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Proviral Integration Site *Mis-1* in Rat Thymomas Corresponds to the *pvt-1* Translocation Breakpoint in Murine Plasmacytomas

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Two loci independently implicated in T- and B-lymphocyte neoplasia are shown to be equivalent. The *Mis-1* locus is a common proviral integration site in retrovirally induced rat T lymphomas, while the *pvt-1* locus on murine chromosome 15 frequently translocates to the κ locus in plasmacytomas bearing 6;15 translocations. By comparing cloned sequences, we show that *pvt-1* is the murine homolog of *Mis-1*.

Cellular oncogenes can be activated by mechanisms as disparate as chromosome translocation and the nearby integration of retroviral DNA (for a review, see reference 27). Both mechanisms are exemplified by the c-*myc* oncogene, which is activated by translocation to an immunoglobulin locus in many murine and human B-lymphoid tumors (for reviews, see references 10, 11, and 16) or by retroviral insertion in avian bursal lymphomas (8) and some murine (1, 13, 19) and rat (20) T lymphomas. Such findings have stimulated the search for new oncogenes by the identification of common proviral integration sites (3, 12, 14, 17, 21, 22) and translocation breakpoints (2, 5, 24, 28) in tumors. An intriguing convergence of these approaches has emerged from independent studies in our laboratories.

The locus we have designated *Mis-1* (Moloney integration site) was isolated as a common proviral integration site in rat T lymphomas induced by Moloney murine leukemia virus (12). On the other hand, the *pvt-1* locus on chromosome 15 was identified by its frequent translocation to the C_κ locus in murine plasmacytomas bearing variant (6; 15) translocations (2, 28). The detection of several proviral integrations within *pvt-1* in murine T lymphomas (7) and the observation that the murine homolog of *Mis-1* also maps to chromosome 15 (9) encouraged us to examine the relationship between these two loci. We report here that *Mis-1* is equivalent to *pvt-1*. The identification of this locus in different species by two independent approaches underlines its significance for both B- and T-lymphocyte neoplasia.

The rat clone MO-1 was isolated from a bacteriophage library prepared from a partial *Eco*RI digest of Lewis rat liver DNA cloned in Charon 4A as described previously (12). Rat probe 4 was used for hybridization at high stringency. Filters were hybridized at 41°C in 50% formamide–3× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–Denhardt solution and then washed at 60°C in 2× SSC for 15 min, in 0.1× SSC–0.1% sodium dodecyl sulfate for 1 h, and in 0.1× SSC for 5 min before autoradiography. The clone thus obtained was characterized by restriction analysis as described previously (12). The mouse clone M1 was isolated from a library prepared from high-molecular-weight DNA extracted from Ti-8, a cell line from an X-ray-induced thymoma of a C57BL/6 mouse (18). Ti-8 DNA partially digested with *Sau*3A was cloned into *Bam*HI-digested lambda 47.1 arms as described previously (12). A total of 750,000 phage plaques were screened by using rat probe 4 at low stringency. Filters were hybridized at 41°C in 30% formamide–3× SSC–Denhardt solution and then washed at 55°C in 2× SSC–0.1% sodium dodecyl sulfate for 1 h and in 2× SSC for 5 min at 65°C before autoradiography. Two positive clones were obtained, and clone M1 was further characterized by restriction analysis.

The previously defined murine *pvt-1* locus is depicted in Fig. 1A, and the rat *Mis-1* locus (clone MO-1) is shown at the bottom of Fig. 1B. The fact that *Mis-1* is related to *pvt-1* became apparent when unique-sequence rat probes 2 and 4 of *Mis-1* (Fig. 1B) were found to hybridize at high stringency to *Eco*RI fragments from the *pvt-1* cosmids cW.28 and cA4.5; the hybridizing fragments lie between −27 and −12 kilobases (kb) on the *pvt-1* map (data not shown). To facilitate the comparison, DNA from clone M1 (Fig. 1B), the murine homolog of *Mis-1*, was used. A direct comparison of M1 with cA4.5 shows that they share a single *Kpn*I and eight small *Eco*RI fragments, and unique-sequence rat probes 3, 4, 6, and 7 hybridize to the same fragments in the two murine clones (Fig. 2). These results establish that M1 maps within the *pvt-1* locus, spanning the region from −20 to −5 kb (Fig. 1B). Furthermore, the unique-sequence mouse probes a and b hybridize to the expected single, or contiguous, *Eco*RI fragments in rat genomic DNA (Fig. 3), indicating that the rat *Mis-1* and mouse *pvt-1* regions are equivalent, and not merely closely related, regions.

