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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.

Keywords

Murine Leukemia Virus; Retrovirus; Fv1 restriction; APOBEC3; Tetherin

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.

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 (Fv1ⁿ) and that in BALB/c mice (Fv1^b), and MLV isolates that could grow well in cells from Fv1ⁿ mice were

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called “N-tropic”, while Fv1^b cells were permissive for “B-tropic” MLVs. Resistance to virus of the opposite tropism was semidominant, as Fv1ⁿ/Fv1^b cells (derived from BALB/c x NIH Swiss F1 mice) were resistant to both B-tropic and N-tropic MLVs. This key facet of the system implied that the Fv1ⁿ allele somehow blocks replication of B-tropic MLV and *vice versa*. (In addition to Fv1ⁿ 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 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 Fv1ⁿ 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 hr. 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 Trim5 α , a restriction factor in primates and other mammals with no sequence similarity to Fv1. While Trim5 α 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 Trim5 α , 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.

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 with retroviral replication was first discovered in 2002, when Sheehy et al. reported that APOBEC3G (hA3G) could block the replication of a class of mutants of HIV-1 (Sheehy et al., 2002), namely mutants with defects in the *vif* gene. Later studies showed that the viral “accessory protein” Vif binds to hA3G and targets it for proteasomal degradation (Yu et al., 2003).

hA3G is very potent in its ability to interfere with HIV-1 replication. For this reason, the entire APOBEC3 family has been the subject of intense interest since these initial findings. Briefly, the APOBEC3s are cytidine deaminases. In general, they act on cytidines within single-stranded (ss) DNA, converting them to uridines. As the ssDNA intermediate in retroviral replication is the “minus” strand, complementary to the viral RNA, this transition mutation results in the replacement of guanines with adenines in the sequence of the “sense” or coding strand in proviral DNA. In the absence of Vif, hA3G can cause G:A mutations at extraordinary frequencies.

In the absence of Vif, hA3G can be incorporated into assembling HIV-1 particles. However, there is still some confusion about the mechanism (or mechanisms) by which it blocks HIV-1 replication. On the one hand, when these virions infect a new host cell, the hA3G deaminates cytidines to uridines in the newly synthesized (-) strand ssDNA, leading to high levels of G to A mutations. On the other hand, the encapsidated hA3G can also block DNA synthesis and DNA integration. The key to reconciling these multiple modes of action may lie in the finding that hA3G can exist as a monomer or dimer or, alternatively, as a larger

oligomer. In the former state, it binds to and releases from ssDNA very rapidly and deaminates cytidines in the DNA. However, in the latter state, its off-rate is far slower, and it simply acts as a “roadblock” impeding the progression of reverse transcriptase (Chaurasiya et al., 2014). Thus the nature of the restriction may be a function of the hA3G concentration or of unknown variables determining the oligomeric state of the hA3G.

Unlike humans, mice contain only one APOBEC3 gene. Mouse APOBEC3 (mA3) is a cytidine deaminase; when it is tested against HIV-1, its activity is very similar to that of hA3G, except that it is unaffected by Vif (Mariani et al., 2003). However, its effects on MLV are quite different. Its ability to block MLV infection is easily detectable, but is far lower than its activity against HIV-1 (Browne and Littman, 2008; Rulli et al., 2008). Remarkably, the restriction of Moloney and many other MLV isolates by mA3 is not accompanied by detectable G to A mutation (Browne and Littman, 2008; Rulli et al., 2008). Rather, MLV particles whose replication is blocked by mA3 are evidently unable to synthesize DNA when inoculated onto new host cells; thus, mA3 blocks MLV infection at or before the initiation of reverse transcription (Sanchez-Martinez et al., 2012). While these results were obtained in highly artificial systems, using cultured cells and plasmid transfections, they seem to apply well to natural restriction of retroviruses by the naturally expressed mA3 in mice (Low et al., 2009; Santiago et al., 2008; Takeda et al., 2008). Moreover, the mechanism of restriction of the betaretrovirus mouse mammary tumor virus (MMTV) by mA3 seems to be completely analogous to the restriction of MLV (a gammaretrovirus) described here (MacMillan et al., 2013; Okeoma et al., 2010; Okeoma et al., 2007; Okeoma et al., 2009).

How MLV particles carrying mA3 evade its mutagenic activity is not known. The deaminase activity of the encapsidated mA3 can be readily detected biochemically in these particles (Nair et al., 2014). Recent studies raise the possibility that the relative resistance of MLV to mA3 is due to the action of “glycoGag” (Kolokithas et al., 2010; Stavrou et al., 2013). This is an alternative form of the MLV Gag protein. It has an N-terminal extension relative to the normal Gag protein; this extension includes a signal sequence, leading to its glycosylation. Its function is not understood, but it was observed many years ago that it is unimportant for MLV replication in cultured cells but strongly facilitates virus replication in mice (Corbin et al., 1994; Fan et al., 1983; Schwartzberg et al., 1983). It was recently reported that the selective pressure for viral glycoGag expression in infected mice depends upon the expression of mA3 in the mice (Stavrou et al., 2013). This striking observation strongly suggests that protection of MLV particles against mA3 restriction is somehow mediated by their glycoGag protein.

