

# Innate immunity against retroviral pathogens: from an ambiguous genetic self to novel therapeutic approaches

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## Summary

Almost half of the human genome is derived from exogenous genetic invaders, most of them related to retroviruses. This is the consequence of longstanding interactions between retroelements and higher organisms, governed by a delicate equilibrium between virus-based evolutionary forces and control by host defense mechanisms. Insight into these longstanding genetic conflicts is suggesting leads for novel therapeutic strategies to fight HIV infection.

In 2001, the Human Genome Organisation (HUGO) and the Human Genome Project (HGP) consortia provided first drafts of the sequence of the human genome [1, 2]. Completed in 2004, this effort opened new avenues for understanding the genetic bases of both physiological and pathological process, hence to move towards a more global comprehension of health and disease. As insights into the content of the human genome were gained, it quickly became apparent

that only a fraction, barely two percent, encodes for proteins, which had been traditionally considered as the main executive arms of the cell. More strikingly even, close to 50 percent of human DNA was found to derive from genetic invaders called transposons, most of them retroelements functionally related to the human immunodeficiency virus (HIV). Found in the genomes of all species, transposons are motors of evolution, yet their uncontrolled spread can be fatal to their host organisms. Their presence in higher species thus reflects a delicate equilibrium between occasional permissiveness and tight restriction, through innate immunity mechanisms also engaged in protection against their exogenous viral counterparts.

*Key words: genome; retroelement; HIV; innate immunity; evolution*

## Internal mobility

Mobile genetic elements were discovered some sixty years ago by Barbara McClintock based on her observation of changing colour patterns in maize. Her results and their implications were long held in such scepticism that she even came to renounce publishing in the scientific literature. After progress in the understanding of gene regulation revealed the seminal importance of her discoveries, she was ultimately awarded the Nobel Prize in Physiology and Medicine, some thirty years later. And it was another two decades before the sequencing of the human genome revealed that close to half of its DNA comes from such mobile elements. These can be classified in two general categories [3, 4]. A small minority (about 5%) are DNA transposons that replicate

by a “cut-and-paste” mechanism, encode for a recombinase that mediates their “hopping” along the cell genome. These elements do not get amplified during this process, and no currently active DNA transposon has been detected in the mouse or human genomes. This somewhat belittles their perceived impact on recent evolution, hence their relevance to the present discussion. By contrast, the vast majority (almost 95%) of mobile elements harboured by the genomes of higher species are retrotransposons, which replicate through a “copy-and-paste” mechanism leading to an amplification that explains their prevalence. The biology of these retrotransposable elements and the intricacy of their interactions with higher species is the topic of this review.

## Biology of retrotransposons

The DNA of retrotransposons is transcribed by the cellular machinery into RNA molecules that are copied by the group-defining reverse transcriptase back into DNA, which can become integrated again into the genome of the cell. Because the RNA intermediates can be generated by multiple rounds of transcription, this process results in a marked amplification of retroelements once they invade the genome of a species, unless and until control mechanisms are in place. Furthermore, the reverse transcriptase enzyme being error-prone, retrotransposons and other retroelements can evolve rapidly when confronted with selective pressures.

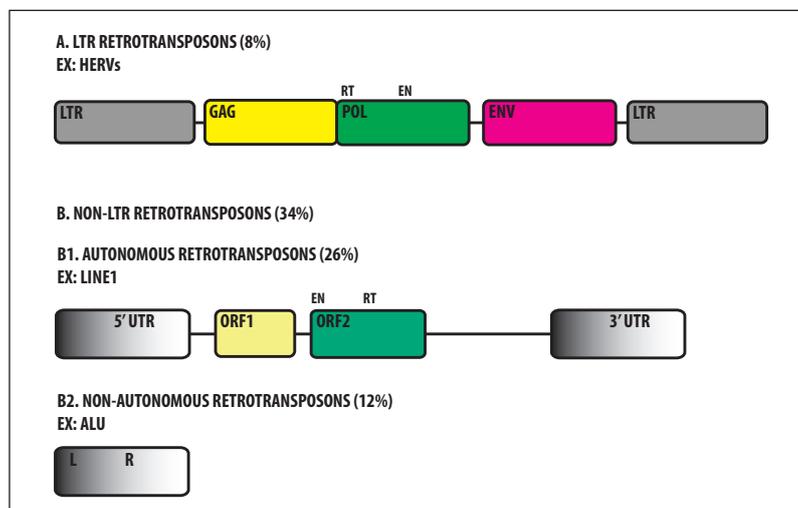
Retrotransposons are further classified into Long Tandem Repeat (LTR)-containing retroele-

