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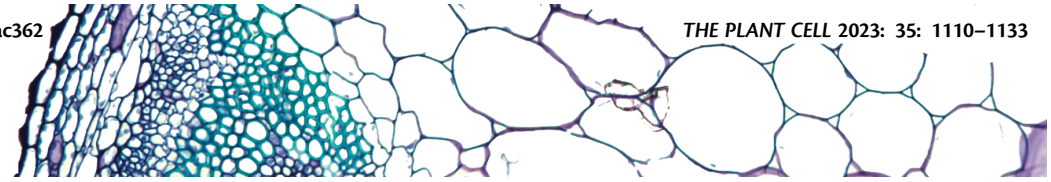

Research Article

Auxin contributes to jasmonate-mediated regulation of abscisic acid signaling during seed germination in Arabidopsis

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Abstract

Abscisic acid (ABA) represses seed germination and postgerminative growth in *Arabidopsis thaliana*. Auxin and jasmonic acid (JA) stimulate ABA function; however, the possible synergistic effects of auxin and JA on ABA signaling and the underlying molecular mechanisms remain elusive. Here, we show that exogenous auxin works synergistically with JA to enhance the ABA-induced delay of seed germination. Auxin biosynthesis, perception, and signaling are crucial for JA-promoted ABA responses. The auxin-dependent transcription factors AUXIN RESPONSE FACTOR10 (ARF10) and ARF16 interact with JASMONATE ZIM-DOMAIN (JAZ) repressors of JA signaling. ARF10 and ARF16 positively mediate JA-increased ABA responses, and overaccumulation of ARF16 partially restores the hyposensitive phenotype of JAZ-accumulating plants defective in JA signaling in response to combined ABA and JA treatment. Furthermore, ARF10 and ARF16 physically associate with ABSCISIC ACID INSENSITIVE5 (ABI5), a critical regulator of ABA signaling, and the ability of ARF16 to stimulate JA-mediated ABA responses is mainly dependent on ABI5. ARF10 and ARF16 activate the transcriptional function of ABI5, whereas JAZ repressors antagonize their effects. Collectively, our results demonstrate that auxin contributes to the synergetic modulation of JA on ABA signaling, and explain the mechanism by which ARF10/16 coordinate with JAZ and ABI5 to integrate the auxin, JA, and ABA signaling pathways.

Introduction

Flowering plants proliferate through sexual reproduction and seed production. The maintenance of seed vigor and successful germination determine the propagation and survival of plant populations. Seed germination and subsequent postgerminative growth are strictly regulated and require the precise coordination of multiple environmental and internal cues, including phytohormones. For example, abscisic acid (ABA) suppresses seed germination and postgerminative growth in *Arabidopsis thaliana* (Gubler et al., 2005; Finkelstein et al., 2008; Hauser et al., 2011; Nakashima and Yamaguchi-Shinozaki, 2013). When the concentration of ABA is elevated, the PYRABACTIN RESISTANCE (PYR)/PYR1-LIKE (PYL)/REGULATORY COMPONENT OF ABA RECEPTOR (RCAR) family of proteins perceive ABA and, in

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turn, suppress the type 2C PROTEIN PHOSPHATASE (PP2Cs) coreceptors, resulting in the release of SUCROSE NONFERMENTING1-RELATED KINASE2 (SnRK2s) from PP2C-SnRK2 complexes (Ma et al., 2009; Miyazono et al., 2009; Nishimura et al., 2009; Park et al., 2009; Santiago et al., 2009; Cutler et al., 2010). The activated SnRK2s subsequently phosphorylate and stimulate downstream components of ABA signaling, thus leading to the transcriptional reprogramming in response to ABA (Kobayashi et al., 2005; Furihata et al., 2006; Fujii et al., 2007; Fujii and Zhu, 2009; Nakashima et al., 2009; Yu et al., 2015).

Previous studies using genetic and molecular approaches have identified several crucial transcriptional components that modulate ABA signaling during seed germination in Arabidopsis. Among them, the ABSCISIC ACID INSENSITIVE5 (ABI5) transcription factor, which belongs to the basic leucine zipper (bZIP) family and is strongly responsive to ABA, is a master positive regulator of ABA-repressed seed germination and postgerminative growth. The seed germination of the loss-of-function *abi5* mutant is hyposensitive to ABA, compared with that of wild type (Finkelstein, 1994; Finkelstein and Lynch, 2000a; Lopez-Molina and Chua, 2000; Söderman et al., 2000; Lopez-Molina et al., 2001, 2002; Brocard et al., 2002; Finkelstein et al., 2005; Skubacz et al., 2016). Mechanistic investigations have revealed that ABI5 is tightly modified at the posttranslational level and acts as a key node of convergence for ABA signaling and other signaling pathways (Stone et al., 2006; Garcia et al., 2008; Miura et al., 2009; Lee et al., 2010; Liu and Stone, 2010; Lim et al., 2013; Albertos et al., 2015; Yu et al., 2015; Zhou et al., 2015; Kim et al., 2016; Yang et al., 2016; Lynch et al., 2017; Ji et al., 2019; Ju et al., 2019; Pan et al., 2020; Yang et al., 2021; Peng et al., 2022). For instance, the SnRK2 protein kinases phosphorylate and stabilize ABI5 in response to ABA, while the phosphatase PROTEIN PHOSPHATASE6 (PP6) dephosphorylates ABI5 and attenuates its accumulation (Kobayashi et al., 2005; Furihata et al., 2006; Fujii et al., 2007; Fujii and Zhu, 2009; Nakashima et al., 2009; Dai et al., 2013; Zhou et al., 2015). PSEUDO-RESPONSE REGULATOR5 (PRR5) interacts with and stimulates ABI5 to integrate the circadian clock and ABA signaling during seed germination (Yang et al., 2021).

The phytohormone auxin functions as a critical signaling molecule in plants. It is perceived by the F-box proteins TRANSPORT INHIBITOR RESPONSE1 (TIR1)/AUXIN SIGNALING F-BOX (AFB; Dharmasiri et al., 2005a, b; Kepinski and Leyser, 2005; Vanneste and Friml, 2009; Chapman and Estelle, 2009; Peer, 2013). Upon auxin perception, auxin/indole-3-acetic acid (Aux/IAA) proteins, which are crucial repressors of auxin signaling, are recruited for degradation via the $SCF^{TIR1/AFB}$ complex-26S proteasome pathway (Vanneste and Friml, 2009; Chapman and Estelle, 2009; Peer, 2013; Kong et al., 2016). The degradation of Aux/IAA repressors alleviates the inhibition of AUXIN RESPONSE FACTOR (ARF) transcription factors to activate or repress the expression of downstream auxin-responsive genes (Ulmasov et al., 1999a, b; Vanneste and Friml, 2009; Chapman and Estelle, 2009; Peer, 2013).

Auxin plays essential regulatory roles in multiple aspects of plant growth and development, and in stress responses (Benková et al., 2003; Woodward and Bartel, 2005; Reinhardt, 2003; Kepinski and Leyser, 2005; Bohn-Courseau, 2010). For example, auxin promotes ABA signaling to maintain seed dormancy and repress germination (Brady et al., 2003; Liu et al., 2007, 2013). Increases in auxin biosynthesis or signaling dramatically enhance ABA responses and inhibit seed germination, whereas disruption of critical auxin biosynthesis-related YUCCA (YUC) enzymes, TIR1/AFB receptors, or downstream ARF10 and ARF16 transcription factors strongly attenuates ABA signaling and seed dormancy (Liu et al., 2007, 2013).

In addition to auxin, the phytohormone jasmonic acid (JA) is also involved in seed germination and synergistically modulates ABA signaling (Wilen et al., 1991; Krock et al., 2002; Preston et al., 2002; Norastehnia et al., 2007; Barrero et al., 2009; Dave et al., 2011, 2016; Pan et al., 2020). Treatment with exogenous JA, such as methyl jasmonate (MeJA), the precursor of the active conjugate jasmonoyl-isoleucine (JA–Ile), enhances ABA signaling to delay seed germination in Arabidopsis (Dave et al., 2011; Pan et al., 2020). Consistently, endogenous CORONATINE-INSENSITIVE1 (COI1)/JASMONATE ZIM-DOMAIN (JAZ)-mediated JA signaling positively modulates ABA responses (Ju et al., 2019; Pan et al., 2020; Hu et al., 2022).

Although both auxin and JA are known to stimulate ABA responses during seed germination, their possible synergistic effects on ABA signaling and the underlying molecular mechanisms remain unknown. In this study, we found that treatment with exogenous auxin and JA synergistically activated ABA responses during seed germination. We also discovered that auxin biosynthesis and TIR1/AFB-mediated auxin signaling are critical for the JA-induced increase in ABA signaling. Further analyses revealed that the auxin-dependent ARF10 and ARF16 transcription factors interact with several JAZ repressors of JA signaling in yeast (*Saccharomyces cerevisiae*) and *in planta*. Phenotypic observations demonstrated that ARF10 and ARF16 are required for JA-enhanced ABA signaling, and that overaccumulation of ARF16 partially rescues the hyposensitive seed germination phenotype of JAZ-accumulating plants with impaired JA signaling (such as the *coi1-16* and *coi1-2* mutants) in response to simultaneous ABA and JA treatment. Furthermore, our results showed that ARF10 and ARF16 physically associate with ABI5, and that ARF16 positively regulates JA-mediated ABA signaling mainly through functional ABI5. Mechanistic investigations revealed that ARF10 and ARF16 stimulate the function of ABI5 to modulate the transcription of downstream target genes, whereas JAZ proteins interfere with the regulatory effects of ARF10 and ARF16. Together, our results indicate that auxin contributes to the synergetic regulation of JA on ABA signaling, and provide a mechanistic understanding of how ARF10/16 transcription

factors function together with JAZ and ABI5 to integrate the auxin, JA, and ABA signaling pathways during seed germination in Arabidopsis.

Results

Exogenous auxin and JA synergistically stimulate ABA signaling to inhibit seed germination

Previous studies have shown that exogenous auxin (e.g. IAA) or JA (e.g. MeJA) enhance ABA responses during seed germination (Staswick et al., 1992; Ellis and Turner, 2002; Liu et al., 2007, 2013; Dave et al., 2011; Pan et al., 2020). To investigate the potential synergistic effects of auxin and JA on ABA signaling and further dissect the underlying molecular mechanisms, we first verified the potential of auxin and JA to stimulate ABA responses. We evaluated germination and cotyledon greening of wild-type seeds on half-strength Murashige and Skoog (MS) medium containing 0.3 μM ABA with 1 μM IAA or 10 μM MeJA. Similar to the results of previous studies (Liu et al., 2013; Pan et al., 2020), the seeds on medium supplemented with both ABA and IAA or MeJA exhibited much lower percentages of germination and expanded greening cotyledons compared with seeds on medium with ABA, IAA, or MeJA alone (Figure 1, A–C).

To better understand the roles of auxin and JA in regulating ABA signaling, we then analyzed the performance of wild-type seeds on medium containing 0.3 μM ABA, 1 μM IAA, and 10 μM MeJA. As shown in Figure 1A, the seeds simultaneously treated with ABA, IAA, and MeJA displayed significantly lower germination percentages than those treated with ABA and IAA or MeJA. Moreover, the percentages of expanded green cotyledons were much lower upon simultaneous exposure to ABA, IAA, and MeJA than upon exposure to ABA and IAA or MeJA (Figure 1, B and C). These observations suggest that exogenous auxin and JA work synergistically to enhance ABA responses to suppress seed germination.

To eliminate the effects of nitrate and/or sucrose on seed germination (Garciarrubio et al., 1997; Finkelstein and Lynch, 2000b; Dekkers et al., 2004; Alboresi et al., 2005; Dave et al., 2011), we also investigated the performance of wild-type seeds on water agar medium containing ABA, IAA, and MeJA. As shown in Supplemental Figure 1, the percentages of germinated seeds and expanded greening cotyledons were significantly lower on medium with ABA, IAA, and MeJA than on medium containing ABA and IAA or MeJA. Collectively, these results provide further evidence that exogenous auxin and JA synergistically stimulate ABA signaling during seed germination.

Auxin biosynthesis and TIR1/AFB-mediated auxin signaling are critical for JA-stimulated ABA signaling

Because exogenous auxin works synergistically with JA to activate ABA responses during seed germination, we wondered whether endogenous auxin contributes to the JA-induced increase in ABA signaling. To test this, we analyzed whether crucial components of auxin biosynthesis, perception, or signaling mediate JA-enhanced ABA-mediated inhibition of seed germination. The YUC flavin monooxygenases catalyze a rate-limiting step in tryptophan-dependent auxin biosynthesis, and the endogenous auxin level is reduced in the *yuc1 yuc6* double mutant and the *yuc1 yuc2 yuc6* triple mutant (Zhao et al., 2001; Cheng et al., 2006). Compared with seeds of wild type, seeds of the *yuc1 yu6* double mutant and the *yuc1 yuc2 yuc6* triple mutant showed significantly higher percentages of germination and expanded greening cotyledons on half-strength MS medium containing 0.3 μM ABA and 10 μM MeJA (Figure 2, A–C; Supplemental Figure 2). Conversely, the progeny of auxin-overproducing *iaaM-OX* transgenic plants (Romano et al., 1995; Cheng et al., 2006) exhibited dramatically lower percentages of germination and expanded greening cotyledons on medium supplemented with both ABA and MeJA, compared with seeds of wild type (Figure 2, A–C).

The F-box proteins TIR1 and AFB are auxin receptors that positively regulate auxin signaling (Dharmasiri et al., 2005a; Kepinski and Leyser, 2005). To examine whether TIR1 and AFB receptors modulate JA-promoted ABA responses during seed germination, we assayed the loss-of-function *tir1 afb1 afb2* triple mutant and *tir1 afb1 afb2 afb3* quadruple mutant (Dharmasiri et al., 2005a, b; Kepinski and Leyser, 2005; Liu et al., 2013). Phenotypic analyses showed that compared with wild type, the *tir1 afb1 afb2* triple mutant and the *tir1 afb1 afb2 afb3* quadruple mutant displayed much higher percentages of seed germination and cotyledon greening on medium containing 0.3 μM ABA and 10 μM MeJA (Figure 2, A–C; Supplemental Figure 2). Aux/IAA proteins are crucial suppressors of auxin signaling and directly link auxin perception with downstream transcription factors (Sabatini et al., 1999; Nagpal et al., 2000; Vanneste and Friml, 2009; Chapman and Estelle, 2009; Kong et al., 2016). AXR3/IAA17 and AXR2/IAA7 are extensively studied repressors of TIR1/AFB-mediated auxin signaling, and gain-of-function auxin-resistant *axr3-1* and *axr2-1* mutants display auxin-insensitive phenotypes and show constitutive repression of auxin-responsive genes (Sabatini et al., 1999; Nagpal et al., 2000; Liu et al., 2013). Further analyses showed that in the presence of both ABA and MeJA, the percentages of seed germination and expanded greening cotyledons were much higher in the *axr3-1* and *axr2-1* mutants than in wild type (Figure 2, A–C; Supplemental Figure 2). In addition, parallel experiments showed that the auxin-related mutant seeds behaved similarly on water agar medium supplemented with ABA and MeJA (Supplemental Figure 3). These results demonstrate that auxin biosynthesis and TIR1/AFB-mediated auxin signaling are positively involved in JA-stimulated ABA repression of seed germination.

To further investigate the regulatory relationship between auxin and JA in mediating ABA responses during seed germination, we analyzed the phenotypes of mutant seeds impaired in JA signaling on medium supplemented with both ABA and IAA. The F-box protein COI1 is the JA receptor and positively modulates JA signaling (Xie et al., 1998; Yan

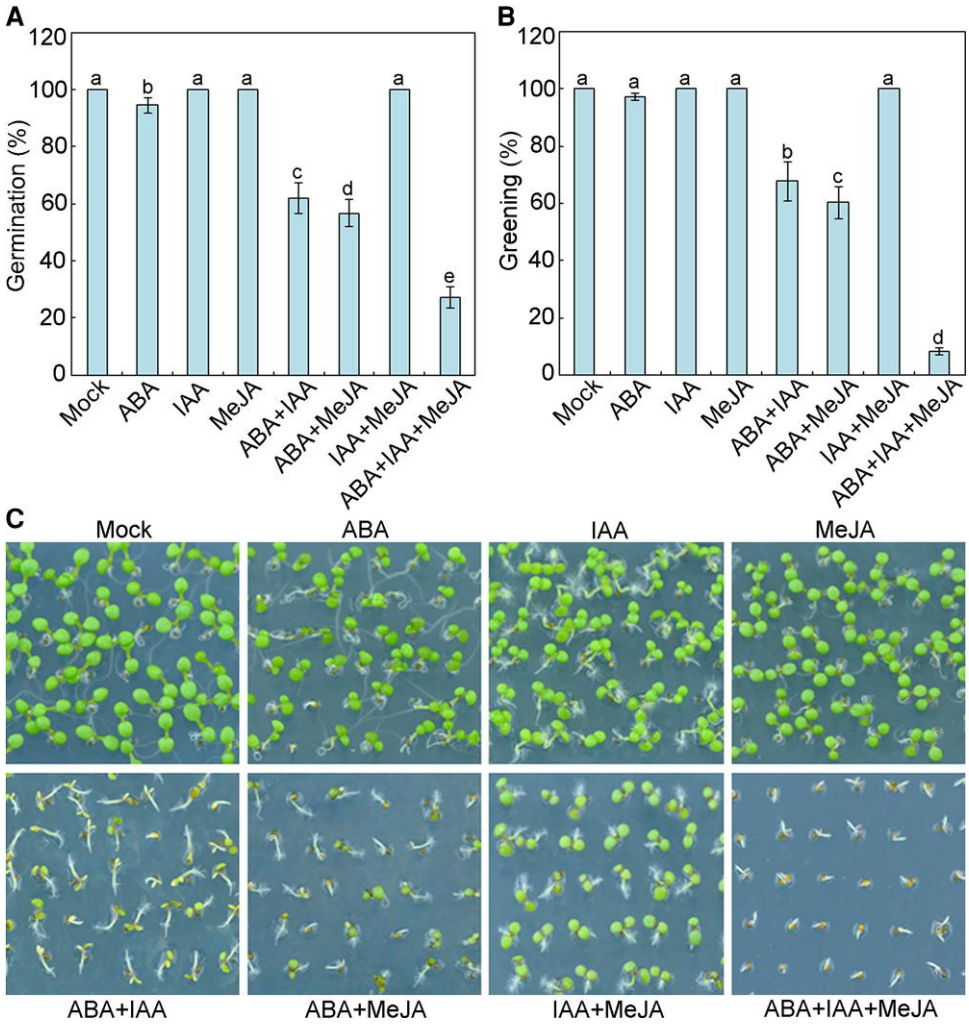

Figure 1 Auxin and JA synergistically stimulate ABA signaling during seed germination. A, Germination of wild-type seeds on half-strength MS medium containing 0.3 μM ABA, 1 μM IAA, and/or 10 μM MeJA. Seed germination was recorded 2 d after stratification at 4°C. In the mock treatment, an equal volume of 10% (v/v) ethanol was added. B, Cotyledon greening of wild type on half-strength MS medium with 0.3 μM ABA, 1 μM IAA, and/or 10 μM MeJA. Cotyledon greening was scored 5 d after stratification. Experiments were performed five times by analyzing different batches of seeds. Each batch of seeds was pooled from more than 80 independent plants. For each biological replicate, more than 120 seeds were examined. Values are means ± standard deviation (SD). Bars with different letters are significantly different from each other ($P < 0.05$). Data were analyzed by analysis of variance (ANOVA). C, Seedlings of wild type 6 d after germination on half-strength MS medium with 0.3 μM ABA, 1 μM IAA, and/or 10 μM MeJA.

et al., 2009). As shown in Supplemental Figure 4, the seeds of two leaky loss-of-function *COI1* mutants *coi1-16* and *coi1-2* showed significantly higher percentages of germination and expanded greening cotyledons on half-strength MS medium containing 0.3 μM ABA and 1 μM IAA, compared with seeds of wild type. These results show that COI1-mediated endogenous JA signaling is required for auxin-increased ABA responses during seed germination. Taken together, auxin and JA function synergistically to enhance ABA signaling and inhibit seed germination.

