Brief Definitive Report

GENETIC MAPPING OF THE ECOTROPIC VIRUS-INDUCING LOCUS *Akv-2* OF THE AKR MOUSE

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Chromosomal genes for the induction of infectious murine leukemia viruses (MuLV) have now been identified and mapped in several inbred mouse strains. In the AKR mouse, the prototype for high virus, high leukemic mice, two independently segregating loci for ecotropic virus inducibility have been defined (1). One of these, designated *Akv-1*, was mapped to mouse chromosome 7 (2) and has been shown to contain proviral DNA sequences (3).

Genetic mapping studies with other virus-positive strains suggest that there is much heterogeneity in proviral integration sites in mice with high ecotropic virus-inducing loci. Such high virus strains generally have two to four unlinked virus loci (4), and mapping studies have localized a number of these to nonallelic sites in the mouse genome (5; and C. A. Kozak and W. P. Rowe. Unpublished data.). Recent studies with congenic mice carrying single high virus loci suggest that some of this heterogeneity can be attributed to viral reinfection of germ line cells (6). Analysis of the chromosomal distribution of both established as well as newly acquired viral loci should help identify common or preferred regions of integration and clarify the relationship, if any, between these chromosomal sites and different patterns of virus expression.

In this report, we describe the genetic mapping of the second AKR locus for ecotropic virus, *Akv-2*, by a combination of somatic cell hybridization and standard breeding techniques. Previous mendelian crosses using NIH Swiss mice partially congenic for *Akv-2* (NIH.*Akv-2*) showed that this locus was not linked to a number of genes for morphological or biochemical phenotypes (R. Risser and W. P. Rowe. Unpublished data.). In the present study, analysis of interspecific Chinese hamster/NIH.*Akv-2* somatic cell hybrids showed that virus inducibility segregated with the expression of mouse *Sod-1* (superoxide dismutase), an isozyme marker recently mapped to chromosome 16 by somatic cell genetic techniques (7). Mendelian crosses were then used to position *Akv-2* near the *md* locus, which is at the centromeric end of chromosome 16.

Materials and Methods

Two families of congenic mice (Nos. 4–2 and 1–7) carrying the *Akv-2* locus on an NIH Swiss genetic background were used. Both originated from the progeny of the same C57BR × (C57BR × AKR)F_1 backcross as illustrated for family 4–2 in Fig. 1 of ref. 6. Family 1–7 was initiated with a virus-positive female of the same backcross; after an additional backcross to C57BR, the line was carried by mating virus-positive males to NIH Swiss or NFS/N females for 15 generations. The animals used in this study were from the 4th inbred generation. The family 4–2 mice were derived by mating virus-positive males to NIH Swiss females for four

generations followed by inbreeding. Mice used for the cell fusion experiment were of the 12th inbred generation; those used for sexual crosses were the 15th generation of the same subline.

The cells used for somatic cell hybridization were spleen cells and peritoneal macrophages from three 4-wk-old animals of a family 4–2 litter in which early virus expression was suppressed by maternal antibody. No infectious virus was detected in these mouse cell populations; mixed cultures with SC-1 cells (8) showed no virus by the XC test after four weekly passages. The cells were fused with Chinese hamster lung fibroblasts of the line E36, and hybrid clones were isolated in HAT selective medium by methods previously described (9). Hybrid clones were expanded in nonselective medium and subclones were isolated in nonselective medium or medium that contained 8-azaguanine: 33 primary clones and 41 secondary clones derived from 10 primary hybrids were characterized for mouse chromosome content and virus inducibility. Cell extracts of each clone were analyzed for the expression of 13 isozyme markers in starch gels (10). Segregation of an enzyme marker on the mouse X chromosome, hypoxanthine phosphoribosyltransferase, was monitored by growth in selective media. Methods used for karyotypic analysis have been described previously (11). Virus production was induced by treating hybrid cells in subconfluent growth with 20 μg/ml IdU (5-iododeoxyuridine) for 48 h. Ecotropic virus was assayed by the XC test in induced and uninduced hybrid cells after cocultivation with SC-1 cells for 7–10 d.

