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Genetic Mapping of a Cellular DNA Region Involved in Induction of Thymic Lymphomas (*Mlvi-1*) to Mouse Chromosome 15

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***Mlvi-1* defines a genetic locus representing a common domain for proviral DNA integration in Moloney murine leukemia virus-induced rat thymic lymphomas. Cellular sequences homologous to *Mlvi-1* are present in mouse DNA, and we have used hamster-mouse somatic cell hybrids to chromosomally map *Mlvi-1* in the mouse genome. Results demonstrated that *Mlvi-1* maps to mouse chromosome 15 and that it is distinct from the *Mlvi-2* integration region and from the cellular oncogenes c-*myc* and c-*sis*, which also map to this chromosome. Therefore, *Mlvi-1* may contain novel sequences involved in the establishment and maintenance of virus-induced murine tumors, many of which contain abnormalitites of chromosome 15.**

The DNA of normal somatic cells contains various transforming genes or oncogenes that have been implicated in the induction or progression of neoplastic diseases. Such oncogenes are evolutionarily conserved among species and presumably play some important role in normal cellular or develomental processes (2, 10). Many lines of evidence have now shown that tumorigenesis is associated with the alteration or transcriptional activation of these oncogenes by point mutation, chromosomal translocation, or gene amplification (1, 20, 23–25, 29). Alternatively, oncogene activation and neoplastic disease can also follow the chromosomal integration of a nonacute transforming retrovirus near these potentially transforming host genes. The major retroviral sequences necessary for transformation in avian leukosis virus have been localized to the U3 region of retroviral long terminal repeats (LTRs) (21, 22, 31–33). Recent studies with murine nontransforming retroviruses also argue for the importance of the proviral LTR sequences in determining oncogenicity (3, 6, 15). These viral sequences can alter the transcriptional activity of nearby cellular oncogenes since LTRs contain promoter sequences as well as *cis*-active enhancers (14, 30; L. A. Laimins, P. Tsichlis, and G. Khoury, Nucleic Acids Res., in press). Tumor induction by this type of insertional mutagenesis was first demonstrated in chickens, in which it was shown that the majority of bursal lymphomas induced by avian leukosis virus contain proviral integrations near the c-*myc* oncogene (9, 16, 18).

In both rats and mice, the Moloney murine leukemia virus (MoMuLV) induces thymic lymphomas after a long latency period. We have identified and molecularly cloned three cellular regions termed *Mlvi-1*, *Mlvi-2*, and *Mlvi-3* which represent common domains for proviral integration in virus-induced thymic lymphomas in rats (34, 35; P. Tsichlis, unpublished data). Cellular sequences in *Mlvi-1* were rearranged in 7 of 16 tumors examined, whereas *Mlvi-3* was rearranged in 3 of 16 tumors. Of the seven tumors carrying rearrangements in *Mlvi-1*, six were also found to have a rearranged *Mlvi-2* locus with both events present in the same population of tumor cells (P. N. Tsichlis, P. G. Strauss, and M. Lohse, manuscript in preparation). However, the data suggest that the three *Mlvi* domains clearly represent distinct chromosomal regions. Molecular clones of *Mlvi-1*, *Mlvi-2*, and *Mlvi-3* have different restriction maps and show no homology to one another by hybridization (34; P. Tsichlis, unpublished data). Furthermore, analysis of rat-mouse somatic cell hybrids indicates that the *Mlvi-1*, *Mlvi-2*, and *Mlvi-3* genetic loci are present on different rat chromosomes (P. Tsichlis, M. Lohse, C. Szpierer, and G. Levan, unpublished data). Since MoMuLV does not itself contain a transforming gene, it is likely that *Mlvi-1*, *Mlvi-2*, and *Mlvi-3* define distinct cellular sequences involved in the induction of thymic lymphomas in the rat.

The mouse genome contains sequences that are homologous to all three of these provirus integration domains. We have now been able to map the mouse homologs of *Mlvi-1* and *Mlvi-2* to specific chromosomes by using somatic cell hybrids. We have previously described the chromosomal mapping of the mouse *Mlvi-2* homolog to chromosome 15 (36). We now report that the mouse *Mlvi-1* homolog also maps to chromosome 15. Furthermore, our data suggest that these two loci are in different regions of this chromosome.

The *Mlvi-1* region was originally cloned as a provirus-host junction fragment from *Sac*I-digested DNA from an MoMuLV-induced rat thymoma (34). This clone, λC1C$_3$1A, contained proviral LTR sequences and approximately 9 kilobases (kb) of cellular sequences (Fig. 1). A *Sac*I-to-*Eco*RI fragment was subsequently subcloned into pBR322. The *Pvu*II-to-*Pvu*II fragment of this subclone, pTS26 P/P, was free of viral LTR and repetitive cellular sequences and was used to probe for homologous sequences in mouse, hamster, and hybrid cell DNAs.

