REVIEW

For reprint orders, please contact: reprints@futuremedicine.com

Understanding restriction factors and intrinsic immunity: insights and lessons from the primate lentiviruses

Andrea Kirmaier¹, Annabel Krupp^{1,2} & Welkin E Johnson^{*1}

ABSTRACT: Primate lentiviruses include the HIVs, HIV-1 and HIV-2; the SIVs, which are endemic to more than 40 species of nonhuman primates in Africa; and SIVmac, an AIDS-causing pathogen that emerged in US macaque colonies in the 1970s. Because of the worldwide spread of HIV and AIDS, primate lentiviruses have been intensively investigated for more than 30 years. Research on these viruses has played a leading role in the discovery and characterization of intrinsic immunity, and in particular the identification of several antiviral effectors (also known as restriction factors) including APOBEC3G, TRIM5 α , BST-2/tetherin and SAMHD1. Comparative studies of the primate lentiviruses and their hosts have proven critical for understanding both the evolutionary significance and biological relevance of intrinsic immunity, and the role intrinsic immunity plays in governing viral host range and interspecies transmission of viruses in nature.

Primate lentiviruses

By the end of the 20th century, HIV had spread from central Africa to encompass the entire globe. Currently, it is estimated that approximately 0.8% of the human population between the ages of 15 and 49 years is infected [1]. Both HIV-1 and HIV-2 are derived from SIVs, which were transmitted from natural hosts (African primates) to humans, probably in the early-to-mid 20th century [2–4]. The four currently known subtypes of HIV-1 (M, the pandemic strain; N; O; and P) are thought to be the result of four single, independent transmissions of SIV from chimpanzees (SIVcpz) into humans. In the case of groups M and N, SIVcpz is probably the direct ancestor. The HIV-1 group O and P viruses cluster closest with SIV of gorillas (SIVgor), which itself is derived from SIVcpz [5,6], indicating that both chimps and gorillas are reservoirs for lentiviruses capable of infecting humans. Another reservoir species, the sooty mangabey, harbors viruses (SIVsmm) closely related to HIV-2 groups A through I, with each of the nine groups likely originating from an independent transmission of SIVsmm [7–12]. While HIV-1 M has spread globally, even the most frequently found clades of HIV-2, A and B, are mostly confined to western Africa. All other subtypes are rare, sometimes consisting of a single infected individual. But, in all cases, it is possible that the viruses underwent a significant period of adaptation to humans as hosts between the time of the original transmissions and the emergence of HIV/AIDS in the 1980s [13,14].

At present, over 40 naturally occurring types of SIV have been identified in primate populations in Africa [14,15]. There is serological evidence that humans are frequently exposed to retroviruses from multiple nonhuman primate reservoirs, including viruses that were already the sources of HIV-1 groups M, N (SIVcpz from chimpanzees), O and P (SIVgor from gorilla) and HIV-2 groups

KEYWORDS

• APOBEC3G • BST-2/tetherin
• cross-species transmission
• emerging viruses • HIV
• host genetics • intrinsic immunity • primate lentiviruses • restriction factor • SAMHD1 • SIV
• TRIM5

¹Biology Department, Boston College, 550 Higgins Hall, 140 Commonwealth Ave., Chestnut Hill, MA 02467, USA

²Institut für Klinische und Molekulare Virologie, Friedrich-Alexander-Universität, Erlangen-Nuremberg, Schlossgarten 4, 91054 Erlangen, Germany

*Author for correspondence: Tel.: +1 617 552 4223; welkin.johnson@bc.edu

A through I (SIVsmm from sooty mangabeys) [2–4,12,16–17]. In fact, cross-species transmission appears to be a common theme in the natural history of these viruses. The host and virus phylogenetic trees have different topologies, indirect evidence that the distribution of extant viruses among modern African primates must reflect, at least in part, a history of interspecies jumps (Figure 1) [13–14,18].

Phylogenetic analyses also suggest a role for recombination in cross-species transmission and emergence of several SIV lineages, such that some species of SIV appear to be descended from two or more distinct founder viruses [14]. Recombination between retroviruses requires that coinfection of the same cell in a single individual with both parental viruses must have occurred [22]. In addition to HIV-1 and HIV-2,

there is evidence that SIVcpz of chimpanzees (*Pan troglodytes* sp.), SIVgor of gorillas (*Gorilla gorilla gorilla*), SIVmus-1 and SIVmus-2 [23] of the mustached monkey (*Cercopithecus cephus*), SIVgsn [24] of the greater spot-nosed monkey (*Cercopithecus nictitans*), SIVagmSab of the sabaues subspecies of African green monkey (*Chlorocebus sabaues*) [25], SIVdrl of drills (*Mandrillus leucophaeus*) and SIVmnd-2 of mandrills (*Mandrillus sphinx*) are the results of natural cross-species transmissions. Phylogenetic comparisons suggest that emergence of at least five of these, SIVcpz [26], SIVagmSab [27], SIVrcm, SIVdrl and SIVmnd-2 [28], involved recombination between two or more parental SIV strains (reviewed in [29]). The relative importance of recombination for interspecies transmission and emergence of new viruses remains an open question.

Figure 1. Tanglegram of primate species and primate lentiviruses. (Left) Primate species; (right) primate lentiviruses. Dotted lines connect the primate lentivirus(es) to their respective hosts. Subsets of the HIV-1 and HIV-2 types infecting humans are highlighted in blue (HIV-1) and green (HIV-2), respectively; viruses isolated from captive macaques are highlighted in red. The primate phylogeny is derived from [19]. The primate lentivirus phylogeny is based on *pol* sequences obtained from the Los Alamos National Library HIV sequence database. Neighbor-joining trees were generated using the Tree Builder algorithm in Geneious (Auckland, New Zealand), pruned with Mesquite [20] and rendered using FigTree [21].

Intrinsic immunity: early insights from animal models of aids

Beginning in the 1970s, and prior to the emergence of AIDS in humans or the discovery of HIV-1, AIDS-like symptoms began to affect macaques housed in National Primate Research Centers in the USA (**Figure 2**). Shortly after the detection and isolation of HIV-1, the cause of AIDS in macaque colonies was identified as an HIV-like virus, now known as SIVmac [30]. This was followed fairly quickly by the first description of HIV-2 [31], and the discovery of naturally occurring SIVs in many African primates. In fact, SIVmac and HIV-2 are both descended from SIVsmm [14,32]. Experimental infection of macaques quickly became the predominant experimental model for preclinical AIDS research, and the discovery of several intrinsic immune effectors can be traced from early work with the SIV/macaque model.

• SHIVs

Despite the obvious relatedness of HIV-1 to the newly discovered SIVs, researchers discovered that HIV-1 could not replicate in monkey cells [33]. This prompted the creation of recombinant SIV/HIV chimeric viruses (SHIV), in which subsets of viral genes from one parent virus were replaced with the homologous regions of the other [34–36]. While *env* and some of the accessory gene loci of SIV could be substituted with HIV-1 sequence and still form viruses that replicated in monkey cells, replacement of the SIV *gag* gene with HIV-1 *gag* resulted in a severe restriction of replication in monkey cells. The capsid (CA)

domain within *gag* turned out to be the determinant of the species-specific patterns of SIV and HIV-1 infectivity, and subsequent work determined that the CA-dependent block manifests after viral entry, but prior to integration [37].

Hofmann and colleagues conducted a broad survey of primate cell lines, comparing single-cycle infectivity of HIV-1 and SIVmac [38]. They tested cell lines of human, Old World monkey, New World monkey, prosimian, rodent, lagomorph, carnivore and bovid origin, using viruses pseudotyped with a heterologous envelope protein to circumvent cell-specific or species-specific differences in receptor expression. They found that the early block to HIV-1 in rhesus macaque cells was part of a larger pattern in which almost all Old World monkey cells were refractory to HIV-1 infection. By contrast, New World monkey cells were generally susceptible to single-cycle HIV-1 infection, but were not readily infected with SIVmac (with the exception of cell lines from owl monkeys). Similar blocks were seen in cells from rabbits and cows, and prosimian cells were resistant to both virus types [38].

