-specific envelope probe (1). This probe detected the endogenous C57BL/6 ecotropic provirus and 3 to 10 non-germ line fragments in *Eco*RI-digested DNA from each of the tumors. Illustrative results are shown in Fig. 1A. Since *Eco*RI cuts once in F-MuLV, the tumor-specific bands presumably represent F-MuLV-flanking cellular DNA junction fragments and indicate that the tumors are clonal or oligoclonal.

Tumor 580-3 was chosen to clone F-MuLV-cellular DNA junction fragments because this tumor had a small number of F-MuLV inserts (Fig. 1A, lane 4) and was documented to be free of MCF virus by virologic (8) and blot hybridization (30) assays. Tumor 580-3 arose in a 5-month-old C57BL/6 mouse which had been inoculated as a neonate with biologically cloned F-MuLV. This mouse had splenomegaly and enlarged, green lymph nodes typical of chloroleukemia. Microscopic examination of blood and fixed tissues indicated that the tumor involved the splenic red pulp, lymph nodes, and periportal areas of the liver and was composed of immature myeloid cells, many of which had indented or doughnut-shaped nuclei (Fig. 2B).

DNA from tumor 580-3 was digested to completion with *Eco*RI and ligated to *Eco*RI arms of bacteriophage λgtWES. Recombinant bacteriophage were selected with the ecotropic virus *env* probe (1). Recombinant phage carrying a 7.2-kb *Eco*RI insert were recovered. The 7.2-kb insert was subcloned in pBR322 and mapped with restriction enzymes (Fig. 3B). The left-hand 5.3 kb of this cloned fragment closely resembled the published map of F-MuLV (3) (Fig. 3A). A few restriction enzyme cleavage sites were different, however: the proviral fragment we cloned lacks a *Pst*I site at 8.3 kb and a *Hin*cII site a 7.0 kb on the virus map and has novel sites for *Kpn*I at 3.3 kb and for *Hin*cII at 3.8 kb. These differences are likely to be due to minor variations in

