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Antiretroviral Restriction Factors

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Abstract

Studies of retroviruses have been instrumental in revealing the existence of an array of antiviral proteins, or restriction factors, and the mechanisms by which they function. Some restriction factors appear to specifically inhibit retrovirus replication, while others have a broader antiviral action. Here, we briefly review current understanding of the mechanisms by which several such proteins exert antiviral activity. We also discuss how retroviruses have evolved to evade or antagonize antiviral proteins, including through the action of viral accessory proteins. Restriction factors, their viral targets and antagonists have exerted evolutionary pressure on each other, resulting in specialization and barriers to cross-species transmission. Potentially, this recently revealed intrinsic system of antiviral immunity might be mobilized for therapeutic benefit.

Introduction

Although direct antiviral mediators are known or suspected to exist for a variety of viral families, a significant impetus for the study of such proteins has come from recent discoveries made in the field of retrovirology. Most modern retroviruses are specialists, having adapted to colonize a single or narrow range of host species and/or cell types, and for many years, this host range was thought to be determined primarily by their ability (or inability) to parasitize the particular variants of required molecules (e.g. receptors) that are present in a candidate host cell type or species. Indeed, it was almost completely unappreciated that viruses have driven the evolution of defense mechanisms that directly inhibit specific processes in the retrovirus life cycle. In recent years, this view has changed, and we now know of several specific antiretroviral proteins encoded by host cells (Figure 1), and we have come to appreciate that variation in host defenses is a critical determinant of host range. The study of known antiretroviral defenses is currently one of the most vibrant areas of virology, particularly since some defense mechanisms have broader activity, and target a variety of virus families.

While several antiretroviral gene products have arisen and evolved independently, and have nothing in common in the mechanisms by which they function, they often share some key features. First, they are encoded by functionally autonomous, individual genes and inhibit retrovirus replication in cell culture. Second, some are type-I interferon (IFN α/β) stimulated genes (ISGs) in at least some cell types. Third, retroviruses are often found to have evolved

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to resist the action of antiretroviral proteins in their natural host species, sometimes (e.g. in the HIV/SIV group of retroviruses) by encoding accessory genes that antagonize the inhibitor's function. Fourth, the sequence of antiretroviral genes tend to be unusually variable in primates, and have evolved via diversifying selection - i.e. they exhibit high dN/dS ratios over at least a portion of their coding sequence. The unusually high variability in antiretroviral genes means that retroviruses are often adapted to evade particular variants of these proteins that are present in their natural hosts, and barriers to cross species transmission are thus imposed. Fifth, antiretroviral proteins tend to function in unanticipated, unique and interesting ways. Here, we review our current understanding of several of the presently known antiretroviral proteins.

APOBEC3 proteins

A key cellular antiretroviral factor is Apolipoprotein B editing catalytic subunit-like 3G (APOBEC3G)[1]. This is a member of a family of enzymes with cytidine deaminase activity whose number has expanded during mammalian evolution to 7 members that are well conserved in primates[2,3]. In addition to APOBEC3G, other members of the family, including APOBEC3F and 3H, are active against retroviruses, whereas others inhibit retrotransposons and some even target viruses from other families (reviewed in[4]). APOBEC3 proteins inhibit virus infectivity by an unusual mechanism. The proteins themselves are incorporated into retroviral particles via interaction with RNA[5-7]. While, intuitively, it seems likely that some specific interaction with viral RNA drives APOBEC3 incorporation (since viral RNA represents only a small percentage of the total RNA in the cell cytoplasm), APOBEC3 proteins are incorporated into retroviruses whose sequence is highly divergent. Upon infection of a new target cell, the viral capsid core, which contains APOBEC3 as well as viral components, is released into the target cell cytoplasm. Thereafter, reverse transcription and digestion of the viral RNA results in the single minus-strand DNA intermediate that becomes a target for APOBEC3 catalyzed deamination [8-11]. The viral genome thus becomes 'poisoned' with G-to-A mutations that inactivate the virus (Figure1). Additionally, it has been suggested that APOBEC3 proteins inhibit virus infectivity via an unknown mechanism that is independent of enzymatic activity[12]. This, might be a consequence APOBEC3 binding to viral RNA, which could interfere with reverse transcription.

