Retrotransposons and germ cells: reproduction, death, and diversity

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Abstract

The evolutionary success of retrotransposable elements is reflected by their abundance in mammalian genomes. To restrict their further advance, a number of defence mechanisms have been put in place by the host. These seem to be particularly effective in the germ line while somatic lineages might be more permissive to new insertions, as recent work by Kano and colleagues suggests.

Introduction and context

Large chunks of the mammalian genome (approximately 40%) are made up of retrotransposable elements [1]. Retrotransposons multiply by making more copies of themselves within a host genome, but at the same time they have to keep the host alive to guarantee its and therefore their own reproductive success. Host and transposons probably adapt to cooperate with each other while at the same time trying to outdo each other in order to gain the upper hand. One of the largest groups of these 'selfish genes' in humans, and the only such group that is active, is the L1 retrotransposon family (a subfamily of long interspersed nucleotide elements, or LINEs) with a full length of approximately 6 kb and about 500,000 copies in the genome, most of which, however, are truncated forms [1]. Their transcript encodes two proteins called ORF1 and ORF2, which are responsible for retrotransposition via a 'copy and paste' mechanism that can cause various types of insertion mutations in the host genome. These include target site deletions, alteration of expression of nearby genes, exon-shuffling, and even the creation of new genes [1].

In somatic cells, expression of L1 retrotransposons is attenuated by DNA methylation in order to maintain genomic integrity [1]. However, in mice (and probably also in other mammals), primordial germ cells between E11.5 and E13.5 (and early embryos) undergo genome-wide demethylation during a process called epigenetic reprogramming [2]. This substantial loss of DNA methylation, which comprises many genomic elements, including L1 retrotransposons [3], lifts this key epigenetic silencing mechanism from L1 elements at a particularly vulnerable time when new insertions would affect the integrity of the germ line genome. So, are retrotransposons roaming freely during these critical windows in development, or are other mechanisms curtailing their movements?

In male germ cells, a pathway involving small RNAs – the so-called piRNAs, which are bound by the Piwi (P element-induced wimpy testis) clade of Argonaute proteins – has been shown to keep L1 elements in check [4]. The knockout of two Piwi members – Mili and Miwi2 – leads to loss of L1 DNA methylation in testes and to sterility, a phenotype strikingly similar to loss of Dnmt3L in mouse male germ cells [5-7]. It has therefore been proposed that *de novo* methylation of transposons in male germ cells, which starts around day E14.5, is guided by piRNAs [8,9]. Mili is also expressed in female germ cells, but the function of piRNAs in the female germ line is unclear. Female germ cells undergo *de novo* methylation much later – during oocyte growth – and remain in meiotic arrest, a non-dividing state less favourable for L1 retrotransposition [5,10].

In addition to epigenetic silencing of transposons, there could be other layers of protection, especially during the genome-wide erasure of DNA methylation, including post-transcriptional regulation or interference with other aspects of the life cycle of the retrotransposon. Given the incomplete knowledge we have of the mechanisms that may interfere with retrotransposon mobility in germ cells, an important question to ask is how common retrotransposition is in germ cells and early embryos.

Major recent advances

The Kazazian lab [11] has been using a system in which an L1 transcription unit is expressed from its own promoter in transgenic mice or rats, and transposition events that create new insertions in the genome are monitored by the loss of an intron. Recent work by Hiroki Kano and colleagues [12] based on this transgenic system has now shown that retrotransposition in germ cells is in fact uncommon but that most new insertions that are detectable in mouse tissues were created by transposition events in early embryos, leading to somatic mosaicism.

First, the authors detected expression at the RNA level of the L1 transgene during spermatogenesis and also in ovaries (they did not investigate expression in oocytes themselves) and showed L1 transgene expression at least in late-stage germ cells. However, despite this expression, the frequency of finding new transposon insertions in the next generation was low, suggesting that protection mechanisms, inhibiting the transposon life cycle at a post-transcriptional level, are in place. Furthermore, most new insertions that were found were mosaic in the offspring (i.e., had presumably not occurred in germ cells but rather in early embryos after fertilization); notably, the authors observed that retrotransposition events in the offspring can occur even without the transmission of the transgene.