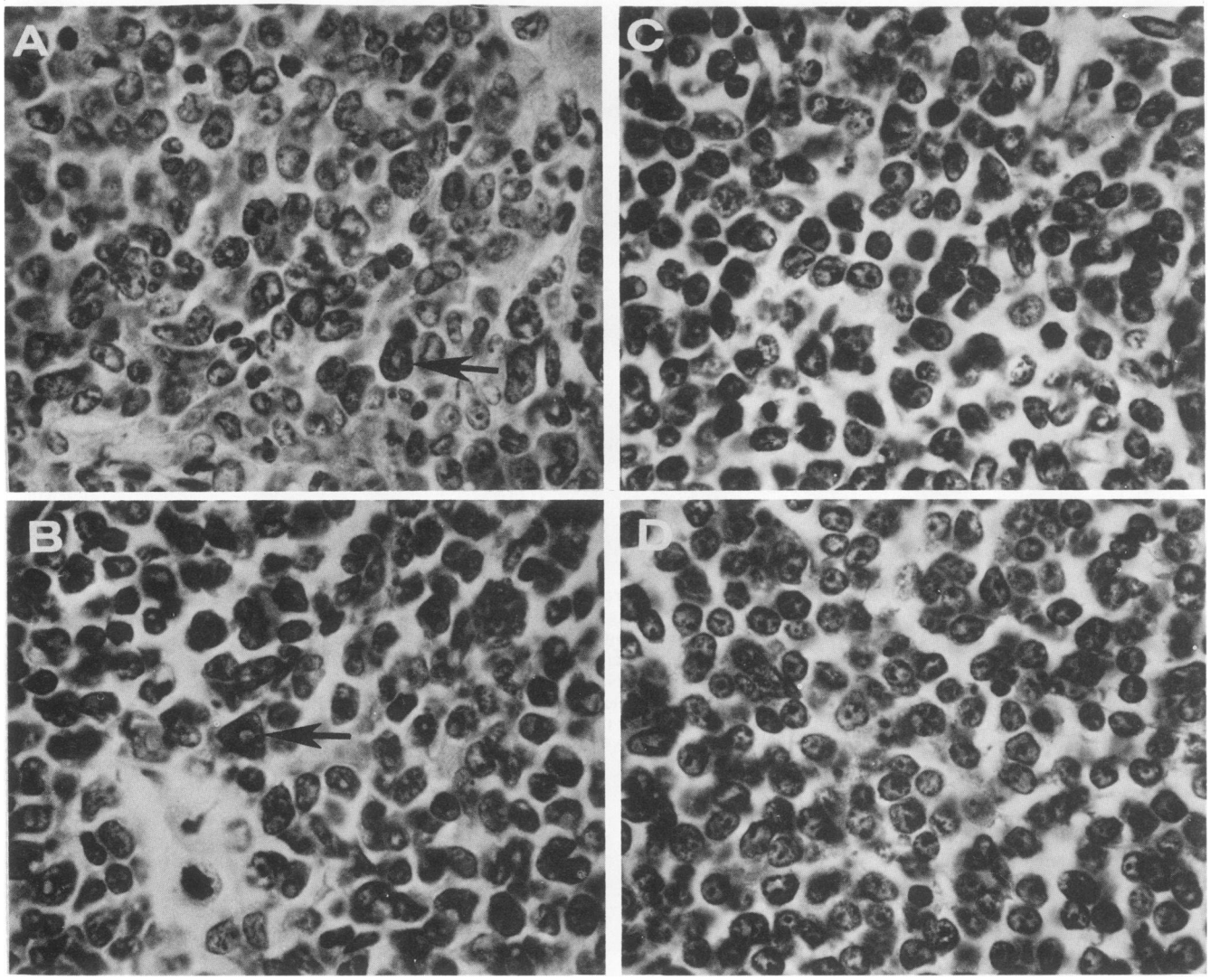

FIG. 2. Light micrographs of hematoxylin & eosin-stained sections from tumors 580-1 (A), 580-3 (B), 875-3 (C), and 906-8 (D). Arrows identify doughnut-shaped nuclei characteristic of myeloid precursors in panels A and B. Magnification, ×400.

different strains of F-MuLV (see below). The right-hand 1.9 kb of the cloned insert does not correspond to F-MuLV and presumably represents flanking cellular DNA.

Two regions of the 7.2-kb virus-cellular DNA junction fragment were subcloned in pUC8: a 0.8-kb *Bam*HI fragment from the envelope region (designated p0.8) and a 1.8-kb *Bam*HI-*Eco*RI fragment from the flanking cellular DNA region (designated p1.8). The p0.8 plasmid hybridized to a cloned endogenous ecotropic virus envelope gene fragment (1) and was used in Southern blots of genomic DNA to identify DNA fragments containing F-MuLV *env* sequences. At moderate stringency (0.1× SSC, 55°C wash) this probe detected F-MuLV and endogenous ecotropic proviruses, while at higher stringency (0.1× SSC, 65°C wash) it was specific for F-MuLV sequences. The F-MuLV *env* probe detected the same proviral inserts in tumor 580-3 DNA as did the ecotropic *env* probe. The p1.8 plasmid was used for the examination of flanking-sequence DNA. Under stringent conditions (0.1× SSC, 65°C wash), p1.8 hybridized to a single 2.0-kb *Eco*RI fragment in normal C57BL/6 liver DNA and to two bands in DNA from tumor 580-3: the germ line 2.0-kb band and a 7.2-kb band, as expected (Fig. 4C, lane 2).

Data from restriction enzyme analysis of the cloned junction fragment and of genomic DNA from normal mice and from tumor 580-3 were used to construct a map of normal cellular DNA in the region of the cloned provirus insertion. This map is shown in Fig. 3C.

Screening additional tumors for alterations in the *Fis-1* region. The restriction enzyme *Eco*RV generated the largest fragment (17 kb) of normal cellular DNA reactive with p1.8. This fragment extended approximately 8 kb to either side of the proviral insertion site in tumor 580-3. *Eco*RV was used to screen 34 additional F-MuLV-induced tumors for rearrangements in the *Fis-1* region. These tumors included 6 erythroid leukemias, 19 lymphomas, and 9 myelogenous leukemias from a variety of mouse strains [two BALB/c, five C57BL/6, two (BALB/c × C57BL/6)F_1, seven BALB/c × (BALB/c × C57BL/6), one (NFS/N × C3H)F_1, and 17 NFS/N × (AKR/N × C3H)]. Two tumors besides 580-3 had readily detectable non-germ line fragments with the p1.8 flanking-sequence probe (Fig. 1B, lanes 5 and 7). Both tumors were lymphomas which arose in NFS/N × (AKR/N × C3H) mice 2 months after neonatal inoculation with F-MuLV. These mice had small or normal-sized thymuses but grossly en-