The weapon chosen by lentiviruses to combat APOBEC3 inhibition is a small viral protein named Vif[1]. Vif proteins block APOBEC3 protein incorporation into virions by reducing the overall levels of APOBEC3 proteins in the virus-producing cell (Figure 1). This is achieved by simultaneous interaction of Vif with APOBEC3 proteins and the Cul5 and elongin B and C proteins, causing APOBEC3G ubiquitination and degradation in the proteasome[8,13-16]. The adaptation of various lentiviruses to their host species is reflected in the specificity with which the Vif protein from a particular lentivirus antagonizes the APOBEC3 proteins expressed in its host. Thus, HIV-1 Vif protein is very efficient in targeting human APOBEC3 proteins but has no activity against proteins from lower primates[17,18]. Conversely, some Vif proteins are active against APOBEC3 proteins from a wider range of hosts; For example, Vif proteins from the SIVsm lineage have a broad ability to antagonize APOBEC3G from non-natural host species, perhaps providing an explanation for why this lineage of viruses (originally derived from sooty mangabeys) has been able to colonize both human and macaques.

TRIM5/TRIMCyp proteins

A second intrinsic antiretroviral defense mechanism is TRIM5 α [19], a member of the tripartite motif protein family that derive their name from the presence of three characteristic

protein domains at their N-terminus, namely RING, B-Box and coiled coil domains. The C-terminus of TRIM proteins is very heterogeneous and, in the case of TRIM5 α , is a SPRY/B30.2 domain. This C-terminal domain recognizes and binds to the incoming viral capsid that forms the shell surrounding the viral enzymes and nucleic acids[20,21]. Thus, the avoidance of inhibition by TRIM5 α in a given host species requires the selection of capsid protein sequences or structures that are not recognized by TRIM5. As such, the HIV-1 capsid has evolved to avoid detection by human TRIM5 α , but is sensitive to TRIM5 α inhibition from some other species such as rhesus macaques[19,22-25]. In certain species (e.g. Owl monkeys and some macaques) the TRIM5 C-terminal SPRY domain has been replaced by cyclophilin A (CypA) following LINE-mediated retrotransposition of CypA-coding sequences into the TRIM5 locus[26-32]. Due to the ability of CypA to interact with HIV-1 and other lentiviral capsids, the resulting TRIMCyp fusion proteins are generally strong inhibitors of HIV-1 infectivity. Surprisingly, the TRIMCyp protein in macaques has acquired mutations in the CypA-coding region that render it incapable of binding the HIV-1 capsid, although it inhibits infection mediated by other CypA-binding lentiviral capsids such as HIV-2, FIV and SIVagmTan[31,32]. Interestingly, chimeric viruses expressing capsid proteins from 'fossilized' lentiviruses found in rabbits and gray mouse lemurs are also sensitive to inhibition by TRIMCyp proteins[33] suggesting that CypA binding by lentiviral capsids is an ancient phenomenon that was exploited by hosts in the evolution of antiviral defenses. The mechanism by which infection is inhibited by TRIM5 proteins is not completely understood. It has been shown that the RING domain is required for full antiviral potency, but mutants lacking this domain can retain vestigial activity[34,35]. The RING domain has ubiquitin ligase activity and while blocking proteasome activity does not alleviate virus inhibition (suggesting that proteasomal-mediated degradation is not required for TRIM5 antiviral activity) it does change the precise step at which infection is blocked[36,37]. The coiled coil mediates TRIM5 dimerization and recently it has been shown that the B-box (with Arg121 in human TRIM5 α playing a particularly important role) is important for formation of higher order multimers[38,39]. Interestingly, formation of higher order multimers enhances the antiviral activity of TRIM5[40]. In vitro, TRIM5 proteins can assemble into a hexagonal lattice[41] that is complementary to the hexagonal lattice assembled using the HIV-1 capsid protein and it is possible that assembly into this higher order structure facilitates capsid recognition or even the capsid disruption that accompanies inhibition by TRIM5 (Figure 1).

A very recent study[42] has suggested that TRIM5 has a second role in innate immune system signaling by acting together with the ubiquitin-conjugating enzyme UBC13-UEV1A to catalyze the formation of free K63-linked ubiquitin chains. These chains activate the TAK1 kinase complex, which in turn activates the AP-1 and NF κ B pathways. Signaling is dependent on the ubiquitin ligase activity of TRIM5 and appears to be enhanced following infection with lentiviruses and, surprisingly, even vesicular stomatitis virus and Newcastle disease virus, two viruses that were not thought to interact with TRIM5[42]. The significance of this activity in the overall role of TRIM5 in inhibiting virus replication remains to be determined.

