

Evolutionary conflicts between viruses and restriction factors shape immunity

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Abstract | Host restriction factors are potent, widely expressed intracellular blocks to viral replication that are an important component of the innate immune response to viral infection. However, viruses have evolved mechanisms that antagonize restriction factors. Through evolutionary pressure for both host survival and virus replication, an evolutionary ‘arms race’ has developed that drives continuous rounds of selection for beneficial mutations in the genes encoding restriction factors and their viral antagonists. Because viruses can evolve faster than their hosts, the innate immune system of modern-day vertebrates is for the most part optimized to defend against ancient viruses, rather than newer viral threats. Thus, the evolutionary history of restriction factors might, in part, explain why humans are susceptible or resistant to the viruses present in the modern world.

Fv1 locus

(Friend virus susceptibility 1 locus). A genomic region in mice containing the *Fv1* gene. Different *Fv1* alleles encode versions of a restriction factor that protect mice from murine leukaemia viruses.

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Restriction factors are proteins of the innate immune system encoded in the germline genome that inhibit the replication of viruses during their life cycle in host cells. These host proteins are dedicated antiviral factors that are often induced by interferon (IFN) signaling as part of the innate immune response. They are antagonized by viral factors and are rapidly evolving. The term ‘restriction factor’ was historically adopted by laboratories studying retroviruses following the characterization of the mouse *Fv1* locus, which conferred resistance to murine retroviruses¹. However, this term can also be applied more broadly to host-encoded gene products that inhibit the intracellular replication of any animal virus. Recent work has shown that host susceptibility to viral infection and disease is determined, in part, by the components of the innate immune system (such as restriction factors) and the viral proteins that have evolved to evade or destroy these host defences. In this Review, we describe the general characteristics of restriction factors and show how the evolutionary conflict between viruses and restriction factors has shaped the immune systems of modern-day vertebrates. We use examples of host restriction factors that block primate lentiviruses, although we believe that many of the principles are generally applicable to other viruses and other hosts. These topics are of particular relevance today, as there have been many recent discoveries of restriction factors and determinants of viral susceptibility.

Characteristics of restriction factors

Classical innate immunity against viruses is mediated by specialized cells such as natural killer cells, dendritic cells and macrophages. By contrast, restriction factors are germline-encoded factors that mediate a ‘cell-intrinsic’ immune response. They are part of the broader innate immune repertoire of cellular molecules that detect and respond to viral infections in the absence of previous exposure. Typically, viral infections are detected by cytoplasmic or membrane-bound pattern-recognition receptors (PRRs), such as Toll-like receptors (TLRs), which trigger an IFN response that induces a programme of expression of IFN-stimulated genes with broad-ranging effects on cell growth and metabolism (reviewed in REFS 2,3). Many of these IFN-stimulated genes are restriction factors that specifically inhibit viral growth within infected cells. TABLE 1 lists the general features of the restriction factors that target retroviruses and other viruses that are described in this Review. TABLE 1 is not a comprehensive list of restriction factors, but contains some of the best-studied examples.

There are several distinguishing characteristics of restriction factors that allow one to make inferences about their role in the evolution of both the host and the virus. Typically, we define a host gene as a restriction factor gene if it encodes a protein that: has antiviral activity as its major biological function; is induced by IFNs or by virus infection; is antagonized by a viral protein; and shows evolutionary ‘signatures’ of genetic conflict (positive selection).

Table 1 | Characteristics of some well-studied examples of restriction factors

Restriction factor	IFN induced?	Viral targets*	Viral lifecycle stage inhibited	Viral antagonists	Under positive selection?
Fv1	No	Retroviruses	Capsid uncoating ⁹³	None known	Yes ⁹⁴
TRIM5α and TRIM-CYP	Yes	Retroviruses	Capsid uncoating ^{9,93}	None known (escape through capsid mutations)	Yes ^{53,95}
APOBEC3 family	APOBEC3A	Retroviruses, retrotransposons, hepadnaviruses	Reverse transcription ^{80,93}	Vif (lentiviruses), Bet (spumaviruses), Gag (gammaretroviruses)	APOBEC3DE, APOBEC3G, APOBEC3H ^{45,49,50}
SAMHD1	Yes	Retroviruses	Reverse transcription ⁸⁸	Vpx (some SIVs), Vpr (some SIVs)	Yes ^{81,82}
ZAP	Yes	Retroviruses, filoviruses, alphaviruses	Viral protein translation ⁹³	None known	Yes ⁹⁶
Tetherin	Yes	Retroviruses, flaviviruses, herpesviruses, rhabdoviruses, paramyxoviruses, arenaviruses	Budding ⁹³	Nef (some SIVs), Vpu (HIV-1), Env (HIV-2), glycoprotein (Ebola virus), K5 (KSHV)	Yes ^{97,98}
Viperin	Yes	Orthomyxoviruses, flaviviruses, herpesviruses, alphaviruses, paramyxoviruses	Budding ⁹⁹	None known	Yes ¹⁰⁰
MxA and Mx1	Yes	Orthomyxoviruses, paramyxoviruses, hepadnaviruses, rhabdoviruses, alphaviruses, bunyaviruses, togaviruses, picornaviruses	Nucleocapsid transport or another early lifecycle step ¹⁰¹	None known	ND
IFITM1, IFITM2 and IFITM3	Yes	Orthomyxoviruses, flaviviruses, coronaviruses	Endosomal fusion or uncoating ⁹⁹	None known	ND
PKR	Yes	Poxviruses	Viral protein translation ¹⁰¹	K3L and E3L (vaccinia virus), TRS1 and IRS1 (HCMV) ¹⁰² , and many others ¹⁰³	Yes ¹⁰⁴

HCMV, human cytomegalovirus; IFITM, interferon-induced transmembrane protein; KSHV, Kaposi's sarcoma-associated herpesvirus; ND, not determined; PKR, RNA-activated protein kinase; SAMHD1, SAM domain- and HD domain-containing protein 1; SIV, simian immunodeficiency virus; TRIM, tripartite motif-containing protein; ZAP, zinc-finger antiviral protein (also known as ZC3HAV1). *Viruses are listed by family, which refers to a group of viruses with similar genomic structures and replication strategies.

The majority of true restriction factors share these features, as described in detail below. However, the exceptions to these definitions are also highlighted in TABLE 1, as they can be enlightening with regard to understanding the additional cellular roles that restriction factors might have.

Expression and activity of viral restriction factors. Many restriction factors are IFN-stimulated genes (TABLE 1), which is consistent with their fundamental role in antiviral responses. The IFN-mediated induction of many restriction factors is also an indication that their major activity is in combating pathogens, rather than some central metabolic or developmental role in the organism. Moreover, as many restriction factors cause destructive events, such as protein modifications or nucleotide mutations, their expression needs to be tightly controlled to avoid deleterious effects on cell growth in the absence of viral challenge. However, IFN-mediated induction is not a universal property of restriction factors, as some are expressed constitutively. In cases in which expression of the protein is constitutive, it is probable that the restriction factor also has a role in restricting endogenous events. For example, in the APOBEC3 family of cytidine deaminases, APOBEC3G is constitutively expressed by many cell types, including T cells and germ cells^{4,5}. Although it has a well-characterized role in T cells in the inhibition of retroviruses through the hypermutation of viral

genomes during reverse transcription, we suggest that APOBEC3G might have an even more ancient role in protecting the host genome in germ cells from endogenous retrotransposons, which do not induce an IFN response⁶.

