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Review

miRNAs in the biogenesis of *trans*-acting siRNAs in higher plants

Edwards Allen*, Miya D. Howell

Monsanto Company, 700 Chesterfield Pkwy W, Chesterfield, MO 63017, USA

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ABSTRACT

Multicellular eukaryotes utilize many complex small RNA mechanisms to regulate gene expression from DNA modifications to RNA stability. RNA interference also regulates exogenous gene expression by degrading invading pathogen RNAs or preventing expression of foreign DNA incorporated into the host genome. Here we review the mechanisms for *trans*-acting (ta)-siRNA biogenesis and function, including pathways that utilize components of the miRNA and transitive RNAi defense. There are several distinguishing features of ta-siRNA pathways including the requirement for a miRNA-guided cleavage event that sets a processing register, RDR6 dependent dsRNA production, and DCL4 dependent processing to create unique, phased 21 nucleotide small RNAs. These phased small RNAs function to suppress target genes that only show similarity at the ta-siRNA recognition site, and act in *trans* to repress expression non-cell autonomously of specific target genes. Since the advent of high throughput sequencing technologies, phased siRNAs have been identified in a number of organisms [Heisel SE, Zhang Y, Allen E, Guo L, Reynolds TL, Yang X, et al. Characterization of unique small RNA populations from rice grain. PLoS One 2008;3:e2871. Zhao T, Li G, Mi S, Li S, Hannon GJ, Wang XJ, et al. A complex system of small RNAs in the unicellular green alga *Chlamydomonas reinhardtii*. Genes Dev 2007;21:1190–203. Johnson C, et al. Clusters and superclusters of phased small RNAs in the developing inflorescence of rice. Genome Res 2009;19:1429–40. Zhu QH, Spriggs A, Matthew L, Fan L, Kennedy G, Gubler F, et al. A diverse set of microRNAs and microRNA-like small RNAs in developing rice grains. Genome Res 2008;18:1456–65. Howell MD, Fahlgren N, Chapman EJ, Cumbie JS, Sullivan CM, Givan SA, et al. Genome-wide analysis of the RNA-DEPENDENT RNA POLYMERASE6/DICER-LIKE4 pathway in Arabidopsis reveals dependency on miRNA- and ta-siRNA-directed targeting. Plant Cell 2007;19:926–42.]. These include transcripts generated either from non-protein-coding or protein-coding transcripts, long imperfect dsRNA or through an unknown mechanism; therefore some of these may not necessarily be classified as canonical ta-siRNAs.

Contents

* Corresponding author. Tel.: +1 636 737 6724.
E-mail address: ed.m.allen@monsanto.com (E. Allen).

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1. *Trans*-Acting siRNA discovery

1.1. Classification and function of miRNAs

Endogenous small RNAs in plants are generally classified into groups primarily based on factors required for their biogenesis and their function, including miRNAs, heterochromatin associated (hc)-siRNAs, *trans*-acting (ta)-siRNAs, repeat associated (ra)-siRNAs and naturally occurring antisense (nat)-siRNAs. The workhorses of the group, miRNAs and hc-siRNAs, function to post-transcriptionally or transcriptionally regulate gene expression, respectively. Early studies associated plant miRNA function with regulation of many critical regulatory genes, primarily transcription factors required for proper patterning or timing of developmental processes [6–8]. miRNA genes are transcribed by RNA Pol II to generate imperfect self-complementary foldback structures that are subsequently processed by DICER-Like1 (DCL1) to generate double-stranded fragments, abrogating the need for an RNA-dependent RNA polymerase (RdRP) [9]. The resulting miRNA/miRNA* duplex fragments are approximately 21-nt in length and contain 2 nucleotide 3′ overhangs [8,10]. The majority of miRNAs are recognized by the AGO1 effector complex due to their 5′ terminal uracil specificity [11–13]. miRNAs target transcripts with little to no similarity outside the miRNA recognition site, and generally function to cleave transcripts between bases 10 and 11 relative to the 5′ end of the miRNA, resulting in two specific RNA fragments that may subsequently undergo degradation by exoribonucleases [14–16]. Efficient cleavage requires association with AGO1 and near perfect complementarity of the miRNA to the target, although mispairs are generally tolerated towards the end of the miRNA:target duplex. Rules have been established that effectively predict miRNA targets [17–19]. miRNAs act cell autonomously, resulting in specific and localized gene regulation [6]. More recently, miRNAs have been shown to repress translation in plants [20,21].

