

piRNA Rules of Engagement

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piRNAs are known to silence transposable elements, but not all piRNAs match transposon sequences. Recent studies from Shen et al. (2018) and Zhang et al. (2018) identify rules for piRNA target recognition in *Caenorhabditis elegans*. Permissive pairing rules allow targeting of essentially all germline mRNAs, while protective mechanisms prevent silencing self-genes.

Germ cells are under constant attack by foreign invaders, such as transposons with the ability to self-replicate and take up residence at new locations in the genome. In animals, a major line of defense against foreign genes involves PIWI proteins and their associated Piwi-interacting RNAs (piRNAs) (Iwasaki et al., 2015). Although their role as master regulators of transposons and pseudogenes in the germline is well characterized, additional roles for piRNAs in germ cells, as well as in somatic cells, such as neurons (e.g., Kim et al., 2018), have recently come to light. *C. elegans* contains thousands of distinct piRNAs, each produced from its own autonomous transcript (Iwasaki et al., 2015). Unlike in insects and mammals, most piRNAs in *C. elegans* lack homology to transposons and rarely interact with perfect complementarity to target mRNAs (Lee et al., 2012). Thus, identifying the targets of *C. elegans* piRNAs has proved challenging. Now, two independent studies published in *Cell* (Shen et al., 2018) and *Science* (Zhang et al., 2018) identify targeting rules of the piRNA pathway and uncover features that help to distinguish self-genes from non-self invaders in the worm.

Many organisms, including flies, fish, and mammals, use an elaborate mechanism, termed the ping-pong cycle, to amplify the piRNA signal (Iwasaki et al., 2015). Although an analogous piRNA amplification mechanism is absent in *C. elegans*, piRNAs nonetheless propagate the silencing signal via a specialized class of small interfering RNAs (siRNAs) called 22G-RNAs. piRNA targets are handed off by the lone *C. elegans* PIWI protein, PRG-1, to a complex containing an RNA-dependent RNA polymerase

that spawns out 22G-RNAs antisense to the mRNA target. Although 22G-RNAs provide a signature for piRNA targeting, there are additional triggers for 22G-RNA formation, and thus they are not always a reliable readout for piRNA activity. Furthermore, once a piRNA initiates silencing, the downstream 22G-RNAs provide a memory of targeting that can be transmitted from one generation to the next in absence of the piRNA trigger (Iwasaki et al., 2015). This phenomenon likely explains why loss of piRNAs has only a modest impact on germline development in the worm, whereas in many other animals it leads to sterility. piRNA target identification is further complicated by the 22G-RNA memory of their activity, because the impact of loss of *piwi/prg-1* on gene expression is at least partially masked by epigenetic mechanisms.

Using complementary approaches, Shen et al. (2018) and Zhang et al. (2018) set out to identify how piRNAs engage their targets in *C. elegans*. Shen et al. (2018) analyzed, transcriptome-wide, the targets of PIWI/PRG-1 using a method in which the PRG-1-piRNA-mRNA complexes were co-immunoprecipitated and the associated RNAs ligated to one another and subjected to high-throughput sequencing (CLASH). piRNA-mRNA hybrids captured by the method allowed for identification of direct interactions between piRNAs and their mRNA targets. Surprisingly, piRNAs were found to interact with essentially all germline mRNAs. Two approaches were then used to analyze base-pairing between piRNAs and target mRNAs. The first approach involved analysis of the hybrid data to identify pairing patterns between the piRNAs and associated mRNA fragments. In the second approach, an

endogenous piRNA locus was edited using CRISPR/Cas9 to produce a synthetic piRNA targeting a GFP transgene. Mutations were then introduced into the synthetic piRNA sequence to assess which positions along the piRNA were required for target recognition. A similar GFP synthetic piRNA-based approach was used by Zhang et al. (2018). Additionally, to explore endogenous targeting rules, Zhang et al. (2018) deleted an endogenous piRNA locus or, conversely, introduced a synthetic piRNA targeting endogenous genes and used the loss or gain, respectively, of 22G-RNAs as a readout for piRNA activity. Together, these experiments revealed that *C. elegans* piRNA target recognition is strikingly similar to that of miRNAs: pairing in the seed sequence (positions 2–8 relative to the 5' end of the piRNA or miRNA) is the primary determinant of target recognition. Seed pairing, however, is not sufficient. Additional base-pairing outside of the seed is also important, particularly near the 3' end of the piRNA (positions 14–19), which overlaps with a region important for supplementary pairing by miRNAs (positions 13–16) (Bartel, 2009) (Figure 1A).