ARF10 and ARF16 transcription factors physically interact with JAZ repressors of JA signaling

Having ascertained that auxin acts together with JA to stimulate the ABA-induced delay of seed germination, we followed up by investigating the underlying molecular mechanisms. Previous studies have revealed that JAZ proteins are critical repressors of JA signaling and negatively modulate JA responses (Chini et al., 2007; Thines et al., 2007). Importantly, JAZ repressors interact with several transcriptional regulators to integrate JA with other signaling pathways (Hou et al., 2010; Zhu et al., 2011; Ju et al., 2019; Zhou et al., 2019; Pan et al., 2020). Because auxin is crucial for JA-regulated ABA responses, we hypothesized that key components of auxin signaling may also directly associate with JAZ proteins to mediate auxin-JA crosstalk. To test this idea, we used JAZ1 as the representative to screen for JAZ-interacting proteins potentially involved in auxin signaling from an Arabidopsis cDNA library in a yeast two-hybrid system. The full-length JAZ1 was ligated with the Gal4 DNA-binding domain of

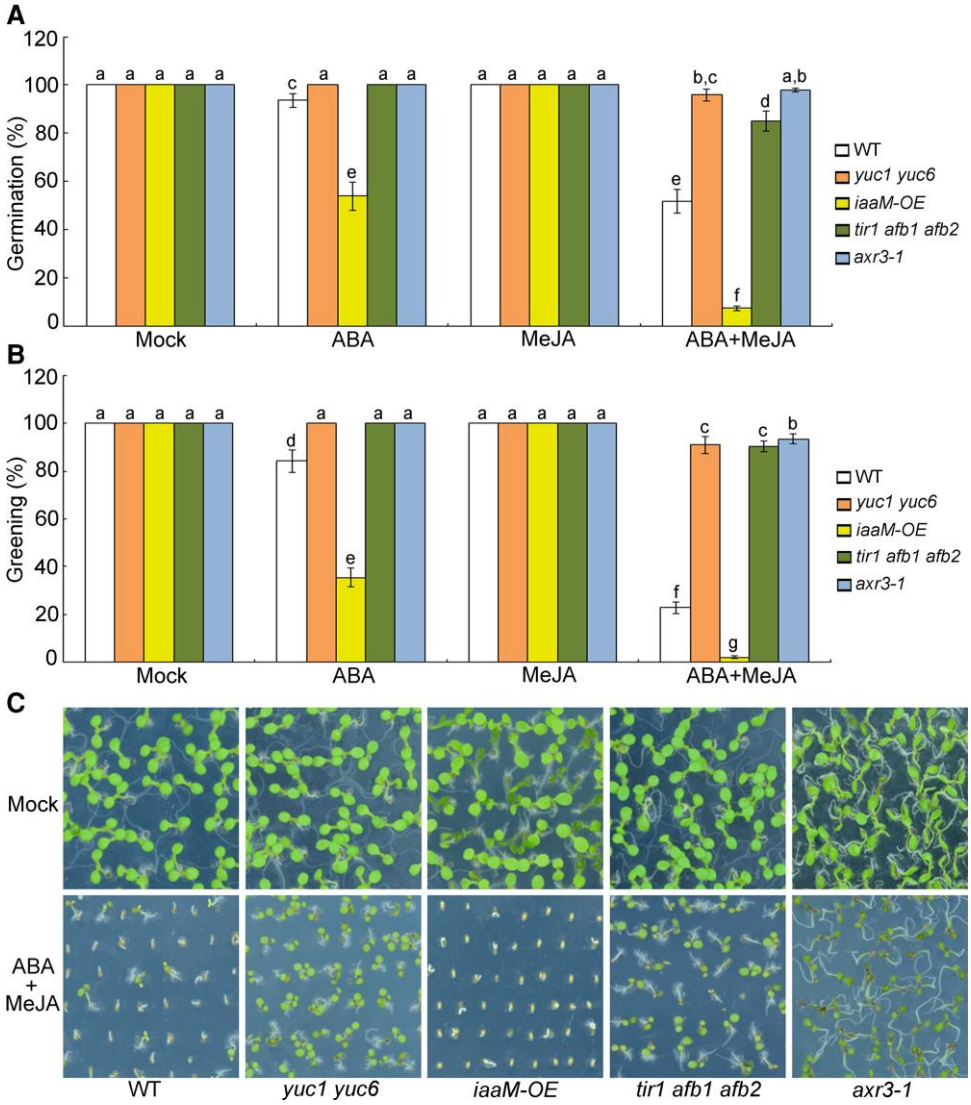

Figure 2 Auxin biosynthesis and TIR1/AFB-mediated auxin signaling are required for JA-enhanced ABA signaling. A, Germination of the wild type (WT), *yuc1 yuc6*, *iaaM-OE*, *tir1 afb1 afb2*, and *axr3-1*. Seed germination was recorded 2 d after stratification on half-strength MS medium supplemented with 0.3 μM ABA and/or 10 μM MeJA. In the mock treatment, an equal volume of 10% (v/v) ethanol was added. B, Cotyledon greening of the WT, *yuc1 yuc6*, *iaaM-OE*, *tir1 afb1 afb2*, and *axr3-1*. Cotyledon greening was scored 4 d after stratification on half-strength MS medium with 0.3 μM ABA and/or 10 μM MeJA. Experiments were performed five times by analyzing different batches of seeds. Each batch of seeds was pooled from more than 80 independent plants. For each biological replicate, more than 120 seeds were examined. Values are means ± SD. Bars with different letters are significantly different from each other ($P < 0.05$). Data were analyzed by analysis of variance (ANOVA). C, Seedlings of WT, *yuc1 yuc6*, *iaaM-OE*, *tir1 afb1 afb2*, and *axr3-1* 4 d after germination on half-strength MS medium containing 0.3 μM ABA and 10 μM MeJA.

the bait vector (BD-JAZ1). After screening, independent clones encoding ARF16, a key transcription factor modulating auxin responses, were isolated four times by prototrophy for histidine (His) and adenine (Ade) (Supplemental Table 1). To confirm the JAZ1–ARF16 interaction, the full-length *ARF16* sequence was cloned and fused to the Gal4 activation domain of the prey vector (AD-ARF16). The protein–protein interaction was reconstructed when the BD-JAZ1 and AD-ARF16 vectors were co-transformed into yeast (Figure 3A). To analyze whether ARF16 specifically binds to JAZ1, we tested whether it also interacts with other Arabidopsis JAZ proteins. Besides JAZ1, ARF16 also associates with JAZ3, JAZ4, JAZ7, JAZ9, and JAZ11 in yeast (Figure 3A). Moreover, we assayed the interactions of ARF10 (the close homolog of ARF16) with JAZ proteins and found that it exhibited interactions with JAZ1, JAZ4, JAZ7, and JAZ9 in yeast (Figure 3A).

To identify the region(s) of ARF16 responsible for the interaction with JAZ proteins, we performed additional directed yeast two-hybrid analyses. Five truncated variants of the *ARF16* sequence were produced, and each one was fused to the Gal4 activation domain of the prey vector (Supplemental Figure 5A; Tiwari et al., 2003). The results showed that deletion of the C-terminal domain of ARF16

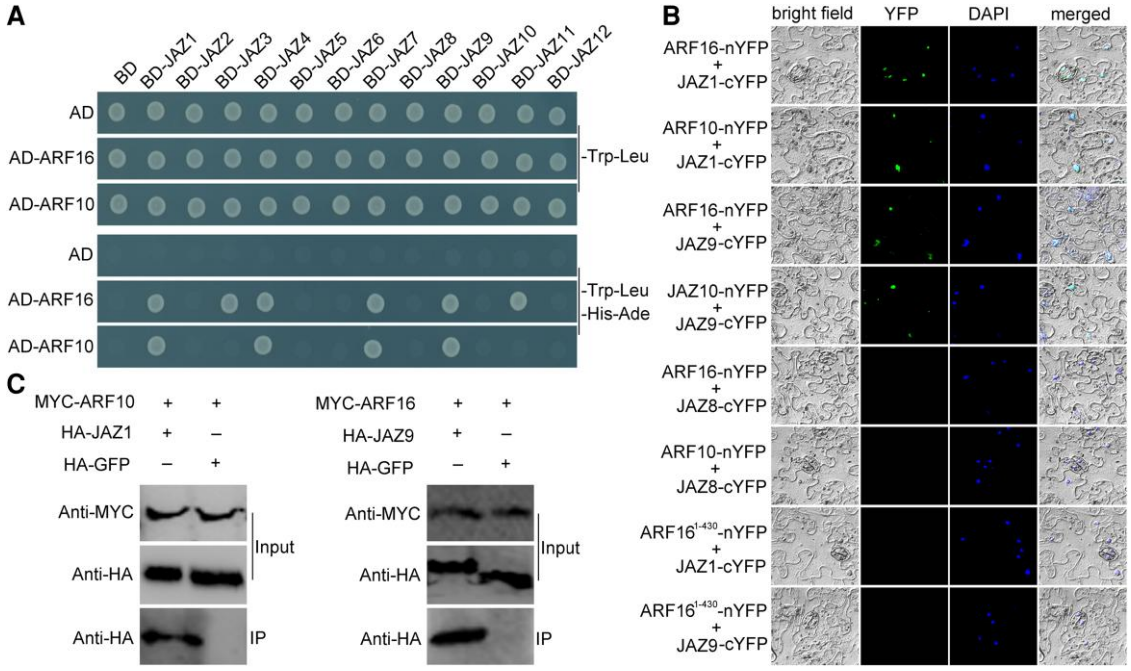

Figure 3 Physical interactions of JAZ repressors with ARF10 and ARF16. A, Yeast two-hybrid screening assays showing interactions of JAZ repressors with ARF10 or ARF16. Interactions are indicated by the ability of yeast cells to grow on dropout medium lacking Leu, Trp, His, and Ade for 4 d after plating. Empty pGBKT7 (BD) and pGADT7 (AD) were used as negative controls. B, Bimolecular fluorescence complementation (BiFC) assays. Fluorescence was detected in the nuclear compartment of transformed *N. benthamiana* cells, resulting from the complementation of JAZ1-cYFP (or JAZ9-cYFP) with ARF10-nYFP or ARF16-nYFP. No signal was observed for the negative controls in which JAZ8-cYFP was co-expressed with ARF10-nYFP or ARF16-nYFP, and $ARF16^{1-430}$-nYFP (the sequence encoding the N-terminal part of ARF16 fused with nYFP) was co-expressed with JAZ1-cYFP or JAZ9-cYFP. Nuclei are indicated by DAPI staining. C, Co-immunoprecipitation (Co-IP) assays. MYC-fused ARF10 or ARF16 was immunoprecipitated using an anti-MYC antibody (1:250), and the coimmunoprecipitated protein was detected using an anti-HA antibody (1:10,000). Protein input for MYC-fused ARF10 or ARF16 in immunoprecipitated complexes was also detected and is shown. Experiments were repeated three times with similar results.

(amino acids 545–670) did not affect its associations with JAZ1 and JAZ9 (Supplemental Figure 5A). Further mapping revealed that both the N-terminal domain (NTD) and the middle region (MR) of ARF16 (amino acids 1–544) are required for the interactions with JAZ1 and JAZ9 (Supplemental Figure 5A). We conducted similar analyses to identify the fragments of JAZ1 essential for binding to ARF10 and ARF16. We generated six fragments of the *JAZ1* sequence: three encoding portions of the N terminus ($JAZ1^{1-180}$, $JAZ1^{1-120}$, and $JAZ1^{1-60}$) and three encoding portions of the C terminus with the Jas domain ($JAZ1^{161-253}$, $JAZ1^{121-253}$, and $JAZ1^{61-253}$). As shown in Supplemental Figure 5B, deletion of the C-terminal residues of JAZ1 (amino acids 61–253, including the ZIM and Jas domains) did not interfere with its direct associations with ARF10 and ARF16 in yeast. In contrast, the removal of the N-terminal portion of JAZ1 (amino acids 1–60) eliminated its ability to interact with ARF10 and ARF16 (Supplemental Figure 5B). These observations demonstrate that the N-terminal region of JAZ1 is critical for its interaction with ARF10 and ARF16.

To further verify the interactions of ARF10 and ARF16 with JAZ proteins in plant cells, we performed bimolecular fluorescence complementation (BiFC) assays in *Nicotiana benthamiana*. The sequence encoding the N-terminal fragment of yellow fluorescent protein (nYFP), driven by the Cauliflower mosaic virus (CaMV) 35S promoter (*Pro35S*), was fused with full-length *ARF10* and *ARF16* sequences to produce ARF10-nYFP and ARF16-nYFP. The sequence encoding the C-terminal fragment of YFP (cYFP) under the control of *Pro35S* was ligated with full-length *JAZ1* and *JAZ9* sequences to generate JAZ1-cYFP and JAZ9-cYFP. When JAZ1-cYFP was co-expressed with ARF10-nYFP or ARF16-nYFP in leaves of *N. benthamiana*, strong YFP fluorescence was observed in the nuclear compartment of transformed cells, as revealed by 4′,6-diamidino-2-phenylindole (DAPI) staining (Figure 3B; Supplemental Figure 6). Similarly, YFP signal was also detected in *N. benthamiana* leaf cells when JAZ9-cYFP was co-infiltrated with ARF10-nYFP or ARF16-nYFP (Figure 3B; Supplemental Figure 6). No fluorescence was detected in the negative controls, i.e. when JAZ8-cYFP (JAZ8 fused with cYFP) was co-expressed with ARF10-nYFP or ARF16-nYFP, or when $ARF16^{1-430}$-nYFP (the N-terminal region of ARF16 fused with nYFP) was co-expressed with JAZ1-cYFP or JAZ9-cYFP (Figure 3B; Supplemental Figure 6). In addition to BiFC assays, co-immunoprecipitation (CoIP) assays with total plant

protein were conducted to further corroborate physical associations between JAZ proteins and ARF10 or ARF16 (Figure 3C). Collectively, these results demonstrate that the ARF10 and ARF16 transcription factors physically interact with several JAZ repressors of JA signaling, suggesting that ARF10 and ARF16 function together with JAZ proteins to mediate the convergence of the auxin and JA signaling pathways.

ARF10 and ARF16 are essential for JA-enhanced ABA signaling during seed germination

The ARF10 and ARF16 transcription factors are two important regulators of auxin-mediated processes in Arabidopsis (Wang et al., 2005; Liu et al., 2007, 2013; Kieffer et al., 2010). Interestingly, ARF10 and ARF16 were shown to positively modulate ABA responses during seed germination (Liu et al., 2007, 2013). The loss-of-function *arf10 arf16* double mutant is dramatically less sensitive to ABA, whereas two transgenic lines expressing *miRNA160*-resistant forms of *ARF10* and *ARF16* (termed *mARF10* and *mARF16*, respectively), which have silent mutations in the miRNA target site, are hypersensitive to ABA (Wang et al., 2005; Liu et al., 2007, 2013). Because ARF10 and ARF16 directly associate with JAZ repressors of JA signaling, we wondered whether they are required for JA-activated ABA signaling during seed germination. To test this idea, we investigated the performance of wild type, *arf10 arf16*, *mARF10*, and *mARF16* seeds on half-strength MS medium containing 0.3 μM ABA with or without 10 μM MeJA. As expected, wild-type seeds simultaneously treated with ABA and MeJA had significantly lower percentages of germination and expanded green cotyledons compared with those treated with ABA or MeJA alone (Figure 4, A–C). Similar to the results of previous studies (Liu et al., 2007, 2013), we also found that seeds of the *arf10 arf16* double mutant germinated and grew faster than the wild-type seeds upon ABA treatment (Figure 4, A–C). Furthermore, compared with wild type, the *arf10 arf16* double mutant exhibited much higher percentages of seed germination and expanded greening cotyledons on medium containing both ABA and MeJA (Figure 4, A–C). In contrast, the progeny of ARF10- and ARF16-overaccumulating transgenic plants (*mARF10* and *mARF16*, respectively) displayed significantly lower percentages of seed germination and cotyledon greening in the presence of both ABA and MeJA compared with wild type (Figure 4, A–C). Taken together, these findings demonstrate that the ARF10 and ARF16 transcription factors are necessary for JA-stimulated ABA signaling during seed germination.

Overaccumulation of ARF16 partially rescues the hyposensitive phenotype of *coi1-16*, *coi1-2*, and *JAZ1-ΔJas* under combined ABA and JA treatment

JAZ proteins repress JA signaling by associating with and inhibiting multiple transcription factors (Kazan and Manners, 2013; Chini et al., 2016; Guo et al., 2018a; Howe et al., 2018). JAZ repressors are degraded when the concentration of JA is elevated, but remain stable in *coi1* mutants, where they constitutively repress JA responses (Chini et al., 2007; Thines et al., 2007; Katsir et al., 2008; Fonseca et al., 2009; Guo et al., 2018b). Recent studies revealed that COI1/JAZ-mediated JA signaling is positively involved in the ABA-induced delay of seed germination (Ju et al., 2019; Pan et al., 2020). The *coi1* mutants and transgenic plants overexpressing *JAZ* with a deleted *Jas* domain (*JAZ-ΔJas*, which highly accumulates JAZ proteins) are hyposensitive to ABA (Ju et al., 2019; Pan et al., 2020).

Because ARF10 and ARF16 interact with JAZ repressors and are required for JA-increased ABA signaling, we hypothesized that the ABA-hyposensitive phenotype of *coi1* mutants and *JAZ-ΔJas* plants may partially result from attenuation of these two factors by JAZ proteins. To verify this, we analyzed whether overaccumulation of ARF10 or ARF16 could rescue the hyposensitive phenotype of *coi1* and/or *JAZ1-ΔJas* seeds germinating on half-strength MS medium containing both ABA and MeJA. The *coi1-16*, *coi1-2*, and a *JAZ1-ΔJas* transgenic plant were each crossed with *mARF16* to generate *coi1-16 mARF16*, *coi1-2 mARF16*, and *JAZ1-ΔJas mARF16*, respectively. In addition, *coi1-16* was also crossed with *mARF10* to generate *coi1-16 mARF10*. The seed germination phenotypes of wild type, *mARF16*, *coi1-16*, and *coi1-16 mARF16* were examined initially. As expected, the germination percentages of *coi1-16* seeds were noticeably increased, whereas those of *mARF16* seeds were obviously reduced, compared with that of wild type, on medium containing both 0.3 μM ABA and 10 μM MeJA (Figure 5, A–C). Importantly, the percentages of seed germination and expanded greening cotyledons were significantly lower in *coi1-16 mARF16* than in *coi1-16* upon treatment with ABA and MeJA simultaneously (Figure 5, A–C). Likewise, *coi1-2 mARF16* seeds germinated and grew slower than *coi1-2* seeds in response to ABA with or without MeJA (Supplemental Figure 7). As shown in Supplemental Figure 8, the percentages of seed germination and expanded greening cotyledons were also lower in *JAZ1-ΔJas mARF16* than in *JAZ1-ΔJas* in the presence of both ABA and MeJA. Similarly, the seeds of *coi1-16 mARF10* displayed lower percentages of germination and expanded greening cotyledons on medium containing ABA and MeJA, compared with seeds of *coi1-16* (Supplemental Figure 9). These results demonstrate that the overaccumulation of ARF16 or ARF10 partially suppresses the hyposensitive phenotype of *coi1-16*, *coi1-2*, and/or *JAZ1-ΔJas* in response to both ABA and JA during seed germination.

