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# 'There and back again': revisiting the pathophysiological roles of human endogenous retroviruses in the post-genomic era

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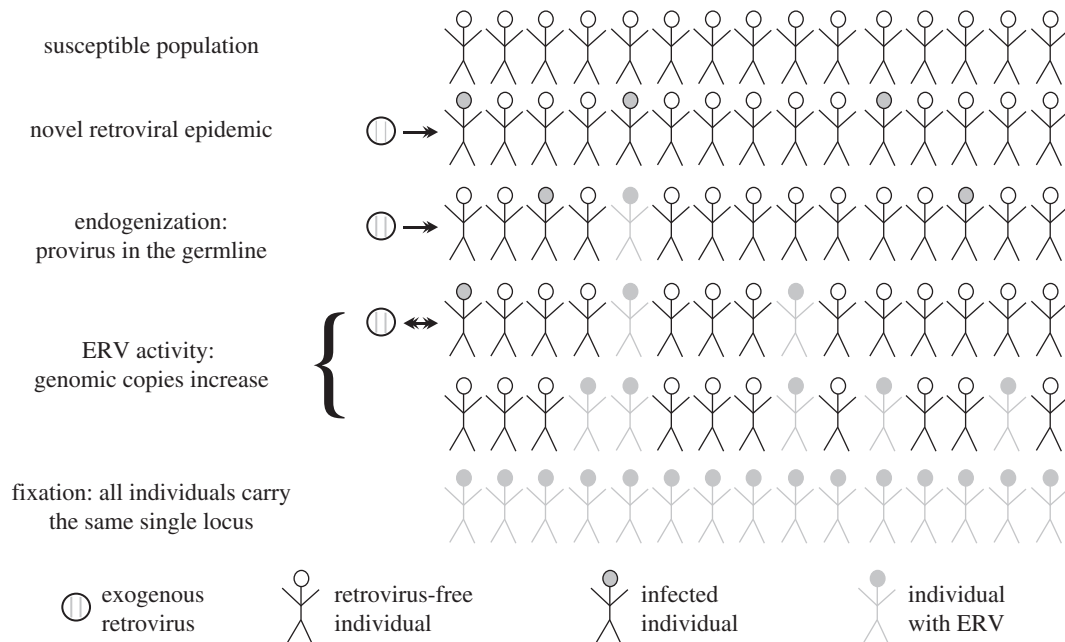
Almost 8% of the human genome comprises endogenous retroviruses (ERVs). While they have been shown to cause specific pathologies in animals, such as cancer, their association with disease in humans remains controversial. The limited evidence is partly due to the physical and bioethical restrictions surrounding the study of transposons in humans, coupled with the major experimental and bioinformatics challenges surrounding the association of ERVs with disease in general. Two biotechnological landmarks of the past decade provide us with unprecedented research artillery: (i) the ultra-fine sequencing of the human genome and (ii) the emergence of high-throughput sequencing technologies. Here, we critically assemble research about potential pathologies of ERVs in humans. We argue that the time is right to revisit the long-standing questions of human ERV pathogenesis within a robust and carefully structured framework that makes full use of genomic sequence data. We also pose two thought-provoking research questions on potential pathophysiological roles of ERVs with respect to immune escape and regulation.

## 1. Introduction

Transposable elements (TEs) comprise almost half of the human genome. A significant proportion of them (almost 8% of the human genome) are the descendants of occasional germline invasions by exogenous retroviruses (XRVs) [1,2]. We call them endogenous retroviruses (ERVs) and they can be identified as DNA segments within animals' germline genomes that are similar to known retroviral sequences.

More than 10 years have passed since the first complete human genome sequence [3], and thousands of full human genome sequences have since become available [4]. One might think that we now have the technology to understand the role, if any, of ERVs in disease. However, mapping individual ERVs to their genomic positions remains a major experimental and biocomputing challenge, whereas studies of human ERV (HERV) expression are small with no clear consensus [5,6]. The fact that extensive research on HERVs has not shown clear pathologies, in contrast to ERVs in several other animals, suggests that research in HERVs still needs to be performed, but only within a rigorous and robust experimental framework. We argue that researchers have been 'there' (i.e. searched for potential pathogenic roles of ERVs) and failed likely owing to technological restrictions and fragmentary knowledge of cancer and autoimmunity, but now it is time to 'go back again'.

Here, we summarize what we consider to be the most promising lines of ERV research that might illuminate the role of HERVs in pathophysiology. We emphasize the most important questions of the role of HERVs on human pathophysiology and describe some of the challenges that need to be tackled in order to have clearer understanding of HERVs' ecology. We start with a brief introduction to ERV taxonomy and the most important HERV families.



**Figure 1.** Population genetics of endogenous retroviruses. It has been suggested that ERVs that we currently identify in animal genomes are the result of ancient retroviral epidemics. Thus, the first step of ERV colonization involves the establishment of an epidemic in a susceptible population. Endogenization starts when retroviruses integrate into the host's germline and pass to the offspring through inheritance. The copies of the ERV in the germline constitute a family and may increase through time (ERV activity). Some of these copies become extinct or drift to fixation (i.e. every individual has the same locus).

## 2. Classification of endogenous retroviruses

The classification system of ERVs divides them into three classes (I, II, III) [7]. If the XRVs were included based on their phylogeny, then class I would include the gammaretroviruses, II the betaretroviruses and III the spumaviruses. Well-defined groups within these classes determined by phylogenetic analysis are termed 'families'. These families generally represent a single invasion followed by a copy-number expansion within the host's genome [8]. ERV families have traditionally been named after the amino acid carried by the tRNA complementary to the primer binding site (PBS) of the ERV genome. This nomenclature still holds for historical reasons, even if members of the same family do not have the same PBS complementary to the amino acid that names their family [9]. HERVs are classified into 31–40 families [10,11].

## 3. 'Popular' human endogenous retrovirus families

### (a) HERV-T: the typical

HERV-T is a typical example of a small-to-medium-sized HERV family (approx. 60 copies or loci) in the human genome; others include HERV-S, HERV-K(HML5) and HERV-P. Most of these typical families have fewer than approximately 80 copies, an *env* gene and proliferate primarily by reinfection in the human genome (rather than retrotransposition or complementation) [12,13]. They have largely ceased generating new loci since approximately 35 Ma. Consistent with this observation is that the phylogenetic trees are star-like [14], indicating that ancient proliferation was followed by cessation of activity for tens of millions of years (figure 1).

### (b) HERV-L: the old

The oldest ERV family in the human genome is HERV-L. This family has members across a wide range of mammalian species,

and is therefore believed to have infected the common ancestor of mammals [15]. The most striking characteristic of this ERV is that no one has ever found an *env* gene, meaning that it has been replicating through retrotransposition. ERV-L had bursts of replication in the mouse, the simian clade of primates and most recently in the elephant [15]. In humans, HERV-L ceased replicating approximately 30–40 Ma.

### (c) HERV-H: the abundant

The most abundant ERV family in humans is HERV-H, which accounts for about one-third of pol-containing ERV loci [9]. It invaded the primates about 30 Ma, around the separation of Old and New World monkeys [16,17]. Unlike HERV-L, there are HERV-H loci with *env* genes [18], which have been shown to be immunosuppressive [19]. It seems, however, that HERV-H replicated mostly through retrotransposition, with some re-infection [20] and trans-complementation [12].

### (d) HERV-W: the indispensable

This is a relatively small family that entered the genome of primates before the separation of Old and New World monkeys [21] and was active for only a short period, about 5 Myr [22]. This family, as well as HERV-FRD, have received increased research attention because their *env* genes have been co-opted (*syncytin-1* and *-2*) to benefit their hosts [23,24]. HERV-W is also the only HERV family commonly copied by long interspersed nuclear elements (LINEs) [25].

### (e) HERV-K: the last (but not the least)

All but one of the HERV families have ceased replicating within the human genome. The family that might still expand, as evidenced by multiple human-specific insertions, and insertional polymorphism within the human population, is termed HERV-K (HML-2) [26–30], hereafter referred to as HK2. By using the consensus of different full-length HK2 loci, a functional infectious virus has been reconstructed *in vitro* [31,32].

HK2 is the only family that has loci with all of their open reading frames (ORFs) intact [27,30,33–35], compared with the rest of the families that have been inactivated with frameshifting indels and premature stop codons (see §4).

#### 4. Human endogenous retroviruses are inactivated, downregulated or become replication defective through random knock-out mutations, hypermutation and silencing mechanisms

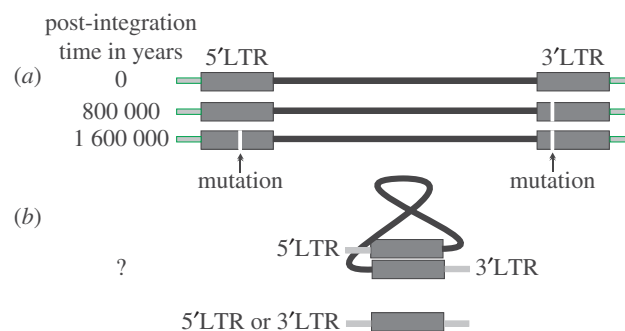
Most HERV loci are replication defective owing to mutations acquired during host germline cell division, which can cause premature stop codons [2] or frameshifts. The exception to this rule are those ERV genes that have been co-opted to provide a functional role to the host, and thus maintain coding capacity owing to purifying selection (e.g. *syncytin-1*) [36]. Another exception is some HK2 loci, which are very recent integrations [30]; even if HK2 loci have intact ORFs, they are transcriptionally and translationally inactive or replication defective. This can result from various mechanisms, including histone and DNA methylation [37,38], antiretroviral hypermutation [39,40] and potentially through RNA silencing as in other retrotransposons [41–44].