The alignment and relative orientation of the rat and mouse clones were confirmed by showing that various probes from the region −23 to −10.5 kb of *pvt-1* hybridized to the expected *Eco*RI fragments of the rat clone MO-1 (data not shown). These results position the MO-1 rat sequences on the *pvt-1* map (Fig. 1B) and indicate that sequences within this region are colinear and extensively conserved between the mouse and the rat.

To date, proviral insertions within the *pvt-1*/*Mis-1* locus have been detected in 6 of 33 murine T lymphomas (7) and 11 of 20 rat thymomas (12). In the mouse, four proviral inserts and five translocation breakpoints lie within an 8-kb region

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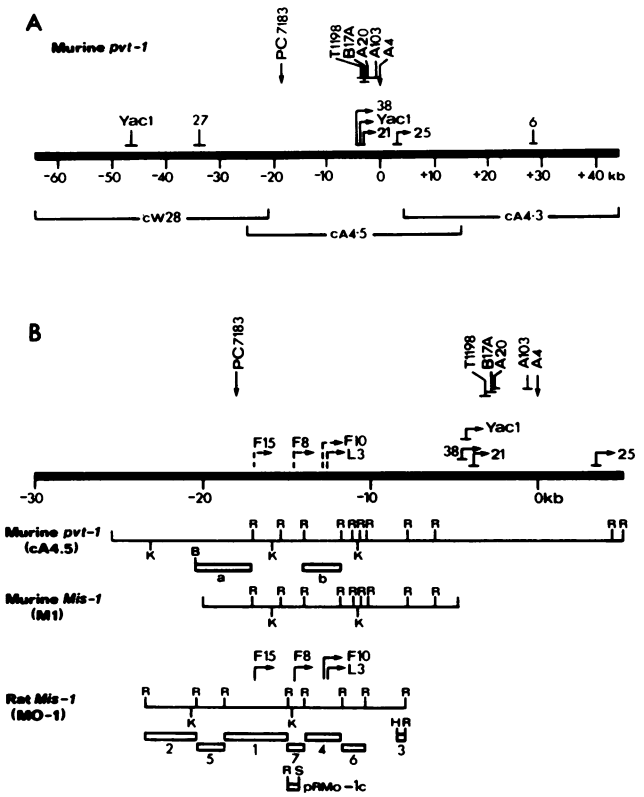

FIG. 1. The murine *pvt-1* locus and its relationship to the rat *Mis-1* locus. (A) Cloned 108-kb region across the murine *pvt-1* locus, showing chromosome 15 breakpoints in six plasmacytomas (2) (top) and proviral insertions in six T lymphomas (7); arrows indicate the probable transcriptional orientation, when known. The isolation and characterization of this region are described in references 2, 7, and 28. The positions of three cosmid clones are shown below the bar. (B) Expanded map of the *pvt-1* locus. The positions and partial restriction maps of the murine clones cA4.5 and M1 are shown below the bar. A restriction map of the rat MO-1 clone is also shown. Its indicated position and orientation with respect to the murine clones was inferred from hybridization data by using unique-sequence rat probes (Fig. 2) and converse experiments with murine probes (see text). The numbered bars indicate the various rat probes; bars a and b indicate mouse probes. The position of the pRMo-1C fragment, previously cloned (12), is illustrated. The position and transcriptional orientation of four proviruses that map within MO-1 are shown (12). The broken arrows above the top bar indicate their inferred position and orientation relative to the murine *pvt-1* map. Abbreviations for restriction endonucleases are as follows: R, *Eco*RI; K, *Kpn*I; H, *Hin*dIII; S, *Sal*I; B, *Bam*HI.