ARF10 and ARF16 physically interact with ABI5

In addition to ARF10 and ARF16, the ABI5 and ABI3 transcription factors have recently been shown to interact with JAZ repressors and integrate the JA and ABA signaling pathways during seed germination (Ju et al., 2019; Pan et al., 2020). These findings prompted us to query whether ARF10 and ARF16 also function together with ABI5 and ABI3. To test this idea, we first analyzed direct associations of ARF10 and

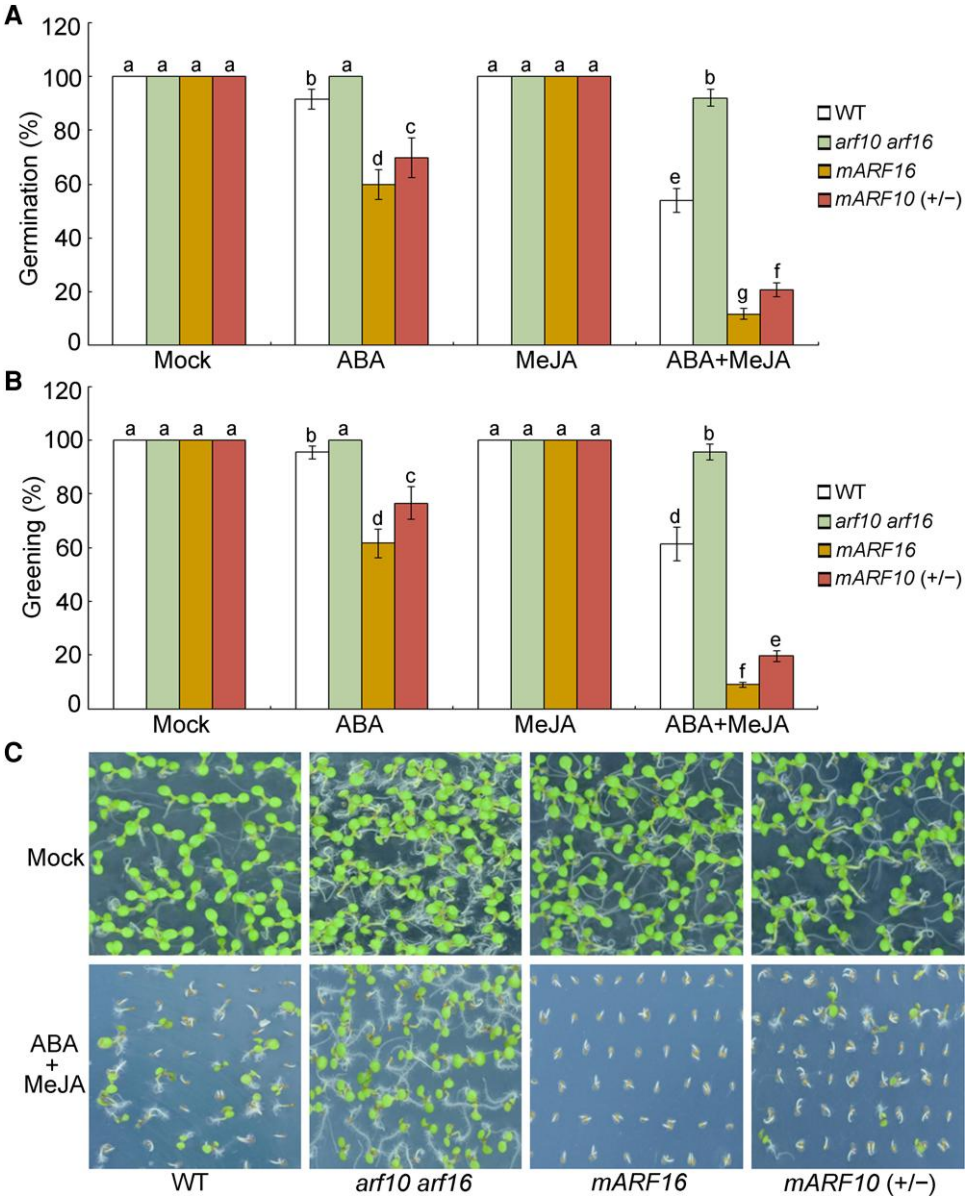

Figure 4 ARF10 and ARF16 are necessary for JA-stimulated ABA signaling during seed germination. A, Germination of the wild type (WT), *arf10 arf16*, *mARF10*, and *mARF16*. Seed germination was recorded 2 d after stratification on half-strength MS medium containing 0.3 μM ABA and/or 10 μM MeJA. In the mock treatment, an equal volume of 10% (v/v) ethanol was added. B, Cotyledon greening of the WT, *arf10 arf16*, *mARF10*, and *mARF16*. Cotyledon greening was scored 5 d after stratification on half-strength MS medium with 0.3 μM ABA and/or 10 μM MeJA. Experiments were performed five times by analyzing different batches of seeds. Each batch of seeds was pooled from more than 80 independent plants. For each biological replicate, more than 120 seeds were examined. Values are means ± SD. Bars with different letters are significantly different from each other ($P < 0.05$). Data were analyzed by analysis of variance (ANOVA). C, Seedlings of WT, *arf10 arf16*, *mARF10*, and *mARF16* 5 d after germination on half-strength MS medium containing 0.3 μM ABA and 10 μM MeJA.

ARF16 with ABI5 (or ABI3) in the yeast two-hybrid system. The full-length *ABI5* or *ABI3* sequence was ligated with the Gal4 DNA-binding domain of the bait vector to generate BD-ABI5 or BD-ABI3. As shown in Figure 6A, ARF10 and ARF16 strongly interacted with ABI5 in yeast. However, no obvious interaction of ARF10 or ARF16 with ABI3 was detected. ABI4 is another crucial transcription factor positively involved in ABA signaling during seed germination (Finkelstein et al., 1998; Söderman et al., 2000). We also fused the full-length *ABI4* sequence to the Gal4 DNA-binding domain of the bait vector to generate BD-ABI4. Experiments showed that ABI4 did not interact with ARF10 and ARF16 in yeast (Figure 6A), further supporting the specificity of the interactions of ABI5 with ARF10 and ARF16. We also investigated which domain of ARF16 mediates its association with ABI5 and found that the middle region of ARF16 (amino acids 357–544) is sufficient for the interaction (Supplemental Figure 10A). A mutated form of ARF16 with the middle

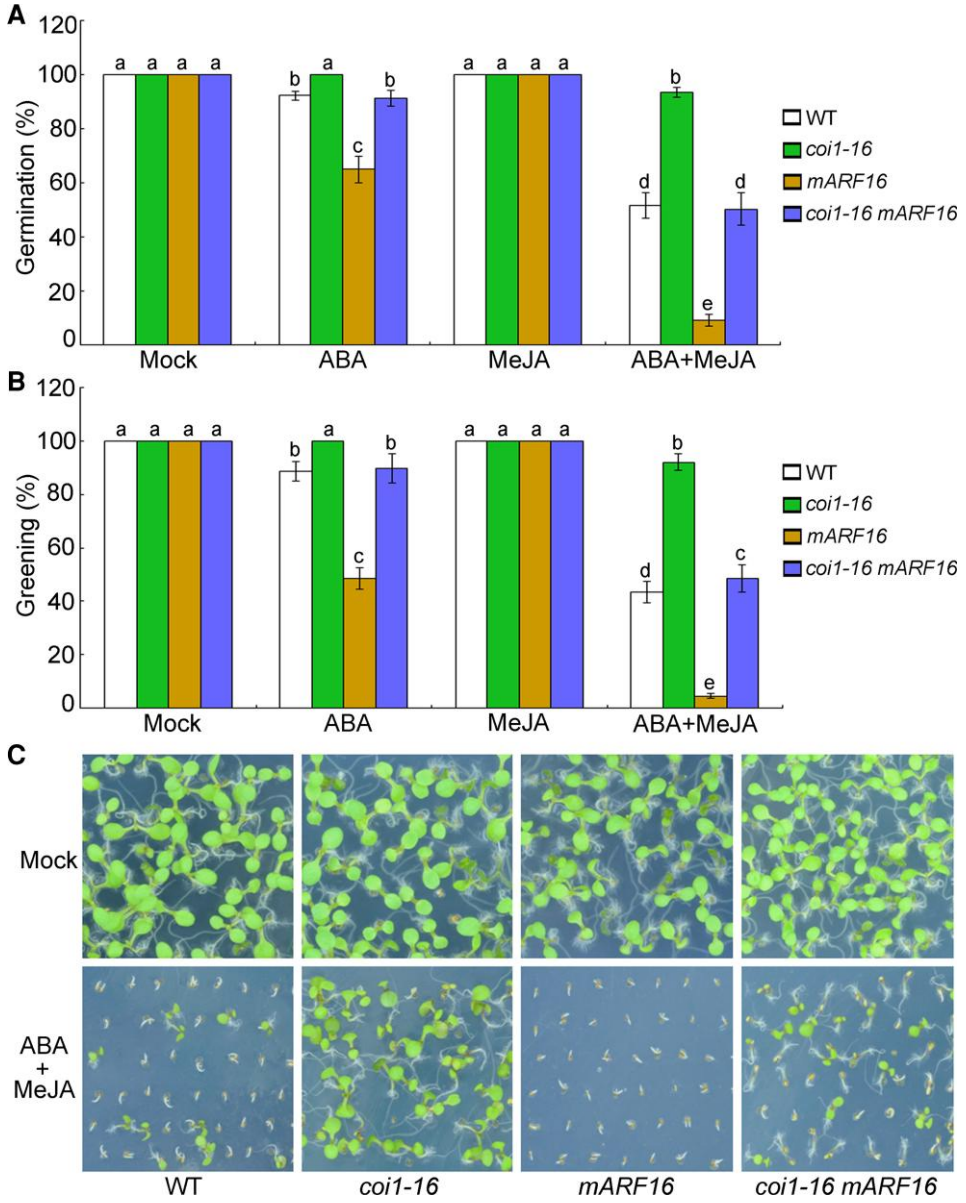

Figure 5 Overaccumulation of ARF16 partially rescues the hyposensitive phenotype of *coi1-16* under combined ABA and JA treatment. A, Germination of the wild type (WT), *coi1-16*, *mARF16*, and *coi1-16 mARF16*. Seed germination was recorded 2 d after stratification on half-strength MS medium containing 0.3 μM ABA and/or 10 μM MeJA. In the mock treatment, an equal volume of 10% (v/v) ethanol was added. B, Cotyledon greening of the WT, *coi1-16*, *mARF16*, and *coi1-16 mARF16*. Cotyledon greening was scored 4.5 d after stratification on half-strength MS medium with 0.3 μM ABA and/or 10 μM MeJA. Experiments were performed five times by analyzing different batches of seeds. Each batch of seeds was pooled from more than 80 independent plants. For each biological replicate, more than 120 seeds were examined. Values are means ± SD. Bars with different letters are significantly different from each other ($P < 0.05$). Data were analyzed by analysis of variance (ANOVA). C, Seedlings of WT, *coi1-16*, *mARF16*, and *coi1-16 mARF16* 4.5 d after germination on half-strength MS medium containing 0.3 μM ABA and 10 μM MeJA.

region deleted (ARF16$^{1-430\ 545-670}$; also designed as ARF16$^{\Delta MR}$) did not interact with ABI5 (Supplemental Figure 10A). Similarly, to identify the region of ABI5 critical for binding to ARF16, we fused five truncated sequences of *ABI5* to the Gal4 DNA-binding domain of the bait vector (Supplemental Figure 10B). The results showed that the amino acid residues 165–220 of ABI5 are responsible for the ARF16–ABI5 interaction (Supplemental Figure 10B).

The interaction between ARF16 and ABI5 was further verified by BiFC and CoIP assays *in planta*. For the BiFC assays, the full-length *ABI5* sequence was fused to the sequence encoding the C-terminal YFP fragment to generate ABI5-cYFP. When ARF10-nYFP or ARF16-nYFP was co-expressed with ABI5-cYFP in *N. benthamiana* leaves, strong YFP fluorescence was detected in the nucleus of the transformed cells, as revealed by DAPI staining (Figure 6B; Supplemental

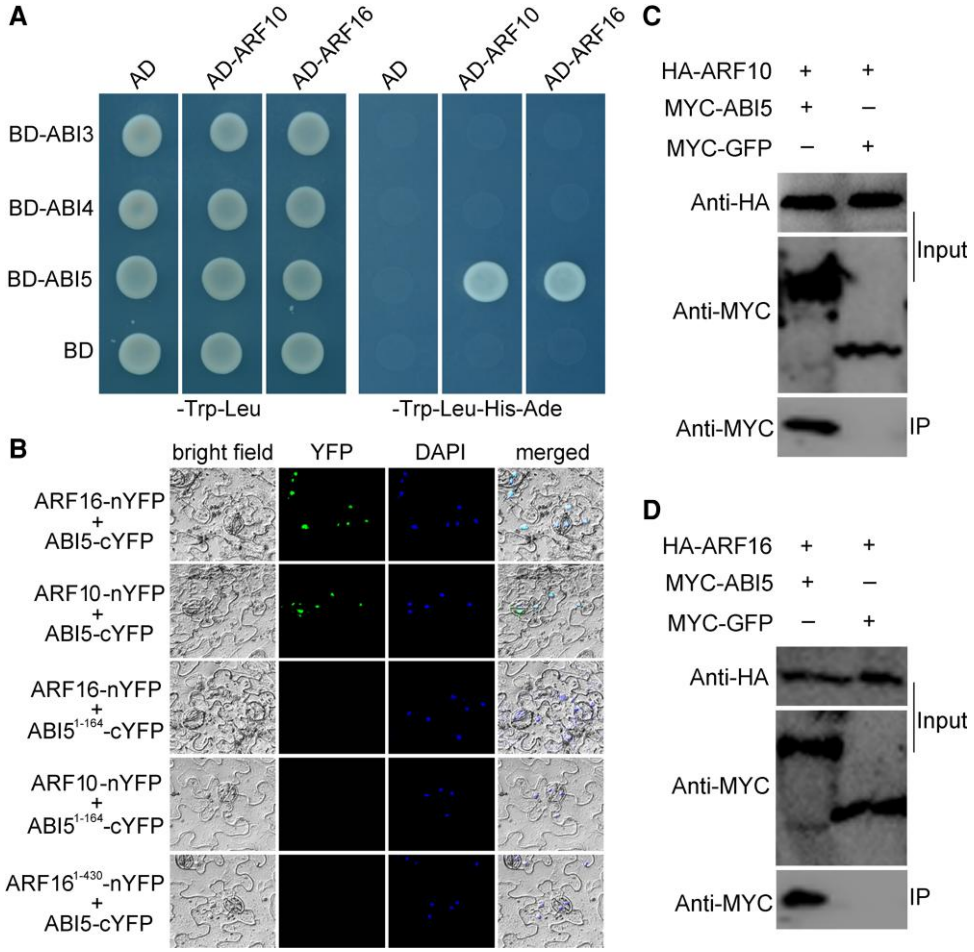

Figure 6 Physical interactions of ABI5 with ARF10 and ARF16. A, Yeast two-hybrid assays showing interactions of ABI5 with ARF10 and ARF16. Interactions are indicated by the ability of yeast cells to grow on dropout medium lacking Leu, Trp, His, and Ade and containing 10 mM 3-amino-triazole for 4 d after plating. Empty pGBKT7 (BD) and pGADT7 (AD) were used as negative controls. B, Bimolecular fluorescence complementation (BiFC) assays. Fluorescence was detected in the nuclear compartment of transformed *N. benthamiana* cells, resulting from the complementation of ABI5-cYFP with ARF10-nYFP or ARF16-nYFP. No signal was observed in the negative controls in which ABI5$^{1-164}$-cYFP (the sequence encoding the N-terminal domain of ABI5 fused with cYFP) was co-expressed with ARF10-nYFP or ARF16-nYFP, and ARF16$^{1-430}$-nYFP (the sequence encoding the N-terminal part of ARF16 fused with nYFP) was co-expressed with ABI5-cYFP. Nuclei are indicated by DAPI staining. C and D, Co-immunoprecipitation (Co-IP) assays. HA-fused ARF10 (C) or ARF16 (D) was immunoprecipitated using an anti-HA antibody (1:250), and the coimmunoprecipitated protein was detected using an anti-MYC antibody (1:10,000). Protein input for HA-fused ARF10 or ARF16 in immunoprecipitated complexes was also detected and is shown. Experiments were repeated three times with similar results.

Figure 11). No YFP fluorescence was detected in negative controls in which ARF16$^{1-430}$-nYFP was co-expressed with ABI5-cYFP, or when ARF10-nYFP or ARF16-nYFP was co-expressed with ABI5$^{1-164}$-cYFP (sequence encoding the N-terminal amino acid residues 1–164 of ABI5 fused to cYFP) (Figure 6B; Supplemental Figure 11). In addition to the BiFC assays, the results of CoIP assays provided further evidence for direct associations of ARF10 and ARF16 with ABI5 in vivo (Figure 6, C and D). Collectively, these findings show that ARF10 and ARF16 physically interact with the ABI5 transcription factor in the nuclei of plant cells.

Genetic interactions of ARF10 and ARF16 with ABI5

The ABI5 transcription factor is a master positive modulator of ABA responses during seed germination and postgerminative seedling growth. The germination of *abi5* mutant seeds is hyposensitive to ABA compared with that of wild-type seeds (Finkelstein, 1994; Finkelstein and Lynch, 2000a). Having ascertained that ARF10 and ARF16 directly associate with ABI5, we investigated whether *ARF10* and *ARF16* genetically interact with *ABI5* to mediate JA-enhanced ABA signaling during seed germination. For these analyses, we generated *abi5-1 mARF16* and *abi5-7 mARF16* plants by crossing *mARF16* with *abi5-1* or *abi5-7*. *abi5-1* is a loss-of-function mutant of *ABI5* in the Wassilewskija (Ws) background (Finkelstein, 1994; Finkelstein and Lynch, 2000a) that was introduced into the Columbia (Col) background by backcrossing it with wild-type Col six times (Hu et al., 2019; Pan et al., 2020; Yang et al., 2021). Similar with the results of a previous study

(Pan et al., 2020), compared with seeds of wild type, seeds of *abi5-1* and *abi5-7* had much higher percentages of germination and expanded greening cotyledons in response to both 0.3 μM ABA and 10 μM MeJA on half-strength MS medium (Figure 7, A–C; Supplemental Figure 12). Moreover, similar to *abi5-1* and *abi5-7* seeds, the progeny of *abi5-1 mARF16* and *abi5-7 mARF16* were hyposensitive to ABA and MeJA, with much higher percentages of seed germination and expanded greening cotyledons than those of wild type and *mARF16* (Figure 7, A–C; Supplemental Figure 12). These results show that the hypersensitivity of *mARF16* to both ABA and MeJA during seed germination requires ABI5, suggesting that the ability of ARF16 to mediate JA-activated ABA responses mainly depends on a functional ABI5.