1.2. Biogenesis factors distinguish phased trans-acting siRNAs from miRNAs

Previous guidelines for miRNA annotation required classification by DCL1 dependence, origination from an imperfect foldback structure, conservation, and specific targeting of an unrelated mRNA [22]. Early studies to identify miRNAs were limited by the availability of characterized silencing mutants, sequenced genomes and target prediction tools. As the small RNA sequence databases grew, a few sequences (miR175 and miR389) appeared to fit the accepted criteria for miRNAs, but were associated with abundant nearby siRNAs uncharacteristic of canonical miRNAs [8,23]. Similar to miRNAs, they targeted unrelated endogenous genes for suppression. Surprisingly, these siRNAs required additional factors, which should not be necessary for a sequence originating from an RNA foldback structure [18,24,25]. The precursor transcripts from which the siRNAs derived were elevated in *sgs3* and *rdr6* mutants, genes originally associated with viral defense (VIGS, virus induced gene silencing) and transgene silencing (PTGS, post-transcriptional gene silencing) [26]. Unlike small RNAs typical of PTGS, the siRNAs from these transcripts were in register with each other, indicative of sequential processing. The key to this mystery came when miRNA target sites were identified [18,24,25,27], explaining the dependence on DCL1 for generation of an initiator miRNA. The components of PTGS/VIGS were required for secondary production of dsRNA after the miRNA cleavage event. Previous attempts to identify miRNA target sites from these transcripts had failed because these were non-coding non-annotated transcripts, or analysis did not allow for mispairing at critical sites (see mechanism section). In recent years, considerable advances have been made in understanding the biogenesis and function of ta-siRNAs.

1.3. Revised miRNA and ta-siRNA classification

The current criteria for plant miRNA annotation now require precise excision from a stem–loop hairpin precursor. The criteria ensures the presence of a miRNA and miR* sequence on opposite stem-arms, their base-pairing interactions, and a low number of asymmetric bulges [28]. Aided by the availability of additional mutants in *Arabidopsis thaliana*, miRNAs are further distinguished by the lack of dependence on DCL4, RDR6, SGS3, or other components of the small RNA processing machinery (Box 1) [28]. As small RNA discovery expands to new organisms lacking annotation, it is critical that sequence information is not discarded when exploring small RNA function. Since the discovery of ta-siRNAs, several labs have developed models and algorithms to predict phased siR-

Box 1: Factors required for ta-siRNA function in plants

RDR6 (RNA-dependent RNA polymerase6): First identified as a factor required for RNA-mediated virus induced silencing. Also known as SGS2 (suppressor of gene silencing2) and SDE1 (silencing defective1). Required for production of double-stranded RNA from a single-stranded precursor. *rdr6* mutants have downward-curled and elongated leaves, with abaxial trichomes appearing early.

DCL4 (DICER-like4): Processes phased 21 nucleotide small RNAs from dsRNA precursor. *dcl4* mutants also display similar phenotypes to *rdr6* mutants, leading to accelerated juvenile-to-adult phase change in *Arabidopsis*.

SGS3 (suppressor of gene silencing3)phosphate deficiency: SGS3 was identified in screens for mutations deficient for post-transcriptional gene silencing. Interacts and colocalizes with RDR6, and recently shown to bind 5′ overhang containing dsRNA. Also shows potential role in de novo DNA methylation in *Arabidopsis*. Point mutation sgs33 mutant reduces ta-siRNA accumulation.

AGO7 (argonaute7, zippy): Selectively binds miR390 for initiation of ta-siRNA biogenesis in TAS3 transcripts. Data suggests AGO7 is not required for initial cleavage but rather recruitment of RDR6. AGO7 mutants (zip-1) show similar rosette phenotypes to rdr6 and dcl4 mutants.

DRB4 (DICER RNA binding factor4): Specifically interacts with DCL4. drb4 mutants phenocopy dcl4 mutants and display elongated and downwardly curled rosette leaves with increased anthocyanin. Mutants display reduced accumulation of both TAS1 and TAS3 ta-siRNAs.