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Only those genes of the KIAA1245 gene subfamily that contain HERV(K) LTRs in their introns are transcriptionally active

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Abstract

Insertion of LTRs into some genome locations might seriously affect regulation of the neighboring genes expression. This hypothesis is widely accepted but, however, not confirmed directly. Earlier, we have identified a family of closely related genes highly similar to the KIAA1245 mRNA counterpart. This family included a subfamily of genes some of which contained and the others lacked an LTR in their structure. We compared transcription of several closely related genes of the subfamily differing in the presence or absence of LTRs. Only LTR-containing genes were transcribed in transformed cell lines, tumorous and embryonic human tissues, whereas LTR-lacking genes remained silent. Since the genes were in the same intracellular microenvironment, we suggested that this effect was most probably due to intrinsic *cis*-characteristics of integrated LTRs and confirmed this by demonstrating high enhancer activity of KIAA1245 LTRs. The expression of the LTR-containing genes in embryonic tissues might suggest their involvement in evolutionary events during primate speciation.
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Introduction

Retroelements (REs) that replicate and transpose through RNA intermediates are the only known transpositionally active group of transposable elements in mammals. In vertebrates, REs occupy up to 30–40% of genetic information (Weiner et al., 1986). REs can affect genome properties in many ways including numerous host DNA rearrangements due to recombination events (Kazazian and Moran, 1998; Lei and Vorechovsky, 2005), transductions of 5′ or 3′ RE flanking sequences into new genomic loci, by creating pseudogenes (Boeke and Stoye, 1997; Brosius, 1999; Elrouby and Bureau, 2001; Goodier et al., 2000) or by causing RNA recombinations (Lei and Vorechovsky, 2005). Being mobile carriers of transcription regulatory elements, REs can affect expression of many host genes by providing transcriptional regulatory signals. Furthermore, recently expanded gene classes, such as those involved in immunity or response to external stimuli, have transcripts enriched in REs, whereas REs are excluded from mRNAs of highly conserved genes with basic functions in development or metabolism. These data, although indirectly, support the view that REs played a significant role in the diversification and evolution of mammalian genes (van de Lagemaat et al., 2003).

The potential ability to affect gene regulation, in particular those genes involved in embryo development, makes REs likely candidates for taking part in speciation processes (Hedges and Batzer, 2005; Kazazian, 2004; Sverdlov, 1998, 2000). In particular, REs might well be at least partly responsible for phenotypic differences between *Homo sapiens* and its closest relatives, *Pan paniscus* and *Pan troglodytes* chimpanzee species (Kazazian, 2004; Mayer and Meese, 2005; Sverdlov, 2000). These differences can be suggested to arise not only from changes in gene content but also from variations in regulation of some genes common to the related species (Han and Boeke, 2005; King and Wilson, 1975; Sverdlov, 2000). Perhaps, the most likely candidates for such a role are endogenous retroviral

Abbreviations: REs, retroelements; HERV, human endogenous retrovirus; HERV(K), the K family of human endogenous retroviruses; LTR, long terminal repeat; Myr, million of years; Mya, million of years ago; Mb, mega base pairs.

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long terminal repeats (LTRs) that flank retroviral cores in genomic DNA (Bannert and Kurth, 2004; Leib-Mosch and Seifarth, 1995). Their structure harbors functional enhancers, promoters, and polyadenylation signals required for retroviral gene expression (Gifford and Tristem, 2003; Urnovitz and Murphy, 1996). It was recently suggested that LTRs may drive transcription of unique host non-repetitive sequences because 5′-termini of various mRNAs were found to harbor LTR-related sequences (van de Lagemaat et al., 2003).

HERV LTRs were reported to be involved in transcription regulation of cellular downstream genes (Abrink et al., 1998; Di Cristofano et al., 1995; Kowalski and Mager, 1998; Larsson and Andersson, 1998; Samuelson et al., 1996; Ting et al., 1992). There were also reports on HERV germ line insertions that changed tissue specificity of expression of human genes (Pi et al., 2004; Schulte et al., 1996), although this kind of data should be considered with caution. As an example, it was reported that an integration of an endogenous retrovirus into the 5′-flanking region of the human amylase gene led to its expression in the human salivary gland, apart from the pancreas where this gene is normally expressed (Ting et al., 1992). However, a more detailed analysis (Samuelson et al., 1996) revealed that the retroelement was not required for amylase transcription in the primate salivary gland. Of course, this example in no way excludes the possibility of strong direct effect of HERVs on nearby gene expression.

