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SHORT COMMUNICATION

Abscisic acid signaling is involved in regulating the mitogen-activated protein kinase cascade module, AIK1-MKK5-MPK6

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ABSTRACT

Abscisic acid (ABA) plays roles in plant growth and development and in stress responses. Recently, we found that ABA regulates ABA-insensitive protein kinase 1 (AIK1), a mitogen-activated protein kinase kinase kinase. Compared with wild-type, *aik1–1* showed downregulation of ABA-responsive genes (*RD29A, MYC2, ABI3* and *ABI4*). Under ABA treatment, the transcript level of *KRP1* (Kip-related protein, a cyclin-dependent kinase inhibitor) was lower in *aik1–1* than in wild-type. The activity of ABA-activated MPK6 was decreased in *abi1 abi2*, and *abi1 abi2 hab1*, and increased in *snrk2.2 snrk2.3* and *pyr1 pyl1 pyl2 pyl4* mutants. These results indicated that AIK1-MKK5-MPK6 functions in ABA responses and requires ABA-responsive gene expression to regulate ABA-inhibited root growth and cell division. The ABA signaling pathway regulates this MAPK cascade.

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The phytohormone abscisic acid (ABA) plays a central role in plant development and in the response to abiotic stresses.[1-3] Previous studies have revealed the molecular mechanism of ABA action in Arabidopsis.[4-8] Abscisic acid triggers downstream responses by binding to the cytosolic receptors (ABAR) PYR/PYL/RCARs (pyrabactin resistance/pyrabactin resistance-like/regulatory component of ABARs), which then sequester the negative regulators PP2C (clade A type 2C protein phosphatases), leading to the activation of SnRK2s (group III sucrose non-fermenting-1-related protein kinases 2).[9,10] The SnRK2s activate the expression of downstream ABA-responsive genes via regulation of ABA-responsive element-binding factors (AREB or ABF) (e.g. AREB1/ABF2, AREB2/ABF4 and ABF3).[11-13] More than 10% of the genes in the Arabidopsis genome are induced by ABA,[14] and components of the MAPK (mitogen-activated protein kinase) cascade are also involved in the response to ABA.[15-17]

Recently, the ABA-activated AIK1-MKK5-MPK6 cascade was shown to be involved in regulating ABA responses such as primary root growth and stomatal movement.[18] However, the molecular mechanism by which ABA activates this MAPK cascade and how this cascade transfers the ABA signal to the downstream components remain unclear. To test how AIK1 regulates ABA responses, we analyzed *aik1–1* and wild-type plants to determine the transcript levels of ABA-inducible genes such as *RD29A*[19] (response-to-dehydration 29A), *ABI3*[20] (ABA-insensitive 3), *ABI4*[21], and *MYC2*[22] (a basic helix-loop-helix transcription factor). As reported previously, the transcript levels of all these ABA-responsive genes markedly increased in wild-type seedlings in response to ABA. The *aik1–1* mutant with a disrupted *AIK1* gene also showed upregulated transcript levels of *RD29A, ABI3, ABI4*, and *MYC2* in the presence of ABA. However, compared with wild-type seedlings, *aik1–1* seedlings showed lower transcript levels of these genes at different time points (Fig. 1A–D). Our previous study showed that abscisic acid induces *AIK1* at the kinase activity level and at the transcriptional level, and that AIK1 modulates ABA signaling by sequentially relaying and amplifying intracellular signals through the AIK1-MKK5-MPK6 cascade.[18] We hypothesize that there is at least one component upstream or downstream of AIK1 or MPK6 that responds to ABA and regulates the transcriptional activity of *AIK1*. For example, the transcription factor MYC2 was reported to interact with MPK6 and was phosphorylated by MPK6 in response to blue light.[23]

The inhibition of primary root growth is a classic response to ABA mediated by changes in both cell extensibility and cell division.[3] We found that the decreased sensitivity to ABA during root tip growth in the *aik1* mutant was related to the function of AIK1 to regulate root cell division and elongation during the ABA response.[18] Kip-related proteins (KRPs) are cyclin-dependent kinase inhibitor proteins that negatively regulate cell division and promote endoreduplication. The expression of *KRP1* was found to be highly induced by ABA, leading to inhibition of cell division.[24,25] The transcript level of *KRP1* increased in response to ABA in both wild-type and *aik1–1* plants; however, the magnitude of this increase was smaller in *aik1–1* than in wild-type plants (Fig. 2). This result was consistent with the phenotype of increased total number of cells from the root tip to the elongation zone in *aik1*, compared with wild-type seedlings.[18]