ments (8.5% of the human genome, representing over a quarter million copies) and LTR-less or non-LTR retroelements (35% of the human genome, 2.4 million copies) (fig. 1). LTR-retrotransposons are endogenous retroviruses (ERVs), that is, retroviruses that once infected the germ line of their host species or of one of its ancestors, and thus became a stable component of the genome of this lineage. The timing of such invasion can often be traced through phylogenetic studies (table 1) [5, 6]. Rare are the endogenous retroviruses (none in humans) that conserve the ability to perform a full extra-cellular cycle, but their genomes carry the hallmarks of retroviruses, with sequences (or remnants thereof) encoding the structural Gag components, a reverse transcriptase, an integrase and for some an envelope [7]. Many of these elements have become inactive over time through the accumulation of mutations, but they are also kept at bay by host defence mechanisms. Non-LTR retrotransposons lack several prototypic features of exogenous retroviruses, amongst which an envelope coding sequence. This group comprises autonomous and non-autonomous members, the prototypes of which are L1 elements and *Alu* repeats, respectively, found each at close to one million copies in the human genome.

**Figure 1**

Main families of Retrotransposons.

The retrotransposons are classified in two major classes. (A) The Long Tandem Repeat retrotransposons have two identical tandem repeats (*LTR*) at each end of their sequence, serving as promoters and transcriptional termination signals. They have three main genes for structural proteins (*Gag*), the replication enzymes (*Pol*), namely reverse transcriptase (*RT*) and endonuclease (*EN*), and often the remnants of the envelope (*Env*). (B) The non-LTR retrotransposons are further segregated into autonomous and non autonomous. The autonomous non-LTR retrotransposons (B1) have a 5' untranslated region (5' UTR) driving transcriptional activity, 2 genes (*Orf1* and *Orf2*) of which *Orf2* contains the endonuclease (*EN*) and reverse transcriptase (*RT*) activities, and a 3' UTR. The non-autonomous retrotransposons (B2) have dual monomers for transcriptional activity but no genes. Adapted from Kazazian [3].



**Table 1**

Superfamilies of LTR-containing retroelements.

The LTR-retrotransposons are classed in six major superfamilies and based on sequence analysis are related to the retroviral subfamilies, suggesting different infectious events. Reproduced from Bannert et al. [6].

Element	Characteristics
Class I ERV	Similar to type C or retroviruses
Class II ERV	Similar to type B or retroviruses
Class III ERV	Distantly related to spuma retroviruses
MER4	Non-autonomous class I-related ERVs
MST	Named for common MstII restriction site
MLT	Mammalian LTR retro-transposons

## Origin of retrotransposons

Phylogenetic analyses of the reverse transcriptase sequences of endogenous and exogenous retroviruses demonstrate a clear evolutionary relationship, pointing to a likely common ancestor hundreds of millions years ago [8, 9]. Similar studies also indicate a common origin for all classes of retrotransposons (LTR-retrotransposons, LINE – including L1- and SINE – including *Alu*- elements), and it is tempting by extension to relate the origin of retrotransposons to bacterial and mitochondrial genetic elements that encode a re-

verse transcriptase, or to the eukaryotic telomerase. However, the latter connections, albeit plausible, remain speculative. According to the sequence divergence between members of one class and their functional consensus sequence, and taking as a reference the average mutation rate of eukaryotic genomes (e.g. divergence between humans and old-world monkeys reflects 25 mio years of evolution), one can estimate the periods that have witnessed retrotransposition activity [1]. Interestingly, over the last 100 million years,

LTR-retrotransposons were very active up to 50 million years ago (mya), but since then have progressively lost most activity in primates, with the last new insertion dated back to 0.2 mya in the precursor of modern humans [1, 6]. Although also active 100 mya, non-LTR retrotransposons peaked later (approx 50 to 25 mya) in primates, but then declined to become almost completely controlled in modern humans (only 80 active L1 elements out of the million or so present in the

human genome, with only one human birth in a 100 carrying a new insertion) [6, 10]. Interestingly, these most recent bursts of retrotransposon activity were synchronous with the development of mammals (approximately 100 mya) and the speciation of hominids (50 mya). Of note, retrotransposons have remained far more active in rodents, causing close to 10% of spontaneous mutations in inbred strains of mice.

