The Arabidopsis Mediator Complex Subunit MED19a is Involved in ABI5-mediated ABA Responses

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Abstract Arabidopsis Mediator complex subunit 19a (MED19a), which mediates interactions between transcriptional regulators and RNA polymerase II, plays a critical role in plant response to infection by pathogens. However, the roles of MED19a in other signaling pathways are unknown. Here, we report that MED19a plays an important role in regulation of abscisic acid (ABA)-mediated transcriptional regulation in Arabidopsis. Plants deficient in MED19a showed reduced sensitivity to ABA inhibition of seed germination, cotyledon greening, root growth, and stomatal opening. MED19a-deficient mutants also had reduced resistance to drought stress, evidenced by high water-loss rates and low survival rates. Molecular genetic analysis revealed that MED19a mutants had down-regulated ABA-induced genes, including Em1, Em6, and RD29B, and MED19a could occupy the promoters of Em1 and Em6 in an ABA-dependent manner. Furthermore, MED19a interacted with the transcription factor ABA-insensitive 5 (ABI5) in split-luciferase complementation assays and co-immunoprecipitation assays. An analysis of double mutants (med19a-2 and abi5-7) suggested that the action of MED19a in ABA signaling was dependent upon ABI5. Furthermore, MED19a and ABI5 influenced each other in recruiting the promoters of the target genes Em1 and Em6, which are involved in embryonic development. Altogether, these results indicate that MED19a acts as a positive regulator in ABI5-mediated ABA responses.

Keywords: ABA responses, ABI5, Mediator complex, MED19a, Transcription regulation

Introduction

Plant hormone abscisic acid (ABA) is considered to be a crucial hormone in diverse plant development processes, including seed dormancy and germination, root elongation and in responding to drought stress (Cohen et al. 2015; Linster et al. 2015; Zhao et al. 2016; Trivedi et al. 2016). Upon ABA accumulation, ABA can change a variety of plant physiological and growth processes to adapt to environment changes. Until now, the underlying mechanisms of ABA regulation of stress-related gene expression have been elucidated by characterizing a number of ABA-deficient and ABA-insensitive mutants in Arabidopsis. ABA-INSENSITIVE1 (ABI1) and ABI2, two phosphatase 2C (PP2C) family proteins, play negative roles in ABA signaling (Merlot et al. 2001; Yu and Setter 2003). In addition, three other ABI transcription factors ABI3, ABI4, and ABI5 play positive roles in ABA signaling (Giraudat et al. 1992; Finkelstein et al. 1998; Finkelstein and Lynch 2000; Nambara et al. 2010; Li et al. 2011). Through searching the interaction factors of the PP2C proteins, pyrabactin resistance (PYR)/PYR1-like (PYL), also called regulatory components of ABA receptor (RCAR), was identified as an ABA receptor. It is considered that, upon ABA treatment, PYL protein could interact with ABI1 and/or ABI2. The interaction could then reduce the phosphatase activity of ABI1/ABI2 and then relieve their inhibition of the downstream target protein kinases. This leads to the accumulation of the phosphorylated SNF1-related protein kinases (SnRK2s), and SnRK2s will phosphorylate a variety of ABA-responsive factors, including ion channels and transcriptional factors such as the ABA-responsive element (ABRE) binding (AREB) protein 1 (AREB1), AREB2, and ABRE-binding factor 3 (ABF3) (Fujii et al. 2009; Ma et al. 2009; Melcher et al. 2009; Miyazono et al. 2009; Park et al. 2009; Santiago et al. 2009; Cutler et al. 2010; Yoshida et al. 2010; Chai et al. 2011). ABI5 is one of the transcription factors that can be phosphorylated by SnRK2s. ABI5 can directly bind to the ABA-responsive element (AREB) cis-element in the promoter sequence of ABA-responsive genes, such as Arabidopsis EARLY METHIONINE-LABELED 1 (AtEm1) and AtEm6 to modulate their expression during ABA response (Carles et
Mediator is a conserved multisubunit complex that connects the transcriptional regulators with RNA polymerase II in eukaryotes (Conaway and Conaway 2011). There are 21 conserved and 6 plant-specific subunits in Arabidopsis (Bäckström et al. 2007). Of them, several Mediator subunits have been reported to play important roles in the activation of signaling pathways, including plant development and in response to biotic stress (Dhawan et al. 2009; Kidd et al. 2009; Kim et al. 2011; Chen et al. 2012; Bonawitz et al. 2012; Caillaud et al. 2013; Lai et al. 2014; Zhu et al. 2014; Li et al. 2015; Chhun et al. 2016). MED12 and MED13 are required for early embryo patterning and pathogen resistance (Gillmor et al. 2010; Ito et al. 2011; Zhu et al. 2014). MED14 plays an important role in plant cell proliferation, while MED15 is a key factor in regulating glycolysis-related and fatty acid biosynthetic genes during embryogenesis. Also, MED16 integrates cellular and environmental cues into the circadian clock (Autran et al. 2002; Knight et al. 2008; Kim et al. 2016). On the other hand, MED14, MED15, and MED16 are all necessary in plant systemic acquired resistance (SAR) (Canet et al. 2012; Zhang et al. 2012; Zhang et al. 2013). MED17, MED18, and MED20a are reported to be key factors in production of small and long noncoding RNAs (Kim et al. 2011). HaRxL44, an effector from Hyaloperonospora arabidopsidis (Hpa), could interact with MED19a and degrade MED19a in a proteasome-dependent manner. Mutants of MED19a could elevate JA/ET signaling and reduce SA-triggered immunity in Arabidopsis (Caillaud et al. 2013). MED25, the most researched mediator subunit in Arabidopsis, was first studied for its role in flowering and was later found to regulate JA-mediated responses (Kidd et al. 2009; Chen et al. 2012). MED33a and MED33b both are required for phenylpropanoid homeostasis (Bonawitz et al. 2012). CDK8, the kinase mediator subunit, exhibited a negative role in resistance to B. cinerea infection but exhibited a positive role in plant A. brassicicola resistance (Zhu et al. 2014).