During an acute viral infection, each productively infected cell generates many infectious particles, leading to exponential viral growth. Therefore, restriction factors must have extremely potent antiviral activity to have any significant effect on viral loads (the restriction factors included in TABLE 1, for example, decrease viral infectivity by tenfold or more in single-round viral infectivity assays, although the level of restriction observed will vary depending on the system). This antiviral activity can be demonstrated experimentally through overexpression of a restriction factor, which causes a decrease in viral growth, or knockdown of a restriction factor, which causes an increase in viral growth. For example, SAMHD1 (SAM domain- and HD domain-containing protein 1) was recently defined as a restriction factor that is present in monocytes. Decreasing its endogenous expression in monocytes using RNA interference enhanced the replication of HIV-1, and exogenously expressing SAMHD1 in terminally differentiated myeloid cells restricted HIV-1 replication^{7,8}. In addition, the antiviral activity of restriction factors is sometimes specific to families of viruses. For example, TRIM5α (tripartite motif-containing protein 5α) seems to be active only against retroviruses,

Pattern-recognition receptors (PRRs). Host receptors (such as Toll-like receptors (TLRs) or NOD-like receptors (NLRs)) that can sense pathogen-associated molecular patterns and initiate signalling cascades that lead to an innate immune response. PRRs can be membrane bound (such as TLRs) or soluble cytoplasmic receptors (such as RIG-I, MDA5 and NLRs).

Positive selection
Selection to increase the frequency of mutations that confer a fitness advantage.

Endogenous retrotransposons
Mobile elements present in the genome that move locations using a mechanism involving reverse transcription.

Box 1 | Coordination of restriction factors with other arms of the immune system

The relationship between restriction factors and the rest of the innate immune system is a growing area of research. In many ways, restriction factors are similar to pattern-recognition receptors (PRRs) because they recognize structural patterns on pathogens. In fact, TRIM5 α (tripartite motif-containing protein 5 α) — which binds to a viral capsid lattice structure⁸⁶ and accelerates capsid uncoating to cause viral restriction — has recently been shown to also function as a PRR for retroviruses²⁰. After binding to retroviral capsids, TRIM5 α causes the activation of nuclear factor- κ B (NF- κ B) signalling and a distinct innate immune response. Moreover, even in the absence of retroviral capsids, TRIM5 α has been shown to have a role in innate immune responses, as it functions as a constitutive signalling intermediate in the NF- κ B cascade¹⁹. Similarly, tetherin has been shown to activate NF- κ B in addition to its viral restriction function⁸⁷.

SAMHD1 (SAM domain- and HD domain-containing protein 1) also functions as both a restriction factor and a mediator of the innate immune response to non-viral events. SAMHD1 protects dendritic cells and monocytes from HIV-1 infection by decreasing the level of cellular dNTPs to below the level required for the synthesis of viral DNA^{7,8,88}. In addition, SAMHD1 has a role in the innate immune response even in the absence of retroviral infection, as genetic mutations in *SAMHD1* (and in *TREX1* (3' repair exonuclease 1)) are associated with autoimmunity in humans²¹. Similarly to *TREX1*, *SAMHD1* might have a protective role against autoimmune responses by preventing the accumulation of inappropriate retrotransposon by-products, such as single-stranded DNA^{88,89}. Studying retroviral restriction factors will undoubtedly contribute to our understanding of many aspects of the innate immune system.

because it inhibits viral replication by means of a specific interaction with retroviral capsid proteins⁹. By contrast, tetherin (also known as BST2) can restrict enveloped viruses across several virus families, because it is nonspecifically incorporated into the cell and virus membranes and prevents efficient viral release by tethering enveloped viruses to the cell¹⁰ (TABLE 1).

We propose that the major biological activity of restriction factors is to inhibit viral replication. In many cases in which restriction factor function can be examined by gene knockout in mice, ablation of the restriction factor has no untoward effect on mouse development. For example, mice lacking the single mouse *ApoBec3* gene are viable, and the only reported phenotype is that they are more susceptible to murine retroviruses than are their wild-type counterparts¹¹. In fact, natural mutations in *ApoBec3* and the *Mx* locus that abolish function exist in some inbred mouse strains^{12,13}. Similarly, mice with natural or engineered mutations in the tetherin, viperin (also known as *Rsad2*) or interferon-induced transmembrane protein 3 (*Ifitm3*) genes are also viable but are more sensitive to some viral infections^{14–18}. However, it is possible that some restriction factors have other cellular roles in addition to viral restriction. For example, TRIM5 α has a more general role in antiviral signalling in addition to its specific role in retroviral restriction^{19,20}, and mutations in human *SAMHD1* are associated with autoimmune disease²¹ (BOX 1). However, perhaps as a result of the duplication of many restriction factor genes within a host (described further below), restriction factors can undergo subfunctionalization, in which one gene retains an essential cellular function whereas its paralogue becomes a dedicated antiviral factor.

Viral antagonists of restriction factors. Viruses have evolved antagonists to restriction factors. These viral proteins are often encoded by 'accessory genes' that are not needed for viral replication except in the presence of restriction factors²². Restriction factors such as tetherin that inhibit the replication of multiple virus families can be antagonized by diverse viral proteins from the different virus families (TABLE 1). In cases in which there is no known viral antagonist to a particular restriction

factor, it is possible that the virus can escape restriction through mutation of the viral protein targeted by the restriction factor, as is the case for lentiviral evasion of TRIM5 α -mediated restriction through viral capsid mutations^{23,24}. It is also theoretically possible that a recently evolved restriction factor might not yet have selected for a viral antagonist. However, in most cases, we think that the inability to identify a viral antagonist is more likely to be attributable to the fact that the relevant sets of viruses and host species have yet to be examined.

Viral antagonists can overcome restriction factors using several mechanisms. For example, viral antagonists can couple the restriction factor to protein degradation pathways; cause the mislocalization of the restriction factor and thus downregulate functional expression; or function as mimics of the restriction factor substrate (FIG. 1). To antagonize the restriction factor SAMHD1, the Vpx protein encoded by HIV-2 and related primate lentiviruses targets SAMHD1 for ubiquitylation followed by proteasomal degradation (FIG. 1a) by simultaneously binding to SAMHD1 and an adaptor protein in the cullin 4 ubiquitin ligase complex^{7,8}. The lentiviral Vif protein antagonizes APOBEC3G by a similar mechanism^{25,26}. By contrast, the lentiviral Vpu protein antagonizes the restriction factor tetherin by altering its normal subcellular localization (FIG. 1b). Through a direct protein–protein interaction, Vpu sequesters tetherin in the *trans*-Golgi network and redirects it from the cell membrane to endosomes, where it is unable to restrict viral budding from the cell membrane²⁷. A third mechanism of antagonism is illustrated by K3L, a poxvirus-encoded antagonist of the host antiviral RNA-activated protein kinase (PKR) pathway. Following recognition of double-stranded RNA, PKR inhibits protein translation by phosphorylating eukaryotic initiation factor 2 subunit- α (eIF2 α). K3L is structurally homologous to eIF2 α and competes for binding to PKR (FIG. 1c). By acting as a mimic of eIF2 α , K3L prevents the phosphorylation of eIF2 α and the translational shutoff that the PKR pathway would otherwise induce (reviewed in REF. 28). Viruses might also use other strategies that have not yet been characterized to allow viral replication despite the presence of restriction factors. A key feature common to all these modes of antagonism

Paralogue

A gene that is homologous to another gene within a species. Paralogues are generated through gene duplication and then divergence.

