Minireview

Epigenetic transmission of piRNAs through the female germline
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Abstract

In Drosophila, small RNAs bound to Piwi proteins are epigenetic factors transmitted from the mother to the progeny germline. This ensures ‘immunization’ of progeny against transposable elements.

The silencing of mobile elements in germ cells depends on a distinct class of RNAs, the 24-to-30 nucleotides long, Piwi-interacting RNAs (piRNAs), which are associated with Argonaute proteins of the Piwi subfamily [1,2]. These small RNAs guide the cleavage of complementary RNA, or target DNA for methylation, and protect the germline against mutations caused by active transposons [2-4]. In Drosophila, the three Piwi proteins expressed in the germline are Piwi, Aubergine (Aub) and Argonaute3 (Ago3). Work from the laboratory of Gregory Hannon (Brennecke et al. [5]) published recently in Science now provides evidence that piRNAs bound to Piwi proteins serve as epigenetic factors that are transmitted through the maternal germline. By piRNA sequencing, Hannon and colleagues show that the maternally deposited piRNAs loaded onto Piwi proteins affect transposon suppression in a heritable fashion, and that these piRNAs can serve as maternal suppressors of hybrid dysgenesis.

This study explains the nature of maternal effects that were noticed long ago in crosses between Drosophila strains that differ in the presence of particular transposable elements, the so-called dysgenic crosses. Hybrid dysgenesis is observed in the female progeny of crosses between males that harbor certain active transposable elements and females that lack functional elements. It is associated with mutations, chromosome aberrations and female sterility, and is attributed to mobilization in the dysgenic progeny of the paternally inherited transposons [6,7]. The genetically identical progeny of the reciprocal cross is fertile, strongly suggesting transmission of epigenetic transposon suppressors through the maternal germline. Experimental data suggested that these maternal effects are mediated by RNA [8]. The first evidence for the role of maternally transmitted short RNAs in transposon silencing was obtained in Drosophila virilis. Hybrid dysgenesis in D. virilis is characterized by mobilization of several families of transposable elements, including retrotransposons of the Penelope family. RNAs derived from these retroelements in the D. virilis genome were shown to contribute to maternal repression of Penelope [9].

Germ cells are specified by a special region of cytoplasm, the germplasm, which is localized at the posterior pole of the oocyte. Germplasm-specific structures, the polar granules, are essential for germline determination and are rich in RNAs and RNA-binding proteins. Drosophila Aub and Piwi have been shown to be maternal components of the polar granules [10,11]. The identification of Piwi proteins as components of the germplasm led to the realization that short RNAs might physically migrate from the mother to the germline of her daughters.

Mechanisms of piRNA production
Before discussing the work of Brennecke et al., we shall briefly give some background on the mechanism of piRNA production. In Drosophila, most piRNAs are derived from the transcripts of mobile elements. Transposable element repression is provided by two classes of piRNAs: ‘primary piRNAs’ encoded by specific genomic loci (‘master loci’), and
secondary piRNAs' generated by a 'ping-pong' amplification mechanism that reproduces the original piRNAs [1,4]. In the fly, most primary piRNAs match defective transposons and derive from discrete pericentromeric and telomeric heterochromatic loci enriched in damaged repeated sequences. Primary piRNAs are believed to be processed from long single-stranded transcripts corresponding to these loci. The processing mechanism, as yet unknown, is independent of Dicer [12] but might involve Piwi proteins.

