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 retroelements of this family 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.

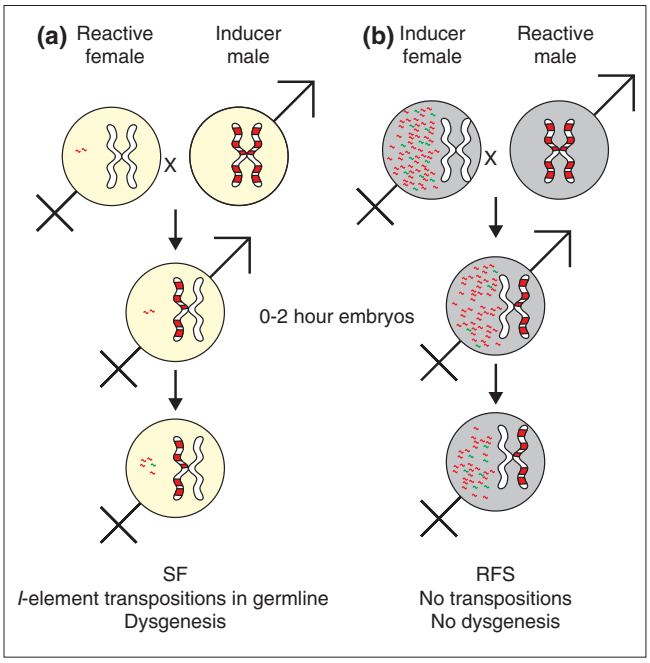

Figure 1
The I-R hybrid dysgenic system. Crossing schemes represent **(a)** dysgenic and **(b)** non-dysgenic crosses. Despite identical genomes in SF and RSF females (chromosomes depicted schematically), the pools of their ovarian *I*-specific piRNAs (short wavy lines) are different. The approximate ratios of *I*-specific piRNAs in the ovaries of I and R mothers, in 0-2 hour embryos, and in the ovaries of SF and RSF daughters are shown. piRNAs that are antisense with respect to the *I*-element are in red; sense ones are in green.

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 germplasm 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 RSF 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 the R strain lacking active *I*-elements, no ping-pong amplification occurs. However, most of the *I*-specific piRNAs present at a low level in the ovaries of the R strain were also derived from the 42AB locus [5]. Although SF females fail to suppress paternal *I*-element activity, the appearance of the sense piRNAs corresponding to active elements in their ovaries clearly indicates that the maternal antisense piRNAs transmitted from the R mother do activate biogenesis of secondary *I*-specific piRNAs. Ten generations are enough to repress the enhanced activity of the invading *I*-element in dysgenic crosses. During this period, the amount of *I*-specific piRNAs is adjusted to a level sufficient for the activity of the *I*-element to be suppressed, and the R strain turns into an I strain. Brennecke *et al.* [5] have underlined the role of maternal antisense piRNAs in transposon silencing, but it remains unclear why transgenes containing transcribed fragments of the *I*-element in sense and antisense orientations and introduced into the R strain exert similar effects on *I*-element suppression in SF daughters [17].

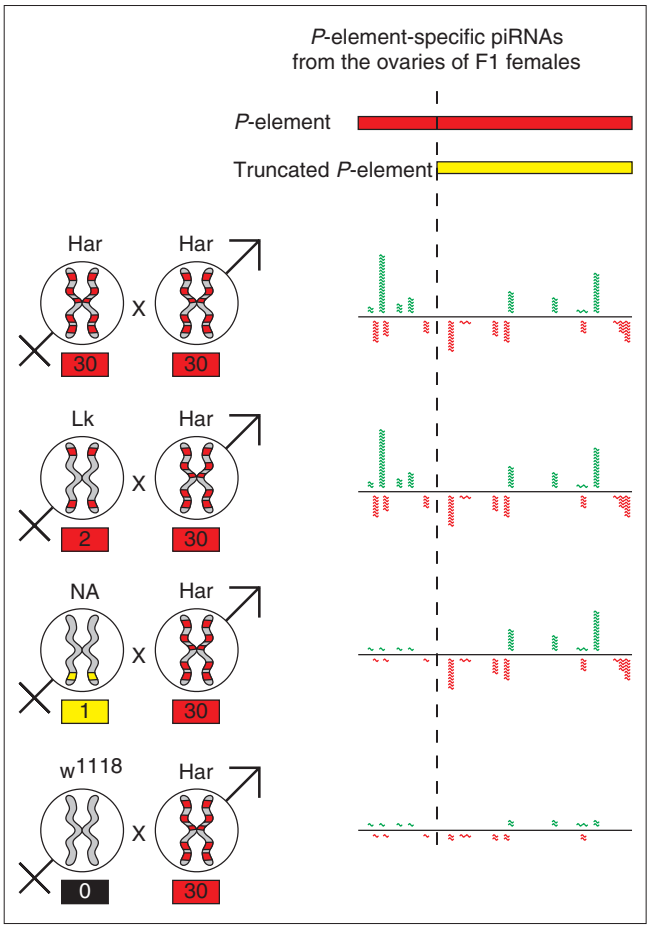

Figure 2
Maternal piRNAs suppress hybrid dysgenesis in P-M crosses. Crossing schemes on the left represent crosses of males of a strong P strain (*Har*) to females from different strains: w^{1118} is an M strain lacking *P*-elements; *Lk* carries two *P*-element copies in the subtelomeric region; *NA* possesses a truncated *P*-element in the subtelomeric region of the X chromosome. The numbers in the rectangles beneath each cross are the *P*-element copy number per haploid genome. The *P*-specific piRNA density across the *P*-element in the ovaries of F1 daughters of each cross is depicted schematically on the right. piRNAs (wavy lines) that are antisense with respect to the *P*-element are in red; sense ones are in green. The truncated *P*-element in the *NA* strain is shown at the top in relation to a full-length *P*-element.

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 *Har* × *Har* cross). M mothers and their early embryos lacked such piRNAs (Figure 2, the w^{1118} × *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

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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