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Role of miRNAs and siRNAs in biotic and abiotic stress responses of plants ☆

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ABSTRACT

Small, non-coding RNAs are a distinct class of regulatory RNAs in plants and animals that control a variety of biological processes. In plants, several classes of small RNAs with specific sizes and dedicated functions have evolved through a series of pathways. The major classes of small RNAs include microRNAs (miRNAs) and small interfering RNAs (siRNAs), which differ in their biogenesis. miRNAs control the expression of cognate target genes by binding to reverse complementary sequences, resulting in cleavage or translational inhibition of the target RNAs. siRNAs have a similar structure, function, and biogenesis as miRNAs but are derived from long double-stranded RNAs and can often direct DNA methylation at target sequences. Besides their roles in growth and development and maintenance of genome integrity, small RNAs are also important components in plant stress responses. One way in which plants respond to environmental stress is by modifying their gene expression through the activity of small RNAs. Thus, understanding how small RNAs regulate gene expression will enable researchers to explore the role of small RNAs in biotic and abiotic stress responses. This review focuses on the regulatory roles of plant small RNAs in the adaptive response to stresses. This article is part of a Special Issue entitled: Plant gene regulation in response to abiotic stress.

1. Introduction

Small non-coding RNAs, which consist of 20–24 nucleotides (nt), have been increasingly investigated as important regulators of protein-coding gene expression; these small RNAs function by causing either transcriptional gene silencing (TGS) or posttranscriptional gene silencing (PTGS) [1]. They were first reported in the nematode *Caenorhabditis elegans* [2] and are responsible for the phenomenon known as RNA interference (RNAi), co-suppression, gene silencing, or quelling [3–6]. Shortly after these reports were published, researchers demonstrated that PTGS in plants is correlated with the activity of small RNAs [7]. These small RNAs regulate various biological processes, often by interfering with mRNA translation. In plants, two main categories of small regulatory RNAs are distinguished based on their biogenesis and function: microRNAs (miRNAs) and small interfering RNAs (siRNAs). Recently, miRNAs and siRNAs have been shown to be highly conserved, important regulators of gene expression in both plants and animals [8,9]. The modes of action by which small RNAs control gene expression at the transcriptional and posttranscriptional levels are now being developed into tools for molecular biology research. One important goal of this research is to determine how a stress affects small RNAs and how small RNAs in turn regulate plant responses to a stress.

Plants have evolved sophisticated mechanisms to cope with a variety of environmental stresses. Many plant genes are regulated by stresses such as drought, salt, cold, heat, light, and oxidative stress [10,11]. Recent evidence indicates that plant miRNAs and siRNAs play a role in biotic and abiotic stress responses [12–14]. The first indication for such roles came from bioinformatics/in silica analysis of miRNAs and their target genes, and cloning of miRNAs from stress-treated *Arabidopsis thaliana* plants, which revealed new miRNAs that had not been previously cloned from plants grown in unstressed conditions [15,16]. Understanding small RNA-guided stress regulatory networks can provide new insights for the genetic improvement of plant stress tolerance. Many studies have also revealed complexity and overlap in plant responses to different stresses, and understanding this complexity and overlay will likely lead to new ways to enhance crop tolerance to diseases and environmental stresses. Researchers recently demonstrated that manipulation of miRNA/siRNA-guided gene regulation can help in the engineering of stress-resistant plants [17–19]. In this review, we consider what are currently known and unknown about roles of miRNAs and siRNAs in plant responses to biotic and abiotic stresses. The responses of small RNAs to different types of stresses are discussed in detail.

2. Classes of small RNAs

Independent approaches combining traditional cloning, computational prediction, and high throughput sequencing of small RNA libraries have identified several classes of small RNAs with specific sizes and functions in plants. These classes of small RNAs include

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miRNAs, repeat-associated small interfering RNAs (ra-siRNAs), natural antisense transcript-derived small interfering RNAs (nat-siRNAs), trans-acting small interfering RNAs (ta-siRNAs), heterochromatic small interfering RNAs (hc-siRNAs), secondary transitive siRNA, primary siRNAs, and long small interfering RNAs (lsiRNAs) [20–22]. The biogenesis and functions of most of these small RNA classes have been well characterized in the model plant *A. thaliana*. In general, small RNAs are generated from at least partially double-stranded RNA precursors by the action of ribonuclease III-like Dicer proteins (DCL) [23]. The small RNA duplexes generated by DCL activity have a characteristic 2-nucleotide overhang at the 3′ end because of an offset cutting of the DCLs. In plants, these 3′ overhangs are stabilized by 2′-O-methylation [24]. Generation of lsiRNAs depends on the DCL and ARGONAUTE (AGO) subfamily protein AGO7. This differs from the generation of the 25- to 31-nt animal PIWI interacting RNAs, which are independent of the Dicer and AGO subfamily protein. Only one strand of the processed small RNA duplex subsequently associates with an RNA-induced silencing complex (RISC) that scans for nucleic acids complementary to the loaded small RNA to execute its function [25,26]. In plants, small RNAs act in gene silencing by mediating RNA slicing [27], translational repression [28], and histone modification and DNA methylation [29,30]. The first two mechanisms control gene expression posttranscriptionally, whereas the latter two affect gene expression at the transcriptional level (Fig. 1).

