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Minireview

Perpetually mobile footprints of ancient infections in human genome

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Abstract Up to 1% of the human genome is represented by human endogenous retroviruses (HERVs) and their fragments that are likely footprints of ancient primate germ-cell infections by retroviruses that occurred 10–60 million years ago. HERV solitary long terminal repeats (LTRs) can be often met in close vicinity to functional genes. The LTRs comprise a set of regulatory sequences like promoters, enhancers, hormone responsive elements and polyadenylation signals that might come out as new regulatory signals to resident genes and thus change their regulation in evolution. Moreover, the LTRs have a potential for chromatin remodeling that can also modulate gene expression. This review describes the integration specificity and distribution of the HERVs and LTRs in the human genome and discusses possible functional consequences of their integration in the vicinity of genes.

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Key words: Human endogenous retrovirus; Long terminal repeat; Transposable element; Retrotransposon; Regulation; Evolution; Human genome; Mapping; Transcription; Repression; Methylation

1. Introduction

The great progress in the Human Genome Project opens prospects of integral vision of the whole genome structural organization and functional interplay of its elements. In particular, there is hope that new approaches and techniques will offer a much deeper insight into the role of DNA transposable elements (TEs) that occupy 35% (!) of the human genome [1]. Until recently the interplay of retroelements and genomes could be studied for just a few TEs that more or less accidentally came into the view of researchers. Currently more profound though far from comprehensive knowledge is rapidly accumulating. Even this fragmented information uncovers many interesting things, allowing one to hypothesize about a more general picture of the role and function of these genome argonauts, their ancestry and their involvement in the genome evolution, regulation, and pathology. In this Minireview I am going to focus on one of the TE types, human endogenous retroviruses (HERV), location of their long terminal repeats (LTRs) in the genome and their possible role in gene regulation.

2. After-effects of ancient viral infections

Up to 1% of the human genome likely represent footprints of ancient germ-cell infections by retroviruses [2–6]. The HERVs and their parts are related to primate and murine retroviruses and they are the most sophisticated entities among different TEs (Fig. 1). Similar to abundant interspersed repeats of *Alu* or *LINE* types, the HERVs are retroelements that spread throughout genomes by a process termed retroposition, involving transcription of active element(s) residing in the genome, reverse transcription of the RNA intermediate formed and insertion of the cDNA copy into a new genomic site. Since the original TE is not eliminated in this process, retroposition leads to the expansion of the repeats in the genome.

Various in primary structures and abundance, HERVs are thought to have been inserted into the germline at different times between <10 and 60 million years ago [5,6]. Along with near full-length HERV elements, the HERV superfamily includes also solitary HERV LTRs with no retroviral genes attached. As a rule, the number of these solo LTRs is considerably higher than that of their full-length counterparts. For example, one of the HERV families, HERV-K, comprises as little as 30–50 near full-size members per haploid genome in humans, whereas HERV-K-related LTRs have been estimated to be present in about 10 000–25 000 copies [5].

Some HERVs are transcriptionally active and although genomes of many HERVs are corrupted by termination codons, deletions or frame shift mutations, recent studies reveal HERV protein expression or virus-like particle formation. HERVs as well as other TEs can be considered as specialized intragenomic parasites whose jumps over the genome can cause a multitude of genetic variations, ranging from minor changes in tissue specificity of the gene expression to dramatic alterations in development (reviewed in [1–11]). The newly inserted elements mostly cause deleterious effects including hereditary diseases due to insertional mutations. However, sometimes the hosts exploit the capacity of TEs to generate variations for their own benefit. The retroelements can come out as traveling donors of sequence motifs for nucleosome positioning, DNA methylation, transcriptional enhancers, poly(A) addition sequences, splice sites, and even amino acid codons for incorporation into open reading frames of encoded proteins [8,9].