## Impact of retrotransposons

Retrotransposons have shaped the genomes of higher organisms by exerting direct influences on their architecture, by modulating their expression, and by triggering the emergence of defence mechanisms.

1. Retrotransposons as architects of the human genome: Through their “copy-and-paste” replication mechanism, retroelements have filled genomes with hundred of thousands to millions of near-identical DNA sequences. This constitutes a most favourable ground for rearrangements of several kinds: homologous recombination, duplications, deletions and translocations (fig. 2). As such, retrotransposons are formidable evolutionary forces. Comparing the human and chimpanzee genomes at orthologous loci reveals species-specific losses of up to several megabases, and insertions of several tens of thousands of base pairs [11], involving both coding sequences and regulatory elements. Duplications have promoted

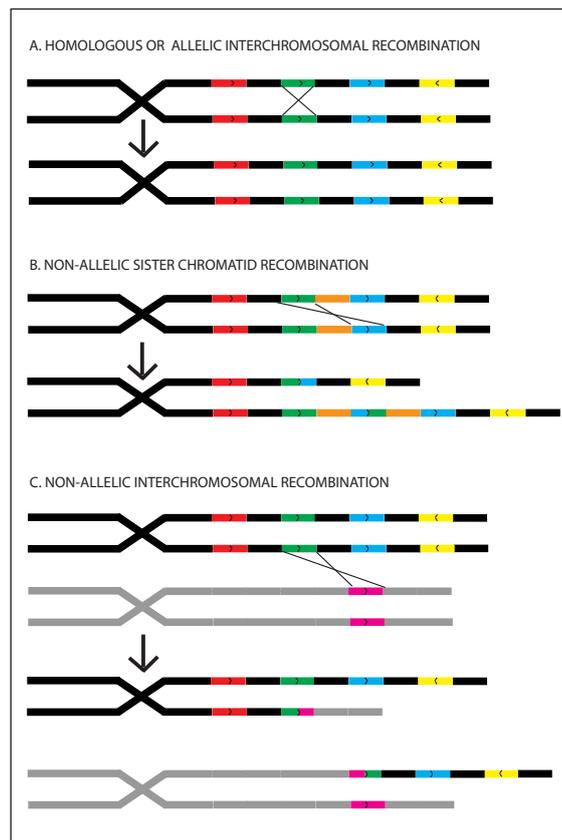
the expansion of gene classes. For instance, the some 600 human genes coding for zinc-finger transcription factors are often found in clusters enriched in SINE retrotransposons, and most likely derived from a common ancestor. Conversely, retrotransposon-induced deletions account for almost 50 human diseases [4], including familial hyperlipidaemia, due to the deletions in the LDL receptor gene, and acute myelogenous leukaemia, secondary to a deletion in the MLL gene [12].

2. Retrotransposons as contributors to the coding potential of the host: retrotransposons tend to integrate in gene-rich regions, and sometimes become part of the exons of existing genes or even contribute new genes. One of the most striking examples of the latter situation is that of syncytin in mice and humans. This protein is essential for the formation of the placental syncytiotrophoblast, and perhaps crucial for maternofoetal immune tolerance [13]. Remarkably, its various forms are all encoded by the *env* gene of endogenous retroviruses that independently invaded the germ cells of mice and humans, and likely substituted for a resident gene until then responsible for this mammal-defining function. Also, retroelement-derived sequences are over-represented in mRNAs of rapidly changing genes such as those involved in immune or stress responses, suggesting that retrotransposons have favoured the rapid evolution of these genes [14]. As well, the inclusion of sequences adjacent to those of retroelements during their copy-and-paste replication (a process called transduction) can cause the fusion of gene fragments resulting in novel proteins, as for example the HIV1 restriction factor TRIMcyp of owl monkeys [15]. In fact, it is suggested that up of 0.1% of human protein coding regions contain transposable elements [16].