2.1. miRNAs

2.1.1. Biogenesis of miRNAs

miRNAs are small regulatory RNAs of 20–22 nt that are encoded by endogenous *MIR* genes. Their primary transcripts form precursor RNAs, which have a partially double-stranded stem-loop structure and which are processed by DCL proteins to release mature miRNAs [26]. In the miRNA biogenesis pathway, primary miRNAs (pri-miRNAs) are transcribed from nuclear-encoded *MIR* genes by RNA polymerase II (Pol II) [31] leading to precursor transcripts with a characteristic hairpin structure (Fig. 1A). In plants, the processing of these pri-miRNAs into pre-miRNAs is catalyzed by DCL1 and assisted by HYPONASTIC LEAVES 1 (HYL1) and SERRATE (SE) proteins [26]. The pre-miRNA hairpin precursor is finally converted into 20- to 22-nt miRNA/miRNA* duplexes by DCL1, HYL1, and SE. The duplex is then methylated at the 3′ terminus by HUA ENHANCER 1 (HEN1) and exported into the cytoplasm by HASTY (HST1), an exportin protein [32,33]. In the cytoplasm, one strand of the duplex (the miRNA) is incorporated into an AGO protein, the catalytic component of RISC, and guides RISC to bind to cognate target transcripts by sequence complementarity (Fig. 1A). In addition to the control of targets at the posttranscriptional level, miRNAs regulate gene expression by causing epigenetic changes such as DNA and histone methylation [29,34,35].

Functional analysis of conserved miRNAs revealed their involvement in multiple biological and metabolic processes in plants. They regulate various aspects of developmental programs including auxin signaling, meristem boundary formation and organ separation, leaf development and polarity, lateral root formation, transition from juvenile-to-adult vegetative phase and from vegetative-to-flowering phase, floral organ identity, and reproduction. They also regulate plant responses to biotic and abiotic stresses, and the miRNA pathway itself (Table 1).

2.1.2. Role of miRNAs in plant stress responses

Environmental stress causes plants to over- or under-express certain miRNAs or to synthesize new miRNAs to cope with stress. Several stress-regulated miRNAs have been identified in model plants under various biotic and abiotic stress conditions, including nutrient deficiency [36], drought [37–39], cold [40], salinity [37,41], bacterial infection [42], UV-B radiation [43], and mechanical stress [44]. Although stress regulation may imply a potential function of the regulated miRNA in stress responses, it is obvious that the fact that a miRNA is differentially regulated in response to an environmental stress does not necessarily mean that the miRNA is involved in stress adaptation responses.

In a recent report, the levels of 117miRNAs under salinity, drought, and low-temperature conditions were analyzed using miRNA chips representing nearly all known miRNAs identified in *Arabidopsis*[37]. Seventeen stress-inducible miRNAs were detected, and the results were confirmed by detecting their expression patterns and analyzing the *cis*-regulatory elements in their promoter sequences [37]. Jones-Rhoades and Bartel [15] identified novel *Arabidopsis* miRNAs that were predicted to target genes such as superoxide dismutases, laccases, and ATP sulfurylases (APS). The expression of one particular miRNA (*miR395*) was increased upon sulphate starvation, showing that miRNAs can be induced by environmental factors and not only by developmental processes. *miR395* targets the genes that encode ATP sulfurylases APS1, APS3, and APS4. These enzymes catalyze the first step of inorganic sulfate assimilation [15,16]. Sunkar and Zhu [16] constructed a library of small RNAs from *Arabidopsis* seedlings exposed to different abiotic stresses including cold, dehydration, high salt, and abscisic acid (ABA), and identified several new miRNAs that are responsive to abiotic stress [16]. For example, *miR393* was upregulated by cold, dehydration, salinity, and ABA treatments; *miR397b* and *miR402* were slightly upregulated by general stress treatments while *miR319c* was induced by cold but not by the other treatments;*miR389a*, however, was downregulated by all of the stress treatments. The results indicated that stress-induced miRNAs target negative regulators of stress responses or positive regulators of processes that are inhibited by stresses and that several of the newly identified miRNAs exhibit tissue- or developmental stage-specific expression patterns. More recently, genome-wide profiling and analysis of miRNAs were carried out in drought-challenged rice [39]. Lu et al. [44] also identified 48 miRNA sequences from the *Populus* genome and found that most of these *Populus* miRNAs target developmental and stress/defense-related genes. The authors also found that plant miRNAs can be induced by mechanical stress and may function in critical defense systems for structural and mechanical fitness [44].

A number of miRNAs have been linked to biotic stress responses in plants, and the role of these miRNAs in plants infected by pathogenic bacteria, viruses, nematodes, and fungi has been reported [12,45]. Moreover, miRNAs are also important in regulating plant–microbe interactions during nitrogen (N) fixation by *Rhizobium* and tumor formation by *Agrobacterium* [45]. Additionally, Mishra et al. [46] observed a significant increase in the GC content of stress-regulated miRNA sequences, which further supports the view that miRNAs act as ubiquitous regulators under stress conditions. GC content may also be considered a critical parameter for predicting stress-regulated miRNAs in plants [46].

It is noteworthy that some of the regulations were seen from only a single species and may not be applicable to other species. In fact, it remains to be seen whether some of the observations may even be confirmed by an independent method or reproducible from independent experiments with the same species.

2.1.2.1. miRNAs involved in ABA-mediated stress responses. The phytohormone ABA is involved in plant responses to environmental stresses. The first indication that miRNAs may be involved in ABA-mediated responses came from observations of ABA hypersensitivity in an *Arabidopsis* mutant containing a “pleiotropic recessive *Arabidopsis* transposon insertion mutation,” *hyl1* [47]. Recently, two research groups independently found that either ABA or gibberellin (GA) treatment regulated *miR159* expression [16,48] and controlled floral organ development [48]. In germinating *Arabidopsis* seeds, *miR159* was upregulated in ABA-treated seedlings [49]. Sunkar and Zhu [16] reported that the expression of *miR393*, *miR397b* and *miR402* was upregulated by ABA treatment. In contrast, *miR389a* appears to be