Nevertheless, the responses of *abi5-1 mARF16* and *abi5-7 mARF16* to both ABA and MeJA were clearly different from those of *abi5-1* and *abi5-7*, implying that ARF16 may associate with other components of ABA signaling to modulate JA-increased ABA responses during seed germination. To further analyze the genetic interactions of *ARF10* and *ARF16* with *ABI5*, we crossed the *arf10 arf16* double mutant with *abi5-1* to generate the *arf10 arf16 abi5-1* triple mutant, and examined its seed germination on half-strength MS medium containing 0.6 μM ABA and 20 μM MeJA. Compared with *arf10 arf16* and *abi5-1*, the *arf10 arf16 abi5-1* triple mutant displayed higher percentages of seed germination and expanded greening cotyledons (Supplemental Figure 13), supporting the idea that ARF10 and ARF16 associate with other regulators besides ABI5 to mediate JA-regulated ABA responses.

ARF10 and ARF16 stimulate the transcriptional function of ABI5, but JAZ proteins antagonize their regulatory effects

Having demonstrated that ARF10 and ARF16 physically and genetically interact with JAZ and ABI5 in modulating JA-enhanced ABA responses during seed germination, we further investigated the regulatory relationships among these proteins. Recent studies have shown that several interacting partners of ABI5 exert their regulatory effects mainly by activating or inhibiting the transcriptional function of ABI5 (Lim et al., 2013; Kim et al., 2016; Hu et al., 2019; Ju et al., 2019; Zhao et al., 2019; Pan et al., 2020; Yang et al., 2021). We thus analyzed the possible modulatory effect of ARF10 and ARF16 on the transcriptional function of ABI5 in Arabidopsis mesophyll protoplasts by performing dual-luciferase (LUC) reporter assays (Yoo et al., 2007). The effectors contained an *ABI5*, *ARF10*, *ARF16*, *JAZ1*, *JAZ9*, or *GFP* (green fluorescent protein) gene driven by *Pro35S* (Supplemental Figure 14A). Because *LATE EMBRYOGENESIS ABUNDANT 6* (*EM6*) and *EM1* are direct targets of ABI5 (Finkelstein and Lynch, 2000a; Lopez-Molina and Chua, 2000; Nakamura et al., 2001; Carles et al., 2002; Nakabayashi et al., 2005; Reeves et al., 2011), their promoters were ligated with the *LUC* gene to generate reporter constructs (Supplemental Figure 14A). Predictably, the expression of ABI5 dramatically up-regulated the expression of *LUC* driven by the *EM6* or *EM1* promoter in wild-type mesophyll protoplasts upon 5 μM ABA treatment, compared with the expression of GFP alone (Figure 8A; Zhou et al., 2015; Pan et al., 2018, 2020; Hu et al., 2019). However, *LUC* expression driven by the *EM6* or *EM1* promoter was apparently reduced in ABI5-expressing protoplasts of *arf10 arf16* but increased in protoplasts of *mARF10* and *mARF16*, compared with that in wild-type protoplasts (Figure 8A). These results imply that ARF10 and ARF16 are crucial for ABI5 to activate the transcription of downstream target genes.

To verify the regulatory effect of ARF10 and ARF16 on ABI5, we further compared the ability of ABI5 to stimulate *EM6* and *EM1* promoter activity in wild-type mesophyll protoplasts with or without the co-expression of ARF10 or ARF16. As shown in Figure 8B and Supplemental Figure 14B, the co-expression of ABI5 with ARF10 or ARF16 further enhanced *LUC* expression when compared with the co-expression of ABI5 and GFP. These findings support the notion that ARF10 and ARF16 stimulate the function of ABI5 to modulate the expression of downstream target genes such as *EM6* or *EM1* in response to ABA. Because ARF10 and ARF16 associate with ABI5 and enhance its transcriptional role to activate *EM6* and *EM1*, we wondered whether ARF10 and ARF16 directly modulate the expression of *EM6* and *EM1* by recognizing their promoters. The results based on yeast one-hybrid analyses showed that ARF10 and ARF16 did not bind to the promoter sequences of *EM6* and *EM1* (Supplemental Figures 15 and 16). Previous studies have shown that JAZ proteins are repressors of JA signaling and that they interfere with the transcriptional functions of multiple JAZ-interacting factors (Chini et al., 2007; Thines et al., 2007; Katsir et al., 2008; Fonseca et al., 2009; Kazan and Manners, 2013; Schweizer et al., 2013; Guo et al., 2018a). As JAZ repressors interact with ARF10 and ARF16, we hypothesized that JAZ proteins might affect the function of these two factors. To test this, we co-expressed ARF10 or ARF16 with JAZ1 (or JAZ9) in protoplasts of wild type. The co-expression of ARF10 or ARF16 with JAZ proteins decreased *LUC* expression compared with the expression of ARF10 or ARF16 alone in protoplasts of wild type (Figure 8B; Supplemental Figure 14B). Moreover, the co-expression of ARF10 or ARF16 with JAZ proteins also down-regulated *LUC* expression compared with the expression of ARF10 or ARF16 in ABI5-expressing protoplasts of wild type (Figure 8B; Supplemental Figure 14B). These results suggest that JAZ1 and JAZ9 proteins antagonize the regulatory functions of ARF10, ARF16, and ABI5 to modulate the expression of downstream genes.

To further confirm the modulatory effects among ARF16, ABI5, and JAZ, we investigated whether the protein interactions of ARF16–ABI5 and ARF16–JAZ are important for the regulations. We generated effectors that included a mutated form of ARF16 or JAZ1, disrupting the physical associations

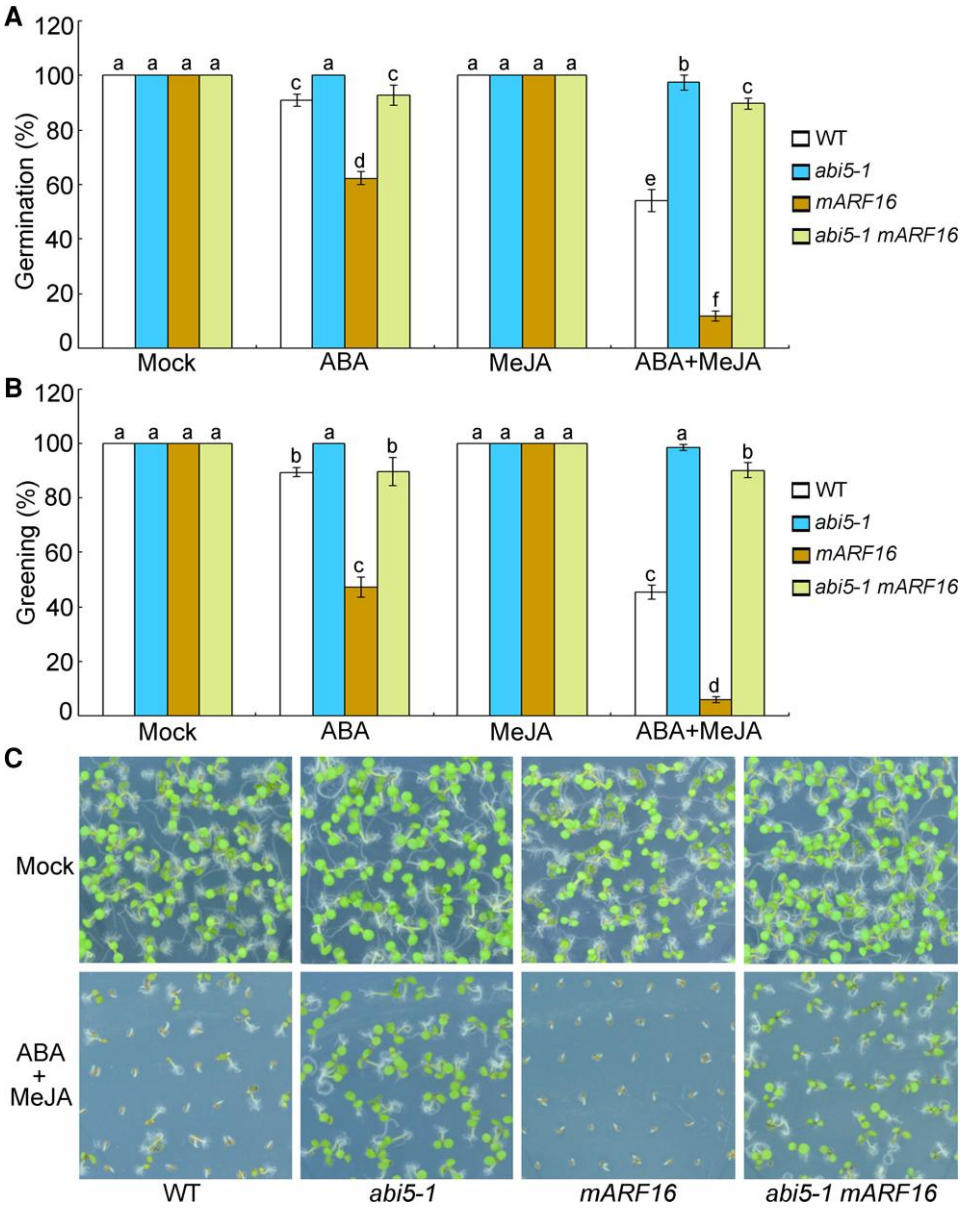

Figure 7 The hypersensitivity of *mARF16* to both ABA and JA during seed germination requires ABI5. A, Germination of the wild type (WT), *abi5*, *mARF16*, and *abi5 mARF16*. Seed germination was recorded 2 d after stratification on half-strength MS medium containing 0.3 μM ABA and/or 10 μM MeJA. In the mock treatment, an equal volume of 10% (v/v) ethanol was added. B, Cotyledon greening of the WT, *abi5*, *mARF16*, and *abi5 mARF16*. Cotyledon greening was scored 4.5 d after stratification on half-strength MS medium with 0.3 μM ABA and/or 10 μM MeJA. Experiments were performed five times by analyzing different batches of seeds. Each batch of seeds was pooled from more than 80 independent plants. For each biological replicate, more than 120 seeds were examined. Values are means ± SD. Bars with different letters are significantly different from each other ($P < 0.05$). Data were analyzed by analysis of variance (ANOVA). C, Seedlings of WT, *abi5*, *mARF16*, and *abi5 mARF16* 4.5 d after germination on half-strength MS medium containing 0.3 μM ABA and 10 μM MeJA.

of ARF16–ABI5 and ARF16–JAZ1 (Supplemental Figures 5, B and 10A). One effector contained a truncated ARF16 fragment with the middle region deleted ($ARF16^{\Delta MR}$) (Supplemental Figures 10, A and 14A). The other effector included a portion of JAZ1 with the N-terminal 60 amino acids removed ($JAZ1^{61-253}$) (Supplemental Figures 5, B and 14A). As shown in Supplemental Figure 14C, the expression of *LUC* driven by the *EM6* promoter displayed no significant change in $ARF16^{\Delta MR}$-expressing wild-type protoplasts compared with that in GFP-expressing protoplasts. More importantly, the co-expression of $ARF16^{\Delta MR}$ with ABI5 did not increase the *LUC* expression level when compared with the expression of ABI5 (Supplemental Figure 14C). These observations suggest that the middle region is essential for ARF16 to stimulate the transcriptional function of ABI5 and mediate the expression of downstream genes. Furthermore,

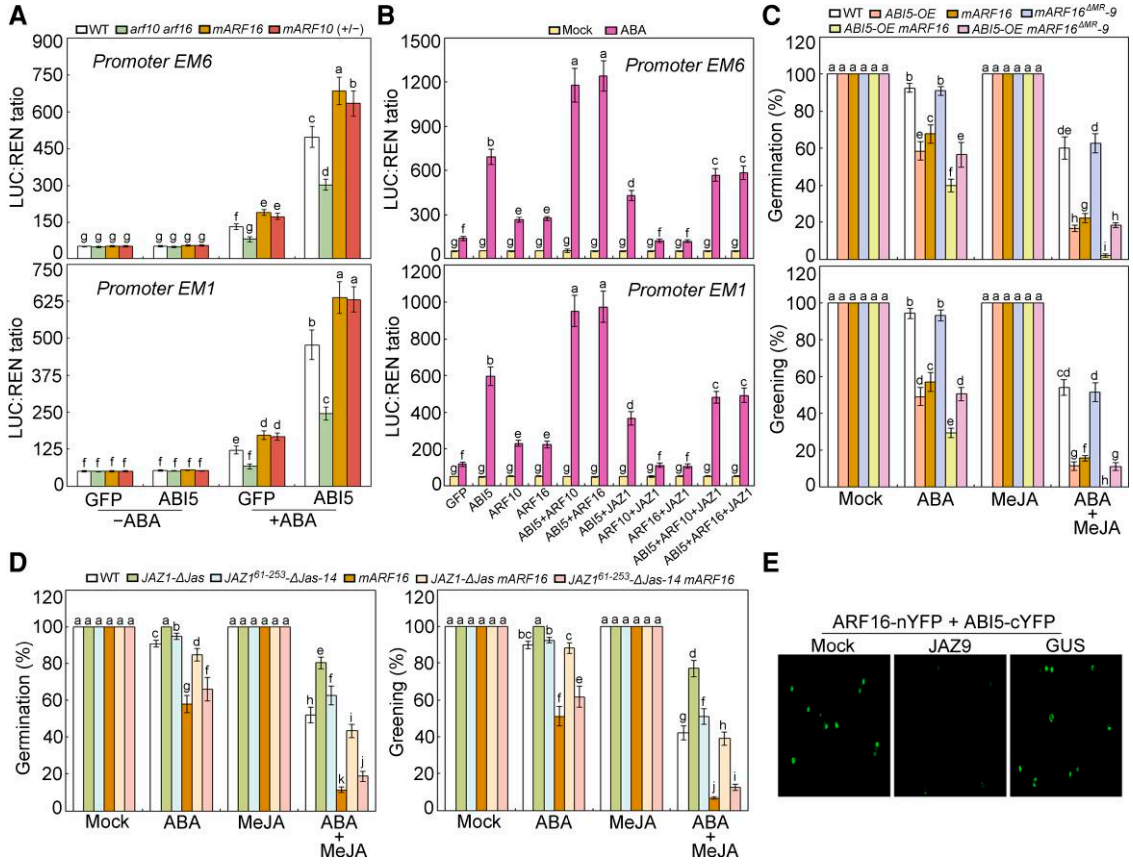

Figure 8 ARF10 and ARF16 activate the transcriptional function of ABI5, whereas JAZ repressors antagonize their effects. A, Transient transcriptional activity assays showing that activation of the *EM6* and *EM1* promoter by ABI5 is reduced in the *arf10 arf16* mutant in response to 5 μM ABA. Error bars indicate SD from three biological replicates using different batches of *arf10 arf16* mutants; each replicate was from different leaves of more than 60 plants. WT, wild type. B, Transient dual-luciferase reporter assays showing that JAZ1 antagonizes ARF10/16 and ABI5 to modulate the expression of *EM6* or *EM1* in response to 5 μM ABA. Error bars show SD from three biological replicates using different batches of wild-type plants; each replicate was from different wild-type leaves of more than 60 plants. C, Germination and greening of WT, *ABI5-OE*, *mARF16*, *mARF16$^{\Delta MR}$-9*, *ABI5-OE mARF16*, and *ABI5-OE mARF16$^{\Delta MR}$-9*. Seed germination and cotyledon greening were recorded 2.5 d and 5.5 d, respectively, after stratification on half-strength MS medium containing 0.3 μM ABA and/or 10 μM MeJA. In the mock treatment, an equal volume of 10% (v/v) ethanol was added. Experiments were performed five times by analyzing different batches of seeds. Each batch of seeds was pooled from more than 80 independent plants. For each biological replicate, more than 120 seeds were examined. D, Germination and greening of WT, *JAZ1-ΔJas*, *JAZ1$^{61-253}$-ΔJas-14*, *mARF16*, *JAZ1-ΔJas mARF16*, and *JAZ1$^{61-253}$-ΔJas-14 mARF16*. Seed germination and cotyledon greening were recorded 2 d and 4.5 d, respectively, after stratification on half-strength MS medium containing 0.3 μM ABA and/or 10 μM MeJA. Experiments were performed five times by analyzing different batches of seeds. Each batch of seeds was pooled from more than 80 independent plants. For each biological replicate, more than 120 seeds were examined. Values are means ± SD. Bars with different letters are significantly different from each other ($P < 0.05$). Data were analyzed by analysis of variance (ANOVA). E, BiFC analyses showing that JAZ9 interferes with the interaction between ARF16 and ABI5. As a negative control, when GUS was co-expressed with ARF16-nYFP and ABI5-cYFP, the fluorescence intensity was not obviously changed. Similar results were obtained from at least three independent replicates.

results showed that the N-terminal 60 amino acids are critical for JAZ1 to affect the transcriptional activity of ARF16. As shown in Supplemental Figure 14D, there was a greater reduction of *LUC* expression in wild-type protoplasts co-expressing ARF16 with JAZ1 than in protoplasts co-expressing ARF16 with JAZ1$^{61-253}$, compared with the expression of ARF16 alone. Taken together, these results imply that the physical interactions are crucial for the regulatory relationships among ARF16, ABI5, and JAZ1.

The importance of the ARF16–ABI5 interaction for regulating downstream genes prompted us to further explore the biological relevance of this interaction in mediating ABA signaling during seed germination. We generated transgenic plants (termed *mARF16$^{\Delta MR}$*) overexpressing a truncated form of *ARF16* (*ARF16$^{\Delta MR}$*) with deletion of the middle region (including the target site of *miRNA160*) under the control of *Pro35S*. Two independent lines (*mARF16$^{\Delta MR}$-5* and *mARF16$^{\Delta M}$-9*) with similar expression levels of *ARF16* (or mutant form of *ARF16*) as those in previously described transgenic plants *mARF16* (Wang et al., 2005) were selected for further phenotypic analyses (Supplemental Figure 17A). Unlike seeds of *mARF16*, the progeny of *mARF16$^{\Delta MR}$-5* and

mARF16$^{\Delta MR}$*-9* displayed germination and greening percentages in response to ABA that were similar to those of the wild-type seeds (Supplemental Figure 17A). This finding shows that overexpression of *ARF16*$^{\Delta MR}$ had little effect on ABA signaling during seed germination. Moreover, we also investigated whether the ABA responses of *ABI5*-overexpressing plants (*ABI5-OE*, containing a functional *ABI5-4xMYC* construct driven by *Pro35S*; Chen et al., 2012) were affected by overexpression of *ARF16* or *ARF16*$^{\Delta MR}$. We introduced the *ARF16* overexpression and *ARF16*$^{\Delta MR}$ overexpression into *ABI5-OE* through genetic crossing to produce *ABI5-OE mARF16* and *ABI5-OE mARF16*$^{\Delta MR}$*-9*, respectively. As shown in Figure 8C, seeds of *ABI5-OE mARF16* germinated and grew slower than did *ABI5-OE* seeds in response to ABA with or without MeJA. However, *ABI5-OE mARF16*$^{\Delta MR}$*-9* behaved similarly as *ABI5-OE* upon ABA and/or MeJA treatment during seed germination (Figure 8C). These observations imply that the increased ABA responses in *ABI5-OE* were enhanced by *ARF16* overexpression but not by *ARF16*$^{\Delta MR}$ overexpression, providing further evidence that the protein–protein interaction is critical for ARF16 to stimulate ABI5 and modulate ABA signaling during seed germination.

