Meiotic phasiRNAs – (more than) the plant version of piRNAs?

Stefanie Dukowic-Schulze[1], Anitha Sundararajan[2], Thiruvarangan Ramaraj[2], Joann Mudge[2], Wojciech Pawlowski[3], Changbin Chen[1]

[1] Department of Horticultural Science, University of Minnesota (Minnesota, USA), [2] National Center for Genome Resources (New Mexico, USA), [3] Department of Plant Breeding and Genetics, Cornell University (New York, USA)

Background

Reproductive phasiRNAs (phased small interfering RNAs) are a class of small RNAs with high abundance in male reproductive tissues in monocot plants. Their temporal occurrence in maize anthers has been characterized by Zhai et al (2015) (**Figure 1**), but their function remains mysterious. This is reminiscent of mammalian pachytene piRNA which are important for male spermiogenesis. Mammalian pachytene piRNAs have been extensively studied, but their function(s) are only beginning to be understood, e.g. elimination of large amounts of mRNA (Gou et al., 2014).

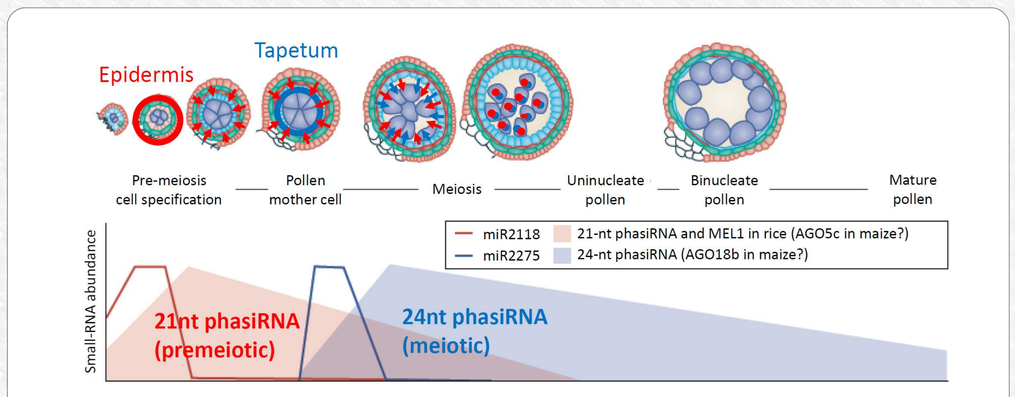
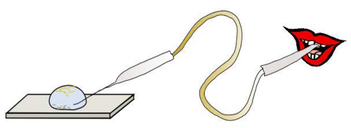


Figure 1: Occurrence of maize phasiRNA during meiosis and male gametogenesis
MiR2118 and miR2275 trigger the production of secondary small RNAs, premeiotic 21nt phasiRNAs and meiotic 24nt phasiRNAs, respectively. 21nt and 24nt phasiRNAs biogenesis differ spatiotemporally, as shown in the anther cross-sections. 21nt-triggering miR2118 emerges in the epidermis, 24nt-triggering miR2775 in the tapetum layer. Modified from Borges & Martienssen (2015).

Figure 2: Sketch of meiocyte collection technique.
Stage-specific male meiocytes from maize are isolated manually via mouth-pipetting (Dukowic-Schulze et al., 2014)



We used isolated zygotene meiocytes from maize (**Figure 2**) for downstream sequencing approaches to gain more insight on a potential role of phasiRNAs in plant monocot meiosis.

Results

We conducted sRNA sequencing and DNA bisulfite sequencing of isolated meiocytes in zygotene, whole corresponding anthers, and 2-week-old seedlings.

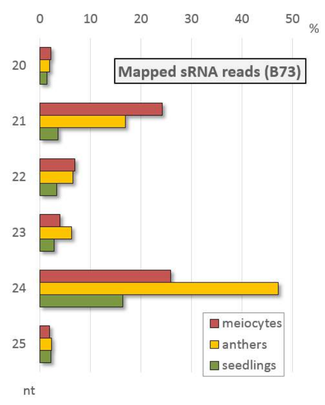


Figure 3: Percentage of aligned reads per nucleotide length.