The number of described cases in which retroelement sequences confer useful traits to the host is growing [11–13]. Retropositions can therefore be considered as a major pacemaker of the evolution that continues to change our genomes [14–16]. In particular, HERV elements could interact with human genome through (i) expression of retroviral genes, (ii) human genome loci rearrangement following the retroposition of the HERVs or (iii) the capacity of LTRs to regulate nearby genes [2–5]. A plethora of solitary LTRs comprises a variety of transcription regulatory elements, such as promoters, enhancers, hormone-responsive elements, and poly-

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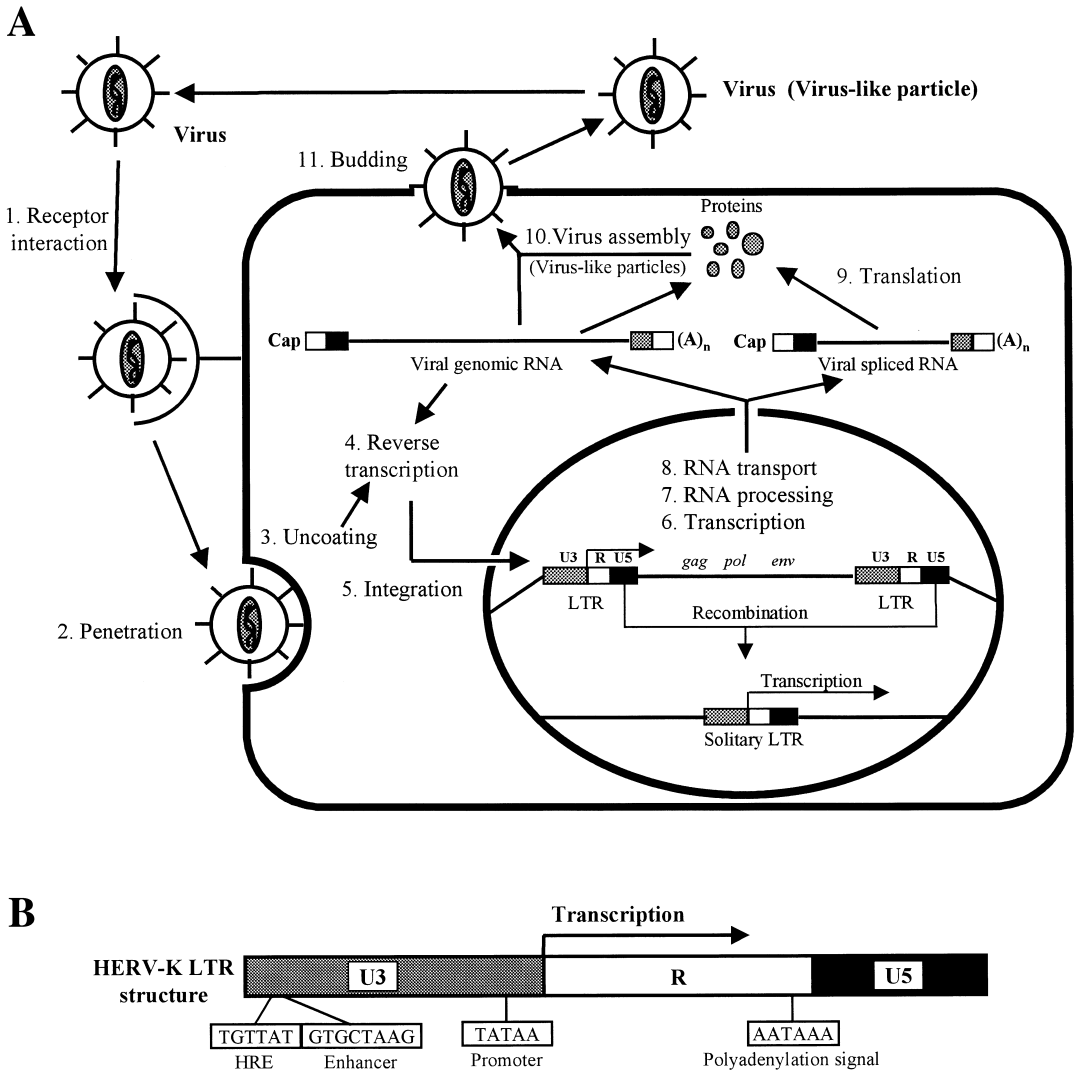