Mediator was also reported to play important roles in plant response to abiotic stress. It was recently reported that MED16, MED14, and MED2 were required for regulating mediator and RNA polymerase II recruitment to CBF-responsive cold-regulated genes. Loss-of-function mutant of MED16, MED14, and MED2 were all insensitive to cold stress (Hemsley et al. 2014). In addition, MED25, which previous has been shown to be a key factor in JA-mediated pathogen resistance, was also required for drought tolerance (Elfving et al. 2011). There are two molecular mechanisms of MED25 in regulating plant response to drought stress. First, the MED25 ACID domain could interact with DREB2A, ZFHD1, and MYB-like proteins, all of which are well-known drought-related transcriptional factors (Elfving et al. 2011). In addition, another report showed that MED25 could regulate ABA-mediated drought tolerance by physically associating with the basic Leu zipper transcription factor ABA-INSENSITIVE5 (ABI5) in promoter regions of ABI5 target genes and showed a negative effect on ABI5-regulated gene transcription (Chen et al. 2012).

As mentioned above, MED19a was a positive factor in Arabidopsis resistance to Hpa by balancing the salicylic acid/jasmonic acid (SA/JA) pathway (Caillaud et al. 2013). In addition, the ABA pathway was shown to play an important role in Hpa resistance (Choi and Hwang 2012), which implied that MED19a might participate in ABA signaling. In this study, we used two transfer DNA (T-DNA) SALK lines of MED19a, med19a-1 and med19a-2 that were previously shown to have low transcript levels (Caillaud et al. 2013). We also constructed two overexpression lines in the background of med19a-1, which we named RE1 and RE2. We were able to find that MED19a acts as a positive factor in ABA signaling by using these materials. In addition, molecular biology and biochemical analyses show that MED19a can occupy the promoters of ABI5 target genes, Em1 and Em6, and positively regulate their expression. Genetic analyses reveal that the MED19a acts as a cofactor of ABI5 in ABA signaling pathway.

**Results**

**MED19a Mutant Decreases ABA Sensitivity**

Each mutant genotype was compared with the WT plants to analyze differences in ABA responses, such as ABA-mediated inhibition of seed germination, cotyledon greening, and seedling growth. In general, the transcript and protein levels of MED19a in RE1 and RE2 plants were higher than in WT plants (Fig. S1). In the absence of ABA, the 5-day cotyledon-greening percentages of all five genotypes were similar (Fig. 1A). In the presence of 0.8 µM ABA, several med19a-1 and med19a-2 mutant plants had green cotyledons, whereas the WT, RE1, and RE2 plants were still only sprouting at 5 days after planting (Fig. 1A). Cotyledon-greening percentages of med19a-1 and med19a-2 were 40.3% and 41%, whereas WT, RE1, and RE2 plants were below to 1% (Fig. 1B). These results indicate that MED19a plays a positive role in ABA-regulated cotyledon greening. The med19a-1, med19a-2, RE1, and RE2 plants were also assessed for their response to ABA during seed germination. In an ABA dose-response assay, seeds were germinated on ABA-free or ABA-containing media, and seed germination (obvious radicle emergence) percentage was scored three days after stratification. There were no clear differences in seed germination among the different genotypes when grown on ABA-free medium. At higher levels of ABA concentration, seed germination rate was lower in WT plants. In the presence