

Common Inbred Strains of the Laboratory Mouse That Are Susceptible to Infection by Mouse Xenotropic Gammaretroviruses and the Human-Derived Retrovirus XMRV[∇]

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Laboratory mouse strains carry endogenous copies of the xenotropic mouse leukemia viruses (X-MLVs), named for their inability to infect cells of the laboratory mouse. This resistance to exogenous infection is due to a nonpermissive variant of the XPR1 gammaretrovirus receptor, a resistance that also limits *in vivo* expression of germ line X-MLV proviruses capable of producing infectious virus. Because laboratory mice vary widely in their proviral contents and in their virus expression patterns, we screened inbred strains for sequence and functional variants of the XPR1 receptor. We also typed inbred strains and wild mouse species for an endogenous provirus, *Bxv1*, that is capable of producing infectious X-MLV and that also contributes to the generation of pathogenic recombinant MLVs. We identified the active *Bxv1* provirus in many common inbred strains and in some Japanese *Mus molossinus* mice but in none of the other wild mouse species that carry X-MLVs. Our screening for *Xpr1* variants identified the permissive *Xpr1^{sxv}* allele in 7 strains of laboratory mice, including a *Bxv1*-positive strain, F/St, which is characterized by lifelong X-MLV viremia. Cells from three strains carrying *Xpr1^{sxv}*, namely, SWR, SJL, and SIM.R, were shown to be infectable by X-MLV and XMRV; these strains carry different alleles at *Fv1* and vary in their sensitivities to specific X/P-MLV isolates and XMRV. Several strains with *Xpr1^{sxv}* lack the active *Bxv1* provirus or other endogenous X-MLVs and may provide a useful model system to evaluate the *in vivo* spread of these gammaretroviruses and their disease potential in their natural host.

Xenotropic mouse gammaretroviruses were first isolated by Levy and Pincus from the NZB strain of laboratory mice (25). The virus was termed xenotropic because of its inability to infect cells of the various inbred mouse strains, although it could infect rat cells. Subsequent studies isolated this virus type from additional strains of inbred and outbred laboratory mice (23) and showed that these viruses are infectious for cells of multiple mammalian species (24, 33).

The various inbred strains of laboratory mice carry up to 15 endogenous proviruses related to infectious xenotropic mouse leukemia viruses (X-MLVs) (8, 34), and some of these germ line X-MLV proviruses, or XMVs, are capable of producing infectious virus or viral proteins (reviewed in reference 40). These inbred mouse strains differ in their abilities to produce infectious X-MLVs because of the different XMVs that they carry and because of the presence of regulatory genes that alter virus expression and spread. Two strains, F/St and NZB, have a “high-virus” phenotype; that is, they are viremic with X-MLVs from an early age, and virus can readily be isolated from cells and tissues of these strains (6, 25, 31). A second and larger set of inbred strains, which includes common strains such as C57BL and BALB/c, carry XMVs that produce virus after chemical or immunological stimulation (1, 9, 27, 38). A third set of strains, including Swiss strain-derived mice and strains

such as A and C3H, are not or are rarely capable of producing infectious X-MLV.

Four active XMVs have been identified in the strains that can produce infectious virus. Two of these proviruses are found in NZB mice (6), a third provirus, termed *Bxv1*, is found in several common inbred strains (8, 18, 19), and the fourth is found along with the *Bxv1* provirus in MA/My mice (16). Only one of these proviruses, *Bxv1*, has been mapped in the mouse genome; it has been mapped to a position on distal chromosome 1 (Chr 1), where it was first located by conventional linkage analysis of chemically inducible virus production (18). The provirus at this locus was subsequently identified by Southern blotting of somatic cell hybrids and recombinant inbred mouse strains (11, 8). The *Bxv1* provirus (also termed *Xmv43*) is present in the sequenced C57BL mouse genome and maps at 179 MB on Chr 1 (12). This provirus is biologically important for two reasons: not only does *Bxv1* reliably produce virus in response to specific inducers (18, 19), but it is also involved in virus-induced leukemias. These diseases are associated with the generation of recombinant leukemogenic polytropic MLVs (P-MLVs), and the *Bxv1* provirus has been identified as the source of the long terminal repeat sequences of these pathogenic recombinants (11, 41).

The presence of multiple endogenous XMVs in mice that are not susceptible to X-MLV infection can be explained by looking at the wild mouse progenitors of these laboratory strains. The common laboratory strains represent a mosaic of three house mouse species: *Mus domesticus*, *Mus castaneus*, and *Mus musculus* (49). Two of these 3 species carry XMVs (17, 43), and the 3 species carry 3 different alleles of the XPR1 receptor used for entry by X-MLVs, all 3 of which are permis-

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sive for X-MLVs (28, 47, 48). In contrast, the XPR1 receptor gene originally cloned from NIH 3T3 cells is not permissive for X-MLV entry (2, 42, 50). This suggests that XMVs were originally acquired by X-MLV-susceptible wild mice and that the receptor variants permissive for this virus were lost in the fancy mouse breeding stocks used to generate laboratory strains.

There are hundreds of strains of laboratory mice. While it is generally recognized that these strains are less genetically diverse than wild *Mus* species, it is clear that there are distinct lineages of laboratory strains that can be traced to different fancy mouse and wild mouse progenitors. Few of the hundreds of mouse strains have actually been tested for X-MLV susceptibility, and the observations that X-MLV susceptibility is widespread among wild mice and that these inbred strain lineages represent different mosaics of wild mouse species (49) raised the possibility that X-MLV-permissive *Xpr1* alleles might have been captured in some of these strains. In this study, we screened inbred strains of laboratory mice for the presence of known and novel variants of the *Xpr1* receptor, with the goal of identifying strains susceptible to X-MLV infection. We also identified the inbred strains carrying the inducible *Bxv1* provirus and identified the origin of this provirus in the Japanese house mouse species *Mus molossinus*. The permissive *svx* allele of *Xpr1* (*Xpr1^{svx}*) was identified in 7 inbred strains, including the high-virus, *Bxv1*-positive F/St strain. We also demonstrate that cells from laboratory mouse strains carrying *Xpr1^{svx}* can be efficiently infected with XMRV and X-MLVs.

