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REVIEW ARTICLE

Response of Plant miRNAs Under Abiotic Stress Conditions

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ABSTRACT

MicroRNAs (miRNAs) are endogenous approximate 22 nucleotide (nt) small non-coding regulatory RNAs that play important roles in plants by targeting mRNAs for cleavage or translational repression. Plant miRNAs were described 10 years later than animal miRNAs did; there are some differences between them in terms of biogenesis and mechanism of function. Furthermore, plant miRNAs have been shown to be involved in various stress responses, such as oxidative, mineral nutrient deficiency, dehydration, and even mechanical stimulus. In this review, we focus on the current understanding of biogenesis and regulatory mechanisms of plant miRNAs. We also highlight specific examples of miRNAs, which are important regulators for plant abiotic stress responses.

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INTRODUCTION

Endogenous small non-coding regulatory RNAs can be divided into two major classes: microRNAs (miRNAs) and short-interfering RNAs (siRNAs). miRNAs are about 20-24 nucleotide (nt), single-stranded RNAs processed from typical stem loop precursors by the Dicer-like (DCl) family of enzymes in plants. miRNAs are known to play important regulatory roles in plants by targeting mRNAs for cleavage or translational repression [1]. miRNAs and their targets have been found to affect diverse processes, including organ development such as leaf morphogenesis, floral organ identity, and root development. Plant miRNAs also function in feedback regulation in small RNA pathway and in the biogenesis of certain class of siRNAs, for example transacting siRNAs [2]. Moreover, they are involved in various stress responses [3-6]. Because of sessile nature, therefore, plants have to cope with various adverse environments, such as drought, salinity, and temperature extremes. They have evolved sophisticated mechanisms to adapt to environmental stresses [7]. In contrast to the increasing number of reports demonstrating the roles of miRNAs in development and morphogenesis of plants, relatively less is known on the roles of miRNAs in stress responses of plants. In this review, we focus on the current understanding of miRNAs biogenesis and regulatory mechanisms in the research of plant miRNAs. We also highlight specific examples of miRNAs with a role in abiotic stress responses.

Discovery and characters of plant miRNAs

miRNAs were first described in *Caenorhabditis elegans* [8]. It is lin-4, which are key regulatory molecules in the pathway controlling the timing of larval development in the nematode C. elegans. Plant miRNAs were identified 10 years later. There are several groups found plant miRNAs by cloning small RNAs in *Arabidopsis* [9-11]. They found miRNAs are present in both plant and animal kingdoms, indicating that this class of noncoding RNAs arose early in eukaryotic evolution. According to the miRNA Registry Database (Release 18, November 2011), 18226miRNA genes have been identified in various plants, including 290 from Arabidopsis, 590 from rice (Oryza sativa), from 234 *Populus trichocarpa*, and the rest from *Saccharum officinarum,* Sorghum bicolor, *Medicago truncatula,* Glycine max, respectively (http://www.sanger.ac.uk/cgi-bin/Rfam/mirna/browse.pl) [6]. There are some obvious characters of miRNAs: (1) miRNAs are about 20-24 nucleotides, single-stranded noncoding RNAs [9]; (2) all miRNAs precursors have a well-predicted stem loop hairpin structure, and this fold back hairpin structure has a low free energy [1,12]; (3) the 50 terminal phosphate and 30 terminal hydroxyl of mature miRNAs make them distinct from the most oligonucleotides and degraded fragments of functional RNAs [1]; (4) in addition, most miRNAs are conservative, temporal, and tissue-specific.

Distinctions between animal miRNAs and plant miRNAs

Differences between animal miRNAs and plant miRNAs exist: (1) Plant miRNAs are less conserved than animal miRNAs. Usually, only the mature miRNAs are conserved in plants instead of miRNA precursors that are usually conserved in animals [1]. (2) In animals, the pri-