#### 5. Criticism against the possible role of human endogenous retroviruses and human disease

The role of HERVs in human disease has been investigated for at least three decades. XRVs are often oncogenic, and the initially high expectation to find that HERVs were a cause of human cancer [45,46] or autoimmunity was followed by disappointment and dead-end projects. These unsuccessful attempts gave rise to the term ‘tumour viruses’ as a description of ERVs [47] (a play on the term ‘tumour viruses’) to reflect the lack of evidence of a pathogenic effect.

Before the deciphering of the human genome, our knowledge of HERVs was fragmentary [3,8,11], but since then, as well as the availability of many more animal genomes, the behaviour of ERVs and other TEs as genomic parasites is being clarified [48]. High-throughput sequencing provides an opportunity to study the mobility of these viruses in populations and individuals. For example, it has recently been shown using high-throughput sequencing [49] that *L1* and *Alu* retrotransposons are copying within the brain during development [50,51], though at low levels [52], and within cancer cells [53]. We also know much more about the pathogenesis of cancer and autoimmunity. These advances now allow us to build more solid hypotheses about the role of HERVs in the development of disease than we were able to 10 years ago, as well as test them more rigorously.

ERVs may be involved in pathophysiological mechanisms either through their replication or through expressed gene products. Another potential mechanism would be through ectopic recombination between HERV copies (as it is for other genomic repeats) [54,55], but we will not analyse this in depth here. First, we are going to discuss the replication cycle as a possible pathogenic mechanism operating in the most recently active HERV, HK2. Second, we are going to examine the possible role of *env* genes in human pathophysiology.



**Figure 2.** The fate of ERV long terminal repeats (LTRs). When a retrovirus integrates in the host’s genome (time 0), LTRs are identical. Over time, they accumulate mutations at a host’s substitution rate; thus the divergence of the LTRs from the same locus can be used to estimate how much time has passed since integration. On many occasions, a host genomic repair mechanism uses LTRs from a locus as a template to loop-out the internal region of the ERV resulting in remnant LTRs known as solo-LTRs. (Online version in colour.)

#### 6. The search for a mobile human endogenous retrovirus: HK2

##### (a) Is HK2 active in the human germline?

As we mentioned in §4, HK2 is the only HERV family that has definitely been copying itself after the human and chimpanzee split [26–28,56]. This is proved by multiple human-specific HK2 loci for which LTR-dating supports integration times at least as recent as 1 Ma (figure 2) [57], and by the existence of insertionally polymorphic loci within humans [28]. The unanswered question is whether HK2 contains replication competent loci and still increases its copy number within our genome (germline activity).

Two HK2 loci (K106 and K115) have identical LTRs [58], suggesting that HK2 was producing new germline insertions around (or earlier than) 0.8 Ma, assuming a divergence rate of 0.13% per Myr [56]. However, mutations accumulate stochastically at this rate, and a locus could have identical LTRs but be much older (or younger). Another dating method [59] uses a sample of the 5’ LTR sequences of a specific locus in the population and estimates the coalescent dates of these LTRs. These authors suggested that the convergence date of a population sample of K106 LTRs is between 0.09 and 0.15 Ma, which is after the emergence of anatomically modern humans [58]. Finally, data assuming a neutral model of HERV insertions indicate that the insertional polymorphism data of HK2 are consistent with activity continuing up to any time between the present day and 0.5 Ma [28].

Another group has found HK2 loci in fossil archaic human remains (a Denisovan) that are not in the modern human genome, suggesting that HK2 was active after the modern human–Denisovan split (approx. 0.8 Ma) [60]. However, finding loci in the Denisovan genome, which are not in the modern human reference genome, is not definitive proof of active infections after the human–Denisovan split. Two other processes could result in Denisovan-specific loci. First, at the time of Denisovan–modern human divergence, many HK2 loci were presumably unfixated within the common ancestral population; therefore some will have gone to fixation in the Denisovan and been lost from the human population. A similar process has led to a HERV-K locus being present in the same

genomic location in gorillas and chimpanzees but not in humans [61]. Second, the human reference genome contains mostly fixed HK2 loci and only a minority of unfixed ones. This is because the reference genome (GRCh37) is the consensus of 13 genomes [62] and as such is likely to contain only fixed or very common loci. We expect that at least some polymorphic loci in the modern human population have not been mapped (e.g. some were identified in [63]), so it is probable that some loci of the Denisovan genome would have unfixed orthologous loci in the human population.

If we conservatively summarize the above, then our best estimate of HK2 copying activity in the human genome is that it was active more recently than 0.8 Ma with the most recent integration that went to fixation being around 0.1 Ma. To improve this estimate, we need to map the polymorphic loci in a large sample of humans to test the hypothesis that HK2 is still active or ceased replication 0.1–0.8 Ma using modelling based on population genetics theory [28]. Both mapping polymorphic loci and simulating the human population during the last 1 Myr are extremely challenging. Furthermore, an approach based purely on population genetics cannot definitively prove or reject a very recent (e.g. within the past 0.02 Myr) cessation of activity. If HK2 is actively replicating as a lineage, then there is a substantial probability that it would have pathogenic potential as has been shown for active families in other animals, because replication cannot be decoupled from insertional mutagenesis [2,64]. We note, however, that because HK2 is a family that invaded the genome at least 30 Ma, we do not expect high virulence (i.e. it should not significantly increase mortality and morbidity during the first 30 years of a human's life).

### (b) Theoretical evolution suggests that human endogenous retroviruses could be pathogenic at the post-reproductive age of the host

HK2 is an old family within which is a small branch that contains some recently integrated loci with largely intact *env* genes. The existence of multiple HK2 loci in human genomes means that these loci cannot be strongly deleterious, at least not prior to reproductive age [65]. Population genetics theory predicts that a slightly harmful allele can drift to fixation, especially if the pathogenicity is mainly expressed after the age of reproduction in the same way as a trait associated with senescence [66–75]. HK2 loci could have slight pathogenic potential especially at the post-reproductive age, which also coincides with the higher incidence of autoimmunity and cancer.

### (c) HK2 is upregulated in patients with cancer and other diseases

The observation that cancer cells produce virus-like antigens and particles is very old [76]; however, it is a phenomenon that has only partially been described and its role in cancer biology is still under investigation [77] (see §5).

Since the sequencing of the human genome in 2001 [3], more rigorous studies on the upregulation of HERVs in health and disease have been undertaken [78–87]. HK2 RNA has been found most frequently in the plasma of patients with HIV-1, breast cancer and lymphoma, with titres up to  $10^7$  copies  $\text{ml}^{-1}$  [82,85]. Only rarely and with much lower titres has it been found in patients with rheumatoid arthritis, HCV and/or

normal volunteers [85]. Viral and virus-like particles have been found at a fraction of  $1.16 \text{ g ml}^{-1}$  using electronic microscopy of plasma from patients with breast cancer and HIV-1; they were identified with immunoelectron microscopy and molecular sequencing as being HK2 [76,87]. Upregulation of HK2 expression has been shown to cause an immune response [88–93]. However, the disease spectrum where HK2 is upregulated has not been systematically investigated with a rigorous epidemiological design.

### (d) *In vivo* infectivity of HK2 is still under investigation

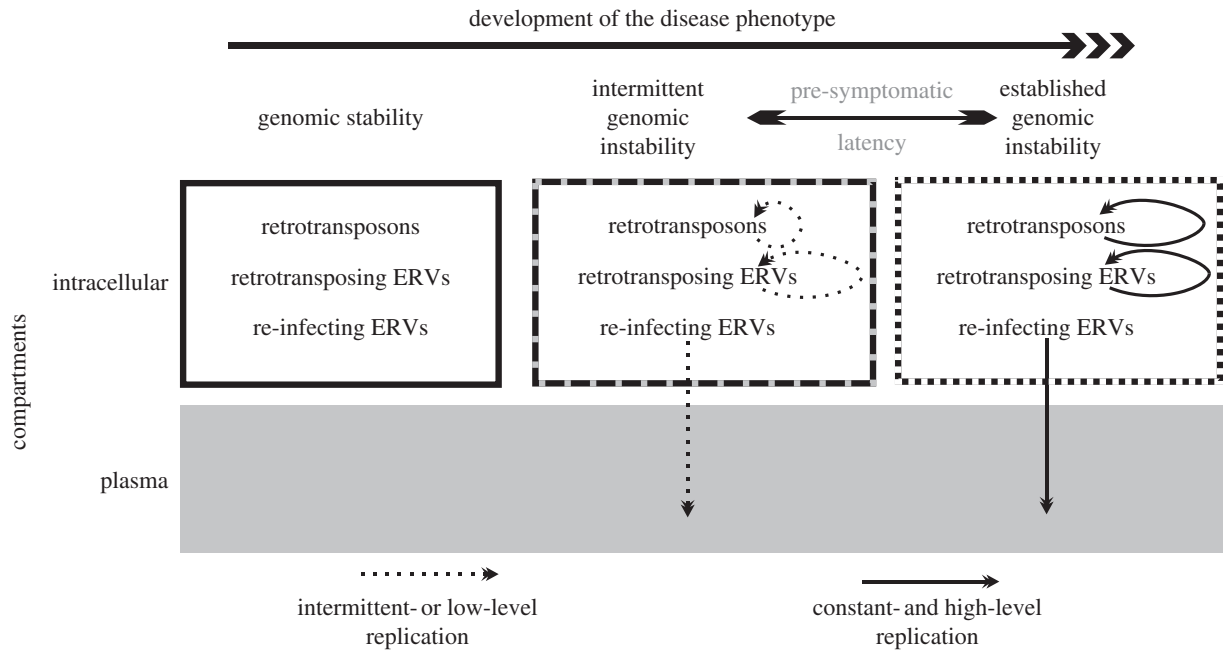
While the evidence of HK2 plasma RNA is robust, suggesting upregulation in certain diseases, there is no definitive evidence for the production of infectious particles. Infectious progenitors of HK2 have been constructed *in vitro* using existing HK2 DNA fragments from the human genome, providing proof of principle of the potential for HK2 infectious particle formation in humans today [31]. Furthermore, pseudo-typed viral particles with the HK2 *env* from locus K108 have been shown to be infectious in cell lines [32,94]. Isolation of sequences from patients and comparison of their mutations with copies in the published human genome have indicated purifying selection, which could result from copying of loci within the individual [87]. However, HK2 is insertionally polymorphic within the human population and many loci are not in the published human genome [28]. Therefore, the identification of new loci under purifying selection in contrast to the published loci is not conclusive proof of infectious activity. A more rigorous experimental design powered by high-throughput sequencing technologies and state-of-the-art molecular evolution analyses would provide a solid answer to the infectious potential of HK2.