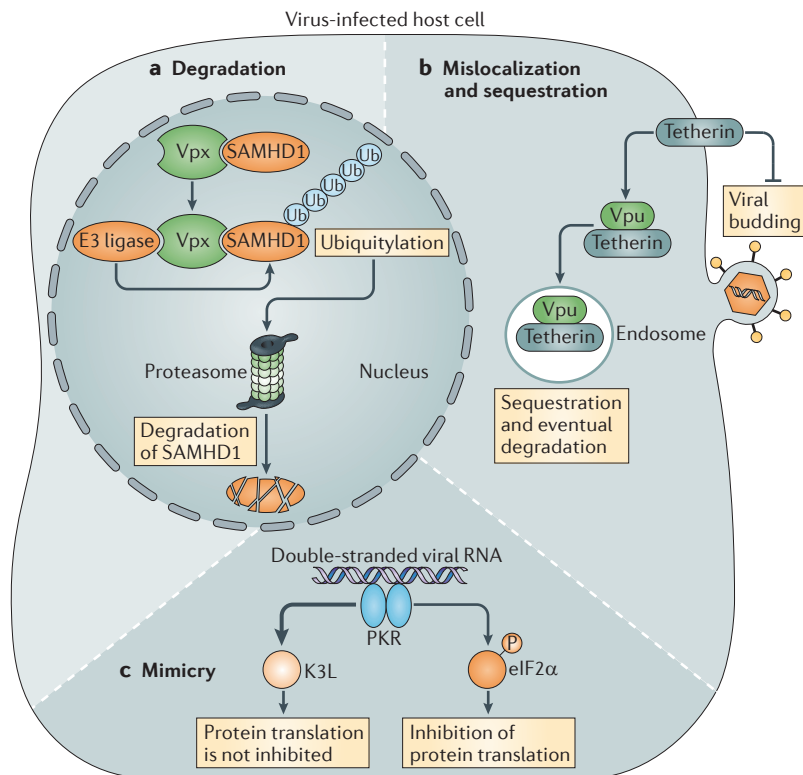


Figure 1 | Mechanisms of viral antagonism of host restriction factors. **a** | Degradation. The lentiviral accessory protein Vpx antagonizes the host restriction factor SAMHD1 (SAM domain- and HD domain-containing protein 1) by targeting it for degradation^{7,8}. Vpx bridges SAMHD1 to an E3 ubiquitin (Ub) ligase complex, which ubiquitylates SAMHD1, thus targeting SAMHD1 for degradation by the proteasome. **b** | Mislocalization and sequestration. The HIV-1 accessory protein Vpu antagonizes the host restriction factor tetherin by promoting its mislocalization, which leads to functional downregulation²⁷. Vpu interacts with tetherin at the plasma membrane and facilitates its trafficking to early endosomes. Tetherin is then either sequestered in the *trans*-Golgi network, where it is unable to restrict viral budding from the cell surface, or degraded in lysosomes. **c** | Mimicry. The poxvirus accessory protein K3L antagonizes the host RNA-activated protein kinase (PKR) pathway by acting as a mimic of the PKR substrate, eukaryotic initiation factor 2 subunit- α (eIF2 α)²⁸. PKR is activated by binding to double-stranded RNA and induces an antiviral signalling pathway that leads to the inhibition of protein translation. By competing for PKR binding, K3L prevents the phosphorylation of eIF2 α , thus pre-empting the antiviral response of host protein translation shut-off.

is often a result of two genetic entities evolving in conflict with one another, as illustrated by the ‘Red Queen’ hypothesis, which describes an evolutionary system in which continuous adaptation is required to maintain the status quo²⁹. Virus–host interactions are examples of ‘Red Queen’ competition, as host restriction factors exert a selective pressure on virus replication and pathogenic viruses exert fitness costs on their hosts. Mutations that allow a restriction factor to evade a viral antagonist provide a means for the host to escape the fitness costs conferred by the virus. This imposes a selective pressure on the viral antagonist to evolve specificity for the new restriction factor encoded by the host species. As a result, a prey–predator-like ‘arms race’ dynamic is established, leading to the rapid evolution of both the host and the virus (FIG. 2a). Thus, nearly all of the restriction factors described in TABLE 1 contain genetic ‘signatures’ of positive selection.

Because viruses have existed throughout vertebrate evolution, the arms race between hosts and viruses is ancient³⁰. In fact, many host restriction factors have evolved under positive selection for many millions of years. Under a long-term or recurrent viral selection pressure, a single amino acid in a restriction factor that directly interacts with a viral antagonist may repeatedly be mutated many times during evolution, or a restriction factor may accumulate mutations at multiple residues to escape from antagonism by many different viruses. This leads to an unusually high ratio of the non-synonymous mutation rate (dN) to the synonymous mutation rate (dS) — dN/dS — at single residues and across entire proteins (FIG. 2b). To estimate the dN/dS ratio of a gene, ancestral gene sequences can be reconstructed using orthologous gene sequences from modern-day species that diverged millions of years ago, and statistical methods are used to calculate the rate of evolution across a phylogenetic tree³¹. This method has shown that many human restriction factors have been evolving under episodic positive selection throughout primate evolution (TABLE 1). Other methods in addition to the calculation of dN/dS can also be used to identify positive selection across different timescales³², and additional background on the relevance and use of measures of positive selection in human evolution can be found in other reviews³³.

is the direct interaction between the viral antagonist and the host restriction factor, which has set the stage for the evolutionary ‘arms race’ that is characteristic of many restriction factors, as described below.

Positive selection as a fundamental principle of virus–host interactions. Many non-coding regions of the genome evolve under neutral selection; for example, non-synonymous (amino acid-altering) and synonymous mutations are predicted to accumulate at the same rate in pseudogenes. Most host protein-coding genes evolve under negative (purifying) selection, which removes non-synonymous mutations from the population to maintain the function of the protein. By contrast, the interactions between restriction factors and viral antagonists evolve under positive selection, a selective regime that results in an excess rate of non-synonymous mutations compared with synonymous mutations (BOX 2). Positive selection

How do hosts keep up in the arms race?

If single nucleotide changes were the only effector mechanism in the co-evolution of hosts and viruses, the host would be at a seemingly enormous disadvantage, because RNA viruses and some small single-stranded DNA viruses have nucleotide substitution rates that are 1,000 times faster than those of their hosts^{34–37}. How then does a host restriction factor ever win an arms race with a virus, especially considering that a host might be simultaneously challenged by many different types of virus? The answer lies in the types of genetic landscape that viruses and hosts can explore.

Limitations on viral evolution. RNA viruses maintain densely packed genomes that include overlapping reading frames and RNA hairpin structures involved in genome packaging and replication. The limitations on the

Neutral selection
Changes in allele frequency that are due to genetic drift rather than a fitness outcome.

Pseudogenes
Nonfunctional remnants of genes.

‘Red Queen’ hypothesis
An evolutionary hypothesis proposed by Leigh Van Valen that states: “For an evolutionary system, continuing development is needed just in order to maintain its fitness relative to the systems it is co-evolving with.”

Box 2 | Selective sweeps are the mechanism of adaptation

An important aspect of the positive selection of restriction factors is that the selection (and therefore evolution) of an advantageous mutation acts on a population level. The cost of a viral infection must affect the ability of the population to reproduce before it will exert a selective pressure on the population to evolve. During a population-wide infection, some individuals may carry a previously neutral genetic mutation that now confers those individuals with a reproductive advantage in the face of infection, and this advantageous genotype can rapidly rise in frequency until the mutation reaches a frequency of 100% (known as fixation). In a classic selective sweep, surrounding regions of the genome are inherited together (known as hitchhiking) with the genomic sequence that confers the fitness advantage, thus decreasing genetic diversity near the region of the genome under positive selection (reviewed in REFS 90–92). Therefore, genomic loci that are under positive selection in a population are predicted to have skewed allele frequencies across long genetic distances surrounding the selected locus. Eventually, if a mutation reaches fixation within a species, between-species comparisons will reveal an excess of non-synonymous mutations in this region relative to the number expected under neutral selection⁹⁰.

genome size of RNA viruses necessitate that many viral proteins carry out multiple functions³⁸. This constrains their evolutionary potential, as mutations to optimize one function (for example, adaptation to a polymorphism in a host restriction factor) might compromise another function of that protein (for example, capsid assembly). In addition, the small viral genome size generally prevents gene duplication-driven mechanisms of adaptive evolution, whereas these mechanisms can occur in host genomes. One possible exception to the lack of gene duplication in viruses is the pair of homologous genes *vpr* and *vpx* in some lentiviruses³⁹.

Host heterozygosity. Genetic polymorphisms in genes encoding host restriction factors can be maintained as a result of population-level adaptation against viruses. Balancing selection in restriction factors may result when multiple viruses co-infect a population, such that different host haplotypes are advantageous against different viruses, essentially maintaining polymorphism within the population (known as frequency-dependent selection). Several of the best-known examples of genes under balancing selection are genes involved in immunity. These include: MHC genes⁴⁰, which maintain multiple alleles that present a variety of antigens and therefore protect against a variety of pathogens; the glucose-6-phosphate dehydrogenase gene⁴¹, a housekeeping gene that contains polymorphisms that are associated with clinical disorders and also with malaria resistance; and *TRIM5*, which has been suggested to be under balancing selection in Old World monkey populations⁴².

