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Antiretroviral restriction factors in mice

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ABSTRACT

One of the most exciting areas in contemporary retrovirus research is the discovery of "restriction factors". These are cellular proteins that act after virus entry to inhibit infection by or replication of retroviruses (and other viruses and intracellular pathogens). We briefly discuss here three antiretroviral restriction factors in mice: Fv1, APOBEC3, and tetherin, touching on both biological and molecular aspects of these restriction systems.

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One of the most exciting areas in contemporary retrovirus research is the discovery of antiviral "restriction factors". These are components of the innate immune system by which the host organism interferes with viral infection or replication at points in the life cycle after entry into the host cell. The analysis necessarily includes the study of the countermeasures employed by the viruses to evade the restriction factors. We will briefly review the three best-characterized restriction factors in mice: Fv1, APOBEC3, and BST2/Tetherin (summarized in Table 1). There are analogous systems in humans and it seems likely that other restriction factors remain to be discovered. Dissection of the restriction mechanisms inevitably sheds light on the details of viral replication, and moreover carries the hope that the restrictions might somehow be exploited to bolster antiviral defenses.

1. Fv1 restriction

Fv1 restriction was the first antiretroviral restriction factor to be discovered. Fv1 was originally identified as a gene controlling the susceptibility of mice to Friend murine leukemia virus (MLV)-induced leukemia (Lilly, 1970). It was soon found that the gene was operative not only in mice, but also in cultured mouse cells (Pincus et al., 1971; Rowe et al., 1973). The two principal alleles of Fv1 were that in NIH Swiss mice ($Fv1^n$) and that in BALB/c mice ($Fv1^b$), and MLV isolates that could grow well in cells from $Fv1^n$ mice were called "N-tropic", while $Fv1^b$ cells were permissive for "B-tropic" MLVs. Resistance to virus of the opposite tropism was semidominant, as $Fv1^n/Fv1^b$ cells (derived from BALB/c × NIH Swiss F1 mice) were resistant to both B-tropic and N-tropic MLVs. This key facet of the system implied that the $Fv1^n$ allele somehow blocks replication of B-tropic MLV and *vice versa*. (In addition to $Fv1^n$ and $Fv1^b$, a distinct Fv1 allele, "$Fv1^{nr}$", is present in some strains of inbred mice, including 129 mice. $Fv1^{nr}$ restricts some N-tropic MLVs, as well as B-tropic MLVs (Pincus et al., 1971)). On the other hand, some MLV isolates that had been passaged in the laboratory, such as Moloney MLV, had become insensitive to Fv1 restriction: these were called NB-tropic MLVs.

The Fv1 gene is present in many species of mice. Interestingly, the Fv1 alleles isolated from mouse species other than *Mus musculus* show remarkable diversity in the "target" viruses that they restrict (see below) (Yap et al., 2014).

Research into the mechanisms of Fv1 restriction has yielded many surprises. When it was first investigated, the only known controls on the host-range of animal viruses involved the cell entry step: it was thus a natural assumption that Fv1 restriction somehow interfered with the entry of the restricted virus into the host cell. When this assumption was found to be incorrect, the only remaining paradigm for the restriction seemed to be by analogy to bacteriophage lambda repression, in which the restrictive protein binds to cis-acting sequences in the viral genome, preventing their transcription. However, this expectation had to be discarded when it was found that MLVs could "donate" their tropism (e.g., the sensitivity of N-tropic MLV to $Fv1^b$ restriction) to replication-defective acute transforming viruses (Bassin et al., 1975). In fact, MLVs could

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Table 1
Key features of the three antiretroviral restriction systems in mice discussed in this review.

Restriction factor	MLV replication step targeted	Mechanism of action	Where found	Polymorphic in mice
Fv1	Between DNA synthesis and integration	Fv1 protein binds to CA in assembled cores	Some mouse species	Yes
APOBEC3	Before or at DNA synthesis	?	All placental mammals	Yes
BST2/Tetherin	Virus release	Tethering	All mammals?	Yes

undergo phenotypic mixing with respect to their tropism: MLV particles produced in cells containing both N- and B-tropic MLVs were found to be phenotypically sensitive to both $Fv1^b$ and $Fv1^n$ restriction, although they were still genetically either N- or B-tropic, like their parents (Rein et al., 1976). Further studies showed that there was a direct linkage between the viral capsid protein $p30^{CA}$ and the tropism of the virus (Hopkins et al., 1977; Rommelaere et al., 1979; Schindler et al., 1977). Viral tropism is largely determined by the identity of residue 110 of $p30^{CA}$, together with neighboring amino acids (Jung and Kozak, 2000; Kozak and Chakraborti, 1996; Stevens et al., 2004).