In contrast, the subsequent ‘ping-pong’ amplification of primary piRNAs is well documented [1,4]. Briefly, piRNAs corresponding to the antisense strand of the retrotransposon preferentially bind Piwi/Aub protein and show a strong bias for uridine at the 5’ end; sense piRNAs, by contrast, associate with Ago3 and show enrichment for adenine at position 10. Aub/Piwi cleaves transposon mRNA between positions 10 and 11 of the guide antisense piRNA, generating the 5’ end of a sense Ago3-associated piRNA. The mature sense piRNA is capable of guiding cleavage of the antisense transposon transcript, thus creating additional copies of the original antisense piRNA. This pathway generates a pool of piRNAs that can guide degradation of retrotransposon mRNA. The anti-mobile element activity of Piwi proteins and their associated small RNAs is confirmed by the retrotransposon activation observed in mutants lacking Piwi proteins [13,14]. Transposon mobilization in the germline is believed to induce DNA breaks that activate the DNA-damage response, resulting in defects in progression through meiosis [15]. This phenotype always accompanies piRNA pathway mutations.

**A role for piRNAs in I-element-mediated hybrid dysgenesis**

The study of Brennecke et al. [5] focuses on two well characterized dysgenic systems in *D. melanogaster*, I-R and P-M, relating to derepression of the non-LTR (long terminal repeat) retrotransposon I and the DNA transposon P, respectively. Crosses of I (inducer) males carrying active I-elements to R (reactive) females lacking functional I-elements yield dysgenic daughters (SF) with a sterility syndrome and elevated mutation rates due to mobilization of the I-element. These traits are not seen in the female progeny of the reciprocal cross (termed RSF) (Figure 1). To elucidate the nature of the maternally transmitted determinants responsible for this effect, Brennecke et al. [5] sequenced short RNAs from the ovaries of I and R females, from 0-2-hour embryos resulting from dysgenic and nondysgenic crosses, and from ovaries of SF and RSF females. This revealed a similarity between the short RNA populations from maternal ovaries and early embryos (in which zygotic transcription is not yet activated), clearly indicating the maternal origin of the embryonic small RNAs. Aub- and Piwi-associated piRNAs, and to a lesser extent Ago3-bound piRNAs, were found in both maternal ovaries and embryos, consistent with the observed deposition of Piwi and Aub in early embryonic germ cells (pole cells) [5]. The number of piRNAs in maternal ovaries was comparable with that in the early embryos, underlining the large scale of transmission of this maternal information.

A comparison of ovarian piRNA populations between I and R strains revealed a strong similarity in content. The most pronounced difference was the amount of I-specific piRNA, which was 20-fold lower in the R strain than in the I strain [5]. This difference is maintained in the corresponding embryonic libraries. These data clearly indicate that it is the piRNAs bound to Piwi proteins that provide maternal inheritance of transposon suppression, and that this inheritance is realized through direct transmission of maternal piRNAs via the germlasm that is incorporated into the embryonic germ cells.

Brennecke et al. [5] go on to uncover the reason for the dysgenic syndrome manifested in SF daughters. Genomes of SF and RSF flies are identical, and piRNA levels corresponding to many transposable elements are known to be intermediate in SF and RSF ovaries when compared with I and R ovarian libraries [5]. In the RSF females, the number of I-specific piRNAs is just half that of their I mother as a result of ‘dilution’ of the inducer genome by the R genome lacking functional I-elements (Figure 1). However, I-specific
piRNAs are much less abundant (namely, sevenfold) in the ovaries of SF daughters than in the ovaries of RSP females (Figure 1). This low level of piRNAs allows the mobilization of the paternally inherited I-elements and results in sterility - in other words, the dysgenic syndrome.

Despite the absence of a functional I-element in R strains, their genomes contain remnants of ancestral I-related elements located in pericentromeric heterochromatin, including the 42AB locus, which was described previously as one of the master loci [1]. Changes in expression of the I-related damaged copies have been shown to correlate with the reactivity level of R females, which indicates a substantial role for these defective copies in the epigenetic mechanism of transposon suppression [16]. I-specific piRNAs from the ovaries of I strain exhibit a ping-pong signature (adenosine in position 10 of sense piRNAs and 5' uridine in antisense piRNAs). Notably, Brennecke et al. [5] find that most of the sense I-specific piRNAs are derived from modern copies, whereas sequences of antisense piRNAs deviate from them and correspond to ancestral heterochromatic I-elements. A substantial portion of these piRNAs are uniquely matched to 42AB I-related copies. These results confirm the previous observation that the ping-pong cycle takes place between the transcripts of active transposons and heterochromatic piRNA loci [1].