Ultimately, a screen for rhesus macaque cDNAs that could block HIV-1 infection of human cells led to the identification of TRIM5 α as the responsible factor [39]. TRIM5 α is a cytoplasmic protein with retrovirus-specific antiviral activity, and work from a number of laboratories has shown TRIM5 α -mediated restriction of a range of retroviruses, including gammaretroviruses, betaretroviruses, lentiviruses and spumaviruses [39–44]. TRIM5 α belongs to the large family of tripartite motif (TRIM) proteins. TRIM

Figure 2. Timeline of events and discoveries in the field of primate lentivirology from the 1960s to present. Red: viral transmissions, bold: discoveries of viruses, blue: discoveries of host restriction factors. Special interest references are indicated below the timeline.

proteins are involved in many cellular functions, including antiviral defense [45–48]. TRIM5α can restrict diverse lentiviruses; it binds the viral capsid core through directed interactions mediated by its C-terminal SPRY domain, forms a multimeric lattice and causes the premature disassembly and proteasomal degradation of the capsid structure [49–54].

Sequencing of the *TRIM5* coding sequence from multiple species and cell culture experiments confirmed that the species-specific patterns of restriction could be explained by divergence in the amino acid sequences of the SPRY domain of TRIM5α [44,55–58]. The restrictive properties of TRIM5 were also discovered by two groups working with owl monkey cells (*Aotus sp.*) – surprisingly, they found that the *TRIM5* locus of owl monkeys does not encode the α-isoform, which contains a C-terminal SPRY domain [49,59]. As the result of an ancient, LINE-mediated retrotranspositional insertion, owl monkeys instead express a chimeric protein in which the C-terminal SPRY domain has been replaced with a Cyclophilin-A (CypA) domain. Because CypA can bind to the capsid proteins of some lentiviruses, owl monkey TRIM-CypA1 can act as a restriction factor [49,59]. A strikingly similar event occurred in the Old World macaques, also resulting in a TRIM5–CypA chimeric protein (discussed in a later section) [60–64].

• Accessory genes & live-attenuated SIV

All primate lentiviruses share the three canonical *gag*, *pol* and *env* retroviral genes, which encode the basic structural and enzymatic proteins required for infection and replication. Additionally, they possess a variable repertoire of accessory genes (Table 1). *nef*, *vif* and *vpr* are shared among all primate lentiviruses, while *vpu* is only found in certain viruses, such as SIVgsn, SIVgor, SIVcpz and HIV-1. Similarly, a different accessory gene called *vpx* is only found in some viruses, such as SIVrcm, SIVsm, SIVmac and HIV-2. While the accessory proteins are not required for basic replication of the virus in most cell lines, their importance *in vivo* has been proven by both

experimental and naturally occurring accessory gene deletion mutants. For example, SIVmac239 strains deleted in one or more accessory genes are attenuated to various degrees *in vivo*, ranging from mild ($\Delta nef\Delta vpr$), to moderate (any combination involving Δvpx), to severe (any combination involving Δvif) [65–67].

Similar effects were documented in cases of human HIV-1 infection wherein the virus had inactivating mutations in *nef*. For example, the Sydney Blood Bank Cohort comprises a small group of patients unintentionally infected with a naturally occurring *nef*-deleted strain of HIV-1 originating from a single blood donor. All members of the cohort qualified as long-term nonprogressors, initially, and even after 30 or more years of infection with this attenuated HIV-1 strain, a subset of individuals maintain their nonprogressor status [68,69]. In an unrelated longitudinal study, only *nef*-deleted HIV-1 could be recovered from a long-term nonprogressor patient [70]. In both cases, long-term attenuation of HIV was not mediated by adaptive immunity, but rather caused by abnormalities of the viral accessory gene repertoire.

Research on accessory gene function has led directly to the identification of at least three restriction factors, APOBEC3G (and related enzymes of the APOBEC3 cluster), BST-2/tetherin and SAMHD1 [71–75]. In all three cases, the antiviral function of these factors is actively thwarted by the activity of one or more lentiviral accessory proteins. The first restriction factor to be identified was APOBEC3G (A3G), which is targeted for degradation by viral infectivity factor proteins (Vif) [71]. A3G is a cytidine deaminase that incorporates into assembling virions and causes G-to-A hypermutation of the proviral DNA during reverse transcription in the subsequent target cell [76,77]. Additionally, there is evidence that A3G may also inhibit the reverse transcription complex in a deaminase-independent manner [78–80]. Vif recruits a multiprotein E3 ubiquitin ligase complex to mediate proteasomal degradation of A3G, thereby preventing incorporation of A3G into budding virions [81–84].

Table 1. Accessory gene repertoire of representative primate lentiviruses.	
Accessory genome composition	Examples
<i>vif</i> , <i>vpr</i> , <i>nef</i>	SIVagm, SIVmnd-1, SIVcol
<i>vif</i> , <i>vpr</i> , <i>vpx</i> , <i>nef</i>	HIV-2, SIVmac, SIVsmm, SIVmnd-2, SIVdrl, SIVrcm
<i>vif</i> , <i>vpr</i> , <i>vpu</i> , <i>nef</i>	HIV-1, SIVcpz, SIVgor, SIVgsn, SIVmus-1

Exclusion of A3G from virions prevents both inhibition of the reverse transcription complex and hypermutation during reverse transcription.

HIV-1 mutants with an inactivated *vpu* gene display a striking phenotype in cell culture: electron microscopy images reveal mature virions built up in thick layers around cells, unable to separate from the cell surface [72,75]. mRNA microarray experiments identified BST-2, also called tetherin, to mediate this phenotype. BST-2 localizes to the plasma membrane, where it prevents freshly budded virions from moving away from the cell. Vpu counteracts BST-2 through direct binding and endocytosis-mediated removal of BST-2 from the plasma membrane. Not all primate lentiviruses have a *vpu* gene (Table 1), and in such viruses, the anti-BST-2 activity is usually provided by Nef [85,86].

SAMHD1 was identified in proteomic screens as a major Vpx-interacting protein [73,74]. SAMHD1 is a myeloid cell-specific phosphohydrolase, which depletes the intracellular concentration of dNTPs, thus severely inhibiting synthesis of viral DNA by the reverse transcription complex. This phosphorylation-dependent activity is restricted to resting cell populations, and missing in activated cells [87]. Depletion of the dNTP pool would be expected to inhibit other virus types as well, and a diverse panel of retroviruses as well as the DNA virus HSV-1 have indeed been found to be sensitive to SAMHD1-mediated restriction [88,89]. Primate lentiviruses use Vpx (and in some cases, Vpr [90]) to target SAMHD1 for proteasomal degradation.

Restriction factors & intrinsic immunity in primates

Comparative approaches have proven useful in the study of intrinsic immunity and the restriction factors, particularly in the case of primate hosts (including humans) and their lentiviruses (HIV-1, HIV-2 and the SIVs). Molecular evolutionary analyses, including phylogenetic comparisons and statistical tests of positive selection (e.g., d(N)/d(S) estimates), have proven especially informative when combined with cell culture experiments. Such approaches consistently indicate that the restriction factor genes in primates, including those encoding APOBEC3G, TRIM5, BST-2/tetherin and SAMHD1, have been subject to strong, recurring bouts of positive selection throughout much of primate evolution [91]. In several cases, evolutionary analyses have guided experiments to map interacting domains

and key residues involved in virus–host interactions [53,90,92–95]. Dating of signatures of positive selection in host intrinsic immunity genes and cell culture experiments strongly suggest that viruses related to the extant primate lentiviruses were already present several million years ago and infected ancestors of the Old World primates [96], a possibility that is indirectly supported by the presence of endogenous SIV-like sequences in the genomes of some prosimians [97].

Evolutionary studies, genetic surveys, laboratory experiments and animal models of lentiviral infection together suggest that intrinsic immunity has a significant impact on viral host range in nature, and may serve as an important barrier to cross-species transmission of viruses and emergence of new viral diseases. The following sections focus on four restriction factors as examples of how comparative approaches are shedding light on the evolutionary significance and biological relevance of intrinsic immunity.

• TRIM5

TRIM5 belongs to a very old and large gene family, and *TRIM* family members can be found widely among metazoans [98,99]. The mammalian *TRIM5* gene has been most extensively studied in primates, and comparison of the *TRIM5* locus across primate lineages reveals a very high degree of protein sequence diversification, particularly in the C-terminal SPRY domain [55,56]. On at least two separate occasions, the SPRY domain has been replaced with a CypA domain, giving rise to the TRIM-CypA fusions in South American owl monkeys (*Aotus sp.*) and Asian macaques (*Macaca sp.*) [49,60]. The presence of degraded CypA sequences downstream of the *TRIM5* locus in the genomes of some primates suggests that additional TRIM-CypA fusions may have arisen more than once during the evolution of Old World primates [100].