(7), but alignment of the mouse and rat clones places the four rat inserts mapped to date within another cluster some 8 to 13 kb further to the left (Fig. 1B). Interestingly, the rat inserts fall fairly close to the chromosome 15 breakpoint associated with a complex (6;12;15) rearrangement (25) in the NZB murine plasmacytoma PC7183 (Fig. 1B). None of 32 AKR murine T lymphomas examined previously (7) has a rearrangement near those detected in the rat, but this is unlikely to reflect a species difference, since some other murine T lymphomas do bear inserts in this region (L. Villeneuve and P. Jolicoeur, unpublished data). It is noteworthy that all eight proviral integrations so far mapped within *pvt-1/Mis-1* appear to have the same orientation (Fig. 1B). In contrast, insertions within *int-1* and *int-2*, two loci implicated in the induction of mammary carcinomas by mouse mammary tumor virus, fall into two oppositely oriented clusters surrounding a central coding domain (4, 26). The alterations mapped within *pvt-1/Mis-1* are remarkably widespread; the two main clusters themselves span ca. 20 kb, and three apparent proviral integrations map much further to the left or right (Fig. 1A), indicating that the *pvt-1* locus may comprise as much as 70 kb.

Other common proviral integration regions identified in T lymphomas do not appear to fall within the *pvt-1/Mis-1* locus. The *pim-1* locus (3), an insertion region found in many murine T lymphomas induced by murine leukemia virus, has a restriction map unrelated to that of *pvt-1*. Two other insertion sites for Moloney murine leukemia virus in the rat (21, 22), *Mlvi-1* and *Mlvi-2*, have murine homologs on chromosome 15 (23), but comparison of the restriction maps of the cloned murine regions (kindly provided by P. Tsichlis) with that of a 108-kb region spanning *pvt-1* (2) reveals no overlap. Moreover, rat *Mis-1* probes 2 and 4 did not hybridize at low stringency to DNA from rat *Mlvi-1* or *Mlvi-2*. Finally, the *Gin-1* proviral insertion locus recently identified in leukaemias induced by Gross passage A murine leukemia virus does not hybridize to *Mis-1/pvt-1* and indeed maps to a different chromosome (R. Villemur, E. Rassart, Y. Monczak, C. Kozak, and P. Jolicour, manuscript in preparation). Hence, in addition to c-*myc* and *pvt-1/Mis-1*, at least four other loci seem to be implicated in T-lymphoma development. Thus it appears that T-cell neoplasia can occur by diverse pathways, in contrast to the almost invariant activation of *erbB* in avian erythroblastosis (6) and the high frequency of c-*myc* activation in avian bursal lymphomas (8).

The mechanism by which *pvt-1/Mis-1* promotes tumor development remains unknown. Since *pvt-1* and c-*myc* map cytogenetically to the same chromosome band (D2/3) (15) and c-*myc* is expressed in tumors with *pvt-1* alterations, one interpretation is that the translocations and proviral integrations induce c-*myc* expression in *cis*, perhaps by a change in chromatin suprastructure (2, 7). However, we have previously shown that c-*myc* and *pvt-1* are separated by at least 72 kb (2) and conceivably may lie much further apart. Such long-range effects of retroviral integration would not be expected, since murine leukemia virus insertion is generally thought to promote tumorigenesis via local effects on gene transcription (27). Hence *pvt-1/Mis-1* may bear a gene that can promote lymphoid transformation directly, although the search for transcripts within the region in which most of the insertions and breakpoints occur has not yet been productive (J. M. Adams and M. Graham, unpublished results). If *Mis-1/pvt-1* bears an oncogene, it probably is not closely related to known oncogenes, since we have detected no low-stringency homology between the entire region from −23 to +5 kb on the *pvt-1* map with 18 oncogenes (Ki-*ras*, Ha-*ras*, N-*ras*, *rel*, *fos*, *fps*, *src*, *erbB*, *erbA*, *fms*, *raf*, *myc*, *mos*, *abl*, *sis*, *fes*, *int-1*, and *myb*), and much of this region was also checked against *pim-1*, p53, *fgr* IL3, B-*lym*, and *yes*.

Whatever the operative molecular mechanism, the involvement of this locus in tumors of two distinct lymphoid lineages, its conservation between the rat and the mouse, and the similarity of rearrangements induced by proviral integration in the two species, strongly argue that *pvt-1/Mis-1* plays an important role in lymphoma development. It seems significant that two markedly different approaches have independently resulted in the identification and isolation of the same locus.

