

Review

Expression of Retroelements in Mammalian Gametes and Embryos

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Abstract. *Retroelements are genetic mobile elements, expressed during male and female gamete differentiation. Retrotransposons are normally regulated by the methylation machinery, chromatin modifications, non-coding RNAs, and transcription factors, while retrotransposition control is of vital importance in cellular proliferation and differentiation process. Retrotransposition requires a transcription step, by a cellular RNA polymerase, followed by reverse transcription of an RNA intermediate to cDNA and its integration into a new genomic locus. Long interspersed elements (LINEs), human endogenous retroviruses (HERVs), short interspersed elements (SINEs) and SINE-VNTR-Alu elements (SVAs) constitute about half of the human genome, play a crucial role in genome organization, structure and function and interfere with several biological procedures. In this mini review, we discuss recent data regarding retroelement expression (LINE-1, HERVK-10, SVA and VL30) and retrotransposition events in mammalian oocytes and spermatozoa, as well as the importance of their impact on human and mouse preimplantation embryo development.*

Retroelements are genetic mobile elements that constitute 43% of the human and 37% of the mouse genome (1). They have been cited as “junk DNA”, but recent evidence support that they play a crucial role in genome organization, structure, and function during evolution, as they can relocate themselves from one locus to another using an RNA intermediate (2, 3). The retrotransposition process requires a transcription step, by a cellular RNA polymerase, followed by reverse transcription of an RNA intermediate to cDNA and its integration into a new genomic locus (4).

Retroelements are categorized, according to structural and functional criteria, in Long terminal repeats (LTR) and non-LTR as well as in autonomous and non-autonomous. LTR retrotransposons include Human endogenous retroviruses (HERVs), whereas non-LTR retrotransposons contain mainly the classes of Long interspersed elements (LINEs) and Short interspersed elements (SINEs) (5, 6) (Figure 1).

LINEs and HERVs are autonomous retroelements, as they are able to encode reverse transcriptase and proteins necessary for their retrotransposition (7, 8). On the contrary, SVA (SINE-VNTR-Alu) and Alu elements, as non-autonomous, use the LINE-1 protein machinery for their mobilization and relocation (9-11). LINE-1 retroelements move between loci through the target primed reverse transcription (TPRT) mechanism. LINE-1 retrotransposition starts with the binding of RNA polymerase on the promoter of the 5' UTR region and the production of LINE-1 mRNA in the cell nucleus. LINE-1, mRNA moves to the cytoplasm where the ORF1 and ORF2 proteins are expressed. LINE-1 mRNA, ORF1p, and ORF2p are reinserted into the nucleus as a ribonucleoprotein (RNP) complex. ORF2 acts as an endonuclease that breaks the target DNA in the new site of retrotransposons insertion, producing a single stranded edge.

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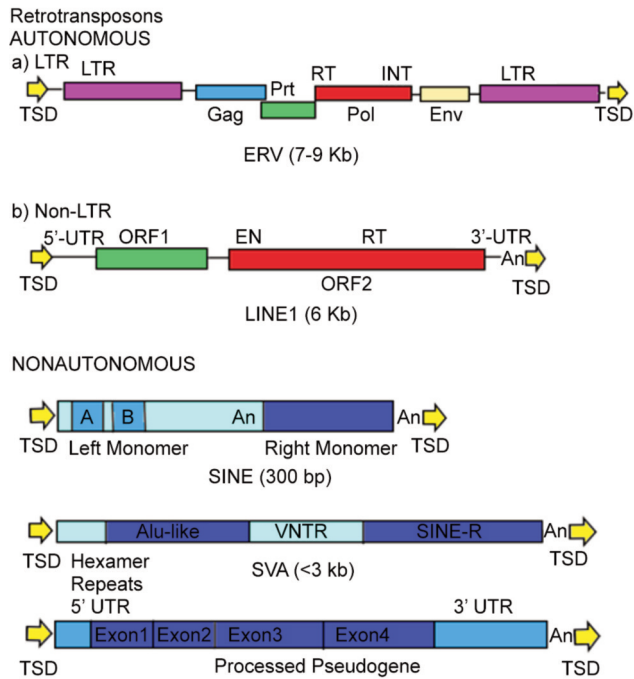


Figure 1. Mammalian retroelements and their nomenclature. TSD: Target site duplications; UTR: untranslated regions; ORF: open reading frames; VNTR: variable number tandem repeats; LTR: long terminal repeats.

The rich in thymine's edge is hybridized with the poly-A region of LINE-1 mRNA. The single strand edge acts as a promoter template for the initiation of reverse transcription and the production of the complementary DNA. At the two ends of the LINE-1 copies there are target site duplications (TSDs) resulting from the duplication of the target site during retrotransposition (12) (Figure 2).