### (e) Upregulation of HK2 could provide mobility/infectiousness of HERV-K

No HK2 locus has been found to be replication competent. However, there are HK2 proviruses with intact ORFs [27,30,33–35], and in mice, recombination between replication-defective ERV loci can lead to replication-competent loci [95,96]. This provides a plausible model for the reconstitution of mobile and/or infectious HK2 within the genome [97]. A recent paradigm of reconstitution of a replication competent ERV from two defective endogenous loci is the accidental laboratory recombination of defective endogenous murine leukaemia viruses (MLVs) which resulted in the xenotropic MLV-related retrovirus [95]. Young *et al.* [96] have recently shown the resurrection of defective ERVs in antibody-deficient mice through recombination, as a result of the upregulation of these ERVs by inflammation induced from microbiota in the gut. More importantly, we know that infectious HK2 can be reconstituted *in vitro* by the consensus of common HK2 loci within the genome [31,32], suggesting that, in principle, naturally occurring recombination could restore replication and infectivity.

## 7. Molecular basis of a possible HK2 connection with the development of cancer

HK2 loci produce one of two non-standard retroviral proteins, Np9 and Rec through alternatively spliced mRNA [81,98]. Rec



**Figure 3.** Model of ERV upregulation with respect to disease. HK2 RNA has been isolated from the plasma of patients with lymphoma and breast cancer. It is thought that upregulation of HK2 in these diseases could be connected with genomic instability which, however, affects most of the mobile elements. HK2 is isolated from the plasma because it is a re-infecting ERV and can form retrovirus-like particles.

is analogous to the Rev protein of HIV-1 and when highly expressed has been shown to promote tumour development in mice [99,100]. A region of the transmembrane domain of *env* has been found to be immunosuppressive in several retroviruses—the so-called immunosuppressive domain [101–103]. Heidmann and co-workers showed that tumours expressing *env* proteins of mouse mammary tumour virus can escape the immune response (at least transiently), whereas tumours having knocked-out *env* genes were efficiently recognized by the immune system and rejected successfully [104]. It must be noted, however, that evidence for immunosuppressive properties of the *env* of HK2 is scarce [105]. The emergence of replication competent/retrotransposing HK2 could be implicated in pathogenesis through insertional mutagenesis (extensively reviewed in [106]), a process which has been recently shown to be common for *L1* and *Alu* retrotransposons [50]. Finally, another possible pathogenic mechanism would be through promoter activity of LTRs; for example, de-repression of one of the oldest HERVs has been recently connected with the development of Hodgkin's lymphoma [107].

The expression of oncogenic and immunosuppressive proteins by HK2 could contribute to the development of the malignant phenotype, complementing and enhancing the other numerous well-characterized factors (e.g. oncogenes). Because evolution of a cell towards the cancerous phenotype is a multi-stage, multi-factorial process, HK2 could be one of the many components enhancing the development of the selfish phenotype by increasing the aggressiveness or immune-stealth [108] property of the cell [109–111].

Perhaps the upregulation of HK2 expression, independently of any HK2 activity in the early stages of tumorigenesis, provides the conditions for further involvement of HK2 in the latter stages of the development of the malignant phenotype. Very few apparently healthy individuals have detectable HK2 in the plasma [85]. This could be due to an inability to suppress ERV and retrotransposon expression/replication [112], which happens at an early

stage throughout the development of the full disease phenotype. Quasi-defective antiviral (e.g. TRIM5 $\alpha$  [113–115], RNase-L [116,117]), silencing mechanisms or predisposition to genomic instability could allow upregulation/replication of HK2 well before the full development of the disease phenotype (pre-symptomatic latency; figure 3). An epidemiological study to define how widespread and early this phenomenon is, and what the significant underlying confounders are, could elucidate the mechanism associating HK2 with cancer.

## 8. The immune response against the upregulation of HK2

ERV antigens are host antigens and believed not to promote immune response upon their expression [118]. However, if an ERV antigen was not presented during the development of the immune system, then its later expression could promote an immune response [118]. Immune responses against HK2 antigens have been reported in patients with upregulated HK2 [119]. Nixon's group showed that stronger T-cell responses against HK2 antigens are related to slower progression to AIDS [88–91]. Some evidence suggests that this is not the consequence of immune deterioration in cases progressing to AIDS, but is rather the result of the immune system being tolerant to HERV antigens (not able to promote HERV-specific immune response). This group has also shown recently that CD8 T-cell responses against HK2 antigens resulted in the elimination of HIV-1 infected cells as a result of the upregulation of HK2 by HIV infections; this suggests that a HERV-based vaccine to provide long-term control of HIV might be possible [88]. Given that immunosuppression increases the incidence of specific cancers, a study to describe immune escape of HK2 as a result of generalized immunosuppression or HK2-specific immunotolerance with respect to the development of cancer, could provide insights on the immune escape of malignant cells [92,120].

## 9. Technical challenges in the study of HK2 mobility

As we have already noted, our ability to study TEs has greatly improved during the past five years. This is firstly due to the availability of a high-quality human reference genome [3] and secondly due to the development of higher throughput, lower-cost sequencing technologies. A HERV that is able to replicate within the human genome has never been described so far; determining whether HK2 is mobile within the genome could have potential clinical implications. To show mobility, we need to see insertional polymorphism within the same individual, i.e. we have to identify somatic integrations as loci present in a tissue but not in another tissue and exclude the possibility of deletion. Somatic integrations are expected to be random; therefore unless novel HK2 integrations are followed by clonal expansion of the cells, the recovery of a single locus against thousands of germline (i.e. within every cell) copies is a major experimental and bioinformatics challenge. Let us assume that we extract the genome of 1000 cells within which there have been 100 random novel somatic integrations in total. There are approximately 90 full-length HK2 loci in the human genome [26], so for the sake of simplicity, we will consider there to be 100. Assuming that solo LTRs (i.e. the naturally occurring product of recombinational deletion of full-length loci; figure 2) are 10 times more frequent [121], we expect that each one of the 100 novel integrations would have to be identified against a background of 1100 loci, each one having 1000 copies, in total 1 100 000 standard fixed HK2 copies. The problem becomes even more difficult if we consider 5000–6000 copies of SVAs, active transposons carrying a truncated LTR of HK2 [122]. Current high-throughput sequencing approaches are not expected to recover novel somatic integrations (though some researchers have been able to recover rare integrations [50,52]), unless there is a significant favourable bias for the novel integrations either owing to massive proliferation of the cell carrying the novel HK2 locus or through a novel experimental protocol which would enrich the novel and deplete the fixed HK2 loci. With respect to the bioinformatics challenge, the difficulty of mapping the high-throughput sequencing reads uniquely and identifying breakpoints is compounded by the fact that at least 50% of the human genome comprises repetitive regions. Longer, better and more reads per locus would help to establish which loci could be uniquely mapped within highly repetitive elements. We expect that the recent and forthcoming updates of high-throughput sequencing technologies will be able to dramatically improve this within 2013.

## 10. *Env* genes and their role in human disease

HK2 is the only HERV family that could be pathogenic through replication competent loci. On the other hand, replication-defective HERVs can contribute in pathophysiological mechanisms via their gene products. Here, we focus on the role of HERV *env* (envelope) genes and especially their immunosuppressive properties with respect to recent evolutionary insights pertaining to the mechanism of immune repression. Retroviral *env* genes have also been implicated in carcinogenesis as growth stimulators [123,124], but we shall not refer to this mechanism.

### (a) The burden of *env* genes

We have recently shown that the number of ERV loci generated following the initial germline invasion of a mammalian host by the virus is strongly inversely correlated to the integrity of the *env* gene, with the largest ERV families generally being derived from elements with deleted (or non-functional) envelopes [48]. The main way for a locus to produce many copies in the genome and be active in the long term is to lose its *env* gene and become a retrotransposon. There appears to be a trade-off between the integrity of the *env* gene and stable parasite–host coexistence, with the cost here being the loss of the ability to move between hosts. ERVs can be placed on a spectrum, with elements with intact *env* being the least adapted to intracellular life, and ERVs with no envelope at all being the most adapted to stable coexistence with the host.

In addition to being crucial for retroviral reinfection [12,13], several *env* genes have been independently co-opted by their mammalian hosts throughout their evolution, and contribute to two immune-related functions (other envelopes are involved in antiviral immunity [125]): development of the syncytiotrophoblast and immunotolerance of the mother to the paternal antigens of the fetus [126]. In humans, we are aware of two co-opted retroviral *env* genes, *syncytin-1* and *syncytin-2*, both efficiently expressed at the syncytiotrophoblast [23,24]. In what follows, we bring two thought-provoking research questions about the role of *env* genes in cancer and autoimmunity.

### (b) Is immune escape of transmissible cancer related to the evolutionary development of the trophoblast and the co-option of *env* genes?

