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EVIDENCE FOR THE HORIZONTAL ACQUISITION OF MURINE AKR VIROGENES BY RECENT HORIZONTAL INFECTION OF THE GERM LINE

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The AKR inbred strain of laboratory mice *Mus musculus domesticus* was originally derived by brother-sister matings of mice derived from a commercial Pennsylvania mouse dealer in 1928 by Jacob Furth (1). The prototype strain (A) had an infrequent incidence of leukemia, and this characteristic (leukemogenesis) was intentionally selected during inbreeding to derive a strain characterized by a high incidence of lymphoid leukemia (2). The development of lymphoid leukemia in AKR mice is accompanied by the appearance of an endogenous N-tropic murine leukemia virus, AKV, in addition to MCF (mink cell focus) viruses, which appeared to be recombinant between endogenous ecotropic and xenotropic viral genomes (3, 4). At least three unlinked functional virogene loci that specify MuLV functions have been identified (5, 6), and two of these (*Akv-1* and *Akv-2*) specify complete and recoverable viral genomes (5). The endogenous AKV virogenes, though partially homologous to other endogenous MuLV, can be discriminated from non-AKV specific MuLV sequences on the basis of expression, percent hybridization with AKV specific cDNA probes, and more recently by restriction endonuclease cleavage patterns of AKR cell DNA vs. the prototype virus negative NIH Swiss or NFS inbred NIH Swiss strains (5, 7, 8). In addition, two laboratories have independently reported the use of molecularly cloned portions of the AKV *env* gene that are type specific for the AKV virus compared with other murine retroviruses (8, 9). Recent molecular analysis of inbred strains with appropriate viral probes has indicated that high leukemic strains (AKR, C58, C3H/Fg) are characterized by multiple integrations of endogenous ecotropic provirus, whereas low leukemic strains (BALB/c, C57BL/6, DBA2) contain fewer copies of ecotropic proviral DNA, probably one per haploid genome (7, 8). Virus-negative and leukemia-free strains (NIH Swiss, their inbred derivatives, C57L, 129) appear to lack the AKV-specific sequences (7–9).

Steffen et al. (10) have recently described a genetic signature for the presence of endogenous ecotropic provirus in murine genomes by using two restriction enzymes that cleave the unintegrated ecotropic proviral DNA three times (*Bam HI*) and four times (*Kpn I*). Two characteristic internal fragments were produced with each enzyme

digest of AKR cellular DNA hybridized to an AKV virus cDNA probe. The same enzyme fragments were absent in virus-negative NIH Swiss mice DNA. A series of inbred strains and murine subspecies were screened for the presence of these four diagnostic fragments. Both high virus strains (AKR, C3H/Fg, C58/J) and low virus strains (C3H/An, BALB/c, and C57BL/6J) expressed all four restriction fragments, whereas several virus-negative strains (NIH Swiss, C57L, 129, and NZB) each lacked all four bands, as did California- and Massachusetts-caught wild mice (*domesticus*) and a number of subspecies of *Mus musculus*. Interestingly, two animals of the Japanese subspecies *Mus musculus molossinus* each contained the four diagnostic restriction fragments in their cell DNA.

In an independent analysis, Chattopadhyay et al. (8) restricted DNA from *molossinus* mice and detected several virogene copies by using a molecular subclone of the AKV *env* gene specific for ecotropic proviral DNA sequences. This result confirmed their earlier finding of AKR-specific sequences in *molossinus* cell DNA with liquid hybridization and reverse-transcribed cDNA probes (11). Furthermore, a retrovirus immunologically related to AKV has been recovered from *molossinus* (11, 12). The restriction map of the *molossinus* retrovirus is identical to that of AKV (13). These results have strongly indicated that the germ line integrations of AKR and related inbred strains positive for AKV type virus derived these virus from the Asian ancestors (10, 11). Credibility is put to this hypothesis by the fact that apparent interbreeding of Japanese pet mice and European mice occurred in the United States by mouse fanciers at the turn of the century when these strains were developed (14). Steffen et al. (10) hypothesized that the progeny of certain of these matings between European *domesticus* and Japanesse *molossinus* were used to derive certain inbred strains, and more specifically those that contain the AKV provirus.

