JOURNAL OF VIROLOGY, May 1989, p. 2366–2369
0022-538X/89/052366-04$02.00/0
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Vol. 63, No. 5

The *Mlvi-1* Locus Involved in the Induction of Rat T-Cell Lymphomas and the *pvt-1/Mis-1* Locus Are Identical

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Received 17 November 1988/Accepted 27 January 1989

Mlvi-1 defines a locus of proviral integration in rat thymomas induced by Moloney murine leukemia virus. pvt-1/Mis-1 represents an independently identified locus which becomes rearranged either by chromosomal translocation in murine plasmacytomas or by provirus insertion in retrovirus-induced murine and rat thymic lymphomas. Although it had been claimed that pvt-1/Mis-1 and Mlvi-1 represent two different loci, we present here evidence showing that they are identical. This finding demonstrates the need for rigorous characterization of any newly identified common regions of integration in retrovirus-induced neoplasms.

The main factor in oncogenesis by nontransforming retroviruses is the induction of insertion mutations mediated by the integration of the provirus into the cellular genome (22). Two lines of evidence led to this conclusion. It was shown that the provirus may integrate in the domain of cellular proto-oncogenes and alter their expression. In addition, it was shown that provirus integration in the genome of virus-induced tumor cells could occur in regions of integration which were common among tumors. In both cases, the integrated proviruses appear to activate neighboring cellular genes by mechanisms such as promoter insertion, enhancer insertion, and truncation of the normal gene (2, 14, 22).

The importance of provirus integration in the induction of retrovirus-induced rodent hematopoietic tumors was demonstrated first in MoMuLV-induced thymic lymphomas in rats (19). These studies revealed that in these tumors the MoMuLV provirus integrates reproducibly in a common region of integration which was named *Mlvi-1* (Moloney leukemia virus integration locus 1) (19, 21). The effects of provirus integration in the *Mlvi-1* locus have been elusive until very recently. We now know that provirus insertion in this locus, which maps at least 300 kilobases 3′ to the *myc* proto-oncogene, affects the expression of this gene probably in *cis* (P. A. Lazo and P. N. Tsichlis, submitted for publication). In addition, the same integrated provirus is associated with the expression of an RNA message which contains sequences located immediately upstream of the cluster of the *Mlvi-1* proviruses (P. N. Tsichlis and P. A. Lazo, submitted for publication). Following the description of *Mlvi-1*, at least 15 additional common regions of integration were identified in retrovirus-induced murine and rat hematopoietic tumors (3–5, 11, 13, 15, 18, 21, 23, 24, 26, 27). The gene(s) that may be deregulated because of provirus integration in most of these loci has not been identified yet. This introduces a major difficulty in determining the relationship between new and previously discovered common loci of integration. This difficulty is compounded by the fact that there are no established rules of identity for such loci.

In this report, we present data showing that *Mlvi-1* was rediscovered twice, and we suggest certain rules that may prevent this problem in the future.

The description of *Mlvi-1* was followed by the discovery of the *Mis-1* locus, which was rearranged because of provirus integration in MoMuLV-induced rat thymic lymphomas (11). This, in turn, was followed by the discovery of the *pvt-1* locus, which was rearranged in murine plasmacytomas carrying the 6;15 plasmacytoma variable translocation (4) and in virus-induced AKR thymomas (5). Although *Mlvi-1*, *Mis-1*,

