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RESEARCH HIGHLIGHT

RNA-directed DNA methylation and seed development: an unexpected difference between *Arabidopsis thaliana* and *Brassica rapa*

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Linked article: This is a Research Highlight about Jeffrey W. Grover *et al*. To view this article visit https://doi.org/10.1111/tpj.13910.

Transposable elements are silenced during the reproductive phase by Polymerase IV-derived small interfering RNAs (p4-siRNAs), which trigger RNA-directed DNA Methylation (RdDM) (Mosher and Melnyk, 2010). However, it was a puzzle as to how important this process was in Arabidopsis, as plants mutated in components required for RdDM had no striking phenotypes (Mosher *et al*., 2009). The highlighted paper (Grover *et al*., 2018) shows that the story is quite different in an Arabidopsis relative, *Brassica rapa*, where there are striking seed size defects in RdDM mutants when they are transmitted maternally, as shown in Figure 1.

Rebecca (Becky) Mosher, now an Associate Professor at the University of Arizona, has worked in the epigenomics area for about 10 years. She first became interested in the role of RdDM during seed development when she was a postdoc in David Baulcombe's lab. Becky knew there was slight reduction in seed weight in RdDM mutants in Arabidopsis, but the tiny Arabidopsis seeds meant it would be difficult to tease out the cause (i.e., a gametophytic or sporophytic contribution). So her initial impetus to work on *Brassica rapa* was practical: bigger seeds that would allow genetic dissection of this subtle phenotype.

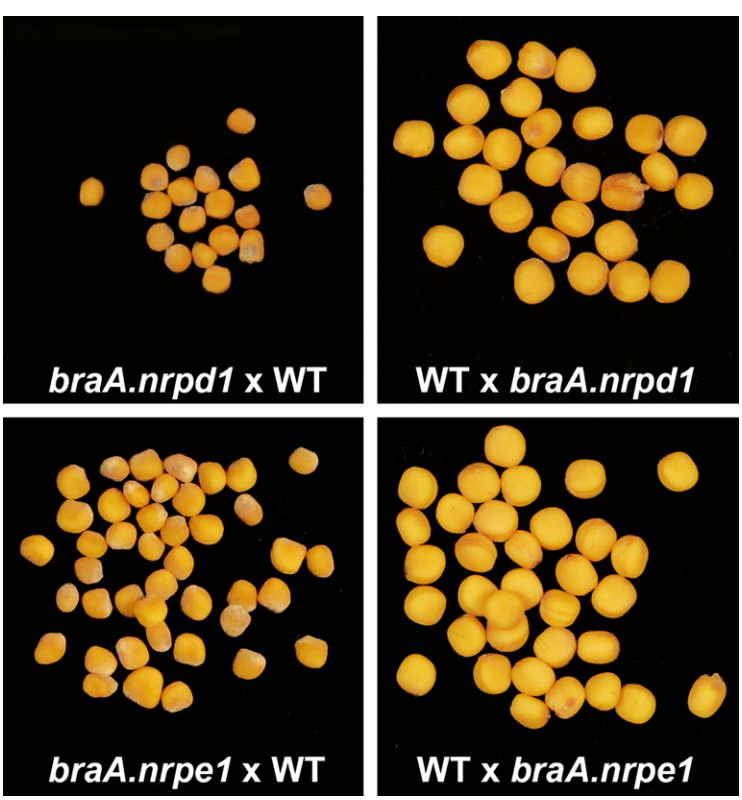

Figure 1. Mutations in the RNA-directed DNA methylation components BraA.NRPD1 and BraA.NRPE1 cause reduced seed development when they are transmitted maternally (left panels), but not paternally (right panels). Image credit: Timmy Kendall.

