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Mouse Chromosomal Mapping of a Murine Leukemia Virus Integration Region (*Mis-1*) First Identified in Rat Thymic Leukemia

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We have previously identified a region of genomic DNA which constitutes the site of frequent provirus integration in rat thymomas induced by Moloney murine leukemia virus (Lemay and Jolicoeur, Proc. Natl. Acad. Sci. USA 81:38–42, 1984). This genetic locus is now designated *Mis-1* (Moloney integration site). Cellular sequences homologous to *Mis-1* are present in mouse DNA. Using a series of hamster-mouse somatic cell hybrids, we mapped the *Mis-1* locus to mouse chromosome 15. Frequent chromosome 15 aberrations have been described in mouse thymomas. *Mis-1* represents a putative new oncogene which might be involved in the initiation or maintenance or both of these neoplasms.

The genome of normal somatic cells contains several genes homologous to known viral oncogenes which appear to be implicated in the development of some neoplastic diseases (for a review see reference 2). Under physiological conditions, these genes are nononcogenic and in fact might be essential to normal cellular or developmental processes or both. In some non-virus-induced tumor cells, the oncogene ras^H has been found to be altered by point mutation (33, 42), whereas in others, transcription of oncogenes has been shown to be activated by chromosome translocation (17, 38, 43) or gene amplification (1, 6, 18, 36, 37). In tumors induced by nondefective retroviruses which do not carry oncogenic sequences, transcriptional activation of cellular sequences such as c-*myc* in avian bursal lymphoma (15, 29) and c-*erb* in erythroleukemia (13) results from promoter insertion or insertion mutagenesis by proviruses. Activation of c-*myc* by provirus integration has also been reported in some rat (41) and mouse (7) T-cell leukemias induced by murine leukemia viruses (MuLVs). We and others have shown that the primary determinant of leukemogenicity and of disease specificity of these MuLVs maps within the U3 long terminal repeat sequences, more specifically within the tandem direct repeats (4, 5, 10, 11, 16, 27) known to be enhancer elements (25, 28). U3 long terminal repeat sequences have also been shown to harbor determinants of oncogenicity in avian retroviruses (35, 44). It appears likely that the proviral U3 long terminal repeat sequences act in *cis* at the chromosomal level to activate nearby cellular oncogenes.

In an effort to identify new cellular oncogenes which might be activated by such a mechanism in MuLV-induced tumors, we initially searched for common integration sites of proviruses in Moloney MuLV-induced rat thymomas. We found one sequence, previously designated RMoInt-1, which appears to be the site of relatively frequent integration of proviruses (26). This sequence is part of a locus, now designated *Mis-1* (Moloney integration site). We found that a rat probe pMo-1C, which was derived from *Mis-1*, hybridized to mouse DNA at high stringency. Recently, proviruses integrated into this locus have been identified in some MuLV-induced mouse thymoma DNAs (manuscript in preparation), indicating that the same region is implicated in this disease in mice. Using somatic cell hybrid DNAs, we mapped the mouse *Mis-1* homolog to chromosome 15.

The pMo-1C fragment corresponds to a 0.7-kilobase-pair (kbp) *Eco*RI-*Sal*I fragment of rat *Mis-1* (Fig. 1). This fragment has been subcloned into pBR322 and is free of reiterated sequences (26). When used as a probe under stringent conditions on mouse DNA cleaved with *Eco*RI, it hybridized with a single 2.2-kbp fragment (Fig. 2, lane g). Under these conditions of hybridization, this probe did not hybridize significantly to E36 Chinese hamster cell DNA (Fig. 2, lane a). To map this sequence to a specific chromosome, we cleaved DNAs from a series of hamster-mouse somatic cell hybrids which had lost different mouse chromosomes with *Eco*RI and analyzed them by the Southern blotting technique with the rat pMo-1C fragment or fragment 4 as a probe. The cell hybrids were generated by fusion of Chinese hamster cells (E36) with peritoneal cells or spleen cells of BALB/c, A/J, and NFS.Akv-2 congenic mice. The characterization of these hybrids and their use in the chromosomal mapping of other cellular genes have been described elsewhere (19–22). A total of 22 hybrid cell DNAs were analyzed by this method. Figure 2 shows a representative example of the results. All hybrid DNAs analyzed could be scored as positive or negative for the presence of the 2.2-kbp *Eco*RI fragment homologous to pMo-1C or fragment 4. The mouse chromosomal content of these hybrids revealed that all clones positive for the presence of *Mis-1* also carried mouse chromosome 15 (Table 1). The hybrid cell DNAs which did not harbor the 22-kbp *Eco*RI *Mis-1* fragment were also negative for chromosome 15. Therefore, these results indicate that the mouse *Mis-1* homolog maps to chromosome 15.