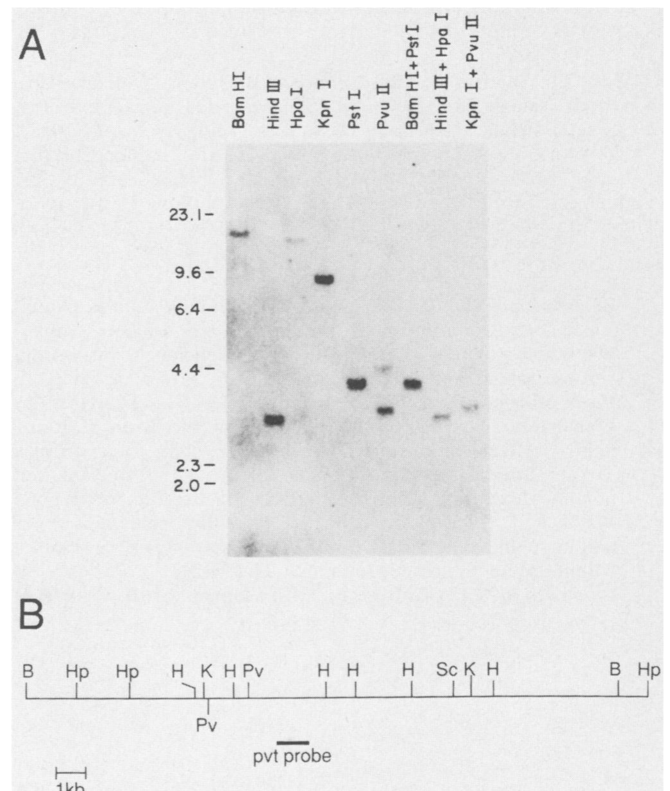

FIG. 1. (A) Hybridization of mouse probe E from the *pvt-1* locus to genomic DNA from rat thymoma 2774. Sizes are in kilobases. (B) Restriction map of the rat *Mlvi-1* locus. *Bam*HI (B), *Hpa*I (Hp), *Hin*dIII (H), *Kpn*I (K), *Pvu*II (Pv), *Sac*I (Sc).

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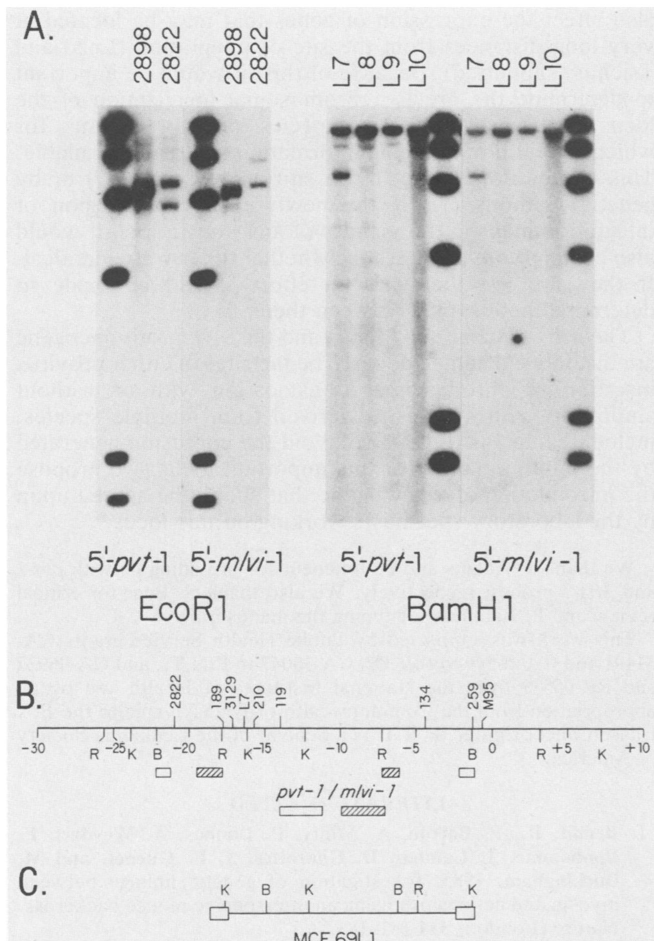

FIG. 2. (A) Southern blot analysis of tumor DNA digested with the indicated restriction enzyme and hybridized to the indicated probe. (B) Restriction map of the *pvt-1*/*Mlvi-1* locus showing the proviral insertions and using endonucleases *Eco*RI (R), *Bam*HI (B), and *Kpn*I (K). The probes are indicated as boxes under their corresponding positions in the maps. (C) Restriction map of MCF69L1 proviral DNA.

and *pvt-1* were all mapped to murine chromosome 15 (4, 8, 9), it was claimed that they were different from each other because either the clones representing them did not cross-hybridize or their restriction endonuclease maps were different. Shortly later, it was reported that *Mis-1* and *pvt-1* were identical. It was claimed again, however, that both were different from *Mlvi-1* (25).