In primates, the TRIM5 SPRY domain has evolved under strong positive selection for at least 23 million years, and substitutions in positively selected sites affect virus-target specificity in cell-culture experiments, consistent with a protein engaged in long-standing or repeating cycles of genetic conflict with viral pathogens [55]. Positively selected residues largely fall into variable ‘patches’ in the SPRY domain coding sequence, and in some lineages these same variable regions have also undergone changes in length due to in-frame duplications of short stretches of sequence [55,56]. For example, the

V1 region of African green monkey TRIM5 α and related species has a duplication resulting in 60 additional nucleotides (Figure 3) [55,56]. It is therefore likely that positive selection has acted upon both kinds of variation – amino-acid substitutions and length differences in variable regions – in shaping the interactions of primate TRIM5 with retroviral pathogens.

Among Old World primates, the *TRIM-CypA* allele has only been found in Asian macaques, suggesting an origin subsequent to the migration of macaque ancestors out of Africa. Although the CypA domain of macaque TRIM-CypA is much younger than the SPRY domain, there is also evidence for functional diversification of the CypA domain in the macaque lineage [101]. Some residues in the TRIM5 coiled-coil (CC) domain also appear to have evolved under positive selection [55,58]. Selection on residues in the SPRY domain can be attributed to direct interaction with the capsid proteins of retroviral pathogens; selection on the CC domain is harder to explain, but it is worth noting that the CC domain of

another TRIM protein, TRIM25, is bound by a viral antagonist – the NS1 protein of influenza A virus [102]. As with TRIM25, perhaps the CC domain of primate TRIM5 is (or was) also the target of an unknown virus-encoded antagonist that drove diversification of these residues.

Altogether, rhesus macaques possess at least three classes of *TRIM5* alleles, encoding three distinct TRIM5 proteins that differ in target recognition properties, referred to as rhTRIM5^Q, rhTRIM5^{TFP} and rhTRIM5-CypA [60–64]. The rhTRIM5^Q and rhTRIM5^{TFP} alleles contain C-terminal SPRY domains, and differences in specificity map to a complex Q/TFP polymorphism at position 339–341. The third, rhTRIM5-CypA, expresses a fusion protein in which the SPRY domain is replaced with a CypA domain – TRIMCypA fusions inhibit lentiviruses by interacting with a specific loop in lentiviral capsids (the 4–5 loop, also known as the CypA-binding loop). At least four *TRIM5* alleles have been reported in crab-eating macaques (*Macaca fascicularis*), mfTRIM5^{TFP}, mfTRIM5^Q, mfTRIM5-CypA1 and mfTRIM5-CypA2 [101]. In captive pigtailed macaques (*Macaca nemestrina*) TRIM5 α -encoding allele(s) have not been found, and it is possible that the TRIM5-CypA allele (mnTRIM5-CypA) became fixed in this species [62–63,101].

Of the two allele types with SPRY domains, the Q at position 339 (rhTRIM5^Q alleles) is ancestral, whereas the TRIM5^{TFP} allelic lineage arose sometime in the past 10 million years (Figure 3) [51]. Mutations that sensitize the macaque virus SIVmac239 to restriction by TRIM5^{TFP} are closely clustered in one region of CA (see below) [51].

The first *in vivo* evidence that TRIM5 has an impact on lentivirus host switching came from retrospective analysis of two experimental cohorts of SIVsmm-infected rhesus macaques [103]. Genotyping of over 40 SIVsmE543-3-infected rhesus macaques in the first cohort revealed a significant correlation between plasma viral loads and *TRIM5* genotype. Specifically, animals expressing alleles that were restrictive in cell culture experiments (genotypes rhTRIM5^{TFP/TFP}, rhTRIM5^{TFP/CypA} and rhTRIM5^{CypA/CypA}) had the lowest viral levels in plasma, whereas animals homozygous for the nonrestrictive rhTRIM5^Q allele had the highest levels of viremia. Heterozygous animals (animals with one restrictive allele and one permissive allele)

Figure 3. Some key events in the evolution of primate restriction factors, overlaid on a tree depicting evolution of New World and Old World primates. Branches are not to scale.

had intermediate viral loads. Notably, over time, viral loads increased in a small number of animals with restrictive *TRIM5* genotypes. Virus sequences recovered at late time points from these monkeys shared an R97S substitution at the base of the helix 4–5 surface loop of the viral CA protein. This was confirmed as a rhTRIM5^{TFP} escape mutation, and cell culture experiments confirmed that this residue functions as an important determinant of TRIM5 sensitivity [51,103]. Arginine is also found at the homologous position in HIV-1 CA, and comparison of the HIV-1 and SIVmac CA crystal structures indicate that the R-to-S change results in a rearrangement of hydrogen bond networks at or near the base of the CypA binding loop [51].

A second cohort, consisting of four rhesus macaques infected with a primary SIVsmm isolate (SIVsmE041) was also analyzed [103]. All four animals possessed at least one restrictive *TRIM5* allele. While three of the four monkeys have controlled SIVsmE041 replication for 7 years postinfection [103] [KIRMAIER A, HALL LR, KAUR A, JOHNSON WE, UNPUBLISHED DATA], virus rebounded in the fourth animal around 4 months post-infection. Viral sequences recovered from this animal's plasma revealed the same R97S escape mutation in CA [103].

The importance of the R97S mutation was confirmed by two related studies [51,104]. Efforts to map the binding site of rhTRIM5 α on CA demonstrated that viruses with either a serine or arginine at CA position 97 scored the highest impact on differential recognition by rhTRIM5 α [51]. During experimental infection of rhesus macaques with SIVsmm two changes in capsid emerged: P37S and R98S (equivalent to R97S in SIVmac) [104]. When the two mutations were introduced into the SIVsmE543–3 clone, the virus displayed partial resistance to rhTRIM5^{TFP} *in vitro*, and *ex vivo* it replicated significantly better in rhesus macaque primary blood mononucleated cells than the parental SIVsm clone [104]. Thus, multiple independent lines of evidence show that the emergence of SIVsmm as pathogenic SIVmac in rhesus macaques in the 1970s involved adaptation of CA to evade recognition by rhesus TRIM5 proteins. The outcome of experimental cross-species transmission of SIV in rhesus macaques is therefore largely dependent on the *TRIM5* genotype of the recipient monkey, and provides direct proof that adaptation in CA facilitates escape from TRIM5-mediated restriction *in vivo*.

• APOBEC3G

Comparison of the complete *A3G* coding sequences from 11 primate species, including New World monkeys, Old World monkeys and hominids, suggests that *A3G* has been evolving under positive selection for at least 33 million years [92]. Since APOBEC3 is encoded in all placental mammals [105,106], the theoretical upper limit of APOBEC3-mediated host–virus genetic conflict could be up to 100 million years before present [19]. *A3G* has also provided evidence for the existence of ancient primate lentiviruses. For example, a multiresidue insertion event in a subset of Old World monkeys (*Colobinae*), which allows the evasion of Vif-mediated degradation, suggests that SIV-like viruses may have circulated among the ancestors of modern primates as far back as 12 million years ago [96].

Like TRIM5, APOBEC3G of any given primate species usually does not restrict the primate lentivirus naturally infecting that species, indicating Vif-specific adaptation and resistance to *A3G* of the native host [107–109]. Within primates, interspecies variation at amino acids 128, 129 and 130 affect sensitivity to different Vif proteins, suggesting that these are important determinants of *A3G* antilentiviral activity [76,108–114] and that different Vif proteins may bind the same or overlapping elements of *A3G* proteins [112–113,115].

Evidence for the *in vivo* significance of the *A3G*–Vif interaction for host range determination comes from the study of SIVagm in African green monkeys (*Chlorocebus sp.*). The four subspecies of African green monkey are each infected with a different subtype of SIVagm. *A3G* in these subspecies has been subject to recent diversifying selection in wild populations, with nonsynonymous SNPs clustering at surfaces targeted by Vif (*A3G* residues 128 and 130). These mutations in *A3G* were selected to allow evasion of SIVagm Vif proteins, implicating Vif as the selective pressure. *A3G*-driven evolution of Vif is a naturally occurring phenomenon, and the specificity of Vif proteins from the four SIVagm subtypes reflects adaptations to subspecies-specific *A3G* variants in African green monkey populations exposed to SIV in the wild rather than in the laboratory, demonstrating a case of contemporary selection and adaptation between primate lentiviruses and their natural primate hosts [116].

