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piRNAs — guardians of the germline

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By 2001, RNA interference — the sequence-specific inhibition of gene function by homologous double-stranded RNA (dsRNA) — had been observed in a wide range of eukaryotes. The phenomenon had been linked to transposon-repression and anti-viral defence, especially in plants, but the full spectrum and functional relevance of this mechanism in animals was unknown. Then, Gvozdev and colleagues demonstrated homology-dependent silencing of testis-specific Stellate genes mediated by small RNAs generated from both strands of the *Suppressor of Stellate* repeat locus in the *Drosophila melanogaster* male germline. Interestingly, relief of *Stellate* silencing also led to de-repression of retrotransposons and other genomic tandem repeats. This work marked the discovery of piRNAs, although it would take another five years until they gained this name.

In 2006, four studies used RNA sequencing to identify a class of 26–30-nucleotide-long RNAs that specifically associated with mammalian PIWI-clade Argonaute proteins in mouse, rat and human male germ cells — hence the name 'piRNAs', for PIWI-interacting RNAs. PIWI proteins had been genetically linked to germ cell and stem cell maintenance and to meiosis, although their biochemical function remained unknown.

At the time, the related AGO-clade subfamily of Argonaute proteins had been shown to act in RNA interference and microRNA-mediated gene regulation using 21–22-nucleotide RNAs as targeting guides. However, piRNAs seemed distinct. For example, there was little evidence for overlapping complementary RNAs or potential fold-back structures, suggesting that piRNAs might not be derived from dsRNA precursors. Zamore and colleagues then provided evidence that Dicer endonuclease activity — which is essential for microRNA and short interfering RNA biogenesis — was dispensable for piRNA generation in *D. melanogaster*. This finding led to the realization that piRNAs represented a novel class of Dicer-independent small silencing RNAs.

Yet, the mechanism governing piRNA biogenesis remained elusive until 2007, when two groups independently described an intricate piRNA amplification loop, the so-called 'piRNA ping-pong cycle'. Sequencing of small RNAs associated with all three *D. melanogaster* PIWI-clade proteins — Piwi, Aubergine (Aub) and Argonaute 3 (Ago3) — revealed that each protein binds to specific piRNA populations: Piwi-bound and Aub-bound piRNAs were mainly antisense to transposon sequences and harboured a strong preference for having a 5′ terminal uridine. Ago3-associated piRNAs, on the other hand, were biased for transposon sense strands and had a preference for an adenine at nucleotide 10, with no preference for uridine at the 5′ end. Most strikingly, the 5′ ends of Ago3-bound piRNAs were typically offset by precisely ten nucleotides from the 5′ ends of complementary Aub-bound piRNAs. This suggested a model in which an antisense piRNA, complexed with Aub, would recognize and cleave a sense transposon transcript. The cleaved product would then be processed into an Ago3-bound sense piRNA, which could seek out target transcripts. Ago3-directed cleavage triggers generation of the original antisense piRNA, capable of both silencing the target element and further amplifying the response. The majority of the initial antisense precursor RNAs were derived from discrete genomic loci, so-called 'piRNA clusters', which are comprised mainly of defective transposon sequences in the fly.

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Together, these studies established the piRNA pathway as a transposon surveillance mechanism. Although a plethora of later studies provided additional exciting insights into the piRNA pathway and its function as a safeguard of genome integrity and fertility, many questions regarding the precise molecular mechanisms of piRNA generation and their diverse silencing functions are still unanswered and the subject remains an active area of research.

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MILESTONE STUDIES Aravin, A. A. et al. Double-stranded RNA-mediated silencing of genomic tandem repeats and transposable elements in the *D. melanogaster* germline. *Curr. Biol.* **11**, 1017–1027 (2001) | Girard, A. et al. A germline-specific class of small RNAs binds mammalian Piwi proteins. *Nature* **442**, 199–202 (2006) | Aravin, A. et al. A novel class of small RNAs bind to MILI protein in mouse testes. *Nature* **442**, 203–207 (2006) | Lau, N. C. et al. Characterization of the piRNA complex from rat testes. *Science* **313**, 363–367 (2006) | Grivna, S. T. et al. A novel class of small RNAs in mouse spermatogenic cells. *Genes Dev.* **20**, 1709–1714 (2006) | Vagin, V. V. et al. A distinct small RNA pathway silences selfish genetic elements in the germline. *Science* **313**, 320–324 (2006) | Gunawardane, L. S. et al. A slicer-mediated mechanism for repeat-associated siRNA 5′ end formation in *Drosophila*. *Science* **315**, 1587–1590 (2007) | Brennecke, J. et al. Discrete small RNA-generating loci as master regulators of transposon activity in *Drosophila*. *Cell* **128**, 1089–1103 (2007).