During studies on the induction of the rat thymic lymphomas, we identified a new common region of integration (*Mlvi-4*) which, similar to *Mlvi-1*, *Mis-1*/*pvt-1*, and *myc*, was also mapped to murine chromosome 15 (J. Lee et al., manuscript in preparation). This clustering of provirus insertions led us to examine the relative map position of these loci in the rat genome. In the course of these studies, *pvt-1* probe E (4) was hybridized to normal rat genomic DNA digested with multiple restriction endonucleases in an effort to generate a restriction map of the rat *Mis-1*/*pvt-1* locus. To our surprise, the sizes of the detected rat *pvt-1* restriction fragments were identical to those of comparable *Mlvi-1* fragments as suggested by comparison with the *Mlvi-1* restriction map (Fig. 1). The tentative conclusion that the *Mis-1*/*pvt-1* locus was identical to the *Mlvi-1* locus was confirmed with the following experiments. Genomic DNA from AKR thymic lymphomas induced by a mink cell focus-forming virus (15–17) was digested with *Eco*RI or *Bam*HI and, after agarose gel electrophoresis and transfer to nitrocellulose, it was hybridized to two *Mlvi-1* probes and two *pvt-1* probes (Fig. 2B). Hybridization was done at 42°C in 4× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–50% formamide–15 mM sodium citrate (pH 7.0)–1× Denhardt solution–500 μg of salmon testis DNA per ml–10% dextran sulfate–1% sodium dodecyl sulfate. Filters were washed twice with 0.1% SSC–1% sodium dodecyl sulfate at room temperature and then twice at either 55°C for the *pvt-1* probes or 42°C for the *Mlvi-1* probes. A representative sample of the results is shown in Fig. 2A, while Fig. 2B shows a compilation of the data in diagrammatic form. The sizes of the germ line *Mlvi-1* and *pvt-1* restriction endonuclease fragments detected with these probes were identical. Furthermore, with these probes it was shown that all the tumors carrying a rearrangement of the *Mlvi-1* locus also carried a rearrangement of the *pvt-1* locus and that the *Mlvi-1* and *pvt-1* rearranged bands were identical in size. The only exception was tumor 2822, in which the sum of the sizes of the *Mlvi-1* and *pvt-1* rearranged bands equaled the size of the germ line band plus 8.8 kilobases (the size of the MCF provirus) (Fig. 2C), indicating that the provirus had integrated in the region between the two probes.

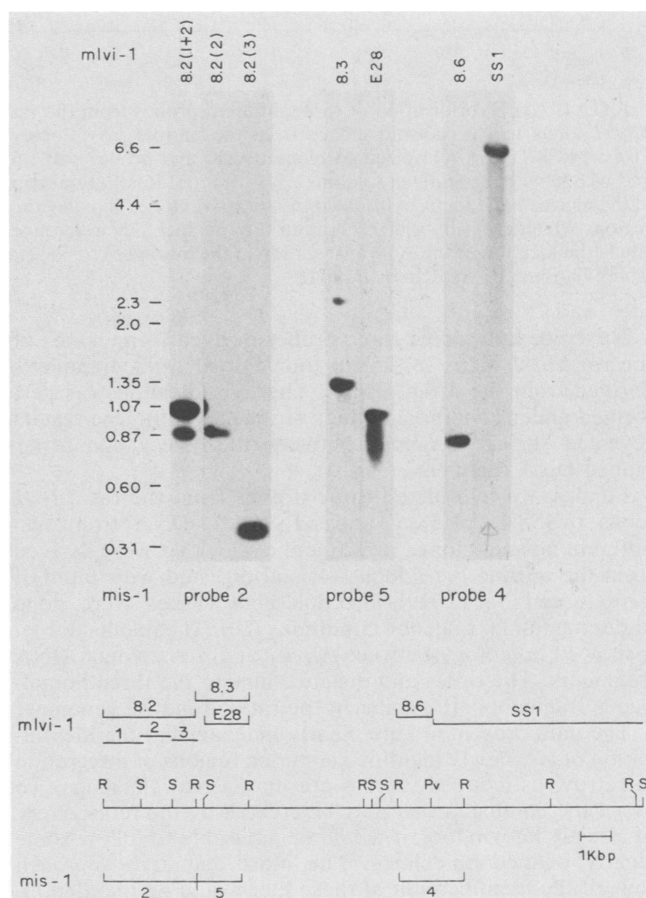

FIG. 3. Hybridization of specific restriction fragments from the rat *Mis-1* locus to fragments from the rat *Mlvi-1* locus. The locations of the fragments from *Mis-1* and *Mlvi-1* are indicated in reference to their common restriction map at the bottom of the figure.