Fig. 1. A: Life cycles of retroviruses and endogenous retroviruses. Stages 1, 2 and 3 are characteristic of retroviruses, other stages are common for both types of retroelements. The recombination between two LTRs of an endogenous retrovirus leading to the formation of a solo LTR is depicted. B: Scheme of a HERV-K LTR with positions of putative hormone responsive element (HRE), enhancer, TATA box and polyadenylation signal. The arrow marks the transcription start point.

adenylation signals. Therefore the LTRs are potentially able to cause significant changes in expression patterns of neighboring genes.

3. HERV solo LTRs most probably appear due to recombinational excision of the HERV genomes

The history of TE appearance and amplification in the genome is reflected in particular in the existence of distinct subgroups within families of related retroelements. The members of the subgroups share common structural features and can be distinguished from those of other subgroups by characteristic (diagnostic) differences in their sequences [17–20]. To explain the existence of the subgroups, in the case of *Alu* or *LINE* repetitive elements it was suggested that the majority of the members of these families are incapable of duplicating and only a small number of them, referred to as master or source genes, is being actively amplified [17–20]. According to this hypothesis, each subfamily of a retroposon family is a generation of a specific master gene. New subfamilies are formed when master genes are changed giving rise to a multitude of mutated master gene(s) copies. The subfamilies were also described for endogenous retroviruses HERV-H [21] and their LTRs [22] as well as for HERV-K [23] and HERV-K LTRs [24,25]. No doubt that more detailed analysis will reveal similar subdivisions for other families of HERVs. It was proposed that LTRs are formed through retrotransposition of nearly full-size HERVs that were transcribed, spliced, reverse transcribed, and reinserted into the genome [5,26,27]. Subsequent homologous recombinations between the LTRs of the newly appeared endogenous retrovirus result in the deletion of the viral genes and leave solitary LTRs. There are good examples in favor of such a mechanism [27,28]. This model implies that the master genes of LTRs are functional HERVs having potentials for transcription, synthesis of cDNA, containing two fully functional LTRs and capable of forming functional integration complex. The model looks attractive, however, other mechanisms implicated in the LTR retroposition should not be ruled out [5].

4. De novo integration selectivity of TEs: pre-evolutional opportunities

It would be natural to believe that retroposons randomly jumped into any site where they could use their integration machinery, and then the evolution selected the subsets of primary integrants characteristic of the hosts that did not suffer from the invasions or even benefited by them. But lengthy coexistence of master elements and host genomes could sometimes elaborate more sophisticated TE-genome relations facilitating better maintenance of viable TE populations at the expense of retropositions. Transposons could possibly maintain an active form in the host genome for millions of years due to coadaptation with the genome, diminishing deleterious effects of fresh integrations by means of specific integration site choice. Integration targeted at most painless genomic loci such as non-coding regions, untranslated regions (UTRs), and introns of genes as well as intergenic space was proposed to be a widespread strategy adopted by retroelements to successfully proliferate within the host genome [9,29].

Another way to diminish deleterious changes in the host genome are the reiterative insertions into or next to pre-existing retroelements [9]. In such a way the newly inserted TEs remain phenotypically invisible. The extent of reiterative integration into pre-existing elements is sometimes very impressive. For example, over 50% of the genome in maize is represented by retroelements [9]. However, the maize genome was not knocked out because highly repetitive elements were mostly targeted at regions between genes and a multitude of them was nested within other elements [9,30]. In this regard it is worth mentioning that different retroelements like *Alu* or LTRs may affect nucleosome positioning in the neighboring regions [31,32]. The nucleosome positioning information is perhaps intrinsic to these elements and may code the sites of de novo retroelement integration. For instance, HIV retrovirus frequently integrates directly into or within one nucleosome distance from *L1H* or *Alu* repetitive elements [33,34]. Another reason for reiterative insertions might be that different retroelements can consecutively use one and the same feature of the host chromatin structure [35]. Frequent coincidences of HERVs and their LTRs with *Alu* and *LINE* repeats in human genome were noticed [5,24,28,36], probably reflecting the bias in their selectivity similar to retroviruses.