Co-first author Jeff Grover, a Ph.D. student, was responsible for all of the NGS sequence analysis, while co-first author Timmy Kendall, a senior scientist in the Mosher lab, was responsible for all the genetic crosses and plant dissections, as well as library preparation and RT-PCR experiments. How did the collaboration with the groups at Southern Cross University and UC-Berkeley come about? A few years ago, Becky heard a seminar by Michael Freeling, where he mentioned that the different sub-genomes of *Brassica rapa* showed differences in the amounts of 24 nucleotide siRNAs (e.g., Woodhouse *et al.*, 2014). Becky thought that having RdDM mutants in *B. rapa* might be an interesting way to test whether those siRNAs influenced genome dominance, so she invited Michael to join her NSF Plant Genome Grant to address those questions. As part of that project, Michael and Diane Burgess, an Associate Specialist in the Freeling lab, annotated the transposable elements in the R-o-18 genome. Graham King and Research Fellow Abdul Baten joined the project because they had an unpublished genome assembly of R-o-18 that proved instrumental for the analyses in Grover *et al.* (2018).

Although there are publicly available *B. rapa* genomes, i.e. for Chiifu, a Chinese cabbage type, and for FPsc (Fast Plant self-compatible), an oilseed type, there is considerable diversity between *B. rapa* varieties, especially in genomic regions that generate siRNAs. There is an extensive TILLING population available for R-o-18 (Stephenson *et al.*, 2010), from which Becky's lab identified mutants for *RDR2*, which encodes an RNA-dependent RNA polymerase, and for *NRPD1*, which encodes the largest subunit of Pol IV. They then used RNA-seq to identify loci that produce small RNAs, showing that p4-siRNAs were strongly reduced in the *nrpd1* mutant and eliminated in the *rdr2* mutant. In addition, they identified a mutant for *NRPE1*, which encodes the largest subunit of Pol V. Pol V produces transcripts that are required for p4-siRNA activity, and thus influences RdDM without eliminating p4-siRNA production.

Brassica rapa mutants lacking RdDM had smaller siliques and produced fewer viable seeds; however, those seeds that were viable grew into normal plants. Early on, seed development appeared normal, but mature siliques could have a range of seed phenotypes: aborted, shriveled, or normal but smaller. No one developmental stage was affected – it was stochastic. Reciprocal crosses (see Figure 1) showed that the maternal genotype was responsible for both the reduced seed number and for seed size/quality. To distinguish siRNA production from the paternal and maternal genomes, they carried out reciprocal interspecific crosses and sequenced siRNAs from developing seeds, showing that the paternal genome did not substantially contribute to the small RNA transcriptomes. Lastly, by genotyping progeny from heterozygotes, they showed that the seed defects are due to loss of p4-siRNA in maternal sporophytic tissues and not to loss in the gametophytes.

One hypothesis is that p4-siRNAs are required for integument development and disruption of this tissue causes defects in the growing embryo and endosperm. Alternatively, the integuments might be the source of p4-siRNAs that then function in the growing embryo and endosperm. In Arabidopsis, small RNAs are produced in the vegetative cell of the pollen grain and are then transferred to the sperm cells to silence transposons (Martínez *et al.*, 2016). An analogous situation was proposed between the endosperm and embryo (e.g. Mosher and Melnyk, 2010), and thus, it was a surprise to find that in *B. rapa* seed development depended on the maternal sporophytic genotype.

But why would sperm cells or the developing embryo need to depend on adjacent cells or tissues to generate siRNAs to silence their transposons? Becky terms this "epigenetic altruism" – the idea being that transposons that are active and therefore potentially damaging if inserted into essential genes, could be identified in a cell or tissue that has no future in the next generation; this altruistic partner would then synthesize siRNAs to help silence the transposons in their neighbor. They plan to test if the siRNAs are made in maternal sporophytic tissue and act in the endosperm or embryo; one approach to this question is to use grafting experiments between wild type and mutant plants. They are interested in determining if the seed size phenotype results from a loss of DNA methylation at many sites in the genome, or instead, if mis-expression of one or a few regulators, or perhaps even mis-expression of only one allele (i.e. the male-derived one) causes the phenotype. And lastly, they want to understand why the Arabidopsis and *B. rapa* phenotypes are so different – could the difference be related to a recent history of outcrossing in *B. rapa*, or to its recent genome duplication? The highlighted paper should stimulate interest in exploring the roles of siRNAs in seed development and should encourage others to consider experiments in *B. rapa* – unexpected findings might await.

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