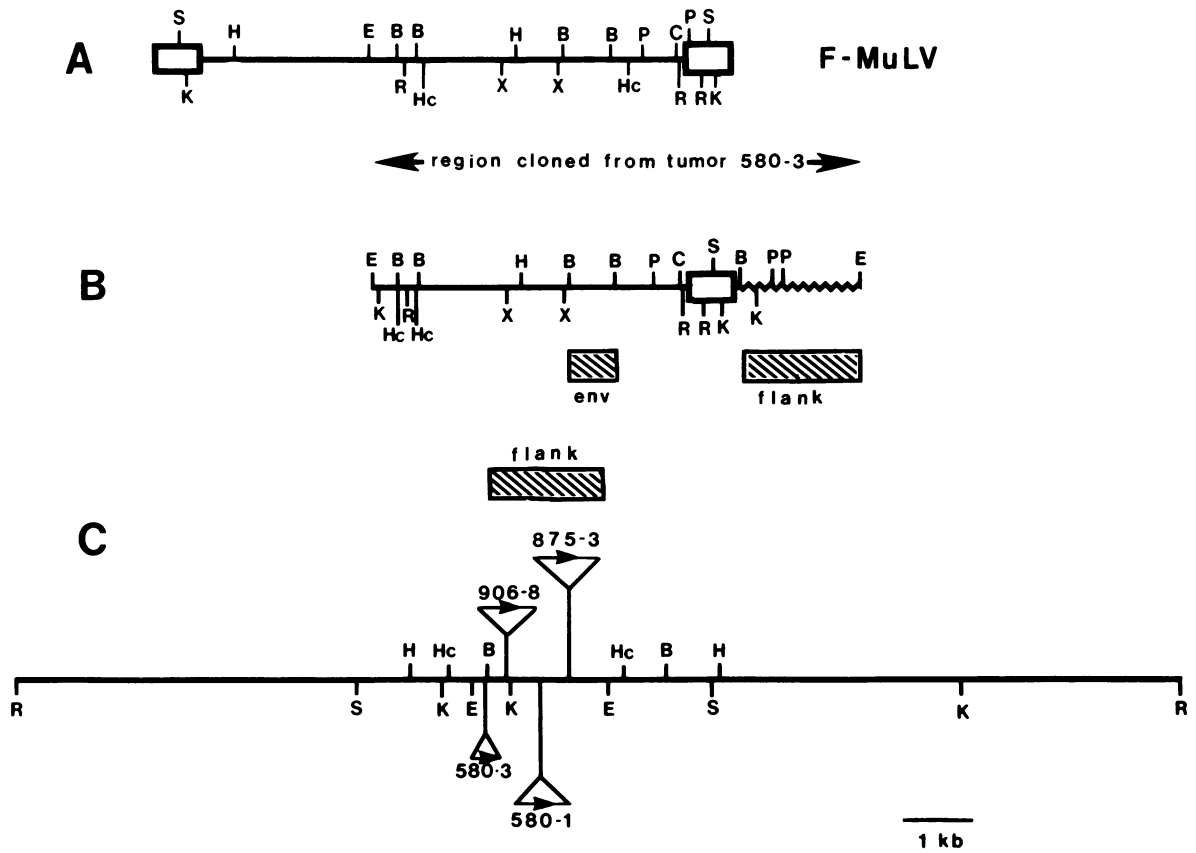

FIG. 3. Restriction endonuclease map of the *Fis-1* region. (A) Map of F-MuLV (from references 3 and 11). (B) Region cloned from tumor 580-3. Solid line, F-MuLV; open box, long terminal repeat; wavy line, flanking cellular DNA; cross-hatched boxes, portions of envelope and flanking cellular regions subcloned for use as hybridization probes. S, *Sst*I; H, *Hin*dIII; E, *Eco*RI; K, *Kpn*I; B, *Bam*HI; Hc, *Hin*cII; R, *Eco*RV; X, *Xba*I; C, *Cla*I; P, *Pst*I. (C) Map of genomic DNA from *Fis-1* region. Triangles indicate position and orientation of proviral inserts in different tumors.

larged white lymph nodes. Histologic examination of tumor tissue showed fairly uniform populations of blast cells expanding white pulp areas of the spleen and effacing the normal architecture of lymph nodes. Wright-Giemsa and hematoxylin & eosin stains showed that these cells had morphologic characteristics of lymphoblasts (Fig. 2C and D). In addition to lymphoma, one of these two mice (tumor 875-3) was severely anemic (hematocrit, 17) and had numerous erythroblasts in the spleen. This mouse probably had erythroleukemia as well as nonthymic lymphoma. The diagnosis of erythroleukemia as well as lymphoma in the spleen of this mouse was supported by blot hybridization data which indicated that the spleen contained an additional clonally expanded population of cells. Using enzymes which generate provirus-cell DNA junction fragments detectable with the F-MuLV *env* probe, we found only two prominant proviral insertions in lymph node DNA (Fig. 4D, lane 3), compared with more than four proviral inserts in spleen DNA from this mouse (Fig. 4B, lane 3). Similar results were obtained with other enzymes. Since the *Fis-1* rearrangement was seen in DNA from a lymph node which appeared to be free of erythroblasts (Fig. 4C, lane 3), this rearrangement occurred in the lymphoma in mouse tumor 875-3.

One additional tumor (580-1) was found in which the *Fis-1* region was altered, although in this tumor the signal from the non-germ line fragment was faint (Fig. 1B, lane 3). Longer exposure of Southern blots from this tumor revealed the additional fragments more clearly (Fig. 5). When tumor 580-1 was grown in vitro, a myeloid cell line grew out which no longer had detectable non-germ line p1.8-reactive fragments. The simplest explanation for these findings is that not all of the tumor cells in tumor 580-1 had the *Fis-1* rearrangement. Tumor 580-1 was a myelogenous leukemia which arose in a 5-month-old C57BL/6 mouse inoculated at birth with F-MuLV. Grossly and microscopically this tumor resembled tumor 580-3: the lymph nodes were large and green, tumor expanded the splenic red pulp as well as lymph nodes, and the malignant cells were large and had folded or doughnut-shaped nuclei (Fig. 2A).