Despite the fact that the human genome is consisted of numerous mobile elements, the majority of them are inactive due to mutations acquired over long evolutionary processes. Nevertheless, several copies of LTR retroelements and LINEs are intact and competent for retrotransposition (13). Due to their capability to mobilize, retrotransposons need to be restrained and controlled to a certain extent, during cellular proliferation and differentiation, as they are found to be associated with several diseases. They are normally regulated by methylation and piwi RNAs and any imbalance in those mechanisms may lead to mutagenesis and disorders with serious consequences for the host cell (14-17).

In normal somatic cells, retroelement RNA expression is suppressed, while it appears to be unrestricted in the developing germ cells (18). Methylation of LINES at the CpG-rich 5' UTR results in transcriptional repression in somatic cells, whereas aberrant hypomethylation leads to RNA expression in various tumors, causing genomic instability (19, 20).

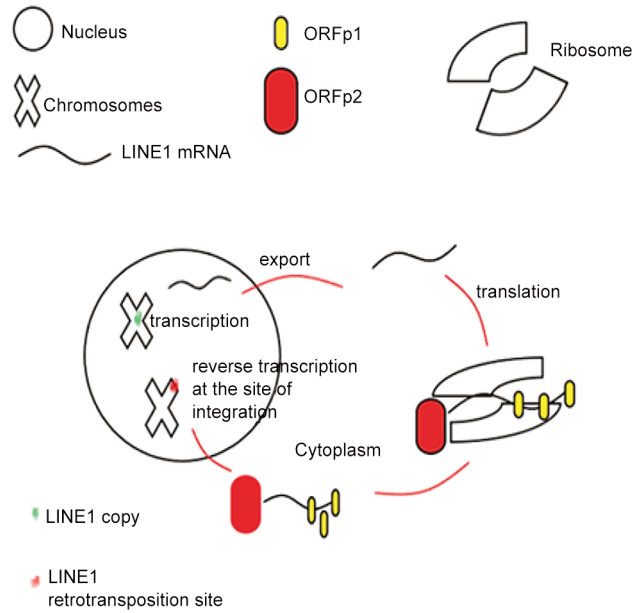


Figure 2. LINE-1 retrotransposition into a new genomic locus.

Due to the retroelements' nature and interference with several biological and genomic functions, this review is focused on the impact of retrotransposons in human gametes and early embryos, based on our previous work concerning this issue.

Human Oocytes. In oocytes, methylation is established during oogenesis and regulated by methyltransferases Dnmt3A, Dnmt3B, and DnmtL (21). Oocytes display a temporal hypomethylated profile. Georgiou *et al.*, studied the RNA expression of LINE-1, HERV-K10, and SVA retroelements as well as the existence of *de novo* retrotransposition events at the germinal vesicle (GV) stage of the oocytes. The results showed that these retroelements, and unexpectedly, the HERV-K10-related human specific retrotransposons SVA, are expressed, and also that retrotransposition events occur in the human oocyte (22).

Expression of LINE-1, HERV-K10, and SVA retroelements in human oocytes was also investigated by Georgiou *et al.*, by performing an RT PCR assay with two sets of primers for LINE-1 and HERV-K10, using HeLa cells and lymphocytes as controls (22-24). A different pattern of retrotransposon expression was documented in the oocytes compared to control cells (22). Interestingly, a higher LINE-1 RNA expression was observed in comparison with HERV-K10s and SVAs in HeLa cells and lymphocytes. These results could depict a retrotransposon expression hierarchy in somatic cells (LINE-1>HERV-K10>SVA). In contrast, oocytes indicated a reversed expression pattern with a higher

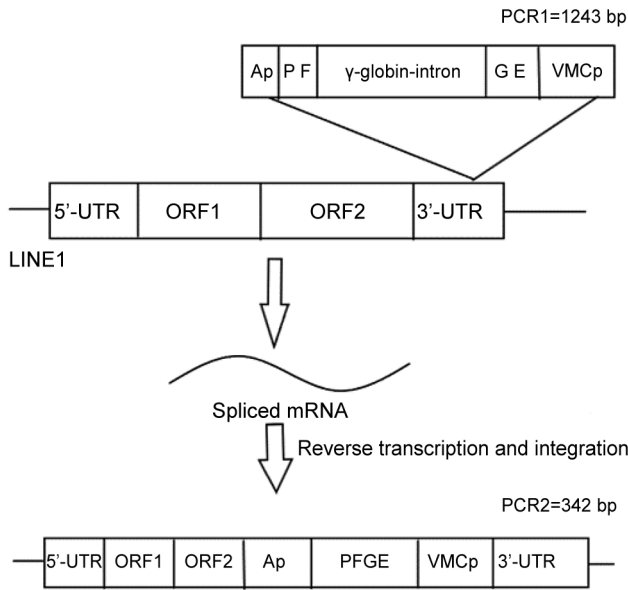


Figure 3. Schematic presentation of retrotransposition cassette for human LINE-1. The EGFP retrotransposition cassette is tagged in the 3' UTR of LINE-1 in the antisense orientation. EGFP gene is disrupted by an γ -globin intron and expressed only when the steps of transcription, splicing, reverse transcription, and integration into chromosomal DNA are completed, mirroring retrotransposition events.

