

RESEARCH HIGHLIGHT

piRNAs and siRNAs collaborate in *Caenorhabditis elegans* genome defense

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Abstract

Caenorhabditis elegans piRNAs promote genome surveillance by triggering siRNA-mediated silencing of nonself DNA in competition with licensing programs that support endogenous gene expression.

Keywords piRNA, siRNA, Piwi, *C. elegans*, germline, genome surveillance, epigenetics, gene silencing, transposable elements.

Piwi-interacting RNAs (piRNAs) are a conserved class of small RNAs that defend against selfish genetic elements in the animal germline. The Piwi Argonautes associate with piRNAs to recognize and silence complementary transcripts. piRNA sequence diversity is immense, enabling targeting of various transposons and repetitive sequences, but mechanisms of target selection are incompletely resolved. Here, we discuss four recent publications that provide important insights into piRNA-mediated genome surveillance mechanisms in *Caenorhabditis elegans* [1-4].

The canonical piRNA pathway promotes genome integrity

In the germline, piRNAs act to silence mobile elements that can be deleterious to the genome. In flies and mammals, these elements can become trapped after integration into genomic piRNA clusters; this chance event induces biogenesis of piRNAs from that element, enabling silencing in *trans* of copies located elsewhere in the genome. Additionally, through an amplification loop termed the ping-pong cycle, the piRNA genome surveillance system can be tuned to recognize and selectively repress actively transcribed mobile elements (reviewed in

[5]). The cycle is primed by primary piRNAs generated through largely unknown mechanisms from sense or antisense transcripts corresponding to target elements. Loaded into a Piwi Argonaute, primary piRNAs direct the cleavage of complementary transcripts of the opposite sense; these cleavage products are in turn incorporated into distinct Piwi Argonautes as secondary piRNAs to direct the generation of still more piRNAs. Thus, active mobile elements provide substrate transcripts for this amplification loop. In fly, the ping-pong cycle not only amplifies piRNA silencing, but also provides a mechanism for epigenetic transmission of silencing to progeny. Maternally inherited Piwi-piRNA complexes are required for continued genome surveillance in developing progeny and may indeed serve as primary piRNAs to trigger silencing in the filial germline.

C. elegans piRNAs act through secondary siRNAs

The *C. elegans* genome encodes two highly homologous Piwi Argonautes, PRG-1 and PRG-2; the latter is dispensable for the piRNA pathway and may represent a pseudogene [6,7]. PRG-1 binds and is required for the production of 21U RNAs, a population of 21 nucleotide small RNAs with a 5' uridine that exhibit the high sequence diversity, genomic clustering, germline enrichment, and terminal methylation characteristic of piRNAs [6,7]. Unlike canonical piRNAs, however, the mechanism of action of 21U RNAs is poorly understood: their targets and functions are largely unknown, and they exhibit no evidence of a ping-pong amplification cycle. Rather, they were previously shown to act upstream of an endogenous siRNA pathway [7], but the specifics of the targeting mechanism and the nature of the secondary siRNAs were not reported.

Recent studies by Bagijn *et al.* [1] and Lee *et al.* [2] provide new clarity to these pathways through deep sequencing of small RNAs in *prg-1* mutant strains and transgenic sensor strains engineered to express complementary 21U RNA target sites. Independently, these two groups show that PRG-1 and the 21U RNA pathway trigger the biogenesis of secondary 22G RNAs of the worm Argonaute (WAGO) pathway in order to effect target silencing. The WAGO 22G RNAs, which associate

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with the worm-specific WAGO clade of Argonautes, represent a point of convergence for multiple *C. elegans* small RNA pathways, including both the primary endogenous siRNA (26G RNA) pathway and exogenous RNA interference (RNAi).

Through activity that is independent of PRG-1 slicer endonuclease function, 21U RNAs guide PRG-1 to target transcripts with up to three or four mismatches, promoting the association of factors involved in WAGO 22G RNA biogenesis to mount a localized silencing response [1,2]. Whereas earlier reports identified only a single transposon silenced by the *C. elegans* piRNA pathway [6,7], Bagijn *et al.* and Lee *et al.* identify numerous additional *C. elegans* piRNA pathway targets that include multiple transposable elements and pseudogenes [1,2], strengthening the previously tenuous connection between 21U RNAs and transposon defense. They further show that many factors required for WAGO 22G RNA biogenesis are also necessary for silencing of 21U RNA genomic targets, indicating that 22G RNAs mediate the silencing effects of piRNAs.