Additional experiments were carried out to determine whether the rearranged p1.8-reactive fragments in tumors 875-3, 906-8, and 580-1 resulted from insertions of proviruses. Putative sites of integration were determined from the size of the new *Eco*RV fragment(s) and the position of *Eco*RV sites within F-MuLV. For tumors 875-3 and 580-1, the predicted insertion sites are in the middle of the cloned flanking sequence, resulting in two new bands detectable with the p1.8 probe. In the case of tumor 906-8, the predicted integration site is very close to the left-hand end of the cloned flanking sequence (Fig. 3C). The presence of F-MuLV proviruses at these sites was confirmed in two ways. First, DNAs were digested with *Eco*RI, *Bam*HI, *Hin*dIII, *Sst*I, and *Hin*cII and probed with the flanking-sequence probe. In each case, we observed p1.8-reactive bands with

the sizes expected if an intact F-MuLV had inserted at the indicated position (Fig. 4; Table 1). For tumor 906-8, faint bands of the size expected for the left-hand junction fragments were seen for enzymes *Eco*RI and *Bam*HI; a faint band was not seen with *Hin*dIII, but its predicted size coincides with that of the right-hand p1.8-reactive fragment. Additional evidence that the rearranged fragments were due to F-MuLV insertions came from experiments in which membranes which had been hybridized to the flanking-sequence probe were stripped and rehybridized to the F-MuLV *env* probe p0.8. In all cases, one of the Friend *env*-reactive fragments comigrated with the expected virus-cellular DNA junction fragment (Fig. 4). Results similar to those shown in Fig. 4 were obtained for tumor 580-1. These data confirm that tumors 875-3, 906-8, and 580-1 have proviral copies of F-MuLV inserted in the *Fis-1* region.

To see if alterations in the *Fis-1* region were common in non-F-MuLV-induced neoplasms, DNAs from 9 myelogenous leukemias arising in BXH-2 mice and 20 lymphomas arising in MCF247-inoculated AKR/N mice were cut with *Eco*RV and examined with the p1.8 probe. Only the germ line p1.8-reactive fragments were detected.

Chromosomal position of *Fis-1*. As a first step in analyzing the *Fis-1* insertion site, we determined its chromosomal assignment by using mouse-Chinese hamster somatic cell hybrids. The flanking-sequence probe detected a 2.6-kb

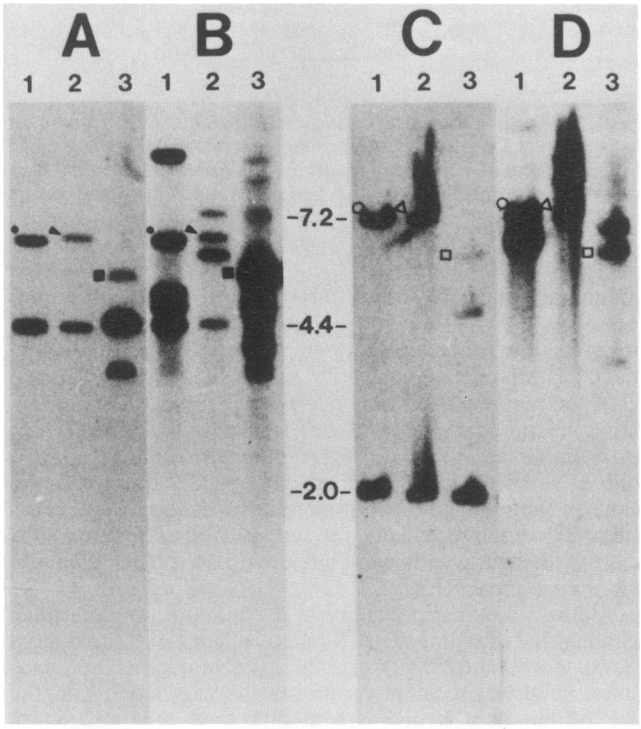

FIG. 4. Blot hybridization of tumor DNA with flanking-sequence and F-MuLV *env* probes. DNA (15 μg) was cut with *Hin*dIII (A and B) or *Eco*RI (C and D), electrophoresed, and transferred as described in the legend to Fig. 1. Membranes were hybridized to the flanking-sequence probe (A and C) and then stripped and rehybridized to the F-MuLV *env* probe (B and D). Lane 1, Tumor DNA from mouse tumor 906-8; lane 2, tumor DNA from mouse tumor 580-3; lane 3 in panels A and B, spleen DNA from mouse tumor 875-3; lane 3 in panels C and D, lymph node DNA from mouse tumor 875-3. Comigrating fragments are identified by circles, triangles, or squares. Numbers in middle indicate kilobases.