Two other oncogenes (*myc* [9] and *sis* [23]), three loci representing preferred integration sites for Moloney MuLV (*Mlvi-1* [24] and *Mlvi-2* [45]) or mouse mammary tumor virus (*Int-1* [30, 32]), and one locus (*pvt-1*) representing a 6;15 chromosomal breakpoint (8) and a preferred integration site for MuLV (14) have already been mapped on chromosome 15. To determine whether *Mis-1* might represent one of these loci, a molecular clone spanning nearly 20 kbp of rat cellular

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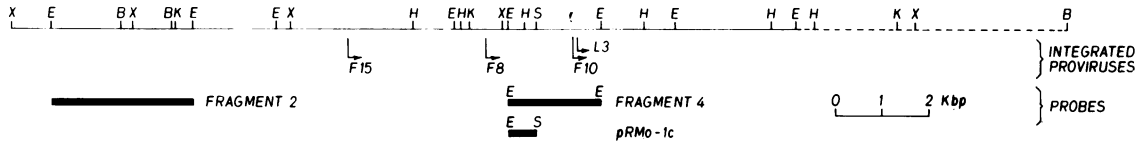

FIG. 1. Restriction endonuclease map of rat *Mis-1*. A partial map previously derived (26) was completed with data obtained from a rat cloned DNA fragment. Closed boxes represent fragments used as probes. Arrows represent integration sites and transcriptional orientations of proviruses previously mapped. Dotted line indicates that not all restriction sites have been mapped in this region. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII, K, *Kpn*I; S, *Sal*I; X, *Xba*I.

DNA surrounding the pMo-1C fragment was isolated and hybridized with a mouse c-*myc* probe or a v-*sis* probe (data not shown). No cross-hybridization was observed indicating that *Mis-1* is distinct from *myc* and *sis*. Also, DNAs (kindly provided by P. Tsichlis) from the *Mlvi-1* and *Mlvi-2* loci, spanning 10 and 22 kbp, respectively, were hybridized with pMo-1C probe and probe 2; no cross-hybridization could be detected (data not shown). Moreover, *Mlvi-1*, *Mlvi-2*, and *Mis-1* have different restriction endonuclease maps. These results indicate that *Mlvi-1*, *Mlvi-2*, and *Mis-1* represent distinct sequences. Comparison of the fragments detected in mouse DNA with pMo-1C (*Bam*HI, 20 kbp; *Hin*dIII, 1.3 and 3.3 kbp; *Pst*I, 8.5 kbp; *Kpn*I, 5.1 kbp.) with the *Int-1* restriction map strongly suggests that these two loci are distinct. Comparison of rat *Mis-1* with mouse *pvt-1* maps revealed discrepancies, and too few restriction endonuclease sites have been located at this point to allow direct comparison. These results suggest that *Mis-1* is a novel locus, representing a putative oncogene, which might be implicated in retrovirus-induced thymic leukemia in rats (26) and mice (in preparation). Whether integration within this region acts in *cis* on *Mis-1* or at a long distance on well-known oncogenes such as c-*myc* or c-*sis* remains unknown. The facts that *Mis-1* rearrangements are associated with T-cell leukemia and that this locus maps on mouse chromosome 15 are interesting in view of the fact that frequent chromosome 15 aberrations are found in mouse thymoma cells. Trisomy 15 has been described in most spontaneous AKR thymomas (12, 47), in 7,12-dimethylbenz[a]anthracene-induced CFW/D and C57BL/Ka thymomas (3, 49), in X-ray-induced C57BL/Ka thymomas (3), and in virus-induced CFW/D thymomas (3, 48). And X;15 and 6;15 translocations have been described in 7,12-dimethylbenz[a]anthracene-induced SJL thymomas (40) and in plasmacytoma variants (8, 31), respectively. A deletion of chromosome 15 has also been observed in few translocation-negative murine plasmacytomas (46, 50). Therefore, there is a likely possibility that *Mis-1*, possibly with other loci, is involved in the initiation or maintenance or both of neoplasms associated with chromosome 15 aberrations.