Causality in cancer has been dogmatically connected with acquired mutations (and replicating HK2 elements can be considered simply as mutations that could lead to cancer). A striking exception to this paradigm is transmissible cancer (as distinct from cancer associated with viral infection), of which two examples are known: canine transmissible venereal tumour and the Tasmanian devil facial tumour. In both cases, the genomes of neoplasms from different hosts are clearly more related to each other than to the genomes of the hosts in which they are found [127–129]. Therefore, it is believed that these tumours are serially transplanted between individuals, causing epidemics in dogs and Tasmanian devils.

The most exotic characteristic of transmissible cancer is that the infected individual's immune system fails to identify the transplanted cancer cells as foreign tissue (immune-tolerance to alloantigens) constituting a natural instance of allogeneic transplantation [129]. Transmission of cancer in humans has been observed as a rare phenomenon, predominantly as a side-effect of medically driven allogeneic tissue transplantation (usually followed by extensive immunosuppression to protect the allogeneic transplant from rejection) [130]. These rare cases support the policy of preventing individuals with cancer from donating their organs unless the primary tumour is within the central nervous system (where the probability of distant metastases is minimal and therefore the probability of cancer transplantation is negligible) [131]. Apart from the scarce medical cases of accidental cancer transplantation, there is an infrequent but well-

described paradigm of naturally occurring allogeneic tumours in humans: gestational trophoblastic disease (GTD).

GTD occurs when a potentially malignant tumour, comprising a mixture of self and alloantigens, arises from the trophoblast of the placenta, during or after pregnancy [132]. The trophoblastic tumour develops by taking advantage of the naturally occurring immune-stealth cloak that placental mammals were forced to evolve to protect the embryo developing in the uterus, hiding and tolerating the fetus's alloantigens [133]. This immunotolerance is so efficient that it can sustain a totally allogeneic embryo as occurs in gestational surrogacy [134]. Apart from the well-developed immune-stealth cloak, GTD takes advantage of another two naturally occurring characteristics of the syncytiotrophoblast: tissue penetration and vascular remodelling [135]. The efficiency of these properties can be seen in ectopic pregnancy where the syncytiotrophoblast is accidentally implanted outside the uterus [136]. In such cases, the syncytiotrophoblast efficiently attaches to almost any tissue in the abdomen (e.g. intestines, peritoneum, spleen, liver, diaphragm), enhances circulation through vascular remodelling and then cannot be separated from the attached organs without extensive bleeding. Dramatically, the treatment of abdominal pregnancy is an operation where the surgeon is forced to concurrently remove the underlying tissues, a situation very similar to the removal of abdominal malignant tumours [137]. The adjuvant treatment is performed with methotrexate, a widely known antineoplastic substance [138]. It therefore seems that placental mammals have co-opted three crucial malignant attributes as a cost for well-protected offspring delivery: immune-tolerance of alloantigens, tissue penetration and vascular remodelling.

Heidmann and co-workers [104] have shown that it is possible to transplant allogeneic cancerous cell-lines in immunocompetent mice by engineering cancerous cells to express retroviral Env proteins. As discussed above, these genes are known to have been co-opted independently multiple times within the placental mammal lineage, where they are expressed in the placenta and play a crucial role for the normal formation of the syncytiotrophoblast [23,135, 139–141]. While not all retroviral *env* genes have been co-opted for their immunosuppressive features (the most striking property of which is the promotion of cell fusion; hence they are called *syncytins*), their contribution to the immune-stealth cloak of the trophoblast is considered crucial for gestational immune tolerance [126]. It is thus reasonable to hypothesize that ectopic upregulation of immunosuppressive *syncytin* might contribute to the immune escape of human cancers (other than GTD).

Apart from showing the contribution of *syncytins* to the syncytiotrophoblast and thus possibly to allogeneic cancers in humans, Heidmann's experiments show a remarkable similarity in natural history to the canine transmissible venereal tumour: the *env*-expressing tumours grow, plateau and then regress [104,129]. This is in striking contrast to what happens in the Tasmanian devil, where the transmissible cancer is more aggressive, leading to a deadly and generalized disease [129]. This difference might be because the Tasmanian devil is a marsupial and is not likely to have developed a regulatory system for trophoblast-like activity. In addition, a possible insight into the regulation mechanisms in placental mammals comes from recent studies on repeated miscarriages in humans and the rapidly emerging field of clinical reproductive immunology [142]. While still considered too controversial to

form a standard treatment guideline [143,144], it seems likely that a natural killer (NK) cell response in humans does not allow normal implantation of the placenta, resulting in recurrent miscarriages at least in some patients [145]. Interestingly, NK responses against the facial tumour could not be induced in Tasmanian devils after active immunization with facial cancer cells [146].

We could test the hypothesis for an *env*-induced immune-stealth cloak in transmissible cancers by implementing the methods used to discover and characterize *syncytins* [23,24, 139–141]: *in silico* search for co-opted (or recently introduced) retroviral *env* genes within the tumour genomes, and *in vitro* verification of expressed and upregulated Env proteins in the tumour. The immunosuppressive properties of the Env protein could be verified in the same way as for the other *env* genes discussed previously [104].

### (c) Is *env* upregulation in autoimmunity a last resort for immune regulation?

The upregulation of HERVs has been described in a variety of diseases considered to be autoimmune, such as multiple sclerosis, rheumatoid arthritis and systemic lupus erythematosus [147–150]. The proposed underlying mechanism of association is that upregulated HERV-derived antigens are triggering an immune response that either attacks the antigen-producing cells, or cells producing similar antigens (molecular mimicry) [151], or that truncated *env* genes act as superantigens that trigger a non-specific autoimmune response [152]. While each of these hypotheses could be valid, the difficulty in proving such a mechanism is substantial. A significant correlation between the immune response against ERV antigens and autoimmune disease has been documented, although not always consistently found [153], but this correlation does not prove that ERV antigens were the trigger for disease onset. It might simply result from a general disruption of immune tolerance. The problem is further complicated by the fact that even for well-established auto-antibodies, the specificity remains low in the vast majority of diseases [154]. For example, there are a wide variety of auto-antibodies (e.g. anti-nuclear, anti-dsDNA) produced in inflammatory diseases, some of which are more commonly detected in older people with no clinical autoimmunity [155]. Thus, distinguishing cause from effect with respect to the role of HERVs and autoimmune diseases remains an open challenge.

On the other hand, HERVs could be involved in autoimmune disorders as a last resort for immune regulation if cells exploit immunosuppressive *env* antigens, such as HERV-H [19] and *syncytin-2* [126]. Both these *env* antigens have been shown to be able to protect against rejection of allografts in mice [19,126]; therefore, the cells that produce these antigens could escape the irregular and prolonged immune responses which are the hallmark of autoimmunity. We propose that at least a proportion of the population of endogenous *env* genes (some of them having ORFs in the human genome [156]) could serve as a downregulatory mechanism of immunity and act as a population of expendable loci with common action rather than a single locus. Thus, although Env is among the viral proteins that may trigger, or be a target of, immune responses, these same proteins could serve an anti-autoimmune role.

This scenario could also explain the co-option of *env*, a major evolutionary paradox, as placental mammals exist throughout eutherian mammals, yet at the same time, the crucial *syncytin* gene has been derived independently multiple times from different ERV families to serve a similar purpose, presenting a very extreme example of convergent evolution [23,24,135,139–141,157,158]. Eutherian mammals have gradually acquired an array of innate immune genes (*tetherin*, *TRIM5 $\alpha$* , *APOBEC*, etc.) [125,159] in an evolutionary arms race between the host and its pathogens. If cell-mediated immune responses developed similarly, then the requirement of regulatory mechanisms against cell immunity would have increased. The population of endogenized *env* genes, some of them having immunosuppressive properties, could have provided the pool from which a true immunoregulatory gene could be selected. Eventually, one *env* gene prevailed and was co-opted to serve a specific immunoregulatory purpose (tolerance to fetal antigens), whereas the rest continued to degrade naturally (as described in §3) but were still able to provide immunosuppressive

protection in extreme conditions. Intriguingly, we have never been able to identify an *env* gene for ERV-L, the oldest ERV family that we know within mammals. This is in line with our hypothesis: if, at the time of ERV-L's invasion, the cost of carrying *env* genes was not counterbalanced by their potential benefits in immunotolerance, this would lead to faster extinction of *env*-carrying families; more recently, as cell immunity became more intensive, *env* genes became useful at least transiently and eventually some of them became co-opted. Testing for recently mobile HERVs might be the most direct way to examine their association with cancer, but perhaps ancient integrations also contain clues for the underlying processes and could still remain as participants in certain present-day cancers.