3. Retrotransposons as modulators of transcriptional activity: the spread of retroelements litters the genome with cis-acting regulatory sequences, comprising promoters, enhancers, splice-acceptor/donor and poly-adenylation signals as well as other transcriptional or posttranscriptional regulatory elements. Recent work suggests that LINE1 may have a role in chromosome X-inactivation (reviewed in [17]). However, if

**Figure 2**

Examples of structural sequence alterations caused by retrotransposons. The accumulation of near identical sequences can cause structural anomalies. In so-called homologous or allelic recombination (A), the retrotransposons (green) on both strands may serve as a template for exchange of chromatid fragments without any consequence for the resulting sister chromatids. In non-allelic sister chromatid recombination (B), a misalignment of the two sister chromatids during meiosis due to accumulation of near-identical sequences (multiple integration sites of a retrotransposons: red, green, blue, yellow) may result in a non-homologous recombination. This will cause duplication and deletion, respectively of the intercalated sequence (orange) on each sister chromatids. If this sequence contains a gene or a gene cluster, this will lead to gain or loss of gene(s). In non-allelic interchromosomal recombination (C), the misalignment between chromatids of different chromosomes will result in translocations with the potential new genes (fragments of 2 different genes brought together) or in the truncation of a given gene. Adapted from Deininger et al. [12].



**Table 2**

Positive and negative effects of retroelements as transcriptional modulators of host genes. Blue indicates presumed favourable effects of retroelement integration, red indicates detrimental integration site. Adapted from Banerj et al. [6].

Element	Gene	Transcriptional modification	Functional role
HERV-E	Endothelin-B receptor	Promoter regulation	Placenta
HERV-L	$\beta$ -Galactosyltransferase	Promoter regulation	Colon, mammary gland
LTR + LINE2	Chaperonin	Promoter dysregulation	McKausick-Kaufman syndrome
LINE1	Factor VII	Premature Poly-A truncation	Haemophilia
Alu	Pro-opiomelanocortin	Enhancer positive regulation	Endocrine regulation
Alu	NF1	Alternative splicing signal	Neurofibromatosis

some of these irruptions of regulatory elements can be beneficial (SINE retrotransposons can act as physiological enhancers as was demonstrated for the pro-opiomelanocortin gene [18]), one can easily imagine the deleterious effects of random insertions of transcriptional modifiers in a well-oiled transcriptional landscape (table 2). Progress in the understanding of epigenetics (changes in phenotype/gene expression that occur due to changes other than in the genomic sequence) reveals that retroelements can be associated with modifications of chromatin architecture [3] that

influence neighbouring genes. In the murine genome, some members of the B1 SINE family can recruit transcriptional repressors [19], and recent work suggests that SINE partake in homeostatic responses such as heat shock by acting as transcriptional repressors upon cellular stress [20]. Moreover, retroelements can shuttle genes out of an unfavourable environment; X-to-autosomal retrotransposition is not uncommon and is proposed as a mechanism to dodge the silencing of house-keeping genes by X chromosome inactivation in somatic cells [21].

## Controlling retroelements

As useful as retroelements can be, their uncontrolled spread would have rapidly lethal consequences. Correspondingly, all species have evolved sophisticated mechanisms to control this process. In primates, the emergence of several of these retroelements' restriction factors coincided with the drop in retroelement activity some 50 mya.

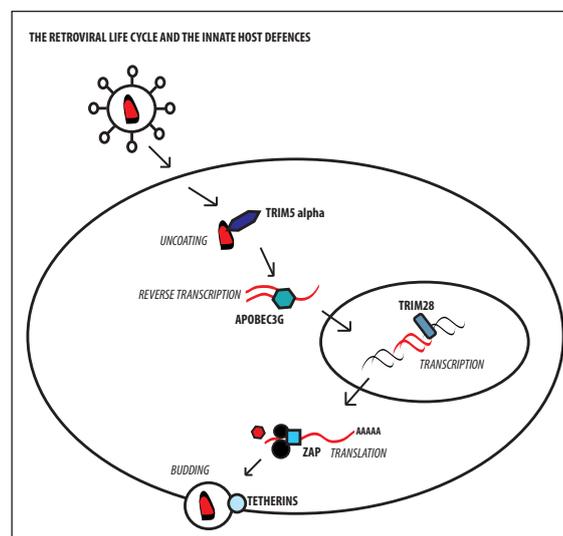
The exogenous retroviral replication cycle provides a convenient background to describe the