Heterozygosity for a restriction factor may be advantageous to a population on a short timescale, as host polymorphisms would force a virus to evolve the ability to target multiple alleles of a given host factor. This was recently suggested for the restriction factor APOBEC3G in African green monkeys⁴³, a primate species naturally infected with simian immunodeficiency virus (SIV). APOBEC3G is polymorphic in African green monkeys, and some individuals have a single amino acid change that renders APOBEC3G resistant to its viral antagonist, Vif. In an experimental infection of African green monkeys with SIV, the virus from a monkey that was heterozygous for the Vif-resistant allele of APOBEC3G was unable to evolve

the ability to antagonize APOBEC3G, whereas the virus from a monkey that was homozygous for the Vif-resistant allele was quickly able to evolve the ability to antagonize APOBEC3G. This suggests that maintaining polymorphism in a restriction factor can be functionally beneficial.

Gene duplication and innovation. The duplication of restriction factor genes is another evolutionary strategy for accelerating host adaptation to a virus. By duplicating a restriction factor, the host can simultaneously explore multiple evolutionary trajectories. For example, in primate, artiodactyl (cloven-hoofed mammalian), canine and feline species, the APOBEC3 repertoires include many more paralogues than in rodents. In these lineages, the ancestral mammalian state — which was a single APOBEC3 gene — has been expanded to a family of APOBEC3 genes^{44–48}. Most primate genomes now encode seven APOBEC3 gene paralogues, which vary in terms of their antiviral activities and retroelement targets, suggesting that they are adapted to different viruses. Several APOBEC3 genes (including APOBEC3DE, APOBEC3G and APOBEC3H) show evidence of positive selection in primates^{45,49,50}, but the specific residues that are under positive selection vary between the APOBEC3 genes, further supporting the idea that each paralogue has evolved to target different viruses. In this way, increasing the copy number of a given restriction factor probably gives the host the flexibility to rapidly evolve in response to several different viruses, leading to large families of related restriction factors. Other restriction factor families that are the result of gene duplications include the *Mx1* gene family, which comprises two paralogues in some mice⁵¹; the IFITM gene family, which comprises at least four paralogues in humans and five paralogues in mice⁵²; and the *Trim5* gene family, which comprises eight paralogues in mice and cows and three paralogues in rats^{53,54}.

Alternatively, multiple members of a restriction factor family could evolve to target the same virus in different ways, thereby constraining viral evolution such that the virus must maintain multiple defence strategies. An example of this is the pair of human paralogues APOBEC3F and APOBEC3G. APOBEC3F and APOBEC3G deaminate cytosine bases in the viral genome within different preferential sequence contexts⁵⁵. Thus, primate lentiviruses (such as HIV-1) have had to evolve multiple mechanisms to antagonize these APOBEC3 proteins; for example, Vif binds to APOBEC3F and APOBEC3G using distinct domains^{56–58}. In this way, the host limits the ability of the virus to evolve while increasing antiviral activity.

Although *TRIM5* is not duplicated in primates, it has undergone gene innovation in macaques and owl monkeys, which have independently gained additional exons through the insertion of a cyclophilin A gene (*CYPA*; also known as *PPIA*) into non-coding segments of the *TRIM5* gene^{59–64}. In both species, a TRIM–CYP fusion protein with potent antiviral activity is produced, although the viral targets are not identical. Moreover, TRIM–CYP and TRIM5α can both be expressed by the same individual, allowing for the restriction of multiple lentiviruses. By restricting viral replication in this manner, the host also slows down the evolution of the virus.

dN/dS

The ratio of the rate of non-synonymous mutations to the rate of synonymous mutations. Values greater than one are indicative of positive selection; values less than one are indicative of negative selection; and values near one are indicative of neutral selection.

Selective sweep

A decrease in diversity in the genomic region surrounding an allele under positive selection.

Hitchhiking

Genetic linkage between loci under positive selection and nearby loci not under positive selection.

Balancing selection

Selection to maintain polymorphism owing to frequency-dependent selection or heterozygote advantage.

Frequency-dependent selection

Selection to maintain mutations at an intermediate frequency to confer a fitness advantage.

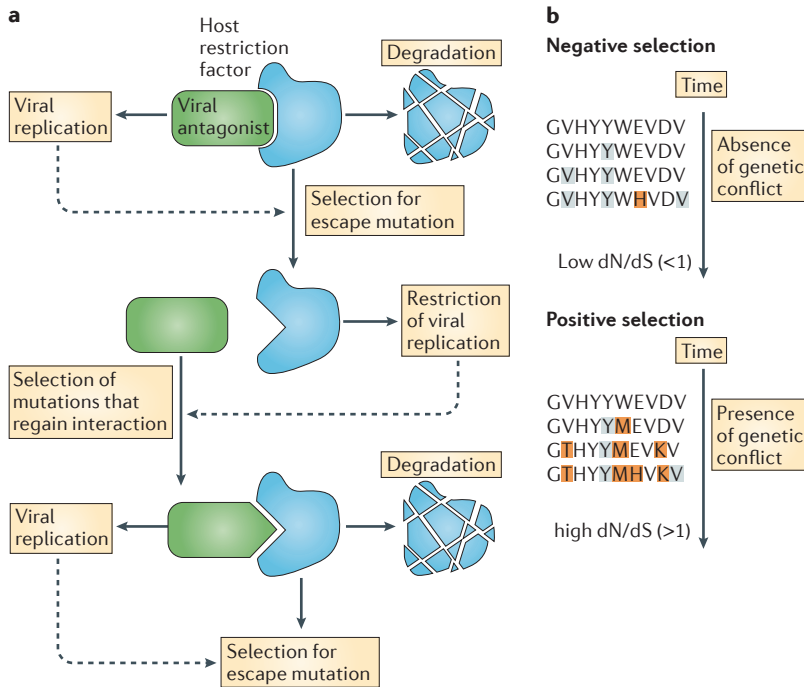


Figure 2 | Genetic conflict between virus and host. **a** | A host restriction factor (blue) that is antagonized by a viral factor (green) cannot restrict viral replication, and the host is susceptible to viral infection. This exerts a fitness cost on the host, and escape mutations will be selected for in the host factor. In return, when the host factor restricts viral replication, a fitness cost is exerted on the virus. Mutations that allow the virus to regain restriction factor antagonism (for example, by re-forming a protein–protein interface between the viral antagonist and the host factor) are selected for in the virus. This back-and-forth fitness adaptation in the virus and host leads to a conflict that is visible on the genetic level. **b** | Over time, in the absence of genetic conflict, most genes evolve under negative (purifying) selection. This leads to a lower rate of non-synonymous mutations (dN) than of synonymous mutations (dS) in the host gene, and the dN/dS ratio is predicted to be less than one. In the presence of a genetic conflict, such as that caused by a viral antagonist, the host gene will rapidly accumulate non-synonymous mutations, and the dN/dS ratio is predicted to be greater than one. Grey boxes represent synonymous changes, and orange boxes represent non-synonymous changes.

Just as restriction factor families expand when an increase in antiviral activity is advantageous in the presence of viruses, restriction factor families can also contract in the absence of a selective pressure. For example, the restriction factor APOBEC3H from macaques and chimpanzees has potent antiviral activity against lentiviruses⁶⁵. However, two independent loss-of-function mutations in *APOBEC3H* are present at high frequencies in humans, despite the conservation of antiviral activity in other primates⁶⁵. This suggests that restriction factors such as APOBEC3H, which is a highly active DNA mutator, might impose a cost on the host genome and therefore might be selected against in the absence of the relevant viral pressure.