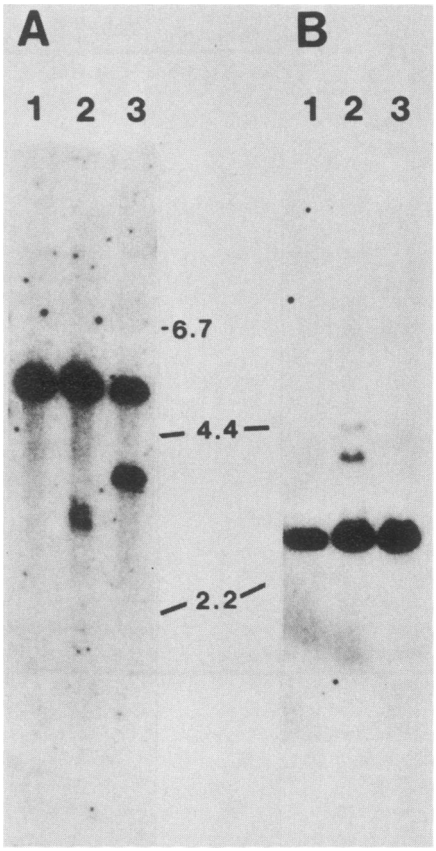

FIG. 5. Blot hybridization of DNA from tumor 580-1 with the flanking-sequence probe. DNA (15 μg) was cut with *Sst*I (A) or *Bam*HI (B), electrophoresed, and transformed as described in the legend to Fig. 1. (A) Lane 1, Control C57BL/6 DNA; lane 2, DNA from tumor 580-1; lane 3, DNA from tumor 580-3. (B) Lane 1, DNA from tumor 577-3 (a C57BL/6 myelogenous leukemia with no alteration of the *Fis-1* region); lane 2, DNA from tumor 580-1; lane 3, DNA from tumor 580-3.

TABLE 1. Size of non-germ line restriction enzyme fragments reactive with the flanking-sequence probe

Tumor	Enzyme	Fragment size (kb)	
		Observed	Predicted[a]
906-8	*Eco*RI	7.2 (3.8)[b]	7.1 (3.8)[b]
	*Hin*dIII	6.4	6.6 (6.7)
	*Bam*HI	4.5 (4.0)	4.4 (3.9)
875-3	*Eco*RI	6.2, 4.7	6.2, 4.7
	*Hin*dIII	5.5, 3.6	5.7, 3.4[c]
	*Bam*HI	4.9, 3.4	4.8, 3.5
580-1	*Eco*RI	6.8, 4.5	6.6, 4.2
	*Hin*dIII	6.3, 3.3	6.1, 3.0[c]
	*Bam*HI	4.4, 3.7	4.5, 3.9
	*Sst*I	3.0, 2.8	3.0, 2.8[d]
	*Hin*cII	6.6, 4.6	6.3, 4.7

[a] Based on the assumption that a copy of F-MuLV is inserted in the position and orientation shown in Fig. 3C.

[b] Numbers in parentheses indicate size of left-hand junction fragments, only short segments of which react with the flanking-sequence probe, resulting in weaker signals.

[c] Assumes a *Hin*dIII site at 1.2 kb as reported for F-MuLV.

[d] Assumes an *Sst*I site at 0.4 kb in the 5′ long terminal repeat as reported for F-MuLV (3).

TABLE 2. Association of *Fis-1* with mouse chromosome 7

Mouse chromosome	No. of hybrid clones in each category (*Fis-1* chromosome/retention)				% Discordant
	+/+	−/−	+/−	−/+	
1	8	3	8	3	50
2	9	5	7	1	36
3	6	2	9	4	62
4	6	4	11	2	56
5	2	5	15	1	70
6	9	5	8	1	39
7	17	6	0	0	0
8	5	5	12	1	56
9	7	6	10	0	43
10	4	5	13	0	59
11	0	6	17	0	74
12	9	3	6	3	43
13	7	6	6	0	38
14	8	6	9	0	39
15	14	1	1	5	29
16	6	5	9	1	48
17	12	2	4	4	36
18	6	6	9	0	43
19	8	5	8	1	41
X	11	3	6	3	39

*Bam*HI fragment in mouse DNA compared with a 5.3-kb *Bam*HI fragment in Chinese hamster DNA (data not shown). A series of mouse-Chinese hamster somatic cell hybrids which had been typed for mouse chromosomes were tested for the presence of the mouse *Fis-1* sequence. Only mouse chromosome 7 showed complete concordance with the mouse *Fis-1* fragment (Table 2), indicating that *Fis-1* is on mouse chromosome 7.