MATERIALS AND METHODS

Viruses, cells, and virus infectivity. CAST-X is an X-MLV isolated in our laboratory from the spleen of a CAST/EiJ mouse (46). Cz524 is a novel MLV isolated from the spleen of a CZECHII/EiJ mouse (47). The human xenotropic murine leukemia virus-related virus, XMRV (7), was kindly provided by R. Silverman (Cleveland Clinic, Cleveland, OH). AKR6 X-MLV, CasE#1 (5), Moloney mink cell focus-inducing virus (MoMCF), and Friend MCF (FrMCF) were originally obtained from J. Hartley (NIAID, Bethesda, MD).

Susceptibility to MLVs was tested in NIH 3T3 cells, in *Mus dunni* cells (20) obtained from M. Lander (NIAID, Bethesda, MD), and in cultured fibroblasts developed from tail biopsy specimens from adult mice (21). Retrovirus stocks carrying the LacZ reporter were generated for the various MLVs by virus infection of GP2-293 cells transfected with pCL-MFG-LacZ (47); the virions produced by these cells represent a mix of infectious virus and particles carrying LacZ. To test for virus susceptibility in a single-round assay, cells were infected with appropriate dilutions of these pseudotype virus stocks in the presence of 4 to 8 μ g/ml Polybrene. One day after infection, cells were fixed with 0.4% glutaraldehyde and assayed for β -galactosidase activity using as the substrate 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-Gal, 2 mg/ml; ICN Biochemicals, Aurora, OH). Infectious titers were expressed as the number of foci of blue cells per milliliter of virus.

Genomic DNA and RNA. DNAs were extracted from mice obtained from several sources. The following mice were obtained from M. Potter (NCI, Bethesda, MD): *M. domesticus* mice trapped at various sites (Abu Rawash, Egypt; Haven's Farm, Davidsonville, MD; Lewes, DE; J. J. Downs, Ridgely, MD; Watkin's Star, Centreville, MD), *M. molossinus* mice (MOL/Li), *M. castaneus* mice (CAST/Ncr, CAS/Li), *M. musculus* mice (Vejrumbro, Denmark; Skive, Denmark), and *M. spretus* mice (Spain). Three wild-trapped mice from Lake Casitas, CA, were obtained from S. Rasheed (University of Southern California, Los Angeles). CAST/Rp mice were obtained from R. Elliott (Roswell Park Cancer Institute, Buffalo, NY), and SAMP8 and SAMR1 samples were obtained from R. Carp (New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY). F/St mice were obtained from H. Morse (NIAID, Bethesda, MD) in the early 1980s; this strain is now extinct. 129-GIX+ mice were obtained from E. Boyse (Memorial Sloan-Kettering Cancer Center, NY). SIM.R and SIM/Ut mice (44) were obtained from A. Axelrad (University of Toronto, Toronto, Canada). AKR/N and NFS/N mice were obtained from the

Small Animal Section, NIH (Bethesda, MD). NFS/N-*Xpr1^{svx}* congenics were developed in our laboratory and carry the *Xpr1* allele from *M. domesticus* (formerly *Mus praetextus*), provided by M. Potter. DBA/2J, DBA/1J, C58/J, C57BL/10J, and SWR/J mice were obtained from the Jackson Laboratory (Bar Harbor, ME).

DNAs were also isolated from the cell lines NZB-Q and SC-1, obtained from J. Hartley (NIAID, Bethesda, MD), and from cells of *M. domesticus* (formerly *M. praetextus*) obtained from J. Rodgers (Baylor College of Medicine, Houston, TX). *Mus fragilicauda* DNA was provided by J. Hartley. The Chinese hamster/mouse somatic cell hybrid BE7-2 was produced by the fusion of E36 cells and primary fibroblasts of BALB/c cells, and its mouse chromosome content was determined by examining Giemsa-stained metaphase spreads and by testing for marker loci (14).

Additional DNAs provided by H. Morse and S. Chattopadhyay (NIAID, Bethesda, MD) include *Mus abboti* and *M. musculus* mice trapped in Vejrumbro, Denmark; Brno, Czechoslovakia; Belgrade, Yugoslavia; and Viborg, Denmark. DNA from *M. macedonicus* was provided by R. Elliott. All other DNAs from common inbred and wild-derived strains were purchased from the Jackson Laboratory (Bar Harbor, ME).

Cloning and sequencing of *Xpr1*, *Fv1*, and the *Bxv1* insertion site. The full-length *Xpr1* genes were amplified by reverse transcription-PCR (RT-PCR) from cells established from SWR tail biopsy specimens and from the cell line SJLSV, a simian virus 40 (SV40)-transformed line of SJL fibroblasts produced by B. Knowles (Institute of Medical Biology, Singapore). Primers used were 5'-ATG AAGTTCGCCGAGCACCTCTC and 5'-AGTGTAGCTTCGTCATCTGT GTC. A segment of *Xpr1* containing the third and fourth putative extracellular loops (ECL3 and ECL4, respectively) was amplified by RT-PCR from NZB mouse spleen RNA using the forward primer 5'-CGAGTATTTACTGCTCCC TTCC and Ex13R, 5'-CGGAAAACCTCAAGGGGG. *Xpr1* exon 13 sequences, containing ECL4, were amplified from the DNA of multiple strains and species using primers Ex13F (5'-GCCTATTACTACTGTGCC) and Ex13R. All PCR products were cloned into pCR2.1-TOPO and sequenced.

PCR primers for the *Bxv1* provirus-cell junction fragment and the empty locus at the *Bxv1* insertion site were designed based on *Mus musculus* chromosome 1 clone RP24-65D16 (GenBank accession no. AC115959). The *Bxv1* provirus is present in the reverse orientation at coordinates 107533 to 116189. A *Bxv1* provirus-cell junction fragment was PCR amplified using primers 5'-AGGAGT AGGAACAGGGACTACAGC (*Bxv1*ENFP; nucleotide coordinates 108635 to 108612) and 5'-GATCTAGAGTCACCTAGGCAACAAGC (*Bxv1*FLRP; nucleotide coordinates 106765 to 106740). The empty locus at the *Bxv1* insertion site was amplified using primers 5'-CCATGCTAAATACCTCAGACTCC AGTG (*Bxv1*FLFP; nucleotide coordinates 116359 to 116333) and 5'-GATCT AGAGTCACCTAGGCAACAAGC (*Bxv1*FLRP; nucleotide coordinates 106765 to 106740) (Fig. 1). Representative PCR products were cloned into pCR2.1-TOPO and sequenced.