Mapped sRNA reads from the maize inbred line B73 showed an abundance of 21 and 24nt long sRNAs in meiocytes and anthers (**Figure 3**). This is due to ~300 high-expressing loci of 21 and 24nt sRNAs each, which mostly constitute the phasiRNAs already described in maize anthers by Zhai et al. (2015). They are distributed across the whole 10 chromosomes of maize (**Figure 4**)

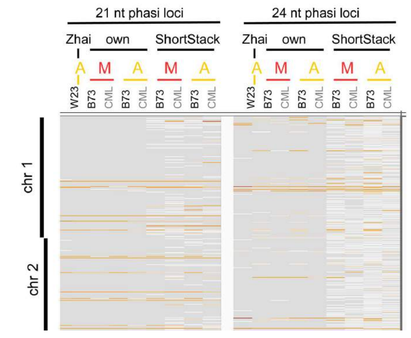


Figure 4: Example locations and strength of phasiRNA loci.
Data from Zhai et al. (2015) in anthers (A), and from own data including meiocytes (M). PhasiRNA loci defined via an arbitrary threshold (≥ 2 RPM, reads per million) or via the tool ShortStack (Axtell, 2013). Background in gray, phasiRNA loci in white (low coverage) to red (high coverage). Maize inbred lines W23, B73 and CML228.

PhasiRNA genomic loci rarely overlap transposable elements (TEs) (**Figure 5A**) but have increased DNA methylation in the CHH context (**Figure 5A**), as well as a peculiar pattern of GC content (**Figure 5B**). Specific to meiocytes, DNA methylation occurs *in cis* at genomic loci of 21nt phasiRNAs in narrow regions (< 500bp) (**Figure 5B**) while it covers broader regions around genomic loci of 24nt phasiRNAs (<5000 bp) (**Figure 6A**). In contrast to 21nt phasiRNAs, there seem to be two subclasses of 24nt phasiRNAs of which one shows the canonical increase of ubiquitous DNA methylation at TEs (**Figure 6B**), similar to genomic loci of 24nt siRNAs with high abundance in seedlings (**Figure 6C**).

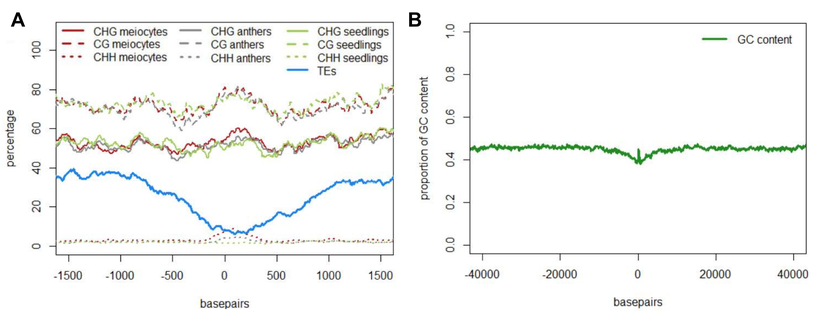


Figure 5: Features at 21nt phasiRNA loci.
Plots show the averaged occurrence of features of interests on ~300 phasiRNA loci.
- **A:** TEs and DNA methylation in the CHG, CG and CHH context in meiocytes, anthers and seedlings.
- **B:** GC content at broad scale.

