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

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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 into 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 leukemia 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 multicycle 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 sacrificed when they developed splenomegaly, lymphadenopathy, or respiratory

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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 EcoRI, electrophoresed through 0.6% agarose, transferred to nitrocellulose (34), and hybridized to a 32P-labeled ecotropic env-specific probe (1). The nitrocellulose membrane was washed in 0.1× SSC at 35°C. Lanes: 2, tumor 577-3; 3, tumor 580-1; 4, tumor 580-3. (B) DNA (15 μg) was cut with EcoRV, 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 EcoRI and ligated to EcoRI arms of bacteriophage λgtWES. Phage plaques were screened with a 32P-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 EcoRI-digested DNA from each of the tumors. Illustrative results are shown in Fig. 1A. Since EcoRI 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 insertions (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 EcoRI and ligated to EcoRI arms of bacteriophage λgtWES. Recombinant bacteriophage were selected with the ecotropic virus env probe (1). Recombinant phage carrying a 7.2-kb EcoRI 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 PstI site at 8.3 kb and a HincII site 7.0 kb on the virus map and has novel sites for KpnI at 3.3 kb and for HincII at 3.8 kb. These differences are likely to be due to minor variations in
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 BamHI fragment from the envelope region (designated p0.8) and a 1.8-kb BamHI-EcoRI 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 EcoRI 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 EcoRV 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. EcoRV 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)F1, seven BALB/c × (BALB/c × C57BL/6), one (NFS/N × C3H)F1, 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-
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 prominent 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 EcoRV fragment(s) and the position of EcoRV 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 EcoRI, BamHI, HindIII, SstI, and HincII 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 EcoRI and BamHI; a faint band was not seen with HindIII, 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 EcoRV 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 HindIII (A and B) or EcoRI (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 SsrI (A) or BamHI (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.](attachment:image)

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

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<th>Tumor</th>
<th>Enzyme</th>
<th>Fragment size (kb)</th>
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<tr>
<td></td>
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<tr>
<td>906-8</td>
<td>EcoRI</td>
<td>7.2 (3.8)</td>
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<td></td>
<td>HindIII</td>
<td>6.4</td>
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<td></td>
<td>BamHI</td>
<td>4.5 (4.0)</td>
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<tr>
<td>875-3</td>
<td>EcoRI</td>
<td>6.2, 4.7</td>
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<tr>
<td></td>
<td>HindIII</td>
<td>5.5, 3.6</td>
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<tr>
<td></td>
<td>BamHI</td>
<td>4.9, 3.4</td>
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<tr>
<td>580-1</td>
<td>EcoRI</td>
<td>6.8, 4.5</td>
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<tr>
<td></td>
<td>HindIII</td>
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<tr>
<td></td>
<td>BamHI</td>
<td>4.4, 3.7</td>
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<tr>
<td></td>
<td>SsrI</td>
<td>3.0, 2.8</td>
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<tr>
<td></td>
<td>HincII</td>
<td>6.6, 4.6</td>
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* 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.

* Assumes a HindIII site at 1.2 kb as reported for F-MuLV.

* Assumes an SsrI site at 0.4 kb in the 3' long terminal repeat as reported for F-MuLV (3).
BamHI fragment in mouse DNA compared with a 5.3-kb BamHI fragment in 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 by EcoRV 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 indispensable 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 KpnI 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 tumorigenesis by long-latency retroviruses but known to be on other chromosomes. These include myc (10), Myb (28), erbB and erbB2 (31, 39), Int-1 (22), Mvi-1 (15), Mvi-2 (38), and Mis-1 (R-My-int-1) (C. Kozak and P. Jolicoeur, unpublished data). Fis-1 also appears to be distinct from Pik-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 fres, 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 lymphomas (20); mouse mammary tumor virus is commonly found integrated near Int-2 in mammary carcinomas in BR6 and GR mice (25) but

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<th>Mouse chromosome</th>
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<td>(Fis-1 chromosome/retention)</td>
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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


35. Steffen, D. 1984. Proviruses are adjacent to c-myc in some