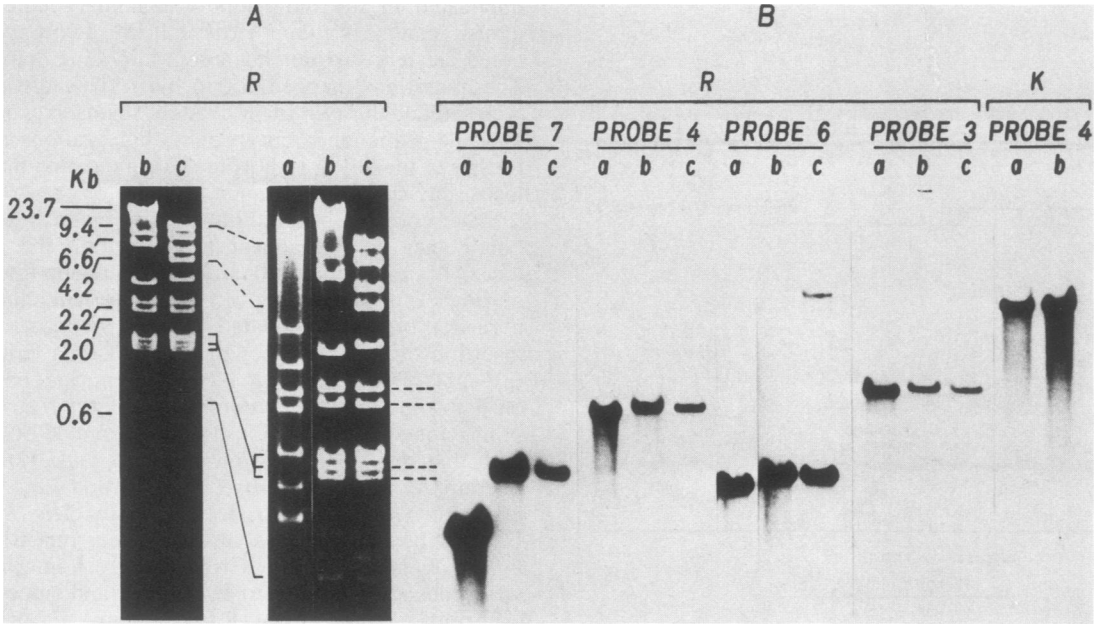

FIG. 2. Agarose gel electrophoresis and Southern blots showing the identity of *Mis-1* and *pvt-1*. (A) Agarose gel electrophoresis of *Eco*RI-digested DNA from rat clone MO-1 (lane a), mouse clone M1 (lane b), and mouse clone cA4.5 (lane c). DNA was run on a 1.5% agarose gel, stained with ethidium bromide, and photographed under UV light. *Hin*dIII-digested λ DNA provided markers. Fragment sizes are in kilobases (Kb). (B) Southern blots showing the identity of *Mis-1* and *pvt-1*. DNAs from rat MO-1 (lane a), mouse M1 (lane b), and mouse cA4.5 (lane c) were digested with *Eco*RI (R) or *Kpn*I (K), transferred to nitrocellulose, and hybridized with ^{32}P-labeled probes 3, 4, 6, and 7 from the rat clone MO-1 (Fig. 1B) at high stringency, as described in the text.

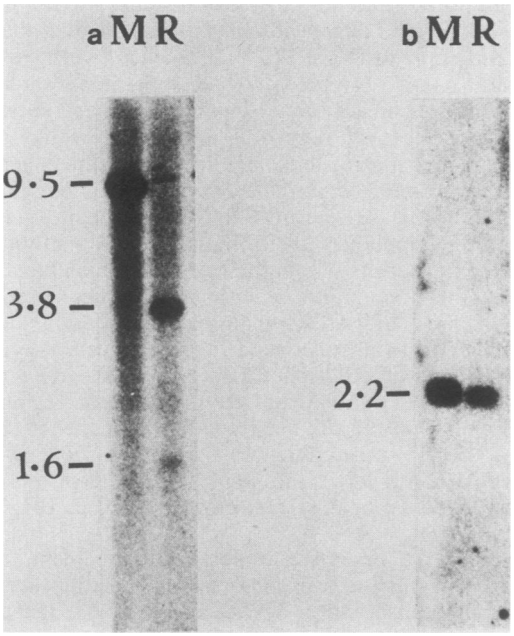

FIG. 3. Genomic Southern analysis, confirming the equivalence of rat *Mis-1* and mouse *pvt-1*. Genomic DNA from BALB/c mouse (M) and Lewis rat (R) was restricted with *Eco*RI, transferred to nitrocellulose, and hybridized as described previously (7) with mouse probes a and b (see Fig. 1B). Filters were washed in 2× SSC at 65°C before autoradiography.

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