PUBLISHED: 5 MAY 2015 | ARTICLE NUMBER: 15055 | DOI: 10.1038/NPLANTS.2015.55



NON-CODING RNAS

# The small mysteries of males

Cells associated with the male germlines of grasses produce huge amounts of small RNAs. A large survey of two types of small RNA in maize uncovers unique characteristics associated with male fertility, but the molecular mechanism by which these germline-associated small RNAs function remains unclear.

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Gene expression in eukaryotes is strongly influenced by small non-coding RNAs. In plants, 21-nt microRNAs (miRNAs) and 24-nt heterochromatic short interfering RNAs (het-siRNAs) are two common and well-understood types, abundant in vegetative tissues. Writing in *Proceedings of the National Academy of Sciences of the USA*, Zhai *et al.*[1] document in exquisite detail the tissue-specific expression patterns of two much less understood types of endogenous small RNA loci: 21-nt pre-meiotic and 24-nt meiotic phased siRNAs (phasiRNAs). They find that both types of phasiRNA accumulate to high levels in distinct tissues and at specific developmental times during maize anther development. Pre-meiotic and meiotic phasiRNAs are likely to be critical factors required for male fertility in grasses.

Phased siRNAs arise from double-stranded RNA (dsRNA) precursors with a consistent starting point. Owing to the processive nature of Dicer-like proteins (endonucleases that release small RNA duplexes from the termini of dsRNAs), siRNAs are arranged in regular intervals relative to the dsRNA terminus, and thus are said to be 'in phase' with each other. The first phasiRNAs identified were vegetatively expressed, predominantly 21 nt in length, and had dsRNA termini defined by miRNA-mediated cleavage of a primary transcript[2].

The first descriptions of germline-associated phasiRNA accumulation in grasses came from rice. Johnson *et al.*[3] found hundreds of large clusters of phasiRNAs specifically from rice inflorescence samples. Some of these clusters are 21-nt phasiRNAs, but many others are 24-nt phasiRNAs. The biogenesis of 21-nt phasiRNAs depends on the microRNA miR2118 and the Dicer-like protein DCL4, whereas that of 24-nt phasiRNAs depends on miR2275 and DCL5 (also known as DCL3b)[4]. The genomic locations that spawn these inflorescence-associated phasiRNAs in rice are startling in their lack of informative annotations:


**Figure 1 |** More similar than we think? Grass phasiRNAs and mammalian piRNAs are both associated with male fertility.

they come from intergenic, non-repetitive regions scattered about all of the chromosomes.

The work of Zhai *et al.*[1] extends the previous data in two ways. First, the existence of both miR2118-initiated 21-nt and miR2275-initiated 24-nt phasiRNAs is documented in developing maize anthers (see Fig. 1), suggesting that these types of phasiRNA are a general property of grass species. Second and more importantly, the timing and cell-type specificities of the phasiRNAs are characterized. The miR2118-dependent 21-nt phasiRNAs appear early in anther development and emanate from the anther epidermis; hence, these are pre-meiotic phasiRNAs. In contrast, the miR2275-dependent 24-nt phasiRNAs appear later in anther development at the time of meiosis, and emanate from the tapetal cell layer; hence, they are the meiotic phasiRNAs. Evidence from comparing *in situ* hybridizations of the initiating miRNAs and phasiRNAs suggests that phasiRNAs may move from their cells of origin into adjacent cell layers.

What are the biological functions of pre-meiotic and meiotic phasiRNAs? Again, some clues have been provided by previous work in rice. Small RNAs function in complexes with specific Argonaute (AGO) proteins. The rice AGO protein meiosis arrested at leptotene 1 (MEL1), which specifically binds the miR2118-dependent 21-nt phasiRNA population[5], is required for fertility[6]. In *mel1* mutants, pollen mother cells are defective and have aberrant patterns of histone modifications. Female germ cell development is also affected by the *mel1* mutation. Zhai *et al.*[1] observe that a maize *MEL1* homologue, *ZmAGO5c*, is most highly expressed in anthers at the 0.7-mm stage — exactly when the 21-nt pre-meiotic phasiRNAs accumulate to their highest levels. Similarly, the expression pattern of *ZmAGO18b* correlates well with that of the 24-nt meiotic phasiRNAs. However, there is not yet any direct evidence of the functionality of these maize AGOs, nor indeed any for the functions of the pre-meiotic or meiotic phasiRNAs in maize.

AGO-bound small RNAs seek targets, and once targets are found, the AGO proteins mediate various forms of repression. For 21-nt miRNAs, the targets are usually mature mRNAs, which are repressed at the post-transcriptional level. For 24-nt het-siRNAs, the targets are nascent long non-coding RNAs, whose associated chromatin is modified. Zhai *et al.*[1]

were unable to find evidence of miRNA-like activities for the phasiRNA populations. PhasiRNAs may instead condition het-siRNA-like chromatin modifications. First, the meiotic phasiRNAs are 24 nt in length, the same size as het-siRNAs. Second, recent evidence indicates that other 21–22 nt siRNAs with properties similar to phasiRNAs can also direct chromatin modifications[7]. Future experiments to examine chromatin modifications at phasiRNA-targeted loci would allow testing of this hypothesis.

Another mystery is the apparently divergent role of miR2118 in grasses as opposed to dicots. In dicots, miRNAs related to miR2118 target nucleotide-binding site leucine-rich repeat (*NBS-LRR*) mRNAs in vegetative tissues, causing high levels of *NBS-LRR*-derived phasiRNA accumulation[8]. As Zhai *et al.*[1] suggest, this may reflect neo-functionalization of the miR2118 superfamily in one of the two lineages.

Finally, Zhai *et al.*[1] point out some interesting parallels between the grass pre-meiotic and meiotic phasiRNAs and mammalian Piwi-associated RNAs (piRNAs). Like the maize phasiRNAs, mammalian piRNAs are expressed in two distinct waves in testis. The first wave (pre-pachytene piRNAs) consists of shorter RNAs (26–27 nt) and the second wave (pachytene piRNAs) comprises longer RNAs (29–30 nt). The piRNAs come from dispersed, non-repetitive clusters in the genome. Loss of the key binding partners (various Piwi proteins) leads to failure of meiosis and male sterility. Finally, like the grass phasiRNAs, the molecular functions of mammalian piRNAs remain largely obscure. If these parallels are more than just coincidence, they may reflect convergent evolution of small RNA systems involved in regulating male reproductive development. Future work should continue to investigate these small (RNA) mysteries of males. ❐

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