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## References

- Jern P, Coffin JM. 2008 Effects of retroviruses on host genome function. *Annu. Rev. Genet.* **42**, 709–732. (doi:10.1146/annurev.genet.42.110807.091501)
- Boeke JD, Stoye JP. 1997 Retrotransposons, endogenous retroviruses, and the evolution of retroelements. In *Retroviruses* (eds JM Coffin, SH Hughes, HE Varmus), pp. 343–436. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Lander ES *et al.* 2001 Initial sequencing and analysis of the human genome. *Nature* **409**, 860–921. (doi:10.1038/35057062)
- Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, Kang HM, Marth GT, McVean GA. 2012 An integrated map of genetic variation from 1,092 human genomes. *Nature* **491**, 56–65. (doi:10.1038/nature11632)
- Ioannidis JP. 2005 Why most published research findings are false. *PLoS Med.* **2**, e124. (doi:10.1371/journal.pmed.0020124)
- Ioannidis JP. 2008 Why most discovered true associations are inflated. *Epidemiology* **19**, 640–648. (doi:10.1097/EDE.0b013e31818131e7)
- Stocking C, Kozak CA. 2008 Murine endogenous retroviruses. *Cell Mol. Life Sci.* **65**, 3383–3398. (doi:10.1007/s00018-008-8497-0)
- Tristem M. 2000 Identification and characterization of novel human endogenous retrovirus families by phylogenetic screening of the human genome mapping project database. *J. Virol.* **74**, 3715–3730. (doi:10.1128/JVI.74.8.3715-3730.2000)
- Jern P, Sperber GO, Blomberg J. 2004 Definition and variation of human endogenous retrovirus H. *Virology* **327**, 93–110. (doi:10.1016/j.virol.2004.06.023)
- Bannert N, Kurth R. 2006 The evolutionary dynamics of human endogenous retroviral families. *Annu. Rev. Genomics Hum. Genet.* **7**, 149–173. (doi:10.1146/annurev.genom.7.080505.115700)
- Katzourakis A, Tristem M. 2005 Phylogeny of human endogenous and exogenous retroviruses. In *Retroviruses and primate genome evolution* (ed. ED Sverdlov), pp. 186–203. Austin, TX: Landes Bioscience.
- Belshaw R, Katzourakis A, Paces J, Burt A, Tristem M. 2005 High copy number in human endogenous retrovirus families is associated with copying mechanisms in addition to reinfection. *Mol. Biol. Evol.* **22**, 814–817. (doi:10.1093/molbev/msi088)
- Belshaw R, Pereira V, Katzourakis A, Talbot G, Paces J, Burt A, Tristem M. 2004 Long-term reinfection of the human genome by endogenous retroviruses. *Proc. Natl Acad. Sci. USA* **101**, 4894–4899. (doi:10.1073/pnas.0307800101)
- Katzourakis A, Rambaut A, Pybus OG. 2005 The evolutionary dynamics of endogenous retroviruses. *Trends Microbiol.* **13**, 463–468. (doi:10.1016/j.tim.2005.08.004)
- Benit L, Lallemand JB, Casella JF, Philippe H, Heidmann T. 1999 ERV-L elements: a family of endogenous retrovirus-like elements active throughout the evolution of mammals. *J. Virol.* **73**, 3301–3308.
- Anderssen S, Sjøttem E, Svineng G, Johansen T. 1997 Comparative analyses of LTRs of the ERV-H family of primate-specific retrovirus-like elements isolated from marmoset, African green monkey, and man. *Virology* **234**, 14–30. (doi:10.1006/viro.1997.8590)
- Mager DL, Freeman JD. 1995 HERV-H endogenous retroviruses: presence in the New World branch but amplification in the Old World primate lineage. *Virology* **213**, 395–404. (doi:10.1006/viro.1995.0012)
- de Parseval N, Casella J, Gressin L, Heidmann T. 2001 Characterization of the three HERV-H proviruses with an open envelope reading frame encompassing the immunosuppressive domain and evolutionary history in primates. *Virology* **279**, 558–569. (doi:10.1006/viro.2000.0737)
- Mangeny M, de Parseval N, Thomas G, Heidmann T. 2001 The full-length envelope of an HERV-H human endogenous retrovirus has immunosuppressive properties. *J. Gen. Virol.* **82**, 2515–2518.
- Goodchild NL, Freeman JD, Mager DL. 1995 Spliced HERV-H endogenous retroviral sequences in human genomic DNA: evidence for amplification via retrotransposition. *Virology* **206**, 164–173. (doi:10.1016/S0042-6822(95)80031-X)
- Kim HS, Takenaka O, Crow TJ. 1999 Isolation and phylogeny of endogenous retrovirus sequences belonging to the HERV-W family in primates. *J. Gen. Virol.* **80**, 2613–2619.
- Costas J. 2002 Characterization of the intragenomic spread of the human endogenous retrovirus family HERV-W. *Mol. Biol. Evol.* **19**, 526–533. (doi:10.1093/oxfordjournals.molbev.a004108)
- Mi S *et al.* 2000 Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. *Nature* **403**, 785–789. (doi:10.1038/35001608)
- Blaise S, de Parseval N, Benit L, Heidmann T. 2003 Genomewide screening for fusogenic human endogenous retrovirus envelopes identifies *syncytin 2*, a gene conserved on primate evolution. *Proc. Natl Acad. Sci. USA* **100**, 13 013–13 018. (doi:10.1073/pnas.2132646100)
- Pavlicek A, Paces J, Elleder D, Hejnar J. 2002 Processed pseudogenes of human endogenous retroviruses generated by LINEs: their integration, stability, and distribution. *Genome Res.* **12**, 391–399. (doi:10.1101/gr.216902).
- Subramanian RP, Wildschutte JH, Russo C, Coffin JM. 2011 Identification, characterization, and comparative genomic distribution of the HERV-K