Further evidence for the importance of *A3G* antagonism comes from the finding that the Vif protein in some primate lentivirus lineages has

evolved alternative or modified A3G interaction sites; for example, A3G of the mantled colobus monkey (*Colobus guereza*) features a multiresidue insertion at amino acid 66 and binding of SIVcol Vif is affected by this A3G domain rather than the 128/129/130 site [112]. This pattern could have occurred either because emergence of SIVcol (after cross-species transmission of a virus from another host) acquired the ability to antagonize this unique form of A3G, or alternatively, this novel A3G variant arose in the host population and rendered a previous allele of SIVcol Vif nonfunctional.

Gorilla A3G resists degradation by most lentiviral Vifs and should therefore effectively serve as a barrier to cross-species transmission of SIV [113]. Yet gorillas did acquire SIVcpzPtt once [5,6], implying that this virus was able to overcome gorilla A3G. Two SIVcpz Vifs indeed exhibit some activity in the presence of gorilla A3G [113], suggesting that partial resistance to gorilla A3G might have lowered the genetic barrier SIVcpzPtt had to overcome during its emergence as SIVgor.

SIVsmm is the ancestor of both SIVmac in macaques and HIV-2 in humans. Compton *et al.* reported that the primary clone SIVsmE041 is resistant to rhesus and human A3G, a property that could have aided in its cross-species transmission and emergence [112]. However, a survey of the rhesus macaque A3G locus revealed the existence of three A3G alleles, distinguished by unique mutations at amino acid position 59/60 (A3G^Y, A3G^{LL} and A3G^{LR}). SIVsmE041 Vif is not able to degrade A3G^{LR}. By contrast, SIVmac, the virus that emerged after transmission of SIVsmm into captive macaque colonies, efficiently degrades all three rhesus macaque A3G alleles. A G17E substitution in the Vif protein of all SIVmac isolates is responsible, and represents an adaptation that must have occurred during emergence of SIVmac [117]. These results establish that A3G can serve as barrier to cross-species transmission and emergence of primate lentiviruses.

• BST-2/tetherin

The discovery of BST-2's antiviral activity and its antagonist HIV-1 Vpu immediately raised the question of whether all primate lentiviruses are affected by BST-2, and in particular those that do not encode Vpu. Vpu is found in the *Cercopithecine* lineage of SIVs but is not encoded in the SIVsm/SIVmac/HIV-2 lineage. As it turned out, in the majority of primate

lentiviruses, including many *Cercopithecine* viruses, Nef counteracts BST-2 instead [85,86]. So why did HIV-1 evolve to use Vpu? Human BST-2 has a five amino acid deletion in its cytoplasmic domain encompassing the Nef binding site, rendering it resistant to most lentiviral Nefs. The ancestors of HIV-1 and HIV-2 (SIVcpz and SIVsm) consequently had to re-establish BST-2 antagonism, which was accomplished by Vpu in HIV-1 and by the envelope protein in HIV-2 [118,119]. Independent acquisition of BST-2 antagonism in three different lentiviral accessory genes emphasizes the significance of BST-2 as a component of host antiviral defense. The five amino acid deletion in human BST-2 is also found in Neanderthal and Denisovan genomes, suggesting the deletion occurred >800,000 years ago; it is interesting to speculate that deletion of the Nef-binding site in the *Homo sp.* lineage reflects prior selection by an ancient virus with Nef or Nef-like activity [120].

Rapid acquisition of anti-BST-2 activity by *nef*-deleted SIVmac after passage in three rhesus macaques further highlights the severe selective pressure exerted on viruses by BST-2. Viruses recovered from these animals had both acquired changes in the viral Env glycoprotein that specifically counteract rhesus BST-2 [121,122]. The changes in Env conferring anti-BST-2 activity were not in the same sites that HIV-2 Env uses to interact with human BST-2, a case of convergent evolution in the same gene of two viruses to gain resistance to BST-2-mediated inhibition.

Analyses of the primate BST-2 locus spanning 33 millions years of evolution revealed that the strongest signatures of selection fall into the Nef-interacting domain of BST-2 [123]. Among the many enveloped viruses that could have influenced the evolution of primate BST-2, viruses encoding Nef or a Nef-like protein may have played a significant role in shaping this locus for tens of millions of years.

• SAMHD1

Some (but not all) primate lentiviruses use Vpx to counteract the effects of SAMHD1 [73–74,124]. The *vpx* and *vpr* accessory genes arose by gene duplication/recombination sometime during evolution of the primate lentiviruses [125]. So far, at least two studies have examined the *SAMHD1* gene of primates for evidence of positive selection [90,93]. Both studies identified clusters of positive selection, and site-directed mutagenesis confirmed that selected residues

are determinants of sensitivity/resistance to Vpx proteins. Curiously, HIV-1 (and related SIVs such as SIVcpz) does not encode Vpx or a Vpx-like activity, and HIV-1 replication in terminally differentiated myeloid cells can be significantly enhanced by SIV Vpx *in trans* [124] – it remains an open question as to why the HIV-1 lineage has not adapted to overcome SAMHD1, and whether there may be a selective advantage to not having a SAMHD1 antagonist and/or to limiting replication in specific myeloid cell types.

Future perspective

With respect to intrinsic immunity and the restriction factors, most research efforts have focused primarily on the molecular and biochemical details of mechanism, and to a lesser extent, regulation. Far less is known about the biological significance of intrinsic immunity in nature, particularly at the organismal and population levels, and with respect to emerging viral diseases and the jumping of viruses between hosts.

Molecular evolutionary studies and primate models together suggest that intrinsic/innate effectors such as TRIM5, APOBEC3, BST-2 and SAMHD1 may have their greatest impact as determinants of viral host range and the ability of viruses to transmit, spread and adapt to new host populations [91]. Although experimental infection of rhesus macaques has provided some insight, the degree to which restriction impacts cross-species transmission in outbred host populations in nature remains unknown and will be difficult to extrapolate from cell culture studies and animal models. Is sensitivity to any one restriction factor enough to ensure that a particular cross-species exposure is unlikely to result in spread and emergence of a new viral disease, and what is the effect of multiple restrictions functioning in tandem? An intensified focus on the genetics of interspecies transmission may help researchers determine if there are fundamental

rules that can be used to help predict the likelihood of specific zoonoses and new emerging viral diseases. Understanding the genetics of restriction and intrinsic immunity also has practical benefits for animal models of AIDS, most of which are currently based on genetically outbred primates – for example, as some of the blocks to HIV-1 infection have been identified, several groups have been able to engineer simian-tropic HIV-1 strains that circumvent restriction and replicate in macaques [126–128]. Moreover, because genetic variation in restriction factor genes is likely to be a confounding element in small, preclinical vaccine and pathogenesis studies in macaques, identifying and accounting for the contributions of different loci can be used to improve the statistical evaluation of outcomes.

Five restriction factors with known activity against lentiviruses (TRIM5, APOBEC3G, BST-2, SAMHD1 and, most recently, Mx2) can be used to illustrate the complexity of the problem. First, all five factors work by distinct mechanisms, attacking different weaknesses in the retroviral replication cycle. Unlike cell culture studies, where individual restrictions are studied in isolation, in nature two or more factors may come into play at once. When SIVsmm emerged as SIVmac in macaque colonies, the virus had to adapt to overcome blocks imposed by rhesus orthologs of *TRIM5* and *APOBEC3G* [103,117]. Similarly, it is likely that emergence of HIV-1 in humans required, at a minimum, adaptation of Vpu as an antagonist of human BST-2, and it is likely that other adaptive changes were required [14].

A survey of several different organisms reveals that the known restriction factors are often distributed across multiple chromosomes (Table 2). In organisms with multiple functionally distinct alleles at such loci, random reassortment will contribute to phenotypic diversity at the population level (Table 3). In rhesus macaques, for example, multiple alleles have been described for

Table 2. Chromosomal locations of restriction factors in five mammalian species.

Locus	<i>Homo sapiens</i>	<i>Pan troglodytes</i>	<i>Macaca mulatta</i>	<i>Callithrix jacchus</i>	<i>Rattus norvegicus</i>
<i>APOBEC3G</i>	chr. 22	chr. 22	chr. 10	chr. 1	chr. 7 [†]
<i>BST-2</i>	chr. 19	chr. 19	chr. 19	chr. 22	chr. 16
<i>Mx2/MxB</i>	chr. 21	chr. 21	chr. 3	chr. 21	chr. 11
<i>SAMHD-1</i>	chr. 20	chr. 20	chr. 10	chr. 5	chr. 3
<i>TRIM5</i>	chr. 11	chr. 11	chr. 14	chr. 11	chr. 1

[†]The common rat encodes only one allele of *APOBEC3*. chr.: Chromosome.

Table 3. Hypothetical numbers of restriction factor genotypes based on numbers of functionally distinct alleles at five independently assorting loci.