Consistently, to further investigate the physiological role of ARF16–JAZ1 interaction, we generated transgenic plants (known as *JAZ1*$^{61-253}$*-ΔJas*) overexpressing a mutated form of *JAZ1* with deletion of the N-terminal 60 amino acids and the Jas domain. *JAZ1*$^{61-253}$*-ΔJas-12* and *JAZ1*$^{61-253}$*-ΔJas-14* with similar expression levels of *JAZ1* (or mutant form of *JAZ1*) as those in *JAZ1-ΔJas* plants (Han et al., 2018) were selected for further analyses. The *JAZ1*$^{61-253}$*-ΔJas-14 mARF16* plants were also generated through genetic crossing *JAZ1*$^{61-253}$*-ΔJas-14* with *mARF16*. The ABA sensitivity of *JAZ1*$^{61-253}$*-ΔJas-12* and *JAZ1*$^{61-253}$*-ΔJas-14* was analyzed by using wild type and *JAZ1-ΔJas* as controls. As shown in Supplemental Figure 17B, although the progeny of *JAZ1*$^{61-253}$*-ΔJas-12* and *JAZ1*$^{61-253}$*-ΔJas-14* had higher germination and greening percentages than the wild type, seeds of *JAZ1*$^{61-253}$*-ΔJas-12* and *JAZ1*$^{61-253}$*-ΔJas-14* were much more sensitive to ABA compared with the seeds of *JAZ1-ΔJas*. Moreover, the germination and greening percentages of *JAZ1*$^{61-253}$*-ΔJas-14 mARF16* seeds were significantly lower than those of *JAZ1-ΔJas mARF16* in the presence of ABA with or without MeJA (Figure 8D). These results further support our proposal that the N-terminal 60 amino acids of JAZI (mediating the ARF16–JAZ1 interaction) are required for its repressive effect on the transcriptional function of ARF16 in modulating ABA signaling during seed germination.

Because JAZ repressors directly interact with ARF10/16 and ABI5 transcription factors (Figure 3; Ju et al., 2019), we performed BiFC assays to further investigate the possible effect of JAZ9 on the physical association between ARF16 and ABI5. When JAZ9 was co-expressed with ARF16-nYFP and ABI5-cYFP in leaves of *N. benthamiana*, the YFP fluorescence signal was dramatically decreased (Figure 8E; Supplemental Figure 18). As a negative control, when GUS (β-Glucuronidase) was co-expressed with ARF16-nYFP and ABI5-cYFP, the fluorescence intensity was not obviously changed (Figure 8E; Supplemental Figure 18). These observations imply that JAZ9 affects the physical association between ARF16 and ABI5.

Auxin and JA synergistically suppress seed germination under salinity and osmotic stress conditions

As auxin and JA work together to enhance the ABA-induced repression of seed germination, we queried whether auxin and JA modulate seed germination under particular natural conditions. Interestingly, several previous studies have shown that the biosynthesis and signaling of auxin or JA are implicated in seed germination under high salinity conditions (Ye et al., 2009; Iglesias et al., 2010, 2014; Park et al., 2011; Chen et al., 2015; Ding et al., 2016; Ebel et al., 2018; Ribba et al., 2020). For example, the germination of transgenic seeds overexpressing *YUC3* (encoding an auxin biosynthetic enzyme) was more severely repressed by high salinity than that of wild-type seeds (Zhao et al., 2001; Park et al., 2011). In contrast, seeds of the *tir1 afb2* mutant defective in auxin signaling displayed higher germination and greening percentages than those of wild type upon high salinity treatment (Iglesias et al., 2010, 2014). To further investigate the regulatory effects of auxin and JA on seed germination, we evaluated the performance of wild-type seeds treated with auxin and/or MeJA under salinity and osmotic stress conditions. Seeds were assayed on water agar medium containing 100 mM NaCl or 200 mM mannitol with 1 μM IAA and/or 10 μM MeJA. As shown in Figure 9, A and B, the wild-type seeds on medium supplemented with both NaCl and IAA or MeJA displayed much lower percentages of germination and expanded greening cotyledons compared with seeds on medium with NaCl, IAA, or MeJA alone. Similarly, the percentages of germination and expanded green cotyledons were much lower upon treatment with mannitol and IAA or MeJA than upon treatment with mannitol, IAA, or MeJA alone (Figure 9, C and D). Moreover, upon simultaneous exposure to NaCl (or mannitol), IAA, and MeJA, the seeds exhibited significantly lower germination and greening percentages than those treated with NaCl (or mannitol) and IAA or MeJA (Figure 9, A–D). These results imply that auxin and JA inhibit seed germination synergistically under salinity and osmotic stress conditions.

To verify the roles of auxin and JA in mediating seed germination, we further analyzed the phenotypes of several mutant plants with impaired endogenous auxin or JA signaling, including *axr3-1*, *arf10 arf16*, *coi1-16*, and *coi1-2*, on medium supplemented with 150 mM NaCl or 250 mM mannitol. As shown in Figure 9, E–H, the seeds of *axr3-1*, *arf10 arf16*, *coi1-16*, and *coi1-2* germinated and grew faster than did wild-type seeds upon NaCl or mannitol treatment. These observations further support the idea that auxin and JA promote the delay of seed germination under salinity and osmotic stress conditions.

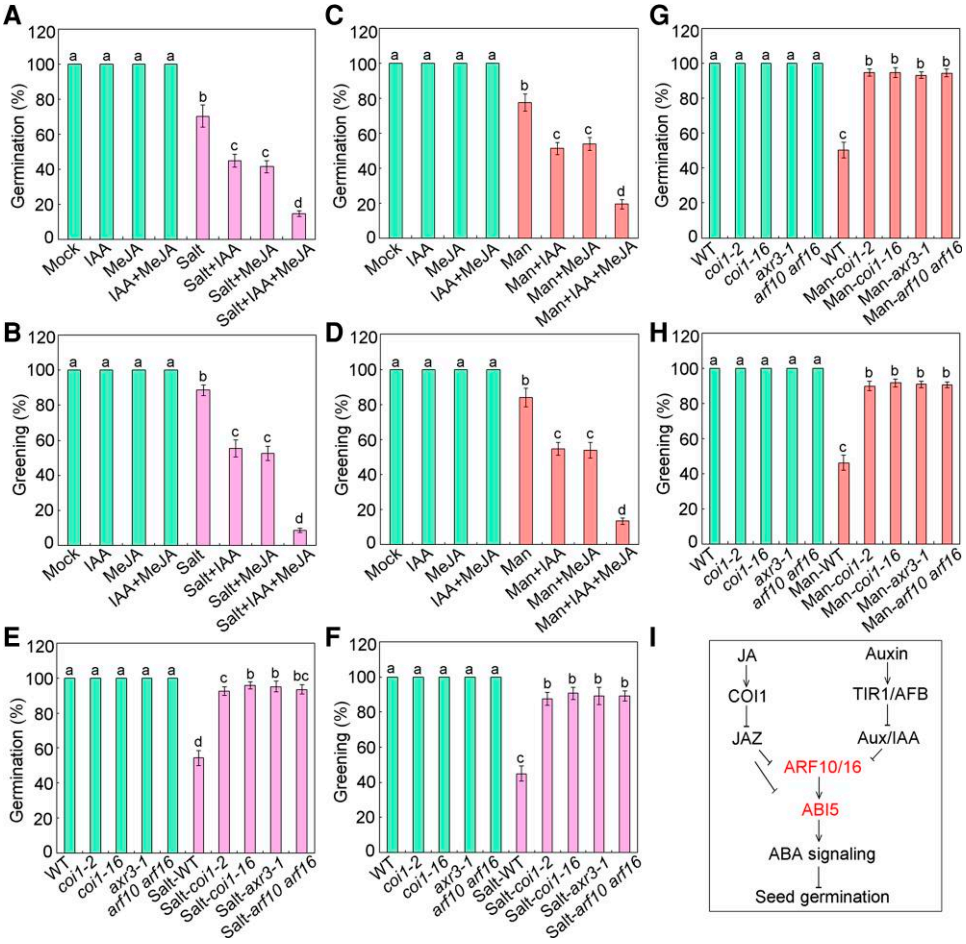

Figure 9 Auxin and JA synergistically repress seed germination under salinity and osmotic stress conditions. A and B, Germination and greening of the wild-type (WT) seeds on water agar medium containing 100 mM NaCl (Salt), 1 μM IAA, and/or 10 μM MeJA. In the mock treatment, an equal volume of 10% (v/v) ethanol was added. Seed germination and cotyledon greening were recorded 2 d and 5 d, respectively, after stratification at 4°C. The columns in green show the percentages of germination and greening without NaCl treatment, and those in pink show the percentages upon NaCl treatment. C and D, Germination and greening of the WT seeds on water agar medium containing 200 mM mannitol (Man), 1 μM IAA, and/or 10 μM MeJA. Seed germination and cotyledon greening were recorded 2 d and 5 d, respectively, after stratification at 4°C. The columns in green show the percentages of germination and greening without Man treatment, and those in orange show the percentages upon Man treatment. E and F, Germination and greening of WT, *coi1-2*, *coi1-16*, *axr3-1*, and *arf10 arf16* under salinity stress conditions. Seed germination and cotyledon greening were recorded 2 d and 5 d, respectively, after stratification on water agar medium containing 150 mM NaCl (Salt). G and H, Germination and greening of WT, *coi1-2*, *coi1-16*, *axr3-1*, and *arf10 arf16* under osmotic stress conditions. Seed germination and cotyledon greening were recorded 2 d and 5 d, respectively, after stratification on water agar medium containing 250 mM mannitol (Man). Experiments were performed five times by analyzing different batches of seeds. Each batch of seeds was pooled from more than 80 independent plants. For each biological replicate, more than 120 seeds were examined. Values are means ± SD. Bars with different letters are significantly different from each other ($P < 0.05$). Data were analyzed by analysis of variance (ANOVA). I, A simplified model for the interactions of ARF10/16 with JAZ and ABI5 in integrating the auxin, JA, and ABA signaling pathways. When the concentrations of auxin and JA are elevated, the degradation of Aux/IAA and JAZ proteins releases ARF10/16 to form a complex with ABI5. ARF10/16 stimulates ABI5 to enhance ABA signaling and maintain proper seed germination and postgerminative growth.

Discussion

Seed germination and subsequent postgerminative seedling growth are strictly regulated by a delicate phytohormone balance. The phytohormone ABA plays a predominant role in repressing these physiological processes in Arabidopsis (Gubler et al., 2005; Finkelstein et al., 2008; Nakashima and Yamaguchi-Shinozaki, 2013). Auxin and JA have been shown to promote the ABA-induced delay of seed germination (Brady et al., 2003; Liu et al., 2007, 2013; Dave et al., 2011, 2016; Ju et al., 2019; Pan et al., 2020). Despite recent advances in our understanding of auxin or JA-enhanced ABA signaling, the possible synergistic effects of auxin and JA on ABA signaling were yet to be investigated. Moreover, little was known about the exact molecular mechanisms underlying the convergence of the ABA, auxin, and JA signaling pathways during

seed germination. The results of the present study show that exogenous auxin and JA synergistically activate ABA responses to suppress seed germination. The wild-type seeds simultaneously treated with ABA, IAA, and MeJA displayed dramatically lower percentages of germination and expanded cotyledon greening compared with those treated with combined ABA and IAA or MeJA (Figure 1, A–C; Supplemental Figure 1). However, compared with seeds of wild type, those of lines with defective endogenous auxin biosynthesis, perception, or signaling were much less sensitive to both ABA and MeJA treatment (Figure 2, A–C; Supplemental Figures 2 and 3). Likewise, blocking COI1-mediated endogenous JA signaling also conferred germinating seeds with hyposensitivity to both ABA and auxin (Supplemental Figure 4). Collectively, these results demonstrate that auxin and JA exhibit synergetic effects on stimulating ABA signaling to suppress seed germination and postgerminative growth in Arabidopsis. Furthermore, auxin and JA also inhibit seed germination synergistically in particular natural situations, such as salinity and osmotic stress conditions (Figure 9, A–H).

The results of previous studies have provided evidence for crosstalk between the auxin and JA signaling pathways. For instance, JA stimulates the biosynthesis of auxin by upregulating the auxin biosynthetic gene *Anthranilate Synthase α1* (*ASA1*) during lateral root formation (Stepanova et al., 2005; Dombrecht et al., 2007; Sun et al., 2009). Auxin antagonizes JA-induced senescence in a process fine-tuned by WRKY57 (Jiang et al., 2014; Hu et al., 2017). MYC2, a central transcription factor mediating versatile aspects of JA responses, binds to the promoters of auxin-responsive *PLETHORA* (*PLT*) genes and represses their expression, illustrating a molecular framework for JA-induced repression of root growth through its interaction with auxin (Boter et al., 2004; Lorenzo et al., 2004; Dombrecht et al., 2007; Chen et al., 2011; Fernández-Calvo et al., 2011). Recently, two studies have revealed a synergistic relationship between auxin and JA signaling in activating root stem cells and promoting regeneration (Zhang et al., 2019; Zhou et al., 2019). Nevertheless, the detailed molecular mechanisms of how auxin coordinates with JA to regulate crucial physiological processes, such as the ABA-induced repression of seed germination, remained largely unknown. In this study, we show that the auxin-responsive transcription factors ARF10 and ARF16 interact with several JAZ repressors of JA signaling (Figure 3, A–C). Further mapping revealed that the N-terminal amino acids from positions 1 to 544 of ARF16 are responsible for the interaction (Supplemental Figure 5A). Moreover, the N-terminal region of JAZ1 mediates its association with ARF16 (Supplemental Figure 5B), similar to previous findings that the N-terminal fragments of JAZ1 and JAZ10 are involved in the interactions with MYC2 or TARGET OF EAT1 (TOE1) (Chung et al., 2010; Moreno et al., 2013; Goossens et al., 2015; Zhai et al., 2015; Chini et al., 2016). After BLAST analysis, we found that the NTDs of those ARF16-binding JAZ proteins (JAZ1, JAZ3, JAZ4, JAZ7, JAZ9, and JAZ11) share several similar residues which may be critical for the ARF16–JAZ interactions (Figure 3A; Supplemental Figure 19). These results raise the possibility that auxin-JA integration is mediated by direct interactions among key components of the auxin and JA signaling pathways.

As repressors of JA signaling, JAZ proteins interact with multiple transcription factors and inhibit their functions to negatively modulate various JA processes, such as root elongation, anthocyanin accumulation, trichome development, male fertility, and stress responses (Boter et al., 2004; Lorenzo et al., 2004; Dombrecht et al., 2007; Cheng et al., 2009; Niu et al., 2011; Fernández-Calvo et al., 2011; Qi et al., 2011, 2015; Song et al., 2011; Hu et al., 2013; Kazan and Manners, 2013; Schweizer et al., 2013; Boter et al., 2015; Zhai et al., 2015; Guo et al., 2018b; Han et al., 2020, 2022; Hu et al., 2022; Serrano-Bueno et al., 2022). Furthermore, JAZ repressors also associate with several transcriptional regulators to integrate JA and other hormone signaling pathways (Hou et al., 2010; Zhu et al., 2011; Pan et al., 2020). Because ARF10 and ARF16 directly bind JAZ proteins, we performed genetic analyses to dissect the biological significance of their physical interactions. The results of the phenotypic analyses indicate that ARF10 and ARF16 are critical for JA-stimulated ABA signaling during seed germination (Figure 4, A–C). Moreover, the overaccumulation of ARF16 partially rescued the hyposensitive phenotype of JAZ-accumulating plants, such as *coi1-16* and *JAZ1-ΔJas*, to combined ABA and MeJA treatment (Figure 5, A–C; Supplemental Figures 7 and 8). Importantly, our results show that JAZ proteins attenuate the transcriptional functions of ARF10 and ARF16 (Figure 8B; Supplemental Figure 14) and interfere with the physical association between ARF16 and ABI5 (Figure 8E; Supplemental Figure 18). Furthermore, the N-terminal 60 amino acids of JAZ1 (mediating its interaction with ARF16) are critical for its repressive effect on ARF16 in modulating ABA signaling during seed germination (Figure 8D; Supplemental Figures 5B and 14D, and 17B). These findings suggest that ARF10 and ARF16 act as crucial regulators downstream of the COI1/JAZ-mediated JA signaling pathway to activate ABA responses. Our study thus reveals a critical signaling module in which JAZ repressors of the JA pathway directly regulate auxin-responsive ARF10 and ARF16 transcription factors to integrate JA and auxin signaling and promote responses to ABA.

The bZIP family transcription factor ABI5 is a master regulator that promotes the ABA-induced delay of seed germination in Arabidopsis (Finkelstein, 1994; Finkelstein and Lynch, 2000a; Lopez-Molina and Chua, 2000; Lopez-Molina et al., 2001, 2002; Brocard et al., 2002; Finkelstein et al., 2005; Skubacz et al., 2016). Moreover, ABI5 is a key node of convergence for ABA signaling and other signaling pathways (Lim et al., 2013; Hu and Yu, 2014; Guan et al., 2014; Yu et al., 2015; Kim et al., 2016; Yang et al., 2016; Liu and Hou, 2018; Yang et al., 2021). Very recently, ABI5 and ABI3 have been shown to interact with several JAZ repressors to mediate

JA-stimulated ABA signaling (Ju et al., 2019; Pan et al., 2020). In this study, we found that ARF10 and ARF16 also physically associate with ABI5 in yeast and *in planta* (Figure 6, A–D). We also observed that the ability of ARF16 to activate JA-mediated ABA responses is mainly dependent on ABI5. Similar to the progeny of the ABA-hyposensitive *abi5* mutants, seeds of *abi5 mARF16* were much less sensitive to combined ABA and MeJA treatment than seeds of wild-type and *mARF16* plants (Figure 7, A–C; Supplemental Figure 12). Further investigations demonstrated that ARF10 and ARF16 enhance the transcriptional function of ABI5 to mediate the expression of downstream target genes. As shown in Figure 8, A and B, the expression of ARF10 or ARF16 enhances the activity of ABI5 to promote the expression of downstream *EM6* and *EM1* genes in response to ABA. Moreover, the middle region is essential for ARF16 to stimulate the transcriptional function of ABI5 and mediate ABA signaling during seed germination (Figure 8C; Supplemental Figures 10A and 14C, and 17A). Thus, ARF10 and ARF16 interact with ABI5 to modulate JA-promoted ABA signaling during seed germination.