PCR primers for the 3' half of the *Fv1* gammaretrovirus restriction gene, MHRF and GT17a (45), generate fragments of 510 bp for *Fv1ⁱⁿ* and *Fv1^{rr}* and 1.8 kb for *Fv1^b*. The 510-bp PCR product from SWR/J mice was cloned into pCR2.1-TOPO and sequenced.

Nucleotide sequence accession numbers. The sequences of the *Xpr1* genes for 19 mouse strains (129, AKR, DBA, F/St, FVB, HRS, LP, LT, MA/My, NOD, PWD, RBA, RIIS, SOD, SWR, YBR, NZB, SIM.R, SJL) were deposited in GenBank under accession no. HQ323662 to HQ323680. Sequences for the empty Chr 1 *Bxv1* site in NFS/N and the SWR *Fv1* gene were deposited in GenBank under accession no. HQ323681 and HQ323682.

RESULTS

Inbred strain distribution of the inducible XMV locus *Bxv1*. We designed primers to identify cell-virus junction fragments diagnostic of the *Bxv1* provirus, as well as primers to identify the empty locus (Fig. 1). Primers were initially tested on DNAs from mouse strains previously shown to lack *Bxv1*, NFS and NIH 3T3, and on DNAs from C57BL and BALB/c, the strains in which *Bxv1* was initially discovered (18). We also tested a hamster/mouse somatic cell hybrid, BE 7-2, carrying fragments of two mouse chromosomes, Chr 1 and Chr 15. This hybrid, like mouse cells carrying *Bxv1*, produces infectious X-MLV following induction (14), and it contains a single XMV provirus (11). The 1.8-kb cell-virus junction fragment was identified

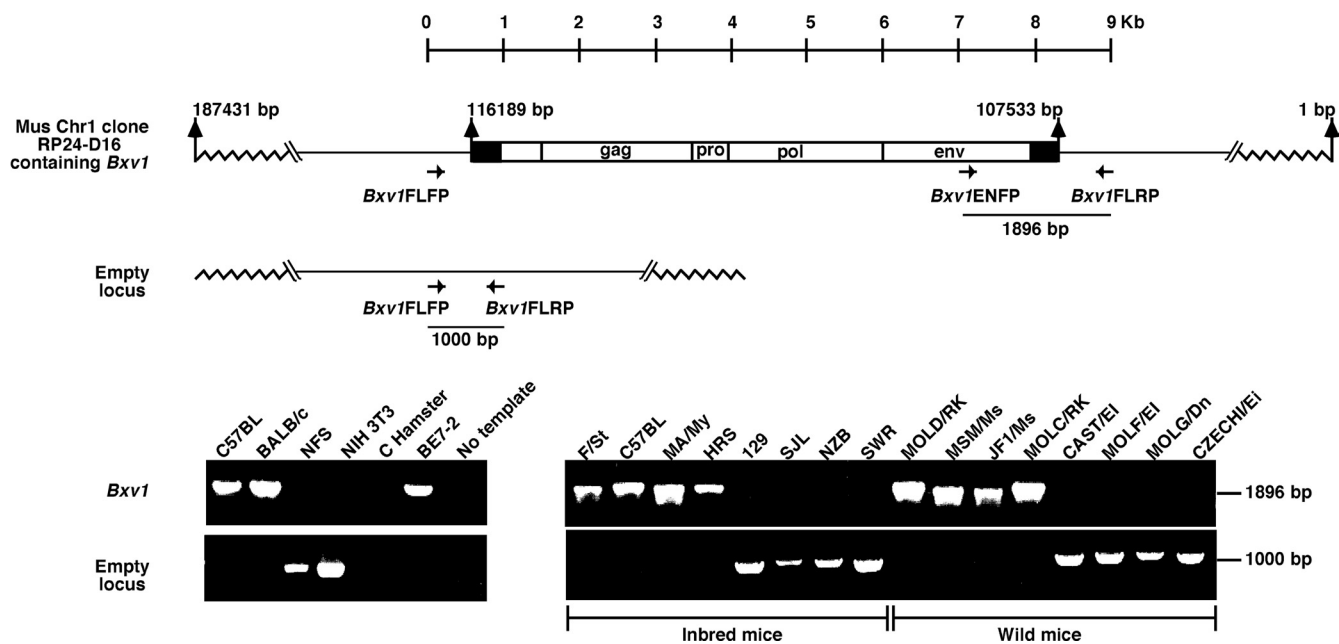


FIG. 1. Analysis of the *Bxv1* provirus in common strains of laboratory mice and wild-derived mouse strains. (Top) *Bxv1* provirus integration site and the empty locus on Chr 1 with PCR primers used to amplify these locus alternatives. For clarity, the *Bxv1* provirus is diagrammed in the forward orientation, as opposed to its reverse orientation in GenBank accession no. AC115959. (Bottom left) PCR screening of mouse DNAs previously shown to carry *Bxv1* (C57BL, BALB/c) or to lack this provirus (NFS, NIH 3T3) and a hamster/mouse somatic cell hybrid, BE7-2, that carries a Chr 1 fragment containing *Bxv1*. (Bottom right) PCR results for representative common inbred strains and wild-derived mice.

only in DNAs from the hybrid cell line and from mice known to carry *Bxv1*, whereas the 1.0-kb fragment diagnostic of the empty locus was found only in DNAs from mice or cells lacking *Bxv1* (Fig. 1), thus confirming that these primers identify the *Bxv1* locus. The sequence of the 1.9-kb cell-virus junction fragment produced by *Bxv1*ENFP and *Bxv1*FLRP was identical to the corresponding Chr 1 segment in the sequenced C57BL genome (GenBank accession no. AC115959), and the sequence of the *Bxv1*FLFP/*Bxv1*FLRP product from the *Bxv1*-negative NFS/N mouse represents the provirus-free insertion site (GenBank accession no. HQ323681).

We analyzed 50 laboratory mouse strains, most of which fall into several inbred strain categories defined by their related breeding histories (3) (Fig. 2). The majority of the analyzed strains originated from the fancy mouse colonies of breeder Abbie Lathrop and from the early lines developed by William Castle; one set of Lathrop mice provided to William Castle gave rise to many common strains, such as DBA and BALB/c, and additional Lathrop mice produced the C57/C58-related strains. A third set of strains was derived from Swiss mice. Additional strains were developed from stocks in New Zealand, and we also included strains of other or uncertain ancestry in this analysis. We did not test the various strains derived by intercrossing other inbred strains.