- (HML-2) group of human endogenous retroviruses. *Retrovirology* **8**, 90. (doi:10.1186/1742-4690-8-90)
27. Barbulescu M, Turner G, Seaman MI, Deinard AS, Kidd KK, Lenz J. 1999 Many human endogenous retrovirus K (HERV-K) proviruses are unique to humans. *Curr. Biol.* **9**, 861–868. (doi:10.1016/S0960-9822(99)80390-X)
  28. Belshaw R, Dawson AL, Woolven-Allen J, Redding J, Burt A, Tristem M. 2005 Genomewide screening reveals high levels of insertional polymorphism in the human endogenous retrovirus family HERV-K(HML2): implications for present-day activity. *J. Virol.* **79**, 12 507–12 514. (doi:10.1128/JVI.79.19.12507-12514.2005)
  29. Hughes JF, Coffin JM. 2004 Human endogenous retrovirus K solo-LTR formation and insertional polymorphisms: implications for human and viral evolution. *Proc. Natl Acad. Sci. USA* **101**, 1668–1672. (doi:10.1073/pnas.0307885100)
  30. Turner G, Barbulescu M, Su M, Jensen-Seaman MI, Kidd KK, Lenz J. 2001 Insertional polymorphisms of full-length endogenous retroviruses in humans. *Curr. Biol.* **11**, 1531–1535. (doi:10.1016/S0960-9822(01)00455-9)
  31. Dewannieux M, Harper F, Richaud A, Letzelter C, Ribet D, Pierron G, Heidmann T. 2006 Identification of an infectious progenitor for the multiple-copy HERV-K human endogenous retroelements. *Genome Res.* **16**, 1548–1556. (doi:10.1101/gr.5565706)
  32. Lee YN, Bieniasz PD. 2007 Reconstitution of an infectious human endogenous retrovirus. *PLoS Pathog.* **3**, e10. (doi:10.1371/journal.ppat.0030010)
  33. Mayer J, Sauter M, Racz A, Scherer D, Mueller-Lantzsch N, Meese E. 1999 An almost-intact human endogenous retrovirus K on human chromosome 7. *Nat. Genet.* **21**, 257–258. (doi:10.1038/6766)
  34. Ono M, Yasunaga T, Miyata T, Ushikubo H. 1986 Nucleotide sequence of human endogenous retrovirus genome related to the mouse mammary tumor virus genome. *J. Virol.* **60**, 589–598.
  35. Ono M. 1986 Molecular cloning and long terminal repeat sequences of human endogenous retrovirus genes related to types A and B retrovirus genes. *J. Virol.* **58**, 937–944.
  36. Blaise S, de Parseval N, Heidmann T. 2005 Functional characterization of two newly identified human endogenous retrovirus coding envelope genes. *Retrovirology* **2**, 19. (doi:10.1186/1742-4690-2-19)
  37. Leung DC, Lorincz MC. 2012 Silencing of endogenous retroviruses: when and why do histone marks predominate? *Trends Biochem. Sci.* **37**, 127–133. (doi:10.1016/j.tibs.2011.11.006)
  38. Rowe HM, Trono D. 2011 Dynamic control of endogenous retroviruses during development. *Virology* **411**, 273–287. (doi:10.1016/j.virol.2010.12.007)
  39. Armitage AE *et al.* 2008 Conserved footprints of APOBEC3G on hypermutated human immunodeficiency virus type 1 and human endogenous retrovirus HERV-K(HML2) sequences. *J. Virol.* **82**, 8743–8761. (doi:10.1128/JVI.00584-08)
  40. Goila-Gaur R, Strebel K. 2008 HIV-1 Vif, APOBEC, and intrinsic immunity. *Retrovirology* **5**, 51. (doi:10.1186/1742-4690-5-51)
  41. Chuma S, Pillai RS. 2009 Retrotransposon silencing by piRNAs: ping-pong players mark their sub-cellular boundaries. *PLoS Genet.* **5**, e1000770. (doi:10.1371/journal.pgen.1000770)
  42. Phalke S, Nickel O, Walluscheck D, Hortic F, Onorati MC, Reuter G. 2009 Retrotransposon silencing and telomere integrity in somatic cells of *Drosophila* depends on the cytosine-5 methyltransferase DNMT2. *Nat. Genet.* **41**, 696–702. (doi:10.1038/ng.360)
  43. Suzuki S *et al.* 2007 Retrotransposon silencing by DNA methylation can drive mammalian genomic imprinting. *PLoS Genet.* **3**, e55. (doi:10.1371/journal.pgen.0030055)
  44. Barbot W, Dupressoir A, Lazar V, Heidmann T. 2002 Epigenetic regulation of an IAP retrotransposon in the aging mouse: progressive demethylation and de-silencing of the element by its repetitive induction. *Nucleic Acids Res.* **30**, 2365–2373. (doi:10.1093/nar/30.11.2365)
  45. Rous P. 1911 A sarcoma of the FOWL transmissible by an agent separable from the tumor cells. *J. Exp. Med.* **13**, 397–411. (doi:10.1084/jem.13.4.397)
  46. Weiss RA, Vogt PK. 2011 100 years of Rous sarcoma virus. *J. Exp. Med.* **208**, 2351–2355. (doi:10.1084/jem.20112160)
  47. Voisset C, Weiss RA, Griffiths DJ. 2008 Human RNA ‘rumor’ viruses: the search for novel human retroviruses in chronic disease. *Microbiol. Mol. Biol. Rev.* **72**, 157–196. (doi:10.1128/MMBR.00033-07)
  48. Magiorkinis G, Gifford RJ, Katzourakis A, De Ranter J, Belshaw R. 2012 Env-less endogenous retroviruses are genomic superspreaders. *Proc. Natl Acad. Sci. USA* **109**, 7385–7390. (doi:10.1073/pnas.1200913109)
  49. Ewing AD, Kazazian Jr HH. 2010 High-throughput sequencing reveals extensive variation in human-specific L1 content in individual human genomes. *Genome Res.* **20**, 1262–1270. (doi:10.1101/gr.106419.110)
  50. Baillie JK *et al.* 2011 Somatic retrotransposition alters the genetic landscape of the human brain. *Nature* **479**, 534–537. (doi:10.1038/nature10531)
  51. Coufal NG *et al.* 2009 L1 retrotransposition in human neural progenitor cells. *Nature* **460**, 1127–1131. (doi:10.1038/nature08248)
  52. Evrony GD *et al.* 2012 Single-neuron sequencing analysis of L1 retrotransposition and somatic mutation in the human brain. *Cell* **151**, 483–496. (doi:10.1016/j.cell.2012.09.035)
  53. Iskow RC, McCabe MT, Mills RE, Torene S, Pittard WS, Neuwald AF, Van Meir EG, Vertino PM, Devine SE. 2010 Natural mutagenesis of human genomes by endogenous retrotransposons. *Cell* **141**, 1253–1261. (doi:10.1016/j.cell.2010.05.020)
  54. Feschotte C, Gilbert C. 2012 Endogenous viruses: insights into viral evolution and impact on host biology. *Nat. Rev. Genet.* **13**, 283–296. (doi:10.1038/nrg3199)
  55. Hughes JF, Coffin JM. 2001 Evidence for genomic rearrangements mediated by human endogenous retroviruses during primate evolution. *Nat. Genet.* **29**, 487–489. (doi:10.1038/ng775)
  56. Lebedev YB, Belonovitch OS, Zybrowa NV, Khil PP, Kurdyukov SG, Vinogradova TV, Hunsmann G, Sverdlov ED. 2000 Differences in HERV-K LTR insertions in orthologous loci of humans and great apes. *Gene* **247**, 265–277. (doi:10.1016/S0378-1119(00)00062-7)
  57. Dangel AW, Baker BJ, Mendoza AR, Yu CY. 1995 Complement component C4 gene intron 9 as a phylogenetic marker for primates: long terminal repeats of the endogenous retrovirus ERV-K(C4) are a molecular clock of evolution. *Immunogenetics* **42**, 41–52. (doi:10.1007/BF00164986)
  58. Jha AR, Nixon DF, Rosenberg MG, Martin JN, Deeks SG, Hudson RR, Garrison KE, Pillai SK. 2011 Human endogenous retrovirus K106 (HERV-K106) was infectious after the emergence of anatomically modern humans. *PLoS ONE* **6**, e20234. (doi:10.1371/journal.pone.0020234)
  59. Jha AR *et al.* 2009 Cross-sectional dating of novel haplotypes of HERV-K 113 and HERV-K 115 indicate these proviruses originated in Africa before *Homo sapiens*. *Mol. Biol. Evol.* **26**, 2617–2626. (doi:10.1093/molbev/msp180)
  60. Agoni L, Golden A, Guha C, Lenz J. 2012 Neandertal and Denisovan retroviruses. *Curr. Biol.* **22**, R437–R438. (doi:10.1016/j.cub.2012.04.049)
  61. Barbulescu M, Turner G, Su M, Kim R, Jensen-Seaman MI, Deinard AS, Kidd KK, Lenz J. 2001 A HERV-K provirus in chimpanzees, bonobos and gorillas, but not humans. *Curr. Biol.* **11**, 779–783. (doi:10.1016/S0960-9822(01)00227-5)
  62. Anon. 2010 E pluribus unum. *Nat. Methods* **7**, 331. (doi:10.1038/nmeth0510-331)
  63. Kidd JM *et al.* 2010 A human genome structural variation sequencing resource reveals insights into mutational mechanisms. *Cell* **143**, 837–847. (doi:10.1016/j.cell.2010.10.027)
  64. Tarlinton RE, Meers J, Young PR. 2006 Retroviral invasion of the koala genome. *Nature* **442**, 79–81. (doi:10.1038/nature04841)
  65. Williams GC. 1957 Pleiotropy, natural selection and the evolution of senescence. *Evol. Int. J. Org. Evol.* **11**, 398–411. (doi:10.2307/2406060)
  66. Hirsch HR. 1980 Evolution of senescence: influence of age-dependent death rates on the natural increase of a hypothetical population. *J. Theor. Biol.* **86**, 149–168. (doi:10.1016/0022-5193(80)90072-7)
  67. Gould KG, Flint M, Graham CE. 1981 Chimpanzee reproductive senescence: a possible model for evolution of the menopause. *Maturitas* **3**, 157–166. (doi:10.1016/0378-5122(81)90007-4)
  68. Hirsch HR. 1982 Evolution of senescence: natural increase of populations displaying Gompertz- or power-law death rates and constant or age-dependent maternity rates. *J. Theor. Biol.* **98**, 321–346. (doi:10.1016/0022-5193(82)90268-5)
  69. Mueller LD. 1987 Evolution of accelerated senescence in laboratory populations of *Drosophila*.

