

Research Highlight

A novel reporter mouse to monitor in vivo retrotransposition in the germline[†]

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The germline genome is constantly challenged by retrotransposons, the harmful mobile genetic elements that can copy and paste themselves in the genome to introduce insertional mutations [1]. In humans, the most successful retrotransposons is the long-interspersed element type 1 (LINE-1, or L1) family that comprises ~17% of the human genome. Mobilization of L1 can generate insertional mutations that drive tumorigenesis and human genetic diseases [2]. Because of the danger for mutations to be passed along to the next generation, retrotransposons such as L1 have to be especially tightly controlled in the germline. To cope with this threat, germ cells have developed the PIWI-interacting RNA (piRNA) pathway as a germ cell-specific small RNA-based immune system to suppress retrotransposon activities [3,4]. The central players in this pathway are PIWI proteins and their associated 24–32 nt piRNAs wherein the PIWI protein uses piRNA as a guide to recognize and suppress retrotransposon expression through transcriptional and posttranscriptional gene silencing.

The timing and frequency of how individual L1s propagate and mobilize within the genome during germ cell development is unknown because the mobilization of individual L1s is extremely difficult to track due to the abundance of preexisting L1 copies in the genome. In a report by Newkirk et al. [5], a research group led by Wenfeng An solved this mystery by elegantly tracking the mobilization of a single copy L1 reporter transgene in a piRNA-deficient background in mice. This allows for the first time the accurate monitoring of L1 transposition in developing germ cells at different stages of spermatogenesis.

To address the above question, Newkirk et al. generated a mouse line with a stable inheritance of a single copy of L1 reporter transgene. This mouse model is advantageous over previous L1 reporter transgenic lines (e.g. CAG-L1 [6]) in that it contains a transgene with an endogenous mouse L1 promoter, codon-optimized L1 ORF1 and ORF2 sequences, and an EGFP-based retrotransposition indicator cassette. The stable single copy

of L1 transgene in mice is important in that it faithfully mimics the endogenous L1 promoter DNA methylation dynamics, which is not represented by mouse models with high copy L1 transgenes or human tandem L1 transgenes. These features together ensure the optimal expression and insertion of L1 transgene that recapitulates that of endogenous L1s.

Although piRNAs are essential for retrotransposon silencing and male fertility in mice, a long puzzling question for reproductive biologists in the piRNA field is that whether piRNA deficiency-triggered elevated L1 activity indeed directly drives germ cell demise. It has been noticed in various piRNA mutants that the defective piRNA production and L1 ORF1 protein overexpression start in fetal germ cells, whereas germ cell arrest does not occur until meiosis [1]. It is unclear why there is a delay from L1 expression to germ cell death. Understanding the timing and frequency of L1 mobilization (insertion) in piRNA-deficient background holds the key to solve this puzzle. To do this, the authors crossed the newly developed L1 reporter line (referred to as SN1) to *Mov10l1^{-/-}* mice that are deficient in piRNAs. *MOV10l1* is an RNA helicase critical for piRNA biogenesis and *Mov10l1^{-/-}* mice show L1 upregulation and germ cell arrest at meiotic prophase I [7,8]. By tracing de novo insertions of SN1 L1 reporter transgene in control and *Mov10l1^{-/-}* germ cells, the authors showed that at postnatal day 7, despite a 37-fold increase in ORF1 mRNA levels and a significant elevation in ORF1 protein expression, L1 transposition rate is low in *Mov10l1^{-/-}* spermatogonia. This low L1 insertions rate is similar to that of the control. Strikingly, the dramatic L1 insertions occur days later in *Mov10l1^{-/-}* spermatocytes at the onset of meiosis. At P14, new SN1 L1 insertions increase by 144-fold in *Mov10l1^{-/-}* spermatocytes. This delayed onset of L1 retrotransposition correlates perfectly with the timing of germ cell death (Figure 1). Would this increased retrotransposition be powerful enough to drive the meiotic arrest in *Mov10l1^{-/-}* germ cells? By estimating the frequency of retrotransposition, the number of new insertions per cell was 2.25 for endogenous L1s. This seemingly low