Bxv1 was identified in 17 strains, most of which are Lathrop or Castle strain-derived mice (Fig. 1, 2). *Bxv1* was not found in any of the New Zealand or Swiss strain-derived mice. This distribution is consistent with previous analyses that typed 12 strains for the presence or absence of the Chr 1 *Bxv1* induction locus (18, 19, 51) and 7 strains for the presence or absence of diagnostic *env* fragments by Southern blotting (8) (Fig. 2).

Previous testing had also identified a *Bxv1*-like locus in F/St mice, and although the Chr 1 location initially determined for this virus induction locus did not clearly identify it as *Bxv1* (31), the present analysis confirms that *Bxv1* is present in F/St (Fig. 1).

Wild mouse origin of the *Bxv1* provirus. Laboratory mice are a mosaic of the wild mouse species of house mice, and 3 of the 4 house mouse species (*M. molossinus*, *M. castaneus*, *M. musculus*) carry X-MLV-related sequences and are capable of producing infectious virus (4, 17, 26, 43, 46, 47). It is therefore possible that the *Bxv1* provirus originated in one of these wild mouse species. A previous attempt to identify *Bxv1* in *M. molossinus* by screening backcross mice for inducible Chr1-linked proviral loci was unsuccessful because these mice carry too many active proviruses (16). We screened house mouse species and several other *Mus* species that had not been previously typed for endogenous MLVs for the presence of *Bxv1* by PCR (Fig. 1, 3). We identified *Bxv1* in 4 wild mouse DNAs, all of which were *M. molossinus*, although 3 additional breeding lines of *M. molossinus* mice did not have this provirus.

None of the other house mouse species carries *Bxv1*. *M. molossinus* is thought to be a natural hybrid of *M. castaneus* and *M. musculus* (52), both of which species carry active X-MVs; however, the *Bxv1* provirus was not found in any mouse of these 2 species (Fig. 1 and 3). The absence of this provirus in these 2 species and the fact that some, but not all, *M. molossinus* mice carry *Bxv1* indicate that this X-MV insertion arose relatively recently in Japanese mice. It is likely that Asian mouse X-MLVs, as well as the ecotropic MLVs carried by these mice (17, 39), were introduced into fancy mouse colonies because of inclusion of Japanese mice in fancy mouse colonies;