Loci	Number of alleles			
<i>APOBEC3G</i>	1	3	2	3
<i>BST-2</i>	1	1	2	3
<i>Mx2</i>	1	1	2	3
<i>SAMHD1</i>	1	1	2	3
<i>TRIM5</i>	1	3	2	3
Composite genotypes	1	36	243	7776

TRIM5 (three alleles), *BST-2* (two alleles) and *APOBEC3G* (three alleles). Because the genes are located on three different chromosomes, there can be up to 108 ($6 \times 3 \times 6$) composite genotypes involving just these three restrictions in rhesus macaques. As other restriction factor genes are discovered and studied, this number is likely to increase. Other hypothetical examples are given in Table 3. Thus, this sort of intrinsic immune complexity may be what many viruses encounter in nature when they invade new, naive host populations.

The existence of multiple independent restrictions resembles HAART, in which HIV-infected individuals are treated with a combination of inhibitors that target two or more distinct steps in the viral replication cycle. Just as resistance to HAART results from selection of multiple, separate adaptations on the part of the virus (often at a cost to viral fitness), the ability of a virus to become fully adapted to a new host is likely to require combinations of multiple, independent adaptations to circumvent intrinsic immune effectors acting on different parts

EXECUTIVE SUMMARY

Primate lentiviruses

- The primate lentiviruses include HIV-1 and HIV-2 in humans, SIVmac and related viruses found in captive macaques, and approximately 40 species of SIV endemic to African primates.
- The distribution of lentiviruses among modern primates is consistent with a natural history of interspecies transmission, adaptation and emergence of new viral lineages.
- Recombination may have played an important role in cross-species transmission of several primate lentiviruses.

Restriction factors (intrinsic immune effectors)

- The restriction factors include various intrinsic antiviral effectors, such as APOBEC3G, TRIM5α, BST-2/tetherin and SAMHD1.
- Restriction factors come in many different forms, act by different mechanisms and exploit weaknesses at different steps in the viral replication cycle.
- As a result of positive selection, restriction factor coding sequences in primates often exhibit significant interspecies divergence and dN/dS values >1.
- Primate restriction factors often exhibit significant nonsynonymous polymorphisms.
- Many of the known restriction factor genes map to different chromosomes, allowing for independent reassortment and formation of complex genotypes.

Viral accessory proteins

- Primate lentiviruses encode various accessory proteins (e.g., Vif, Vpu, Vpx, Vpr and Nef) that function as antagonists of host-encoded restriction factors.
- When primate lentiviruses jump between host species, viral accessory proteins can adapt to accommodate divergent restriction factor protein sequences found in the new host.
- Accessory proteins can also acquire new functions (neofunctionalization), at times facilitating adaptation to a new host.

of the viral replication cycle. We know that for APOBEC3G, BST-2 and TRIM5 one or two point mutations in viral genes suffice to abrogate restriction [94,103,117,129]. The presence of at least four restriction factors (APOBEC3G, BST-2, SAMHD1 and TRIM5; the recently discovered MxB [130–132] may be a fifth, and there are likely to be other undiscovered blocks to lentiviral infection) likely exerts a combined pressure on a newly transmitted virus, analogous to how HAART would in an HIV patient. The situation in outbred populations raises several important questions: given the number of restriction factors and the complexity of the genetics of restriction, is interspecies transmission of lentiviruses more likely between closely related hosts? In this regard, it is worth noting that HIV-1, which emerged from ape reservoirs, has spread much more efficiently in humans than HIV-2, which emerged from a more distant primate (sooty mangabeys). When viruses do successfully cross species lines, does emergence require simultaneous or near-simultaneous acquisition

of multiple resistance mutations, and to what degree does recombination enhance this likelihood? Such questions are relevant to understanding interspecies jumping, adaptation and emergence of all kinds of viruses [133] – perhaps further investigation of the primates and their lentiviruses will help provide the answers.

Acknowledgements

The authors apologize to all their colleagues whose important work could not be directly cited. The authors thank Kevin McCarthy and Ted Diehl for helpful discussions and comments.

Financial & competing interests disclosure

Research in the Johnson laboratory is supported in part by NIH grants AI095092 and AI083118. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:
• of interest

- WHO. *Global Report: UNAIDS Report on the Global AIDS Epidemic*. WHO Press, Geneva, Switzerland (2012).
- Korber B. Timing the ancestor of the HIV-1 pandemic strains. *Science* 288(5472), 1789–1796 (2000).
- Lemey P, Pybus O, Wang B, Saksena N, Salemi M, Vandamme A-M. Tracing the origin and history of the HIV-2 epidemic. *Proc. Natl Acad. Sci. USA* 100(11), 6588–6592 (2003).
- Wertheim J, Worobey M. Dating the age of the SIV lineages that gave rise to HIV-1 and HIV-2. *PLoS Comput. Biol.* 5(5), e1000377 (2009).
- Van Heuverswyn F, Li Y, Neel C *et al.* Human immunodeficiency viruses: SIV infection in wild gorillas. *Nature* 444(7116), 164–164 (2006).
- Plantier J-C, Leoz M, Dickerson JE *et al.* A new human immunodeficiency virus derived from gorillas. *Nat. Med.* 15(8), 871–872 (2009).
- Hirsch V, Olmsted R, Murphey-Corb M, Purcell R, Johnson P. An African primate lentivirus (SIVsm) closely related to HIV-2. *Nature* 339(6223), 389–392 (1989).
- Gao F, Yue L, White A *et al.* Human infection by genetically diverse SIVSM-related HIV-2 in west Africa. *Nature* 358(6386), 495–499 (1992).
- Chen Z, Telfer P, Gettie A *et al.* Genetic characterization of new west African simian immunodeficiency virus SIVsm: geographic clustering of household-derived SIV strains with human immunodeficiency virus type 2 subtypes and genetically diverse viruses from a single feral sooty mangabey troop. *J. Virol.* 70(6), 3617–3627 (1996).
- Chen Z, Luckay A, Sodora DL *et al.* Human immunodeficiency virus type 2 (HIV-2) seroprevalence and characterization of a distinct HIV-2 genetic subtype from the natural range of simian immunodeficiency virus-infected sooty mangabeys. *J. Virol.* 71(5), 3953–3960 (1997).
- Santiago ML, Range F, Keele BF *et al.* Simian immunodeficiency virus infection in free-ranging sooty mangabeys (*Cercopithecus atys atys*) from the Tai Forest, Cote d'Ivoire: implications for the origin of epidemic human immunodeficiency virus type 2. *J. Virol.* 79(19), 12515–12527 (2005).
- Ayoub A, Akoua-Koffi C, Calvignac-Spencer S *et al.* Evidence for continuing cross-species transmission of SIVsmm to humans. *AIDS* 27(15), 2488–2491 (2013).
- Marx P, Apetrei C, Drucker E. AIDS as a zoonosis? Confusion over the origin of the virus and the origin of the epidemics. *J. Med. Primatol.* 33(5–6), 220–226 (2004).
- Sharp PM, Hahn BH. Origins of HIV and the AIDS pandemic. In: *HIV: From Biology to Prevention to Treatment*. Bushman F, Nabel G, Swanstrom R (Eds). Cold Spring Harbor Perspectives in Medicine, NY, USA (2011).
- VandeWoude S, Apetrei C. Going wild: lessons from naturally occurring T-lymphotropic lentiviruses. *Clin. Microbiol. Rev.* 19(4), 728–762 (2006).
- Ahuka-Mundele S, Mbala-Kingebeni P, Liegeois F *et al.* Identification and molecular characterization of new simian T cell lymphotropic viruses in nonhuman primates bushmeat from the Democratic Republic of Congo. *AIDS Res. Hum. Retroviruses* 28(6), 628–635 (2012).
- Aghokeng AF, Ayoub A, Mpoudi-Ngole E *et al.* Extensive survey on the prevalence and genetic diversity of SIVs in primate bushmeat provides insights into risks for potential new cross-species transmissions. *Infect. Genet. Evol.* 10(3), 386–396 (2010).
- Charleston MA, Robertson DL. Preferential host switching by primate lentiviruses can account for phylogenetic similarity with the primate phylogeny. *System. Biol.* 51(3), 528–535 (2002).
- Bininda-Emonds ORP, Cardillo M, Jones KE *et al.* The delayed rise of present-day