various mechanisms at play in the control of retroelements (fig. 3). The TRIM5 $\alpha$  restriction factor interferes with the un-coating of the viral genome after entry, thus preventing reverse transcription and integration [22–24]. TRIM5 $\alpha$  is an important barrier to the cross-species transmission of retroviruses. Human TRIM5 $\alpha$  protects from infection with Murine Leukaemia Virus (MLV) and Equine Immune Anaemia Virus (EIAV), whereas the African green monkey agmTRIM5 $\alpha$  restricts not only these two pathogens but also HIV1, HIV2 and SIVmac. As a corollary, retroviruses have evolved to evade the TRIM5 $\alpha$  orthologue encoded by their cognate host: HIV1 and HIV2, for instance, are not blocked by human TRIM5 $\alpha$ , although the mechanism of their escape is incompletely understood. In two primate species (rhesus macaque and squirrel monkey), a retroelement-driven transduction event led to a fusion between TRIM5 $\alpha$  and cyclophilin A (CypA), resulting in a fusion protein that can capture the capsid protein of HIV1, which has affinity for CypA. Thus, a retroelement-restricting factor arose through the action of another retroelement.

Polynucleotide cytidine deaminases of the APOBEC family are another group of restriction factors that target the early steps of retroviral replication, in their case reverse transcription [25, 26]. Various members of the family act on distinct subsets of retroelements, including exogenous and endogenous retroviruses, non-LTR retrotransposons and hepatitis B virus [27–29]. Again, viruses have evolved mechanisms to surmount cytidine deaminase-mediated restriction in their cognate host. The Vif protein of primate

**Figure 3**

The retroviral life cycle and the innate host defences. The retroviral life cycle serves as a model for the interaction between retroelements and the cellular defences. In the early stages of the replication cycle, cellular defences act on the capsid at postentry (TRIM5 $\alpha$ ) and at reverse transcription (cytidine deaminases such as APOBEC3G). Transcription of the integrated genome can be silenced (TRIM28), or translation of its RNA impaired (Zap). Late replication events (budding) can also be blocked (Tetherins).



lentiviruses, for instance, is the “antidote” against the APOBEC3F and 3G proteins present in these species.

Hosts have also developed mechanisms to limit the impact of retroelements once these are integrated in the genome. As a general rule, the DNA of LTR and non-LTR retrotransposons is methylated during the early embryonic period, which results in its irreversible silencing. Similarly, murine leukaemia virus is silenced in embryonic cells through the recruitment of a transcriptional repressor complex that acts by remodelling its chromatin environment [30]. This complex comprises a member of a large family of DNA-

binding repressors known as KRAB-ZFPs. As KRAB-ZFPs differing in their DNA-binding specificity are encoded in the hundreds by the genomes of higher species, it is tempting to speculate that, by analogy, other members of this family are involved in controlling endogenous retroelements related to MLV. The Zap restriction factor is another example of antiviral effectors, which acts at the posttranscriptional level by inducing the rapid degradation of the MLV RNA [31] in rats. Finally, Tetherin is a cellular protein that can block the release of retroviral particles at the cell membrane [32].

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## Genetic conflicts

Reverse transcriptase being devoid of proof-reading capacity, the replication of retroelements is error-prone, which promotes its escape from the control of restriction factors. In return, genes encoding restriction factors show the marks of intense positive selection, with accumulation of non-synonymous mutations at positions encoding for charged amino acids, predicted to reside at protein-protein or protein-nucleic acid interfaces. The TRIM5 $\alpha$  and APOBEC3G genes, for instance, have accumulated such mutations at far greater rates than housekeeping genes during the last 30 millions years of primate evolution [33, 34]. TRIM5 $\alpha$  predictably exhibits species-specific differences over its retroviral capsid-binding domain, reflecting its selection towards the control of distinct sets of retroviruses. For the cytidine deaminases APOBEC3G, differences between orthologues are noted throughout its sequence, perhaps consistent with a much broader spectrum of restriction (MLV, HIV, HBV, LINE1, Alu).