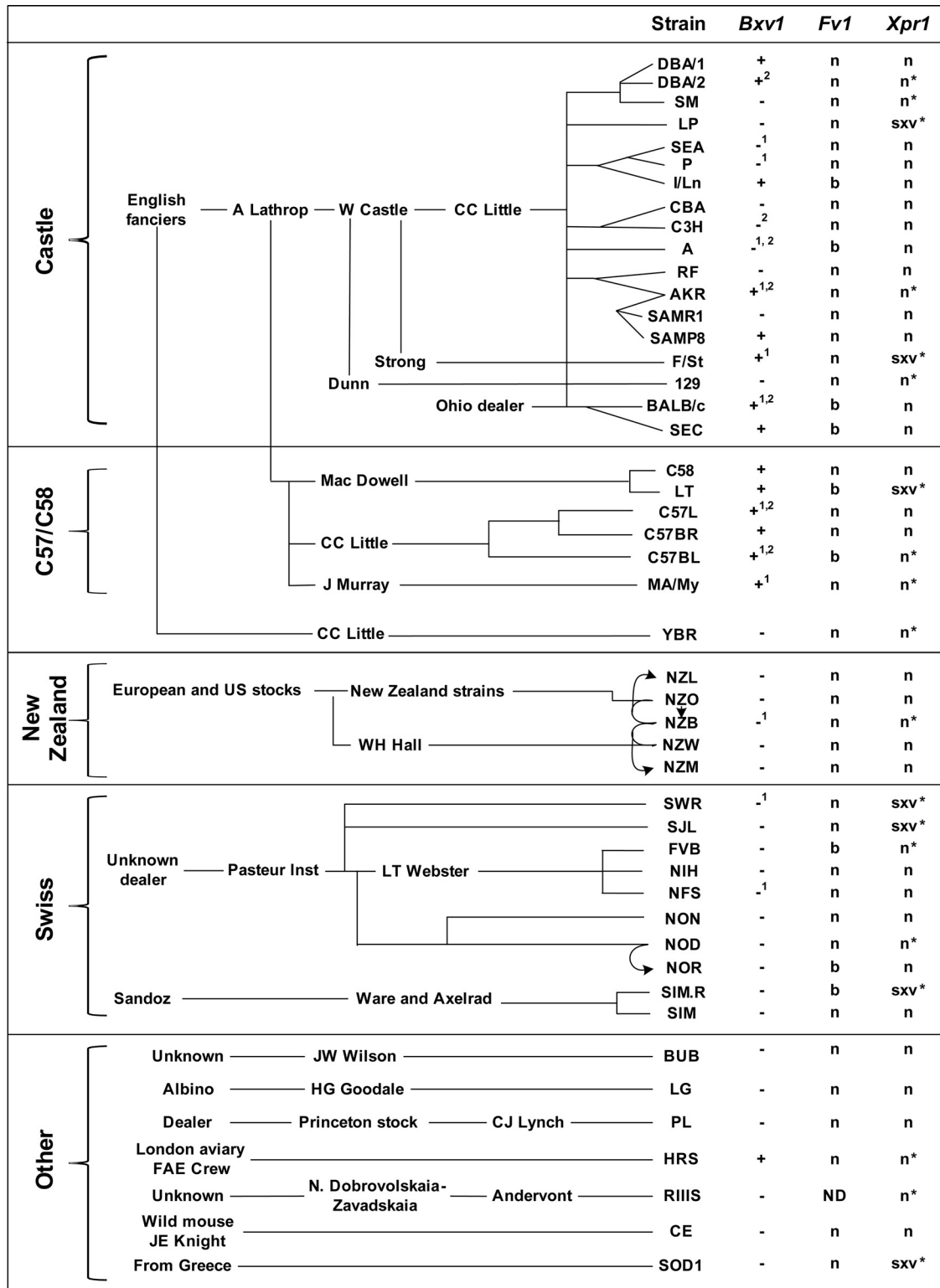


FIG. 2. Distribution of allelic variants of *Bxv1*, *Fv1*, and *Xpr1* in inbred strains of the laboratory mouse. The organization of strains into groups based on their related breeding histories is derived from the analysis done by Beck and associates (3). The *Bxv1* provirus insertion is identified by + and the empty locus by -; numbers identify strains previously typed for *Bxv1* as a Chr 1-linked induction locus (superscript "1") (18, 19, 51) or by Southern blotting (superscript "2") (8). *Fv1* typing identified the presence of the "n" or "b" restriction fragments that distinguish *Fv1ⁿ* and *Fv1^b* from *Fv1ⁿ* (45). Identical results were obtained for strain sublimes SJL/BmJ and SJL/J, for AKR/N and AKR/J, and for C57BL/6 and C57BL/10. *Xpr1* variants confirmed by sequencing are indicated with asterisks; all others were typed by PCR. SAMR1 and SAMP8 are hybrids of AKR and an unknown mouse. NOR was derived from a cross between NOD and C57BLKS/J. NZB was derived from NZO; NZM is a hybrid of NZB and NZW. NZL was developed from NZO with contributions from NZB, C57BL, and 129.

SPECIES	<i>Bxv1</i>	STRAINS/SPECIES
<i>M. castaneus</i>	+	
	-	CAS/Li, CAST/Ncr, CAST/EiJ, CAST/Rp, CASA/RkJ
<i>M. domesticus</i>	+	
	-	<i>M. domesticus</i> (Abu Rawash, Haven's Farm, Lewes, J.J. Downs, Watkin's Star, <i>praetextus</i> , Cal WM), CALB/RkJ, PERA/EiJ, PERC/EiJ, SF/Cam EiJ, RBA/DnJ, RBF/DnJ
<i>M. molossinus</i>	+	MOLC/RkJ, MOLD/RkJ, MSM/Ms, JF1/Ms
	-	MOL/Li, MOLF/EiJ, MOLG/DnJ
<i>M. musculus</i>	+	
	-	<i>M. musculus</i> (Belgrade, Brno, Skive, Vejrumbro, Viborg), PWD/PhJ, PWK/PhJ, CZECHI/EiJ, CZECHII/EiJ
Other	+	
	-	<i>M. abboti</i> , <i>M. fragilicauda</i> , <i>M. macedonicus</i> , <i>M. spretus</i>
House mouse	+	
	-	KK/H1J

FIG. 3. Distribution of *Bxv1* in wild mouse species and wild-derived inbred strains. DNAs were screened by PCR for the *Bxv1* insertion (+) or for the empty locus (-). A total of four wild-trapped California mice, along with California mouse-derived SC-1 cells, were tested.

in fact, Lathrop started her mouse colony with a pair of Japanese waltzing mice (22, 29, 37). It is also probable that the additional active proviruses found in laboratory mouse strains like NZB and MA/My may have been acquired from these Asian mice.

DNAs from *M. molossinus* and from the common laboratory strains were also typed for alleles of the *Fv1* gammaretrovirus restriction gene, a gene that targets the virus capsid and inhibits replication postentry. DNAs were typed using PCR primers that generate products of different sizes for *Fv1^b* (1.8 kb) and for *Fv1^m* or *Fv1^{nr}* (510 bp) (45). Among the laboratory strains, 28 of the 39 mice typed in this way as *Fv1^m* were found to lack *Bxv1*, but 6 of the 10 *Fv1^b* mice have *Bxv1* (Fig. 2). All *M. molossinus* DNAs tested (MOLC/RkJ, MOLD/RkJ, MSM/Ms, JF1/Ms, MOLF/EiJ) produced the longer *Fv1^b*-like PCR product, suggesting the possibility that the *Bxv1* virus is B tropic. However, *M. molossinus* cells have not been tested for *Fv1*-type restriction, and although PCR suggests that this *Fv1* resembles the longer *Fv1^b* at its 3' end, our previous sequence analysis of the *M. molossinus* *Fv1* gene identified *Fv1^{nr}* codons at key sites in the coding region (GenBank accession no. FJ603569). Furthermore, the *Fv1* tropism of the virus produced by *Bxv1* has not been determined, and the *Bxv1* capsid sequence has novel substitutions at key *Fv1* target sites, so its sensitivity to the laboratory mouse *Fv1* variants cannot be predicted. It is therefore not yet clear in what way the acquisition of the *Bxv1* provirus was influenced by *Fv1*.

***Xpr1* receptor alleles in viremic strains of laboratory mice.** Multiple host factors can influence the expression of proviral loci and the spread of virus *in vivo*, and the detection of infectious X-MLV or viral proteins depends on what proviruses are present and can also be influenced by genes at or near the major histocompatibility complex (MHC) locus and the *Fv1* gene (32, 51). For mice carrying active XMVs, another contributing factor is likely to be the XPR1 receptor used by X-MLVs for entry. Because functional variants of the XPR1 receptor for X-MLVs exist in the various mouse species that contributed to the laboratory mouse genome, we screened inbred strains for variants of *Xpr1*. Two inbred strains, NZB and F/St, are unusual among laboratory strains in that they constitutively express infectious X-MLV throughout life (6,

31). X-MLVs are readily isolated from lymphoid organs of these mice. NZB carries two active XMVs, and the high-virus expression pattern has been attributed to the constitutive expression of one of those XMVs (6), although the presence of a permissive XPR1 variant could also be a contributing factor. Neither of the XMV loci in NZB loci is *Bxv1*, as indicated by previous genetic analysis of this strain (16) and by our present study (Fig. 1). To determine if a permissive receptor contributes to the observed high-virus phenotype, we sequenced a segment of the NZB *Xpr1* gene encoding the receptor determinants in XPR1 ECL3 and ECL4 (28, 46). This sequencing identified the NZB receptor as the restrictive allele *Xpr1^m* (GenBank accession no. HQ323678), indicating that the viremia observed in these mice has no contribution from receptor-mediated secondary spread.