A question that we will address with respect to these conclusions is whether ecotropic proviruses were in fact transmitted vertically from the *molossinus* progenitors or rather were acquired by horizontal infection into the germ line of *domesticus* progenitors of AKR. One prediction of the virus acquisition by sexual descent model is a relative consanguinity between derived strains of *molossinus* and AKV-containing strains. Alternatively, if the virus were acquired horizontally by infection, no such consanguinity of *molossinus* and inbred strains would be expected. A second prediction of the sexual descent model is a possible retention of the chromosomal site of integration (seen in *molossinus*) in the derivative inbred strains. The presence of an identical site of AKV integration in both AKR mice and in *molossinus* would support the concept of vertical descent from a common ancestor. Nonidentity of integration sites would be consistent with horizontal infection, especially in light of the apparent rarity of viral excision from chromosomes in various retroviral systems (15–17).

We present here estimations of genetic distance based on 51 randomly selected biochemical loci between *molossinus*, AKR, C58, NIH Swiss, and 12 other mouse strains. The results show a rather large genetic distance between *molossinus* and all inbred strains regardless of their virogene disposition. Our second approach was the examination of cell DNA from AKR and *molossinus* for similarities of integration sites using a restriction enzyme analysis. A type-specific, cloned ecotropic MuLV *env* DNA probe (9) was used to analyze the cellular DNA sequences that flank integrated proviruses. Flanking cellular sequences in AKR were not identical to those found in *molossinus* using three different restriction endonucleases (*Eco RI, Sac I,* and *Xba I*).

These data support a model involving the horizontal acquisition of endogenous ecotropic proviruses by the progenitors of AKR inbred mice.

Materials and Methods

Mice. AKR/N mice were obtained from the National Institutes of Health. MOLO is an outbred colony of *molossinus* maintained at The Jackson Laboratory, Bar Harbor, ME. The colony was derived from 16 mice collected from a feral Japanese population collected by Professor Fusanori Hamajima, Fukuoka University, Kyushu, Japan, and transferred through Dr. Michael Potter, National Cancer Institute, to The Jackson Laboratory. MOLI is an inbred strain derived from MOLO by J. E. Womack. The Swiss mice and their inbred derivatives have been described previously (18, 19). The remaining inbred strains were obtained from The Jackson Laboratory.

Allozyme (Allelic Isozyme) Analysis. 51 isozyme systems were run on starch or acrylamide gels and were developed histochemically or autoradiographically using standard isozyme procedures (20–22).

Preparation and Cleavage of DNA. High molecular weight mouse liver DNA was extracted and purified from fresh tissue as previously described (9). *Eco RI, Xba I, Pst I,* and *Sac I* were obtained from New England Biolabs (Beverly, MA) and used as specified by the supplier. The extent of digestion of cellular DNA was monitored by adding lambda DNA to an aliquot of the reaction mixture and evaluating its cleavage by gel electrophoresis. Restricted DNA samples were resolved electrophoretically in 0.5–1% neutral horizontal slab gels as described (9, 23) and transferred to nitrocellulose membranes as outlined by Southern (24).

Preparation of Specific MuLV DNA Probes. A recombinant Charon 4a phage containing an infectious AKV provirus (25) was generously supplied by Dr. Doug Lowy of the National Cancer Institute. A portion of the *env* gene region from the Charon 4A phage-AKV ecotropic MuLV recombinant specific for ecotropic MuLV genomes and not xenotropic or amphotropic MuLV genomes was isolated and subcloned in *Escherichia coli* K-12 with a pBR322 vector by H. Chan as previously described (9). This subgenomic fragment includes the *env* DNA between the 6.45 kb *Bgl II* site and the 6.95 kb *Bam HI* site (see below).

Recombinant plasmid DNA containing the AKV specific *env* region were labeled by the nick-translation procedure (26) and had specific activities of 8×10^7 to 10×10^7 cpm/μg. Procedures for hybridization to cell DNA previously transferred to nitrocellular sheets have been described (27, 28). Labeled DNA (10×10^6 to 20×10^6 cpm/filter) was incubated with the nitrocellular sheet at 60°C for 24–48 h (27). Subsequent to the hybridization, the filters were extensively washed, air dried, and exposed to Kodak XR-2 x-ray film (Eastman Kodak Co., Rochester, NY).