HERV-K10s and SVAs expression (22, 25). Increased RNA expression of HERV-K10 retrotransposons might concern envelope proteins contributing to the development of the oocyte immunosuppression, which is required for sperm-oocyte binding and fusion (22, 26).

Subsequently, a cassette based on the CMV promoter and EGFP expression in mouse VL30 and human LINE-1 was used, to investigate whether retrotransposition events occur in the human oocyte. In this cassette (kindly provided by Professor Kazazian), EGFP gene was transcriptionally regulated by a CMV promoter and its expression was interrupted by a γ -globin intron cloned in opposite orientation to that of EGFP (Figure 3) (27, 28). The cassette was cloned into the 3'UTR region of the retrotransposon. EGFP expression can be detected only when retrotransposon transcripts undergo splicing, reverse transcription, and integration into the chromosomal DNA. In order to find retrotransposition events, they performed a PCR analysis for the EGFP gene. The VL30 retrotransposition-positive oocytes exhibited ooplasm granulation. In addition to these results, abnormal oocyte morphology associated to LINE-1 retrotransposition was observed, potentially due to the high concentration of the microinjected unmethylated LINE-1 DNA. Probably, retrotransposition-mediated diseases are attributed to events that escape control mechanisms. On the contrary, controlled retrotransposition may benefit oocyte development (22, 29).

Human Spermatozoa. Lazaros *et al.* studied the phenomenon of exogenous DNA internalization in human spermatozoa. This investigation revealed that LINE-1, HERV-K10, and SVA retrotransposons are expressed in human spermatozoa. Furthermore, reverse transcriptase exists in human spermatozoa and *de novo* retrotransposition events can occur, while integration of exogenous retroelements can be incorporated in the sperm genome (30).

Human spermatozoa from normozoospermic and oligozoospermic men were collected and tested for the expression of LINE-1, HERV-K10, and SVA retrotransposons using RT PCR. The results showed that specific LINE-1, HERV-K10, and SVA transcripts were present in all samples regardless of semen sample quality (30).

The methylation of two CpG LINE-1 retrotransposon sites in spermatozoa of normozoospermic and oligozoospermic men was also studied using the COBRALINE-1 assay previously described for cancer detection (31). Firstly, DNA from human spermatozoa was extracted and treated with bisulfide. Bisulfide treatment affects unmethylated cytosines of denaturated DNA but not including the methylated ones. Specifically, unmethylated cytosines are modified to uracils, while methylated cytosines remain unaltered. A PCR analysis for LINE-1 was conducted and restriction enzymes were used to cut the LINE-1 sequence in specific sites depending on the methylation pattern of the two CpG sites in each LINE-1 copy. The results indicated that LINE-1 CpG methylation levels are statistically different between normozoospermic and oligozoospermic men.

Retrotransposon expression is subjected to constant control in order to ensure normal cell function. Methyltransferases and piwi RNAs regulate retroelement expression (32) and are expected to be expressed at the highest level in human spermatozoa (33). Nevertheless, the study showed that LINE-1, HERV-K10, and SVA retroelements are expressed both in oligozoospermic and normozoospermic samples. Their expression is probably attributed to hypomethylation of specific sperm head loci and temporal genome hypomethylation of certain spermatozoa, or the retroelement transcripts may potentially represent RNA molecules expressed at an earlier developmental stage (30).

Subsequently, the human spermatozoa were incubated with EGFP-tagged retrotransposons as mentioned above (22, 34) and checked for retrotransposition events, using confocal microscopy, fluorescence-activated cell sorting (FACS) and PCR analysis. A ratio of 17% of the samples analysed by FACS were positive for retrotransposition, whilst 83% were negative (30). In human sperm samples, the limited population of EGFP-positive cells, is probably due to the highly compacted DNA of sperm cells, that does not favor the process of plasmid DNA integration (30).

Table I. Documented retrotransposition events in gamete cells and embryos.