Lessons from the evolution of restriction factors

Studying the evolution of restriction factors can help us to understand why humans are susceptible to viruses that exist today, as our immune responses to contemporary viruses have been shaped by our evolutionary responses to previous infections. The modern innate immune system is generally not yet optimized against

modern viruses, but rather was selected for by previous rounds of co-evolution with ancient viruses. By determining the types of viral infection that occurred in the past and how they were eliminated, we can form new ideas about how to manipulate the immune system to our advantage in the ongoing battle against viruses.

Identifying previous viral infections. Paleovirology is the study of ancient, extinct viruses (paleoviruses) and their effects on modern-day host–virus interactions⁶⁶. We know that ancient retroviruses infected primates because there are remnants of viral sequences in primate genomes⁶⁷. However, many other retroviruses did not become endogenous in the host genome, and so we have no direct evidence of their existence. In fact, no endogenous lentiviral sequences have been found in primate genomes except in a single genus of prosimians^{68–70}. However, by identifying signatures of positive selection in host restriction factors, we can infer the existence of many additional paleoviruses, as well as the historical timeframe and species in which the infection took place. By combining evolutionary analyses with functional tests, we can determine the type of virus that is likely to have driven selection in the host (FIG. 3).

One of the clearest examples in which a paleovirus was identified by examining positive selection comes from the analysis of APOBEC3DE, a member of the APOBEC3 family in primates that restricts endogenous retrotransposons. *APOBEC3DE* has rapidly evolved in primates, particularly in the chimpanzee and bonobo lineages⁵⁰. Since its divergence from the human gene, *APOBEC3DE* in the chimpanzee lineage has accumulated 24 mutations, of which 23 are non-synonymous changes. These changes have broadened the antiviral activity of chimpanzee APOBEC3DE to include the ability to restrict lentiviruses. Human APOBEC3DE, by contrast, has not evolved the ability to restrict lentiviruses. Therefore, by identifying the adaptive consequences of rapid evolution in chimpanzee *APOBEC3DE*, we suggest that a lentivirus infected the common ancestor of chimpanzees and bonobos in the past; this infection probably occurred approximately 2–5 million years ago, after the chimpanzee–bonobo ancestor diverged from humans. Similarly, the acquisition of a TRIM–CYP fusion gene with antilentiviral functions in owl monkeys 2–6 million years ago strongly argues for such a challenge occurring in this lineage of primates, which is both phylogenetically and geographically distinct from the primates that are known to be infected with lentiviruses currently. By studying the evolution of restriction factors, we can form a more accurate picture of the ancient history of retroviral infections in primates.

Predicting viral pathogenicity. In the virus–host arms race, positive selection occurs when the reproductive fitness of either party is challenged. If a virus is not pathogenic to the host, it is not likely to exert a selective pressure on the host. Therefore, adaptive changes in host restriction factors would not be expected to occur during a non-pathogenic infection. Mildly pathogenic viruses would be expected to impart weak selective pressures that might increase the allele frequency of a

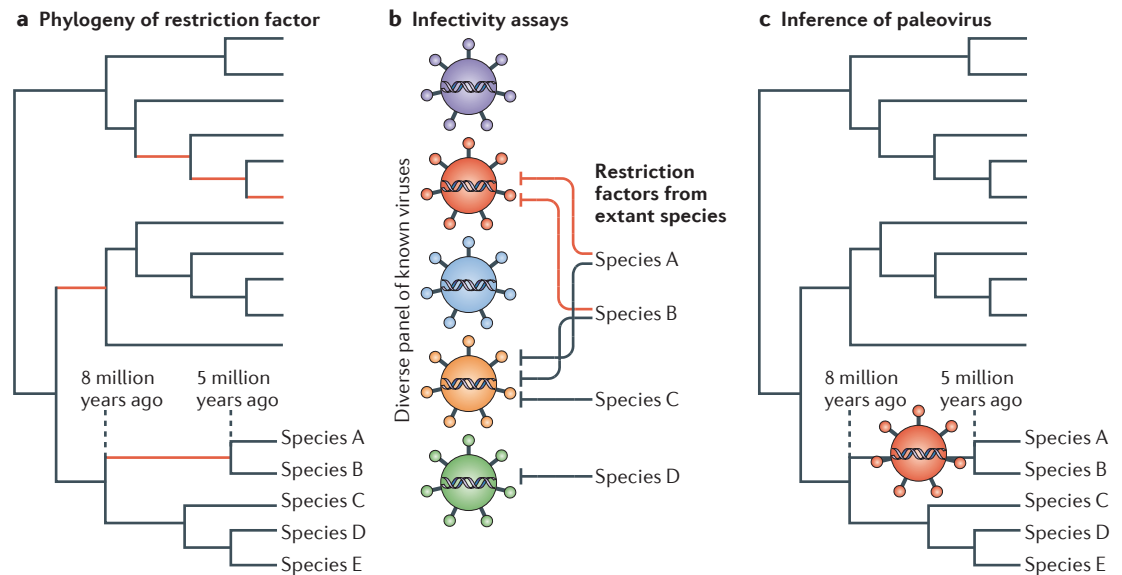


Figure 3 | Identifying paleoviruses using positive selection. **a** | Phylogeny of a restriction factor. Lineages under positive selection (shown in red) are identified using likelihood methods to calculate the ratio of the rate of non-synonymous mutations (dN) to the rate of synonymous mutations (dS) of the restriction factor for each branch. **b** | Infectivity assays. Restriction factor orthologues from extant species are tested for antiviral activity against a panel of viral targets, with the goal of finding species-specific antiviral activity. **c** | Inference of a paleovirus. In lineages that are under positive selection, a paleovirus similar to the virus against which the restriction factor has gained species-specific activity is predicted to have existed during the time of selection.

selected mutation but would not drive polymorphisms to fixation⁷¹. For example, simian foamy viruses (SFVs) are considered to be non-pathogenic in their natural hosts^{72,73}. Interestingly, SFVs have co-evolved with their hosts for more than 30 million years⁷⁴, demonstrating that there might not be a selective pressure to stop SFV replication. Furthermore, the rate of evolution of SFVs is many times slower than for other RNA viruses⁷⁴, which suggests that the arms race between virus and host has slowed down considerably in this case.

Natural infection of African green monkeys by SIV is also thought to be non-pathogenic, as infection does not cause immunodeficiency despite high viral replication levels⁷². Surprisingly, polymorphisms in the African green monkey *APOBEC3G* gene that allow evasion from antagonism by host-specific SIV Vif proteins were found in the grivet and sabaues subspecies⁴³, suggesting a recent selective pressure on *APOBEC3G*. Furthermore, the SIV strains that circulate in these subspecies have regained the ability to antagonize *APOBEC3G*. This suggests that there is an arms race between SIV and African green monkeys that implies some degree of SIV pathogenesis in African green monkeys. For example, SIV might formerly have been pathogenic to African green monkeys, or pathogenesis might be present even now in an unmeasured or mild form. In this manner, the evolution of a host restriction factor and the reciprocal viral evolution can inform our views of viral pathogenesis.

Explaining why humans are susceptible to modern-day viruses. HIV-1 and HIV-2 are the result of multiple cross-species transmission events of SIV from chimpanzees and sooty mangabeys, respectively, into humans⁷⁵.

Primate restriction factors have been shown to have an important *in vivo* role in preventing lentiviral cross-species viral transmission events. For example, experimental infection of rhesus macaques — which are not infected with SIV in the wild — with HIV or SIV can mimic a cross-species transmission event. During experimental HIV-1 infection, rhesus macaque TRIM5α and APOBEC3G completely restrict viral replication^{23,24}. Furthermore, naturally occurring polymorphisms in rhesus macaque *TRIM5* attenuate viral replication by 100- to 1,000-fold during experimental infection with SIV from sooty mangabeys⁷⁶. These host genes involved in susceptibility or resistance to SIV infection may help to explain the dynamics of lentiviral zoonoses.