Results

Computation of Genetic Distance between Operative Mouse Strains as a Measure of Consanguinity. Genetic distance is a statistical method developed by Nei (29, 30) to measure the degree of allelic substitution between populations within species or between species based on the electrophoretic mobility of soluble proteins. The distance estimate, D, is defined as the average number of gene differences per locus between individuals from two test populations. Within the limits of Nei's assumptions, the genetic distance estimates increase proportionately with the amount of time the populations have been reproductively isolated. The statistic is designed to handle populations (like *molossinus*) that are polymorphic in nature, as well as monomorphic inbred lines like AKR or C58.

The genetic distance estimates are based on comparisons of 51 allozyme (allelic isozyme) loci for 17 strains of mice. The allozyme phenotypes of seven relevant inbred strains (AKR, C58, BALB/c, C57BL/6, C57L, DBA2, and MOLI) are presented in Table I. These enzyme phenotypes were determined in our laboratory for this analysis,

TABLE I

Allozyme Genotype of M. musculus molossinus and Selected Inbred Strains of M. musculus domesticus

Gene	MOLO	MOLI	AKR	C58	BALB/c	C57BL/6	C57L	DBA2
Amy-1	b	b	a	a	a	—	a	a
Apk	b	b	a	a	a	a	a	a
Acp-1	a	a	a	a	a	a	a	a
Acp-2	a	a	a	a	a	a	a	a
Ada	a	a	a	a	a	a	a	a
Ak-1	a	a	a	a	a	a	a	a
Ak-2	a	a	a	a	a	a	a	a
Aprt	a	a	a	a	a	a	a	a
Car-2	a	a	a	a	a	c	b	a
Es-1	c	c	b	b	b	a	a	b
Es-2	d	d	b	b	b	b	b	b
Es-3	b, c	b	c	c	a	a	a	c
Es-6	b	b	a	a	a	a	a	—
Es-8	a	a	a	a	a	a	a	—
Es-10	a, c	c	a	a	a	a	a	a
Gapdh	a	a	a	a	a	a	a	a
Glo-1	a	a	a	a	a	a	—	a
Gdc-1	b	b	b	—	b	b	b	b
G6pd	a	a	a	a	a	a	a	a
Gpd-1	a	a	b	a	a	a	a	a
Gpi-1	a	a	a	a	a	a	a	a
Gpt-1	c	c	a	a	a	a	a	a
Got-1	a	a	a	a	a	a	a	a
Got-2	b	b	b	b	b	b	b	a
Gr-1	a	a	a	—	a	a	—	—
Hbb	p, d	d	d	s	d	s	s	a
Hk-1	a	a	a	a	a	a	a	a
Hprt	a	a	a	a	a	a	a	a
Idh-1	a, b, c	c	b	a	a	a	b	b
Idh-2	b	b	b	b	b	b	b	b
Ldh-1	a	a	a	a	a	a	a	a
Ldh-2	a	a	a	a	a	a	a	a
Mod-1	a	a	b	b	a	b	b	a
Mod-2	a	a	b	b	b	b	c	a
Mor-1	a	a	a	a	a	a	a	—
Mor-2	a	a	a	a	a	a	a	a
Mpi-1	a	a	b	b	b	b	b	b
Np-1	b	b	a	a	a	a	a	a
Pep-1	a	a	a	a	a	a	a	a
Pep-2	a	a	a	a	a	a	a	a
Pep-3	a, b	a	b	a	a	a	a	b
Pep-4	a	a	a	a	a	a	a	a
Pep-7	a	a	a	a	a	a	a	a
Pgd	b	b	b	b	b	b	b	—
Pgm-1	b	b	a	a	a	a	a	b
Pgm-2	a	a	a	a	a	a	a	a
Pyp	a	a	a	a	a	a	a	a
Sod-1	b	b	a	a	a	a	a	a
Sod-2	b	b	a	a	a	a	a	a
Tpi	a	a	a	—	a	a	—	—
Xdh	a	a	a	—	a	a	—	—

The 51 loci are structural genes for murine enzymes. The alleles encode electrophoretic variants certain of which are depicted in Fig. 1. Gene names, genetic location, and pertinent references ae listed in refs. 19 and 34. Enzyme phenotypes were scored in our laboratories and where applicable conformed to published allele distributions of these inbred strains (31-33). MOLO is an outbred population of *Mus musculus molossinus* collected by Professor Hamajima (35). MOLI is an inbred derivative of this strain developed by J. E. Womack.