TRIM28/KAP1 and ZFP809

Although TRIM5 is the best studied of the TRIM proteins by virtue of its activity against HIV-1, it is by no means the only TRIM protein with direct antiviral or signaling activity. An intriguing cellular antiretroviral activity, particularly evident in embryonic stem cells, takes the form of a protein complex that includes the TRIM28/KAP1 protein[43]. Although exhibiting a broadly similar domain organization to TRIM5, TRIM28 acts completely differently. Specifically, it suppresses the transcription of retroviruses by recruiting a histone H3 K9 methyltransferase (ESET), a histone deacetylase (the NuRD complex) and

heterochromatin associated protein (HP1). This repressive complex is specifically targeted to proviral DNA (Figure 1) via the tRNA primer binding site (PBS) sequence, and sequence-specific DNA binding activities associated with TRIM28. For one PBS sequence, namely Pro tRNA, a TRIM28-associated DNA binding protein (ZFP809) has been identified that binds the Pro tRNA sequence in proviral DNA and is required for provirus silencing[44]. Since TRIM28 also mediates silencing of proviruses with Lys1,2 tRNA PBS sequences[45], this finding strongly suggests the existence of alternative TRIM28 complexes with other associated DNA binding proteins that target different tRNA sequences. That this retroviral silencing activity is somewhat confined to embryonic cells suggests that it may have evolved to target endogenous retroviruses in particular. Consistent with this idea, endogenous retrovirus expression is dramatically upregulated in TRIM28-knockout murine embryos[46].

Tetherin

Another antiretroviral protein, tetherin[47,48], is an unusual type II transmembrane protein that has both a transmembrane anchor close to its N-terminus and a glycosylphosphatidylinositol (GPI) lipid anchor at its C-terminus. The bulk of the intervening extracellular domain forms a dimeric coiled-coil structure[49-51], with three extracellular cysteines forming disulfide bonds with corresponding cysteines in the tetherin dimer[52]. This configuration appears to allow tetherin to cause retention of nascent virions on cell surfaces through a direct tethering mechanism[52-54]. The aforementioned tetherin configuration is required for virion retention, but entire tetherin domains can be substituted with protein domains that have similar predicted structures but different amino acid sequences, with minimal impact on function[52]. Indeed, a synthetic tetherin-like protein, comprised of structurally similar but unrelated protein domains, can recapitulate tetherin's activity[52]. These findings, and observations that tetherin can inhibit the release of virions of various virus families, whose proteins have no structural or sequence similarity[55-58], indicate that specific recognition events are likely dispensable for function. Rather, tetherin membrane anchors are efficiently incorporated into the lipid envelope of virion particles [52-54] and a likely scenario is that one pair of tetherin membrane anchors is incorporated into the lipid envelope of assembling particles, while the other remains in the plasma membrane of the infected cell. In this manner, the tetherin protein would span both virion and cell membranes after completion of budding. The extended conformation of the tetherin protein could serve to spatially separate the two pairs of membrane anchors, promoting their partition into virion and cell membranes as budding proceeds (Figure 1). An alternative possibility is that non-covalent interactions between tetherin dimers, some of which are incorporated into the virions promote adhesion of the nascent virion to the host cell membrane.

A variety of HIV and SIV proteins have been shown to possess tetherin antagonist activity[47,48,59,60]. The HIV-1 Vpu protein can be co-immunoprecipitated with tetherin [61] and causes its downregulation from the cell surface[48], by inhibiting its progress through the secretory pathway or by inducing its internalization[61-63]. Vpu can also reduce the overall steady state level of tetherin[64]. Most SIVs lack a Vpu protein and instead employ another accessory protein, Nef [59,60], that induces tetherin internalization from the cell surface by recruiting the AP2-clathrin adapter complex[65]. In a few instances, HIV/SIV Env proteins can also antagonize tetherin by sequestering it within intracellular compartments[66,67]. Tetherin antagonists found in HIVs and SIVs have clearly evolved to act in particular primate host species. Indeed, species-specific actions of Vpu and Nef have been used to determine that these two proteins target the tetherin transmembrane helices and cytoplasmic tail, respectively[59,60,68,69]. Analyses of Vpu and Nef proteins from many SIVs has revealed that these proteins effectively exchanged the role of tetherin antagonist as primate lentiviruses were passed from species to species and encountered tetherin proteins

with different transmembrane and cytoplasmic tail sequences[70]. Most strikingly, SIVs found in chimpanzees (SIVcpz), employ the Nef protein as a tetherin antagonist, while their immediate descendent, HIV-1, employs Vpu. The acquisition of tetherin antagonist activity by HIV-1 Vpu almost certainly occurred because human tetherin harbors a deletion of 5 key residues in its cytoplasmic tail that are required for Nef sensitivity[59,60,70]. This and other gain of function events that accompanied primate lentivirus transmission to new species underscore the flexibility of function in HIV/SIV accessory proteins.

