The rat *MIS1/Pvt-1* locus is syntenic with *MYC* on chromosome 7

S. Ingvarsson,¹ Z. Wirschubsky,¹ J. Szpirer,² G. Levan,³ G. Klein,¹ and J. Sümegi¹

¹ Department of Tumor Biology, Karolinska Institutet, Stockholm, ² Département de Biologie Moléculaire, Université Libre de Bruxelles, Rhode-St-Genèse, and ³ Department of Genetics, University of Göteborg

Abstract. Mouse *Pvt-1* and rat *MIS1* are frequent proviral integration sites in retrovirally induced lymphomas. The *Pvt-1* locus is also involved in mouse plasmacytoma (6;15) and in the variant Burkitt lymphoma (2;8) translocations. We show that the *Pvt-1/MIS1* locus is syntenic with *MYC* on rat chromosome 7. This is consistent with a postulate of close linkage and, possibly, a functional relationship between the *MYC* protooncogene and the *MIS1/Pvt-1* locus.

Three B-cell derived tumors, human Burkitt lymphoma (BL), murine plasmacytoma (MPC), and rat immunocytoma (RIC) carry consistent chromosomal translocations. In the majority, the *MYC* gene located on human (HSA) chromosome 8; mouse (MMU) 15, and rat (RNO) 7, becomes juxtaposed to the immunoglobulin heavy chain (IGH) locus on chromosomes 14, 12, and 6, respectively. In the variant translocations that occur in about 10% of the BLs and MPCs, the *MYC* gene is juxtaposed to either the kappa or lambda light chain locus-carrying chromosomes. The breakpoints on HSA 8 and MMU 15 in the IGH variant translocations are more than 72 kb away from the *MYC* locus (Cory et al., 1985). The region involved in these translocations has been designated *Pvt-1*. The same region has also been implicated in murine T-cell leukemogenesis, since six of 33 murine T-lymphomas examined had a rearranged *Pvt-1* region, due to retroviral integration in the vicinity (Graham et al., 1985). In independent experiments on Moloney-induced rat thymomas, a common proviral

integration site, designated *MIS1*, was found in 11 of 20 cases (Lemay and Jolicoeur, 1984). It was recently shown that probes derived from the *MIS1* locus hybridized with *Pvt-1* sequences (Villeneuve et al., 1986), and that the homolog of the rat *MIS1* locus mapped to mouse chromosome 15 (Jolicoeur et al., 1985). In view of the absence of variant translocations in the RICs, the question arose as to whether the *Pvt-1* locus involved in variant translocations may have dissociated from *MYC* in the rat. We have therefore determined the location of this locus and have found that it is syntenic with *MYC*.

Materials and methods

Rat × mouse hybrids. High molecular weight DNA was prepared from a panel of somatic cell hybrid clones which segregate rat chromosomes. The hybrids were derived from the fusion of normal rat hepatocytes from Sprague-Dawley (S-D) rats with the mouse hepatoma line BWTG563 (Szpirer et al., 1984). The rat chromosome constitution of the hybrid clones is shown in Table I.

Southern blot analysis and probes used. Twenty micrograms of high molecular weight DNA were digested to completion with restriction endonucleases *EcoRI* or *EcoRV*. The fragments were separated in a 0.8% gel and transferred to a nylon filter. As a size marker, *HindIII*-cleaved lambda phage DNA was used. The rat *MIS1* probe 4 (Villeneuve et al., 1986) was ³²P-labeled by the "oligolabeling" method (Feinberg and Vogelstein, 1984) to a specific activity of 5 × 10⁸ cpm/μg. The probe was used at a concentration of 3 × 10⁶ cpm/ml. The filters were washed in 0.2 × SSC, 0.2% SDS at 65°C and autoradiographed.

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Request reprints from: Dr. S. Ingvarsson, Department of Tumor Biology, Karolinska Institutet, S-10401 Stockholm (Sweden).

Table I. Correlation of the presence of MIS1 with rat chromosomes in rat × mouse somatic cell hybrids

Hybrid	Rat chromosomes																					
	MIS1 ^a	X	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
LB20	+	+	—	+	—	—	—	(—)	+	—	—	—	—	+	+	—	—	+	(+)	+	+	+
LB161	+	+	—	+	+	+	+	+	+	—	+	+	—	(—)	+	+	+	+	+	+	+	(+)
LB210B	—	+	—	—	—	+	—	—	—	—	—	—	—	—	+	+	—	+	—	+	—	—
LB210C	—	+	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	+	—	+	—	—
LB210D	—	+	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	+	—	(—)
LB210I	—	+	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—	+	—	+	—	—
LB251	+	+	+	+	(—)	+	—	(—)	(+)	—	—	+	—	+	+	—	—	—	+	—	+	—
LB330	—	+	—	+	(—)	+	—	+	—	—	—	+	—	+	—	—	—	+	(+)	—	—	—
LB330TG2	—	—	—	—	—	+	—	+	—	—	—	+	—	+	—	—	—	—	—	—	—	—
LB330TG6	—	—	—	+	—	+	—	+	—	—	—	+	—	+	—	—	—	—	—	—	—	—
LB510	+	+	+	+	+	+	(—)	—	+	—	—	—	—	+	+	+	+	+	+	+	—	(—)
LB600	+	+	+	+	+	+	+	(+)	+	—	(—)	+	+	+	+	+	+	+	+	+	+	(—)
LB630	+	+	(—)	—	+	+	(+)	+	+	—	+	—	+	+	+	(+)	+	+	—	+	+	(—)
LB780	+	+	—	+	+	+	+	—	+	—	—	+	+	—	+	—	—	—	+	+	—	(+)
LB810	+	+	—	+	+	+	—	+	+	+	—	+	+	+	+	+	+	+	+	—	+	(+)
LB1040	+	+	—	—	+	+	(—)	+	+	—	—	+	+	+	—	—	+	+	—	+	—	+
Number of discordant clones		5	6	4	2	5	5	7	0	8	7	6	4	5	5	6	3	6	4	6	3	4

^a MIS1 was detected by the 2.1-kb *EcoRI* fragment derived from the rat MIS1 fragment no. 4. Average number of rat chromosomes per cell: +, ≥0.6; (+), 0.3–0.5; (-), 0.1–0.2; -, 0.

