

Association of the lethal yellow (A^y) coat color mutation with an ecotropic murine leukemia virus genome

(retroviruses/agouti locus)

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ABSTRACT The dilute (d) coat color mutation on chromosome 9 is closely associated with an ecotropic murine leukemia virus (MuLV) genome [Jenkins, N. A., Copeland, N. G., Taylor, B. A. & Lee, B. K. (1981) *Nature* 293, 370-374]. DBA/2J mice homozygous for the reverse mutation to wild type at the dilute locus (d^{+2J}) lack ecotropic virus-specific sequences, suggesting that the dilute mutation was caused by virus integration. In the experiments described here, we analyzed the ecotropic MuLV DNA content of mice that collectively carry 10 different alleles at the agouti coat color locus (of chromosome 2) to determine whether any of these alleles also are associated with ecotropic virus sequences. Of the 10 alleles analyzed, one allele, lethal yellow (A^y), which is carried congenically and heterozygously on C57BL/6J, 129/Sv, and LT/Sv mice, was closely associated with an ecotropic MuLV provirus. The close association of this provirus with the A^y allele suggests that this mutation also may be caused by virus integration. Furthermore, this association may be useful for molecular cloning and characterizing the many alleles at this locus.

Retroviruses replicate by conversion of their single-stranded RNA genome to a double-stranded DNA copy that is then integrated at random sites in host chromosomes. Structurally, the integrated proviral DNA is similar to both prokaryotic and eukaryotic transposable elements (1-5). Like transposable elements, retroviruses also can act as insertional mutagens by interrupting normal gene expression after viral DNA integration (6) or by activating expression of cellular DNA sequences that flank the provirus (7-10). That retroviruses may be normal mutagenic agents of mice was first suggested by our recent experiments involving the dilute coat color mutation of DBA/2J mice (11). In view of these experiments, we were interested in determining if any other mutant alleles carried by inbred mouse strains also are associated with ecotropic murine leukemia virus (MuLV) DNA sequences. The agouti locus is particularly amenable to these types of experiments because at least 17 different alleles have been described at this locus, and many of these alleles are carried congenically on standard inbred mouse strains (12).

MATERIALS AND METHODS

High molecular weight DNAs were isolated from spleens of mice of each of the indicated strains as described (13). DNAs (10 μ g per lane) were digested to completion with *Pvu* II, electrophoresed through 0.6% agarose gels, transferred to nitrocellulose filters, and hybridized in a total volume of 7-12 ml (13) with a 32 P-labeled pBR322 containing ecotropic viral DNA-specific probe (5 \times 10⁶ cpm/ml) (14).

After hybridization, the filters were washed, air dried, and autoradiographed at -70°C with Kodak XAR-5 x-ray film and

Du Pont Lightning Plus intensifying screens (13). Bands were detectable by overnight exposure, with optimal exposures normally requiring 2-3 days.

RESULTS

High molecular weight DNAs were isolated from spleens of mice that collectively carry 10 different alleles at the agouti locus, digested with *Pvu* II, electrophoresed through 0.6% agarose gels, blotted onto nitrocellulose paper, and hybridized with a 32 P-labeled probe specific for ecotropic MuLV DNA sequences. The hybridization probe, representing a 400-base-pair *Sma* I fragment from the *env* gene of cloned AKR ecotropic MuLV DNA (spontaneously produced by AKR mice) subcloned into the *Eco*RI site of plasmid pBR322 (14), was kindly provided by D. R. Lowy (National Institutes of Health, Bethesda, MD). For each distinct ecotropic provirus structurally similar to the prototype provirus found in AKR mice, digestion with *Pvu* II will produce a single detectable 3' cell-virus junction fragment containing 3.0 kilobases (kb) of viral DNA (15). *Pvu* II was used in these experiments because we have found this enzyme to generate the smallest and most diagnostic viral DNA junction fragments (13).

