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A Preferred Region for Integration of Friend Murine Leukemia Virus in Hematopoietic Neoplasms Is Closely Linked to the *Int-2* Oncogene

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Gene mapping experiments show that *Fis-1*, a preferred integration region for Friend murine leukemia virus in hematopoietic neoplasms, is extremely closely linked to *Int-2*, a preferred integration region for mouse mammary tumor virus in mammary carcinomas. Studies at the RNA and DNA level prove that these loci are distinct.

We recently identified a preferred integration region for Friend murine leukemia virus (F-MuLV) in mouse genomic DNA in lymphomas and myeloid leukemias induced by this virus (10). Of 35 F-MuLV-induced hematopoietic neoplasms, 4 had an apparently intact proviral copy of F-MuLV in slightly different positions within a 1.5-kilobase (kb) region of mouse chromosome 7, designated *Fis-1* for Friend integration site. The F-MuLV proviruses at this site all had the same orientation with respect to genomic DNA. We were interested in determining whether *Fis-1* was related to any of the oncogenes or putative oncogenes known to be on mouse chromosome 7. We report here that, on the basis of studies of sexual crosses, *Fis-1* is very closely linked to *Int-2*, a common integration region and putative oncogene for mouse mammary tumor virus in mammary carcinomas (8). However, molecular studies show that *Fis-1* and *Int-2* are separated by at least 25 to 30 kb and that provirus insertions in the *Fis-1* region do not induce *Int-2* mRNA.

Inbred mouse strains differ with respect to the size of the *Hin*cII restriction fragment, which hybridizes to a *Fis-1* region probe, and with respect to the size of the *Pst*I restriction fragment, which hybridizes to an *Int-2* region probe (Fig. 1A; Table 1). These restriction fragment length polymorphisms were used to monitor the inheritance of alleles at *Fis-1* and *Int-2* in the offspring of doubly heterozygous mice. No recombinants were found between *Fis-1* and *Int-2* in 31 progeny of a (C57BL/10J × NFS/N)F_1 mouse backcrossed to C57BL/10J, indicating that these loci are fairly closely linked (16 backcross mice inherited both the *Fis-1*^{a} and *Int-2*^{b} alleles from the NFS/N parent, and 15 mice inherited neither of these NFS/N alleles). To pursue this linkage, we examined a series of recombinant inbred (RI) strains derived from mouse strains which differed at *Fis-1* and *Int-2*.

Because there are multiple chances for recombination to occur during the breeding of RI strains, analysis of such strains is a powerful way of detecting meiotic recombination between closely linked loci (11). No recombinants were detected between *Fis-1* and *Int-2* in 51 RI strains (18 AKXL strains, 26 BXD strains, and 7 CXB strains [Table 1]). This is equivalent to there being no recombinants in ca. 200 backcross mice (11). These results would be unlikely ($P < 0.05$) if *Fis-1* and *Int-2* were separated by more than 1.6 centimorgans (9) (1 centimorgan corresponds to ca. 1,000 kb). One other locus was found to be linked to *Fis-1* and *Int-2*; this was an endogenous xenotropic retrovirus envelope-related sequence characterized by a 6.6-kb *Pvu*II fragment designated XP-9 (13). This XP-9 fragment segregated in the BXD RI strains, and only one (BXD-19) of 26 strains was recombinant for *Fis-1* and this XP-9 fragment (Table 1). These results indicate that the most likely distance between *Fis-1* and the gene encoding this endogenous retroviral sequence is ca. 1 centimorgan, with 95% confidence limits (9) of 0.03 to 6.96 centimorgans (about 30 to 7,000 kb).

To find whether *Fis-1* and *Int-2* were close to one another on a molecular scale, we cloned 30 kb of genomic DNA from