Such laxity in piRNA targeting requirements raises the question of how selectivity is achieved for 21U RNA-triggered repression. The answer may lie with the CSR-1 22G RNAs, another class of endogenous siRNAs required for chromosome segregation. The CSR-1 22G RNAs are primarily antisense to germline-expressed genes and may recruit the Argonaute CSR-1 to protein-coding genomic loci to promote proper chromatin organization through embryonic mitotic divisions [8]. Intriguingly, Bagijn *et al.* observe that transcripts silenced by the 21U RNA pathway are significantly depleted of protein-coding genes [1]. Similarly, Lee *et al.* show that 21U RNAs that map to CSR-1 22G RNA targets trigger a less robust secondary siRNA response compared with those mapping to WAGO 22G RNA targets [2]. Consistent with this finding, a *gfp::histone* fusion transgene shows inconsistent targeting by 21U RNA-dependent secondary siRNAs: the exogenous sequence encoding the GFP moiety is robustly targeted by 22G RNAs, whereas the endogenous sequence encoding the histone moiety largely evades 21U RNA targeting [2]. This leads the authors to suggest that CSR-1-dependent 'licensing' may protect endogenous protein-coding sequences (self) from piRNA-mediated silencing. Unlicensed, nonself genes, in contrast, are silenced by PRG-1-dependent WAGO 22G RNAs. Once such a piRNA immune response is mounted against nonself genes, this silencing is heritable and no longer requires 21U RNAs [2-4].

siRNAs enforce an epigenetic identity of self versus nonself

The mechanism of 21U RNA-dependent, epigenetic memory is further explored by Ashe *et al.* [3] and

Shirayama *et al.* [4]. In related studies, these two groups describe a phenomenon of heritable transcriptional and post-transcriptional silencing initiated by piRNAs. This pathway is also triggered by exogenous RNAi, which likewise engages the WAGO 22G RNA pathway [3]. Trans-generational silencing is observed under diverse circumstances. Shirayama *et al.* show that single-copy transgenes that include lengthy foreign sequences show permanent, PRG-1-dependent silencing that they call RNA-induced epigenetic silencing [4]. Ashe *et al.* demonstrate heritable epigenetic silencing of a single-copy 21U RNA target transgene as well as a transgene targeted by canonical exogenous RNAi [3]. Trans-generational silencing requires an intact WAGO 22G RNA response, including genes involved in nuclear RNAi, and correlates with *de novo* production of filial 22G RNAs. Chromatin factors are also necessary for trans-generational silencing, solidifying a connection between chromatin modification and epigenetic inheritance of silencing [3,4]. Heritably silenced single-copy alleles show enrichment for H3K9me3, a histone mark associated with silenced chromatin [4]. This finding correlates with recent work showing that exogenous RNAi of diverse endogenous loci results in trans-generational silencing that requires the WAGO 22G RNA pathway and results in H3K9me3 accumulation [9,10].

Study of transgenic *C. elegans* is greatly confounded by germline silencing. While the advent of single-copy transgene insertion has revolutionized *C. elegans* transgenesis, even these non-repetitive transgenes can be subject to piRNA-dependent silencing [4]. How, therefore, does silencing machinery recognize self versus nonself? The studies discussed here propose a memory of self genomically encoded in mismatch-tolerant piRNAs. Presumably, piRNA sequences, constrained only by selection against sequences that silence mRNAs, evolve rapidly, enabling targeting of diverse foreign genetic material [1]. An unpaired DNA silencing response may also aid in recognition of foreign sequences, as stable silencing of a piRNA sensor transgene was achieved when present in a heterozygous state over multiple generations [3].

There is, however, competition between the silencing and licensing signals. Endogenous genes targeted by exogenous RNAi generally recover expression after several generations, and even low-copy transgenes containing foreign DNA may become resistant to permanent silencing after propagation for years [4]. Continued propagation of foreign DNA seems to confer self-identity and thus unchecked expression; this process is likely enhanced by experimental selection by investigators for transgenic animals that maintain robust transgene expression. Intriguingly, silenced single-copy *gfp* transgenes can be activated in *trans* by the presence of ancient,

licensed transgenes carrying *gfp* [4]. The agents responsible for this antagonism may well be CSR-1 22G RNAs. Thus, *C. elegans* may distinguish self from nonself through piRNA-mediated surveillance and the activities of competing, complementary siRNA pathways.

Questions remain regarding the function of *C. elegans* piRNAs. What are the consequences of loss of 21U RNAs over many generations? How is genome integrity affected by desilencing of the suite of elements regulated by PRG-1, only a minority of which are transposons? What other factors influence the outcome when silencing and licensing programs clash? Importantly, are analogous epigenetic programs somehow enacted in higher organisms, which lack the arsenal of Argonaute proteins and RNA-dependent RNA polymerases that generate *C. elegans* siRNAs? And finally, whole areas of piRNA biology remain largely uncharted - most notably, primary piRNA biogenesis - and await further study in *C. elegans* and higher organisms.

Abbreviations

GFP, green fluorescent protein; piRNA, Piwi-interacting RNA; RNAi, RNA interference; siRNA, small interfering RNA; WAGO, worm Argonaute.

Competing interests

The authors declare that they have no competing interests.

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