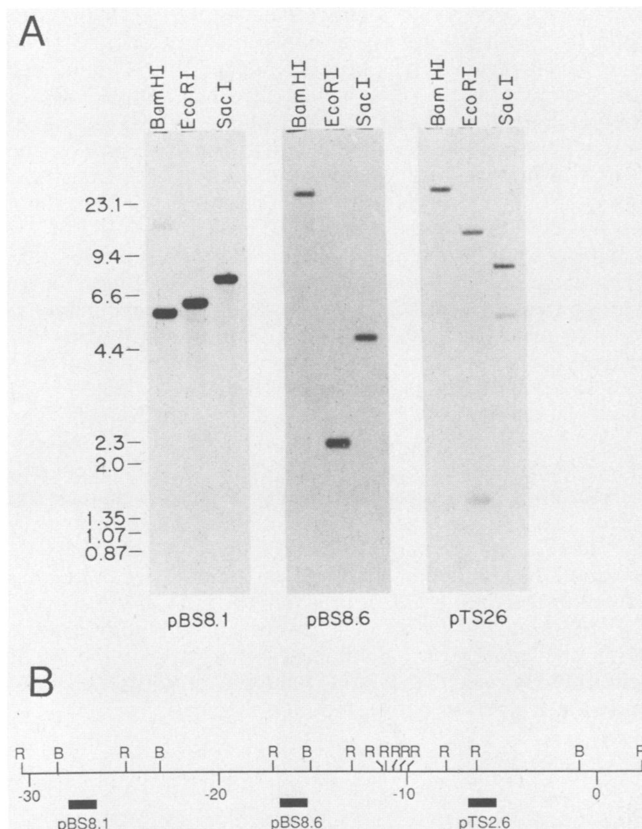

FIG. 4. (A) Hybridization of three different probes from the rat *Mlvi-1* locus to two cosmid clones from the murine *pvt-1* locus. Probe pBS8.1 was hybridized to clone cW28, and probes pBS8.6 and pTS26 were hybridized to clone cW17 (2). (B) Restriction map of the murine *pvt-1* locus hybridized to rat *Mlvi*-1 clones showing the regions which cross-hybridize between the two loci. The reference site is the location of the A4 translocation in the murine *pvt-1* locus. Abbreviations: R, *Eco*RI; B, *Bam*HI.

Next, we hybridized three probes derived from clones of the rat *Mis-1* locus (8, 25) to four cloned DNA fragments derived from the *Mlvi-1* locus. The hybridization was performed under conditions of high stringency (10). The results revealed strong homology between the *Mlvi-1* and *Mis-1* cloned DNA fragments (Fig. 3).

Finally, we hybridized three probes from the rat *Mlvi-1* locus (pBS8.1, pBS8.6, and pTS26) to DNA from two different cosmid clones (cW28 and cW17) that were derived from the murine *pvt-1* locus (4) and digested with *Bam*HI, *Eco*RI, and *Sac*I. Hybridization and washes were done under medium stringency conditions (20). The results in Fig. 4 showed homology between *Mlvi-1* and *pvt-1* cloned DNA fragments. The order and distance among the three homologous fragments are similar in the rat and mouse genomes.

The data presented here clearly indicate that the identification of any newly identified common regions of integration in retrovirus-induced tumors presents a real challenge. To avoid the confusion that may be created by the rediscovery of already known loci, it would be advisable to follow some clearly defined guidelines. The most definitive approach toward the identification of these loci would be the identification and characterization of the gene(s) whose expression may be affected by the integrated provirus. However, this may be a difficult task which may take a long and serious effort to be realized. Furthermore, provirus integration may also affect the expression of genes that may be located at very long distances from the site of integration (Lazo and Tsichlis, submitted). Because of this, it would be important to determine the precise chromosomal localization of the locus in at least one of two species (mice or humans) for which a wealth of mapping information is already available. This can be done either by in situ hybridization (7) or by genetic methods (1). If the newly discovered region of integration maps in the vicinity of any known loci, it would also be important to determine whether the two are identical. If they are not identical, an effort should be made to determine the distance between them.

The *Mlvi-1*/*Mis-1*/*pvt-1* locus and the *myc* proto-oncogene are the only elements known to be the sites of either provirus insertion or chromosomal translocation with or without amplification in neoplasms derived from multiple species, including humans (6, 12). To avoid the confusion generated by the multiple names of this important locus, we propose the introduction of a new name that should be agreed upon by the laboratory specialists working on this locus.

We thank J. Adams and P. Jolicoeur for providing us with *pvt-1* and *Mis-1* probes, respectively. We also thank S. Bear for critical review and P. Bateman for typing the manuscript.

This work was supported by Public Health Service grants CA-31491 and CA-16599 to P.V.O., CA-38047 to P.N.T., and CA-06927 and RR-05539 from the National Institutes of Health and by an appropriation from the Commonwealth of Pennsylvania to the Fox Chase Cancer Center. P.N.T. is a Scholar of the Leukemia Society of America.

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