The results of these experiments are shown in Fig. 1. Mice that carry either the agouti alleles a (nonagouti), a^c (extreme nonagouti), or A (agouti) were negative for ecotropic virus, indicating that none of these alleles are associated with ecotropic viral DNA sequences. The black-and-tan allele (a^t) is carried on the MWT/Le inbred strain (related to C57BL/6J mice). MWT/Le mice only carry the normal endogenous ecotropic provirus of C57BL/6J mice. Likewise, C57BL/6J or C3HeB/FeJ mice congenic for five other agouti alleles A^{w-j} (white bellied agouti-J), A^i (intermediate agouti), A^{vy} (viable yellow), A^{iy} (intermediate yellow), or A^{sy} (sienna yellow) carried only the normal endogenous ecotropic provirus of C57BL/6J or C3HeB/FeJ mice (Fig. 1). Because neither of these endogenous ecotropic proviruses are linked to the agouti locus (16), these results demonstrate that these agouti alleles also are not associated with ecotropic MuLV DNA sequences. However, C57BL/6J mice congenic and heterozygous for the lethal yellow (A^y) allele carried an additional ecotropic provirus (characterized by a 4.7-kb *Pvu* II fragment) that was not carried by normal C57BL/6J mice (Fig. 1). The 4.7-kb band appeared to be half as intense as the 5.2-kb band, which supported the hypothesis that this provirus was associated with the A^y allele.

To further demonstrate that the additional provirus identified by *Pvu* II digestion of C57BL/6J- A^y/a DNA is associated with the A^y allele, we analyzed the ecotropic MuLV DNA content of one litter of C57BL/6J mice in which the A^y allele was segregating. In all cases, a 4.7-kb fragment was identified after *Pvu* II digestion of C57BL/6J- A^y/a DNA (Fig. 2). *Pvu* II diges-

Abbreviations: MuLV, murine leukemia virus; kb, kilobases.

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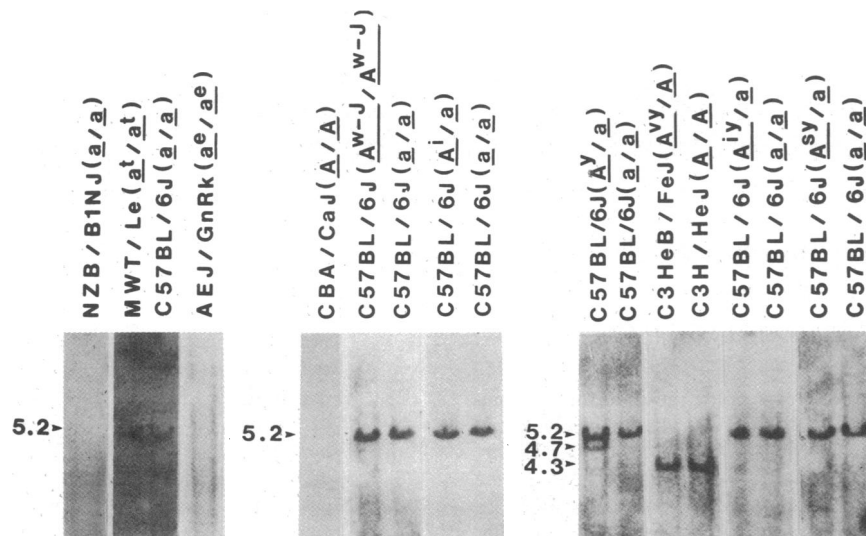


FIG. 1. Characterization of the endogenous ecotropic MuLV DNA sequences of inbred mouse strains that collectively carry 10 different alleles at the agouti locus. High molecular weight DNAs were isolated from spleens of mice of each of the indicated strains and analyzed. The size (in kilobases) of the viral DNA-containing fragments were calculated by using ^{32}P -labeled *Hind*III-digested phage λ DNA electrophoresed in parallel lanes of the same gels.

tion of C57BL/6J-*a/a* DNA only generated the 5.2-kb *Pvu* II fragment characteristic of normal C57BL/6J DNA. Because the A^y allele was transferred to C57BL/6J mice by 65 successive backcrosses and has been maintained since by more than 40 generations of brother-sister matings, these results confirm the close association of this ecotropic provirus to the A^y allele.

Approximately 20 years ago, L. C. Stevens began to transfer the A^y allele from the C57BL/6J strain to the 129/Sv strain. After nine successive backcrosses to 129/Sv, the strain has been maintained since by more than 40 generations of brother-sister matings. DNAs from three litters of 129/Sv mice in which the A^y allele was segregating were digested with *Pvu* II and analyzed with the ecotropic virus-specific hybridization probe. Representative results obtained from one litter are shown in Fig. 2. Again, *Pvu* II digestion of 129/Sv- A^y/A^w DNA generated a 4.7-kb fragment. 129/Sv- A^y/A^w mice were negative for ecotropic virus in agreement with our previously reported results (13).