Several lines of evidence indicate that APOBEC3 proteins are incorporated into retrovirus particles by binding to the RNA during particle assembly (Svarovskaia et al., 2004). All APOBEC3 proteins are composed of either one or two domains with a typical “deaminase fold”, consisting of 6 helices and 6 β -strands in arranged in $\alpha 1$ - $\beta 1$ - $\beta 2$ - $\beta 3$ - $\alpha 2$ - $\beta 4$ - $\alpha 3$ - $\beta 5$ - $\alpha 4$ - $\beta 6$ - $\alpha 5$ - $\alpha 6$ configuration, with $\beta 1$, $\beta 2$, $\beta 3$ being anti-parallel and $\beta 4$, $\beta 5$, $\beta 6$ in parallel orientation. Each such domain contains both the sequence H-X-E, where E is the deaminase active site if the domain has deaminase activity, and the zinc finger motif P-C-X₂₋₄-C, which is important for RNA-binding and encapsidation of the protein (Vasudevan et al., 2013).

mA3, like hA3G, has two such domains. In hA3G, the N-terminal domain lacks deaminase activity, but is necessary for hA3G encapsidation, while the C-terminal domain is an active deaminase. Interestingly, the functional arrangement of mA3 is opposite to that of hA3G: the C-terminal domain is necessary and sufficient for encapsidation, while the N-terminal domain carries the cytidine deaminase activity (Hakata and Landau, 2006; Nair et al., 2014).

What is the selective pressure responsible for the maintenance of mA3 in the mouse genome? It is noteworthy that some mouse strains that contain high levels of xenotropic MLV throughout life have an APOBEC3 gene with an insertion raising its expression level (Sanville et al., 2010). However, as the APOBEC3 gene family is present in all placental mammals, it is incomparably more ancient than the contemporary MLVs discussed here. mA3 has also been shown to block intracellular retrotransposition by MusD elements; these closely resemble retroviruses, but lack *env* genes and replicate without an extracellular phase (Esnault et al., 2006; Ribet et al., 2004). Non-LTR retrotransposons in the mouse genome also contain clusters of G to A mutations, undoubtedly the result of mA3 activity (Carmi et al., 2011). It is clear that mA3 and infectious elements have a long mutual history: like Fv1, mA3 has undergone positive selection during mouse evolution (Sanville et al., 2010). In fact, many of the endogenous MLV genomes in the mouse genome were subjected to G to A mutagenesis by mA3 when they infected the mouse germline (Jern et al., 2007); these are genomes that do not encode glycoGag (Jern et al., 2007; Nitta et al., 2012). (G to A mutations are also induced in AKV, an endogenous MLV, by mA3 (Langlois et al., 2009)). It seems plausible that mA3 has functioned throughout mouse evolution as an inhibitor of retrotransposons (and possibly other elements as well). More generally, perhaps APOBEC3 proteins have served as a first line of defense against invaders of the genome throughout mammalian evolution.

CD317/BST2/Tetherin

The third “restriction factor” reviewed here has been variously called CD317, BST2, HM1.24, and tetherin. Its restriction activity was recognized when its properties were found to be congruent with those of a putative target of the HIV-1 “accessory protein” Vpu (Strebel et al., 1988). Thus, it was known that Vpu could dramatically facilitate the release of HIV-1 virions from some cell lines, while release from other cells was Vpu-independent (Geraghty et al., 1994; Sakai et al., 1995). Heterokaryons between cells of these two types resembled the former cells in that efficient virus release required Vpu (Varthakavi et al., 2003). The effect of Vpu was not limited to HIV-1, but extended to a variety of other viruses (Gottlinger et al., 1993). In some cell lines, treatment with interferon α induced a requirement for Vpu for virus release (Neil et al., 2007). Two papers in 2008 reported that “tetherin” was present in some cells but not others, was interferon-inducible in some cells, interfered with the release of a variety of enveloped viruses from the cell surface, and was antagonized by Vpu, just as predicted from the earlier studies (Neil et al., 2008; Van Damme et al., 2008).

Tetherin is a protein with a highly unusual structure. It is a type II membrane protein, with a cytoplasmic domain at its N-terminus and a glyco-phosphatidyl inositol (GPI) anchor at its C-terminus. Between these two termini, there is a highly extended α -helical extracellular

domain (Swiecki et al., 2011; Yang et al., 2010); the protein is a disulfide-linked dimer and is glycosylated (Andrew et al., 2009; Perez-Caballero et al., 2009). Several lines of evidence indicate that it interferes directly with virus particle release by virtue of its unusual topology: evidently, one end of the protein is embedded within the host cell, while the other is embedded within the virion (Perez-Caballero et al., 2009; Venkatesh and Bieniasz, 2013). Comparison of the tetherins encoded in different strains of laboratory mice demonstrates a strong correlation between the level of cell-surface display of tetherin and its antiviral activity (Barrett et al., 2012)

While the bulk of studies on tetherin focus on its ability to restrict HIV-1 replication, it has been shown that naturally expressed mouse tetherin can significantly impair the replication of both MLV (Goffinet et al., 2010) and MMTV (Jones et al., 2012). It has been suggested that MLVs are frequently able to bypass this restriction in mice because tetherin is not constitutively expressed on the lymphoid target cells (Liberatore and Bieniasz, 2011; Swiecki et al., 2012). On the other hand, it is widely expressed following interferon treatment, and type I interferon expression is induced in mice by some MLVs (Liberatore and Bieniasz, 2011). The biological role(s) of tetherin is not fully understood, as there are indications that it participates in signaling cascades within the immune system (Cao et al., 2009) and can either increase (Swiecki et al., 2012) or decrease (Cao et al., 2009) interferon production. Many of these issues will undoubtedly be clarified in the next few years.

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Highlights

- We briefly discuss “restriction factors” that block retrovirus replication in mice.
- The restriction factors reviewed are Fv1, APOBEC3, and Tetherin.
- We review their modes of action and evolutionary origins.

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