The second high-virus strain, F/St, produces detectable levels of X-MLV at 1 week of age and high levels from 4 weeks (31). This mouse carries one active XMV that was identified as the *Bxv1* provirus (Fig. 1), but this XMV is not associated with viremia in the other strains that carry it. We sequenced exon 13 of F/St *Xpr1*, which contains ECL4, and found that these mice carry the permissive *Xpr1^{svv}* allele (15, 28) (GenBank accession no. HQ323665). While this might suggest an explanation for the F/St high-virus phenotype, analysis of genetic crosses between F/St and other inbred strains have shown that viremia requires *Bxv1* and a recessive gene linked to the major histocompatibility locus (51). *Xpr1* is closely linked to *Bxv1* on Chr 1; its role in viremia cannot be evaluated from the existing data on F/St mice, and this strain has recently become extinct.

***Xpr1* variants in other inbred strains.** Rather than sequence XPR1 from dozens of mouse strains, we first screened DNAs of laboratory mouse strains by PCR for the presence of the permissive *Xpr1^{svv}* allele. There are five functionally distinct *Xpr1* alleles in *Mus*; 4 of these variants are found in the house mouse species and inbred strains, and only 1 of these variants, *Xpr1^{svv}*, encodes a fully permissive receptor (15). The other 3 house mouse receptors (*Xpr1^m*, *Xpr1^r*, and *Xpr1^c*) restrict 2 or more of the viruses that rely on XPR1 for entry, and all 3 of these restrictive receptors have deletions in the ECL4 segment of the receptor (48) (Fig. 4). The deletion found in *M. castaneus* (*Xpr1^c*) removes 5 codons, and the different deletions

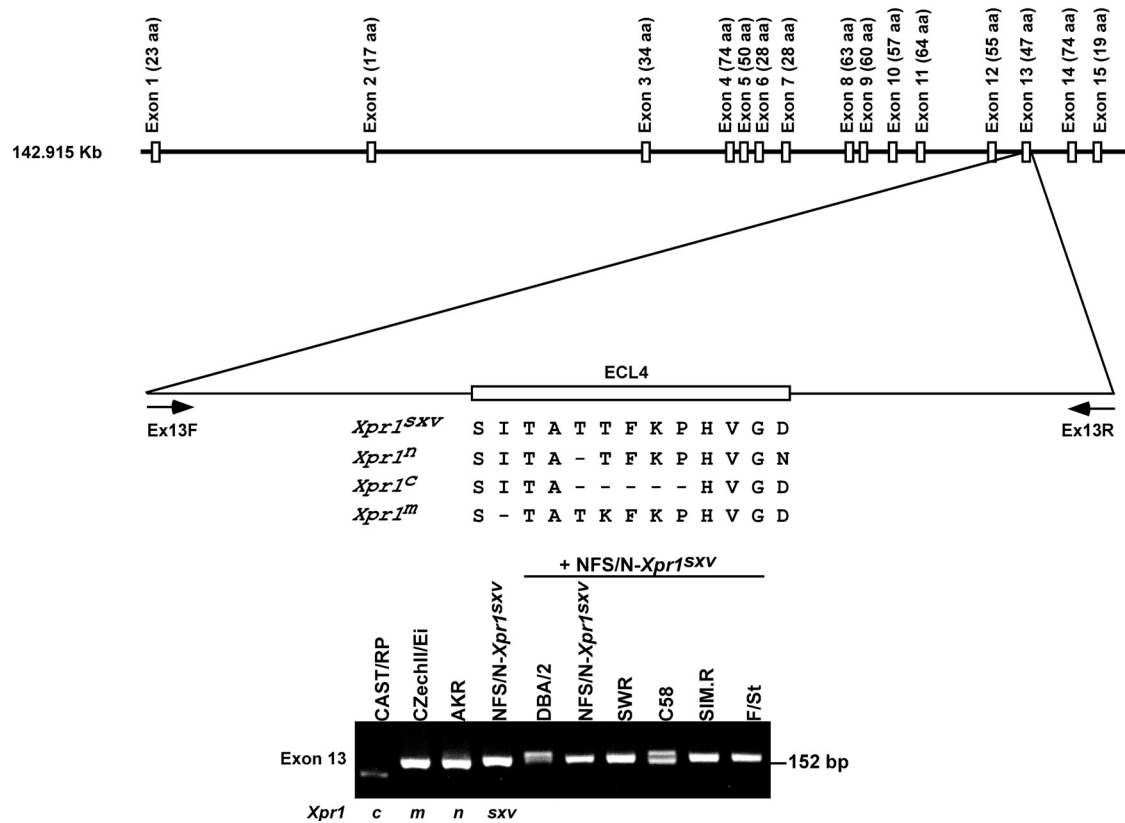


FIG. 4. *Xpr1* variants in laboratory mouse strains. The exon structure of the *Xpr1* gene is indicated at the top. Exon 13 contains the receptor determining the putative ECL4 loop and contains the indicated deletions or substitutions that distinguish the 4 house mouse alleles of *Xpr1*. aa, amino acids. At the bottom are shown PCR products using exon 13 primers, Ex13F and Ex13R. The first 4 lanes contain DNAs from mice carrying the 4 indicated alleles. The remaining 6 lanes contain DNAs of the indicated strains mixed with DNAs from mice carrying *Xpr1^{sxv}*. Doublets are indicative of mice carrying the *Xpr1ⁿ* deletion.

found in NIH 3T3 (*Xpr1ⁿ*) and *M. musculus* (*Xpr1^m*) remove single codons. The *m*, *n*, and *svx* alleles cannot be reliably distinguished by PCR product size differences, but mixtures of DNAs carrying the *m*, *c*, and *n* alleles with *Xpr1^{svx}* DNA produce PCR product doublets. Because we were specifically interested in identifying the permissive *Xpr1^{svx}* allele in laboratory mouse strains, we did an initial PCR screen by mixing DNAs from each mouse strain with DNA from a mouse carrying wild-mouse *Xpr1^{svx}* (Fig. 4).