DNAs from Chinese hamster E36 cells and BALB/c mouse liver were digested with *Sst*I, *Eco*RI, *Kpn*I, *Hin*dIII, *Hpa*I, or *Pst*I. These DNAs were electrophoresed on agarose gels, blotted onto nitrocellulose filters (26), and hybridized against pTS26 P/P DNA as described elsewhere (35). Although the mouse DNA produced distinct single bands with each of the enzymes, sequences homologous to *Mlvi-1* could not be seen in hamster DNA under these hybridization conditions as shown for *Eco*RI-digested DNAs in Fig. 2.

DNAs from various hamster-mouse somatic cell hybrids were analyzed for mouse *Mlvi-1* specific sequences after digestion with *Kpn*I or *Eco*RI (Fig. 2). These enzymes generated *Mlvi-1* specific fragments of 20 kb (*Kpn*I) and 10

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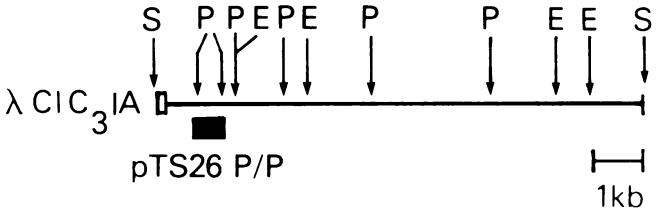

FIG. 1. Restriction endonuclease map of λC1C$_3$1A, a *Sac*I clone of a provirus-host junction DNA function from the MoMuLV-induced rat thymoma C$_3$. Restriction endonuclease cleavage sites are as follows: S, *Sac*I; E, *Eco*RI; and P, *Pvu*II. The *Sac*I site at the 5′ end cleaves within the MoMuLV LTR sequences. The open box at the 5′ end of the clone represents the remaining LTR sequences (U5 and part of U$_3$). The shaded bar between the two *Pvu*II sites at the 5′ end of the clone, pTS26 P/P, represents a single copy element in both the rat and mouse genomes and was used as a hybridization probe in these experiments.

kb (*Eco*RI) in the mouse controls. A total of 16 hybrid lines were analyzed after digestion with both enzymes, and 14 of the 16 hybrids contained *Mlvi-1* sequences. Analysis of the mouse chromosome content of the 16 hybrids showed a correlation between the presence of mouse chromosome 15 and the DNA fragments containing the *Mlvi-1* homolog (Table 1). One apparent discrepancy was observed with chromosome 15. Hybrid line HM25 contained the murine *Mlvi-1* homolog, but mouse chromosome 15 was not identified in this cell line by karyotypic analysis. However, HM25 DNA contained mouse c-*myc* sequences, indicating that this hybrid line contains at least a fragment of the distal end of chromosome 15 (13). Thus, it can be concluded that the murine *Mlvi-1* homolog is present on chromosome 15 and probably maps to the distal end of this chromosome.

In a previous study, we demonstrated that *Mlvi-2*, a second common domain for provirus integration in MoMuLV-induced rat thymic lymphomas, also maps to mouse chromosome 15 (36). However, in contrast to *Mlvi-1*, *Mlvi-2* could not be detected in hybrid HM25, suggesting that *Mlvi-2* maps to the centromeric end of chromosome 15. Thus, the separation of *Mlvi-1* and *Mlvi-2* in a hybrid apparently carrying a chromosome 15 translocation confirms that the two domains define distinct chromosomal regions in mice.

The genetic mapping of *Mlvi-1* to chromosome 15 raised the possibility that this region of integration could contain the cellular oncogenes c-*myc* or c-*sis*. Both of these oncogenes have been mapped to chromosome 15 (5, 13, 19), and several recent studies have suggested that aberrations involving c-*myc* are involved in some rat and mouse viral leukemias (4, 28). Our studies of rat thymomas also suggest that rearrangements c-*myc* may coexist in the same tumors with rearrangements in *Mlvi-1* (P. N. Tsichlis, P. G. Strauss, and M. Lohse, manuscript in preparation). However, *Mlvi-1* is distinct from the *Myc* and *Sis* genetic loci since *Mlvi-1* (λC1C$_3$1A) does not cross-hybridize with molecular clones of v-*sis* (8, 11), human c-*myc* (37) (data not shown), or rat-*myc* (P. Tsichlis, unpublished observation). Furthermore, approximately 30 kb of rat cellular DNA sequences surrounding the sites of provirus integration in *Mlvi-1* have been cloned. These sequences do not overlap with approximately 12 kb of cloned rat cell DNA surrounding the rat c-*myc* homolog. In mice, no overlap could be detected in the restriction maps of the cellular sequences containing *Mlvi-1*,