miRNA is cleaved by the DICER family member Drosha to produce a 60-70 nt pre-miRNA [13]. In plants, the primary miRNAs are cleaved by Dicer-like 1 (DCL1) protein to produce a 60-300 nt miRNA precursor [14, 15]. (3)Plant miRNAs are cleaved into miRNA: miRNA* duplex possibly by DCL1 in the nucleus [16, 1]. In contrast, in animals, the formation of miRNA: miRNA* duplex and mature miRNAs was controlled by Dicer in the cytoplasm [17, 1]. (4) In animals, the miRNA-miRNA* duplex is transported out of the nucleus by Exportin-5 [18, 19]. Plants have an Exportin-5 homolog, HASTY (HST) [20, 21]. (5) Animal miRNAs usually bind to target mRNAs through imperfect complementarity at multiple sites located at the 30 untranslated regions (UTR), and repress gene expression. However, in plants, most target mRNAs only contain one single miRNA complementary site located in the open reading frame of the target, and most corresponding miRNAs typically perfectly complement to these sites and cleave the target mRNAs [1, 22].

Biogenesis and mechanism of miRNAs in plants

In *Arabidopsis,* the maturation of the miRNA from miRNA genes is also a stepwise process. First, a miRNA gene is transcribed to a primary miRNA (pri-miRNA), which is usually a long sequence of more than several hundred nucleotides. This step is controlled by Pol II enzymes [1, 23]. Second, it is found that DCL1 processes pri-miRNA to a stem loop intermediate called miRNA precursor or pre-miRNA in plants [14, 1]. Third, plant miRNAs are cleaved into miRNA: miRNA* duplex possibly by DCL1 in the nucleus [16], then HASTY, the plant ortholog of Exportin-5, is responsible for exporting the miRNA: miRNA* duplex from the nucleus to the cytoplasm [24]. The mature miRNA derives from one strand of the imperfect and the miRNA* is from the other strand [1].The biogenesis of miRNAs in Arabidopsis requires two other proteins, HYL1 and HEN1 [21, 11, 25], HYL1 is required for miRNA but not siRNA biogenesis. HYL1 is a nuclear protein present in a protein complex [21]. HYL1 has been shown to bind double-stranded RNA specifically [26]. HEN1, a plant-specific methyltransferase provides stability to the miRNA duplex by adding methyl groups to the 30 ends [27]. Finally, mature miRNAs are incorporated into the RNA-induced silencing complex (RISCs) complex. The miRNA of miRNA:miRNA* duplex is preferentially loaded into the RISCs, and then the miRNA* appears to be peeled away and degraded [28-30]. ARGONAUTE (AGO) proteins are core component of RISCs. AGO proteins are characterized by two domains: an 20 kDa N-terminal PAZ domain, a hydrophilic cleft in the PAZ domain which binds to the 30 end of single-stranded RNA molecules; an 40 kDa C-terminal PIWI domain, which has a structure similar to that of RNase H. AGO1 protein interferes with the function of RISC [31-33]. In the RISC complex, miRNAs can direct the RISC to regulate gene expression by either of two posttranscriptional mechanisms: mRNA cleavage or translational repression [1]. In plants, most target mRNAs only contain one single miRNA complementary site, and most corresponding miRNAs typically perfectly complement to these sites and cleave the target mRNAs [1]. This general paradigm has exceptions already. Near-perfect base-pairing between plant miR172 and its target sequence in APETALA2 (AP2) would be expected to confer degradation of the AP2 mRNA. Instead, AP2 protein expression is inhibited without a detectable decrease in AP2 mRNA levels [34, 35]. This suggests that miRNAs may be involved in more complicated mechanisms to control gene expression in plants than in animals.