The autophosphorylation and MBP-phosphorylation kinase activity of AIK1 was very high in *in vitro* analyses. The kinase activity assay indicated that both AIK1 and SnRK2.6 could be dephosphorylated by the negative regulator of ABA signaling,

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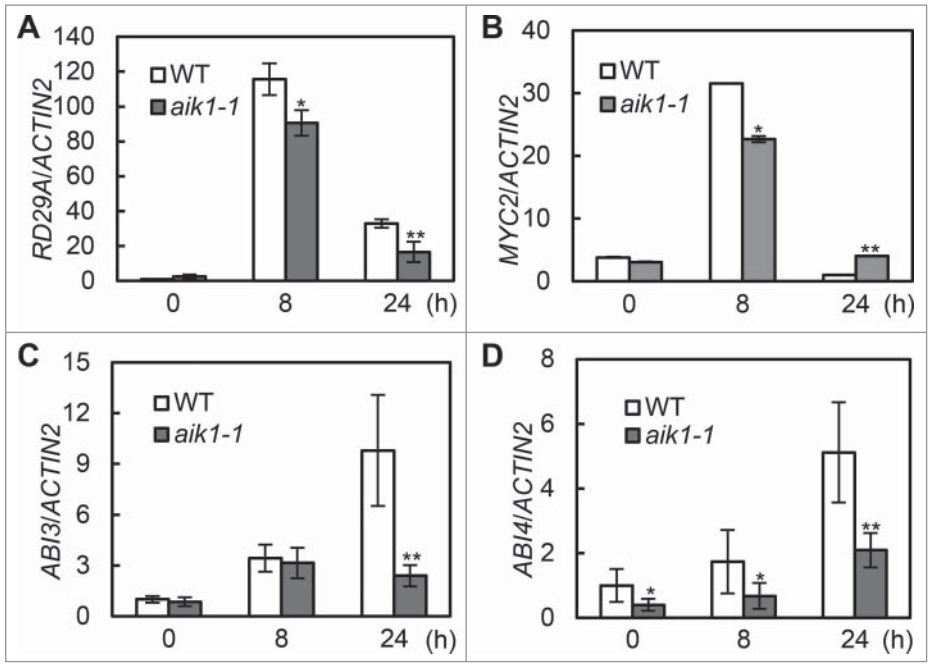

Figure 1. Transcript levels of abscisic acid (ABA)-responsive genes as determined by real-time PCR in seedlings of wild-type (WT) and *aik1–1* seedlings. After growth on Murashige & Skoog medium for 12 d, plants were treated with 100 μM ABA, and then RNA was extracted. Gene transcript levels are shown in relative units with levels in WT plants set to 1. Values are mean ± SD of 3 independent biologic determinations. Asterisks indicate significant differences from the WT at $^{*}P < 0.05$ and $^{**}P < 0.01$ by Student's *t*-test.

ABI1. Interestingly, we detected 3 dephosphorylated forms of AIK1, and each of them showed autophosphorylation activity.[18] The function of these dephosphorylated forms of AIK1 is unknown, and whether one or all of them are able to phosphorylate MBP *in vivo* remains unclear. Further research will focus on these issues.

Apart from the strictly controlled kinase activity of AIK1 regulated by ABA, ABA-activated MPK6 (a component-downstream of AIK1) showed decreased activity in *aik1*.[18] The activity of MPK6 was evaluated in the mutants *abi1 abi2, abi1 abi2 hab1*[26], *snrk2.2 snrk2.3*[27], and *pyr1 pyl1 pyl2 pyl4*[28], to examine whether disruption of ABA signaling affects the ABA-activated MAPK pathway. In wild-type seedlings, MPK6 activity showed a transient (5 min and 15 min) increase in response to ABA. Compared with wild-type, *abi1 abi2* and *abi1 abi2 hab1* seedlings showed decreased MPK6 activity, while *snrk2.2 snrk2.3* and *pyr1 pyl1 pyl2 pyl4* seedlings showed increased MPK6 activity (Fig. 3). In *Arabidopsis*, ABI1 negatively regulates the ABA signaling pathway, the ABA-activated AIK1-MKK5-MPK6 cascade, and also the kinase activity of SnRK2.6 and AIK1. Therefore, it was unexpected that MPK6 activity was lower in *abi1 abi2* and *abi1 abi2 hab1* seedlings than in wild-type. In contrast, ABA-stimulated MPK6 activation was increased in *snrk2.2 snrk2.3* and *pyr1 pyl1 pyl2 pyl4* seedlings, compared with that in wild type. We speculated that the decrease in AIK1-activated MPK6 activity in mutant lines of PP2Cs may result from the blocking of ABA signaling. However, the mutations in SnRK2s and ABA receptors may impair downstream ABA signaling, leading to activation of AIK1-MKK5-MPK6.