The studies of de novo integration specificity may help to better understand the intimate TE-genome interactions. Although not available for HERVs at this point, the results for other retroelements demonstrate that transcriptionally active genome regions might be preferred targets for retrovirus integration [37] and that the site selection during retroposition can be influenced by many factors, including transcriptional status of DNA region, DNA methylation, association of DNA with histones or other proteins, DNA bending, nucleosome positioning, peculiarities of retroelement integration machinery and its interaction with the host cell factors [33,35,38–46]. A good example of retroelement–host interaction gives the study of de novo insertions of Ty1 [47] and Ty3 [48] yeast retrotransposons that are analogues of endogenous retroviruses. Most of the integration sites were found clustered upstream of the genes transcribed by RNA polymerase III. The specificity of Ty3 integration was shown to be governed by the interaction of the Ty3 integration complex with some components of the RNA-polymerase III transcription machinery.

Ty transposons reflect important properties of their viral relatives, striving for transcriptionally active regions to insert their cDNA copies [37]. This is not surprising considering that the retroviral genes integrated in the genome need the host transcriptional machinery to express themselves. But although this concept of retrovirus selectivity is currently prevailing [37], practically all genomic regions were reported to be used as primary integration targets, however, with different preferences. There were identified 'hot spots' containing integration sites used up to 280 times more frequently than predicted mathematically [41,43]. A recent study of the de novo retroviral integration demonstrated also preference for scaffold- or matrix-attachment regions (S/MARs) flanked by DNA with high bending potential [35]. The S/MARs are thought to be important functional sequences of the genome that anchor chromatin loops to the nuclear matrix subdividing the genome into functional domains. They often neighbor regulatory elements involved in gene expression and DNA replication.

A cautious generalization from these findings could be that although TEs can integrate into many sites and may prefer non-coding regions, the de novo integration is frequently targeted at the sites in the vicinity of functionally important elements like transcription start points or origins of replication.

5. Can the genome somehow mitigate the effects of TEs or are most mutant germ cells and zygotes doomed to die?

However merciful toward host genomes HERVs may be, their integration into transcriptionally active loci introduces extensive and qualitatively new regulatory information capable of dramatically changing transcription specificity. If only a small proportion of human transposons had a capacity for retropositions, they would form a huge depot of intragenomic insertional mutagens. Many TEs can transpose at high rate when cloned copies are put into host cells [16] and therefore retain a dangerous potential to the genome integrity. However, the observed hazardous insertional events seem to be rather rare: only 1 of 500 new germ line mutations is due to TE insertions in humans [1]. This low value can be explained in several not mutually exclusive ways.

1. Newly transposed HERVs can be inactivated by mutations. The value of the mutation rate (reviewed in [49]), 0.13–0.16% per one million years, was accepted by Britten [50] for mutations in the *Alu* family of retroelements. We have estimated the mutation rate in HERV-K LTRs [25] as 0.26–0.3% and in [22] the rate for HERV-H LTRs was assumed to be 0.2% per one million years. It is not known how many mutations are required to kill the TE ability for retropositions. Nevertheless, the figures above clearly show that the rate of mutation is too low to quickly destroy dangerous TEs. Therefore some immediate first aid to the genome must exist.
2. TE transcription can be repressed. Methylation of CpG sequences within TEs was suggested to play a major role in the repression of their expression and therefore retroposition [1,51,52]. It was reported that 5-methylcytosines in mammalian DNA mostly reside within TE sequences. Such an observation even led Yoder et al. [1] to the hypothesis that suppression of TEs may be the primary