The four groups of HIV-1 — which are each the result of an independent cross-species transmission event to humans from chimpanzees infected with SIV — differ in their global spread, with group M representing the pandemic strain. It has recently been shown that the adaptation of HIV-1 to human-specific mutations in the restriction factor tetherin was achieved only by group M and N viruses and not by the non-pandemic group O and P strains^{77,78}. Clearly tetherin did not prevent any of the four cross-species transmission events, but it has been suggested that overcoming tetherin-mediated restriction was necessary for the efficient replication of HIV-1 group M in humans and therefore for pandemic spread (reviewed in REF. 79).

In studies of humans, the effects of restriction factor expression levels and polymorphisms on HIV-1 susceptibility and disease progression have not yielded a consensus viewpoint (reviewed in REF. 80). However, our immune system may be better at preventing cross-species

Orthologues
Homologous genes in different species that diverge after speciation.

viral transmissions than intra-species viral transmissions because viruses that have crossed the species barrier have already partially adapted to the host. Perhaps for this reason, the evidence for the effects of restriction factors on intra-species viral acquisition is less clear.

Identifying host–virus interaction domains and implications for treatment. The interactions between a virus and a host restriction factor can be mapped down to distinct protein–protein interfaces and, in some cases, to single amino acid residues. Because these interaction domains are directly engaged in genetic conflict, they often contain the residues that are most rapidly evolving. By looking at genetic signatures of positive selection, the sites involved in protein–protein interactions can be predicted and then tested functionally, as was recently done with remarkable accuracy for SAMHD1 (REFS 81,82). Without knowing anything about the domains of SAMHD1 that are required for antagonism by the lentiviral protein Vpx, two groups carried out positive selection analyses of SAMHD1 using the dN/dS test and identified two different regions of SAMHD1 that have evolved very rapidly in primates. When functionally tested, these two regions of SAMHD1 were shown to be required for its degradation by Vpx proteins from different lentiviruses in a virus-specific manner. This information has helped to explain how lentiviruses and SAMHD1 have evolved on a molecular level. By mapping host–virus interactions, the constraints of the evolutionary arms race can be more fully understood.

Moreover, these protein–protein interactions between host restriction factors and viral antagonists provide tempting targets for small-molecule inhibitors. An ideal inhibitor of a viral antagonist would specifically disrupt the ability of the antagonist to bind to the host restriction factor or to other host machinery required for restriction. This would enable a host restriction factor to specifically inhibit viral replication, without any effect on the rest of the immune system of an individual. Inhibitors of viral antagonists could be used as therapeutic treatments in combination with other antiretroviral drugs. Several inhibitors of HIV-1 Vif have been identified^{83,84}, and attempts have been made at disrupting Vpu function⁸⁵. Achieving inhibition of a viral antagonist without disrupting host functions might be difficult because many viral antagonists use or mimic host machinery for their activity. Also, the virus might be able to quickly evolve resistance mutations, as genes encoding viral antagonists often do not have as many functional constraints as more conserved viral genes.

Conclusion

Restriction factors are early, potent and specific cellular blocks against retroviral replication. They have clearly had an important role in innate immunity against viruses throughout primate evolution, and more work needs to be carried out to define how and when they are important in viral zoonoses, global epidemics and the progression to disease. In this way, characterizing the evolution of restriction factor antiviral activity will help us to understand why we are winning or losing current battles against viruses.

1. Pincus, T., Rowe, W. P. & Lilly, F. A major genetic locus affecting resistance to infection with murine leukemia viruses. II. Apparent identity to a major locus described for resistance to friend murine leukemia virus. *J. Exp. Med.* **133**, 1234–1241 (1971).
2. Takaoka, A. & Yanai, H. Interferon signalling network in innate defence. *Cell. Microbiol.* **8**, 907–922 (2006).
3. Wilkins, C. & Gale, M. Recognition of viruses by cytoplasmic sensors. *Curr. Opin. Immunol.* **22**, 41–47 (2010).
4. Refsland, E. W. *et al.* Quantitative profiling of the full APOBEC3 mRNA repertoire in lymphocytes and tissues: implications for HIV-1 restriction. *Nucleic Acids Res.* **38**, 4274–4284 (2010).
5. Koning, F. A. *et al.* Defining APOBEC3 expression patterns in human tissues and hematopoietic cell subsets. *J. Virol.* **83**, 9474–9485 (2009).
6. Stetson, D. B. Connections between antiviral defense and autoimmunity. *Curr. Opin. Immunol.* **21**, 244–250 (2009).
7. Laguette, N. *et al.* SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. *Nature* **474**, 654–657 (2011).
8. Hrecka, K. *et al.* Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. *Nature* **474**, 658–661 (2011).
9. Johnson, W. E. & Sawyer, S. L. Molecular evolution of the antiretroviral TRIM5 gene. *Immunogenetics* **61**, 163–176 (2009).
10. Le Tortorec, A., Willey, S. & Neil, S. J. Antiviral inhibition of enveloped virus release by tetherin/BST2: action and counteraction. *Viruses* **3**, 520–540 (2011).
11. Okeoma, C. M., Lovsin, N., Peterlin, B. M. & Ross, S. R. APOBEC3 inhibits mouse mammary tumour virus replication *in vivo*. *Nature* **445**, 927–930 (2007).
12. Okeoma, C. M., Petersen, J. & Ross, S. R. Expression of murine APOBEC3 alleles in different mouse strains and their effect on mouse mammary tumor virus infection. *J. Virol.* **83**, 3029–3038 (2009).
13. Haller, O., Acklin, M. & Staeheli, P. Influenza virus resistance of wild mice: wild-type and mutant MX alleles occur at comparable frequencies. *J. Interferon Res.* **7**, 647–656 (1987).
14. Tan, K. S. *et al.* *In vivo* and *in vitro* studies on the antiviral activities of viperin against influenza H1N1 virus infection. *J. Gen. Virol.* **93**, 1269–1277 (2012).
15. Swiecki, M., Wang, Y., Gillfillan, S., Lenschow, D. J. & Colonna, M. Cutting edge: paradoxical roles of BST2/tetherin in promoting type I IFN response and viral infection. *J. Immunol.* **188**, 2488–2492 (2012).
16. Barrett, B. S. *et al.* A single nucleotide polymorphism in tetherin promotes retrovirus restriction *in vivo*. *PLoS Pathog.* **8**, e1002596 (2012).
17. Liberatore, R. A. & Bieniasz, P. D. Tetherin is a key effector of the antiretroviral activity of type I interferon *in vitro* and *in vivo*. *Proc. Natl Acad. Sci. USA* **108**, 18097–18101 (2011).
18. Everitt, A. R. *et al.* IFITM3 restricts the morbidity and mortality associated with influenza. *Nature* **484**, 519–523 (2012).
19. Tareen, S. U. & Emerman, M. Human Trim5a has additional activities that are uncoupled from retroviral capsid recognition. *Virology* **409**, 113–120 (2011).
20. Pertel, T. *et al.* TRIM5 is an innate immune sensor for the retrovirus capsid lattice. *Nature* **472**, 361–365 (2011).
21. Rice, G. I. *et al.* Mutations involved in Aicardi–Goutières syndrome implicate SAMHD1 as regulator of the innate immune response. *Nature Genet.* **41**, 829–832 (2009).
22. Malim, M. H. & Emerman, M. HIV-1 accessory proteins — ensuring viral survival in a hostile environment. *Cell Host Microbe* **3**, 388–398 (2008).
23. Hatzioannou, T. *et al.* Generation of simian-tropic HIV-1 by restriction factor evasion. *Science* **314**, 95 (2006).
24. Kamada, K. *et al.* Generation of HIV-1 derivatives that productively infect macaque monkey lymphoid cells. *Proc. Natl Acad. Sci. USA* **103**, 16959–16964 (2006).
25. Yu, X. *et al.* Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif–Cul5–SCF complex. *Science* **302**, 1056–1060 (2003).
26. Mehle, A., Goncalves, J., Santa-Marta, M., McPike, M. & Gabuzda, D. Phosphorylation of a novel SOCS-box regulates assembly of the HIV-1 Vif–Cul5 complex that promotes APOBEC3G degradation. *Genes Dev.* **18**, 2861–2866 (2004).
27. Kueck, T. & Neil, S. J. A cytoplasmic tail determinant in HIV-1 Vpu mediates targeting of tetherin for endosomal degradation and counteracts interferon-induced restriction. *PLoS Pathog.* **8**, e1002609 (2012).
28. Elde, N. C. & Malik, H. S. The evolutionary conundrum of pathogen mimicry. *Nature Rev. Microbiol.* **7**, 787–797 (2009).
29. Van Valen, L. A new evolutionary law. *Evol. Theory* **1**, 1–30 (1973).
30. Han, G.-Z. & Worobey, M. An endogenous foamy-like viral element in the coelacanth genome. *PLoS Pathog.* **8**, e1002790 (2012).
31. Nielsen, R. & Yang, Z. Likelihood models for detecting positively selected amino acid sites and applications to the HIV-1 envelope gene. *Genetics* **148**, 929–936 (1998).
32. Grossman, S. R. *et al.* A composite of multiple signals distinguishes causal variants in regions of positive selection. *Science* **327**, 883–886 (2010).
33. Kelley, J. L. & Swanson, W. J. Positive selection in the human genome: from genome scans to biological significance. *Annu. Rev. Genom. Hum. Genet.* **9**, 143–160 (2008).
34. Hanada, K., Suzuki, Y. & Gojobori, T. A large variation in the rates of synonymous substitution for RNA viruses and its relationship to a diversity of viral infection and transmission modes. *Mol. Biol. Evol.* **21**, 1074–1080 (2004).
35. Jenkins, G. M., Rambaut, A., Pybus, O. G. & Holmes, E. C. Rates of molecular evolution in RNA viruses: a quantitative phylogenetic analysis. *J. Mol. Evol.* **54**, 156–165 (2002).