Of note, phylogenetic analyses indicate that all known anti-retroviral restriction factors emerged long before the emergence of lentiviruses, which appeared only 1 mya [35]. Factors now at play against HIV and other members of the lentiviral family thus arose as barriers against other genetic invaders, a process in which endogenous retroelements most likely played a crucial role. Whether the current HIV pandemic is exerting an additional pressure on restriction factors is hard to say. Studies on cohorts of HIV-infected and -uninfected individuals have failed to identify alleles of APOBEC3G or TRIM5 $\alpha$  predictive of a more lenient clinical course [36]. However, from an evolutionary perspective, the few decades since the start of the HIV epidemics are far too short a time for a noticeable impact. Furthermore, larger scale studies dissecting patient populations according to HIV strains and genetic background may be necessary to see significant patterns emerge.

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## Leads for novel therapeutic strategies

Our growing understanding of the molecular events that have governed hundreds of millions of years of interplay between retroelements and their hosts is suggesting novel therapeutic approaches to counter current infections, notably by HIV. Efforts to identify chemical substances capable of disrupting the APOBEC3G-Vif interaction, hence of exposing HIV to the antiviral action of the cytidine deaminase, have made significant progress [37]. As well, a fusion protein between human TRIM5 $\alpha$  and CypA was recently demonstrated as an effective blocker of HIV infection in a humanised mouse model, suggesting that it could serve to treat HIV1 infection by gene therapy [38].

More promising even, since unlikely to be evaded by the virus extraordinary mutational ability, are strategies aimed at the host cell mecha-

nisms leading to viral latency, the major obstacle to purging HIV from the infected host with currently available therapies. While latency probably reflects a partial control of HIV by endogenous defence mechanisms similar to those engaged against endogenous retroelements, it precludes the long-term success of HAART (highly active antiretroviral therapy) by providing a permanent source of virus that can reactivate infection in the face of fading pharmacological or immune control. Understanding what mechanisms lead to HIV latency and coming up with methods to force its reactivation are considered by many as imperative milestones for HIV research [39]. The continuing study of mechanisms by which endogenous retroviruses are kept at bay in the genome of higher organisms is likely to provide clues towards meeting these goals.