Mahoganoid mice (C3H/HeJ-md), a linkage testing stock, were supplied by The Jackson Laboratory, Bar Harbor, Maine. The mahoganoid locus, which is recessive for black coat color, has been mapped to the centromeric end of chromosome 16 (12). Backcross animals were assayed for virus inducibility by the XC test on IdU-treated cultures of tail tissue taken at 7–10 d of age (13).

Results

74 somatic cell hybrid clones were examined for spontaneous and IdU-activated induction of *Akv-2*. Spontaneous virus production was rarely detected; only four primary and three secondary hybrids produced XC virus without induction. These seven exceptional clones were excluded from the mapping studies because reinfection with exogenous virus can result in the generation of novel proviral insertions in addition to the original *Akv-2* locus.

Of the 67 clones not spontaneously producing virus, 14 of 29 primary clones and 20 of 38 secondary clones were inducible and thus considered to carry *Akv-2*. XC detectable virus was efficiently induced in all positive hybrids (200–800 plaques/60-mm dish). Of the 14 mouse enzyme markers, only SOD-1 on chromosome 16 cosegregated with virus inducibility in these hybrids; 45 of the 49 primary and secondary clones tested for SOD-1 (91%) coordinately expressed the two phenotypes. No such correlation was observed for any of the 13 other markers tested; the concordant expression of these phenotypes with virus inducibility ranged from 36–81%. Thus, these data indicated that *Akv-2* is linked to *Sod-1* on chromosome 16.

Among the virus-inducible clones, one was identified that expressed only two mouse isozymes, SOD-1 and AK-2 (adenylate kinase-2, chromosome 2). Karyotypic analysis of this hybrid showed that it retained four mouse chromosomes—chromosomes 2, 16, 17, and 19. Serial subcloning resulted in the isolation of a clone that retained only mouse chromosome 16. This clone expressed mouse SOD-1 and was inducible for ecotropic virus production. This confirms the assignment of *Akv-2* to chromosome 16, and further indicates that this chromosome carries the integrated proviral sequences.

The inheritance of *Akv-2* was subsequently examined in sexual crosses to confirm its assignment to chromosome 16 and to provide a more precise map location. Mice of the mahoganoid linkage testing stock were backcrossed to NIH.*Akv-2* animals of

family 4–2. Cultured tail biopsies of the parental mahoganoid animals produced only trace amounts of ecotropic virus after treatment with IdU, as is characteristic of their C3H/HeJ genetic background; this pattern was readily distinguishable from the high virus inducibility characteristic of mice carrying *Akv–2*. 11 of 35 backcross animals tested showed high virus inducibility (> 100 plaques/dish) within 7–10 d after removal of IdU, and there was only one discrepancy between virus inducibility and coat color (Table I). Therefore, the *Akv–2* and *md* loci are closely linked at the centromeric end of chromosome 16.

Because novel high virus-inducing loci have been detected in several sublines of NIH.*Akv* congenics (6), a second *Akv–2* congenic family (No. 1–7) was tested by sexual genetics to confirm that the locus on chromosome 16 was indeed derived from the original AKR cross. Again, there was linkage between virus inducibility and the mahoganoid locus (Table I). However, the linkage estimate was different from that obtained in the family 4–2 cross. Because the majority of recombinants were in the virus-positive category, and because some litter variability was seen, it is possible that family 1–7 mice are heterozygous for a novel proviral reinsertion in addition to *Akv–2*. Consequently, we interpret the results of this cross as confirming that *Akv–2* is on chromosome 16 in AKR mice, but we suggest that only the data from the family 4–2 cross be used to position *Akv–2* on this chromosome.