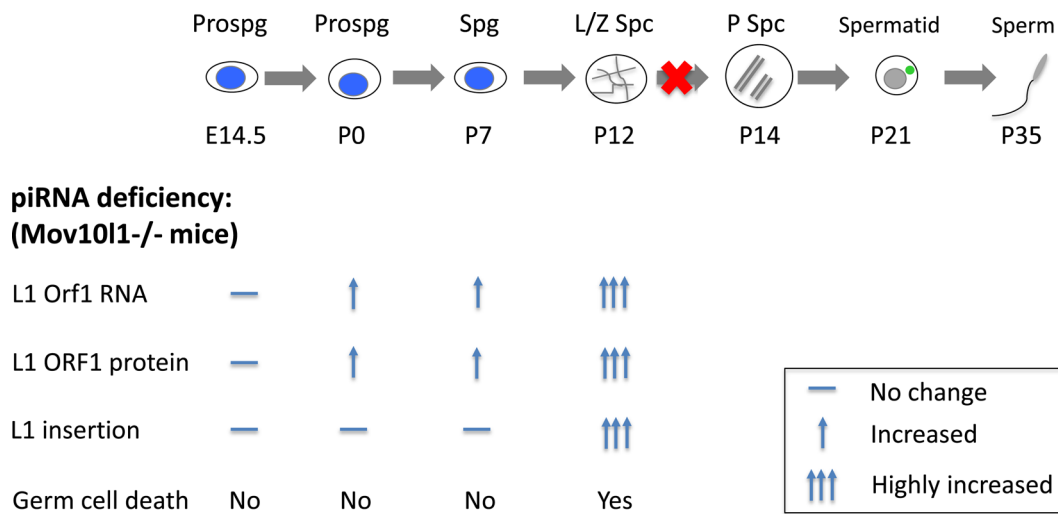


Figure 1. Schematic illustration of the developmental timing of L1 expression and insertion in piRNA-deficient germ cells during spermatogenesis. In piRNA-deficient *Mov10l1*^{-/-} germ cells, L1 RNA and protein upregulation is first seen in fetal/perinatal prospermatogonia (Prospg), which is, however, not accompanied by obvious increased L1 insertions and germ cell death. Drastic L1 overexpression and mobilization occurs in leptotene/zygotene spermatocytes (L/Z Spc), which correlates with the timing of germ cell arrest before the pachytene spermatocyte (P Spc) stage of meiotic prophase I.

numbers of insertions per cell appear unlikely to directly cause germ cell demise. Accordingly, the authors demonstrate that the pharmacological inhibition of L1 retrotransposition in turn did not rescue the meiotic phenotype of *Mov10l1*^{-/-} mice. Thus, the sheer numbers of new L1 insertional mutations in the germline may not be the decisive factor to drive piRNA-deficient germ cell death. However, one cannot exclude the possibilities that other aspects of elevated L1 activities in mutant germ cells may contribute to germ cell arrest. One possibility is that overexpression of L1 ORF2, encoding both an endonuclease and a reverse transcriptase necessary for L1 transposition, may introduce excessive DNA damage without completing L1 insertions in piRNA-deficient germ cells. Another possibility is that L1 insertions may disrupt transcriptomic integrity, which systematically influences germ cell health [9]. These possibilities need to be further investigated.

In summary, Newkirk et al. created an L1 transgenic mouse line uniquely suitable to monitor L1 mobilization in vivo. It reveals the stage-specific increases in L1 retrotransposition during spermatogenesis and confirms that an intact piRNA pathway is critical to prevent L1 mobilization. This SN1 L1 mouse model will also be a useful tool for reproductive biologists to study the mechanism of female germ cell retrotransposon regulation and the effect of genome perturbation by L1 in gametes on embryonic development and offspring health and fertility. Lastly, it can serve as a new model for studying a critical aspect of environmental epigenetics [10], i.e. how environmental factors alter retrotransposon regulation and impart genetic changes through insertional mutagenesis.

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