Fig. 1. Southern blot analysis of 11 LB hybrid cell clone (lanes 1–11), rat (lane 12), and mouse (lane 13) DNAs digested with *EcoRV* and hybridized to MIS1 fragment 4. The rat-specific germ line band is at 6.5 kb. The mouse-specific band is at 7.3 kb. Lane 1, LB20; lane 2, LB210B; lane 3, LB210C; lane 4, LB210D; lane 5, LB210I; lane 6, LB251; lane 7, LB330; lane 8, LB330TG2; lane 9, LB600; lane 10, LB630; lane 11, LB1040; lane 12, S-D liver; lane 13, Balb/c liver.

Fig. 2. Southern blot analysis of mouse (lane 1), rat (lane 2), and 10 LB hybrid cell clone DNAs digested with *EcoRI* and hybridized to MIS1 fragment 4. The mouse-specific germ line band is at 2.3 kb. The rat-specific germ line band is at 2.1 kb. Lane 1, Balb/c liver; lane 2, S-D liver; lane 3, LB20; lane 4, LB161; lane 5, LB210D; lane 6, LB210I; lane 7, LB330; lane 8, LB330TG2; lane 9, LB330TG6; lane 10, LB510; lane 11, LB780; lane 12, LB810.

Results and discussion

A panel of 12 different rat × mouse hybrid DNAs was digested with *EcoRV* and screened for the presence of the rat-specific MIS1 fragment. The results in Fig. 1 show that the 6.5-kb rat-specific band is present in hybrids LB20, LB251, LB600, LB630, and LB1040. This band is absent from the six other hybrid lines tested. Another panel of 10 different rat × mouse hybrid DNAs was digested with *EcoRI* and screened with the same probe. This probe detected a 2.1-kb rat-specific MIS1 fragment in hybrids LB20, LB161, LB510, LB780, and LB810 (Fig. 2). This band is absent from five other hybrids tested. The greater intensity of hybridization observed in rat liver is due to a higher proportion of rat DNA compared to the cell hybrids and to slight variations in the amount of the DNA loaded onto the agarose gel. Only chromosome 7 showed complete concordance with the hybridization pattern (Table I).

The *Pvt-1* and MIS1 loci have recently been shown to be highly conserved between mouse and rat. Both

serve as frequent proviral integration sites in retrovirus-induced mouse and rat T-cell lymphomas (Villeneuve et al., 1986). The mouse *Pvt-1* locus lies more than 72 kb 3' of *Myc* on chromosome 15 (Cory et al., 1985) and is juxtaposed to the *Igk* locus in plasmacytomas carrying the variant 6;15 translocations. Since the *Myc* gene is expressed in mouse T-lymphomas with proviral integration in *Pvt-1* and in 6;15-carrying MPCs with a *Pvt-1/Igk* juxtaposition, it has been suggested (Graham et al., 1985) that the insertions and translocations induce *Myc* expression via long-range effects. An extensive search for *Pvt-1* transcripts has been negative (Graham and Adams, 1986).

In humans, it has been shown that the chromosome 8 breakpoint region involved in the 2;8 variant translocation in BL is homologous to the *Pvt-1* region on MMU 15 (Graham and Adams, 1986). Also, *Myc* and *Pvt-1* are coamplified in the murine tumor ANN-1, but not in human tumors HL60 and N82E, which only show *MYC* amplification (Graham and Adams, 1986). Since most amplification units are of the order of 100–1,000 kb, these results have led to the suggestion that *Pvt-1* lies 100–500 kb 3' of *MYC* on HSA 8 and MMU 15 (Graham and Adams, 1986).

In contrast to BL and MPC, variant translocations have not been found in 20 cytogenetically analyzed RICs (F. Wiener, personal communication). This is surprising, since *MYC* maps to the same chromosome as *MIS1/Pvt-1* in rat (Sümegei et al., 1983), mouse, and human, and since the 10% variant translocations in BL and MPC seem to have been selected for their biological consequences. The absence of variant translocations in RIC may, however, be due to the relatively small number of rat immunocytomas that have been studied.

It is interesting that another locus for provirus insertion in virally-induced rat and mouse T-cell leukemias, *MLvi-1*, maps to RNO 7 and MMU 15 (Tsichlis et al., 1985), similarly to *Myc* and *MIS1/Pvt-1*. Comparison of restriction endonuclease maps and low stringency hybridizations show that the *MLvi-1* and the *MIS1/Pvt-1* loci are not related (Villeneuve et al., 1986). It is intriguing that MMU 15 and RNO 7 contain several preferred regions for provirus insertion which are conducive for the development of T-lymphomas. The possibility that provirus insertion in *MLvi-1*, similar to the *MIS1/Pvt-1* insertions, may deregulate *Myc* expression has, however, not been excluded.

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