When the relationship between the concentration of virus in the inoculum and the number of infections was analyzed quantitatively, another remarkable property of Fv1 restriction was revealed. When virus is added to a cell culture under normal circumstances, the number of infections is a linear function of the virus concentration; this simple linear relationship shows that each infection is initiated by a single virus particle. However, when (for example) $Fv1^b$ cells were infected with N-tropic MLV, the number of infections was found to vary with the square of the virus concentration (Duran-Troise et al., 1977). This means that *two* N-tropic virus particles are required for infection of the $Fv1^b$ cells. Subsequent studies showed that the roles of the two particles are quite distinct. One of them does not contribute genetically to the infection, but in essence renders the cell permissive for normal infection by the other virus. This was termed "abrogation" of Fv1 restriction. The permissive state induced by the abrogating virus lasts less than 18 h. The abrogating virus must be of the restricted tropism, but it need not be fully infectious: particles lacking reverse transcriptase activity can still abrogate Fv1 restriction (Bassin et al., 1980).

The Fv1 gene was finally cloned in 1996 (Best et al., 1996). Surprisingly, its closest relative appears to be the *gag* gene of an endogenous retrovirus family, MERV-L, which is present in many copies in the mouse (and human) genome. The presence of Fv1 in some, but not all, mouse species indicates that it was introduced into the mouse germline roughly 7 million years ago, presumably by infection with an ancient retrovirus. When the Fv1 genes of different mice are compared, a number of residues are found to have a high ratio of non-synonymous to synonymous differences, indicating that they have been subject to positive selection during mouse evolution. This presumably reflects ongoing evolutionary battles between viruses and their hosts (Meyerson and Sawyer, 2011); these battles evidently began well before the appearance of contemporary MLVs (Qi et al., 1998; Yan et al., 2009). Indeed, Fv1 loci isolated from different mice can restrict a wide spectrum of retroviruses, including the lentivirus equine infectious anemia virus and the spumaretrovirus feline foamy virus (Yap et al., 2014). It is striking that several of the residues showing evidence of positive selection have previously been identified as critical for restriction of MLVs (Yan et al., 2009).

The N-terminal portion of the Fv1 gene product contains a coiled-coil domain, and it has been suggested that the ability of the protein to self-associate is critical for its restriction activity (Bishop et al., 2006; Yap et al., 2007). The protein also contains a sequence, called the "major homology region" (MHR), found in the capsid domain of all orthoretroviral Gag proteins (Wills and Craven, 1991). This motif in Gag proteins is known to be important for their assembly into immature virus particles, but beyond this its significance is not well understood. The MHR in Fv1 is also critical for Fv1 restriction and contains residues that have experienced positive selection during evolution (Bishop et al., 2001; Yan et al., 2009). The C-terminal portion of Fv1 contains several regions that differ in sequence in different mouse species; this region is known to be important in the specific recognition of MLV $p30^{CA}$ (Sanz-Ramos and Stoye, 2013; Yap et al., 2014). Interestingly, this overall arrangement of the protein is analogous to that in Trim5α, a restriction factor in primates and other mammals with no sequence similarity to Fv1. While Trim5α was discovered by virtue of its activity against HIV-1, it can also restrict N-tropic, but not B- or NB-tropic, MLVs. In both cases the target of the restriction is the CA molecule, despite the fact that the CA proteins of HIV-1 and MLV show very low resemblance in primary amino acid sequence. Residue 110 of MLV CA is crucial for sensitivity to Trim5α, as well as Fv1.

The mechanism by which Fv1 restriction blocks MLV infection is still not completely clear. Under natural conditions, the Fv1 gene is expressed at such low levels that its protein product is undetectable; this low level presumably explains the fact that infection with a single restricted particle saturates the restriction machinery, rendering the cell transiently permissive (Duran-Troise et al., 1977). In turn, this confounds efforts to analyze the block in infection: biochemical studies are far easier at high multiplicities of infection (moi) than at low moi's, but natural Fv1 restriction is overcome at high moi's. Nevertheless, early studies showed convincingly that upon infection of the restrictive host, an MLV successfully copies its RNA into DNA, as in a normal infection, but that this DNA is not integrated into host chromosomal DNA (Jolicoeur and Baltimore, 1976; Jolicoeur and Rassart, 1980; Yang et al., 1980). Thus the restricted infection could be blocked at integration, or alternatively, at a step required for integration, such as association of the pre-integration complex with mitotic chromosomes or its release from these chromosomes after mitosis (Elis et al., 2012; Schneider et al., 2013).

While the biological properties of Fv1 restriction strongly imply that the Fv1 gene product should specifically interact with CA protein of restricted MLV, even this basic prediction was only confirmed very recently. Several lines of evidence indicate that the Fv1 protein will not interact with free CA, nor with the CA domain of Gag even in the Gag lattice of an immature virus particle, but only with CA protein in the latticework of the core of the mature MLV particle (e.g., (Duran-Troise et al., 1981)). In 2011, Hilditch et al. showed that purified, recombinant CA molecules could be assembled into a lattice underlain by lipid nanotubes *in vitro*; this lattice apparently resembles that in a mature virion closely enough that the restrictive Fv1 protein binds to it (Hilditch et al., 2011). A number of controls all supported the hypothesis that the binding observed in these experiments represents the specific binding leading to restriction *in vivo*. It seems likely that this ability to monitor the Fv1–CA interaction *in vitro* will facilitate further insight into the molecular mechanics of the restriction.

2. APOBEC3

All placental mammals synthesize one or more APOBEC3 proteins; the human genome contains seven APOBEC3 genes (Jarmuz et al., 2002). The ability of these proteins to interfere