piRNAs in the Germ Line
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REVIEW

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Haifan Lin

Small noncoding RNAs have emerged as potent regulators of gene expression at both transcriptional and posttranscriptional levels. Recently, a class of small RNAs that interact with Piwi proteins has been discovered in the mammalian germ line and *Drosophila*. These Piwi-interacting RNAs (piRNAs) represent a distinct small RNA pathway.

The importance of small noncoding RNAs as regulators of transcription, RNA stability, and translation is becoming increasingly evident (*1*). The recently discovered piRNAs differ from the known small interfering RNAs (siRNAs) or microRNAs (miRNAs) in several ways (*2*–*6*). First, piRNAs interact with the Piwi, but not the Argonaute (Ago), subfamily of the Piwi-Ago family proteins. Mouse Piwi (Miwi) is required for piRNA biogenesis and/or stability (*7*), whereas mouse Ago2 is required for the siRNA pathway. Second, piRNAs are 24 to 31 nucleotides (nt) instead of ~21 nt. Third, piRNAs consist of more than 50,000 different species, in contrast to several hundred species of miRNAs. Fourth, most piRNAs match to the genome in clusters of 20 to 90 kbs in a strand-specific manner, with each cluster likely representing a long single-stranded RNA precursor or, more often, two nonoverlapping and divergently transcribed precursors (Fig. 1A) (*2*). In contrast, siRNAs and miRNAs are derived from double-stranded and short-hairpin RNA precursors, respectively. Finally, some piRNAs might positively regulate mRNA stability and translation (see below), in contrast to the negative effect of the siRNAs and miRNAs.

In *Drosophila*, some piRNAs have been called repeat-associated siRNAs ("rasiRNAs") (*3*, *4*, *6*). However, unlike siRNAs, these rasiRNAs bind to Piwi, Aubergine (Aub), and Ago3—three Piwi subfamily proteins, but not to Ago subfamily proteins. Moreover, their production requires neither Dicer-1 nor Dicer-2, which generate miRNAs and siRNAs, respectively (*3*). Thus, by definition, these rasiRNAs are piRNAs.

How are piRNAs produced? They are likely produced from long single-stranded precursors by yet-to-be-identified endonucleases. In *Drosophila*, Piwi, Aub, and Ago3 could be such endonucleases because they have slicing activity (*4*, *6*). For some transposon-derived piRNAs in *Drosophila*, an additional "Ping-Pong" mechanism might be involved in accelerating their processing from precursors (*5*, *6*) (Fig. 1B). This is possible because the Aub- and Piwi-associated piRNAs match the antisense strand of DNA, showing a strong bias for a uracyl (U) at the 5′ ends; yet Ago3-associated piRNAs match the sense strand and complement Aub- and Piwi-associated piRNAs in their first 10 nucleotides, showing a conserved adenine (A) at position 10. Thus, the Ago3-piRNA complex might guide and cleave the 5′ ends of Aub- and Piwi-associated piRNAs, whereas the Aub-Piwi–piRNA complex might guide and cleave the 5′ ends of Ago3-associated piRNAs (Fig. 1B).

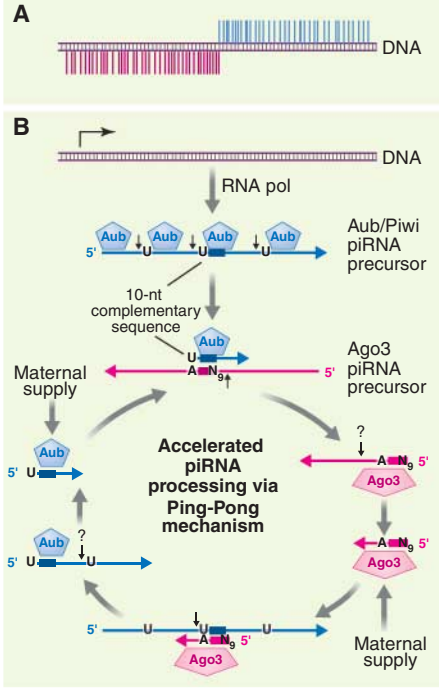

Fig. 1. (**A**) A bidirectional piRNA cluster. (**B**) A proposed piRNA biogenesis pathway. The resulting piRNA-protein complexes then regulate gene expression at the epigenetic or posttranscriptional levels (not shown). N_9, the 9th nucleotide from A_{10} in Ago3-piRNA; the question mark (?), other nuclease.

What are the roles of piRNAs in the germ line? The genomic distribution of piRNAs and the function of their Piwi proteins provide important clues to this question. Most piRNAs map to unique sites in the genome, including intergenic, intronic, and exonic sequences. For example, only 17 to 20% of mammalian piRNAs map to annotated repeats, including transposons and retrotransposons (*2*). Thus, piRNAs may have diverse functions from epigenetic programming and repressing transposition to posttranscriptional regulation. Each of these speculated roles is supported by the known function of their partner Piwi proteins. For instance, Piwi is an epigenetic regulator (*8*). It colocalizes with Polycomb group (PcG) proteins to cluster PcG response sequences in the genome (*9*). Thus, some PIWI-associated piRNAs may be involved in epigenetic regulation. In fact, gypsy piRNAs in *Drosophila* have been shown to downregulate sense gypsy transcripts (*10*). In addition, Piwi prevents retrotransposon transposition in the testicular germ line (*11*), which suggests a second role of Piwi-associated piRNAs. Furthermore, Piwi and Aub appear to positively regulate translation in early *Drosophila* embryos (*12*, *13*). Similarly, Miwi promotes the stability (*14*) and, likely, translation of its target mRNAs (*15*). Thus, some piRNAs may have a third role in positively regulating translation and mRNA stability.

The discovery of piRNAs reveals a new dimension of gene regulation. Future studies on the biogenesis and the potentially diverse functions of piRNAs should substantially advance our understanding of gene regulation that defines the germ line.

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