This study illustrates the connection between a restriction factor and other arms of the innate immune system.

This paper shows the importance of a retroviral restriction factor *in vivo* and emphasizes that the major role of such factors is as antiviral agents.

36. Shackelton, L. A., Parrish, C. R., Truyen, U. & Holmes, E. C. High rate of viral evolution associated with the emergence of carnivore parvovirus. *Proc. Natl Acad. Sci. USA* **102**, 379–384 (2005).
37. Shackelton, L. A. & Holmes, E. C. Phylogenetic evidence for the rapid evolution of human B19 erythrovirus. *J. Virol.* **80**, 3666–3669 (2006).
38. Holmes, E. C. Error thresholds and the constraints to RNA virus evolution. *Trends Microbiol.* **11**, 543–546 (2003).
39. Sharp, P. M., Bailes, E., Stevenson, M., Emerman, M. & Hahn, B. H. Gene acquisition in HIV and SIV. *Nature* **383**, 586–587 (1996).
40. Hughes, A. L. & Yeager, M. Natural selection at major histocompatibility complex loci of vertebrates. *Annu. Rev. Genet.* **32**, 415–435 (1998).
41. Verrelli, B. C. *et al.* Evidence for balancing selection from nucleotide sequence analyses of human G6PD. *Am. J. Hum. Genet.* **71**, 1112–1128 (2002).
42. Newman, R. M. *et al.* Balancing selection and the evolution of functional polymorphism in Old World monkey TRIM5a. *Proc. Natl Acad. Sci. USA* **103**, 19134–19139 (2006).
43. Compton, A. A., Hirsch, V. M. & Emerman, M. The host restriction factor APOBEC3G and retroviral Vif protein coevolve due to ongoing genetic conflict. *Cell Host Microbe* **11**, 91–98 (2012).
44. Conticello, S. G., Thomas, C. J., Petersen-Mahrt, S. K. & Neuberger, M. S. Evolution of the AID/APOBEC family of polynucleotide (deoxy)cytidine deaminases. *Mol. Biol. Evol.* **22**, 367–377 (2005).
45. OhAinle, M., Kerns, J. A., Malik, H. S. & Emerman, M. Adaptive evolution and antiviral activity of the conserved mammalian cytidine deaminase APOBEC3H. *J. Virol.* **80**, 3853–3862 (2006).
46. LaRue, R. S. *et al.* The antiodactyl APOBEC3 innate immune repertoire shows evidence for a multi-functional domain organization that existed in the ancestor of placental mammals. *BMC Mol. Biol.* **9**, 104 (2008).
47. Münk, C. *et al.* Functions, structure, and read-through alternative splicing of feline APOBEC3 genes. *Genome Biol.* **9**, R48 (2008).
48. Bogerd, H. P., Tallmadge, R. L., Oaks, J. L., Carpenter, S. & Cullen, B. R. Equine infectious anemia virus resists the antiretroviral activity of equine APOBEC3 proteins through a packaging-independent mechanism. *J. Virol.* **82**, 11889–11901 (2008).
49. Sawyer, S. L., Emerman, M. & Malik, H. S. Ancient adaptive evolution of the primate antiviral DNA-editing enzyme APOBEC3G. *PLoS Biol.* **2**, E275 (2004).
50. Duggal, N. K., Malik, H. S. & Emerman, M. The breadth of antiviral activity of Apobec3DE in chimpanzees has been driven by positive selection. *J. Virol.* **85**, 11361–11371 (2011).
51. Staeheli, P. & Sutcliffe, J. G. Identification of a second interferon-regulated murine Mx gene. *Mol. Cell. Biol.* **8**, 4524–4528 (1988).
52. Siegrist, F., Ebeling, M. & Certa, U. The small interferon-induced transmembrane genes and proteins. *J. Interferon Cytokine Res.* **31**, 185–197 (2011).
53. Tareen, S. U., Sawyer, S. L., Malik, H. S. & Emerman, M. An expanded clade of rodent Trim5 genes. *Virology* **385**, 473–483 (2009).
54. Sawyer, S. L., Emerman, M. & Malik, H. S. Discordant evolution of the adjacent antiretroviral genes TRIM22 and TRIM5 in mammals. *PLoS Pathog.* **3**, e197 (2007).
55. Bishop, K. N. *et al.* Cytidine deamination of retroviral DNA by diverse APOBEC proteins. *Curr. Biol.* **14**, 1392–1396 (2004).
56. Simon, V. *et al.* Natural variation in Vif: differential impact on APOBEC3G/3F and a potential role in HIV-1 diversification. *PLoS Pathog.* **1**, e6 (2005).
57. Tian, C. *et al.* Differential requirement for conserved tryptophans in human immunodeficiency virus type 1 Vif for the selective suppression of APOBEC3G and APOBEC3F. *J. Virol.* **80**, 3112–3115 (2006).
58. Russell, R. A. & Pathak, V. K. Identification of two distinct human immunodeficiency virus type 1 Vif determinants critical for interactions with human APOBEC3G and APOBEC3F. *J. Virol.* **81**, 8201–8210 (2007).
59. Liao, C. H., Kuang, Y. Q., Liu, H. L., Zheng, Y. T. & 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).
60. Brennan, G., Kozyrev, Y. & Hu, S. L. TRIMCyp expression in Old World primates *Macaca nemestrina* and *Macaca fascicularis*. *Proc. Natl Acad. Sci. USA* **105**, 3569–3574 (2008).
61. Newman, R. M. *et al.* Evolution of a TRIM5-CypA splice isoform in Old World monkeys. *PLoS Pathog.* **4**, e1000003 (2008).
62. Virgen, C. A., Kratovac, Z., Bieniasz, P. D. & Hatzioannou, T. Independent genesis of chimeric TRIM5-cyclophilin proteins in two primate species. *Proc. Natl Acad. Sci. USA* **105**, 3563–3568 (2008).
63. Wilson, S. J. *et al.* Independent evolution of an antiviral TRIMCyp in rhesus macaques. *Proc. Natl Acad. Sci. USA* **105**, 3557–3562 (2008).
64. Sayah, D. M., Sokolskaja, E., Berthou, L. & Luban, J. Cyclophilin A retrotransposition into TRIM5 explains owl monkey resistance to HIV-1. *Nature* **430**, 569–573 (2004).
65. OhAinle, M., Kerns, J. A., Li, M. M., Malik, H. S. & Emerman, M. Antiretroviral activity of APOBEC3H was lost twice in recent human evolution. *Cell Host Microbe* **4**, 249–259 (2008).
66. Emerman, M. & Malik, H. S. Paleovirology — modern consequences of ancient viruses. *PLoS Biol.* **8**, e1000301 (2010). **This paper describes the concept that ancient viral infections can be inferred by their effects on the evolution of restriction factor genes.**
67. Katzourakis, A., Rambaut, A. & Pybus, O. G. The evolutionary dynamics of endogenous retroviruses. *Trends Microbiol.* **13**, 463–468 (2005).
68. Gifford, R. J. *et al.* A transitional endogenous lentivirus from the genome of a basal primate and implications for lentivirus evolution. *Proc. Natl Acad. Sci. USA* **105**, 20362–20367 (2008).
69. Gilbert, C., Maxfield, D. G., Goodman, S. M. & Feschotte, C. Parallel germline infiltration of a lentivirus in two Malagasy lemurs. *PLoS Genet.* **5**, e1000425 (2009).