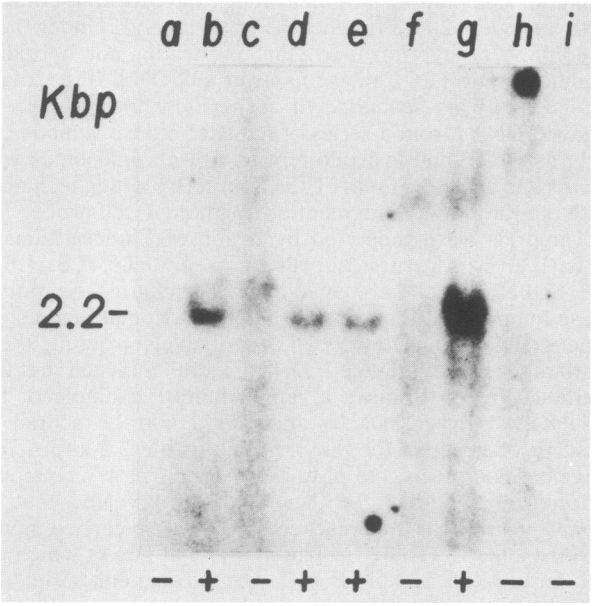

FIG. 2. Hybridization of pMo-1C probe with mouse, Chinese hamster, and hybrid cell DNAs. DNAs (5 μg) were digested with *Eco*RI, run on 1% agarose gel, transferred onto nitrocellulose membrane (39), and hybridized with rat ^{32}P-labeled (34) pMo-1C probe (50% formamide, 3× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], Denhardt solution at 41°C). Filters were washed at 60°C in 2× SSC for 15 min, in 0.1× SSC-0.1% sodium dodecyl sulfate solution for 1 h, and then in 0.1× SSC for 5 min. DNAs were from NIH/Swiss mouse liver (lane g), E36 Chinese hamster cells (a and i), and hybrids HM 20 (b), HM 2 (c), HM 27 (d), HM 30 (e), HM 50 (f),and HM 40 (h).

TABLE 1. Correlation of mouse chromosomes and *Mis-1* in 22 hybrid clones

Mouse chromosome	No. of hybrid clones with *Mis-1*/chromosome retention				% Discordant
	+/+	−/−	+/−	−/+	
1	7	2	9	0	50[a]
2	8	2	7	0	41
3	4	2	8	0	57
4	6	2	11	0	61
5	1	1	14	1	88
6	5	2	8	0	53
7	15	2	4	0	19
8	3	2	12	0	71
9	5	2	13	0	65
10	3	2	15	0	75
11	0	2	15	0	88
12	9	1	5	1	38
13	4	2	8	0	57
14	4	1	13	1	74
15	15	2	0	0	0
16	7	2	7	0	44
17	9	2	6	0	35
18	6	2	9	0	53
19	4	2	10	0	63
X	3	0	9	2	79

[a] Seven hybrids contained *Mis-1* and chromosome 1 (+/+); two hybrids lacked *Mis-1* and chromosome 1 (−/−); nine hybrids contained only *Mis-1* (+/−). Mouse chromosomes were identified in 15 hybrids by Giemsa-trypsin banding followed by staining with Hoechst 33258; 6 hybrids were typed only for the presence of specific mouse markers.

We thank Phil Tsichlis for sending blots of *Mlvi-1* and *Mlvi-2* DNA clones. We are grateful to Stuart Aaronson and Keith Robbins for sending a v-*sis* clone and to Michael Cole for providing a c-*myc* clone.

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ADDENDUM IN PROOF

By molecular hybridization and by direct comparison of cloned DNA from the murine homolog of *Mis-1* with *pvt-1* DNA (8), we recently found that *Mis-1* and *pvt-1* represent the same locus (L. Villeneuve, E. Rassart, P. Jolicoeur, M. Graham, and J. M. Adams, submitted for publication).

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