Although *abi5 mARF16* mimicked the phenotype of *abi5*, the responses of *abi5 mARF16* to both ABA and MeJA differed markedly from those of *abi5* (Figure 7, A–C; Supplemental Figure 12), implying that ARF16 (and/or ARF10) also acts with other regulators besides ABI5 to mediate ABA responses. Consistent with this idea, the percentages of germination and expanded greening cotyledons were higher in the *arf10 arf16 abi5-1* triple mutant than in *abi5-1* and *arf10 arf16* in the presence of ABA and MeJA (Supplemental Figure 13). This speculation is also supported by the finding that the expression of ARF10 or ARF16 alone moderately enhanced the expression of *LUC* driven by the *EM6* or *EM1* promoter in wild-type protoplasts upon ABA treatment, compared with the expression of GFP (Figure 8B; Supplemental Figure 14B). As ARF10 and ARF16 interact with ABSCISIC ACID-RESPONSIVE ELEMENT BINDING FACTOR1 (ABF1) and ABF3 (two close homologs of ABI5) in yeast (Supplemental Figure 20), it is possible that ARF10 and ARF16 may also function through ABF1 and ABF3 to mediate the expression levels of *EM6* and *EM1*. The transcription factors ABI3 and ABI4 are two crucial modulators of ABA signaling (Giraudat et al., 1992; Finkelstein, 1994; Finkelstein et al., 1998, 2002, 2005; Finkelstein and Lynch, 2000a; Lopez-Molina and Chua, 2000; Suzuki et al., 2001; Lopez-Molina et al., 2002). No physical association of ARF10 or ARF16 with ABI3 (or ABI4) was detected in yeast (Figure 6A), suggesting that ARF10 and ARF16 do not directly associate with either of them *via* protein–protein interaction. However, Liu et al. (2013) revealed that ARF10 and ARF16 act upstream of *ABI3* and indirectly modulate its expression to maintain seed dormancy. *EM6* and *EM1* genes are direct downstream targets of ABI5 (Carles et al., 2002). The yeast one-hybrid analyses showed that ARF10 and ARF16 did not recognize the *EM6* and *EM1* promoters (Supplemental Figures 15 and 16). The detailed regulatory relationships of ARF10 and ARF16 with other crucial components in ABA signaling (such as ABI3 and EM6) need to be further dissected. In addition to ARF10 and ARF16, several critical transcriptional regulators have been shown to interact with ABI5 and/or ABI3, such as DELLA proteins, PHYTOCHROME INTERACTING FACTOR1 (PIF1), PRR5, VQ motif-containing proteins, BR-INSENSITIVE1 (BRI1)-EMS-SUPPRESSOR1 (BES1), and Mediator subunit MEDIATOR25 (Chen et al., 2012; Lim et al., 2013; Kim et al., 2016; Pan et al., 2018; Hu et al., 2019; Zhao et al., 2019; Yang et al., 2021). Further research is required to determine whether ARF10 and ARF16 interact with these ABI5-associated regulators and to elucidate the biological significance of their interactions.

The MYC transcription factors MYC2, MYC3, and MYC4 are the most extensively characterized JAZ-interacting factors. They regulate a wide variety of JA-mediated physiological processes, such as the inhibition of root elongation and defense responses (Boter et al., 2004; Lorenzo et al., 2004; Dombrecht et al., 2007; Fernández-Calvo et al., 2011; Zhai et al., 2013; Chini et al., 2016; Wang et al., 2017; Han et al., 2018; Howe et al., 2018). MYC2 is a positive regulator of ABA signaling, and constitutive expression of *MYC2* renders transgenic plants hypersensitive to ABA during seed germination (Lorenzo et al., 2004). Consequently, JA signaling may stimulate ABA-induced repression of seed germination partially through MYC2 and its homologs. Recently, we revealed that the transcription factor INDUCER OF CBF EXPRESSION1 (ICE1) interacts with and antagonizes ABI5 to fine-tune ABA signaling (Hu et al., 2019). ICE1 also functions as a direct downstream target of JAZ repressors in JA signaling (Hu et al., 2013, 2017). Thus, JA signaling may negatively modulate ABA responses via ICE1 during seed germination. These dual (positive or negative) regulatory effects of different JAZ-binding factors on ABA signaling may represent adaptive mechanisms to maintain appropriate JA-mediated ABA signaling levels, thereby ensuring optimal germination and postgerminative seedling growth upon exposure to particular stress conditions. Interestingly, further elucidation of whether those JAZ-related factors associate with ARF10/16 (or other critical components of auxin signaling) and whether they are involved in the convergence of auxin and JA to mediate ABA responses may shed light on the molecular basis of the tight regulation and fine-tuning of ABA signaling networks.

To better understand the molecular mechanism of how auxin contributes to JA-increased ABA signaling during seed germination in Arabidopsis, we propose the following simplified model involving JAZ, ARF10/16, and ABI5 (Figure 9I). Under normal growth conditions, Aux/IAA and JAZ repressors physically interact with ARF10, ARF16, and/or ABI5 transcription factors and attenuate their functions (Figures 3 and 8; Chapman and Estelle, 2009; Vanneste and Friml, 2009; Ju et al., 2019; Pan et al., 2020). When the concentrations of auxin and JA are elevated, the Aux/IAA and JAZ repressors are degraded via the $SCF^{TIR1/AFB}$ and SCF^{COI1}

complex-26S proteasome pathway, respectively (Dharmasiri et al., 2005a, b; Kepinski and Leyser, 2005; Chini et al., 2007; Thines et al., 2007; Chapman and Estelle, 2009; Vanneste and Friml, 2009; Yan et al., 2009; Sheard et al., 2010). The degradation of Aux/IAA and JAZ proteins subsequently releases ARF10/16 to form a transcriptional complex with ABI5 (Supplemental Figures 3, 6, and 8). ARF10/16 stimulate the function of ABI5 to modulate the transcription of downstream target genes (Figure 8), thereby establishing appropriate ABA signaling levels to inhibit seed germination and subsequent seedling growth.

Methods

Materials and plant growth conditions

Common chemicals were obtained from Shanghai Sangon (Shanghai, China), and Taq DNA polymerase was purchased from Takara Biotechnology (Dalian, China). The phytohormones ABA (catalog no. 862169-250MG), MeJA (catalog no. 392707-5ML), and IAA (catalog no. 45533-250MG) were purchased from Sigma-Aldrich (Sigma, St Louis, MO, USA). The wild type and mutant *Arabidopsis thaliana* plants used in this study were in the Col-0 genetic background. Seeds of *iaaM-OX*, *yuc1 yuc6*, *yuc1 yuc2 yuc6*, *tir1 afb1 afb2*, *tir1 afb1 afb2 afb3*, *axr3-1*, *axr2-1*, *arf10 arf16*, *mARF10*, and *mARF16* were kindly provided by Drs Zuhua He and Xiaoya Chen (Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences; Wang et al., 2005; Liu et al., 2013). The *coi1-2* mutant was kindly provided by Dr Daoxin Xie (Tsinghua University). The *coi1-16* and *abi5-7* (SALK_013163C) mutants were obtained from the Arabidopsis Resource Center at Ohio State University (http://abrc.osu.edu). The *abi5-1* mutant has been described previously (Finkelstein, 1994; Finkelstein and Lynch, 2000a; Hu et al., 2019). The transgenic line *ABI5-OE* (Chen et al., 2012; Hu and Yu, 2014; Yang et al., 2021) was kindly provided by Dr Chuanyou Li (Institute of Genetics and Developmental Biology, Chinese Academy of Sciences). To generate *JAZ1-ΔJas* and $JAZ1^{61-253}$*-ΔJas* transgenic plants, the full-length or truncated cDNA of *JAZ1* with the sequence encoding the Jas domain (crucial for the degradation of JAZ proteins) deleted was cloned into the binary vector pOCA30 in the sense orientation under the control of *Pro35S* (Han et al., 2018). To generate $mARF16^{\Delta MR}$ transgenic plants, a truncated form of *ARF16* ($ARF16^{\Delta MR}$) with deletion of the middle region (including the target site of *miRNA160*) was cloned into the binary vector pOCA30 in the sense orientation under the control of *Pro35S* and introduced into wild-type (Col-0) plants using the floral dip method (Clough and Bent, 1998). *coi1-16 mARF16*, *coi1-2 mARF16*, *coi1-16 mARF10*, *JAZ1-ΔJas mARF16*, *abi5-1 mARF16*, *abi5-7 mARF16*, and *arf10 arf16 abi5-1* were generated by genetic crossing using standard techniques. Because the majority of seeds of the quadruple *tir1 afb1 afb2 afb3* mutant were defective, the minority of the normal seeds were selected and assayed. Plants were grown in an artificial growth chamber at 22°C under a 16-h-light (100 mE m^{-2} s^{-1}, white fluorescent bulbs, full wavelength of light), 8-h-dark photoperiod.

Measurement of germination and expanded Cotyledon greening percentages

Analyses of the seed germination and expanded cotyledon greening of wild type and mutant lines were conducted as previously described (Hu and Yu, 2014; Hu et al., 2019). Freshly harvested seeds were stratified for 5 d at 4°C before germination. Germination was determined based on the appearance of the embryonic axis (i.e. radicle protrusion), as observed under a microscope. Seedling greening was determined based on the appearance of expanded green cotyledons. To measure the sensitivity of seed germination and cotyledon greening to phytohormones, seeds were plated on half-strength MS medium or water agar medium containing ABA with or without MeJA and/or IAA. All experiments were conducted more than five times using different batches of seeds as biological replicates. Each batch of seeds was pooled from more than 80 independent plants. For each biological replicate, more than 120 seeds were analyzed.

Yeast two-hybrid assays

The full-length coding sequence (CDS) of *JAZ*, *ABI5*, *ABF1*, *ABF2*, *ABF3*, *ABF4*, *ABI4*, and *ABI3* was each cloned into pGBKT7 (Clontech) to produce bait vectors (BD-JAZ, BD-ABI5, BD-ABF1, BD-ABF2, BD-ABF3, BD-ABF4, BD-ABI4, and BD-ABI3, respectively) containing the Gal4 DNA-binding domain. The full-length CDS of *ARF10* and *ARF16* was each cloned into pGADT7 (Clontech) to generate prey vectors (AD-ARF10 and AD-ARF16, respectively) containing the Gal4 activation domain. To identify the regions critical for interactions, multiple truncated versions of *JAZ1* and *ABI5* sequences were each ligated with pGBKT7 and truncated *ARF16* sequences were each cloned into pGADT7. The Arabidopsis cDNA library was purchased from Clontech (catalog number 630487). Yeast two-hybrid screening was performed via the mating protocol described in Clontech's Matchmaker Gold Yeast Two-Hybrid user manual. To confirm the interactions, the bait and prey vectors were co-transformed into the yeast strain Y2HGold. Physical interactions were indicated by the ability of cells to grow on dropout medium lacking Leu, Trp, His, and Ade with or without 3-aminotriazole for 4 d after plating. The primers used for cloning are listed in Supplemental Data Set 1.

BiFC assays

The cDNA sequences encoding the C-terminal 64 amino acids of enhanced YFP (cYFP) and the N-terminal 173 amino acids of YFP (nYFP) were individually cloned into pFGC5941 plasmids to generate pFGC-cYFP and pFGC-nYFP, respectively (Kim et al., 2008). The full-length CDS of *JAZ1*, *JAZ8*, and *JAZ9* was inserted into pFGC-nYFP to produce an N-terminal in-frame fusion with nYFP (JAZ1-nYFP,

JAZ8-nYFP, and JAZ9-nYFP, respectively). Similarly, the full-length CDS of *ARF10* (or *ARF16*) or the sequence encoding the 430 N-terminal residues of ARF16 was fused with nYFP to generate ARF10-nYFP, ARF16-nYFP, and ARF16$^{1-430}$-nYFP. The full-length CDS of *ABI5* or the sequence encoding the 164 N-terminal residues of ABI5 was inserted into pFGC-cYFP to produce a C-terminal in-frame fusion with cYFP (ABI5-cYFP or ABI5$^{1-164}$-cYFP, respectively). The resulting plasmids were transformed into *Agrobacterium tumefaciens* strain GV3101, and then infiltrated into *Nicotiana benthamiana* leaves as described previously (Hu et al., 2019). The experiments were conducted more than three times using different batches of *N. benthamiana* plants. For each replicate, more than 12 plants were infiltrated and more than 600 cells were investigated. Infected leaves with YFP signals were observed at 48 h after infiltration under a confocal laser-scanning microscope (Olympus Fluoview^{TM} FV1000, Tokyo, Japan). For DAPI staining, infected leaves were stained with DAPI solution (10 mM) for 5 min before observation. The YFP signals were imaged with excitation at 488 nm (the intensity is 24%, and the gains are 1), and the emission signal was collected between 510 and 530 nm. The DAPI signals were imaged with excitation at 405 nm (the intensity is 15%, and the gains are 1), and the emission signal was collected between 420 and 440 nm. Thirty independent fluorescent spots were assessed for fluorescence intensity shown in Supplemental Figure 18. One representative fluorescent spot resulting from the co-expression of ARF16-nYFP with ABI5-cYFP (mock) was used as the control and its relative intensity value was set to 1,000. The primers used for cloning are listed in Supplemental Data Set 1.

Co-IP assays

Full-length CDSs of *ARF10*, *ARF16*, *JAZ1*, *JAZ9*, *ABI5*, and *GFP* were individually inserted into tagging plasmids with MYC or HA tag sequences in the sense orientation behind *Pro35S* (Hu et al., 2013). MYC-fused ARF (or MYC-fused ABI5) and HA-fused JAZ (or HA-fused ARF) were transiently co-expressed in *N. benthamiana* leaves. Infected leaves were sectioned at 48 h after infiltration. Total proteins were prepared from infected leaves with an extraction buffer containing 50 mM Tris–HCl (pH 7.4), 1 mM EDTA, 150 mM NaCl, 10% (v/v) glycerol, 0.1% (v/v) Triton X-100, 1 mM PMSF, and 1× Roche Protease Inhibitor Cocktail. Immunoprecipitation experiments were performed with protein A/G Plus-agarose beads (Santa Cruz Biotechnology, catalog no. D1217) following the manufacturer's protocol. Briefly, MYC-fused ARF was immunoprecipitated using an anti-MYC antibody (Sigma-Aldrich, catalog no. M4439) (1:250) and the coimmunoprecipitated protein was then detected using an anti-HA antibody (Sigma-Aldrich, catalog no. H9658) (1:10,000). To test the ABI5-ARF interaction, HA-fused ARF10 or ARF16 was immunoprecipitated using an anti-HA antibody (1:250) and the coimmunoprecipitated protein was then analyzed using an anti-MYC antibody (1:10,000). The primers used for cloning are listed in Supplemental Data Set S1.

Yeast one-hybrid assays

The yeast one-hybrid assays were performed using the Matchmaker Yeast One-Hybrid System Kit (Clontech) according to the manufacturer's instructions. The putative promoter fragments of *EM6* and *EM1* were cloned into the pAbAi vector to generate pAbAi-pEM6 and pAbAi-pEM1, which were linearized by *Bst*BI, and then transformed into the Y1HGold yeast strain through a polyethylene glycol (PEG)/LiAc-based method. The transformed cells were grown on the SD/-Ura plate for 3 d. AD-ARF10 and AD-ARF16 were then transformed into the strain harboring pAbAi-pEM6 or pAbAi-pEM1 and selected on the SD/-Leu plate. Co-transformed cells were cultured on an SD/-Leu plate containing aureobasidin A (AbA, 200 μg/L) for 2.5 d, and positive clones were spotted in several yeast concentrations from dilution of 10^0 ($OD_{600} = 0.8$) to 10^{-3}. The primers used for cloning are listed in Supplemental Data Set S1.

RNA extraction and Rt-qPCR

Total RNA was extracted from germinating seeds in the various phytohormone treatments using Trizol reagent (Invitrogen, Carlsbad, CA, USA) and RT-qPCR was conducted as described by Yang et al., 2021. Briefly, 1.0 μg DNase-treated RNA was reverse-transcribed in a 20 μl reaction volume with oligo$(dT)_{18}$ primer using Moloney murine leukemia virus reverse transcriptase (Fermentas, Thermo Fisher Scientific, Waltham, MA, USA). Then, 1.0 μl cDNA was used for RT-qPCR with the SYBR Premix Ex Taq kit (Takara) on a Roche LightCycler 480 real-time PCR machine, according to the manufacturer's instructions. Changes in the transcript levels of the target gene were calculated relative to that of At1g13320, which encodes a subunit of Ser/Thr protein phosphatase 2A and is stably expressed in seeds during germination (Czechowski et al. 2005). Three biological replicates for each sample were used for RT-qPCR analyses. The primers used for RT-qPCR analyses to identify the transgenic plants (*mARF16*$^{\Delta MR}$ and *JAZ1*$^{61-253}$-*ΔJas*) are listed in Supplemental Data Set S1.

Transient transactivation assays

The putative promoter sequences of *EM6* (1273 bp) and *EM1* (2000bp) were amplified and cloned into the pGreenII 0800-LUC vector as the reporter (Hellens et al., 2005). Each full-length or truncated CDS of *ABI5*, *ARF10*, *ARF16*, *JAZ1*, *JAZ9*, and *GFP* was ligated into the pGreenII 62-SK vector to produce effectors (Hellens et al., 2005). Combinations of plasmids were transformed into leaf mesophyll protoplasts of wild-type *Arabidopsis* or the *arf10 arf16* mutant using the Sheen laboratory protocol (Sheen, 2001). Transfected cells were cultured for 10–16 h with or without 5 μM ABA, and the relative LUC activity was examined using a Dual-Luciferase Reporter Assay system (Promega, Madison, WI, USA), which measured the activities of firefly LUC and

the internal control *Renilla reniformis* LUC (REN). The primers used for cloning are listed in Supplemental Data Set 1.

Statistical analysis

Statistical analysis was performed by analysis of variance using the least significant difference as a posthoc test. Statistically significant differences were defined as those with $P < 0.05$. The lowercase letters above the column show significant differences ($P < 0.05$) among the different samples. The results of statistical analyses are shown in Supplemental Data Set S2.

Accession numbers

Arabidopsis Genome Initiative numbers for the genes discussed in this article are as follows: *YUC1*, AT4G32540; *YUC2*, AT4G13260; *YUC6*, AT5G25620; *TIR1*, AT3G62980; *AFB1*, AT4G03190; *AFB2*, AT3G26810; *AFB3*, AT1G12820; *AXR2*, AT3G23050; *AXR3*, AT1G04250; *ARF10*, AT2G28350; *ARF16*, AT4G30080; *COI1*, AT2G39940; *JAZ1*, AT1G19180; *JAZ2*, AT1G74950; *JAZ3*, AT3G17860; *JAZ4*, AT1G48500; *JAZ5*, AT1G17380; *JAZ6*, AT1G72450; *JAZ7*, AT2G34600; *JAZ8*, AT1G30135; *JAZ9*, AT1G70700; *JAZ10*, AT5G13220; *JAZ11*, AT3G43440; *JAZ12*, AT5G20900; *ABI5*, AT2G36270; *ABI4*, AT2G40220; *ABI3*, AT3G24650; *ABF1*, AT1G49720; *ABF2*, AT1G45249; *ABF3*, AT4G34000; *ABF4*, AT3G19290; *EM6*, AT2G40170; and *EM1*, AT3G51810.

Supplemental data

The following materials are available in the online version of this article.

Supplemental Figure S1. Responses of the wild-type seeds on water agar medium containing ABA with or without IAA and/or MeJA during seed germination.

Supplemental Figure S2. Responses of *yuc1 yuc2 yuc6*, *tir1 afb1 afb2 afb3*, and *axr2-1* to both ABA and MeJA during seed germination.

Supplemental Figure S3. Responses of *yuc1 yuc6*, *iaaM-OE*, *tir1 afb1 afb2*, and *axr3-1* on water agar medium to both ABA and MeJA during seed germination.

Supplemental Figure S4. Responses of *coi1-16* and *coi1-2* to both ABA and auxin during seed germination.

