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Cellular DNA Region Involved in Induction of Thymic Lymphomas (*Mlvi-2*) Maps to Mouse Chromosome 15

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Two cellular DNA regions representing common domains for proviral DNA integration (*Mlvi-1* and *Mlvi-2*) have been identified in Moloney murine leukemia virus-induced rat thymic lymphomas. Cellular sequences which were free of repeated DNA derived from a clone that defines the *Mlvi-2* integration domain (λCl228) were found to be highly conserved in a variety of vertebrate species that we examined, including mice, hamsters, cats, and humans. In this study, we identified the chromosomal map location of the *Mlvi-2* homologous sequences in mice by using hamster-mouse somatic cell hybrids. The results show that *Mlvi-2* is present on mouse chromosome 15 but is unrelated to the c-*myc* and c-*sis* proto-oncogenes, which map on the same chromosome. Since aberrations on chromosome 15 have been observed reproducibly in mouse thymomas, our data suggest that *Mlvi-2* may define a novel sequence involved in the induction or progression of murine thymic lymphomas.

Two cellular DNA regions, designated *Mlvi-1* and *Mlvi-2*, were originally identified in rats as common domains for proviral DNA integration in Moloney murine leukemia virus (MoMuLV)-induced thymic lymphomas (29, 30). Rearrangement of the DNA sequences in the *Mlvi-2* integration domain was observed in 10 of 16 independently derived thymomas. In the majority of these cases, the rearrangements are due to provirus integration in this cellular DNA domain (P. N. Tsichlis, P. G. Strauss, and L.-F. Hu, submitted for publication). Provirus DNA integration within common DNA substrates, in the MoMuLV-induced rat thymomas and other retrovirus-induced tumors, probably contributes to oncogenesis. This is suggested both from the study of rat thymomas (29, 30) and from the strong evidence of *c-myc* involvement in the induction or progression of avian leukosis virus-induced avian bursal lymphomas (5, 14, 16).

Rats and mice are closely related mammals with substantial regions of chromosomal homology (21, 22). In addition, mice as well as rats are susceptible to MoMuLV-induced leukemogenesis (12), and mice have long been used as a model mammalian system in the study of viral leukemogenesis. Because of genetic similarities between rats and mice and because of similarities between MoMuLV-induced rat thymic lymphomas and murine thymomas, we have initiated studies of the role of *Mlvi-1* and *Mlvi-2* provirus integration domains in the induction or progression of murine thymomas. We began these studies by examining the chromosomal localization of these regions in the mouse genome.

We report here that sequences which are homologous to *Mlvi-2* map to mouse chromosome 15. This is particularly interesting since aberrations of chromosome 15 are reproducibly observed in murine thymic lymphomas (2, 25, 32). This suggests that *Mlvi-2* may contain sequences which play a role either in the induction or in the progression of these tumors.

The *Mlvi-2* provirus integration domain in rats contains sequences that represent single-copy elements that are highly conserved among species. Two *Hin*dIII fragments from rat clone λCl228 that are free of repeated sequences were subcloned in pBR322 (pTS6 and pTS10), ^{32}P labeled by nick translation (18), and hybridized against mouse and hamster DNA digested with *Sac*I, *Eco*RI, *Kpn*I, *Bam*HI, *Hin*dIII, *Hpa*I, and *Pst*I. The results (data not shown) indicate that the *Mlvi-2* sequences are conserved and that they are represented by a single copy in both mouse and hamster DNA. In addition, *Kpn*I digestion generates different-sized restriction fragments that hybridize to both probes (pTS6 and pTS10) in mouse and hamster DNA. A restriction map of λCl228 indicating the origin of the pTS6 and pTS10 probes is shown in Fig. 1C. We took advantage of the different sizes of fragments generated by *Kpn*I digestion of

