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Multiple roles for sRNA during plant reproduction

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Abstract

Germline development and early embryogenesis in eukaryotes are characterized by large-scale genome reprogramming events. In companion cells of the Arabidopsis male gametophyte, epigenome reorganization leads to loss of heterochromatin and production of a distinct small RNA (sRNA) population. A specific class of sRNA derived from transposons appears to be mobile and can accumulate in germ cells. In the germline of maize, rice, and Arabidopsis, specific ARGONAUTE-sRNA silencing complexes appear to play key roles in reproductive development, including meiosis and regulation of germ cell fate. These results reveal new roles for sRNAs during plant reproduction and suggest that mobility of sRNAs could be critical for some of these functions.

Introduction

In eukaryotes, RNA interference (RNAi) plays an important role in gene regulation, development and response to abiotic and biotic stress. Aside from regulating gene expression at the level of messenger RNA, RNAi regulates heterochromatin transcription, and the expression and mobility of transposable elements (TEs) [1]. To control gene expression, heterochromatin organization and TE repression, RNAi relies on the production of small RNAs (sRNA) that provide specificity for target nucleic acid sequences [2–5]. In plants, a common feature in the life cycle of these sRNA is the production of a double stranded RNA precursor followed by cleavage into 20–24nt sRNAs by one of the DICER ribonucleases (DCL) and specific binding to an ARGONAUTE (AGO) protein [6]. The sRNA-AGO silencing complex can then regulate gene and TE expression at the transcriptional and posttranscriptional levels [7].

The mobility of sRNAs from their site of biogenesis to recipient tissue has gained a lot of attention over the last few years. In plants, several recent studies have shown that different classes of sRNAs (small interfering RNA (siRNA), microRNA (miRNA) and trans-acting RNA (tasiRNA)) travel either from cell-to-cell or over long distances to silence specific targets in recipient cells [8–12]. Our understanding of mobile sRNA in plants has dramatically improved over the last few years, particularly with regard to their biogenesis [6]. In contrast, less is known about the biological function of these mobile signals.

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In this review, we focus on the expanding roles of sRNAs during plant reproduction, particularly on their function in the regulation of TEs and heterochromatin. We also emphasize the non-cell autonomous roles of sRNA during this developmental stage (for an extensive review on mobility of sRNA see Voinnet and Brosnan in this issue).

Mobile siRNA in the male germline

In contrast to animals, plants do not specify male and female germlines during embryogenesis; instead they do so at a late stage of their adult development. With regards to male gametogenesis, the anthers host the microspore mother cells which undergo meiosis, giving rise to tetrads of haploid microspores. Enzymes secreted by the surrounding tapetum tissue in the anthers promote the release of individual microspores from the tetrads [13]. Pollen development begins when the microspores start dividing to generate two asymmetric cell types: the vegetative (somatic) and generative (germ) cells. The vegetative cell cytoplasm occupies most of the volume of the bi-cellular pollen grain, and completely encompasses the smaller generative cell. In contrast to the vegetative cell, the generative cell undergoes a second round of mitosis to produce two small sperm cells, resulting in the three-celled mature pollen grain in Arabidopsis [14].

Cytological analysis of Arabidopsis mature pollen grains reveals striking differences between the nuclei of sperm cells and the vegetative cell. While the chromatin of the sperm cells is highly condensed and enriched in the repressive epigenetic modifications histone 3 lysine 9 dimethylation (H3K9me2) and H3K27me1, the chromatin of the vegetative cell nucleus (VN) is largely decondensed with no detectable H3K9me2 and only dispersed H3K27me1 signals [15]. These differences in chromatin structure and histone marks are reflected in the transcriptional status of these cells, as repetitive elements (e.g. retrotransposons) are expressed exclusively in the vegetative cell [16]. Chromatin decondensation and heterochromatic transcription are also observed in leaf cells with lower levels of H3K27me1 [17], and reduced H3K27me1 might be critical for VN function as constitutive expression of the H3K27 methyltransferase ATXR5 results in defective pollen grains and male-sterile plants [18].

Chromatin decondensation in the VN is accompanied by changes in DNA methylation. Cytosines in the CHG and CHH sequence contexts within satellite repeats were found to be hypermethylated by the RNA-directed DNA methylation (RdDM) pathway [15], which depends on 24nt siRNAs [19,20]. Interestingly, CHH cytosines in AtMu1 elements lost methylation along with corresponding 24nt siRNA [16], consistent with the idea that non-CG methylation is guided by 24nt siRNA in the VN. A critical finding concerning the loss of heterochromatin in the VN was the identification of 21nt siRNAs mostly from the large Athila family of retrotransposon. Although Athila transcripts are only detected in the VN, the matching siRNAs accumulate in sperm cells (Figure 1) [16]. 21nt siRNAs, which are involved in post-transcriptional gene silencing, were shown to move from the vegetative cell to the sperm cells and silence a reporter gene [16]. Cytoplasmic bridges connect the vegetative cell and the generative cell and could facilitate movement of these 21nt siRNAs [21], although other mechanisms, such as segregation during cell division, could also contribute. These results reveal a role for VN-specific heterochromatin reprogramming in providing silencing signals for the germline.