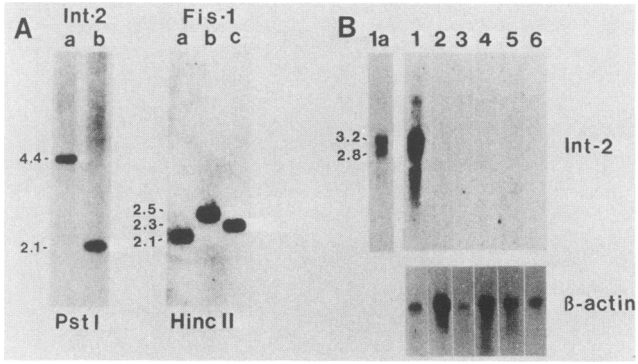

FIG. 1. (A) Restriction fragment length polymorphisms at *Int-2* and *Fis-1*. Left, *Int-2* alleles; right, *Fis-1* alleles. Genomic DNA (10 μg) was digested with *Pst*I or *Hin*cII, electrophoresed in 0.6% agarose, transferred to nitrocellulose, and hybridized to ^{32}P-labeled (5) plasmid subclones Int-2a (4) or p1.8 (10) (*Fis-1*). Fragment sizes are indicated at the left in kilobases. The *Int-2*^{a} allele is carried by C57BL/6, C57BL/10, and C57L mice; the *Int-2*^{b} allele is carried by NFS, AKR, BALB/c, and DBA/2 mice; the *Fis-1*^{a} allele is carried by NFS mice; the *Fis-1*^{b} allele is carried by C57BL/6, C57BL/10, and C57L mice; and the *Fis-1*^{c} allele is carried by AKR, BALB/c, and DBA/2 mice. (B) Lack of expression of *Int-2* mRNA in tumors with F-MuLV proviruses in *Fis-1*. Poly(A)$^+$ RNA (2 to 5 μg) was electrophoresed in formaldehyde agarose gel (7), transferred to nitrocellulose, and hybridized to ^{32}P-labeled int-2f (4) (upper panel) or β-actin (lower panel). Lanes: 1, positive control mammary carcinoma D124, which contains an MMTV insert in *Int-2*; 2, tumor 1707-2; 3, tumor 580-3; 4, tumor 580-1; 5, spontaneous AKR thymic lymphoma; 6, normal AKR thymus. Upper panel, exposure was for 1 week (except lane 1a, for which exposure was 20 h); lower panel, exposure was for 4 h.

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TABLE 1. Linkage of *Fis-1*, *Int-2*, and the locus encoding XP-9[a] in a series of RI strains

Genotype	Strain
AKXL strains	
AKR haplotype (*Fis-1*^{c} *Int-2*^{b})	AKXL-12, AKXL-13, AKXL-25, AKXL-28, AKXL-29, AKXL-38
C57L haplotype (*Fis-1*^{b} *Int-2*^{a})	AKXL-5, AKXL-6, AKXL-7, AKXL-8, AKXL-9, AKXL-14, AKXL-16, AKXL-17, AKXL-19, AKXL-21, AKXL-24, AKXL-37
Recombinant haplotype	None
CXB strains	
BALB/c haplotype (*Fis-1*^{c} *Int-2*^{b})	None
C57BL/6 haplotype (*Fis-1*^{b} *Int-2*^{a})	CXB-D, CXB-E, CXB-G, CXB-H, CXB-I, CXB-J, CXB-K
Recombinant haplotype	None
BXD strains	
C57BL/6 haplotype (*Fis-1*^{b} *Int-2*^{a} XP-9$^-$)	BXD-6, BXD-8, BXD-11, BXD-14, BXD-16, BXD-21, BXD-22, BXD-24, BXD-30
DBA/2 haplotype (*Fis-1*^{c} *Int-2*^{b} XP-9$^+$)	BXD-1, BXD-2, BXD-5, BXD-9, BXD-12, BXD-13, BXD-15, BXD-18, BXD-20, BXD-23, BXD-25, BXD-27, BXD-28, BXD-29, BXD-31, BXD-32
Recombinant haplotype (*Fis-1*^{b} *Int-2*^{a} XP-9$^+$)	BXD-19

[a] AKR and DBA/2 mice have a similar size of xenotropic virus envelope probe-reactive *Pvu*II fragment designated XP-9, but the genes encoding these fragments are known not to be linked (13). The XP-9 fragment described here is that characteristic of DBA/2 mice; data for inheritance of XP-9 in BXD strains are from reference 13, and were confirmed by us for BXD-19.

the *Fis-1* region from a BALB/c myeloma library. The cloned DNA extended about 15 kb to either side of the region of clustered F-MuLV proviral inserts (Fig. 2). The restriction enzyme map of the *Fis-1* region does not overlap with the restriction enzyme map of cloned DNA that extends about 15 kb to either side of the *Int-2* oncogene (4). In addition, no hybridization was detected between lambda clones of the *Fis-1* region and a DNA fragment, int2-f, from the coding region of *Int-2* (4). Therefore, the site of F-MuLV insertions is at least 25 to 30 kb away from *Int-2*. Also, no hybridization was detected between the lambda *Fis-1* clones and cloned DNA from the long terminal repeat-*gag-pol* and *env* regions of endogenous xenotropic-related retroviruses (1).

Close linkage between *Fis-1* and *Int-2* could be explained if F-MuLV proviral insertions at *Fis-1* activated transcription of *Int-2*. However, the *Int-2* coding region probe failed to hybridize to poly(A)$^+$ RNA from three tumors containing F-MuLV proviruses in the *Fis-1* region (Fig. 1B). Thus, proviruses in *Fis-1* do not activate *Int-2* from a distance.