SAMHD1

Since the Vif, Vpu and Nef lentiviral accessory gene products have all been shown to antagonize restriction factors, the finding that Vpx recruits the Cul4 ubiquitin ligase complex by interacting with DDB1[71,72] immediately suggested that it was likely responsible for mediating the removal of an unidentified restriction factor. Interestingly, HIV-1 lacks a Vpx protein but other lentiviruses, including SIV_{MAC} and HIV-2, express Vpx, which is very efficiently incorporated into viral particles. Vpx appears to alleviate an inhibition to the early steps of HIV/SIV infection in dendritic cells (DC) and macrophages[71,73,74]. This effect does not require Vpx expression in these target cells but rather delivery of this protein by incoming viral particles, consistent with the notion that Vpx counteracts a restriction factor. Very recent work has suggested that this factor is the SAM- and HD-domain containing protein 1 (SAMHD1)[75,76]. Mutations in SAMHD1 are associated with Aicardi-Goutieres syndrome, a genetic disorder that mimics congenital viral infection, and SAMHD1 expression is induced in DCs and macrophages in response to IFN γ . SAMHD1 fills all the required criteria to be the restriction factor targeted by Vpx[75,76] in that: 1) it is targeted for proteasomal degradation by Vpx and Cul4, 2) its overexpression in otherwise permissive cells renders them resistant to HIV-1 infection 3) RNAi mediated silencing of SAMHD1 expression in resistant cells increases permissivity to HIV-1 infection and alleviates the requirement for Vpx. Finally, mutations in the putative enzymatically active site of SAMHD1 abolish its antiviral activity[76].

Several questions remain regarding the importance of this factor in viral replication and, in particular, whether there are indeed differences in *in vivo* replication in DCs and macrophages between HIV-2 and SIV_{MAC} (which both express Vpx) and HIV-1 (which does not).

Other antiretroviral proteins

Finally, several other cellular proteins have been shown to have antiviral activity although they have been less well studied. For example, the Zinc finger antiviral protein (ZAP), a CCCH-type ring finger protein, specifically inhibits accumulation of murine leukemia virus (MLV) mRNA in the cytoplasm of infected cells[77]. The protein is actually more potently active against alphaviruses, inhibiting translation of the viral mRNA[78]. It has also been shown that ZAP binds directly to specific sequences in the viral RNA providing a mechanistic explanation of its antiviral activity[79]. Another example consists of the fasciculation and elongation protein ζ -1 (FEZ1) that interacts with protein kinase C. Overexpressed Rat FEZ1 inhibits MLV and HIV-1 infection at a step after reverse transcription, but prior to nuclear entry[80]. Finally, the Moloney leukemia virus 10 (MOV10) protein has a putative RNA helicase domain and colocalizes with APOBEC3G in P bodies[81]. Overexpression of MOV10 in retrovirus producing cells inhibits infectivity of various progeny retroviruses, including HIV-1, mainly by inhibiting reverse transcription once the virus enters target cells[82-84]. However, MOV10 silencing has minimal effects on retrovirus infectivity, and the precise mechanism by which it exerts its activity is yet to be

elucidated. (Of note, MOV10 has also been suggested to be required for hepatitis D virus replication[85].)

Concluding remarks

The discovery and investigation of gene products with antiviral properties is one of the most exciting recent developments in virology. This review is, necessarily, not exhaustive, and new antiviral activities that target retroviruses and other virus families are being discovered with notable regularity. Understanding how the antiviral proteins work, how they have evolved, and how viruses escape them is providing new and unanticipated insights into the complexity of the interactions between viruses and their hosts. Ultimately, studies in this area may enable the use of pharmacological agents to mobilize these specific intrinsic defenses, or antagonize the viral antagonists, and thereby reduce the burden of viral disease in humans.

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* of special interest

** of outstanding interest

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Highlights

- Mammalian cells express a number of antiretroviral proteins, termed restriction factors
- Currently known antiretroviral restriction factors include APOBEC3, TRIM5, KAP1/TRIM28/ ZFP809, SAMHD1 and Tetherin
- These proteins have several unique and interesting ways of inhibiting viral replication
- Retroviruses evolve to evade or antagonize restriction factors, sometimes employing accessory proteins
- Restriction factors can impose barriers to cross-species viral transmission

Figure. Factors and events in the restriction of retroviral replication by host proteins

Key retroviral restriction factors are shown in red, antagonists expressed by the HIV/SIV retroviruses are shown in green. Cellular cofactors, co-opted by viral antagonists are shown in purple. Key events or processes in the restriction of retroviral replication or associated with the action of the antagonists are indicated. The process of APOBEC3-mediated hypermutation is depicted in detail in the inset panel.