In insects and mammals, canonical piRNAs interact with perfect complementarity to their targets, similar to the way siRNAs engage their targets, often deriving from the same or related genes to those that they target (Iwasaki et al., 2015). Pachytene piRNAs in mice, however, do not appear to come from transposons and instead target a broad range of mRNAs, also using miRNA-like pairing rules, to affect widespread mRNA elimination in spermatids (Gou et al., 2014). Some *Drosophila* piRNAs also interact with non-transposon mRNAs through



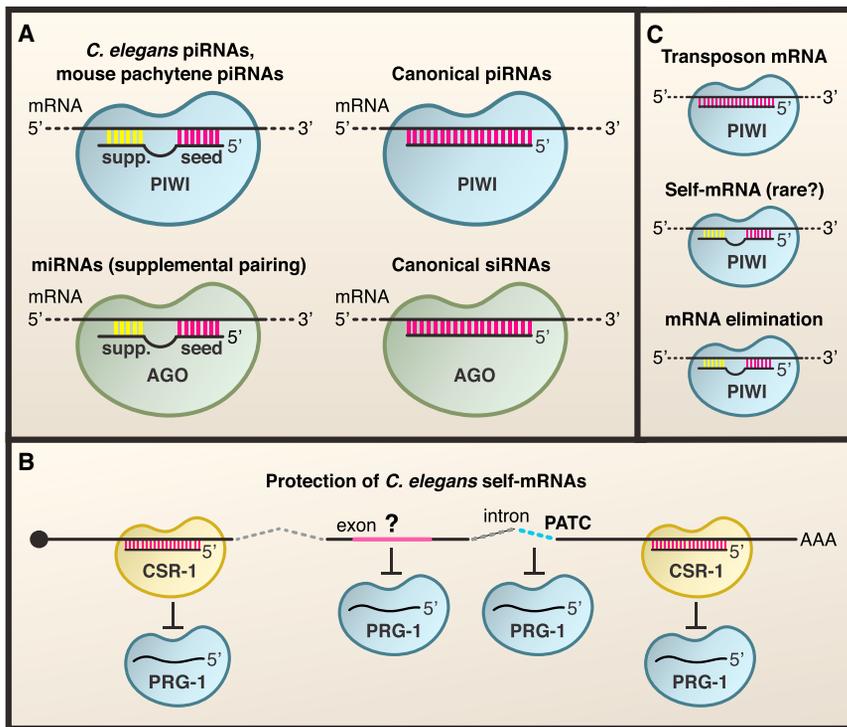


Figure 1. Target Recognition and Regulatory Roles of piRNAs

(A) Canonical piRNAs and siRNAs target mRNAs with perfect complementarity, whereas some piRNAs, similar to miRNAs, require only partial complementarity, particularly in the seed sequence (positions 2–8). Supplementary pairing (supp.) near the 3' end is also important in some instances. (B) *C. elegans* safeguards against silencing of self-mRNAs via *trans*-acting factors (CSR-1) and *cis*-acting sequences (unknown exonic sequences and intronic A_n/T_n clusters, PATC). (C) piRNAs have essential roles in regulating transposons, typically through perfect base-pairing, and have less-understood roles in regulating self-genes and in mRNA elimination, often via imperfect pairing.

imperfect base-pairing (Iwasaki et al., 2015). Thus, the piRNA pairing rules identified in *C. elegans* may be to some extent conserved across animals.

Given the promiscuousness of *C. elegans* piRNAs, how do beneficial self-genes evade silencing? The majority of mRNAs expressed in the germline are also targeted by a distinct 22G-RNA pathway involving the worm-specific Argonaute CSR-1 (Iwasaki et al., 2015). CSR-1 targets tend to be expressed rather than silenced, pointing to a protective role of the pathway that acts in opposition to piRNAs. Indeed, by repeating their PIWI/PRG-1 CLASH experiments in a *csr-1* conditional mutant background, Shen et al. (2018) showed that CSR-1 functions upstream to limit PRG-1 binding to expressed genes. It is unclear how a gene goes about obtaining

protection by CSR-1. However, CSR-1 is not the only safeguard against silencing. Zhang et al. (2018) discovered that periodic adenine/thymine clusters (PATCs) found in the introns and promoters of some germline genes (Frøkjær-Jensen et al., 2016) also confer resistance to piRNAs. Another recent study (Seth et al., 2018) identified a protective role for unknown sequences within the coding regions of some endogenous genes. Thus, both *trans*-acting factors, such as CSR-1, and *cis*-acting intrinsic sequences can safeguard against piRNAs (Figure 1B).

In addition to silencing non-self-genes, Shen et al. (2018) and another study from the Mello group in this issue of *Developmental Cell* (Tang et al., 2018) identified a role for piRNAs in fine-tuning gene expression to control sex determi-

nation in *C. elegans*. Interestingly, piRNAs also regulate sex determination in silkworms (Iwasaki et al., 2015). In both species, specific piRNAs regulate genes involved in dosage compensation. Thus, despite differences between piRNA pathways in worms and other animals, the pathways appear to share many common roles in gene regulation and genome defense (Figure 1C). The extent to which the promiscuous nature of *C. elegans* piRNAs is shared between other species remains to be determined.

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