Role of miRNAs in abiotic stress

Abiotic stresses, such as drought, salinity, and extreme temperatures, regulate the expression of thousands of genes in plants at both transcriptional and posttranscriptional levels. At present, many miRNAs have been predicted and some have been confirmed experimentally to be involved in a variety of abiotic stress responses. Below, we summarize miRNAs that are involved in various stress responses, such as dehydration, mineral-nutrient, and even mechanical stress. miRNAs involved in nutrient stresses. Mineral nutrients are of fundamental importance to plants and indirectly to all living organisms that are dependent on plants. Pi, K, and N are three essential plant macronutrients that are often deficient in soils. miR399 was identified in *Arabidopsis* and rice (*Oryza sativa*). The target of miR399 in *Arabidopsis* is a gene encoding a putative ubiquitin-conjugating enzyme (UBC; At2g33770). Fujii found that there is no substantial regulation of miR399 by salt, drought, or cold stress. Low K or low N did not induce the expression of miR399. But miR399 was highly induced whereas the target UBC mRNA was reduced by low-phosphate (Pi) stress. In transgenic plants with constitutive expression of miR399, UBC mRNA accumulation was suppressed even under high Pi. miR399 down regulates UBC mRNA accumulation by targeting the 50 UTR, and this regulation is important for plant responses to Pi starvation [3]. Furthermore, transgenic Arabidopsis plants with constitutive expression of miR399 accumulated more Pi than the wild-type, and transgenic plants expressing the UBC mRNA without 50UTR (miRNA deregulated) showed less inhibition of primary root growth and reduced induction of a Pi transporter gene by low-Pi stress than those of wild type plants. miR395 is complementary to mRNAs of ATP sulfurylase (APS) proteins. As seen for plants grown in soil, miR395 was not detected in the samples from plants grown in 2 mM SO_4^{2-}. However, miR395 was readily detected in the samples grown in 0.2 or 0.02 mM SO_4^{2-}. The miR395induction is greater than 100-fold that of control. APS1expression decreased in the conditions that induced miR395, and found that its expression as would be expected if APS1 was a cleavage target of miR395 [36]. These results suggest that miRNA have functional roles for plants to cope with fluctuations in mineral-nutrient availability in the soil.

miRNA and oxidative stress

Stress-induced reactive oxygen species (ROS) accumulation is counteracted by intrinsic antioxidant systems in plants that include a variety of enzymatic scavengers [37], Superoxide dismutases (SODs) constitute the first line of defense against highly toxic superoxide radicals. SOD aids in the scavenging of reactive oxygen species (ROS) by converting O_2 to H_2O_2 [38]. On the basis of the metal cofactor used, SODs are classified into three groups: iron SOD (Fe-SOD), manganese SOD (Mn-SOD), and copper-zinc SOD (Cu/Zn-SOD) [38]. miR398 is discovered in Arabidopsis and rice (*Oryza sativa*). The miR398 family is encoded by three loci (MIR398a, MIR398b, and MIR398c) in Arabidopsis. miR398 targets two closely related Cu/Zn-SODs genes: cytosolic CSD1and chloroplast-localized CSD2 [12, 36]. The CSD1 and CSD2 mRNA levels were increased in response to high light, Cu^{2+}, Fe^{3+}, and MV treatments.miR398 was down regulated under oxidative stress conditions. The lack of CSD1 and CSD2 expression in unstressed plants depends on miR398-mediated posttranscriptional regulation, and the stress induction of CSD1

and CSD2 mRNA is mediated by the down regulation of miR398. Additionally, transgenic Arabidopsis thaliana plants over expressing a miR398-resistant form of CSD2accumulate more CSD2 mRNA than plants over expressing a regular CSD2 and are consequently much more tolerantto high light, heavy metals, and other oxidative stresses [4]. Major copper proteins in the cytoplasm of plant cells are plastocyanin, copper/zinc superoxide dismutase and cytochrome c oxidase. Under copper limited conditions, expression of copper/zinc superoxide dismutase is down regulated and the protein is replaced by iron superoxide dismutase in chloroplasts [39]. Presented evidence that microRNA, miR398, mediates this regulation in *A. thaliana* miR398 is involved in the down-regulation of CSD2 mRNA under low-Cu conditions in *A. thaliana.* The expression ofCSD2 is post-transcriptionally suppressed under low-Cu conditions. At the same time the expression of miR398 is induced. These results suggest that miR398 directs degradation of copper/zinc superoxide dismutase mRNA depending on Cu availability [39].