- mammals. *Nature* 446(7135), 507–512 (2007).
- 20 Maddison WP, Maddison DR. Mesquite: a modular system for evolutionary analysis. <http://mesquiteproject.org>
- 21 FigTree. <http://tree.bio.ed.ac.uk/software/figtree>
- 22 Goff SP. Retroviridae. In: *Fields Virology*. Lippincott Williams & Wilkins, PA, USA (2013).
- 23 Aghokeng AF, Bailes E, Loul S *et al*. Full-length sequence analysis of SIVmus in wild populations of mustached monkeys (*Cercopithecus cephus*) from Cameroon provides evidence for two co-circulating SIVmus lineages. *Virology* 60(2), 407–418 (2007).
- 24 Courgnaud V, Salemi M, Pourrut X *et al*. Characterization of a novel simian immunodeficiency virus with a *vpu* gene from greater spot-nosed monkeys (*Cercopithecus nictitans*) provides new insights into simian/human immunodeficiency virus phylogeny. *J. Virol.* 76(16), 8298–8309 (2002).
- 25 Ma D, Jasinska A, Kristoff J *et al*. SIVagm infection in wild African green monkeys from South Africa: epidemiology, natural history, and evolutionary considerations. *PLoS Pathog.* 9(1), e1003011 (2013).
- 26 Bailes E, Gao F, Bibollet-Ruche F *et al*. Hybrid origin of SIV in chimpanzees. *Science* 300(5626), 1713 (2003).
- 27 Jin M, Hui H, Robertson D *et al*. Mosaic genome structure of simian immunodeficiency virus from west African green monkeys. *EMBO J.* 13(12), 2935–2947 (1994).
- 28 Hu J, Switzer W, Foley B *et al*. Characterization and comparison of recombinant simian immunodeficiency virus from drill (*Mandrillus leucophaeus*) and mandrill (*Mandrillus sphinx*) isolates. *J. Virol.* 77(8), 4867–4880 (2003).
- 29 Klatt N, Silvestri G, Hirsch V. Nonpathogenic simian immunodeficiency virus infections. In: *HIV: From Biology to Prevention to Treatment*. Bushman F, Nabel G, Swanstrom R (Eds). Cold Spring Harbor Perspectives in Medicine, NY, USA (2011).
- 30 Daniel M, Letvin N, King N *et al*. Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. *Science* 228(4704), 1201–1204 (1985).
- 31 Clavel F, Guyader M, Guetard D, Salle M, Montagnier L, Alizon M. Molecular cloning and polymorphism of the human immune deficiency virus type 2. *Nature* 324(6098), 691–695 (1986).
- 32 Sharp PM, Rayner JC, Hahn BH. Evolution. Great apes and zoonoses. *Science* 340(6130), 284–286 (2013).
- 33 Himathongkham S, Luciw P. Restriction of HIV-1 (subtype B) replication at the entry step in rhesus macaque cells. *Virology* 219(2), 485–488 (1996).
- 34 Shibata R, Adachi A. SIV/HIV recombinants and their use in studying biological properties. *AIDS Res. Hum. Retroviruses* 8(3), 403–409 (1992).
- 35 Li J, Lord C, Haseltine W, Letvin N, Sodroski J. Infection of cynomolgus monkeys with a chimeric HIV-1/SIVmac virus that expresses the HIV-1 envelope glycoproteins. *J. Acquir. Immune Defic. Syndr.* 5(7), 639–646 (1992).
- 36 Shibata R, Sakai H, Kawamura M, Tokunaga K, Adachi A. Early replication block of human immunodeficiency virus type 1 in monkey cells. *J. Gen. Virol.* 76(Pt 11), 2723–2730 (1995).
- 37 Owens CM, Yang PC, Gottlinger H, Sodroski J. Human and simian immunodeficiency virus capsid proteins are major viral determinants of early, postentry replication blocks in simian cells. *J. Virol.* 77(1), 726–731 (2003).
- 38 Hofmann W, Schubert D, LaBonte J *et al*. Species-specific, postentry barriers to primate immunodeficiency virus infection. *J. Virol.* 73(12), 10020–10028 (1999).
- **Demonstrated species-specific restriction of HIV-1 and SIVmac in numerous mammalian species (both primate and nonprimate), setting the stage for the initial discovery of TRIM5 and TRIMCypA.**
- 39 Stremlau M, Owens CM, Perron MJ, Kiessling M, Autissier P, Sodroski J. The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. *Nature* 427(6977), 848–853 (2004).
- 40 Diehl WE, Stansell E, Kaiser SM, Emerman M, Hunter E. Identification of postentry restrictions to Mason-Pfizer monkey virus infection in New World monkey cells. *J. Virol.* 82(22), 11140–11151 (2008).
- 41 Yap M, Nisole S, Lynch C, Stoye J. Trim5alpha protein restricts both HIV-1 and murine leukemia virus. *Proc. Natl Acad. Sci. USA* 101(29), 10786–10791 (2004).
- 42 Pacheco B, Finzi A, McGee-Estrada K, Sodroski J. Species-specific inhibition of foamy viruses from South American monkeys by New World monkey TRIM5 proteins. *J. Virol.* 84(8), 4095–4099 (2010).
- 43 Yap M, Lindemann D, Stanke N *et al*. Restriction of foamy viruses by primate Trim5alpha. *J. Virol.* 82(11), 5429–5439 (2008).
- 44 Johnson WE, Sawyer SL. Molecular evolution of the antiretroviral *TRIM5* gene. *Immunogenetics* 61(3), 163–176 (2009).
- 45 Fletcher AJ, Towers GJ. Inhibition of retroviral replication by members of the TRIM protein family. *Curr. Top. Microbiol. Immunol.* 371, 29–66 (2013).
- 46 Rhodes DA, Trowsdale J. TRIM21 is a trimeric protein that binds IgG Fc via the B30.2 domain. *Mol. Immunol.* 44(9), 2406–2414 (2007).
- 47 Barr S, Smiley J, Bushman F. The interferon response inhibits HIV particle production by induction of TRIM22. *PLoS Pathog.* 4(2), e1000007 (2008).
- 48 Meroni G. *TRIM/RBCC Proteins*. Springer Science and Business Media, LLC, NY, USA (2012).
- 49 Sayah D, Sokolskaja E, Berthouix L, Luban J. Cyclophilin A retrotransposition into *TRIM5* explains owl monkey resistance to HIV-1. *Nature* 430(6999), 569–573 (2004).
- 50 Wilson SJ, Webb BLJ, Maplanka C *et al*. Rhesus macaque *TRIM5* alleles have divergent antiretroviral specificities. *J. Virol.* 82(14), 7243–7247 (2008).
- 51 McCarthy KR, Schmidt AG, Kirmaier A, Wyand AL, Newman RM, Johnson WE. Gain-of-sensitivity mutations in a trim5-resistant primary isolate of pathogenic SIV identify two independent conserved determinants of Trim5α specificity. *PLoS Pathog.* 9(5), e1003352 (2013).
- 52 Ganser-Pornillos BK, Chandrasekaran V, Pornillos O, Sodroski JG, Sundquist WI, Yeager M. Hexagonal assembly of a restricting TRIM5alpha protein. *Proc. Natl Acad. Sci. USA* 108(2), 534–539 (2011).
- 53 Stremlau M, Perron M, Lee M *et al*. Specific recognition and accelerated uncoating of retroviral capsids by the TRIM5alpha restriction factor. *Proc. Natl Acad. Sci. USA* 103(14), 5514–5519 (2006).
- 54 Rold CJ, Aiken C. Proteasomal degradation of TRIM5α during retrovirus restriction. *PLoS Pathog.* 4(5), e1000074 (2008).
- 55 Sawyer S, Wu L, Emerman M, Malik H. Positive selection of primate TRIM5alpha identifies a critical species-specific retroviral restriction domain. *Proc. Natl Acad. Sci. USA* 102(8), 2832–2837 (2005).
- **Reported extensive genetic variation in primate *TRIM5α*, driven by strong positive selection acting on a specific TRIM5α domain (SPRY), and used evolutionary analysis to guide mapping of viral interacting regions.**