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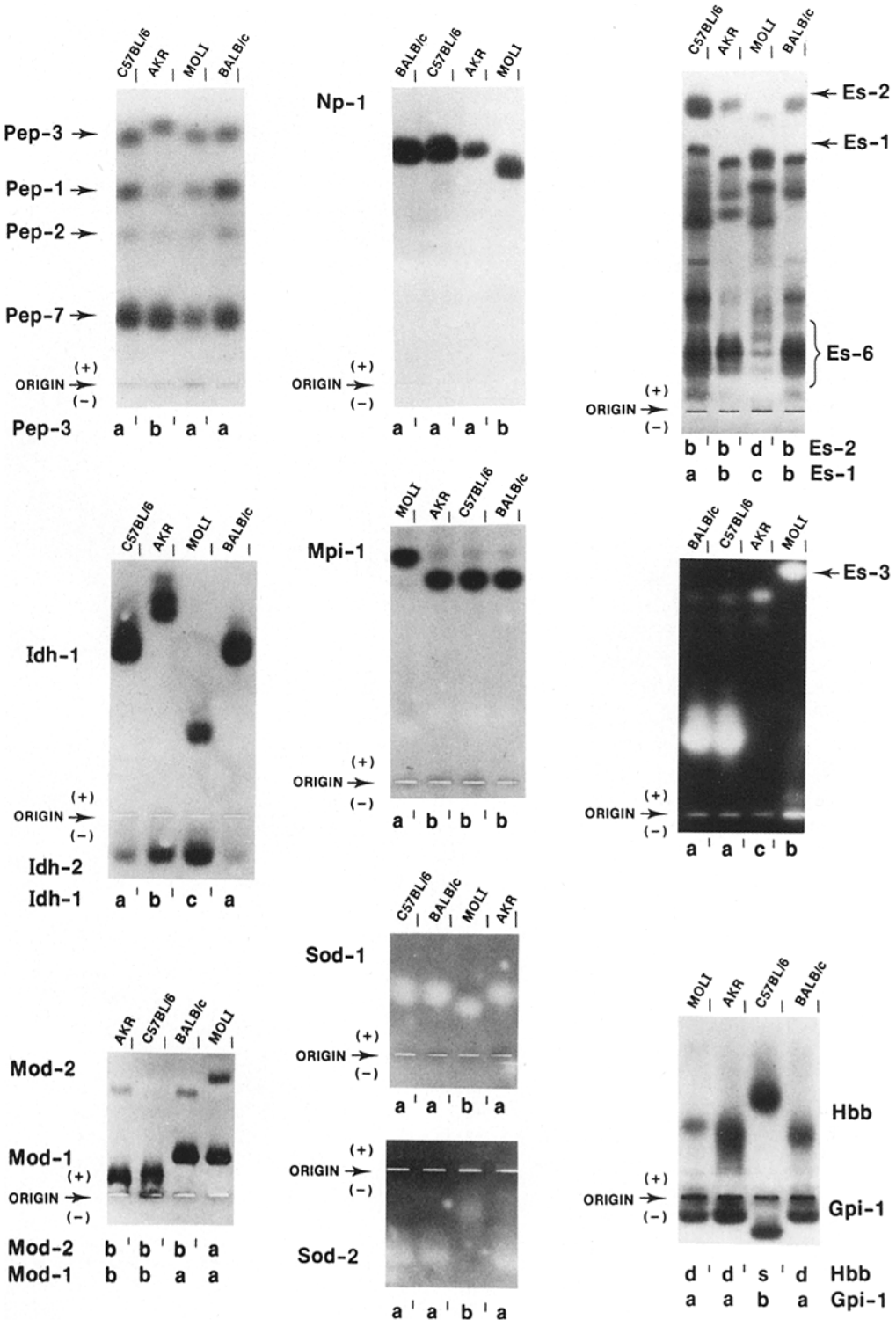

and compared favorably with published genotypes for many of the loci of these strains (31–33, and T. Roderick, personal communication). Table I also lists the allele distribution of a sample of twelve *molossinus* animals (MOLO) from The Jackson Laboratory colony typed in 1975 just after their collection by Professor F. Hamajima. The allele distribution of this strain is also presented in Table I. Fig. 1 presents electropherograms of selected loci which vary between the strains under study.