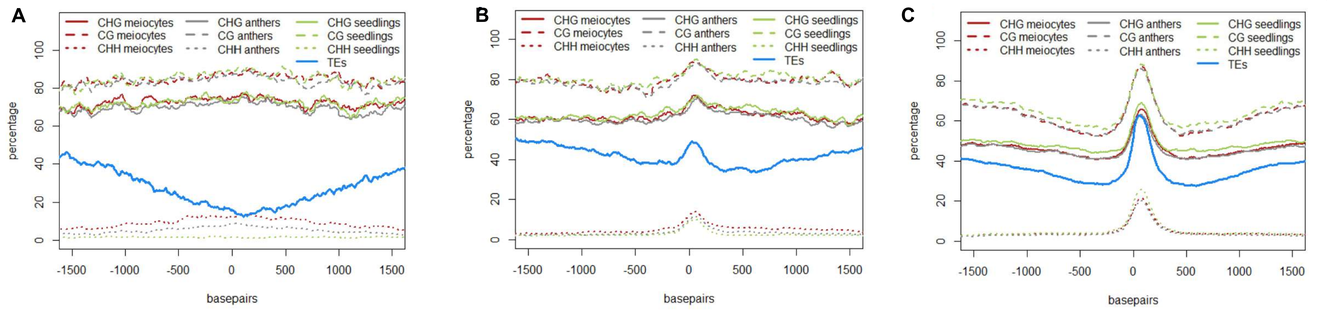


Figure 6: Occurrence of TEs and DNA methylation at 24nt smallRNA loci.
Plots show the percentage of loci with annotated TEs and the averaged percentage of DNA methylation at distinct populations of 24 nt sRNA loci.
- **A:** loci for 24nt phasiRNAs in meiocytes (identified via threshold of 2 RPM)
- **B:** loci for 24nt phasiRNAs/sRNAs in meiocytes (identified by ShortStack)
- **C:** loci for 24nt sRNAs in seedlings (identified by ShortStack)

Summary

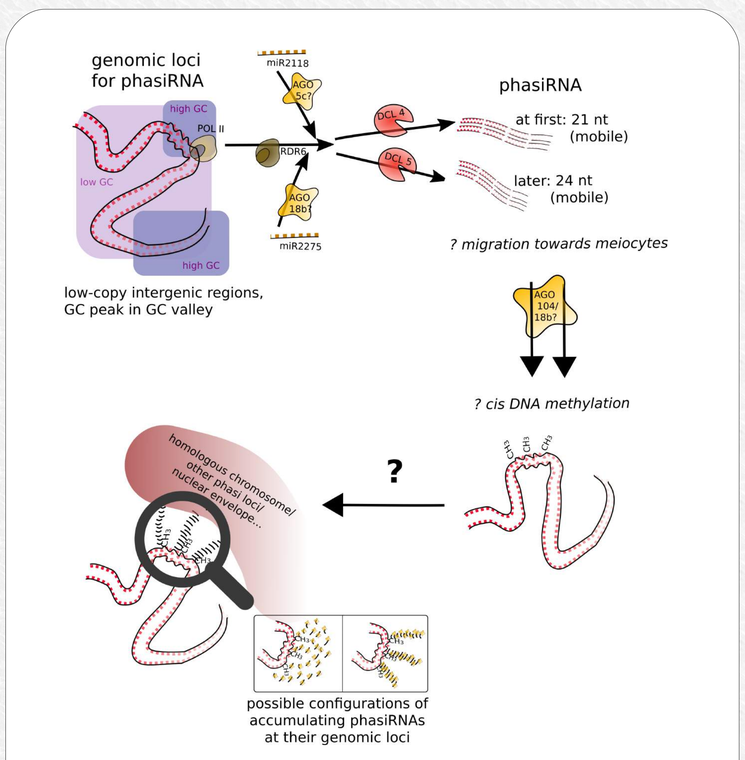


Figure 7: Sketch of phasiRNA regions, biosynthesis and proposed functions
PhasiRNAs are produced in two waves by miRNA triggers. They presumably migrate towards the center of an anther to mediate DNA methylation *in cis* in meiocytes. The vast abundance of phasiRNAs together with increased DNA methylation and peculiar GC pattern could shape recognition hallmarks facilitating meiotic processes like pairing of homologous chromosomes.

Key findings:

- Mammalian pachytene piRNAs and monocot phasiRNAs both ...
  - … occur in intergenic low-copy regions,
  - … peak during meiosis,
  - … likely have functions other than expression decrease of a few specific targets.
- Loci for 21 and 24 nt phasiRNAs show ...
  - … increased DNA methylation in the CHH context specifically in meiocytes,
  - … a peculiar GC content pattern in the DNA sequence.

Resulting hypotheses:

- PhasiRNAs act as mobile signals from outer anther layers, migrating towards meiocytes where they perform their function.
- PhasiRNA loci could be important for meiotic chromosome behavior (localization, pairing, synapsis).

Literature

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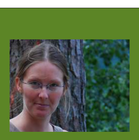

Contact: sdukowic@umn.edu

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