function of cytosine methylation that evolved in mammals as a host defense mechanism. Indeed, methylation is known to play a crucial role in the regulation of gene expression [53], and there are data on suppression of the transcription of some TEs after methylation [51,52,54]. But here there is a serious inconsistency that can undermine the whole idea of the role of methylation in the host defense against TEs [55]. The methylation status of TEs during embryo development is contrary to what should be expected if it was targeted at TE retroposition repression. DNA is least methylated in germ cells and at early stages of embryo development, when transcription of TEs is known to be significant. Only at some stages of the postimplantation period become TEs methylated de novo and their transcription comes down. Therefore it seems unlikely that methylation can completely prevent transposition in the germ line where playing with genetic material is most dangerous for the descendants. Further research is needed to resolve this problem. On the other hand, methylation of TEs seems sufficient to protect somatic cells from mutations due to transpositions.

3. The repression can result from low efficiency of posttranscriptional stages of retropositions. There are no data evaluating this efficiency.
4. There could be a mechanism for excision of most newly inserted TE copies.
5. The simplest explanation is, however, just either inefficiency of mature gamete formation from insertionally mutated germ cells or death of the zygotes formed by these mutant gametes. This would make the repression of the transposition or sophisticated processes of genome repair after the insertion unnecessary.

But in this or other ways the genome has to endure the invaders. Let us consider the distribution of integration sites of TEs in genomes that may be pertinent to the problem.

6. Location of the HERV LTRs in genomes: post-evolutional patterns

I will consider two aspects of the distribution of HERV LTRs in the genome.

6.1. How are HERV LTRs distributed in genomes if considered independent of gene location?

The data on physical distribution of different HERVs and their LTRs in the human genome are not exhaustive. Neither preferred chromosomes nor their particular regions were revealed for the integration of RTVL-H2 LTRs and HERV-A [56], though some clusters of the elements seem to exist on chromosomes 1p and 7q [57]. 25 000 copies of HERV-K-related LTRs were reported to be unevenly distributed over most human chromosomes [5]. A similar uneven distribution was also reported for HERV-K10 sequences [58]. Preferential localization of the low-copy number ERV3 related HERV on Y-chromosome was described [59]. The resources developed by the Human Genome Project enabled us to precisely map HERV-K LTRs on human chromosome 19 [25,60]. There was no correlation between physical neighborhood of the LTRs on the map and the level of their identity. In most cases the LTRs with a high level of identity, adjacent on the nearest-neighbor dendrogram, were located far apart on the chromosome, sometimes even on different chromosome arms. It means that the LTRs were transposed according to the 'shotgun' principle, jumping into any region of the chromosome, rather than by means of a step by step movement along the chromosome, starting from the point of insertion of a predecessor master LTR. Such random jumping is in contrast to the transposition manner of some known DNA transposons [61] that transposed preferentially to nearby chromosomal sites. Random transpositions into different chromosome areas seem to be in conflict with the above mentioned unbalanced chromosomal distribution of HERV-K LTRs [5,58]. This contradiction remains to be explained.

6.2. How are HERV LTRs positioned relative to genes?

The complete sequence of the yeast genome was used to precisely determine positions of the Ty1–Ty5 retrotransposons with respect to genes [29,62,63]. Generally, the Ty1–Ty4 insertions were found upstream of transcription start sites of *Pol*III transcribed genes, whereas Ty5 demonstrated the bias to origins of DNA replication at the telomers and the silent mating locus [63]. Since no more than 5–10% of the estimated total number of human genes is mapped at the chromosome level, it is presently impossible to comprehensively analyze the neighborhood of LTRs and the genes. However, some LTRs and HERVs were shown to be located near or within gene loci [12,13,36,64–67]. We found frequent coincidences in positions of HERV-K LTRs and mapped genes on human chromosome 19 [68] where the situation with mapped genes is slightly better. Although it would be premature to interpret this result as the indication of the regulatory interplay between closely located LTRs and genes, still some of the coincidences seem interesting. Most striking is the frequent coincidence of the LTRs with Zn-finger or Zn-finger-like genes scattered all over the chromosome. The implication of retroviral sequences in expression of Zn-finger genes was described [69,70]. The participation of retroviral regulatory elements in the expression of the Zn-finger genes could thus be a rather common event. Recently a new human Zn-finger gene was also shown to contain an endogenous retroviral sequence [36]. Among other interesting coincidences, the LTRs were often detected in the vicinity of a number of genes (*RRAS, EPOR, JAK3* etc.) implicated at different stages of Jak-Stat signal transduction pathway. The frequent coincidences of the LTRs with the genes of similar or concerted functions might suggest either functional involvement of the LTRs in the expression of the genes or their evolutionary relations.