70. Katzourakis, A. & Gifford, R. J. Endogenous viral elements in animal genomes. *PLoS Genet.* **6**, e1001191 (2010). **This study shows that many different families of virus have left 'fossils' in the genomes of different animals, thus proving their ancient ancestry.**
71. Pritchard, J. K., Pickrell, J. K. & Coop, G. The genetics of human adaptation: hard sweeps, soft sweeps, and polygenic adaptation. *Curr. Biol.* **20**, R208–R215 (2010).
72. Sodora, D. L. *et al.* Toward an AIDS vaccine: lessons from natural simian immunodeficiency virus infections of African nonhuman primate hosts. *Nature Med.* **15**, 861–865 (2009).
73. Meiering, C. D. & Linial, M. L. Historical perspective of foamy virus epidemiology and infection. *Clin. Microbiol. Rev.* **14**, 165–176 (2001).
74. Switzer, W. M. *et al.* Ancient co-speciation of simian foamy viruses and primates. *Nature* **434**, 376–380 (2005).
75. Sharp, P. M. & Hahn, B. H. Origins of HIV and the AIDS pandemic. *Cold Spring Harb. Perspect. Med.* **1**, a006841 (2011).
76. Kirmaier, A. *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**, e1000462 (2010). **This study shows how a host restriction factor can affect virus transmission.**
77. Sauter, D. *et al.* Tetherin-driven adaptation of Vpu and Nef function and the evolution of pandemic and nonpandemic HIV-1 strains. *Cell Host Microbe* **6**, 409–421 (2009). **This paper shows that a deletion in a human restriction factor gene (tetherin) has important consequences for the adaption of HIV-1 to humans.**
78. Sauter, D. *et al.* HIV-1 group P is unable to antagonize human tetherin by Vpu, Env or Nef. *Retrovirology* **8**, 103 (2011).
79. Kirchhoff, F. Immune evasion and counteraction of restriction factors by HIV-1 and other primate lentiviruses. *Cell Host Microbe* **8**, 55–67 (2010).
80. Albin, J. S. & Harris, R. S. Interactions of host APOBEC3 restriction factors with HIV-1 *in vivo*: implications for therapeutics. *Expert Rev. Mol. Med.* **12**, e4 (2010).
81. Lim, E. S. *et al.* 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**, 194–204 (2012).
82. Laguette, N. *et al.* Evolutionary and functional analyses of the interaction between the myeloid restriction factor SAMHD1 and the lentiviral Vpx protein. *Cell Host Microbe* **11**, 205–217 (2012).
83. Nathans, R. *et al.* Small-molecule inhibition of HIV-1 Vif. *Nature Biotech.* **26**, 1187–1192 (2008).
84. Cen, S. *et al.* Small molecular compounds inhibit HIV-1 replication through specifically stabilizing APOBEC3G. *J. Biol. Chem.* **285**, 16546–16552 (2010).
85. Zhang, Q. *et al.* High-throughput assay to identify inhibitors of Vpu-mediated down-regulation of cell surface BST-2. *Antiviral Res.* **91**, 321–329 (2011).
86. Ganser-Pornillos, B. K. *et al.* Hexagonal assembly of a restricting TRIM5a protein. *Proc. Natl Acad. Sci. USA* **108**, 534–539 (2011).
87. Matsuda, A. *et al.* Large-scale identification and characterization of human genes that activate NF- κ B and MAPK signaling pathways. *Oncogene* **22**, 3307–3318 (2003).
88. Lahuouassa, H. *et al.* SAMHD1 restricts the replication of human immunodeficiency virus type 1 by depleting the intracellular pool of deoxynucleoside triphosphates. *Nature Immunol.* **13**, 223–228 (2012).
89. Stetson, D. B., Ko, J. S., Heidmann, T. & Medzhitov, R. Trex1 prevents cell-intrinsic initiation of autoimmunity. *Cell* **134**, 587–598 (2008).
90. Nielsen, R. Molecular signatures of natural selection. *Annu. Rev. Genet.* **39**, 197–218 (2005).
91. Nielsen, R., Hellmann, I., Hubisz, M., Bustamante, C. & Clark, A. G. Recent and ongoing selection in the human genome. *Nature Rev. Genet.* **8**, 857–868 (2007).
92. Biswas, S. & Akey, J. M. Genomic insights into positive selection. *Trends Genet.* **22**, 437–446 (2006).
93. Wolf, D. & Goff, S. P. Host restriction factors blocking retroviral replication. *Annu. Rev. Genet.* **42**, 143–163 (2008).
94. Qi, C. F. *et al.* Molecular phylogeny of Fv1. *Mamm. Genome* **9**, 1049–1055 (1998).
95. Sawyer, S. W., Lu, L., Emerman, M. & Malik, H. Positive selection of primate TRIM5a identifies a critical species-specific retroviral restriction domain. *Proc. Natl Acad. Sci. USA* **102**, 2832–2837 (2005). **This was the first study to show that positive selection analysis could functionally identify the region of a restriction factor responsible for species specificity.**
96. Kerns, J. A., Emerman, M. & Malik, H. S. Positive selection and increased antiviral activity associated with the PARP-containing isoform of human zinc-finger antiviral protein. *PLoS Genet.* **4**, e21 (2008).
97. Lim, E., Malik, H. & Emerman, M. Ancient adaptive evolution of Tetherin shaped Vpu and Nef functions in human immunodeficiency virus and primate lentiviruses. *J. Virol.* **84**, 7124–7134 (2010).
98. McNatt, M. W. *et al.* Species-specific activity of HIV-1 Vpu and positive selection of tetherin transmembrane domain variants. *PLoS Pathog.* **5**, e1000300 (2009).
99. Liu, S. Y., Sanchez, D. J. & Cheng, G. New developments in the induction and antiviral effectors of type I interferon. *Curr. Opin. Immunol.* **23**, 57–64 (2011).
100. Lim, E. S., Wu, L. I., Malik, H. S. & Emerman, M. The function and evolution of the restriction factor viperin in primates was not driven by lentiviruses. *Retrovirology* **9**, 55 (2012).
101. Sadler, A. J. & Williams, B. R. Interferon-inducible antiviral effectors. *Nature Rev. Immunol.* **8**, 559–568 (2008).
102. Marshall, E. E., Bierle, C. J., Brune, W. & Geballe, A. P. Essential role for either TRS1 or IRS1 in human cytomegalovirus replication. *J. Virol.* **83**, 4112–4120 (2009).
103. Langland, J. O., Cameron, J. M., Heck, M. C., Jancovich, J. K. & Jacobs, B. L. Inhibition of PKR by RNA and DNA viruses. *Virus Res.* **119**, 100–110 (2006).
104. Elde, N., Child, S., Geballe, A. & Malik, H. Protein kinase R reveals an evolutionary model for defeating viral mimicry. *Nature* **457**, 485–489 (2009).

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Competing interests statement

The authors declare no competing financial interests.

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