Two feral mouse populations of *domesticus*, Lake Casitas and Bouquet Canyon (36), were also included in the analysis. The remaining seven strains included three outbred Swiss mouse colonies (NIH, Eppley Institute, and CD1) and four inbred Swiss derivatives (NFS, NFR, HSFR, and HSFS) (19). The American Swiss mouse strain was originally established from nine mice brought from Switzerland by Clara Lynch in 1926. The three colonies have been isolated from each other for nearly 45 yr and their genetic structure and relatedness have been discussed in detail elsewhere (19).

The genetic distance computed between all combinations of the 17 mouse strains is presented in Table II. The distance estimations are restricted to the 51 loci listed in Table I. These loci were selected simply because the technology for typing them was available in our laboratories and should therefore be irrelevant to the virus constitution of the strains.

A number of important observations emerge upon examination of Table II. First, the two California feral populations have a distance from each other of 0.019. This value is similar to the average distance between the three outbred Swiss colonies (0.023), which were derived from nine mice and have been isolated for 45 yr (18, 19). Second, the average distance between Swiss outbred and their inbred derivatives (0.055) is over twice the distance between the isolated outbred strains (0.023). This result is not surprising, because the Swiss outbred strains have remained fairly similar during their isolation (19), whereas the inbred strains have (by definition) lost all their variation and as such diverged away from the parent population and from each other (average $D = 0.055$) by random allele fixation during inbreeding. Third, the three Swiss colonies and the two California populations; each *domesticus* subspecies members isolated for at least 200 yr (when mice were introduced from Europe into California), have an average distance from each other of 0.047. Fourth, three of the inbred strains that share a common origin (C58, C57BL/6, and C57/L) but a different virus phenotype (7) have an average distance of 0.099. The distances of these same three strains from other unrelated inbred strains vary from 0.089 to 0.321. Fifth, the distance of *molossinus* strains from all the inbred strains including the virus-positive strains is significantly larger (3–20 times) than any of the above measurements between consanguinous strains. The distances for *molossinus* from AKR, other AKV^+ strains, AKV^- strains, Swiss, and feral California mice are equivalent. The degree of *molossinus* differences are comparable to differences measured between subspecies defined in other systems (29, 30, 39, and S. J. O'Brien, unpublished observations).

Restriction Enzyme Analysis of Murine Cellular DNA with an AKV env Probe. Cellular DNA from livers of weanling mice of four strains (AKR/N, F/St, RF/J, and MOLO) were examined for AKV-related sequences, and their flanks were examined with an

FIG. 1. Allozyme phenotypes of four inbred strains AKR, MOLI, BALB/c, and C57BL/6 at several isozyme systems that vary between these lines. The phenotype designation of the polymorphic system(s) is indicated at the bottom of each photograph.

TABLE II

Genetic Distance (D) between Outbred molossinus (MOLO), Inbred molossinus (MOLI), and 15 Mouse Strains

	molossinus		V⁺, high leukemia		V⁺, low leukemia			V⁻, leukemia negative									
									Swiss outbred			Swiss inbred				Feral	
	MOLO	MOLI	AKR	C58	BALB/c	DBA2	C57BL/6	C57L	CD1	NIH	EPPLEY	HSFS	NFS	HSFR	NFR	LC	BC
MOLO	0.0																
MOLI	0.031	0.0															
AKR	0.377	0.446	0.0														
C58	0.442	0.496	0.091	0.0													
BALB/c	0.346	0.377	0.105	0.067	0.0												
DBA2	0.340	0.417	0.121	0.178	0.147	0.0											
C57BL/6	0.446	0.490	0.182	0.093	0.128	0.304	0.0										
C57L	0.463	0.518	0.161	0.113	0.137	0.229	0.093	0.0									
CD1	0.413	0.503	0.093	0.074	0.136	0.160	0.130	0.191	0.0								
NIH	0.391	0.477	0.133	0.121	0.138	0.164	0.164	0.242	0.025	0.0							
EPPLEY	0.379	0.465	0.093	0.109	0.122	0.107	0.185	0.212	0.020	0.025	0.0						
HSFS	0.441	0.512	0.127	0.163	0.124	0.141	0.187	0.288	0.054	0.053	0.033	0.0					
NFS	0.441	0.519	0.127	0.134	0.124	0.111	0.187	0.255	0.081	0.070	0.056	0.045	0.0				
HSFR	0.562	0.647	0.154	0.134	0.206	0.204	0.217	0.255	0.058	0.071	0.049	0.067	0.067	0.0			
NFR	0.441	0.519	0.182	0.192	0.178	0.111	0.247	0.322	0.060	0.040	0.039	0.045	0.045	0.067	0.0		
LC	0.332	0.397	0.098	0.129	0.104	0.133	0.206	0.221	0.051	0.064	0.023	0.054	0.082	0.098	0.082	0.0	
BC	0.344	0.423	0.114	0.121	0.123	0.178	0.212	0.241	0.050	0.059	0.034	0.077	0.099	0.109	0.099	0.019	