Fifty strains were screened by this method, and 6 strains in addition to F/St were implicated as having *Xpr1^{svx}*. Sequencing confirmed the presence of this permissive allele in all 6 strains. *Xpr1^{svx}* was found in 3 of the 10 Swiss mouse DNAs and in the 3 mouse strains SOD1, LP, and LT/SvEi. We also sequenced exon 13 in 9 additional strains of interest. This segment of *Xpr1* is hypervariable in rodent species (48), and we wanted to identify the presence of any novel polymorphisms due to substitutions that would be missed by the PCR screen for insertions or deletions. The 9 strains selected for sequencing included AKR and HRS, 2 strains that have been used in studies on viral leukemogenesis, and RIHS, a strain that has acquired an XMV insertion in its *Apobec3* gammaretrovirus resistance gene (36). We also sequenced exon 13 in strains expressing high levels of nonectropic MLV envelope (Env) glycoprotein. For example, DBA mice carry a provirus associated with the *Rmcf* locus that

blocks the entry of viruses that use the *Xpr1* receptor (13) and also express high levels of XenCSA and GIX, cell surface antigens related to nonectropic MLV Env glycoprotein (30). 129-GIX+ mice also express the Env glycoprotein-related GIX antigen. Strains selected for sequencing also included 129, FVB, and NOD, because they are used as human disease models or for genetic manipulations, MA/My, a strain that carries two active XMV proviruses (16), and YBR, a strain produced from a different fancy mouse stock (Fig. 2). No novel sequence variants were identified in these additional mice; all of these strains were found to carry the exon 13 sequence of *Xpr1ⁿ*.

In the last few decades, a number of new inbred strains were derived from wild-trapped mice. These strains are unrelated to the fancy mouse-derived older common inbred strains, and they carry the *Xpr1* alleles found in their species of origin. Thus, strains derived from *M. molossinus* and *M. musculus* carry *Xpr1^m* and strains derived from *M. castaneus* carry *Xpr1^c* (48). Two inbred strains derived from wild-trapped *M. domesticus* mice, PWD and RBA, were found by sequencing to carry *Xpr1^{svx}*, as expected (GenBank accession no. HQ323672 and HQ323673).

X-MLV infectivity of cells derived from inbred strains SWR, SJL, and SIM.R. Three of the strains determined to carry the permissive *Xpr1^{svx}* receptor based on initial screening and se-

TABLE 1. Susceptibility of mouse cells to LacZ pseudotypes of X/P-MLVs

Mouse species, cell line, or strain	<i>Xpr1</i> allele	<i>Fv1</i> allele	Log ₁₀ titer ^a						
			X-MLV		XMRV	Cz524	CasE#1	P-MLV	
			CAST-X	AKR6				FrMCF	MoMCF
<i>M. dunni</i>	<i>scv</i>	<i>0</i>	7.1 ± 0.1	6.1 ± 0.3	5.1 ± 0.3	5.9 ± 0.1	6.4 ± 0.2	5.9 ± 0.5	4.6 ± 0.4
NIH 3T3	<i>n</i>	<i>n</i>	<0	<0	<0	<0	<0	5.5 ± 0.3	5.4 ± 0.2
SIM.R	<i>scv</i>	<i>b</i>	5.4 ± 0.2	5.4 ± 0.5	4.1 ± 0.2	4.7 ± 0.4	6.0 ± 0.8	5.4 ± 0.3	5.4 ± 0.6
SWR	<i>scv</i>	<i>n</i>	4.8 ± 0.2	4.0 ± 0.5	3.2 ± 1.5	4.8 ± 0.3	4.2 ± 0.6	5.2 ± 0.4	4.5
SJLSV	<i>scv</i>	<i>n</i>	5.4 ± 0.4	3.9	2.2 ± 0.4	4.5 ± 0.4	3.7 ± 0.4	4.9 ± 0.3	4.8 ± 0.1

^a Titers represent numbers of foci of LacZ-positive cells (log₁₀ in 1.0 ml). Where no standard deviation is given, infectivity was measured only once. <0, no positive cells after infection with undiluted pseudotype virus stock.

quencing of exon 13 were further characterized. We sequenced the entire *Xpr1* gene from SJL and SIM.R. The gene in both strains is identical to that originally cloned from *M. dunni* cells (28). This allele differs from *Xpr1ⁿ* by one insertion, Δ582T, and two substitutions, E500K and N590D.

The presence of a permissive receptor in these mice does not guarantee infectivity, as failure to infect can result from factors such as receptor interference (reviewed in reference 40). Therefore, we examined cells from all three of these strains for X-MLV infectivity. Cultured tail fibroblasts from SIM.R and SWR and an SV40-transformed line of SJL mouse fibroblasts were tested for susceptibility to LacZ retroviral vectors incorporating the Env glycoproteins of 2 mouse X-MLVs and 5 other virus isolates known to use the *Xpr1* receptor. These 5 additional viruses included 2 polytropic MLVs (P-MLVs), 2 wild mouse virus isolates (CasE#1 and Cz524), and XMRV, an X-MLV-related virus originally isolated from human patients with prostate cancer (7). *Xpr1ⁿ* restricts all these viruses except the P-MLVs, while *Xpr1^{scv}* is permissive for all 7 viruses (47) (Table 1).

The cells from these three inbred mouse strains are susceptible to all viruses (Table 1), confirming that these cells all express a fully permissive XPR1 receptor. Some viruses infected these cells with nearly equal efficiencies, but most of the viruses showed infectivity differences that are consistent with *Fv1* restriction. All MLVs are potentially subject to *Fv1* restriction, including X-MLVs (35), and mouse-tropic MLVs have been typed as N or B tropic based on their restriction by *Fv1^b* or *Fv1ⁿ*, respectively, or as NB tropic if unaffected by either allele. SIM.R was developed as a congenic strain of Swiss noninbred mice and carries the *Fv1^b* allele from C57BL/6 (44). The presence of *Fv1^b* in the SIM.R cells and *Fv1ⁿ* in SJLSV was also determined by PCR. A segment of *Fv1* was cloned from SWR cell DNA and identified as *Fv1ⁿ* by sequencing (GenBank accession no. HQ323682).