7. What mechanisms can be used by HERV LTRs in the gene regulation?

HERV LTRs can modulate the gene expression directly as transcription regulatory signals or indirectly by means of chromatin remodeling at the sites of integration.

Possible involvement of HERV LTRs in transcription regulation of cellular downstream genes has been described [12,13,69–74]. There were reports on HERV germ line insertions that changed tissue specificity of the expression of human genes [13,73], though this kind of data should be assessed with caution. For example, it was believed that the integration of an endogenous retrovirus into the 5′-flanking region of the human amylase gene ensured its expression additionally in the human salivary gland, apart from the pancreas where this gene is normally expressed [73]. But more

detailed analysis [75] has revealed that the retroelement is not required for amylase transcription in the primate salivary gland. However, this example in no way excludes the possibility of a strong direct effect of HERVs on nearby gene expression.

HERV LTRs might also affect the transcription regulation through chromatin remodeling as in the case of their relative, mouse mammary tumor virus (MMTV) promoter. Transcriptionally inactive MMTV LTRs as well as *Alu* repeat elements [31] dictate nucleosome phasing [32,76–79] in the way preventing the access of transcription factors to their cognate sites. The association of the glucocorticoid receptor with its target in the LTR bound to core histones leads to nucleosome unraveling and transcription. In general, nucleosome positioning is believed to play an important role in the regulated transcription of eukaryotic genes. The insertion of sequences capable of locally changing chromatin structure can disturb delicate functioning of regulatory factors and affect gene regulation without direct involvement in the process of transcription. The chromatin structure at the sites of the LTR integration remains remodeled irrespective of the LTR methylation status [53,80].

8. Could HERV LTRs influence regulation of more than just neighboring genes?

We do not know how important the involvement of the LTRs is in genome functioning. What we do know is that some elements within the LTRs are highly conserved in evolution [25]. These include in particular TATA box, polyadenylation signal, borders between the R and U3 and U5 regions. There probably exists a kind of selection protecting the elements from mutational erosion. Similar conservatism was also observed for *Alu* repeats [17]. It supports the idea that the LTRs (and perhaps other TEs) are of importance for some genomic purposes. At the present stage of our knowledge we can only guess what these purposes are, but they can probably extend beyond just regulation of particular genes. In this connection let us remember the frequent location of the LTRs in the vicinity of Zn-finger genes. Many Zn-finger proteins function as transcriptional regulators, so changes in their own regulation can have an impact upon expression of multitudes of other genes. In this way effects of the LTRs on regulatory genes might be of importance for the whole genome. Similar global effects of the LTRs can be expected in the case of genes involved in the regulation of cell life, such as signal transduction exemplified above by Jak-Stat pathway.

9. Conclusion

The limited information available at present does not allow us to answer most of the questions put in this review. The whole human genome sequenced and compared to the genomes of other mammals will hopefully tell us the history of the genome invasion by HERVs and LTRs and their subsequent migration, in exactly the same way as, for example, DNA analysis of various human populations uncovered time and place of habitat of 'mitochondrial Eva' and 'Adam' and migration of their descendants all over the earth. And perhaps we will learn about the tragic viral epidemics that struck our ancestors and forever changed their destinies having given rise to *Homo sapiens* lineage in evolution.

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