Genetic distance is computed using the genes presented in Table I. Allele distribution and frequencies of the two California populations (LC and BC) and the outbred Swiss colonies are derived from refs. 19 and 36, respectively. I equals the probability of allelic identity of any randomly selected genes at any locus in each of two test populations. D equals the average number of gene differences per locus between individuals from the test populations. Algebraically: $I = J_{xy}/\sqrt{J_x J_y}$, $D = \ln I$, where J_{xy} is the arithmetic mean of $j_{xy} = \sum_i x_i y_i$ over all loci, J_x is the arithmetic mean of $j_x = \sum_i x_i^2$ over all loci, and x_i (or y_i) is the frequency of the ith allele in the first (or second) population (29, 30). The endogenous AKV type and leukemia status of the studied strains is: V⁺, high leukemia: AKR, C58; V⁺, low leukemia: BALB/c, DBA2, C57BL/6; V⁻, leukemia negative: C57L, CD1, NIH-Swiss, Eppley-Swiss, HSFS, NFS, HSFR, NFR; Bouquet Canyon. The Lake Casitas population has a high leukemia incidence and is infected with an amphotropic and ecotropic virus (37). The AKV virus, however, is apparently not endogenous in this population (38).

AKV-specific *env* probe. The cloned probe (9) was derived from a portion of the *envelop* gene of AKV between a *Bgl II* site (at 6.45 kilobasepair [kb]) and a *Bam HI* site (at 6.95 kb). A restriction map of AKV provirus indicating the portion represented in the clone is presented in Fig. 2. The probe has two distinct advantages. First, it is specific for the AKR-like ecotropic proviral sequences and does not hybridize to xenotropic, MCF, or additional (40) endogenous murine retroviral DNA sequences. Second, the probe permits directional dissection of flanking cell DNA. Thus, an enzymelike *Sac I* or *Xba I*, which cleaves the AKR ecotropic provirus a single time, can be used to characterize sequences that abut proviral DNA. A single reactive band is generated