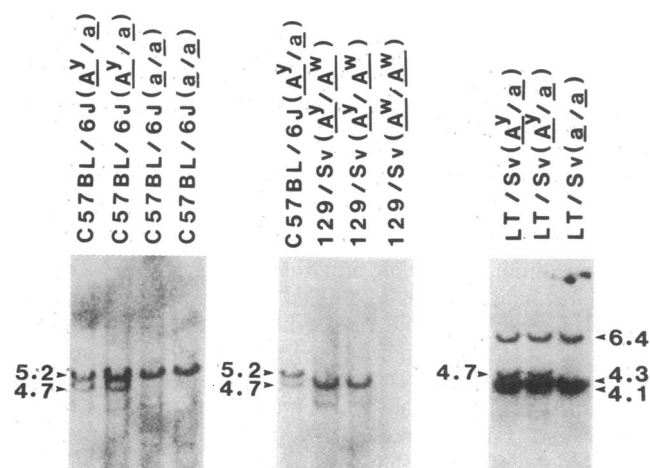


FIG. 2. Segregation of ecotropic MuLV DNA sequences and the A^y allele in C57BL/6J, 129/Sv, and LT/Sv mice. DNAs were isolated from spleens of mice from C57BL/6J, 129/Sv, and LT/Sv litters in which the A^y allele was segregating, digested to completion with *Pvu* II, and analyzed as described in the Fig. 1 legend.

About 3 years ago, L. C. Stevens also began to transfer the A^y allele from the C57BL/6J strain to the LT/Sv strain. Analysis of one litter from the N_6 generation of LT/Sv- A^y/a mice (Fig. 2) again showed that LT/Sv- A^y/a mice carry an extra provirus that is not carried by normal LT/Sv-*a/a* mice. Collectively, these experiments demonstrate that the A^y allele of these three strains is closely associated with an ecotropic provirus.

Because 129/Sv- A^w/A^w mice are negative for ecotropic virus, we used 129/Sv- A^y/A^w mice to characterize the A^y -associated virus in more detail. Restriction enzyme analysis of 129/Sv- A^y/A^w DNA with enzymes that normally produce single detectable internal viral DNA fragments (*Pst* I, *Bam*HI, and *Kpn* I) (15) suggested that the A^y -associated virus is structurally similar to the prototype AKR ecotropic provirus (data not shown). In addition, some 129/Sv- A^y/A^w mice spontaneously produced an ecotropic virus capable of fusing XC cells. (H. R. Bedigian, personal communication). Therefore, the A^y -associated virus appears to be a structurally nondefective ecotropic provirus similar to the prototype provirus found in AKR mice.

Recently, we characterized the endogenous ecotropic MuLV DNA content and integration sites of 54 inbred strains and substrains of mice (13). The endogenous ecotropic viral loci that we identified were designated *Emv-1* through *Emv-14* (for endogenous ecotropic murine leukemia viral locus). In keeping with this nomenclature, we have designated the A^y -associated proviral locus of 129/Sv- A^y/A^w mice *Emv-15*.

DISCUSSION

The lethal yellow allele was first described in 1905 by Cuénot (17) and represents an old mutation of the mouse fancy. It is believed that the A^y allele carried in all strains of laboratory mice originated from this original mutation. Interestingly, the dilute coat color mutation of DBA/2J mice also is an old mutation of the mouse fancy. Moreover, lethal yellow is of interest because it is homozygous lethal, with affected individuals dying on day 6 of gestation (12). In addition, this allele has been associated with an increased susceptibility to both spontaneous and induced pulmonary tumors, to spontaneous hepatomas in males and spontaneous mammary tumors in females, to induced skin tumors, and to spontaneous reticular neoplasms (12). Whether the increased susceptibility to tumors in mice carrying

the A^y allele is related to the ecotropic proviral DNA sequences associated with this allele remains to be elucidated. Another interesting effect of the A^y allele is its ability to decrease by 90% the testicular teratoma incidence of 129/Sv mice (18). Again, it will be important to determine if this effect is mediated by ecotropic proviral DNA sequences.

Because it has not been possible to isolate a spontaneous lethal yellow revertant, we are not yet able to determine if the lethal yellow mutation was induced by virus integration. It is clear from preliminary experiments that ecotropic virus-specific DNA sequences are closely associated with the A^y allele. This association may be particularly useful for molecular cloning and characterizing the many alleles at this locus. Furthermore, this type of experimental approach, involving the use of hybridization probes representative of other classes of endogenous retrovirus-related sequences that are carried by inbred mouse strains, may be of general use for cloning and characterizing other loci that have been identified in mouse chromosomes.

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