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Perspective

Retrotransposon Silencing During Embryogenesis: *Dicer* Cuts in LINE

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Fossilised mobile genetic elements, including Long Interspersed Element-1 (LINE-1 or L1) retrotransposons, comprise at least two-thirds of the human genome [1]. Their molecular history is reminiscent of speciation and natural selection, where, as noted by Carl Sagan, "Extinction is the rule. Survival is the exception" [2]. Broadly, the life cycle of a retrotransposon begins with innovation to evade host genome surveillance, followed by "copy-and-paste" retrotransposition and, finally, quiescence as a result of host defence adaptation. Before being tamed, a new or newly reactivated retrotransposon can undergo massive copy number amplification. For instance, more than one million copies of the primate-specific Short Interspersed Element (SINE) *Alu* comprise 11% of the human genome [3]. Even more impressively, approximately 500,000 copies of a single retrotransposon superfamily, *Gypsy*, occupy nearly half of the maize genome [4]. Thus, retrotransposons can overrun a genome within a brief evolutionary period, making their suppression a high host priority.

Retrotransposition requires transcription of an RNA template for DNA-primed reverse transcription. Several cellular defence mechanisms have evolved to hinder this process, including: 1) promoter methylation and heterochromatinisation, 2) degradation of retrotransposon transcripts via RNA interference (RNAi), and 3) host factor prevention or destabilisation of reverse transcription. To describe in detail just one of a myriad of specific inhibitory pathways, repeat associated small interfering RNAs (rasiRNAs) are present in plant, worm, fly, fish, and mouse gametes and, therefore, represent a highly conserved defence against germ line retrotransposition [5–8]. A plausible model of rasiRNA biogenesis involves bidirectional transcription of opposed retrotransposon promoters [9,10], resulting in the formation of double-stranded RNAs (Figure 1). These are cleaved by *Dicer* (DCR) and then assembled with *Argonaute* (AGO) and other proteins into the RNA-induced silencing complex (RISC) that, in turn, produces RNAi against retrotransposon transcripts [11]. The suppressive influence of rasiRNAs, in concert with other pathways, may explain why retrotransposition is more common during embryogenesis than in gametes [12,13]. Importantly, although rasiRNAs have been found in stem cells and soma, their capacity to suppress retrotransposition during development is relatively unexplored [14–16].

In this issue of *PLOS Genetics*, Ciaudo et al. [17] describe rasiRNA-mediated suppression of LINE-1 activity in mouse embryonic stem cells (mESCs). Focusing on the L1-Tf subfamily, where they previously described an unusual rasiRNA signature mapping to the 5′UTR [15], Ciaudo et al. observed that knock-out of *Dicer* markedly decreases L1-Tf promoter methylation and increases L1-Tf transcription, translation, and copy number in cultured mESCs. In particular, $DCR^{-/-}$ mESCs accumulate a remarkable 860 L1-Tf copies (greater than five megabases of genomic DNA) per cell over 20 passages, versus 255 copies per cell in $DCR^{Flx/Flx}$ controls, based on SYBR-Green qPCR targeting the L1-Tf 5′UTR. High-throughput small RNA sequencing then confirmed that $DCR^{-/-}$ mESCs were depleted of approximately 22 nt molecules found in wild-type mESCs, immunoprecipitated with AGO2 and aligned to L1-Tf, and therefore resembling rasiRNAs. Hence, LINE-1 activation in $DCR^{-/-}$ mESCs coincides with rasiRNA depletion and is also possibly influenced by ablation of *Dicer*-mediated LINE-1 promoter methylation.

Intriguingly, a second class of *Dicer*- and AGO2-independent small RNAs were found to "paint" the L1-Tf 5′UTR. Again, assessing L1-Tf transcription and copy number, Ciaudo et al. found that deletion of XRN2 and DGCR8, respective members of the RNA surveillance and *Drosha*-DGCR8 Microprocessor pathways, led to increased L1-Tf transcription but not copy number amplification. These observations agree with other recent reports of small RNAs immunoprecipitated with DGCR8 and enriched for LINE-1 sequences [18], as well as evidence of elevated L1-Tf expression in $DGCR8^{-/-}$ mESCs [19]. As a final experiment, Ciaudo et al. complemented $DCR^{-/-}$ mESCs with human *Dicer* and found that these cells recapitulated wild-type mESC LINE-1 suppression and differentiated normally, unlike $DCR^{-/-}$ mESCs.