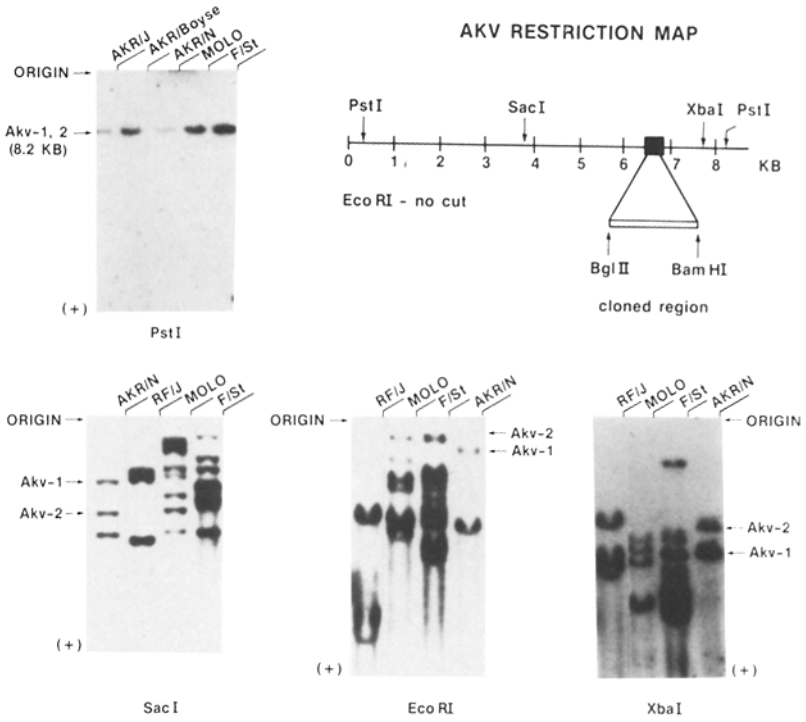

FIG. 2. Restriction enzyme pattern of cell DNA of MOLO, AKR/N, RF/J, and F/St mouse strains using type specific *env* clone of AKV proviral DNA. The restriction map of AKR provirus (9, 13) is presented with the region represented in the probe indicated. The specific fragments in AKR/N that represent *Akv-1* and *Akv-2* have been determined and are so indicated.[1] The molecular weights (kb) of the fragments for *Akv-1* and *Akv-2* were *Sac I*, 8.6, 6.2; *Xba I*, 11, 15; *Eco RI*, 23.5, >24, respectively. A more comprehensive description of AKR virus integrations in various inbred strains and in different colonies of AKR mice is presented elsewhere.[1]

for each integrated provirus consisting of viral sequences containing *env* and the flanking cellular sequences. *Xba I*, which cleaves to the right of the cloned proviral sequence, generates fragments containing cellular sequences located to the 5′ side of proviral DNA, whereas *Sac I*, which cuts to the left of the cloned sequence, produces cleavage products consisting of viral sequences and cellular DNA that flank the 3′ termini (see map, Fig. 2). "No-cut" enzymes (like *Eco RI*) also produce a single band for each integrated provirus containing viral as well as the cellular sequences that flank the 3′ and 5′ termini.

The patterns of restricted DNA of the four studied mouse strains are presented in Fig. 2. The *Pst I* pattern is identical for all AKV-positive mouse strains yielding a single 8.2 kb band. *Pst I* cuts in the long terminal redundancy at either end of integrated provirus (see map, Fig. 2). This result affirms the AKV-MuLV specificity of the probe and also demonstrates that each of the multiple AKV integrations (8, 10, and see below) represent complete ecotropic MuLV copies.

[1] Moore, J. L., H. W. Chan, W. P. Rowe, and M. A. Martin. Heterogeneity of endogenous ecotropic retrovirus genes in inbred AKR mice and AKV congenic mice. Manuscript submitted for publication.

The restriction enzyme patterns of four virus-positive, but otherwise unrelated mouse strains, using *Eco RI*, *Sac I*, and *Xba I*, are presented in Fig. 2. The AKR/N strain exhibits three fragments representing individual chromosomal integrations with *Eco RI* and *Sac I*, and two fragments with *Xba I*. In each case two of the fragments have been specifically identified as respective products of *Akv-1* (murine chromosome 7) and *Akv-2* (chromosome 16) by examination of AKV congenic (on an NIH Swiss-virus negative background) mice (41, 42).[1] The specific *Akv-1* and *Akv-2* fragments are indicated in Fig. 2. The third fragment seen in *Eco RI* and *Sac I* digestions of AKR/N7 has not been thoroughly characterized, but is seen as a third AKV fragment in several AKR strains examined to date.[1]

The restriction enzyme pattern of *molossinus* is very different from AKR/N cell DNA. At least six different genome size (or greater) fragments were detected in MOLO DNA with *Eco RI* and *Sac I*. Furthermore, there is no evidence for a similar integration fragment between AKR/N and MOLO. That is, the *Akv-1* and *Akv-2* fragments have no corresponding counterparts in *Eco RI*, *Xba I*, or *Sac I* digestions of MOLO. The uncharacterized fragment has no identical counterpart in *Sac I* or *Eco RI* digests. In addition, two additional AKV-positive inbred strains, F/St and RF, do not appear to share common restriction with AKR/N or MOLO.

Discussion

The comprehensive molecular analyses of several laboratories (8, 10, 11, 13) have clearly demonstrated the identity of murine ecotropic virus found in inbred mouse strains (notably AKR) and the endogenous MuLV isolated from an outbred strain (MOLO) of *molossinus*. Nonetheless, the genetic distance between strain AKR (or other virus-positive inbred strains) and *molossinus* are large, of the order of magnitude seen between subspecies and even between some species (39, S. J. O'Brien, unpublished observations). These observations preclude the possibility of *molossinus* contributing in any detectable extent to the modern genome of AKR inbred mice as might be expected if they had a common ancestor.