Mouse chromosome 7 also carries the oncogenes c-*fes* and c-Ha-*ras* and a common integration region in mouse mammary tumor virus-induced tumors designated *Int-2*. To determine if *Fis-1* is the same as c-*fes*, c-Ha-*ras*, or *Int-2*, mouse DNA was cut with *Eco*RV and hybridized to v-*fes*, c-Ha-*ras*, and *Int-2* probes. In each case the fragment detected was different in size from the 17-kb fragment detected with the p1.8 flanking-sequence probe. Therefore, these oncogenes are at least 7 to 8 kb away from the site of the F-MuLV insertions described here. In addition, DNA from *Mus pahari*, which has multiple copies of c-Ha-*ras* sequences (2), was hybridized to the p1.8 probe. Only a single band was detected, indicating that *Fis-1* is outside of the region reduplicated in *M. pahari* DNA. For *Int-2*, the restriction enzyme map of 30 kb surrounding the sites of mouse mammary tumor virus insertion (25) was compared with the map of the *Fis-1* region; the maps showed no region of overlap. These data indicate that *Fis-1* is different from c-*fes*, c-Ha-*ras*, and *Int-2*.

DISCUSSION

Our finding that 4 of 35 F-MuLV-induced tumors have a provirus inserted with the same orientation in a 1.5-kb region indicates that provirus insertions in F-MuLV-induced tumors are not completely random. The 1.5-kb insertion region represents about 10^{-6} of the mouse genome. If proviral integration sites in tumors were random, the probability that 3 additional proviruses would be found in this region, of roughly 200 proviruses in the 35 tumors screened, is approximately $(10^{-6})^3$ times the number of combinations of 200 proviruses taken three at a time, or about 10^{-11}. We conclude that proviral integration is nonrandom or that integrations in the *Fis-1* region are selected in tumors. Proviral integration in the same region in tumors of independent origin appears to be a common phenomenon since the first proviral integration site we cloned from a tumor with four proviruses was a common integration site, and similar results have been reported for several other retroviral systems (4, 5, 7, 9, 16, 18–21, 23–25, 29, 35, 37).

The most likely explanation for nonrandom proviral integration regions in tumors is that insertions in these regions provide a growth advantage or in some other way cause or contribute to the tumor phenotype. This hypothesis is supported by the fact that many common integration regions are adjacent to previously identified oncogenes (*myc*, *myb*, *erb*) and are associated with enhanced or altered expression of these genes (4, 7, 9, 18–21, 24, 29, 35). Furthermore, in the most intensively studied systems, the proviral integrations are not random within a common integration region, but cluster to either side of a cellular coding sequence and have preferred orientations, suggesting that activation of a cellular gene is important. Common integration regions not previously identified as oncogenes could represent cellular genes with analogous functions. Thus, experiments of the type described here provide a way to expand our knowledge of potentially oncogenic sequences in mammalian chromosomes.

An alternative explanation for common insertion regions in tumors is that they represent preferred regions for proviral integration which have nothing to do with tumor pathogenesis. While there is no evidence to support preferred integration regions in nontumor tissue (18), the search for such regions has not been intense.

The frequency of alterations in *Fis-1* detected here, ~10%, is comparable to the frequency of integration adjacent to *myc* in mouse thymic lymphomas (4, 18, 29, 35). Since a majority of tumors do not have proviral insertions near *myc* or in the *Fis-1* region, it is clear that these particular insertions are not indispensible for tumor formation.

The proviruses we identified in the *Fis-1* region appear to be intact and nonrecombinant. In contrast, many of the proviruses inserted near *myc* appear to carry deletions or resemble recombinant MCF viruses (4, 29). While the partial proviral segment we cloned differs at a few restriction enzyme sites from the published F-MuLV map (3), it is unlikely that this provirus is a recombinant which arose in mouse tumor 580-3, because mice acutely infected with this same strain of F-MuLV show the *Kpn*I restriction enzyme site at 3.3 kb (J. Silver, unpublished data).

Localization of *Fis-1* to mouse chromosome 7 proves that *Fis-1* is distinct from several oncogenes and common integration regions implicated in tumorgenesis by long-latency retroviruses but known to be on other chromosomes. These include *myc* (10), *Myb* (28), *erbA* and *erbB* (31, 39), *Int-1* (22), *Mlvi-1* (15), *Mlvi-2* (38), and *Mis-1* (R-Mo-*int-1*) (C. Kozak and P. Jolicoeur, unpublished data). *Fis-1* also appears to be distinct from *Pim-1*, a common integration region for Moloney virus in murine lymphomas, based on published restriction enzymes maps (5). The data presented here show that *Fis-1* is distinct from *fes*, Ha-*ras*, and *Int-2*, which are known to be on chromosome 7 (14, 26).