Two of the viruses used here are NB tropic, and these two P-MLVs, FrMCF and MoMCF, infected all 5 mouse cells with roughly equal efficiencies (Table 1). The other 5 viruses have unknown *Fv1* tropism, but all 5 showed reduced LacZ titers in some or all 3 of the *Xpr1^{scv}* laboratory mouse cells relative to titers in *M. dunni*, which lacks a functional *Fv1* gene *Fv1⁰* (45). This was somewhat unexpected, as the LacZ pseudotypes used here were produced by virus infection of GP2-293 cells, which carry ecotropic Moloney MLV *gag-pol* genes and therefore produce NB-tropic MoMLV capsids as well as capsids of the infecting virus. The fact that none of these 5 pseudotypes are

NB tropic indicates that tropism is influenced by the infecting virus. Two of those viruses, CasE#1 and AKR6, more efficiently infected SIM.R than they infected SWR or SJL, suggesting that these viruses are restricted by *Fv1ⁿ* and may therefore be B tropic. CAST-X, Cz524, and XMRV are restricted in all 3 of these cells relative to levels of infection in *M. dunni* cells, although one virus, XMRV, appears somewhat more sensitive to *Fv1ⁿ* than to *Fv1^b*. The restriction of XMRV by *Fv1ⁿ* and *Fv1^b* has been reported previously (10, 47), and these results suggest that CAST-X and Cz524 may have similar *Fv1*-defined tropisms.

DISCUSSION

The acquisition of endogenous nonectropic gammaretroviruses, the time course and tissue specificity of their expression, and the horizontal transmission of these viruses to new hosts are governed by various host factors that restrict or enhance replication and spread. While numerous studies have focused on host restriction genes that can inhibit virus replication or host immune system genes that alter *in vivo* spread, receptor polymorphisms can clearly either facilitate infection or provide an effective host defense against pathogenic and mutagenic viruses. We have identified 5 functional variants of the mouse XPR1 receptor for the xenotropic/polytropic family of gammaretroviruses, 4 of which are capable of restricting infection by multiple members of this family. The distribution of these alleles in wild mice indicates that, not surprisingly, species exposed to infectious virus carry restrictive alleles (48). The first of these alleles to be recognized, *Xpr1ⁿ*, was identified in laboratory mice and was assumed to be representative of all mice; this conclusion, that mice are resistant to infection by xenotropic MLVs, discouraged further analysis of these infectious agents in their natural host. Our findings, that a number of mouse strains carry the permissive allele of the XPR1 receptor and that cells from these mice are as infectable by X-MLVs as cells of the wild mouse species *M. dunni*, mean that laboratory mouse strains can, in principle, be used to develop mouse models to study expression of endogenous proviruses such as *Bxv1* and to describe the course and consequences of exogenous infection by these mouse viruses.

Inbred mouse strains that carry all 5 of the known functional variants of mouse XPR1 are available. Three Eurasian mouse variants (*Xpr1^c*, *Xpr1^m*, *Xpr1^p*) are found in inbred lines recently developed from *M. castaneus*, *M. musculus*, *M. molossinus*, and *Mus pahari*, but it was surprising to also find that the

older common inbred lines carry two variants of *Xpr1*. One of these variants, *Xpr1^{svv}*, is widespread in wild mouse species, while *Xpr1ⁿ* has not been identified in any wild-derived or wild-trapped mouse (48). The X-MLV-restrictive allele, *Xpr1ⁿ*, is carried by the majority of these older strains of laboratory mice, while we identified the X-MLV-permissive allele, *Xpr1^{svv}*, in 3 of 10 Swiss mouse strains and in several strains developed from the Lathrop colonies.

Xpr1ⁿ is the only one of the 5 *Mus Xpr1* alleles to completely restrict X-MLVs, and its origin is unclear. Studies to determine the wild mouse origins of the common inbred strains have concluded that the largest contribution to the inbred mouse genome is from *M. domesticus* (49). It makes sense for *M. domesticus* to carry *Xpr1ⁿ*, as these mice lack endogenous X-MVs, consistent with *Xpr1ⁿ* restriction of X-MLVs. However, our survey of wild mouse species failed to find *Xpr1ⁿ* in any *M. domesticus* mouse or in any wild mouse of any species (48). It is of course possible that *Xpr1ⁿ* is present in some unidentified wild mouse populations that contributed to the laboratory mouse genome. Alternatively, it is possible that this allele arose in the fancy mouse colonies that were maintained by hobbyists for centuries (29). These colonies included mice from multiple species that normally do not interbreed in nature, and these hybrids acquired an increased burden of MLV-endogenous retroviruses of different subtypes that are otherwise segregated in the wild mouse species (17). Artificial breeding protocols might also have resulted in the loss of host factors capable of restricting virus in natural populations, like interfering Env genes. Thus, the appearance of a restrictive receptor allele might have provided a survival advantage leading to its selection and near fixation in these fancy mice.

The XPR1 receptor defines the virus subtypes that can infect the host species. XPR1 therefore controls the type of endogenous retroviruses that accumulate in host genomes and also affects expression of these endogenous viruses in the individual animals. Whether the inbred strains carrying *Xpr1^{svv}* differ from strains carrying *Xpr1ⁿ* in their expression of endogenous viruses like *Bxv1* or whether these strains will permit efficient *in vivo* replication of exogenous X-MLVs is not yet known but is suggested by the fact that one strain viremic with xenotropic MLV, F/St, carries *Bxv1* along with the permissive *Xpr1^{svv}* receptor, although other factors, like MHC haplotype (51), clearly influence this unusual phenotype. While F/St is no longer extant, one additional strain identified here, LT/SvEi, carries the *svv* receptor as well as *Bxv1* and can be examined for X-MLV expression.

The *Xpr1^{svv}* strains identified here could be used to develop mouse models for the analysis of X-MLV replication, tissue tropism, and pathogenesis and could also be used to determine whether the different host range subtypes in the X/P-MLV infectious-virus family have different *in vivo* properties. Previous analyses of the disease process in naturally highly leukemic strains, like AKR, while focused on ecotropic and polytropic MLVs, have recognized that at least one X-MV, *Bxv1*, contributes to leukemogenesis by providing LTR sequences to the pathogenic P-MLV recombinants that arise in these mice (11, 41). These studies can now be expanded to determine how polymorphic variants of the XPR1 receptor affect disease type or latency as well as the type of recombinant viruses that appear. Studies on pathogenesis by exogenous X-MLVs can

make use of the Swiss strain-derived mice carrying *Xpr1^{svv}*. While SIM.R and SJL have not been characterized for their proviral content, SWR and other Swiss strain-derived mice, like NFS, carry only a single X-MV copy (11, 34). The near absence of endogenous X-MVs in these X-MLV-susceptible mice should simplify analysis of exogenous-virus infection and pathogenesis. The analysis of X-MLV expression and pathogenesis in such mouse models could have broader relevance, particularly for studies on the origin, pathogenesis, and trans-species transmission of XMRV.

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