- Proc. Natl Acad. Sci. USA* **84**, 1974–1977. (doi:10.1073/pnas.84.7.1974)
70. Kirkwood TB, Rose MR. 1991 Evolution of senescence: late survival sacrificed for reproduction. *Phil. Trans. R. Soc. Lond. B* **332**, 15–24. (doi:10.1098/rstb.1991.0028)
71. Bell G. 1993 Pathogen evolution within host individuals as a primary cause of senescence. *Genetica* **91**, 21–34. (doi:10.1007/BF01435985)
72. Charlesworth B, Hughes KA. 1996 Age-specific inbreeding depression and components of genetic variance in relation to the evolution of senescence. *Proc. Natl Acad. Sci. USA* **93**, 6140–6145. (doi:10.1073/pnas.93.12.6140)
73. Gagos S, Iliopoulos D, Tseleni-Balafouta S, Agapitos M, Antachopoulos C, Kostakis A, Karayannakos P, Skalkas G. 1996 Cell senescence and a mechanism of clonal evolution leading to continuous cell proliferation, loss of heterozygosity, and tumor heterogeneity: studies on two immortal colon cancer cell lines. *Cancer Genet. Cytogenet.* **90**, 157–165. (doi:10.1016/S0165-4608(96)00049-0)
74. Burstein M. 1998 Evolution and the senescence process. Broadening our view of aging. *J. Gerontol. Nurs.* **24**, 16–19.
75. Caldwell ME, Denicola GM, Martins CP, Jacobetz MA, Maitra A, Hruban RH, Tuveson DA. 2011 Cellular features of senescence during the evolution of human and murine ductal pancreatic cancer. *Oncogene* **31**, 1599–1608. (doi:10.1038/onc.2011.350)
76. Lower R, Boller K, Hasenmaier B, Korbmacher C, Muller-Lantzsch N, Lower J, Kurth R. 1993 Identification of human endogenous retroviruses with complex mRNA expression and particle formation. *Proc. Natl Acad. Sci. USA* **90**, 4480–4484. (doi:10.1073/pnas.90.10.4480)
77. Balaj L, Lessard R, Dai L, Cho YJ, Pomeroy SL, Breakefield XO, Skog J. 2011 Tumour microvesicles contain retrotransposon elements and amplified oncogene sequences. *Nat. Commun.* **2**, 180. (doi:10.1038/ncomms1180)
78. Ono M, Kawakami M, Ushikubo H. 1987 Stimulation of expression of the human endogenous retrovirus genome by female steroid hormones in human breast cancer cell line T47D. *J. Virol.* **61**, 2059–2062.
79. Wang-Johanning F, Frost AR, Johanning GL, Khazaeli MB, LoBuglio AF, Shaw DR, Strong TV. 2001 Expression of human endogenous retrovirus k envelope transcripts in human breast cancer. *Clin. Cancer Res.* **7**, 1553–1560.
80. Wang-Johanning F, Frost AR, Jian B, Epp L, Lu DW, Johanning GL. 2003 Quantitation of HERV-K env gene expression and splicing in human breast cancer. *Oncogene* **22**, 1528–1535. (doi:10.1038/sj.onc.1206241)
81. Buscher K *et al.* 2006 Expression of the human endogenous retrovirus-K transmembrane envelope, Rec and Np9 proteins in melanomas and melanoma cell lines. *Melanoma Res.* **16**, 223–234. (doi:10.1097/01.cmr.0000215031.07941.ca)
82. Contreras-Galindo R, Kaplan MH, Markovitz DM, Lorenzo E, Yamamura Y. 2006 Detection of HERV-K(HML-2) viral RNA in plasma of HIV type 1-infected individuals. *AIDS Res. Hum. Retroviruses* **22**, 979–984. (doi:10.1089/aid.2006.22.979)
83. Contreras-Galindo R, Lopez P, Velez R, Yamamura Y. 2007 HIV-1 infection increases the expression of human endogenous retroviruses type K (HERV-K) *in vitro*. *AIDS Res. Hum. Retroviruses* **23**, 116–122. (doi:10.1089/aid.2006.0117)
84. Wang-Johanning F *et al.* 2007 Expression of multiple human endogenous retrovirus surface envelope proteins in ovarian cancer. *Int. J. Cancer* **120**, 81–90. (doi:10.1002/ijc.22256)
85. Contreras-Galindo R *et al.* 2008 Human endogenous retrovirus K (HML-2) elements in the plasma of people with lymphoma and breast cancer. *J. Virol.* **82**, 9329–9336. (doi:10.1128/JVI.00646-08)
86. Frank O, Verbeke C, Schwarz N, Mayer J, Fabarius A, Hehlmann R, Leib-Mosch C, Seifarth W. 2008 Variable transcriptional activity of endogenous retroviruses in human breast cancer. *J. Virol.* **82**, 1808–1818. (doi:10.1128/JVI.02115-07)
87. Contreras-Galindo RA *et al.* 2011 Characterization of human endogenous retroviral elements in the blood of HIV-1-infected individuals. *J. Virol.* **86**, 262–276. (doi:10.1128/JVI.00602-11)
88. SenGupta D *et al.* 2011 Strong human endogenous retrovirus-specific T cell responses are associated with control of HIV-1 in chronic infection. *J. Virol.* **85**, 6977–6985. (doi:10.1128/JVI.00179-11)
89. Rakoff-Nahoum S *et al.* 2006 Detection of T lymphocytes specific for human endogenous retrovirus K (HERV-K) in patients with seminoma. *AIDS Res. Hum. Retroviruses* **22**, 52–56. (doi:10.1089/aid.2006.22.52)
90. Tandon R *et al.* 2011 Identification of human endogenous retrovirus-specific T cell responses in vertically HIV-1-infected subjects. *J. Virol.* **85**, 11 526–11 531. (doi:10.1128/JVI.05418-11)
91. Garrison KE *et al.* 2007 T cell responses to human endogenous retroviruses in HIV-1 infection. *PLoS Pathog.* **3**, e165. (doi:10.1371/journal.ppat.0030165)
92. Hahn S, Ugurel S, Hanschmann KM, Strobel H, Tondera C, Schadendorf D, Lower J, Lower R. 2008 Serological response to human endogenous retrovirus K in melanoma patients correlates with survival probability. *AIDS Res. Hum. Retroviruses* **24**, 717–723. (doi:10.1089/aid.2007.0286)
93. Boller K, Janssen O, Schuldes H, Tonjes RR, Kurth R. 1997 Characterization of the antibody response specific for the human endogenous retrovirus HTDV/HERV-K. *J. Virol.* **71**, 4581–4588.
94. Dewannieux M, Blaise S, Heidmann T. 2005 Identification of a functional envelope protein from the HERV-K family of human endogenous retroviruses. *J. Virol.* **79**, 15 573–15 577. (doi:10.1128/JVI.79.24.15573-15577.2005)
95. Paprotka T *et al.* 2011 Recombinant origin of the retrovirus XMRV. *Science* **333**, 97–101. (doi:10.1126/science.1205292)
96. Young GR, Eksmond U, Salcedo R, Alexopoulou L, Stoye JP, Kassiotis G. 2012 Resurrection of endogenous retroviruses in antibody-deficient mice. *Nature* **491**, 774–778. (doi:10.1038/nature11599)
97. Stoye JP. 2001 Endogenous retroviruses: still active after all these years? *Curr. Biol.* **11**, R914–R916. (doi:10.1016/S0960-9822(01)00553-X)
98. Armbruester V, Sauter M, Krautkraemer E, Meese E, Kleiman A, Best B, Roemer K, Mueller-Lantzsch N. 2002 A novel gene from the human endogenous retrovirus K expressed in transformed cells. *Clin. Cancer Res.* **8**, 1800–1807.
99. Galli UM, Sauter M, Lecher B, Maurer S, Herbst H, Roemer K, Mueller-Lantzsch N. 2005 Human endogenous retrovirus *rec* interferes with germ cell development in mice and may cause carcinoma *in situ*, the predecessor lesion of germ cell tumors. *Oncogene* **24**, 3223–3228. (doi:10.1038/sj.onc.1208543)
100. Boese A, Sauter M, Galli U, Best B, Herbst H, Mayer J, Kremmer E, Roemer K, Mueller-Lantzsch N. 2000 Human endogenous retrovirus protein cORF supports cell transformation and associates with the promyelocytic leukemia zinc finger protein. *Oncogene* **19**, 4328–4336. (doi:10.1038/sj.onc.1203794)
101. Denner J, Wunderlich V, Bierwolf D. 1986 Suppression of human lymphocyte mitogen response by proteins of the type-D retrovirus PMFV. *Int. J. Cancer* **37**, 311–316. (doi:10.1002/ijc.2910370221)
102. Mathes LE, Olsen RG, Hebebrand LC, Hoover EA, Schaller JP. 1978 Abrogation of lymphocyte blastogenesis by a feline leukaemia virus protein. *Nature* **274**, 687–689. (doi:10.1038/274687a0)
103. Mathes LE, Olsen RG, Hebebrand LC, Hoover EA, Schaller JP, Adams PW, Nichols WS. 1979 Immunosuppressive properties of a virion polypeptide, a 15,000-dalton protein, from feline leukemia virus. *Cancer Res.* **39**, 950–955.
104. Mangeney M, Heidmann T. 1998 Tumor cells expressing a retroviral envelope escape immune rejection *in vivo*. *Proc. Natl Acad. Sci. USA* **95**, 14 920–14 925. (doi:10.1073/pnas.95.25.14920)
105. Denner J. 2010 Immunosuppressive properties of endogenous retroviruses. In *Retroviruses: molecular biology, genomics and pathogenesis* (eds K Reinhard, N Bannert), pp. 41–45. Norfolk, UK: Caister Academic Press.
106. Rosenberg N, Jolicoeur P. 1997 Retroviral pathogenesis: oncogenesis. In *Retroviruses* (eds JM Coffin, SH Hughes, HE Varmus), pp. 475–586. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
107. Lamprecht B *et al.* 2010 Derepression of an endogenous long terminal repeat activates the CSF1R proto-oncogene in human lymphoma. *Nat. Med.* **16**, 571–579. (doi:10.1038/nm.2129)
108. Kudo-Saito C, Shirako H, Takeuchi T, Kawakami Y. 2009 Cancer metastasis is accelerated through immunosuppression during Snail-induced EMT of cancer cells. *Cancer Cell* **15**, 195–206. (doi:10.1016/j.ccr.2009.01.023).