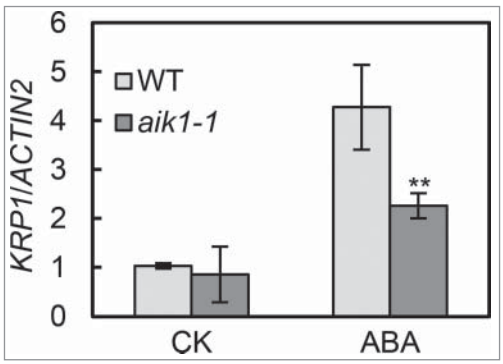

Figure 2. Transcript levels of *KRP1* in wild-type (WT) and *aik1–1* seedlings. After growth on Murashige & Skoog medium for 12 d, plants were treated with 100 μM ABA for 8 h and then RNA was extracted. Gene transcript levels are shown in relative units with level in WT plants set to 1. Values are mean ± SD of 3 independent biologic determinations. Asterisks indicate significant differences from the WT at $^{**}P < 0.01$ by Student's *t*-test.

Alternatively, there may be an ABA-independent pattern of AIK1-MKK5-MPK6 activation. Whether the activity of AIK1 is similar to that of MPK6 in plants with disrupted ABA signaling is unknown. Moreover, there may be some components between the ABA signaling pathway and the AIK1-MKK5-MPK6 cascade that regulate kinase activity. Further research will focus on analyzing the activity of AIK1 in mutant lines of PP2Cs, SnRK2s, and ABA receptors, and on screening to identify the regulators of the ABA-activated AIK1-MKK5-MPK6 cascade.

In conclusion, we found that disruption of AIK1 in the *aik1* mutant reduced the transcript levels of many ABA-responsive genes, and the activity of ABA-activated MPK6 also altered in some ABA signal-disrupted mutants. These results suggest that AIK1 is involved in regulating the expression of some ABA-responsive genes, and reveal the importance of AIK1-MKK5-MPK6 cascade in ABA signal transduction. However, the molecular mechanism of ABA-activated protein kinases in the

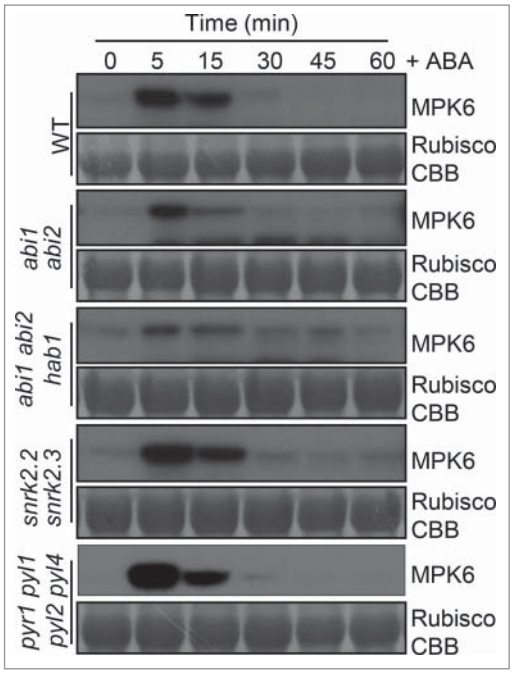

Figure 3. In-gel kinase assays of MPK6 activity. Wild-type (WT), *abi1 abi2, abi1 abi2 hab1, snrk2.2 snrk2.3*, and *pyr1 pyl1 pyl2 pyl4* plants were treated with 100 μM abscisic acid (ABA), then equal quantities of protein were resolved on SDS-PAGE and stained with Coomassie brilliant blue (CBB). Large subunit (LSU) of ribulose-1,5-bis-phosphate carboxylase/oxygenase (Rubisco) is shown as loading control to confirm equal protein loadings.

regulation of plant growth and development is complex. These results are just the beginning in terms of understanding the function of AIK1. Major challenges in the near future are to identify all the components of the AIK1 network that affect its activation and inhibition, and to identify the substrates of the AIK1 cascade or the direct substrates of AIK1.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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