Data from other proviral systems suggests that common integration regions have some specificity for particular cell types or host strains. Thus, Moloney virus has been found integrated near *myc* in T-cell lymphomas (4, 18, 29, 35) but near *Myb* in plasmacytoid lymphosarcomas (20); mouse mammary tumor virus is commonly found integrated near *Int-2* in mammary carcinomas in BR6 and GR mice (25) but

near *Int-1* in mammary carcinomas in C3H mice (23). The results reported here suggest that *Fis-1* insertions are fairly specific for F-MuLV since none were found in 29 tumors of similar type induced by other retroviruses. However, no specificity was noted for any particular mouse strain. Remarkably, the *Fis-1* integrations were seen in both lymphoid and myeloid neoplasms. This lack of tumor type specificity suggests that the putative *Fis-1* oncogene is important in several different hematopoietic lineages.

In mouse DNA, most proviruses inserted adjacent to *myc*, *Int-1*, and *Int-2* are oriented such that viral transcription proceeds away from the activated cellular gene. If that was the case for *Fis-1*, the region 5′ to the provirus might be expected to contain a *Fis-1* oncogene. To pursue the question of whether *Fis-1* insertions are associated with activation of adjacent cellular genes, we are extending the region of cloned *Fis-1* DNA and screening additional F-MuLV-induced tumors for expression of a putative *Fis-1* oncogene.