Experiments	Gametes				Embryos	
	Human oocytes	Mouse oocytes	Human spermatozoa	Mouse spermatozoa	Human embryos	Mouse embryos
LINE-1, SVA, HERV-K10 RNA expression	+	Not tested	+	+	Not tested	Not tested
<i>De novo</i> retrotransposition events	+	+	+	+	Not tested	+
Exogenous DNA integration			+	+		

LINE-1 in Early Human Embryos. Retrotransposable elements are known to produce DNA breaks (35, 36) through retrotransposition mechanisms, creating new integration sites in the genome. An immunohistochemistry analysis of LINE-1 injected embryos, revealed the presence of double strand DNA breaks, as early as in the 4-cell stage embryo. The presence of DNA double strand breaks at this early stage may affect the cleavage rate of mitotic cells (35, 37) and cause high levels of fragmentation in affected embryos, leading to developmental failure. LINE-1 expression is important for preimplantation embryo development as LINE-1 transcripts are expressed in early stage mouse embryos (38). Repression of these transcripts seems to interfere with the normal embryonic development (39).

Mouse Sperm and Preimplantation Embryo Development

The same EGFP-tagged cassettes for human LINE-1, HERV-K10, and mouse VL30 were used in order to study events of *de novo* retrotransposition in mouse spermatozoa and embryos. Once again, the presence of retrotransposition events was evaluated by a PCR analysis for the *EGFP* gene. Mouse spermatozoa were found positive for retrotransposition in a ratio of up to 53%, while the retrotransposition frequency was not statistically different between cassettes carrying retroelements (34).

In both human and mouse spermatozoa, EGFP expression indicated that retroelements can be internalized in sperm cells, irrespective of the sperm origin, penetrate the tightly packed chromatin, interfere with the genome, possibly influence the sequence of certain loci, and give rise to new retrotransposition events (30, 34).

In order to suspend reverse transcriptase activity, Lamivudine, an inhibitor of reverse transcriptase, was used. FACS analysis showed a significant reduction of retrotransposition events at the lower concentration of Lamivudine (15-50 μ M) (34). Sperm samples were also screened for EGFP expression with confocal microscopy, where a fluorescent signal was detected in the heads of the spermatozoa positive for retrotransposition events (34).

These results indicated that sperm cells can integrate exogenous retroelements and favour retrotransposition. A part of the internalized DNA is probably integrated in specific sperm nuclear loci, within undermethylated retrotransposon sequences, suggesting a common site for integration and retrotransposition events (40, 41). Also, the ability of mouse epididymal spermatozoa to uptake and reversely transcribe RNA to cDNA indicated a reverse transcriptase activity (42).

Considering the above results and knowing that spermatozoa are able to bind spontaneously exogenous DNA and internalize a proportion of it into their nucleus (43, 44), Kitsou *et al.* investigated the possibility of mice sperm transferring exogenous human retroelements into the mouse oocyte and assessing the mouse embryo development. The results indicated that mouse spermatozoa not only integrate exogenous retroelements but they also transfer them into the oocyte during fertilization, affecting preimplantation embryo development (34).

Embryos were tested for retrotransposition events with confocal microscopy and PCR analysis (34). The *in vitro* preimplantation development of mouse embryos was scored daily until they entered the blastocyst stage. Embryos positive for retrotransposition events appeared to have accelerated asymmetric cell division, multiple fragments, and the majority of them were arrested and collapsed before blastocyst formation and expansion (34). Thus, the presence of retroelements carried from sperm to embryo can impair preimplantation embryo development.

Under normal conditions, retrotransposition events are potentially necessary for embryogenesis and are controlled by methylation and piwi RNAs (45, 46). Embryos with repressed LINE-1 expression show impaired embryonic development, suggesting that LINE-1 is part of the developmental program of early embryos (47). However, the induction of retrotransposition events from exogenous human and mouse retroelements, may dysregulate the control mechanisms and cause abnormal embryo preimplantation development (48).

Conclusion

The eukaryotic genome includes numerous retroelement copies (49, 50); however, the respective mobilization or transcription ability and retrotransposition activity are restricted to a minority of transposons due to 5' end truncation, point mutations, and sequence rearrangements (51). Retrotransposition events occur in early mouse and human development, in neuronal precursor and adult brain cells, while they are more frequent in germ cells and in embryos at early developmental stages compared to normal somatic cells (48, 52, 53). Retrotransposon transcripts have been also documented to play an important role in male and female gametes (43, 54).

The findings of retrotransposition events both in human spermatozoa and oocytes, indicate that reverse transcription, transcription, and translation machineries, as well as splicing are present in germ cells (4, 28), allowing the occurrence of retrotransposition events even for the non-human and non-autonomous retrotransposons such as VL30 (22, 30) (Table I).

Conflicts of Interest

The Authors declare that they have no conflicts of interest in relation to this study.

Authors' Contributions

E.M., A.C. and I.G. have made substantial contributions to the conception of the work, the acquisition and interpretation of data, E.M. and A.C. have drafted the work, E.M., A.C., K.P., A.Z., and I.G. discussed the manuscript and provided important feedback.

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