Evidence for a reciprocal relationship between rasiRNA depletion and LINE-1 activation significantly advances our understanding of RNAi-mediated control of retrotransposition during mammalian embryogenesis. These data are also important because they address a longstanding question of why rasiRNAs cannot be consistently detected in mammalian somatic cells: small RNAs generated by RNA surveillance and the Microprocessor may cleave the same pool of precursor LINE-1 mRNAs processed by *Dicer* and obscure rasiRNA detection (Figure 1). As Ciaudo et al. note, it is possible that insertional mutagenesis caused by LINE-1 contributes

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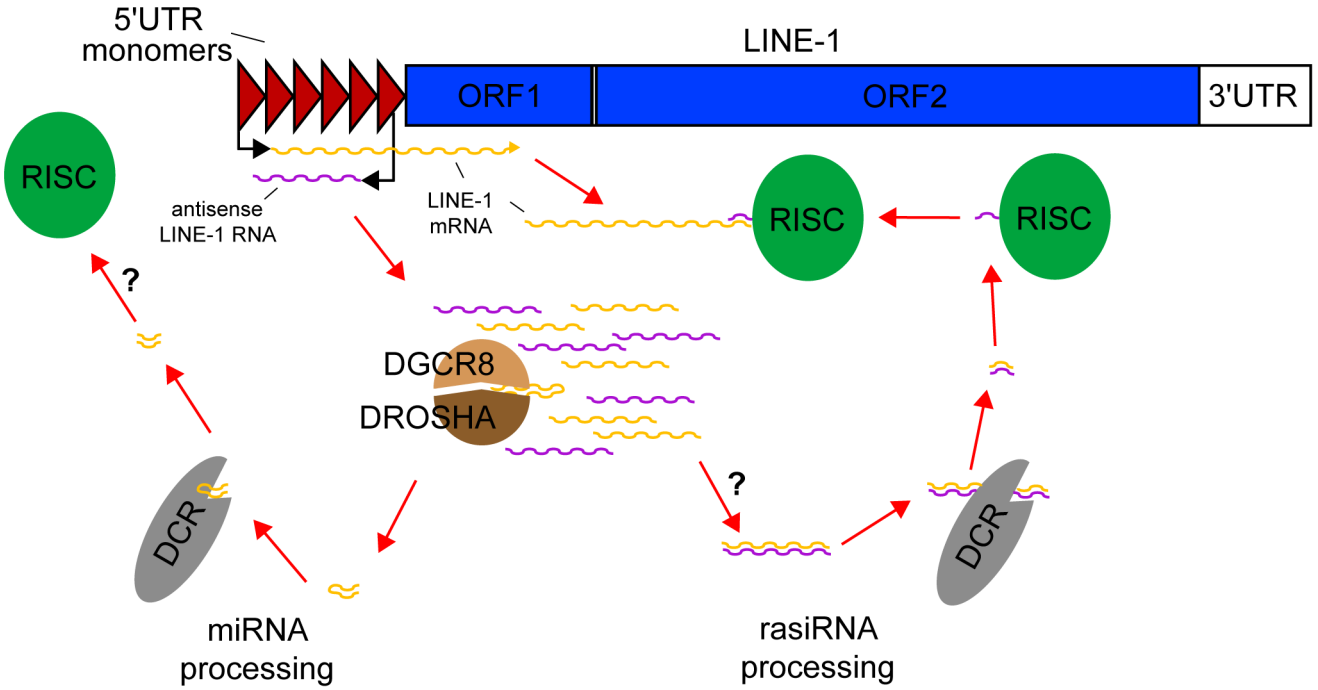

Figure 1. rasiRNAs inhibit LINE-1 expression in mESCs. Mouse LINE-1s are comprised of two ORFs flanked by 5′ and 3′UTRs. Several monomers in the 5′UTR provide promoter activity. Following the LINE-1 expression and copy number variation data of Ciaudo et al., bidirectional transcription of the 5′UTR generates sense and antisense LINE-1 RNAs. The *Drosha*-DGCR8 Microprocessor cleaves these precursors into pre-miRNAs, which are processed into miRNAs by *Dicer*, but may not be loaded into the RISC complex. By contrast, double-stranded RNAs potentially formed by sense/antisense pairing of LINE-1 RNAs are also cleaved by *Dicer* but here generate rasiRNAs, loaded into the RISC complex, which degrade canonical LINE-1 mRNAs. *Dicer* also appears to mediate LINE-1 promoter methylation (not shown).
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to the reported differentiation defects for $DCR^{-/-}$ mESCs [20], though it is unclear why lesser but still substantial LINE-1 activity is tolerated by wild-type mESCs. Interestingly, experiments using engineered LINE-1 reporters have shown elsewhere [16,19] that mutation of *Dicer* or the Microprocessor increases LINE-1 mobilisation in cancer cells, with the latter result at odds with data generated here from mESCs. Future advances in high-throughput sequencing and single cell genomics should enable characterisation of endogenous LINE-1 mobilisation events in stem cells and further delineate the multifaceted roles of *Dicer* and other factors in LINE-1 inhibition.