Kano *et al.* [12] were indeed able to detect transgenic L1 RNA in pre-implantation embryos that had not inherited the transgene from their parents (both from transgenic mothers and fathers). The authors suggest that the L1 RNA produced in germ cells is then carried over by either oocyte or sperm with similar efficiencies into the next generation, where it is reverse-transcribed and then integrates into the host genome during early embryonic development. By breeding transgene-negative animals that had undergone retrotransposition (mediated by parentally inherited transcripts), the authors provide evidence for somatic rather than germ cell retrotransposition as the newly inserted transposon is not inherited in the next generation. They were also able to estimate the frequency of retrotransposition by

RNA carried over to about 1 in 1000 cells by performing quantitative polymerase chain reaction analysis on tissues of transgene-negative, retrotransposition event-positive mice. It is surprising and interesting that transfer of L1 RNA via sperm to offspring is as effective as that through the much larger oocyte, but in both situations it appears that non-genetic information based on RNA can be transmitted from parents to offspring through the germ cells.

While retrotransposition occurs in embryos in the presence of this inherited RNA, a 10-fold-higher retrotransposition frequency is observed in embryos that also inherit the L1 transgene. This could be explained through further transposon transcription in a second phase of genome-wide demethylation after fertilization in the early embryo [13]. It is possible that, during this developmental period, post-transcriptional restrictions to transposition are slowly being lifted as embryonic cells divide and the likelihood that the RNA would invade germ line cells diminishes. Interestingly, however, retrotransposon insertions that occur after fertilisation thus result in somatic mosaicism for the new insertion, with the possibility that this can create increased phenotypic diversity without being detrimental to the host. An intriguing example of this type of somatic retrotransposition and its potential implications comes from studies on human neural progenitor cells, which showed that retrotransposition events of L1s in the fetal brain can create somatic mosaicism [14,15]. This mosaicism has the potential to influence neuron formation and thus create individual characteristics and phenotypic diversity of the brain.

Future directions

The interesting set of experiments reported by Kano *et al.* [12] in 2009 opens up many new questions about the regulation of L1 transposition in germ cells and early embryos. For example, is the L1 transgene transcribed at an early stage of primordial germ cell development, during which DNA methylation is erased? Does it become silenced at later stages when the Piwi/piRNA pathway gets activated? Does the L1 RNA survive for longer periods during gametogenesis because it might be packaged in ribonucleoprotein particles that lead to a more stable protein-RNA complex than RNA itself and thus can avoid defence mechanisms that would usually lead to its destruction? Are there other mechanisms controlling retrotransposable elements in germ cells at the post-transcriptional level in particular? Elucidating the full arsenal of defence mechanisms will be a key future objective to understand the interrelation of transposon expression and their integration in germ

cells. For example, the function of APOBEC (apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like) or ADAR (adenosine deaminases acting on RNA) deaminases may be required for editing of transposon-derived transcripts (reviewed in [14,15]). Furthermore, very little is known about the role of Tex19.1, a protein with an intriguing role in restricting activation of retrotransposons in pluripotent cells [16].

It is surprising that the L1 RNA can apparently be inherited stably from gametes into the early embryo, but it may offer a strategy for retrotransposons to reproduce in cells that can give rise to the germ line (blastomeres of preimplantation embryos) if they are prevented from doing so in the germ cells themselves. Hence, whether there is a trigger or loss of a protective mechanism in the early embryo that allows the L1 transcripts to produce DNA copies and to integrate into the host genome needs to be established. The erasure of DNA methylation in the early embryo may also contribute to increasing L1 transcription; insertions during this developmental period potentially create mosaicism in both future germ cells and in somatic cells and thus can contribute to somatic phenotypic diversity in many tissues without necessarily being transmitted through the germ line.

Finally, it is also possible that our logic in interpreting these results is completely back-to-front: instead of retrotransposons apparently benefitting from epigenetic reprogramming by being expressed and able to transpose, is it possible that epigenetic reprogramming 'exposes' retrotransposons to powerful silencing mechanisms (perhaps based on small RNA pathways)? [17]. In other words, are repeated exposure and resilencing necessary because epigenetic silencing mechanisms are inevitably leaky?

While the cat-and-mouse games that transposons and the host genome play will continue to fascinate biologists for some time to come, an overall balance between the two that ensures prosperity and reproductive success for both is probably a successful aspiration for the future!

Abbreviations

L1, long interspersed nucleotide element-1; LINE, long interspersed nucleotide element; piRNA, Piwi (P element-induced wimpy testis)-interacting RNA; Piwi, P element-induced wimpy testis.

Competing interests

The authors declare that they have no competing interests.

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