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## References

- Lander ES, et al. Initial sequencing and analysis of the human genome. *Nature*. 2001;409:860–921.
- Venter JC, et al. The sequence of the human genome. *Science*. 2001;291:1304–51.
- Kazazian HH Jr. Mobile elements: drivers of genome evolution. *Science*. 2004;303:1626–32.
- Goodier JL, Kazazian HH Jr. Retrotransposons revisited: the restraint and rehabilitation of parasites. *Cell*. 2008; 135:23–35.
- Medstrand P, van de Lagemaat LN, Mager DL. Retroelement distributions in the human genome: variations associated with age and proximity to genes. *Genome Res*. 2002;12:1483–95.
- Bannert N, Kurth R. Retroelements and the human genome: new perspectives on an old relation. *Proc Natl Acad Sci. USA* 2004;101(Suppl 2):14572–9.
- Craig NL, Craigie R, Gellert AM. *Mobile DNA II* (American Society for Microbiology, Washington, DC, 2002).
- Xiong Y, Eickbush TH. Origin and evolution of retroelements based upon their reverse transcriptase sequences. *Embo J*. 1990;9:3353–62.
- Eickbush TH, Jamburuthugoda VK. The diversity of retrotransposons and the properties of their reverse transcriptases. *Virus Res*. 2008;134:221–34.
- Brouha B, et al. Hot L1s account for the bulk of retrotransposition in the human population. *Proc Natl Acad Sci. USA* 2003;100:5280–5.
- Xing J, Witherspoon DJ, Ray DA, Batzer MA, Jorde LB. Mobile DNA elements in primate and human evolution. *Am J Phys Anthropol*. 2007;Suppl 45:2–19.
- Deininger PL, Batzer MA. Alu repeats and human disease. *Mol Genet Metab*. 1999;67:183–93.
- Knerr I, et al. Endogenous retroviral syncytin: compilation of experimental research on syncytin and its possible role in normal and disturbed human placentogenesis. *Mol Hum Reprod*. 2004;10:581–8.
- Van de Lagemaat LN, Landry JR, Mager DL, Medstrand P. Transposable elements in mammals promote regulatory variation and diversification of genes with specialized functions. *Trends Genet*. 2003;19:530–6.
- Sayah DM, Sokolskaja E, Berthoux L, Luban J. Cyclophilin A retrotransposition into TRIM5 explains owl monkey resistance to HIV-1. *Nature*. 2004;430:569–73.
- Gotea V, Makalowski W. Do transposable elements really contribute to proteomes? *Trends Genet*. 2006;22:260–7.
- Lyon MF. Do LINEs Have a Role in X-Chromosome Inactivation? *J Biomed Biotechnol*. 2006;59746.
- Santangelo AM, et al. Ancient exaptation of a CORE-SINE retroposon into a highly conserved mammalian neuronal enhancer of the proopiomelanocortin gene. *PLoS Genet*. 2007; 3:1813–26.
- Roman AC, Benitez DA, Carvajal-Gonzalez JM, Fernandez-Salguero PM. Genome-wide B1 retrotransposon binds the transcription factors dioxin receptor and Slug and regulates gene expression in vivo. *Proc Natl Acad Sci. USA* 2008;105: 1632–7.
- Mariner PD, et al. Human Alu RNA is a modular transacting repressor of mRNA transcription during heat shock. *Mol Cell*. 2008;29:499–509.
- Bradley J, et al. An X-to-autosome retrogene is required for spermatogenesis in mice. *Nat Genet*. 2004;36:872–6.
- Stremlau M, et al. The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. *Nature*. 2004;427:848–53.
- Anderson JL, et al. Proteasome inhibition reveals that a functional preintegration complex intermediate can be generated during restriction by diverse TRIM5 proteins. *J Virol*. 2006;80:9754–60.
- Campbell EM, Perez O, Melar M, Hope TJ. Labeling HIV-1 virions with two fluorescent proteins allows identification of virions that have productively entered the target cell. *Virology*. 2007;360:286–93.
- Harris RS, et al. DNA deamination mediates innate immunity to retroviral infection. *Cell*. 2003;113:803–9.
- Mangeat B, et al. Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. *Nature*. 2003;424:99–103.
- Bogerd HP, et al. Cellular inhibitors of long interspersed element 1 and Alu retrotransposition. *Proc Natl Acad Sci. USA* 2006;103:8780–5.
- Bogerd HP, Wiegand HL, Doehle BP, Lueders KK, Cullen BR. APOBEC3A and APOBEC3B are potent inhibitors of LTR-retrotransposon function in human cells. *Nucleic Acids Res*. 2006;34:89–95.
- Turelli P, Trono D. Editing at the crossroad of innate and adaptive immunity. *Science*. 2005;307:1061–5.
- Wolf D, Goff SP. Embryonic stem cells use ZFP809 to silence retroviral DNAs. *Nature*. 2009;458:1201–4.
- Gao G, Guo X, Goff SP. Inhibition of retroviral RNA production by ZAP, a CCCH-type zinc finger protein. *Science*. 2002;297:1703–6.
- Neil SJ, Zang T, Bieniasz PD. Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. *Nature*. 2008;451: 425–30.
- Sawyer SL, Wu LI, Emerman M, Malik HS. Positive selection of primate TRIM5alpha identifies a critical species-specific retroviral restriction domain. *Proc Natl Acad Sci. U S A* 2005; 102:2832–7.
- Sawyer SL, Emerman M, Malik HS. Ancient adaptive evolution of the primate antiviral DNA-editing enzyme APOBEC3G. *PLoS Biol* 2, E275 (2004).
- Sharp PM, Bailes E, Robertson DL, Gao F, Hahn BH. Origins and evolution of AIDS viruses. *Biol Bull*. 1999;196:338–42.
- Fellay J, et al. A whole-genome association study of major determinants for host control of HIV-1. *Science*. 2007;317:944–7.
- Nathans R, et al. Small-molecule inhibition of HIV-1 Vif. *Nat Biotechnol*. 2008;26:1187–92.
- Neagu MR, et al. Potent inhibition of HIV-1 by TRIM5-cyclophilin fusion proteins engineered from human components. *J Clin Invest*. 2009.
- Richman DD, et al. The challenge of finding a cure for HIV infection. *Science*. 2009;323:1304–7.