Many questions arise concerning the role of these siRNAs. For example, why is there a need for transposon-derived siRNA in the germline? By analogy with Piwi-interacting RNA in *Drosophila* [22], it is possible that these siRNAs act in a genome surveillance pathway preventing the movement of transposons in the germline. Is there anything intrinsic to germ cells that could make them more susceptible to TE movement than somatic cells? One

common theme of germline differentiation, pre- and post-fertilization, is chromatin remodeling through histone replacement. In the Arabidopsis sperm and egg cells, the histone H3 content was recently found to be highly restricted, with the replication-dependent H3.1 variant absent from mature gametes [23]. Canonical or gamete-specific H3.3 variants (replication-independent) are loaded specifically during male and female germline development, most likely to organize chromatin structure and reset the epigenome in preparation for fertilization and development of the totipotent zygote [23–25]. After fertilization, the histone H3 content is rapidly remodeled in the early embryo to reflect the H3 content found in most mature somatic cells [23]. As genome-wide histone replacement is likely to destabilize the epigenome of the gametes and zygote, this could increase the risk of transposition [26–29]. A role for VN-derived 21nt siRNAs loaded in sperm cells could thus be to safeguard the germline pre- and post-fertilization during these large-scale remodeling events. Transient reactivation in the VN reveals TE by generating small RNA that accumulate in the sperm cells [16]. By accumulating in gametes, these small RNA could also distinguish gametes with the same TE repertoire, excluding foreign gametes, which do not, and thus contributing to hybridization barriers between species [30].

Regulation of meiosis, heterochromatin and germ cell fate by germline-specific AGO proteins

The recent identification of germline-specific AGO proteins has revealed many new functions for sRNAs during plant reproduction. In maize and rice, germline-specific AGOs have been implicated in the progression of meiosis and the regulation of sporogenesis [31,32]. MEL1, a rice homolog of the Arabidopsis proteins AGO1, AGO5, and AGO10, is specifically expressed in reproductive organs and regulates chromosome condensation during both male and female meiosis. *mell* loss-of-function mutants do not progress through meiosis normally, ultimately leading to arrested gametes. Although *ago1* mutants in Arabidopsis are known to have a range of severely abnormal phenotypes, *AGO1* expression, unlike *MEL1*, is not restricted to reproductive organs [31]. In contrast, *AGO5* is specifically expressed in the germline of Arabidopsis [33], and is a likely ortholog of rice *MEL1*.

In Arabidopsis, *AGO9* is highly expressed in the ovule, and sequencing of ovule siRNAs associated with AGO9 revealed that most of the 24nt sRNAs originate from TEs and satellite repeats [34]. Maize *AGO104* is a homolog of Arabidopsis *AGO9* and appears to be required for heterochromatic CHG and CHH methylation [32]. In *ago104* loss-of-function mutants, meiosis is severely affected, but in contrast to *mell*, unreduced and viable gametes are still formed [32]. The sRNAs associated with AGO104 in maize have yet to be profiled, but the heterochromatic decondensation in maize *ago104* meiocytes, the meiotic phenotype of *mell* mutants in rice and the identification of sRNA-AGO9 originating from TEs in Arabidopsis suggest a role for ARGONAUTE proteins in the regulation of TEs during meiosis.

Recent transcriptome profiling of Arabidopsis male meiocytes has shown that Copia and Gypsy elements, mostly located in the heterochromatin, are significantly upregulated whereas other TEs remain silent [35,36]. The biological significance of differential regulation of TEs during meiosis is not clear and seems counterintuitive because transposition of the TEs during meiosis would disrupt the integrity of the genome. In mice, de-repression of endogenous retroviruses and retrotransposons during meiosis has been linked with severe meiotic abnormalities [37]. A possible explanation is that their expression is important for chromatin remodeling which is in turn required for homologous recombination during meiosis. Germline specific AGOs such as AGO5 and/or AGO9 might therefore be necessary for posttranscriptional silencing of these upregulated TEs to prevent any transposition into the genome (Figure 1).

Interestingly, both *AGO9* in Arabidopsis and *AGO104* in maize are specifically expressed in somatic companion cells and not in the gametes themselves [32,34]. In *ago9* mutants, somatic cells surrounding the megaspore mother cell are reprogrammed into germ cells without undergoing meiosis [32,34]. The siRNA-AGO9 complex seems to act as a liaison between the somatic companion cells, where certain TEs are reactivated, and the gametes, where these TEs are subsequently silenced. In *ago9* mutants, failure to relay this information results in the initiation of gametogenesis in surrounding somatic cells, and later in de-repression of TEs in the egg cell prior to fertilization [34]. Thus, reactivation of TEs in ovule and pollen companion cells induces the production of siRNAs, respectively 21nt and 24nt in size, which facilitate silencing in the neighboring germline (Figure 1) [16,34].