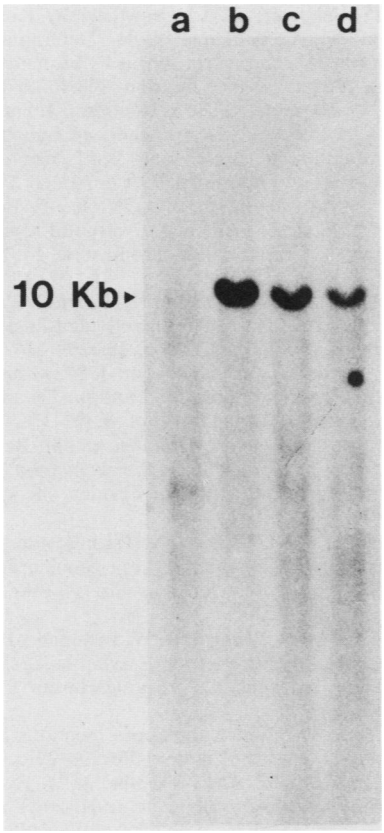

FIG. 2. Hybridization of λC1C$_3$1A with Chinese hamster, mouse, and hybrid cell DNAs digested with *Eco*RI. Lanes a, Chinese hamster; b, BALB/c mouse liver; c, hybrid HM36; d, hybrid HM35.

TABLE 1. Correlation of mouse chromosomes and *Mlvi-1* in 16 hybrid clones

Chromosome	No. of hybrid clones (*Mlvi-1*/chromosome retention)[a]				% Discordant
	+/+	−/−	+/−	−/+	
1	6	2	7	0	47
2	5	2	8	0	53
3	4	2	8	0	57
4	4	2	9	0	64
5	0	2	13	0	87
6	4	2	9	0	60
7	10	1	4	1	31
8	1	2	12	0	80
9	3	2	10	0	67
10	1	2	12	0	80
11	0	2	13	0	87
12	8	1	5	1	40
13	4	2	8	0	57
14	2	2	11	0	73
15	12	2	1	0	7
16	5	2	8	0	53
17	8	1	6	1	44
18	5	2	8	0	53
19	4	2	9	0	60
X	3	1	9	1	64

[a] The first line of data would be read as six hybrids contain *Mlvi-1* and chromosome 1 (+/+); two hybrids lack *Mlvi-1* and chromosome 1 (−/−); seven hybrids contain only *Mlvi-1* (+/−). Mouse chromosomes were identified in 14 hybrids by Giemsa-trypsin banding, followed by staining with Hoechst 33258 (12); four hybrids were typed only for the presence of specific mouse isozyme markers.

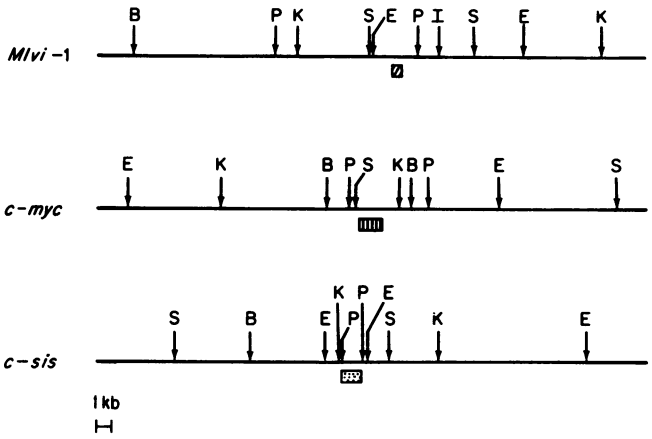

FIG. 3. Restriction endonuclease maps of the murine *Mlvi-1*, c-*myc* and c-*sis* sequences. The maps were generated by single and double restriction endonuclease digestion of mouse NIH 3T3 cell DNA and hybridization to the probes represented by bars under each restriction map. Symbols: , *Mlvi-1* probe pTS26 P/P; , probe derived from the 5′ exon of human c-*myc*; , probe derived from the 3′ exon of human c-*sis*.

c-*myc*, and c-*sis* (Fig. 3). These data suggest that *Mlvi-1* represents a novel cellular sequence distinct from the known cellular oncogenes on this chromosome.