miRNAs involved in ABA stress

The plant hormone abscisic acid (ABA) regulates many important aspects of plant development and many physiological processes, such as seed maturation, germination, synthesis of seed storage proteins and lipids, stomatal closure, pathogen response, and tolerance induction [40,41].In germinating Arabidopsis seeds, miR159 increased accumulation in ABA-treated 1-day-old seedlings.miR159 accumulation was maximal at 4-8 h after ABA addition (approximately 5-fold). Moreover, when seedlings germinated on MS medium were subjected to drought treatment for 8 h, miR159 levels were also increased. Consistent with this, miR159 over-expression suppressesMYB33 and MYB101 transcript levels and renders plants hyposensitive to ABA, whereas transgenic plants over expressing cleavage-resistant forms of MYB33 andMYB101 are hypersensitive, abi3-1 and abi5-4 mutants was used to relate miR159 to known components of the ABA signaling pathway, and found that ABA-inducedmiR159 accumulation requires ABI3 but is only partially dependent on ABI5. These results indicate that ABA induced accumulation of miR159 is a homeostatic mechanism to direct MYB33 and MYB101 transcript degradation to desensitize hormone signaling during seedling stress responses [42]. Sunkar and Zhu reported that the expression of miR393 is strongly up regulated by ABA treatments, whilemiR397b and miR402 are slightly up regulated by ABA stress. In contrast, miR389a appears to be down regulated by ABA stress treatments [43].miRNAs and mechanical stress Lu isolated small RNAs from the developing xylem of *Populus trichocarpa* stems and cloned 22 miRNAs. They examined ptr miRNA transcript levels in tension-stressed and compression-stressed xylem tissues and compared with those in normal developing xylem tissues. The expression of ptr-miR156, 162, 164, 475, 480, and 481 are similarly suppressed by tension and compression stresses transcript levels of ptr-miR408, are up regulated in both tension and compression-stressed tissues. This results show that plant miRNAs can be regulated by mechanical stress and may function in one of the most critical defense systems for structural and mechanical fitness [44]. miRNAs and other abiotic stress. To test whether the expression of some miRNAs is regulated by abiotic stresses, RNA gel blot analysis was performed on seedlings treated with cold, dehydration or NaCl. Result showed that the expression of miR393 is strongly up regulated by cold, dehydration, and NaCl treatments. miR397b and miR402 are slightly up

regulated by all the stress treatments, whereas miR319c appears to beupregulated by cold but not dehydration, NaCl miR389aappears to be down regulated by all of the stress treatments[43]. Jung reported that miRNA417 plays a role as a negative regulator of seed germination in Arabidopsis plants under salt stress conditions [45]. Zhao used microarray to analysis all known rice microRNAs expression profile under drought stress and two drought-induced micro RNAs, miR-169g and miR393, were validated. Only miR-169g showed induction by drought among numerous members of miR169 family, and the induction of miR-169g was more prominent in roots than in shoots. Sequence analysis revealed that miR-169g may be regulated directly by DRE binding transcriptional factors [46].

Discussions and outlook

Past few years have witnessed an explosive increase in research reports on plant miRNAs, more than 700 miRNAs have been identified from a variety of plants. Large numbers of miRNA targets were predicted, some of which were validated or confirmed experimentally. These primary works will provide a foundation for future researches in the field. The study of miRNAs is in its infancy and there are still a number of questions remain to be answered. The function of most miRNAs is currently unknown; there is a large gap between the identification of miRNA genes and the confirmation of their functions. The general model for miRNA biogenesis and activity in plants depicted awaits refinement, for example, the exactly mechanism through which all miRNAs function is still unclear, what are their cofactors is also important. How do miRNAs choose between mRNA cleavage and translation inhibition in plants remain to be verified, where does RISC assembly occur in the nucleus or in the cytoplasm or in both compartments need further study [47]. Current data argue that miRNA scan exhibit their silencing effects by two separate mechanisms: translational repression and mRNA degradation. Moreover, how miRNAs themselves are regulated is currently unknown. The mechanism by which miRNAs they are regulated remains a subject for speculation at present. Finally, the roles of miRNAs in plants are quite diverse, and encompass functions in development, nutrient homeostasis and stress responses. An understanding of miRNA mediated gene regulation could lead to novel strategies for improving plant traits such as stress tolerance. The extent of small RNA involvement in abiotic stress response should becomes clear in the near future. If the breadth of regulation by miRNAs is as predicted, miRNAs may be used as a promising tool to improve plant yields, quality, or resistance to various environmental stresses.

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