Supplemental Figure S5. Yeast two-hybrid assays to identify ARF16 and JAZ1 regions required for their interaction.

Supplemental Figure S6. Negative controls for BiFC assays to analyze the interactions between ARF16 and JAZ proteins.

Supplemental Figure S7. Overaccumulation of ARF16 partially rescues the hyposensitive phenotype of *coi1-2* upon both ABA and MeJA treatment.

Supplemental Figure S8. Overaccumulation of ARF16 partially rescues the hyposensitive phenotype of *JAZ1-ΔJas* under combined ABA and MeJA treatment.

Supplemental Figure S9. Overaccumulation of ARF10 partially rescues the hyposensitive phenotype of *coi1-16* upon both ABA and MeJA treatment.

Supplemental Figure S10. Yeast two-hybrid assays to identify ARF16 and ABI5 regions required for their interaction.

Supplemental Figure S11. Negative controls for BiFC assays to analyze the interaction between ARF16 and ABI5.

Supplemental Figure S12. Responses of *abi5-7*, *mARF16*, and *abi5-7 mARF16* to both ABA and MeJA during seed germination.

Supplemental Figure S13. Responses of *abi5*, *arf10 arf16*, and *arf10 arf16 abi5* to both ABA and MeJA during seed germination.

Supplemental Figure S14. Transient dual-luciferase reporter assays showing the repressive effect of JAZ9 on ARF10/16 activity and the importance of protein interactions for the regulatory relationships among ARF16, ABI5, and JAZ1.

Supplemental Figure S15. Yeast one-hybrid assays testing binding of ARF10 and ARF16 to the promoter region of *EM6*.

Supplemental Figure S16. Yeast one-hybrid assays testing binding of ARF10 and ARF16 to the promoter region of *EM1*.

Supplemental Figure S17. Responses of $mARF16^{\Delta MR}$ and $JAZ1^{61-253}$-*ΔJas* transgenic plants to ABA during seed germination.

Supplemental Figure S18. Quantitative analysis of YFP fluorescence intensity shown in Figure 8E.

Supplemental Figure S19. Alignment of the N-terminal regions of ARF16-interacting JAZ proteins.

Supplemental Figure S20. Yeast two-hybrid assays to analyze the interactions of ARF10 and ARF16 with ABF1, ABF2, ABF3, and ABF4.

Supplemental Table S1. Sequences of amino acids of the ARF16 clones obtained from the yeast two-hybrid screening assays.

Supplemental Data Set S1. Primers used for cloning and RT-qPCR.

Supplemental Data Set S2. The results of statistical analyses performed by ANOVA.

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References

Albertos P, Romero-Puertas MC, Tatematsu K, Mateos I, Sánchez-Vicente I, Nambara E, Lorenzo O (2015) S-nitrosylation triggers ABI5 degadation to promote seed germination and seedling growth. Nat Commun **6**(1): 8669

Alboresi A, Gestin C, Leydecker MT, Bedu M, Meyer C, Truong HN (2005) Nitrate, a signal relieving seed dormancy in *Arabidopsis*. Plant Cell Environ **28**(4): 500–512

Barrero JM, Talbot MJ, White RG, Jacobsen JV, Gubler F (2009) Anatomical and transcriptomic studies of the coleorhiza reveal the importance of this tissue in regulating dormancy in barley. Plant Physiol **150**(2): 1006–1021

Benková E, Michniewicz M, Sauer M, Teichmann T, Seifertová D, Jürgens G, Friml J (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. Cell **115**(5): 591–602

Bohn-Courseau I (2010) Auxin: a major regulator of organogenesis. C R Biol **333**(4): 290–296

Boter M, Golz JF, Giménez-Ibañez S, Fernandez-Barbero-Zorrilla JM, Solana R (2015) FILAMENTOUS FLOWER is a direct target of JAZ3 and modulates responses to jasmonate. Plant Cell **27**(11): 3160–3174

Boter M, Ruíz-Rivero O, Abdeen A, Prat S (2004) Conserved MYC transcription factors play a key role in jasmonate signaling both in tomato and *Arabidopsis*. Genes Dev **18**(13): 1577–1591

Brady SM, Sarkar SF, Bonetta D, McCourt P (2003) The *ABSCISIC ACID INSENSITIVE 3 (ABI3)* gene is modulated by farnesylation and is involved in auxin signaling and lateral root development in *Arabidopsis*. Plant J **34**(1): 67–75

Brocard IM, Lynch TJ, Finkelstein RR (2002) Regulation and role of the *Arabidopsis abscisic acid-insensitive 5* gene in abscisic acid, sugar, and stress response. Plant Physiol **129**(4): 1533–1543

Carles C, Bies-Etheve N, Aspart L, Léon-Kloosterziel KM, Koornneef M, Echeverria M, Delseny M (2002) Regulation of *Arabidopsis thaliana Em* genes: role of ABI5. Plant J **30**(3): 373–383

Chapman EJ, Estelle M (2009) Mechanism of auxin-regulated gene expression in plants. Annu Rev Genet **43**(1): 265–285

Chen Z, Hu L, Han N, Hu J, Yang Y, Xiang T, Zhang X, Wang L (2015) Overexpression of a miR393-resistant form of *Transport Inhibitor Response Protein1 (mTIR1)* enhances salt tolerance by increased osmoregulation and Na+ exclusion in *Arabidopsis thaliana*. Plant Cell Physiol **56**(1): 73–83

Chen R, Jiang H, Li L, Zhai Q, Qi L, Zhou W, Liu X, Li H, Zheng W, Sun J et al. (2012) The *Arabidopsis* mediator subunit MED25 differentially regulates jasmonate and abscisic acid signaling through interacting with the MYC2 and ABI5 transcription factors. Plant Cell **24**(7): 2898–2916

Chen Q, Sun J, Zhai Q, Zhou W, Qi L, Xu L, Wang B, Chen R, Jiang H, Qi J, Li X, Palme K, Li C (2011) The basic helix-loop-helix transcription factor MYC2 directly represses *PLETHORA* expression during jasmonate-mediated modulation of the root stem cell niche in Arabidopsis. Plant Cell **23**(9): 3335–3352

Cheng Y, Dai X, Zhao Y (2006) Auxin biosynthesis by the YUCCA flavin monooxygenases controls the formation of floral organs and vascular tissues in *Arabidopsis*. Genes Dev **20**(13): 1790–1799

Cheng H, Song S, Xiao L, Soo HM, Cheng Z, Xie D, Peng J (2009) Gibberellin acts through jasmonate to control the expression of MYB21, MYB24, and MYB57 to promote stamen filament growth in *Arabidopsis*. PLoS Genet **5**(3): e1000440

Chini A, Fonseca S, Fernández G, Adie B, Chico JM, Lorenzo O, García-Casado G, López-Vidriero I, Lozano FM, Ponce MR, et al. (2007) The JAZ family of repressors is the missing link in jasmonate signaling. Nature **448**(7154): 666–671

Chini A, Gimenez-Ibanez S, Goossens A, Solano R (2016) Redundancy and specificity in jasmonate signaling. Curr Opin Plant Biol **33**: 147–156

Chung HS, Cooke TF, Depew CL, Patel LC, Ogawa N, Kobayashi Y, Howe GA (2010) Alternative splicing expands the repertoire of dominant JAZ repressors of jasmonate signaling. Plant J **63**(4): 613–622

Clough SJ, Bent AF (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J **16**(6): 735–743

Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR (2010) Abscisic acid: emergence of a core signaling network. Annu Rev Plant Biol **61**(1): 651–679

Czechowski T, Stitt M, Altmann T, Udvardi MK, Scheible WR (2005) Genome-wide identification and testing of superior reference genes for transcript normalization in *Arabidopsis*. Plant Physiol **139**(1): 5–17

Dai M, Xue Q, Mccray T, Margavage K, Chen F, Lee J-H, Nezames CD, Guo L, Terzaghi W, Wan J, et al. (2013) The PP6 phosphatase regulates ABI5 phosphorylation and abscisic acid signaling in *Arabidopsis*. Plant Cell **25**(2): 517–534

Dave A, Hernández ML, He Z, Andriotis VM, Vaistij FE, Larson TR, Graham IA (2011) 12-oxo-Phytodienoic acid accumulation during seed development represses seed germination in *Arabidopsis*. Plant Cell **23**(2): 583–599

Dave A, Vaistij FE, Gilday AD, Penfield SD, Graham IA (2016) Regulation of *Arabidopsis* thaliana seed dormancy and germination by 12-oxo-phytodienoic acid. J Exp Bot **67**(8): 2277–2284

Dekkers BJ, Schuurmans JA, Smeekens SC (2004) Glucose delays seed germination in *Arabidopsis thaliana*. Planta **218**(4): 579–588

Dharmasiri N, Dharmasiri S, Estelle M (2005a) The F-box protein TIR1 is an auxin receptor. Nature **435**(7041): 441–445

Dharmasiri N, Dharmasiri S, Weijers D, Lechner E, Yamada M, Hobbie L, Ehrismann JS, Jürgens G, Estelle M (2005b) Plant development is regulated by a family of auxin receptor F box proteins. Dev Cell **9**(1): 109–119

Ding H, Lai J, Wu Q, Zhang S, Chen L, Dai Y-S, Wang C, Du J, Xiao S, Yang C (2016) Jasmonate complements the function of *Arabidopsis* lipoxygenase3 in salinity stress response. Plant Sci **244**: 1–7

Dombrecht B, Xue GP, Sprague SJ, Kirkegaard JA, Ross JJ, Reid JB, Fitt GP, Sewelam N, Schenk PM, Manners JM, et al. (2007) MYC2 differentially modulates diverse jasmonate-dependent functions in *Arabidopsis*. Plant Cell **19**(7): 2225–2245

Ebel C, BenFeki A, Hanin M, Solano R, Chini A (2018) Characterization of wheat (*Triticum aestivum*) *TIFY* family and role of *Triticum Durum Td*TIFY11a in salt stress tolerance. PLoS ONE **13**(7): e0200566

Ellis C, Turner JG (2002) A conditionally fertile *coi1* allele indicates cross-talk between plant hormone signaling pathways in *Arabidopsis thaliana* seeds and young seedlings. Planta **215**(4): 549–556

Fernández-Calvo P, Chini A, Fernández-Barbero G, Chico J-M, Gimenez-Ibanez S, Geerinck J, Eeckhout D, Schweizer F, Godoy M, Franco-Zorrilla JM, et al. (2011) The *Arabidopsis* bHLH transcription factorsMYC3 and MYC4 are targets of JAZ repressors and act additively withMYC2 in the activation of jasmonate responses. Plant Cell **23**(2): 701–715

Finkelstein RR (1994) Mutations at two new *Arabidopsis* ABA response loci are similar to the *abi3* mutations. Plant J **5**(6): 756–771

Finkelstein R, Gampala SS, Lynch TJ, Thomas TL, Rock CD (2005) Redundant and distinct functions of the ABA response loci *ABA-INSENSITIVE (ABI) 5* and *ABRE-BINDING FACTOR (ABF) 3*. Plant Mol Biol **59**(2): 253–267

Finkelstein RR, Gampala SS, Rock CD (2002) Abscisic acid signaling in seeds and seedlings. Plant Cell **14**(suppl 1): S15–S45

Finkelstein RR, Lynch TJ (2000a) The *Arabidopsis* abscisic acid response gene *ABI5* encodes a basic leucine zipper transcription factor. Plant Cell **12**(4): 599–609

Finkelstein RR, Lynch TJ (2000b) Abscisic acid inhibition of radicle emergence but not seedling growth is suppressed by sugars. Plant Physiol **122**(4): 1179–1186

Finkelstein R, Reeves W, Ariizumi T, Steber C (2008) Molecular aspects of seed dormancy. Annu Rev Plant Biol **59**(1): 387–415

Finkelstein RR, Wang ML, Lynch TJ, Rao S, Goodman HM (1998) The *Arabidopsis* abscisic acid response locus *ABI4* encodes an APETALA 2 domain protein. Plant Cell **10**(6): 1043–1054

Fonseca S, Chico JM, Solano R (2009) The jasmonate pathway: the ligand, the receptor and the core signaling module. Curr Opin Plant Biol **12**(5): 539–547

Fujii H, Verslues PE, Zhu JK (2007) Identification of two protein kinases required for abscisic acid regulation of seed germination, root growth, and gene expression in *Arabidopsis*. Plant Cell **19**(2): 485–494

Fujii H, Zhu JK (2009) *Arabidopsis* mutant deficient in 3 abscisic acid-activated protein kinases reveals critical roles in growth, reproduction, and stress. Proc Natl Acad Sci USA **106**(20): 8380–8385

Furihata T, Maruyama K, Fujita Y, Umezawa T, Yoshida R, Shinozaki K, Yamaguchi-Shinozaki K (2006) Abscisic acid-dependent multisite phosphorylation regulates the activity of a transcription activator AREB1. Proc Natl Acad Sci USA **103**(6): 1988–1993

Garcia ME, Lynch T, Peeters J, Snowden C, Finkelstein R (2008) A small plant-specific protein family of ABI five binding proteins (AFPs) regulates stress response in germinating *Arabidopsis* seeds and seedlings. Plant Mol Biol **67**(6): 643–658

Garciarrubio A, Legaria JP, Covarrubias AA (1997) Abscisic acid inhibits germination of mature *Arabidopsis* seeds by limiting the availability of energy and nutrients. Planta **203**(2): 182–187

Giraudat J, Hauge BM, Valon C, Smalle J, Parcy F, Goodman HM (1992) Isolation of the *Arabidopsis ABI3* gene by positional cloning. Plant Cell **4**(10): 1251–1261

Goossens J, Swinnen G, Vanden Bossche R, Pauwels L, Goossens A (2015) Change of a conserved amino acid in the MYC2 and MYC3 transcription factors leads to release of JAZ repression and increased activity. New Phytol **206**(4): 1229–1237

Guan C, Wang X, Feng J, Hong S, Liang Y, Ren B, Zuo J (2014) Cytokinin antagonizes abscisic acid-mediated inhibition of cotyledon greening by promoting the degradation of ABSCISIC ACID INSENSITIVE5 protein in *Arabidopsis*. Plant Physiol **164**(3): 1515–1526

Gubler F, Millar AA, Jacobsen JV (2005) Dormancy release, ABA and pre-harvest sprouting. Curr Opin Plant Biol **8**(2): 183–187

Guo Q, Major IT, Howe GA (2018a) Resolution of growth-defense conflict: mechanistic insights from jasmonate signaling. Curr Opin Plant Biol **44**: 72–81

Guo Q, Yoshida Y, Major IT, Wang K, Sugimoto K, Kapali G, Havko NE, Benning C, Howe GA (2018b) JAZ Repressors of metabolic defense promote growth and reproductive fitness in *Arabidopsis*. Proc Natl Acad Sci USA **115**(45): 10768–10777

Han X, Hu Y, Zhang G, Jiang Y, Chen X, Yu D (2018) Jasmonate negatively regulates stomatal development in *Arabidopsis* cotyledons. Plant Physiol **176**(4): 2871–2885

Han X, Kui M, Xu T, Ye J, Du J, Yang M, Jiang Y, Hu Y (2023) CO Interacts with JAZ repressors and bHLH subgroup IIId factors to negatively regulate jasmonate signaling in Arabidopsis seedlings. Plant Cell **35**(2): 852–873

Han X, Zhang M, Yang M, Hu Y (2020) *Arabidopsis* JAZ proteins interact with and suppress RHD6 transcription factor to regulate jasmonate-stimulated root hair development. Plant Cell **32**(4): 1049–1062

Hauser F, Waadt R, Schroeder JI (2011) Evolution of abscisic acid synthesis and signaling mechanisms. Curr Biol **21**(9): R346–R355

Hellens RP, Allan AC, Friel EN, Bolitho K, Grafton K, Templeton MD, Karunairetnam S, Gleave AP, Laing WA (2005) Transient expression vectors for functional genomics, quantification of promoter activity and RNA silencing in plants. Plant Methods **1**(1): 13

Hou X, Yen L, Lee C, Xia K, Yan Y, Yu H (2010) DELLAs modulate jasmonate signaling via competitive binding to JAZs. Dev Cell **19**(6): 884–894

Howe GA, Major IT, Koo AJ (2018) Modularity in jasmonate signaling for multistress resilience. Annu Rev Plant Biol **69**(1): 387–415

Hu Y, Han X, Yang M, Zhang M, Pan J, Yu D (2019) The transcription factor INDUCER OF CBF EXPRESSION1 interacts with ABSCISIC ACID INSENSITIVE5 and DELLA proteins to fine-tune abscisic acid signaling during seed germination in *Arabidopsis*. Plant Cell **31**(7): 1520–1538

Hu Y, Jiang Y, Han X, Wang H, Pan J, Yu D (2017) Jasmonate regulates leaf senescence and tolerance to cold stress: crosstalk with other phytohormones. J Exp Bot **68**(6): 1361–1369

Hu Y, Jiang L, Wang F, Yu D (2013) Jasmonate regulates the inducer of CBF expression–C-repeat binding factor/DRE binding factor1 cascade and freeding tolerance in *Arabidopsis*. Plant Cell **25**(8): 2907–2924

Hu Y, Yu D (2014) BRASSINOSTEROID INSENSITIVE2 interacts with ABSCISIC ACID INSENSITIVE5 to mediate the antagonism of brassinosteroids to abscisic acid during seed germination in *Arabidopsis*. Plant Cell **26**(11): 4394–4408

Hu S, Yu K, Yan J, Shan X, Xie D (2022) Jasmonate perception: Ligand-receptor interaction, regulation, and evolution. Mol Plant http://dx.doi.org/10.1016/j.molp.2022.08.011

Iglesias MJ, Terrile MC, Bartoli CG, D'Ippólito S, Casalongué CA (2010) Auxin signaling participates in the adaptative response against oxidative stress and salinity by interacting with redox metabolism in *Arabidopsis*. Plant Mol Biol **74**(3): 215–222

Iglesias MJ, Terrile MC, Windels D, Lombardo MC, Bartoli CG, Vazquez F, Estelle M, Casalongué CA (2014) Mir393 regulation of auxin signaling and redox-related components during acclimation to salinity in *Arabidopsis*. PLoS One **9**(9): e107678

Ji H, Wang S, Cheng C, Li R, Wang Z, Jenkins GI, Kong F, Li X (2019) The RCC1 family protein SAB1 negatively regulates ABI5 through multidimensional mechanisms during postgermination in *Arabidopsis*. New Phytol **222**(2): 907–922

Jiang Y, Liang G, Yang S, Yu D (2014) *Arabidopsis* WRKY57 functions as node of convergence for jasmonic acid-and auxin-mediated signaling in jasmonic acid-induced leaf senescence. Plant Cell **26**(1): 230–245

Ju L, Jing Y, Shi P, Liu J, Chen J, Yan J, Chu J, Chen KM, Sun J (2019) JAZ Proteins modulate seed germination through interacting with ABI5 in bread wheat and *Arabidopsis*. New Phytol **223**(1): 246–260

Katsir L, Chung HS, Koo AJ, Howe GA (2008) Jasmonate signaling: a conserved mechanism of hormone sensing. Curr Opin Plant Biol **11**(4): 428–435

Kazan K, Manners JM (2013) MYC2: the master in action. Mol Plant **6**(3): 686–703

Kepinski S, Leyser O (2005) The *Arabidopsis* F-box protein TIR1 is an auxin receptor. Nature **435**(7041): 446–451