TE-derived endosperm siRNAs expressed only from the maternal genome

A hallmark of flowering plants is double fertilization where two haploid sperm cells fuse with a haploid egg cell and a diploid central cell to produce the diploid embryo and triploid endosperm, respectively [38]. Although the extra-embryonic endosperm does not contribute any genetic material to the next generation, it is of vital importance for the development of the embryo [39,40]. Analogous to what happens in the germline, recent work has shown that 24nt siRNAs are highly expressed in the endosperm [41]. Interestingly, these 24nt siRNAs appear to be maternally expressed and depend on RNA polymerase IV (POLIV) for their biogenesis [41,42]. POLIV (along with POLV) is one of the two plant specific DNA-dependent RNA polymerases and has been shown to generate 24nt siRNA which are highly enriched for TEs in heterochromatic regions [42,43]. Genome-wide methylation profiling showed that TE regions (which produce POLIV-dependent siRNA) are partially demethylated in endosperm, but hypermethylated in the embryo [44,45]. Although the biological significance of the POLIV-dependent siRNAs has not yet been determined, one possibility is that these endosperm siRNAs can move into the embryo and regulate TEs (Figure 1).

Mobility of sRNA

Recently, the mobility of siRNA was demonstrated by grafting a sRNA-producing wild type scion to a *dcl2,3,4* triple mutant rootstock (unable to generate 22–24nt siRNAs) [12]. The authors confirmed earlier findings that movement of a silencing signal can occur from sink to source (Figure 2); however movement from shoot to root appears to be more efficient than vice versa [46,47]. Remarkably, sequencing showed that mobile sRNAs are largely derived from TE transcripts and appear to be involved in DNA methylation of genomic sequences coding for these TEs. Long-distance movement of siRNA was also investigated by Dunoyer et al. They showed by bombardment of leaves with labeled 21nt sRNA that double-stranded sRNA duplexes, but not AGO-sRNA complexes, are mobile molecules [8]. Interestingly, bombardment of phloem cells revealed that siRNA can enter the phloem and travel long distance in Arabidopsis. A recent study performed in *Nicotiana benthamiana* suggests that the mobility of siRNA through the phloem is very likely a conserved feature in plants [8,48]. Thus all known size classes of siRNA can move through the plant over a wide range of distances, and in some cases can induce epigenetic changes in recipient cells by *de novo* DNA methylation [47]. This raises the possibility that mobile small RNA might be transmitted through the germline, potentially in response to environmental and developmental signals (see Voinnet and Brosnan this issue).

Conclusion

TEs have long been regarded as parasitic elements with detrimental effects on the host genome. This view is being revisited in light of recent evidence, concerning TE-derived

siRNAs and their potential role in regulating development. A common feature during plant reproduction is the reactivation of TEs to enforce silencing of TEs in other cells or at a later developmental stage. The mobility of siRNA lies at the heart of this pathway and may yet provide clues to whether the movement is limited to the germline or if siRNAs can cross generational boundaries.

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Research highlights

1. Epigenome reprogramming in the vegetative nucleus of pollen grains results in the production of mobile, transposable element (TE)-derived sRNAs, which accumulate in the germline to possibly protect it against movement of TEs.
2. In monocots and dicots, AGO-sRNAs complexes play critical roles in meiosis.
3. Repression of germ cell fate is dependent on AGO9 in Arabidopsis.
4. 24nt sRNAs derived specifically from the maternal genome are produced in the endosperm.

Figure 1. Overview of small RNA (sRNA) functions during different stages of reproduction in Arabidopsis

In the ovules, sRNAs-AGO9 complexes suppress gametogenesis in the somatic cells surrounding the megaspore mother cell. Reactivated transposable elements (TEs) are presumably posttranscriptionally regulated during meiosis. In pollen, reactivation of certain TEs in the VN leads to the production of TE-derived sRNAs that likely move to the sperm cells. Before and after fertilization, maternal expression of TEs leads results in sRNAs that can silence paternal TEs in the endosperm and perhaps also TEs in the embryo. The antipodal cells and synergids of the seven-celled embryo sac are shown in dark- and light grey, respectively. TE = Transposable elements. CC = Central cell. EC = Egg cell. VN = Vegetative nuclei. SC = Sperm cell. PG = Paternal genome. MG = Maternal genome.

Figure 2. Source to sink movement of mobile silencing signals in Arabidopsis

A mobile signal can move from shoot to root (white arrows) or from root to shoot (red arrows). It remains to be determined whether the silencing signal can travel from root to the male or the female germline (inset).