References

1. de Koning AP, Gu W, Castoe TA, Batzer MA, Pollock DD (2011) Repetitive elements may comprise over two-thirds of the human genome. PLoS Genet 7: e1002384. doi: 10.1371/journal.pgen.1002384.
2. Sagan C, Druyan A (2006) The varieties of scientific experience : a personal view of the search for God. New York: Penguin Press. xviii, 284 p. p.
3. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, et al. (2001) Initial sequencing and analysis of the human genome. Nature 409: 860–921.
4. Schnable PS, Ware D, Fulton RS, Stein JC, Wei F, et al. (2009) The B73 maize genome: complexity, diversity, and dynamics. Science 326: 1112–1115.
5. Czech B, Malone CD, Zhou R, Stark A, Schlingeheyde C, et al. (2008) An endogenous small interfering RNA pathway in Drosophila. Nature 453: 798–802.
6. Sijen T, Plasterk RH (2003) Transposon silencing in the Caenorhabditis elegans germ line by natural RNAi. Nature 426: 310–314.
7. Slotkin RK, Vaughn M, Borges F, Tanurdzic M, Becker JD, et al. (2009) Epigenetic reprogramming and small RNA silencing of transposable elements in pollen. Cell 136: 461–472.
8. Watanabe T, Totoki Y, Toyoda A, Kaneda M, Kuramochi-Miyagawa S, et al. (2008) Endogenous siRNAs from naturally formed dsRNAs regulate transcripts in mouse oocytes. Nature 453: 539–543.
9. Speek M (2001) Antisense promoter of human L1 retrotransposon drives transcription of adjacent cellular genes. Mol Cell Biol 21: 1973–1985.
10. Zemojtel T, Penzkofer T, Schultz J, Dandekar T, Badge R, et al. (2007) Exonization of active mouse L1s: a driver of transcriptome evolution? BMC Genomics 8: 392.
11. Hammond SM, Bernstein E, Beach D, Hannon GJ (2000) An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. Nature 404: 293–296.
12. Garcia-Perez JL, Marchetto MC, Muotri AR, Coufal NG, Gage FH, et al. (2007) LINE-1 retrotransposition in human embryonic stem cells. Hum Mol Genet 16: 1569–1577.
13. Kano H, Godoy I, Courtney C, Vetter MR, Gerton GL, et al. (2009) L1 retrotransposition occurs mainly in embryogenesis and creates somatic mosaicism. Genes Dev 23: 1303–1312.
14. Babiarz JE, Ruby JG, Wang Y, Bartel DP, Blelloch R (2008) Mouse ES cells express endogenous shRNAs, siRNAs, and other Microprocessor-independent, Dicer-dependent small RNAs. Genes Dev 22: 2773–2785.
15. Chow JC, Ciaudo C, Fazzari MJ, Mise N, Servant N, et al. (2010) LINE-1 activity in facultative heterochromatin formation during X chromosome inactivation. Cell 141: 956–969.
16. Yang N, Kazazian HH, Jr. (2006) L1 retrotransposition is suppressed by endogenously encoded small interfering RNAs in human cultured cells. Nat Struct Mol Biol 13: 763–771.
17. Ciaudo C, Jay F, Okamoto I, Chen CJ, Sarazin A, et al. (2013) RNAi-dependent and independent control of LINE1 mobility and accumulation in mouse Embryonic Stem Cells. PLoS Genet 9. e1003791 doi: 10.1371/journal.pgen.1003791.
18. Macias S, Plass M, Stajuda A, Michlewski G, Eyras E, et al. (2012) DGCR8 HITS-CLIP reveals novel functions for the Microprocessor. Nat Struct Mol Biol 19: 760–766.
19. Heras SR, Macias S, Plass M, Fernandez N, Cano D, et al. (2013) The Microprocessor controls the activity of mammalian retrotransposons. Nat Struct Mol Biol. doi: 10.1038/nsmb.2658.
20. Kanellopoulou C, Muljo SA, Kung AL, Ganesan S, Drapkin R, et al. (2005) Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. Genes Dev 19: 489–501.