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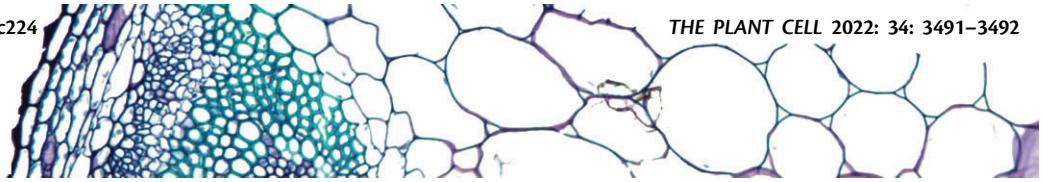

Keep calm and methylate on: Ovule small RNAs methylate protein-coding genes in trans related with fertility

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In Brief

Is it our most important role in life to have kids? As a species, and from a biological point of view, success is measured by the generation of offspring capable of surviving (Darwin, 1859). For organisms with sexual reproduction, gametes are the valuable end-product that creates the next generation. Therefore, the information in germ cells needs to be tightly regulated so the characteristic blueprint of each species lives on in the offspring. Genome stability of germ cells is then critical. Transposons can create genome instability when they change or duplicate their position in the genome. Transposon DNA methylation, triggered by 24-nt small (s) RNAs, helps to maintain genome stability by reducing transposon transcription and activity.

In the new work, **Diane Burgess, Hiu Tung Chow, and colleagues (Burgess, Chow et al., 2022)** explore the 24-nt sRNA population in ovules of *Brassica rapa*, a relative of Arabidopsis. *Brassica rapa* is especially interesting to study because, unlike Arabidopsis, the loss of the proteins involved in the production of 24-nt sRNAs results in a high rate of seed abortion (Grover et al., 2018). Using sRNAseq, RNAseq, and DNA methylation analyses, the authors found that the population of sRNAs is quite different in ovules compared to other tissues. While in leaves most 24-nt sRNAs come from transposon elements, in ovules these 24-nt sRNAs are mainly produced from transposon-free regions called siren loci (Grover et al., 2020). Their production is associated with the putative chromatin remodeler CLSY3 and one of the two copies of the PolIV subunit NRPD1. Siren loci may overlap with transposons (particularly Helitrons and hAT), but most of the 24-nt sRNA are produced from adjacent transposon-free regions and trigger methylation in cis (Figure A), suggesting that their primary role is not transposon silencing.

The sRNA-producing regions of siren loci contain fragments similar to coding genes, raising the possibility that related full-length genes are also methylated by siren sRNAs in trans. And indeed they are, despite mismatches between the sRNA and the target region (Figure B). This phenomenon is mediated by the RNA-dependent RNA polymerase RDR2 and is not specific to ovules as it continues postfertilization in seed coat and endosperm.

In the male germline, tapetum cells produce abundant 24-nt sRNAs that also trigger methylation in trans in the meiocyte (Long et al., 2021). **Burgess, Chow, and colleagues (Burgess, Chow et al., 2022)** also report trans-methylation caused by 24-nt sRNAs in female reproductive tissues, suggesting the possibility of a common mechanism for both germlines. Whether the production of sRNAs in the ovule is cell specific as in the male germline remains to be tested.

Trans-methylation in ovules can lead to transcriptional repression, especially for highly methylated genes, although other regulatory mechanisms, such as posttranscriptional gene repression, cannot be ruled out. Interestingly, multiple siren loci in Arabidopsis and *B. rapa* contain gene fragments from the same families, such as pectin methylesterases (PME). The low sequence similarity and lack of synteny between siren loci from these species suggest either convergent evolution, or maybe that conserved siren loci are rapidly repositioned in the genome. Regarding the role of siren loci, a partial reduction in the activity of a pollen-specific PME reduces fertility in Arabidopsis (Jiang et al., 2005), leading to the hypothesis that siren loci could play a key role in

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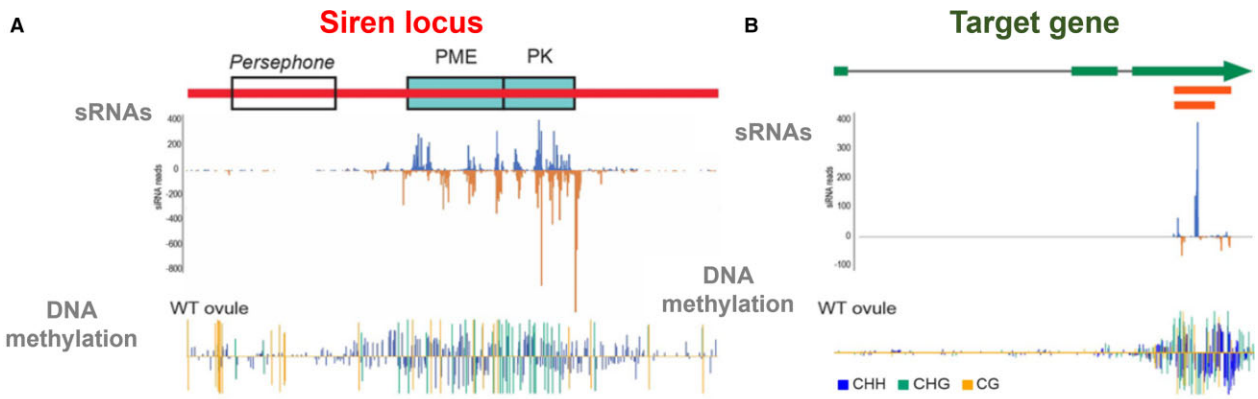

Figure A, Schematic of a siren locus that overlaps with a TE (Persephone) and two gene fragments (PME and PK). Small RNAs from siren loci along the positive (strand (blue) and negative strand (orange) and DNA methylation in CG (orange), CHG (green), and CHH (blue) being H any nucleotide except G, in wild type (WT) ovules. B, Schematic of the PME gene *A01p015570_BraROA*. Regions with high similarity to gene fragments from two different sirens are shown in orange below. Distribution of siRNAs mapping with—one to two mismatches along the positive strand (blue) and negative strand (orange), and DNA methylation in CG (orange), CHG (green), and CHH (blue) in WT ovules. Adapted from Burgess, Chow et al. (2022), Figure 3.

regulating closely related members within this large family. Indeed, a severe seed abortion phenotype has been observed in a *B. rapa* mutant lacking siren sRNAs but with normal production of canonical 24-nt sRNAs (Grover et al., 2020).

The results from **Burgess, Chow and colleagues (Burgess, Chow et al., 2022)** shed light on the complex epigenetic dynamics in the female side of plant reproduction, finding commonalities with the regulation in the male germline. In the ovule, siren loci produce abundant 24-nt sRNAs able to trigger methylation in cis and in trans on coding genes, in a process that for most plants is likely to be essential to ensure proper seed development. Mutants in tomato with reduced accumulation of 24-nt sRNAs usually display low fertility (Gouil et al., 2016), as observed for the *B. rapa* mutant mentioned above. These findings highlight the importance of sRNAs in plant development and open a new avenue to its application in crop breeding to increase fertility and seed production.

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