- 56 Song B, Gold B, O'hUigin C *et al.* The B30.2(SPRY) domain of the retroviral restriction factor TRIM5 α exhibits lineage-specific length and sequence variation in primates. *J. Virol.* 79(10), 6111–6121 (2005).
- 57 Song B, Javanbakht H, Perron M, Park DH, Stremlau M, Sodroski J. Retrovirus restriction by TRIM5 variants from Old World and New World primates. *J. Virol.* 79(7), 3930–3937 (2005).
- 58 Newman R, Hall L, Connole M *et al.* Balancing selection and the evolution of functional polymorphism in Old World monkey TRIM5 α . *Proc. Natl Acad. Sci. USA* 103(50), 19134–19139 (2006).
- 59 Nisole S, Lynch C, Stoye J, Yap M. A Trim5-cyclophilin A fusion protein found in owl monkey kidney cells can restrict HIV-1. *Proc. Natl Acad. Sci. USA* 101(36), 13324–13328 (2004).
- 60 Liao C, Kuang Y, Liu H, Zheng Y, Su B. A novel fusion gene, TRIM5-Cyclophilin A in the pig-tailed macaque determines its susceptibility to HIV-1 infection. *AIDS* 21(Suppl. 8), S19–S26 (2007).
- 61 Wilson S, Webb B, Ylinen L, Verschoor E, Heeney J, Towers G. Independent evolution of an antiviral TRIMCyp in rhesus macaques. *Proc. Natl Acad. Sci. USA* 105(9), 3557–3562 (2008).
- 62 Newman RM, Hall L, Kirmaier A *et al.* Evolution of a TRIM5-CypA splice isoform in Old World monkeys. *PLoS Pathog.* 4(2), e1000003 (2008).
- 63 Brennan G, Kozyrev Y, Hu S. TRIMCyp expression in Old World primates *Macaca nemestrina* and *Macaca fascicularis*. *Proc. Natl Acad. Sci. USA* 105(9), 3569–3574 (2008).
- 64 Virgen C, Kratovac Z, Bieniasz P, Hatzioannou T. Independent genesis of chimeric TRIM5–cyclophilin proteins in two primate species. *Proc. Natl Acad. Sci. USA* 105(9), 3563–3568 (2008).
- 65 Kestler HI, Ringler D, Mori K *et al.* Importance of the *nef* gene for maintenance of high virus loads and for development of AIDS. *Cell* 65, 651–662 (1991).
- 66 Giorgi JV, Hultin LE, Desrosiers RC. The immunopathogenesis of retroviral diseases: no immunophenotypic alterations in T, B, and NK cell subsets in SIVmac239-challenged rhesus macaques protected by SIV delta *nef* vaccination. *J. Med. Primatol.* 25(3), 186–191 (1996).
- 67 Desrosiers RC, Lifson JD, Gibbs JS, Czajak SC, Howe AYM, Arthur LO. Identification of highly attenuated mutants of simian immunodeficiency virus. *J. Virol.* 72(2), 1431–1437 (1998).
- 68 Dyer WB, Geczy AF, Kent SJ *et al.* Lymphoproliferative immune function in the Sydney Blood Bank Cohort, infected with natural *nef*/long terminal repeat mutants, and in other long-term survivors of transfusion-acquired HIV-1 infection. *AIDS* 11(13), 1565–1574 (1997).
- 69 Zaunders J, Dyer WB, Churchill M. The Sydney Blood Bank Cohort: implications for viral fitness as a cause of elite control. *Curr. Opin. HIV AIDS* 6(3), 151–156 (2011).
- 70 Kirchhoff F, Greenough T, Brettler D, Sullivan J, Desrosiers R. Brief report: absence of intact *nef* sequences in a long-term survivor with nonprogressive HIV-1 infection. *N. Engl. J. Med.* 332(4), 228–232 (1995).
- 71 Sheehy A, Gaddis J, Choi J, Malim M. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. *Nature* 418, 646–650 (2002).
- 72 Neil S, Zang T, Bieniasz P. Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. *Nature* 451(7177), 425–430 (2008).
- 73 Hrecka K, Hao C, Gierszewska M *et al.* Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. *Nature* 474(7353), 658–661 (2011).
- 74 Laguette N, Sobhian B, Casartelli N *et al.* SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. *Nature* 74(7353), 654–657 (2011).
- 75 Van Damme N, Goff D, Katsura C *et al.* The interferon-induced protein BST-2 restricts HIV-1 release and is downregulated from the cell surface by the viral Vpu protein. *Cell Host Microbe* 3(4), 245–252 (2008).
- 76 Mangeat B, Turelli P, Caron G, Friedli M, Perrin L, Trono D. Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. *Nature* 424(6944), 99–103 (2003).
- 77 Zhang H, Yang B, Pomerantz R, Zhang C, Arunachalam S, Gao L. The cytidine deaminase CEM15 induces hypermutation in newly synthesized HIV-1 DNA. *Nature* 424(6944), 94–98 (2003).
- 78 Bishop K, Holmes R, Malim M. Antiviral potency of APOBEC proteins does not correlate with cytidine deamination. *J. Virol.* 80(17), 8450–8458 (2006).
- 79 Iwatani Y, Chan DSB, Wang F *et al.* Deaminase-independent inhibition of HIV-1 reverse transcription by APOBEC3G. *Nucleic Acids Res.* 35(21), 7096–7108 (2007).
- 80 Bishop KN, Verma M, Kim E-Y, Wolinsky SM, Malim MH. APOBEC3G inhibits elongation of HIV-1 reverse transcripts. *PLoS Pathog.* 4(12), e1000231 (2008).
- 81 Conticello S, Harris R, Neuberger M. The Vif protein of HIV triggers degradation of the human antiretroviral DNA deaminase APOBEC3G. *Curr. Biol.* 13(22), 2009–2013 (2003).
- 82 Marin M, Rose K, Kozak S, Kabat D. HIV-1 Vif protein binds the editing enzyme APOBEC3G and induces its degradation. *Nat. Med.* 9(11), 1398–1403 (2003).
- 83 Sheehy A, Gaddis N, Malim M. The antiretroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif. *Nat. Med.* 9(11), 1404–1407 (2003).
- 84 Yu X, Yu Y, Liu B *et al.* Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif–Cul5–SCF complex. *Science* 302(5647), 1056–1060 (2003).
- 85 Jia B, Serra-Moreno R, Neidermyer W *et al.* Species-specific activity of SIV Nef and HIV-1 Vpu in overcoming restriction by tetherin/BST2. *PLoS Pathog.* 5(5), e1000429 (2009).
- 86 Zhang F, Wilson S, Landford W *et al.* Nef proteins from simian immunodeficiency viruses are tetherin antagonists. *Cell Host Microbe* 6(1), 54–67 (2009).
- 87 Baldauf H-M, Pan X, Erikson E *et al.* SAMHD1 restricts HIV-1 infection in resting CD4⁺ T cells. *Nat. Med.* 18(11), 1682–1687 (2012).
- 88 Gramberg T, Kahle T, Bloch N *et al.* Restriction of diverse retroviruses by SAMHD1. *Retrovirology* 10(1), 1–1 (2013).
- 89 Kim E, White T, Brandariz-Nunez A, Diaz-Griffero F, Weitzman M. SAMHD1 restricts herpes simplex virus type 1 (HSV-1) in macrophages by limiting DNA replication. *J. Virol.* 87(23), 12949–12956 (2013).
- 90 Lim ES, Fregoso OI, McCoy CO, Matsen FA, Malik HS, Emerman M. The ability of primate lentiviruses to degrade the monocyte restriction factor SAMHD1 preceded the birth of the viral accessory protein Vpx. *Cell Host Microbe* 11(2), 194–204 (2012).
- 91 Johnson WE. Rapid adversarial co-evolution of viruses and cellular restriction factors. *Curr. Top. Microbiol. Immunol.* 371, 123–151 (2013).
- 92 Sara L, Sawyer MEHSM. Ancient adaptive evolution of the primate antiviral DNA-editing enzyme APOBEC3G. *PLoS Biol.* 2(9), e275 (2004).