A second experimental observation bearing on this question is the lack of common integrations (as detected by cellular flanking DNA fragments of the same molecular weight after restriction enzyme digestion) in AKR and MOLO. If AKV were acquired from a common ancestor of MOLO and AKR, a similar flanking sequence might be expected. Similar integration fragments were not evident in our analysis. These negative results are not conclusive, however, because there are additional interpretations. For example, restriction flanks could be modified over time by mutation, recombination with polymorphic restriction sites, viral transposition, virus excision, and reintegration, events that are not without precedence in the development of inbred mouse strains (15–17, 41). None of these explanations, however, is consistent with the large genetic distance discussed above.

The present data appear to exclude the concept of consanguinity by descent of the present AKR, C58, and other virus-positive strains with present-day *molossinus* subspecies. So then, how did AKR get a *molossinus* virus? One explanation we cannot formally exclude is that an F_1 animal between *domesticus* and *molossinus* was consecutively backcrossed to *domesticus* breeders in a manner reminiscent of preparing present-day congenic mice (41, 43). The problem with this idea is that it would require

selection for integrated AKV during the backcross phase of the strain's history. Otherwise, the AKV provirus would be expected to be lost by backcrosses to *domesticus*. We do know that the progenitors of AKR were selected for high leukemia (and therefore probably viremia) during a period of sibling mating (1, 2), but there is no mention of selection during backcrosses to an unrelated (and possibly AKV^-) *domesticus* strain. Even if we postulate selection for *v* (Japanese waltzer allele), this would fix a chromosome 10 segment, a chromosome where AKV is not in AKR or any other inbred strains. The simplest explanation consistent with all the available data is to hypothesize a recent germ line infection of a progenitor of AKR by a *molossinus* virus.

Horizontal virogene transmission is not without precedence. Germ line infection with MuLV has been achieved intentionally under laboratory conditions (44) and horizontal acquisition of retroviral sequences has precedence in nature in at least three systems; namely, RAV-O in chickens, and RD114 and FeLV in cats (45–47). Each of these endogenous virogenes arose precipitously in evolution since closely related species do not harbor homologous gene sequences.

Summary

Several recent reports (8, 10, 11, 13) have established the biological and molecular genetic similarity between the endogenous AKV virus of strain AKR, and an N-ecotropic endogenous virus found in the genome of feral Japanese mice, *Mus musculus molossinus*. The similarities are so striking as to suggest a common origin of these viruses, which are present in some, but not all, inbred mouse strains. The virogenes of AKR mice may have been acquired by either: (*a*) common descent of AKR (and other AKV^+ strains) from a common ancestor of AKR and *molossinus* animals, or (*b*) horizontal germ line infection of the AKR strains by *molossinus* virus at the strain's inception followed by fixation through inbreeding. The sexual descent model carries with it a prediction of relative consanguinity of the AKR strain and *molossinus*, whereas the horizontal infection model does not. We have examined the polymorphic allozyme (allelic isozyme) genotype of 51 nonvirus-related loci in 17 strains of mice including AKR, C58, BALB/c, Swiss, and *molossinus*. By comparing the composite allozyme genotype of different inbred and outbred mouse strains, the "genetic distance" statistic was derived. Genetic distance measures the degree of allelic substitution between populations and increases proportionately with the amount of time the populations have been reproductively isolated. The genetic distance computed between *molossinus* and AKR is large, nearly 5–10 times the distance between known related populations and strains (e.g., C57L vs. C57BL/6). *Molossinus* had a similarly large distance from AKV negative strains (Swiss, C57L) as it did from AKV-positive strains.

Cellular DNA sequences that flank the integrated AKV provirus were analyzed by restriction enzyme digestion of liver DNA from *molossinus*, AKR, and additional inbred strains that express ecotropic murine leukemia virus. The integration flanks of three AKR provirus sequences, *Akv-1*, *Akv-2*, and a third uncharacterized sequence, were not evident in *molossinus* cell DNA, which contained at least six different proviral integration fragments. These data effectively exclude the interpretation of consanguinity of AKR and *molossinus* and support the notion of acquisition of the endogenous virus in AKR by horizontal infection of the *molossinus* virus.

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