Discussion

Research in viral leukemogenesis has focused extensively on the high virus, high leukemic AKR strain. These mice carry 3 virus inducibility loci: two for ecotropic virus (*Akv–1*, *Akv–2*) (1, 14) and one for xenotropic virus (*Bxv–1*) (10), as well as several loci that are expressed only as viral antigens (14, 15). Previous studies have mapped 2 of the virus-inducing loci, *Akv–1* and *Bxv–1*, to mouse chromosomes 7 and 1, respectively, and the work described here provides the chromosomal assignment for the remaining locus. This finding should facilitate a comparative analysis of the chromosomal distribution of viral genes carried in other mouse strains and provide the basis for studies on the role of viral gene expression in leukemogenesis.

The assignment of *Akv–2* to chromosome 16 further emphasizes that multiple integration sites for ecotropic virus exist at nonallelic sites in different mouse strains. In addition to *Akv–1* and *Akv–2*, we have mapped three high ecotropic virus inducibility genes to nonallelic positions in the mouse genome: a locus from C3H/FgLw (chromosome 7, at a site distinct from *Akv–2*) (5), one from C58 (chromosome 8), and one from B10.BR (chromosome 11) (C. A. Kozak and W. P. Rowe. Unpublished data.). The single locus for ecotropic virus inducibility in low virus BALB/c mice was

TABLE I

Segregation of Ecotropic MuLV Induction and Mahoganoid (md) Coat Color in the Cross C3H/HeJ-md × (C3H/HeJ-md × NIH.Akv–2)F$_1$

	Virus inducibility	Coat color	Number of animals in backcross from	
			Family 4–2	Family 1–7
Nonrecombinants	+	Black agouti	10	31
	−	Mahoganoid	24	18
Recombinants	+	Mahoganoid	1	8
	−	Black agouti	0	2
Percentage recombinant			$r = 1/35 = 3 \pm 2.9$	$r = 10/59 = 17 \pm 4.9$

recently mapped to chromosome 5 and shown to be allelic with the ecotropic viral locus in a related strain, C3H/HeJ (16, 17). In contrast, loci for xenotropic viruses show less apparent heterogeneity in their chromosomal distribution. Five different strains separated as inbred lines for many years were recently shown to carry the same locus on chromosome 1 (10). Analysis of additional inducibility loci in feral and inbred mice may help provide some perspective on the accumulation, distribution, and stability of proviral sequences in the genome of this species.

Genetic loci for virus inducibility differ in several respects from classic mendelian genes, and these differences must be considered in genetic mapping studies. Most notably, the appearance of novel proviral reintegrations in high virus mice (6) can interfere with the accurate identification and chromosomal mapping of the original viral loci in several ways. First, the segregation of newly acquired viral loci in sexual crosses can alter observed recombinational distances as suggested by our results with family 1-7 mice. More importantly, we were forced to consider the possibility that the chromosome 16 locus isolated in the congenic mice may in fact represent just such a novel reinsertion rather than *Akv-2*. However, the two congenic families used here were derived from different animals in the first backcross from AKR, and both carry the same locus. This makes it highly probable that the locus identified in this report was present in the original AKR parent.

Genetic studies have never identified more than two genes for virus inducibility in AKR mice (1, 14; W. P. Rowe, M. W. Cloyd, and C. A. Kozak. Unpublished data). However, the biochemical analysis of these mice has identified more than two proviral loci in AKR and has also suggested that different viral loci are present in different AKR sublines (18; H. Chan, M. Martin, S. Staal, and W. P. Rowe. In preparation.). Additional studies are needed to resolve these apparent discrepancies and determine whether the *Akv-1* and *Akv-2* loci defined by our studies are identical in all sublines of AKR mice.

Summary

A combination of somatic cell hybridization and standard mendelian breeding techniques was used to map the AKR ecotropic virus inducibility locus *Akv-2* to the centromeric end of chromosome 16. This assignment of *Akv-2* further emphasizes that endogenous ecotropic retroviruses are inserted at multiple sites in mouse chromosomes.

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