These and other studies on virus-associated leukemogenesis indicate that virus integration into specific chromosomal regions is an important factor in oncogenesis and that sequences on mouse chromosome 15 are probably involved in this process (4, 17, 19, 28). The clustering of at least three genes on chromosome 15 which are associated with thymic lymphomas (*Mlvi-1*, *Mlvi-2*, and *Myc*) and a locus associated with mammary carcinomas (*Int-1*) (17, 19) is particularly intriguing since aberrations of this chromosome are commonly found in murine thymic lymphomas and include trisomy 15 in most AKR thymomas (7, 38) and an X;15 translocation in DMBA-induced SJL thymomas (27). We suggest that *Mlvi-1* and *Mlvi-2* represent unique chromosomal regions which play a role in some of the naturally occurring neoplasms associated with chromosome 15 aberrations.

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LITERATURE CITED

1. **Alitalo, K., M. Schwab, C. C. Lin, H. E. Varmus, J. M. Bishop, and D. George.** 1983. Homogeneously staining chromosomal regions contain amplified copies of an abundantly expressed cellular oncogene (c-*myc*) in malignant neuroendocrine cells from a human colon carcinoma. Proc. Natl. Acad. Sci. U.S.A. **80**:1701–1711.
2. **Bishop, J. M.** 1983. Cellular oncogenes and retroviruses. Annu. Rev. Biochem. **52**:301–354.
3. **Chatis, P. A., C. A. Holland, J. W. Hartley, W. P. Rowe, and N. Hopkins.** 1983. Role for the 3′ end of the genome in determining disease specificity of Friend and Moloney murine leukemia viruses. Proc. Natl. Acad. Sci. U.S.A. **80**:4408–4411.
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. **Crews, S., R. Barth, L. Hood, J. Prehn, and K. Calame.** 1982. Mouse c-*myc* oncogene is located on chromosome 15 and translocated to chromosome 12 in plasmacytomas. Science **218**:1319–1321.
6. **DesGroseillers, L., E. Rassart, and P. Jolicoeur.** 1983. Thymotropism of murine leukemia virus is conferred by its long terminal repeat. Proc. Natl. Acad. Sci. U.S.A. **80**:4203–4207.
7. **Dofuku, R., J. L. Biedler, B. A. Sprengler, and L. J. Old.** 1975. Trisomy of chromosome 15 in spontaneous leukemia of AKR mice. Proc. Natl. Acad. Sci. U.S.A. **72**:1515–1517.
8. **Gelman, E. P., F. Wong-Staal, R. A. Kramer, and R. C. Gallo.** 1981. Molecular cloning and comparative analyses of the genomes of simian sarcoma virus and its associated helper virus. Proc. Natl. Acad. Sci. U.S.A. **78**:3373–3377.
9. **Hayward, W. S., B. G. Neel, and S. M. Astrin.** 1981. Activation of a cellular oncogene by promoter insertion in ALV-induced lymphoid leukosis. Nature (London) **290**:475–480.
10. **Heldin, C. H., and B. Westermark.** 1984. Growth factors; mechanism of action and relation to oncogenes. Cell **37**:9–20.
11. **Josephs, S. F., R. Dalla Favera, E. P. Gelman, R. C. Gallo, and F. Wong-Staal.** 1983. 5′ viral and human cellular sequences corresponding to the transforming gene of simian sarcoma virus. Science **219**:503–505.
12. **Kozak, C. A., J. B. Lawrence, and F. H. Ruddle.** 1977. A sequential staining technique for the chromosomal analysis of interspecific mouse/hamster and mouse/human somatic cell hybrids. Exp. Cell Res. **105**:109–117.
13. **Kozak, C. A., J. F. Sears, and M. D. Hoggan.** 1983. Genetic mapping of the mouse proto-oncogene c-*sis* to chromosome 15. Science **221**:867–869.
14. **Laimins, L. A., G. Khoury, C. Gorman, B. Howard, and P. Gruss.** 1982. Host-specific activation of transcription by tandem repeats from simian virus 40 and Moloney murine sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **79**:6453–6457.
15. **Lenz, Y., D. Celander, R. L. Crowther, R. Patazca, D. W. Perkins, and W. A. Hazeltine.** 1984. Determination of the leukemogenicity of a murine retrovirus by sequences within the long terminal repeat. Nature (London) **308**:467–470.
16. **Neel, B. G., W. S. Hayward, H. L. 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.
17. **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.
18. **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.
19. **Peters, G., C. Kozak, and C. Dickson.** 1984. Mouse mammary tumor virus integration regions *int-1* and *int-2* map on different mouse chromosomes. Mol. Cell. Biol. **4**:375–378.
20. **Reddy, E. P., R. K. Reynolds, E. Santos, and M. Barbacid.** 1982. A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder oncogene. Nature (London) **300**:149–152.
21. **Robinson, H. L., B. M. Blais, P. N. Tsichlis, and J. M. Coffin.** 1982. At least two regions of the viral genome determine the oncogenic potential of avian leukosis viruses. Proc. Natl. Acad. Sci. U.S.A. **79**:1225–1229.
22. **Robinson, H. L., M. N. Pearson, D. W. DeSimone, P. N. Tsichlis, and J. M. Coffin.** 1979. Subgroup-E avian-leukosis-virus-associated disease in chickens. Cold Spring Harbor Symp. Quant. Biol. **44**:1133–1142.
23. **Rowley, J. D.** 1983. Human oncogene locations and chromosome aberrations. Nature (London) **301**:290–291.
24. **Schwab, M., K. Alitalo, H. E. Varmus, J. M. Bishop, and D. George.** 1982. A cellular oncogene (c-Kis-*ras*) is amplified, overexpressed, and localized within karyotypic abnormalities in mouse adrenocortical tumor cells. Nature (London) **303**:497–452.
25. **Shen-Ong, G. L. C., E. J. Keath, S. P. Piccoli, and M. D. Cole.** 1982. Novel *myc* oncogene RNA from abortive immunoglobu-