TABLE 1. Correlation of mouse chromosomes and *Mlvi-2* in 19 hybrid clones

Chromosome	No. of hybrid clones with *Mlvi-2*/chromosome retention:				% Discordant
	+/+[a]	−/−	+/−	−/+	
1	6	4	7	2	47
2	7	5	6	1	37
3	5	6	8	0	42
4	5	6	8	0	42
5	1	6	12	0	63
6	4	4	9	2	58
7	10	4	3	2	26
8	2	6	11	0	58
9	4	6	9	0	47
10	2	6	11	0	58
11	0	6	13	0	68
12	8	5	5	1	31
13	5	5	8	1	47
14	3	5	10	1	58
15	13	6	0	0	0
16	5	6	8	0	42
17	7	2	6	4	53
18	6	4	7	2	47
19	6	5	7	1	42
X	7	4	6	2	42

[a] +/+, Containing *Mlvi-2* and the indicated chromosome; −/−, lacking *Mlvi-2* and the indicated chromosome; +/−, containing *Mlvi-2* and lacking the indicated chromosome; −/+, lacking *Mlvi-2* and containing the indicated chromosome. Mouse chromosomes were identified by Giemsa-trypsin banding followed by staining with Hoechst 33258 (7).

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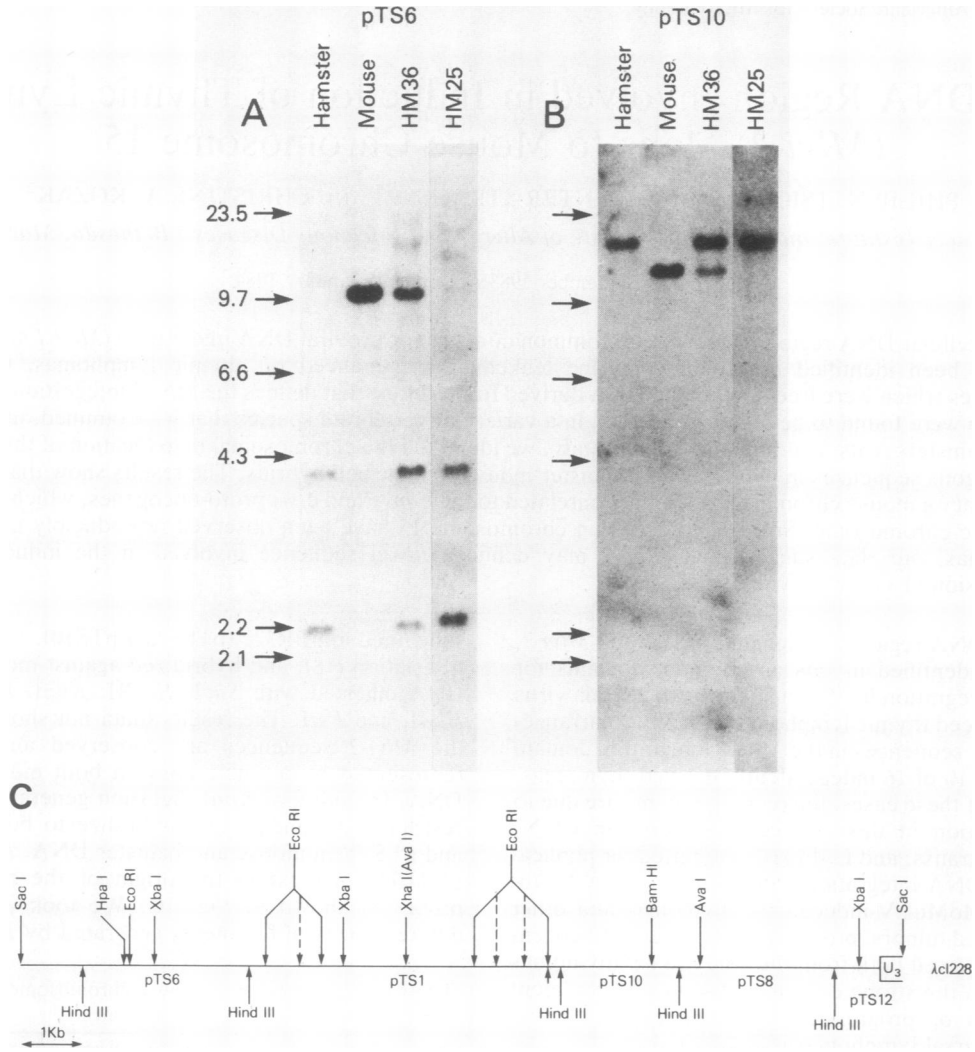