Kieffer M, Neve J, Kepinski S (2010) Defining auxin response contexts in plant development. Curr Opin Plant Biol **13**(1): 12–20

Kim J, Kang H, Park J, Kim W, Yoo J, Lee N, Kim J, Yoon TY, Choi G (2016) PIF1-interacting Transcription factors and their binding sequence elements determine the *in vivo* targeting sites of PIF1. Plant Cell **28**(6): 1388–1405

Kim KC, Lai Z, Fan B, Chen Z (2008) *Arabidopsis* WRKY38 and WRKY62 transcription factors interact with histone deacetylase 19 in basal defense. Plant Cell **20**(9): 2357–2371

Kobayashi Y, Murata M, Minami H, Yamamoto S, Kagaya Y, Hobo T, Yamamoto A, Hattori T (2005) Abscisic acid activated SNRK2 protein kinases function in the gene-regulation pathway of ABA signal transduction by phosphorylating ABA response element-binding factors. Plant J **44**(6): 939–949

Kong X, Zhang L, Ding Z (2016) 26S Proteasome: hunter and prey in auxin signaling. Trends Plant Sci **21**(7): 546–548

Krock B, Schmidt S, Hertweck C, Baldwin IT (2002) Vegetation derived abscisic acid and four terpenes enforce dormancy in seeds of the post-fire annual, *Nicotiana attenuata*. Seed Sci Res **12**(4): 239–252

Lee JH, Yoon HJ, Terzaghi W, Martinez C, Dai M, Li J, Byun MO, Deng XW (2010) DWA1 And DWA2, two *Arabidopsis* DWD protein components of CUL4-based E3 ligases, act together as negative regulators in ABA signal transduction. Plant Cell **22**(6): 1716–1732

Lim S, Park J, Lee N, Jeong J, Toh S, Watanabe A, Kim J, Kang H, Kim DH, Kawakami N, et al. (2013) ABA-insensitive3, ABA-insensitive5, and DELLAs interact to activate the expression of SOMNUS and other high-temperature-inducible genes in imbibed seeds in *Arabidopsis*. Plant Cell **25**(12): 4863–4878

Liu X, Hou X (2018) Antagonistic regulation of ABA and GA in metabolism and signaling pathways. Front Plant Sci **9**: 251

Liu PP, Montgomery TA, Fahlgren N, Kasschau KD, Nonogaki H, Carrington JC (2007) Repression of AUXIN RESPONSE FACTOR10 by microRNA160 is critical for seed germination and post-germination stages. Plant J **52**(1): 133–146

Liu H, Stone SL (2010) Abscisic acid increases *Arabidopsis* ABI5 transcription factor levels by promoting KEG E3 ligase self-ubiquitination and proteasomal degradation. Plant Cell **22**(8): 2630–2641

Liu X, Zhang H, Zhao Y, Feng Z, Li Q, Yang HQ, Luan S, Li J, He ZH (2013) Auxin controls seed dormancy through stimulation of abscisic acid signaling by inducing ARF-mediated *ABI3* activation in *Arabidopsis*. Proc Natl Acad Sci USA **110**(38): 15485–15490

Lopez-Molina L, Chua NH (2000) A null mutation in a bZIP factor confers ABA-insensitivity in *Arabidopsis thaliana*. Plant Cell Physiol **41**(5): 541–547

Lopez-Molina L, Mongrand S, Chua NH (2001) A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the *ABI5* transcription factor in *Arabidopsis*. Proc Natl Acad Sci USA **98**(8): 4782–4787

Lopez-Molina L, Mongrand S, McLachlin DT, Chait BT, Chua NH (2002) *ABI5* Acts downstream of *ABI3* to execute an ABA-dependent growth arrest during germination. Plant J **32**(3): 317–328

Lorenzo O, Chico JM, Sánchez-Serrano JJ, Solano R (2004) JASMONATE-INSENSITIVE1 encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in *Arabidopsis*. Plant Cell **16**(7): 1938–1950

Lynch TJ, Erickson BJ, Miller DR, Finkelstein RR (2017) ABI5-binding Proteins (AFPs) alter transcription of ABA-induced genes via a variety of interactions with chromatin modifiers. Plant Mol Biol **93**(4-5): 403–418

Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, Christmann A, Grill E (2009) Regulators of PP2C phosphatase activity function as abscisic acid sensors. Science **324**(5930): 1064–1068

Miura K, Lee J, Jin JB, Yoo CY, Miura T, Hasegawa PM (2009) Sumoylation of ABI5 by the *Arabidopsis* SUMO E3 ligase SIZ1 negatively regulates abscisic acid signaling. Proc Natl Acad Sci USA **106-**(13): 5418–5423

Miyazono K, Miyakawa T, Sawano Y, Kubota K, Kang H-J, Asano A, Miyauchi Y, Takahashi M, Zhi Y, Fujita Y, et al. (2009) Structural basis of abscisic acid signaling. Nature **462**(7273): 609–614

Moreno JE, Shyu C, Campos ML, Patel LC, Chung HS, Yao J, He SY, Howe GA (2013) Negative feedback control of jasmonate signaling by an alternative splice variant of JAZ10. Plant Physiol **162**(2): 1006–1017

Nagpal P, Walker LM, Young JC, Sonawala A, Timpte C, Estelle M, Reed JW (2000) *AXR2* encodes a member of the aux/IAA protein family. Plant Physiol **123**(2): 563–574

Nakabayashi K, Okamoto M, Koshiba T, Kamiya Y, Nambara E (2005) Genome-wide profiling of stored mRNA in *Arabidopsis thaliana* seed germination: epigenetic and genetic regulation of transcription in seed. Plant J **41**(5): 697–709

Nakamura S, Lynch TJ, Finkelstein RR (2001) Physical interactions between ABA response loci of *Arabidopsis*. Plant J **26**(6): 627–635

Nakashima K, Fujita Y, Kanamori N, Katagiri T, Umezawa T, Kidokoro S, Maruyama K, Yoshida T, Ishiyama K, Kobayashi M, et al. (2009) Three *Arabidopsis* SnRK2 protein kinases, SRK2D/SnRK2.2, SRK2E/SnRK2.6/OST1 and SRK2I/SnRK2.3, involved in ABA signaling are essential for the control of seed development and dormancy. Plant Cell Physiol **50**(7): 1345–1363

Nakashima K, Yamaguchi-Shinozaki K (2013) ABA signaling in stress-response and seed development. Plant Cell Rep **32**(7): 959–970

Nishimura N, Hitomi K, Arvai AS, Rambo RP, Hitomi C, Cutler SR, Schroeder JI, Getzoff ED (2009) Structural mechanism of absicsic acid binding and signaling by dimeric PYR1. Science **326**(5958): 1373–1379

Niu YJ, Figueroa P, Browse J (2011) Characterization of JAZ- interacting bHLH transcription factors that regulate jasmonate responses in *Arabidopsis*. J Exp Bot **62**(6): 2143–2154

Norastehnia A, Sajedi RH, Nojavan-Asghari M (2007) Inhibitory effects of methyl jasmonate on seed germination in maize (*Zea mays*): effect on a-amylase activity and ethylene production. Gen Appl Plant Physiol **33**(1-2): 13–23

Pan J, Hu Y, Wang H, Guo Q, Chen Y, Howe GA, Yu D (2020) Molecular mechanism underlying the synergetic effect of jasmonate on abscisic acid signaling during seed germination in *Arabidopsis*. Plant Cell **32**(12): 3846–3865

Pan J, Wang H, Hu Y, Yu D (2018) *Arabidopsis* VQ18 and VQ26 proteins interact with ABI5 transcription factor to negatively modulate ABA response during seed germination. Plant J **95**(3): 529–544

Park SY, Fung P, Nishimura N, Jensen DR, Fujii H, Zhao Y, Lumba S, Santiago J, Rodrigues A, Chow TF, et al. (2009) Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. Science **324**(5930): 1068–1071

Park J, Kim YS, Kim SG, Jung JH, Woo JC, Park CM (2011) Integration of auxin and salt signals by the NAC transcription factor NTM2 during seed germination in *Arabidopsis*. Plant Physiol **156**(2): 537–549

Peer WA (2013) From perception to attenuation: auxin signaling and responses. Curr Opin Plant Biol **16**(5): 561–568

Peng J, Wang M, Wang X, Qi L, Guo C, Li H, Li C, Yan Y, Zhou Y, Terzaghi W, et al. (2022) COP1 Positively regulates ABA signaling during *Arabidopsis* seedling growth in darkness by mediating ABA-induced ABI5 accumulation. Plant Cell **34**(6): 2286–2308

Preston CA, Betts H, Baldwi IT (2002) Methyl jasmonate as an allelopathic agent: sagebrush inhibits germination of a neighboring tobacco, *Nicotiana attenuata*. J Chem Ecol **28**(11): 2343–2369

Qi T, Huang H, Song S, Xie D (2015) Regulation of jasmonate-mediated stamen development and seed production by a bHLH-MYB complex in *Arabidopsis*. Plant Cell **27**(6): 1620–1633

Qi T, Song S, Ren Q, Wu D, Huang H, Chen Y, Fan M, Peng W, Ren C, Xie D (2011) The jasmonate-ZIM-domain proteins interact with the WD-repeat/bHLH/MYB complexes to regulate jasmonate-mediated anthocyanin accumulation and trichome initiation in *Arabidopsis thaliana*. Plant Cell **23**(5): 1795–1814

Reeves WM, Lynch TJ, Mobin R, Finkelstein RR (2011) Direct targets of the transcription factors ABA-insensitive (ABI)4 and ABI5 reveal synergisticaction by ABI4 and several bZIP ABA response factors. Plant Mol Biol **75**(4–5): 347–363

Reinhardt D (2003) Vascular patterning: more than just auxin. Curr Biol **13**(12): R485–R487

Ribba T, Garrido-Vargas F, O'Brien JA (2020) Auxin-mediated responses under salt stress: from developmental regulation to biotechnological applications. J Exp Bot **71**(13): 3843–3853

Romano CP, Robson PR, Smith H, Estelle M, Klee H (1995) Transgene-mediated auxin overproduction in *Arabidopsis*: hypocotyl elongation phenotype and interactions with the *hy6-1* hypocotyl elongation and *axr1* auxin-resistant mutants. Plant Mol Biol **27**(6): 1071–1083

Sabatini S, Beis D, Wolkenfelt H, Murfett J, Guilfoyle T, Malamy J, Benfey P, Leyser O, Bechtold N, Weisbeek P, et al. (1999) An auxin-

dependent distal organizer of pattern and polarity in the *Arabidopsis* root. Cell **99**(5): 463–472

Santiago J, Dupeux F, Round A, Antoni R, Park SY, Jamin M, Cutler SR, Rodriguez PL, Márquez JA (2009) The abscisic acid receptor PYR1 in complex with abscisic acid. Nature **462**(7273): 665–668

Schweizer F, Fernández-Calvo P, Zander M, Diez-Diaz M, Fonseca S, Glauser G, Lewsey MG, Ecker JR, Solano R, Reymond P (2013) *Arabidopsis* basic helix-loop-helix transcription factors MYC2, MYC3, and MYC4 regulate glucosinolate biosynthesis, insect performance, and feeding behavior. Plant Cell **25**(8): 3117–3132

Serrano-Bueno G, de Los Reyes P, Chini A, Ferreras-Garrucho G, Sánchez de Medina-Hernández V, Boter M, Solano R, Valverde F (2022) Regulation of floral senescence in Arabidopsis by coordinated action of CONSTANS and jasmonate signaling. Mol Plant **11**-(11): 1710–1724

Sheard LB, Tan X, Mao H, Withers J, Ben-Nissan G, Hinds TR, Kobayashi Y, Hsu F-F, Sharon M, Browse J, et al. (2010) Jasmonate perception byinositol-phosphate-potentiated COI1-JAZ co-receptor. Nature **468**(7322): 400–405

Sheen J (2001) Signal transduction in maize and *Arabidopsis* mesophyll protoplasts. Plant Physiol **127**(4): 1466–1475

Skubacz A, Daszkowska-Golec A, Szarejko I (2016) The role and regulation of ABI5 (ABA-insensitive 5) in plant development, abiotic stress responses and phytohormone crosstalk. Front Plant Sci **7**: 1884

Söderman EM, Brocard IM, Lynch TJ, Finkelstein RR (2000) Regulation and function of the *Arabidopsis ABA-insensitive4* gene in seed and abscisic acid response signaling networks. Plant Physiol **124**(4): 1752–1765

Song S, Qi T, Huang H, Ren Q, Wu D, Chang C, Peng W, Liu Y, Peng J, Xie D (2011) The jasmonate-ZIM domain proteins interact with the R2R3-MYB transcription factors MYB21 and MYB24 to affect jasmonate-regulated stamen development in *Arabidopsis*. Plant Cell **23**(3): 1000–1013

Staswick PE, Su W, Howell SH (1992) Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an *Arabidopsis thaliana* mutant. Proc Natl Acad Sci USA **89**(15): 6837–6840

Stepanova AN, Hoyt JM, Hamilton AA, Alonso JM (2005) A link between ethylene and auxin uncovered by the characterization of two root-specific ethylene-insensitive mutants in *Arabidopsis*. Plant Cell **17**(8): 2230–2242

Stone SL, Williams LA, Farmer LM, Vierstra RD, Callis J (2006) KEEP ON GOING, a RING E3 ligase essential for *Arabidopsis* growth and development, is involved in abscisic acid signaling. Plant Cell **18**(12): 3415–3428

Sun J, Xu Y, Ye S, Jiang H, Chen Q, Liu F, Zhou W, Chen R, Li X, Tietz O, et al. (2009) *Arabidopsis ASA1* is important for jasmonate-mediated regulation of auxin biosynthesis and transport during lateral root formation. Plant Cell **21**(5): 1495–1511

Suzuki M, Kao CY, Cocciolone S, McCarty DR (2001) Maize VP1 complements *Arabidopsis abi3* and confers a novel ABA/auxin interaction in roots. Plant J **28**(4): 409–418

Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, Liu G, Nomura K, He SY, Howe GA, Browse J (2007) JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signaling. Nature **448**(7154): 661–665

Tiwari SB, Hagen G, Guilfoyle T (2003) The roles of auxin response factor domains in auxin-responsive transcription. Plant Cell **15**(2): 533–543

Ulmasov T, Hagen G, Guilfoyle TJ (1999a) Activation and repression of transcription by auxin-response factors. Proc Natl Acad Sci USA **96**(10): 5844–5849

Ulmasov T, Hagen G, Guilfoyle TJ (1999b) Dimerization and DNA binding of auxin response factors. Plant J **19**(3): 309–319

Vanneste S, Friml J (2009) Auxin: a trigger for change in plant development. Cell **136**(6): 1005–1016

Wang H, Li Y, Pan J, Lou D, Hu Y, Yu D (2017) The bHLH transcription factors MYC2, MYC3, and MYC4 are required for jasmonate-mediated inhibition of flowering in *Arabidopsis*. Mol Plant **10**(11): 1461–1464

Wang J, Wang L, Mao Y, Cai W, Xue H, Chen X (2005) Control of root cap formation by MicroRNA-targeted auxin response factors in *Arabidopsis*. Plant Cell **17**(8): 2204–2216

Wilen RW, van Rooijen GJ, Pearce DW, Pharis RP, Holbrook LA, Moloney MM (1991) Effects of jasmonic acid on embryo-specific processes in *brassica* and *linum* oilseeds. Plant Physiol **95**(2): 399–405

Woodward AW, Bartel B (2005) Auxin: regulation, action, and interaction. Ann Bot (Lond) **95**(5): 707–735

Xie D, Feys BF, James S, Nieto-Rostro M, Turner JG (1998) *COI1:* an *Arabidopsis* gene required for jasmonate-regulated defense and fertility. Science **280**(5366): 1091–1094

Yan J, Zhang C, Gu M, Bai Z, Zhang W, Qi T, Cheng Z, Peng W, Luo H, Nan F, et al. (2009) The *Arabidopsis* CORONATINE INSENSITIVE1 protein is a jasmonate receptor. Plant Cell **21**(8): 2220–2236

Yang X, Bai Y, Shang J, Xin R, Tang W (2016) The antagonistic regulation of abscisic acid-inhibited root growth by brassinosteroids is partially mediated via direct suppression of ABSCISIC ACID INSENSITIVE 5 expression by BRASSINAZOLE RESISTANT 1. Plant Cell Environ **39**(9): 1994–2003

Yang M, Han X, Yang J, Jiang Y, Hu Y (2021) The *Arabidopsis* circadian clock protein PRR5 interacts with and stimulates ABI5 to modulate abscisic acid signaling during seed germination. Plant Cell **33**(9): 3022–3041

Ye H, Du H, Tang N, Li X, Xiong L (2009) Identification and expression profiling analysis of TIFY family genes involved in stress and phytohormone responses in rice. Plant Mol Biol **71**(3): 291–305

Yoo SD, Cho YH, Sheen J (2007) *Arabidopsis* mesophyll protoplasts: a versatile cell system for transient gene expression analysis. Nature Protoc **2**(7): 1565–1572

Yu F, Wu Y, Xie Q (2015) Precise protein post-translational modifications modulate ABI5 activity. Trends Plant Sci **20**(9): 569–575

Zhai Q, Yan L, Tan D, Chen R, Sun J, Gao L, Dong MQ, Wang Y, Li C (2013) Phosphorylation-coupled proteolysis of the transcription factor MYC2 is important for jasmonate-signaled plant immunity. PLoS Genet **9**(4): e1003422

Zhai Q, Zhang X, Wu F, Feng H, Deng L, Xu L, Zhang M, Wang Q, Li C (2015) Transcriptional mechanism of jasmonate receptor COI1-mediated delay of flowering time in *Arabidopsis*. Plant Cell **27**(10): 2814–2828

Zhang G, Zhao F, Chen L, Pan Y, Sun L, Bao N, Zhang T, Cui CX, Qiu Z, Zhang Y, et al. (2019) Jasmonate-mediated wound signaling promotes plant regeneration. Nat Plants **5**(5): 491–497

Zhao Y, Christensen SK, Fankhauser C, Cashman JR, Cohen JD, Weigel D, Chory J (2001) A role for flavin monooxygenase-like enzymes in auxin biosynthesis. Science **291**(5502): 306–309

Zhao X, Dou L, Gong Z, Wang X, Mao T (2019) BES1 Hinders ABSCISIC ACID INSENSITIVE5 and promotes seed germination in *Arabidopsis*. New Phytol **221**(2): 908–918

Zhou X, Hao H, Zhang Y, Bai Y, Zhu W, Qin Y, Yuan F, Zhao F, Wang M, Hu J, et al. (2015) SOS2-LIKE PROTEIN KINASE5, an SNF1-RELATED PROTEIN KINASE3-type protein kinase, is important for abscisic acid responses in *Arabidopsis* through phosphorylation of ABSISIC SCID-INSENSITIVE5. Plant Physiol **168**(2): 659–676

Zhou W, Lozano-Torres JL, Blilou I, Zhang X, Zhai Q, Smant G, Li C, Scheres B (2019) A jasmonate signaling network activates root stem cells and promotes regeneration. Cell **177**(4): 942–956

Zhu Z, An F, Feng Y, Li P, Xue L, A M, Jiang Z, Kim JM, To TK, Li W, et al. (2011) Derepression of ethylene-stabilized transcription factors (EIN3/EIL1) mediates jasmonate and ethylene signaling synergy in *Arabidopsis*. Proc Natl Acad Sci USA **108**(30): 12539–12544