In previous studies of P-M dysgenesis, it was noticed that active I-elements or homologous transgenes inserted in the subtelomeric region could repress, in the female germline, active P-elements or homologous transgenes [18,19], and that this effect is sensitive to mutations in piRNA pathway genes [20]. Notably, the subtelomeric regions were previously characterized as master loci producing large

piRNAs in P-element-mediated hybrid dysgenesis

Brennecke et al. [5] also studied P-M hybrid dysgenesis, and their results provide perhaps the most pronounced indication so far of a role for maternally inherited piRNAs in the initiation of biogenesis of secondary piRNAs in dysgenic crosses. When P males (containing active P-elements) are crossed with M females lacking such elements, the resulting progeny (GD) exhibit hybrid dysgenesis [6]. Analysis of P-specific piRNAs in the ovaries of P and M mothers and their 0-2-hour embryos by Brennecke et al. [5] revealed strong maternal deposition of these RNAs in the P strain (Figure 2, the \( \text{Har} \times \text{Har} \) cross). M mothers and their early embryos lacked such piRNAs (Figure 2, the \( \mu^{\text{1118}} \times \text{Har} \) cross), and so the daughters of an M female crossed to a P male exhibit severe dysgenic syndrome.

In previous studies of P-M dysgenesis, it was noticed that naturally occurring single P-elements or \( P-lacZ \) transgenes inserted in the subtelomeric region could repress, in the female germline, active P-elements or homologous transgenes [18,19], and that this effect is sensitive to mutations in piRNA pathway genes [20]. Notably, the subtelomeric regions were previously characterized as master loci producing large

\[ P \text{-element-specific piRNAs} \]

from the ovaries of F1 females

\[
\begin{align*}
\text{P-element} & \quad \text{Truncated P-element} \\
\text{Har} & \quad \text{Har} \\
\text{Lk} & \quad \text{Har} \\
\text{NA} & \quad \text{Har} \\
\text{w1118} & \quad \text{Har} \\
\text{0} & \quad \text{Har} \\
\end{align*}
\]
numbers of piRNAs [1]. Brennecke et al. [5] analyzed
P-specific piRNAs in the mothers and early embryos of two
M strains, NA and Lk, containing a single defective or two
full-length P-element copies, respectively, in the
subtelomeric repeats of the X chromosome, and they
revealed maternally deposited P-specific piRNAs. Most
probably, the P-elements in these strains are transcribed and
processed as part of the original subtelomeric piRNA locus.
In the ovaries of NA and Lk dysgenic daughters, which
showed less pronounced dysgenesis, a strong signature of
the ping-pong amplification cycle was revealed. It is
noteworthy that piRNAs corresponding to a P-element
fragment from the NA strain were amplified in the ovaries of
dysgenic daughters despite the presence of full-size P-
elements in their genomes (Figure 2). Two P-elements in the
Lk strain produce enough piRNA to suppress the activity of
the 30-50 genomic copies of the strong P strain. Thus,
maternal small RNAs are essential for priming piRNA
amplification in the progeny.

The study of Brennecke et al. [5] thus has unequivocally
documented the maternal transmission of piRNAs and their
role in suppressing hybrid dysgenesis. In mice, transposon-
specific piRNAs cause methylation of transposon promoter
DNA in the germline [2], and Ronsseray and colleagues [20]
have hypothesized that maternally inherited small RNAs
might modify the chromatin structure of transposable
elements in Drosophila, resulting in transposon silencing.
However, further studies will be necessary to elucidate the
complete pathway of transposon suppression in the
Drosophila germline.

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