FIG. 1. Detection of λCl228 sequences in the cellular DNA of Chinese hamster and mouse cell lines and hybrid cell lines HM36 and HM25. In a Southern blot analysis, *Kpn*I-digested cellular DNA was probed with (A) pTS6 and (B) pTS10. Molecular sizes (in kilobases) are indicated. (C) Restriction map of λCl228 showing the origin of *Hin*dIII subclones pTS6 and pTS10.

mouse and hamster DNA to map chromosomally the *Mlvi-2* homolog in mice by using hamster-mouse somatic cell hybrid lines that lose mouse chromosomes (8–10). Mouse, hamster, and hybrid cell DNA from 19 independent lines was digested with *Kpn*I, transferred onto nitrocellulose filters (24), and hybridized separately to both the pTS6 and pTS10 probes. Some of the results showing the mouse and hamster cell lines (one mouse *Mlvi-2*-positive and one mouse *Mlvi-2*-negative cell line) are shown in Fig. 1. Figure 1A shows hybridization to the pTS6 probe, and Fig. 1B shows hybridization to the pTS10 probe. The patterns of hybridization observed with both probes were identical. Both probes could clearly identify the cell lines that contained the mouse *Mlvi-2* sequence. Comparison of these results with the mouse chromosome content of the 19 independent hybrid cell lines used in this experiment showed that the murine homolog of the rat *Mlvi-2* provirus integration domain maps to mouse chromosome 15 (Table 1). Although most of these hybrids contain only a few mouse chromosomes, 13 of the 19 hybrids contained the murine *Mlvi-2* sequence. This high proportion of *Mlvi-2*-positive lines is consistent with the observation that hamster-mouse hybrids preferentially retain mouse chromosome 15 (3). Most of the other chromosomes showed random segregation patterns, and all showed discordant segregation with *Mlvi-2*. Thus, all hybrids carrying *Mlvi-2* contained chromosome 15, and the six hybrids missing the *Mlvi-2*-reactive fragment also lacked chromosome 15. One positive hybrid contained only chromosomes 1, 3, and 4 in addition to 15, and another positive hybrid contained chromosomes 7, 9, 12, and 15.

These data demonstrate that *Mlvi-2* is present on mouse chromosome 15, which also contains the c-*myc* (6) and c-*sis* (11) proto-oncogenes. Therefore, we questioned the possibility that *Mlvi-2* may be related to c-*myc* or c-*sis*. Sequences in the rat *Mlvi-2* region showed no homology to either of these two proto-oncogenes. Furthermore, our analyses of the hybrid cell lines also suggest that *Mlvi-2* is distinct from both c-*myc* and c-*sis*. The same 19 somatic cell hybrid lines were examined with a probe derived from the 3′ exon of human c-*myc* kindly provided by D. Watson. We identified one *Mlvi-2*-negative hybrid clone (HM25) which contained *myc* sequences (Fig. 2). This cell line lacks a karyotypically identifiable chromosome 15 and retains only mouse chromosome 17. The presence of mouse *myc* sequences in this line