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LITERATURE CITED

1. **Chan, H. W., J. L. Bryan, J. L. Moore, S. P. Staal, W. P. Rowe, and M. A. Martin.** 1980. Identification of ecotropic proviral sequences in inbred strains of mice with a cloned subgenomic DNA fragment. Proc. Natl. Acad. Sci. USA **77**:5779–5783.
2. **Chattopadhyay, S. K., E. H. Chang, M. R. Lander, R. E. Ellis, E. M. Scolnick, and D. R. Lowy.** 1982. Amplification and rearrangement of *onc* genes in mammalian species. Nature (London) **296**:361–363.
3. **Chattopadhyay, S. K., A. I. Oliff, D. L. Linemeyer, M. R. Lander, and D. R. Lowy.** 1981. Genomes of murine leukemia viruses isolated from wild mice. J. Virol. **39**:777–791.
4. **Corcoran, L. M., J. M. Adams, A. R. Dunn, and S. Cory.** 1984. Murine T lymphomas in which the cellular *myc* oncogene has been activated by retroviral insertion. Cell **37**:113–122.
5. **Cuypers, H. T., G. Selten, W. Quint, M. Zijlstra, E. R. Maandag, W. Boelens, P. van Wezenbeek, C. Melief, and A. Berns.** 1984. Murine leukemia virus-induced T-cell lymphomagenesis: integration of proviruses in a distinct chromosomal region. Cell **37**:141–150.
6. **Dickson, C., R. Smith, S. Brooks, and G. Peters.** 1984. Tumorigenesis by mouse mammary tumor virus: proviral refraction of a cellular gene in the common integration region *Int-2*. Cell **37**:524–536.
7. **Fung, Y. T., W. G. Lewis, L. B. Crittenden, and H. Kung.** 1983. Activation of the cellular oncogene *c-erbB* by LTR insertion: molecular basis for induction of erythroblastosis by avian leukosis virus. Cell **33**:357–368.
8. **Hartley, J. W., N. K. Wolford, L. J. Old, and W. P. Rowe.** 1977. A new class of murine leukemia virus associated with development of spontaneous lymphomas. Proc. Natl. Acad. Sci. USA **72**:789–792.
9. **Hayward, W. S., B. G. Neel, and S. Astrin.** 1981. Activation of a cellular onc gene by promoter insertion in ALV-induced lymphoid leukosis. Nature (London) **290**:475–480.
10. **Klein, G.** 1983. Specific chromosomal translocations and the genesis of B-cell-derived tumors in mice and men. Cell **32**:311–315.
11. **Koch, W., G. Hunsmann, and R. Friedrich.** 1983. Nucleotide sequence of the envelope gene of Friend murine leukemia virus. J. Virol. **45**:1–9.
12. **Kozak, C. A., and W. P. Rowe.** 1979. Genetic mapping of ecotropic murine leukemia virus-inducing locus of BALB/c mouse to chromosome 5. Science **204**:69–71.
13. **Kozak, C. A., and W. P. Rowe.** 1980. Genetic mapping of the ecotropic virus-inducing locus *Akv-2* of the AKR mouse. J. Exp. Med. **152**:1419–1423.
14. **Kozak, C. A., J. F. Sears, and M. D. Hoggan.** 1983. Genetic mapping of the mouse oncogenes c-Ha-*ras*-1 and c-*fes* to chromosome 7. J. Virol. **47**:217–220.
15. **Kozak, C. A., P. G. Strauss, and P. N. Tsichlis.** 1985. Genetic mapping of a cellular DNA region involved in induction of thymic lymphoma (*Mlvi-1*) to mouse chromosome 15. Mol. Cell. Biol. **5**:894–897.
16. **Lemay, G., and P. Jolicoeur.** 1984. Rearrangement of a DNA sequence homologous to a cell-virus junction fragment in several Moloney murine leukemia virus-induced rat thymomas. Proc. Natl. Acad. Sci. USA **81**:38–42.
17. **Lenz, J., R. Crowther, A. Straceski, and W. Haseltine.** 1982. Nucleotide sequence of the Akv *env* gene. J. Virol. **42**:519–529.
18. **Li, Y., C. A. Holland, J. W. Hartley, and N. Hopkins.** 1984. Viral integration near *c-myc* in 10 to 20% of MCF 247-induced AKR lymphomas. Proc. Natl. Acad. Sci. USA **81**:6808–6811.
19. **Miles, B. D., and H. L. Robinson.** 1985. High frequency of transduction of c-*erbB* in avian leukosis virus-induced erythroblastosis. J. Virol. **54**:295–303.
20. **Mushinsky, J. F., M. Potter, S. R. Bauer, and E. P. Reddy.** 1983. DNA rearrangement and altered RNA expression of the *c-myb* oncogene in mouse plasmacytoid lymphosarcomas. Science **220**:795–798.
21. **Neel, B. G., W. S. Hayward, H. K. Robinson, J. Fang, and S. M. 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.
22. **Nusse, R., A. Van Ooyen, D. Cox, Y. K. T. Fung, and H. Varmus.** 1984. Mode of proviral activation of a putative mammary oncogene (*Int-1*) on mouse chromosome 15. Nature (London) **307**:131–136.
23. **Nusse, R., and H. E. Varmus.** 1982. Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. Cell **31**:99–109.
24. **Payne, G. S., J. M. Bishop, and H. E. Varmus.** 1982. Multiple arrangements of viral DNA and an activated host oncogene in bursal lymphomas. Nature (London) **295**:209–214.
25. **Peters, G., S. Brookes, R. Smith, and C. Dickson.** 1983. Tumorigenesis by mouse mammary tumor virus: evidence for a common region for provirus integration in mammary tumors. Cell **33**:369–377.
26. **Peters, G., C. Kozak, and C. Dickson.** 1984. Mouse mammary tumor virus integration sites map to different mouse chromosomes. Mol. Cell. Biol. **4**:375–378.
27. **Peters, G., A. G. Lee, and C. Dickson.** 1984. Activation of cellular gene by mouse mammary tumor virus may occur early in mammary tumor development. Nature (London) **309**:273–275.
28. **Sakaguchi, A. Y., P. A. Lalley, B. O. Zabel, R. W. Ellis, E. M. Scolnick, and S. L. Naylor.** 1984. Chromosome assignments of four mouse cellular homologs of sarcoma and leukemia virus oncogenes. Proc. Natl. Acad. Sci. USA **81**:252–259.
29. **Selten, G., H. T. Cuypers, M. Zijlstra, C. Melief, and A. Berns.** 1984. Involvement of *c-myc* in MuLV-induced T cell lymphomas in mice: frequency and mechanisms of activation. EMBO J. **3**:3215–3222.
30. **Silver, J.** 1984. Role of mink cell focus-forming virus in leukemias induced by Friend ecotropic virus. J. Virol. **50**:872–877.
31. **Silver, J., J. B. Whitney, III, C. Kozak, G. Hollis, and I. Kirsch.** 1985. *Erbb* is linked to the alpha-globin locus on mouse chromosome 11. Mol. Cell. Biol. **5**:1784–1786.
32. **Silver, J. E., and T. N. Fredrickson.** 1983. A new gene that controls the type of leukemia induced by Friend murine leukemia virus. J. Exp. Med. **158**:493–505.
33. **Silver, J. E., and T. N. Fredrickson.** 1983. Susceptibility to Friend helper virus leukemias in CXB recombinant inbred mice. J. Exp. Med. **158**:1693–1702.
34. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **38**:503–517.
35. **Steffen, D.** 1984. Proviruses are adjacent to *c-myc* in some

murine leukemia virus-induced lymphomas. Proc. Natl. Acad. Sci. USA **81**:2097–2101.

36. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
37. **Tsichlis, P. N., P. G. Strauss, and L. F. Hu.** 1983. A common region for proviral DNA integration of MoMuLV-induced rat thymic lymphomas. Nature (London) **302**:445–449.
38. **Tsichlis, P. N., P. G. Strauss, and C. A. Kozak.** 1984. A cellular DNA region involved in the induction of thymic lymphomas (*Mlvi-2*) maps to mouse chromosome 15. Mol. Cell. Biol. **4**:997–1000.
39. **Zabel, B. U., R. E. K. Fournier, P. Lalley, S. Naylor, and A. Y. Sakaguchi.** 1984. Cellular homologs of the avian erythroblastosis virus *erb*-A and *erb*-B genes are syntenic in mouse but asyntenic in man. Proc. Natl. Acad. Sci. USA **81**:4784–4787.