109. DeGregori J. 2011 Evolved tumor suppression: why are we so good at not getting cancer? *Cancer Res.* **71**, 3739–3744. (doi:10.1158/0008-5472.CAN-11-0342)
110. Billaud M, Santoro M. 2011 Is co-option a prevailing mechanism during cancer progression? *Cancer Res.* **71**, 6572–6575. (doi:10.1158/0008-5472.CAN-11-2158)
111. Gatenby RA, Gillies RJ. 2008 A microenvironmental model of carcinogenesis. *Nat. Rev. Cancer* **8**, 56–61. (doi:10.1038/nrc2255)
112. Feschotte C. 2008 Transposable elements and the evolution of regulatory networks. *Nat. Rev. Genet* **9**, 397–405. (doi:10.1038/nrg2337)
113. Berthoux L, Sebastian S, Sokolskaja E, Luban J. 2005 Cyclophilin A is required for TRIM5 $\{\alpha\}$ -mediated resistance to HIV-1 in Old World monkey cells. *Proc. Natl Acad. Sci. USA* **102**, 14 849–14 853. (doi:10.1073/pnas.0505659102)
114. Nisole S, Lynch C, Stoye JP, Yap MW. 2004 A Trim5-cyclophilin A fusion protein found in owl monkey kidney cells can restrict HIV-1. *Proc. Natl Acad. Sci. USA* **101**, 13 324–13 328. (doi:10.1073/pnas.0404640101)
115. Sayah DM, Sokolskaja E, Berthoux L, Luban J. 2004 Cyclophilin A retrotransposition into TRIM5 explains owl monkey resistance to HIV-1. *Nature* **430**, 569–573. (doi:10.1038/nature02777)
116. Sato A *et al.* 2010 Association of RNase L with a Ras GTPase-activating-like protein IQGAP1 in mediating the apoptosis of a human cancer cell-line. *FEBS J.* **277**, 4464–4473. (doi:10.1111/j.1742-4658.2010.07833.x)
117. Silverman RH. 2003 Implications for RNase L in prostate cancer biology. *Biochemistry* **42**, 1805–1812. (doi:10.1021/bi021747i)
118. Lower R. 1999 The pathogenic potential of endogenous retroviruses: facts and fantasies. *Trends Microbiol.* **7**, 350–356. (doi:10.1016/S0966-842X(99)01565-6)
119. Herve CA, Lugli EB, Brand A, Griffiths DJ, Venables PJ. 2002 Autoantibodies to human endogenous retrovirus-K are frequently detected in health and disease and react with multiple epitopes. *Clin. Exp. Immunol.* **128**, 75–82. (doi:10.1046/j.1365-2249.2002.01735.x)
120. Shields JD, Kourtis IC, Tomei AA, Roberts JM, Swartz MA. 2010 Induction of lymphoidlike stroma and immune escape by tumors that express the chemokine CCL21. *Science* **328**, 749–752. (doi:10.1126/science.1185837)
121. Belshaw R, Watson J, Katzourakis A, Howe A, Woolven-Allen J, Burt A, Tristem M. 2007 Rate of recombinational deletion among human endogenous retroviruses. *J. Virol.* **81**, 9437–9442. (doi:10.1128/JVI.02216-06)
122. Ostertag EM, Goodier JL, Zhang Y, Kazazian Jr HH. 2003 SVA elements are nonautonomous retrotransposons that cause disease in humans. *Am. J. Hum. Genet.* **73**, 1444–1451. (doi:10.1086/380207)
123. Amanuma H, Katori A, Obata M, Sagata N, Ikawa Y. 1983 Complete nucleotide sequence of the gene for the specific glycoprotein (gp55) of Friend spleen focus-forming virus. *Proc. Natl Acad. Sci. USA* **80**, 3913–3917. (doi:10.1073/pnas.80.13.3913)
124. Wolff L, Ruscetti S. 1988 The spleen focus-forming virus (SFFV) envelope gene, when introduced into mice in the absence of other SFFV genes, induces acute erythroleukemia. *J. Virol.* **62**, 2158–2163.
125. Aswad A, Katzourakis A. 2012 Paleovirology and virally derived immunity. *Trends Ecol. Evol.* **27**, 627–636. (doi:10.1016/j.tree.2012.07.007)
126. Mangeney M, Renard M, Schlecht-Louf G, Bouallaga I, Heidmann O, Letzelter C, Richaud A, Ducos B, Heidmann T. 2007 Placental syncytins: genetic disjunction between the fusogenic and immunosuppressive activity of retroviral envelope proteins. *Proc. Natl Acad. Sci. USA* **104**, 20 534–20 539. (doi:10.1073/pnas.0707873105)
127. Murchison EP *et al.* 2012 Genome sequencing and analysis of the Tasmanian devil and its transmissible cancer. *Cell* **148**, 780–791. (doi:10.1016/j.cell.2011.11.065)
128. Murchison EP *et al.* 2010 The Tasmanian devil transcriptome reveals Schwann cell origins of a clonally transmissible cancer. *Science* **327**, 84–87. (doi:10.1126/science.1180616)
129. Murchison EP. 2008 Clonally transmissible cancers in dogs and Tasmanian devils. *Oncogene* **27**(Suppl. 2), S19–S30. (doi:10.1038/onc.2009.350)
130. Strauss DC, Thomas JM. 2010 Transmission of donor melanoma by organ transplantation. *Lancet Oncol.* **11**, 790–796. (doi:10.1016/S1470-2045(10)70024-3)
131. Warrens AN *et al.* 2012 Advising potential recipients on the use of organs from donors with primary central nervous system tumors. *Transplantation* **93**, 348–353. (doi:10.1097/TP.0b013e31823f7f47).
132. Hoffner L, Surti U. 2012 The genetics of gestational trophoblastic disease: a rare complication of pregnancy. *Cancer Genet.* **205**, 63–77. (doi:10.1016/j.cancergen.2012.01.004)
133. Wang X, Fu S, Freedman RS, Liu J, Kavanagh JJ. 2006 Immunobiology of gestational trophoblastic diseases. *Int. J. Gynecol. Cancer* **16**, 1500–1515. (doi:10.1111/j.1525-1438.2006.00539.x)
134. Brinsden PR. 2003 Gestational surrogacy. *Hum. Reprod. Update* **9**, 483–491. (doi:10.1093/humupd/dmg033)
135. Dupressoir A, Lavielle C, Heidmann T. 2012 From ancestral infectious retroviruses to bona fide cellular genes: role of the captured *syncytins* in placentation. *Placenta* **33**, 663–671. (doi:10.1016/j.placenta.2012.05.005)
136. Gayer G. 2012 Images in clinical medicine. Abdominal ectopic pregnancy. *N. Engl. J. Med.* **367**, 2334. (doi:10.1056/NEJMimc1111814)
137. Poole A, Haas D, Magann EF. 2012 Early abdominal ectopic pregnancies: a systematic review of the literature. *Gynecol. Obstet. Invest.* **74**, 249–260. (doi:10.1159/000342997)
138. Alazzam M, Tidy J, Osborne R, Coleman R, Hancock BW, Lawrie TA. 2012 Chemotherapy for resistant or recurrent gestational trophoblastic neoplasia. *Cochrane Database Syst. Rev.* **12**, CD008891. (doi:10.1002/14651858.CD008891.pub2)
139. Cornelis G, Heidmann O, Bernard-Stoecklin S, Reynaud K, Veron G, Mulot B, Dupressoir A, Heidmann T. 2012 Ancestral capture of *syncytin-Car1*, a fusogenic endogenous retroviral envelope gene involved in placentation and conserved in Carnivora. *Proc. Natl Acad. Sci. USA* **109**, E432–E441. (doi:10.1073/pnas.1115346109)
140. Dupressoir A, Marceau G, Vernochet C, Benit L, Kanellopoulos C, Sapin V, Heidmann T. 2005 *Syncytin-A* and *syncytin-B*, two fusogenic placenta-specific murine envelope genes of retroviral origin conserved in Muridae. *Proc. Natl Acad. Sci. USA* **102**, 725–730. (doi:10.1073/pnas.0406509102)
141. Heidmann O, Vernochet C, Dupressoir A, Heidmann T. 2009 Identification of an endogenous retroviral envelope gene with fusogenic activity and placenta-specific expression in the rabbit: a new “*syncytin*” in a third order of mammals. *Retrovirology* **6**, 107. (doi:10.1186/1742-4690-6-107)
142. Kwak-Kim J. 2012 Clinical reproductive immunology in critical demand. *Am. J. Reprod. Immunol.* **67**, 267. (doi:10.1111/j.1600-0897.2012.01125.x)
143. Coulam CB, Acacio B. 2012 Does immunotherapy for treatment of reproductive failure enhance live births? *Am. J. Reprod. Immunol.* **67**, 296–304. (doi:10.1111/j.1600-0897.2012.01111.x)
144. Bansal AS, Bajardeen B, Thum MY. 2012 The basis and value of currently used immunomodulatory therapies in recurrent miscarriage. *J. Reprod. Immunol.* **93**, 41–51. (doi:10.1016/j.jri.2011.10.002)
145. Tang AW, Alfirevic Z, Quenby S. 2011 Natural killer cells and pregnancy outcomes in women with recurrent miscarriage and infertility: a systematic review. *Hum. Reprod.* **26**, 1971–1980. (doi:10.1093/humrep/der164)
146. Brown GK, Kreiss A, Lyons AB, Woods GM. 2011 Natural killer cell mediated cytotoxic responses in the Tasmanian devil. *PLoS ONE* **6**, e24475. (doi:10.1371/journal.pone.0024475)
147. Moyes D, Griffiths DJ, Venables PJ. 2007 Insertional polymorphisms: a new lease of life for endogenous retroviruses in human disease. *Trends Genet.* **23**, 326–333. (doi:10.1016/j.tig.2007.05.004)
148. Christensen T. 2005 Association of human endogenous retroviruses with multiple sclerosis and possible interactions with herpes viruses. *Rev. Med. Virol.* **15**, 179–211. (doi:10.1002/rmv.465)
149. Baudino L, Yoshinobu K, Morito N, Santiago-Raber ML, Izui S. 2010 Role of endogenous retroviruses in murine SLE. *Autoimmun. Rev.* **10**, 27–34. (doi:10.1016/j.autrev.2010.07.012)
150. Balada E, Vilardell-Tarres M, Ordi-Ros J. 2010 Implication of human endogenous retroviruses in the development of autoimmune diseases. *Int. Rev. Immunol.* **29**, 351–370. (doi:10.3109/08830185.2010.485333)
151. Blank M, Shoenfeld Y, Perl A. 2009 Cross-talk of the environment with the host genome and the immune system through endogenous retroviruses in systemic lupus erythematosus. *Lupus* **18**, 1136–1143. (doi:10.1177/0961203309345728)

152. Stauffer Y, Marguerat S, Meylan F, Ucla C, Sutkowski N, Huber B, Pelet T, Conrad B. 2001 Interferon-alpha-induced endogenous superantigen. A model linking environment and autoimmunity. *Immunity* **15**, 591–601. (doi:10.1016/S1074-7613(01)00212-6)
153. Ruprecht K, Gronen F, Sauter M, Best B, Rieckmann P, Mueller-Lantzsch N. 2008 Lack of immune responses against multiple sclerosis-associated retrovirus/human endogenous retrovirus W in patients with multiple sclerosis. *J. Neurovirol.* **14**, 143–151. (doi:10.1080/13550280801958922)
154. Wiik AS. 2005 Anti-nuclear autoantibodies: clinical utility for diagnosis, prognosis, monitoring, and planning of treatment strategy in systemic immunoinflammatory diseases. *Scand. J. Rheumatol.* **34**, 260–268. (doi:10.1080/03009740500202664)
155. Satoh M *et al.* 2012 Prevalence and sociodemographic correlates of antinuclear antibodies in the United States. *Arthritis Rheum.* **64**, 2319–2327. (doi:10.1002/art.34380)
156. Villesen P, Aagaard L, Wiuf C, Pedersen FS. 2004 Identification of endogenous retroviral reading frames in the human genome. *Retrovirology* **1**, 32. (doi:10.1186/1742-4690-1-32)
157. Cornelis G *et al.* 2013 Captured retroviral envelope *syncytin* gene associated with the unique placental structure of higher ruminants. *Proc. Natl Acad. Sci. USA* **110**, E828–E837. (doi:10.1073/pnas.1215787110)
158. Lavielle C, Cornelis G, Dupressoir A, Esnault C, Heidmann O, Vernochet C, Heidmann T. 2013 Paleovirology of 'syncytins', retroviral *env* genes exapted for a role in placentation. *Phil. Trans. R. Soc. B* **368**, 20120507. (doi:10.1098/rstb.2012.0507)
159. Duggal NK, Emerman M. 2012 Evolutionary conflicts between viruses and restriction factors shape immunity. *Nat. Rev. Immunol.* **12**, 687–695. (doi:10.1038/nri3295)