lin-gene recombination in mouse plasmacytomas. Cell **31**:443–452.
26. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **38**:503–517.
27. **Spira, J., M. Babonits, F. Wiener, S. Ohno, S. Wizschunbski, N. Haran-Ghera, and G. Klein.** 1980. Nonrandom chromosomal changes in Thy-1-positive and Thy-1-negative lymphomas induced by 7,12-dimethylbenzanthracene in SJL mice. Cancer Res. **40**:2609–2612.
28. **Steffen, D.** 1984. Proviruses are adjacent to c-*myc* in some murine leukemia virus-induced lymphomas. Proc. Natl. Acad. Sci. U.S.A. **81**:2097–2101.
29. **Tabin, C. J., S. M. Bradley, C. I. Bargman, R. A. Weinberg, A. G. Papageorge, E. M. Scolnick, P. R. Dhar, D. R. Lowy, and E. H. Chang.** 1982. Mechanism of activation of a human oncogene. Nature (London) **300**:143–149.
30. **Temin, H. M.** 1981. Structure, variation and synthesis of retrovirus long terminal repeat. Cell **27**:1–3.
31. **Tsichlis, P. N., and H. M. Coffin.** 1979. Role of the c region in relative growth rates of endogenous and exogenous avian oncoviruses. Cold Spring Harbor Symp. Quant. Biol. **44**:1123–1132.
32. **Tsichlis, P. N., and J. M. Coffin.** 1980. Recombinants between endogenous avian tumor viruses: role of the c region and other portions of the genome in the control of replication and transformation. J. Virol. **33**:238–249.
33. **Tsichlis, P. N., L. Donehower, G. Hager, N. Zeller, R. Malavarca, S. Astrin, and A. M. Skalka.** 1982. Sequence comparison in the crossover region of an oncogenic avian retrovirus recombinant and its nononcogenic parent: genetic regions that control growth rate and oncogenic potential. Mol. Cell. Biol. **2**:1331–1338.
34. **Tsichlis, P. N., L. F. Hu, and P. G. Strauss.** 1983. Two common regions for proviral DNA integration in MoMuLV induced rat thymic lymphomas. Implications for oncogenesis. UCLA Symp. Mol. Cell. Biol. New Ser. **9**:399–415.
35. **Tsichlis, P. N., P. G. Strauss, and L. F. Hu.** 1983. A common region for proviral DNA integration in MoMuLV-induced rat thymic lymphomas. Nature (London) **302**:445–449.
36. **Tsichlis, P. N., P. G. Strauss, and C. A. Kozak.** 1984. A cellular region involved in the induction of thymic lymphomas (*Mlvi-2*) maps to mouse chromosome 15. Mol. Cell. Biol. **4**:997–1000.
37. **Watson, D. K., M. C. Psallidopoulos, K. P. Samuel, R. Dalla Favera, and T. S. Papas.** 1983. Nucleotide sequence analysis of human c-*myc* locus, chicken homologue, and myelocytomatosis virus MC29 transforming gene reveals a highly conserved gene product. Proc. Natl. Acad. Sci. U.S.A. **80**:3642–3645.
38. **Wiener, F., S. Ohno, J. Spira, N. Haran-Ghera, and G. Klein.** 1978. Cytogenetic mapping of the trisomic segment of chromosome 15 in murine T-cell leukemias. Nature (London) **275**:658–660.