Despite many efforts, the potential of LTRs to change the mode of human gene expression remains mostly hypothetical. It is highly difficult to prove that a gene acquired a new regulatory feature due to an RE integration rather than to accompanying events that escaped the investigator's attention. One of the ways that could help to resolve the problem might be comparative analysis of expression modes of members of one and the same gene family that were recently duplicated in evolution. Being structurally similar, such genes can be different in retroelement integrations. In this case, genes in the same cells with the same set of transcription factors and other possible modulators of gene activity could differ in their functional behavior depending on the presence of REs.

In this report, we describe a study of such a subfamily, part of the KIAA1245 family described by us earlier (Vinogradova et al., 2002). The members of this subfamily are highly similar but some of them carry HERV(K) LTRs in their second intron, whereas the others do not. We demonstrated that only those genes that harbor LTR integrations were transcriptionally active. We also report high enhancer activity of an intronic LTR in a transient expression system.

Results and discussion

Evolutionary tree of the KIAA1245 subfamily members

When studying transcription of HERV(K) LTRs in normal and tumorous tissues, we discovered a transcript that contained an LTR and was highly similar to a fragment of the KIAA1245 mRNA (Nagase et al., 1999). Earlier, an analysis of genomic sequences deposited in GenBank revealed 10 sequences very similar to this mRNA. The sequences were shown to differ in both the degree of identity between the exons and the presence (or absence) of an HERV(K) LTR in the second intron (Vinogradova et al., 2002).

Further analysis revealed quite a number of other sequences in GenBank also similar to different fragments of the KIAA1245 mRNA. For our purposes, we identified two groups of the sequences closely similar but clearly distinct in the region of interest, characterized by the presence or absence of an LTR in the intron between exons 2 and 3. The structure of the region of interest of a typical member of the family is schematized in Fig. 1A.

We demonstrated that this LTR insertion had occurred after the divergence of orangutan from other hominoids but before the divergence of the gorilla lineage, i.e. between 8 and 13 Mya (Vinogradova et al., 2002). An evolutionary analysis of the groups above (see Fig. 1) revealed the existence of at least three extant LTR-containing and two LTR-lacking sequences closely related to the KIAA1245 mRNA (Fig. 1B). The age of the LTR-lacking sequences (AL356957 and AL592309) exceeds 48.3 and 48.8 Mya, respectively. The subgroup of LTR-containing sequences emerged between 8.6 and 48.3 Mya. The youngest AL954711 sequence diverged from the other subgroup sequences not more than 5.8 million years ago.

Differential expression of the LTR-containing and LTR-lacking genes

Below, LTR-containing and LTR-lacking genes should be understood as the members of the KIAA1245 subfamily throughout the text.

We were interested in finding differences among LTR-containing and LTR-lacking genes in their transcriptional activity. To this end, we designed RT-PCR primers of three types (see Fig. 2A and Table 2):

Pr1 and Pr4 primers to amplify mRNAs of all genes of the family, LTR(+),(−).
Pr1 and Pr2 to amplify only LTR-containing members, LTR (+).
Pr1 and Pr3 to selectively amplify only LTR-lacking genes of the family, LTR(−).

The differential primers were designed based on differences in the sequences of the 2nd and 3rd exons that, although conserved, still contain enough differences to allow the design of the required primers.

The primers were targeted at the exon termini bordering the intron as shown in Fig. 2A. Such a choice allowed us to test the specificity of the discriminating primers using total genomic DNA (Fig. 2B). One can see a clear difference in the length of amplicons produced with discriminating primers Pr1–Pr2 and Pr1–Pr3 aimed at distinguishing LTR-containing from LTR-lacking genes, whereas the non-discriminating Pr1–Pr4 primer pair provides the amplification of both types of genes.

Sequencing of the PCR amplification products confirmed that they corresponded to the genes of the KIAA1245

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