- 93 Laguette N, Rahm N, Sobhian B *et al.* Evolutionary and functional analyses of the interaction between the myeloid restriction factor SAMHD1 and the lentiviral Vpx protein. *Cell Host Microbe* 11(2), 205–217 (2012).
- 94 McNatt M, Zang T, Hatzioannou T *et al.* Species-specific activity of HIV-1 Vpu and positive selection of tetherin transmembrane domain variants. *PLoS Pathog.* 5(2), e1000300 (2009).
- 95 Zhang J, Webb D. Rapid evolution of primate antiviral enzyme APOBEC3G. *Hum. Mol. Genet.* 13(16), 1785–1791 (2004).
- 96 Compton AA, Malik HS, Emerman M. Host gene evolution traces the evolutionary history of ancient primate lentiviruses. *Philos. Trans. R Soc. Lond. B Biol. Sci.* 368(1626), 20120496–20120496 (2013).
- 97 Gifford R, Katzourakis A, Tristem M, Pybus O, Winters M, Shafer R. A transitional endogenous lentivirus from the genome of a basal primate and implications for lentivirus evolution. *Proc. Natl Acad. Sci. USA* 105(51), 20362–20367 (2008).
- 98 Meroni G, Diez-Roux G. TRIM/RBCC, a novel class of “single protein RING finger” E3 ubiquitin ligases. *Bioessays* 27(11), 1147–1157 (2005).
- 99 Van Der Aa LM, Levraud J-P, Yahmi M *et al.* A large new subset of TRIM genes highly diversified by duplication and positive selection in teleost fish. *BMC Biol.* 7(1), 7 (2009).
- 100 Malfavon-Borja R, Wu LL, Emerman M, Malik HS. Birth, decay, and reconstruction of an ancient *TRIMCyp* gene fusion in primate genomes. *Proc. Natl Acad. Sci. USA* 110(7), E583–E592 (2013).
- 101 Dietrich EA, Jones-Engel L, Hu S-L. Evolution of the antiretroviral restriction factor TRIMCyp in Old World primates. *PLoS ONE* 5(11), e14019 (2010).
- 102 Gack MU, Albrecht RA, Urano T *et al.* Influenza A virus NS1 targets the ubiquitin ligase TRIM25 to evade recognition by the host viral RNA sensor RIG-I. *Cell Host Microbe* 5(5), 439–449 (2009).
- 103 Kirmaier A, Wu F, Newman RM *et al.* TRIM5 suppresses cross-species transmission of a primate immunodeficiency virus and selects for emergence of resistant variants in the new species. *PLoS Biol.* 8(8), e1000462 (2010).
- First direct, *in vivo* demonstration of a primate restriction factor (TRIM5) selecting for viral adaptations during emergence of a primate lentivirus in a new host.
- 104 Wu F, Kirmaier A, Goeken R *et al.* TRIM5 alpha drives SIVmm evolution in rhesus macaques. *PLoS Pathog.* 9(8), e1003577 (2013).
- 105 LaRue RS, Jónsson SR, Silverstein KA *et al.* The artiodactyl APOBEC3 innate immune repertoire shows evidence for a multi-functional domain organization that existed in the ancestor of placental mammals. *BMC Mol. Biol.* 9(1), 104 (2008).
- 106 Munk C, Willemsen A, Bravo IG. An ancient history of gene duplications, fusions and losses in the evolution of APOBEC3 mutators in mammals. *BMC Evol. Biol.* 12(71), 1–16 (2012).
- 107 Mariani R, Chen D, Schrofelbauer B *et al.* Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. *Cell* 114(1), 21–31 (2003).
- 108 Bogerd H, Doehle B, Wiegand H, Cullen B. A single amino acid difference in the host APOBEC3G protein controls the primate species specificity of HIV type 1 virion infectivity factor. *Proc. Natl Acad. Sci. USA* 101(11), 3770–3774 (2004).
- 109 Xu H, Svarovskaia E, Barr R *et al.* A single amino acid substitution in human APOBEC3G antiretroviral enzyme confers resistance to HIV-1 virion infectivity factor-induced depletion. *Proc. Natl Acad. Sci. USA* 101(15), 5652–5657 (2004).
- 110 Mangeat B, Turelli P, Liao S, Trono D. A single amino acid determinant governs the species-specific sensitivity of APOBEC3G to Vif action. *J. Biol. Chem.* 279(15), 14481–14483 (2004).
- 111 Schrofelbauer B, Chen D, Landau N. A single amino acid of APOBEC3G controls its species-specific interaction with virion infectivity factor (Vif). *Proc. Natl Acad. Sci. USA* 101(11), 3927–3932 (2004).
- 112 Compton AA, Emerman M. Convergence and divergence in the evolution of the APOBEC3G–Vif interaction reveal ancient origins of simian immunodeficiency viruses. *PLoS Pathog.* 9(1), e1003135 (2013).
- 113 Letko M, Silvestri G, Hahn BH *et al.* Vif proteins from diverse primate lentiviral lineages use the same binding site in APOBEC3G. *J. Virol.* 87(21), 11861–11871 (2013).
- 114 Malim MH. APOBEC proteins and intrinsic resistance to HIV-1 infection. *Philos. Trans. R Soc. B Biol. Sci.* 364(1517), 675–687 (2009).
- 115 Goila-Gaur R, Strebel K. HIV-1 Vif, APOBEC, and intrinsic immunity. *Retrovirology* 5(1), 51 (2008).
- 116 Compton AA, Hirsch VM, Emerman M. The host restriction factor APOBEC3G and retroviral Vif protein coevolve due to ongoing genetic conflict. *Cell Host Microbe* 11(1), 91–98 (2012).
- 117 Krupp A, McCarthy KR, Ooms M *et al.* *APOBEC3G* polymorphism as a selective barrier to cross-species transmission and emergence of pathogenic SIV and AIDS in a primate host. *PLoS Pathog.* 9(10), e1003641 (2013).
- Proof that emergence of SIVmm as SIVmac in rhesus macaques required viral adaptation to the accessory factor Vif, and that intraspecies diversity in *APOBEC3G* determines host susceptibility to primate lentiviruses.
- 118 Le Tortorec A, Neil SJD. Antagonism to and intracellular sequestration of human tetherin by the human immunodeficiency virus type 2 envelope glycoprotein. *J. Virol.* 83(22), 11966–11978 (2009).
- 119 Sauter D, Schindler M, Specht A *et al.* Tetherin-driven adaptation of Vpu and Nef function and the evolution of pandemic and nonpandemic HIV-1 strains. *Cell Host Microbe* 6(5), 409–421 (2009).
- 120 Sauter D, Vogl M, Kirchhoff F. Ancient origin of a deletion in human BST2/tetherin that confers protection against viral zoonoses. *Hum. Mutat.* 32(11), 1243–1245 (2011).
- 121 Alexander L, Illyinskii PO, Lang SM *et al.* Determinants of increased replicative capacity of serially passaged simian immunodeficiency virus with *nef* deleted in rhesus monkeys. *J. Virol.* 77(12), 6823–6835 (2003).
- 122 Serra-Moreno R, Bin Jia, Breed M, Alvarez X, Evans DT. Compensatory changes in the cytoplasmic tail of gp41 confer resistance to tetherin/BST-2 in a pathogenic *Nef*-deleted SIV. *Cell Host Microbe* 9(1), 46–57 (2011).
- Demonstration of rapid acquisition of compensatory mutations (in *env*) in an attenuated SIV strain lacking an accessory gene (*nef*), establishing the impact and *in vivo* significance of restriction by BST-2/tetherin.
- 123 Lim ES, Malik HS, Emerman M. Ancient adaptive evolution of tetherin shaped the functions of Vpu and Nef in human immunodeficiency virus and primate lentiviruses. *J. Virol.* 84(14), 7124–7134 (2010).
- 124 Sharkey M. Restriction of retroviral infection of macrophages. *Curr. Top. Microbiol. Immunol.* 371, 105–122 (2013).
- 125 Tristem M, Marshall C, Karpas A, Petrik J, Hill F. Origin of vpx in lentiviruses. *Nature* 347(6291), 341–342 (1990).

- 126 Hatzioannou T, Ambrose Z, Chung N *et al.* A macaque model of HIV-1 infection. *Proc. Natl Acad. Sci. USA* 106(11), 4425–4429 (2009).
- 127 Misra A, Thippeshappa R, Kimata JT. Macaques as model hosts for studies of HIV-1 infection. *Front. Microbiol.* 4, 1–8 (2013).
- 128 Saito A, Akari H. Macaque-tropic human immunodeficiency virus type 1: breaking out of the host restriction factors. *Front. Microbiol.* 4, 187 (2013).
- 129 Barrett BS, Smith DS, Li SX, Guo K, Hasenkrug KJ, Santiago ML. A single nucleotide polymorphism in tetherin promotes retrovirus restriction *in vivo*. *PLoS Pathog.* 8(3), e1002596 (2012).
- 130 Kane M, Yadav SS, Bitzegeio J *et al.* MX2 is an interferon-induced inhibitor of HIV-1 infection. *Nature* 502(7472), 563–566 (2013).
- 131 Goujon C, Moncorgé O, Bauby H *et al.* Human MX2 is an interferon-induced post-entry inhibitor of HIV-1 infection. *Nature* 502(7472), 559–562 (2013).
- 132 Liu Z, Pan Q, Ding S *et al.* The interferon-inducible MxB protein inhibits HIV-1 infection. *Cell Host Microbe* 14(4), 398–410 (2013).
- 133 Holmes EC, Drummond AJ. The evolutionary genetics of viral emergence. *Curr. Top. Microbiol. Immunol.* 351, 51–66 (2007).