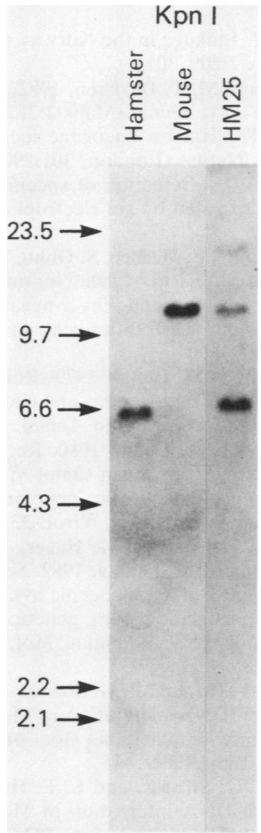

FIG. 2. *Mlvi-2*-negative hybrid clone HM25 contains c-*myc* sequences. The same blot shown in Fig. 1 was probed with c-*myc*. Molecular sizes (in kilobases) are indicated.

indicates that some portion of the noncentromeric end of chromosome 15 is also present in this hybrid and that this chromosomal region does not contain *Mlvi-2*. Analysis of these same somatic cell hybrids suggests that mouse c-*sis* sequences, like c-*myc*, are present in hybrid HM25; therefore, they map at the distal end of chromosome 15 (11). Thus, the separation of *Mlvi-2* from c-*myc* and c-*sis* in hybrid HM25 indicates that *Mlvi-2* is distinct from these two known oncogenes and that *Mlvi-2* maps proximal to c-*sis* and c-*myc*.

During replication, the retrovirus genomic RNA undergoes reverse transcription to form a double-stranded DNA intermediate, the DNA provirus. The provirus DNA integrates into the cellular genome by a process that is site specific with regard to the viral sequences but that appears to be random with regard to the cellular DNA substrate (31). However, although the integration process appears to be random in mass-infected nontumor cells, clonal retrovirus-induced tumors contain a provirus integrated with high frequency within specific cellular DNA domains (4, 5, 13–17. 29, 30). Strong evidence suggests that provirus integration within common cellular DNA regions in retrovirus-induced tumors is a contributing factor in oncogenesis.

In the case of the avian leukosis virus-induced avian bursal lymphomas, the DNA provirus integrates in the domain of c-*myc*, a known oncogene. This provirus integration event is probably responsible for tumor induction since it enhances the transcription of c-*myc*, and this transcriptional enhancement is mediated by control elements within the U_3 region of the provirus long terminal repeat (5, 13, 16), which carries the sole determinant of the viral oncogenic potential (19, 20, 26–28). A number of laboratories have recently demonstrated that c-*myc* may be involved in the induction of several nonvirus-induced malignancies, including mouse plasmacytomas and human Burkitts lymphomas (6). These tumors were shown to carry DNA rearrangements that involve the c-*myc* proto-oncogene, and these rearrangements were found to be the result of tumor-specific chromosomal translocations (6). These results suggest that the development of tumor-specific chromosomal aberrations, reproducibly shown in a variety of tumors including the murine thymic lymphomas, may be a general mechanism in tumor induction or progression (23) or both. The present study was undertaken because *Mlvi-2* represents a common domain for provirus DNA integration in a retrovirus-induced malignancy similarly to c-*myc*, and the murine thymic lymphomas show very distinct chromosomal aberrations. The most common abnormality observed is trisomy of chromosome 15 (2, 32), although Spira et al. (25) have also identified a 15;X translation in 7,12-dimethylbenzanthracene-induced Thy-1-positive tumors in SJL mice. The breakpoint that generates these SJL translocations is very specific and maps to chromosome 15 proximal to c-*myc*. The specificity of the 15;X translocation breakpoint suggests that sequences at this position may be involved in the induction or progression of murine thymomas or both. Although we have not yet determined the exact location of *Mlvi-2* on chromosome 15, our data suggest that, like the breakpoint which generates the SJL translocations, *Mlvi-2* maps proximal to c-*myc*. The involvement of the *Mlvi-2* provirus integration domain in rat thymic oncogenesis and the chromosomal map location of the murine homolog of this DNA region suggest a likely role for *Mlvi-2* in the development of mouse thymomas. Further characterization of the DNA sequences in this region may have broad implications in the study of murine neoplasms.

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