We have sought evidence that proviral insertions in *Fis-1* activate transcription of adjacent genes. To date, we have not detected aberrant virus-cell hybrid mRNAs in tumors with *Fis-1* inserts, nor have we detected poly(A)$^+$ RNA in tumor or normal tissue which hybridizes to a series of unique sequence probes from the *Fis-1* region (Fig. 2). Furthermore, ^{32}P-labeled cDNA made from poly(A)$^+$ RNA from a tumor with a *Fis-1* insert (tumor 1707-2) failed to hybridize to DNA from the lambda *Fis-1* clones (not shown). The latter experiment allowed us to conclude that if tumor 1707-2 contained mRNA from the cloned *Fis-1* region, it was present at less than 1/100th the concentration of F-MuLV mRNA.

The relationship between *Fis-1* and *Int-2* is similar in several respects to the relationship between *pvt* and *myc*. *pvt* is a site of proviral insertion in thymic lymphomas (6). While *pvt* maps to the same chromosomal band on mouse chromosome 15 as *myc* (M. Banerjee, F. Weiner, J. Spira, M. Babonits, M. G. Nilsson, J. Sumegi, and G. Klein, EMBO J., in press), molecular studies show that *pvt* is at least 70 kb from *myc* (3). To date, no *pvt* mRNA has been reported. *pvt-myc* and *Fis-Int* may be dissimilar in that tumors with proviruses in *pvt* were reported to have elevated levels of *myc* mRNA, in contrast to the apparent lack of induction of *Int-2* mRNA by provirus insertions in *Fis-1*. However, there is no direct evidence that the *pvt* proviruses in these tumors are the cause of increased levels of *myc* mRNA. The *pvt* locus is also a site of chromosomal breakage in mouse plasmacytoma variant translocations (3). By analogy, one

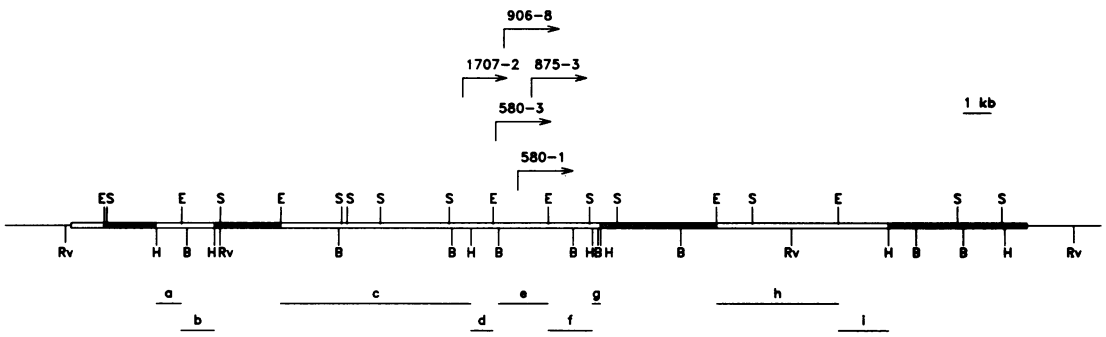

FIG. 2. Restriction enzyme map of *Fis-1* region. Bar region represents DNA from two overlapping lambda Charon 4A clones selected from a BALB/c myeloma library with the *Fis-1* probe p1.8 (10). The single line denotes uncloned genomic DNA whose restriction enzyme sites were determined from genomic Southern blots. Symbols: E, *Eco*RI; H, *Hin*dIII; B, *Bam*HI; S, *Sst*I; Rv, *Eco*RV. Lower lines a through g indicate unique sequence regions subcloned and used as hybridization probes. Cross-hatched segments denote regions which contain repetitive sequences.

might speculate that *Fis-1* or a closely linked gene will be found to be involved in chromosomal translocations. We note that *Fis-1* is very closely linked to *Int-2*, the human homolog of which has been mapped to the same band on human chromosome 11 as *Bcl-1*, a breakpoint in the t(11;14)(q13;q32) translocations characteristic of some human B-cell lymphomas (2, 12).

The relationship between *Fis-1* and *Int-2*, like that between *pvt* and *myc*, is not understood. One intriguing possibility is that the *Fis-Int* and *pvt-myc* regions contain clusters of genes involved in growth control, different members of which may be activated in different tumors by proviral insertion or chromosomal translocation. Another possibility, suggested by the linkage of *Fis-1* to the gene encoding XP-9 in DBA/2 mice, is that this region of mouse chromosome 7 is peculiarly susceptible to proviral insertion for reasons unrelated to tumorigenesis. However, we consider this possibility less likely because (i) the genetic data indicate that the distance between *Fis-1* and the gene encoding the DBA/2 XP-9 fragment could be quite large, making it more probable that their association is coincidental, and (ii) there is no other evidence that proviral insertion sites are clustered except in tumors.

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