SHORT COMMUNICATION

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CPK12

A Ca^{2+}-dependent protein kinase balancer in abscisic acid signaling

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Key words: abscisic acid (ABA), ABA signal transduction, *Arabidopsis thaliana*, Ca^{2+}-dependent protein kinase (CDPK), CPK12

Abbreviations: ABA, abscisic acid; ABF1/ABF4, ABA-responsive transcription factor1/4; ABI2, ABA insensitive 2; Arabidopsis, *Arabidopsis thaliana*; CDPK, Ca^{2+} dependent protein kinase; SnRK2, sucrose non-fermenting-1-related protein kinase 2

Ca^{2+} is believed to be a critical second messenger in ABA signal transduction. Ca^{2+}-dependent protein kinases (CDPKs) are the best characterized Ca^{2+} sensors in plants. Recently, we identified an Arabidopsis CDPK member CPK12 as a negative regulator of ABA signaling in seed germination and post-germination growth, which reveals that different members of the CDPK family may constitute a regulation loop by functioning positively and negatively in ABA signal transduction. We observed that both RNA interference and overexpression of *CPK12* gene resulted in ABA-hypersensitive phenotypes in seed germination and post-germination growth, suggesting a high complexity of the CPK12-mediated ABA signaling pathway. CPK12 stimulates a negative ABA-signaling regulator (ABI2) and phosphorylates two positive ABA-signaling regulators (ABF1 and ABF4), which may partly explain the ABA hypersensitivity induced by both downregulation and upregulation of *CPK12* expression. Our data indicate that CPK12 appears to function as a balancer in ABA signal transduction in Arabidopsis.

Introduction

Abscisic acid (ABA) regulates many processes of plant development, including seed maturation and germination, seedling growth and flowering and plays a vital role in plant adaptation to environment challenges, such as drought, salt and cold stress.[1-5] Ca^{2+} is an important second message involved in ABA signal transduction.[6] The Ca^{2+} sensors identified in plants include calmodulin (CaM) and CaM-related proteins, calcineurin B-like (CBL) proteins and Ca^{2+}-dependent protein kinases (CDPKs).[3,7,8] CDPKs have both kinase and CaM-like domain and are among the best characterized calcium sensors in plants.[9] There are 34 genes predicted to encode CDPKs in Arabidopsis.[9] In recent years, researchers used genetic approaches to identify a set of CDPK members as ABA signaling components, which regulate plant response to abiotic stresses. CPK32 interacts with and phosphorylates an ABA-responsive transcription factor ABF4, and the *CPK32*-overexpression plants show hypersensitive phenotype to ABA and NaCl, indicating that CPK32 is positively involved in ABA signaling.[10] The loss-of-function mutant of the *CPK23* gene shows more tolerant to drought and salt stresses, while the *CPK23*-overexpression lines are hypersensitive to these stresses.[11] CPK23, together with CPK21, is involved in the process of ABA-regulated stomata movement by regulating anion channel SLAC1.[12] When plants face drought stress, CPK23 and CPK21 phosphorylate and activate SLAC1, which leads to depolarization of the membrane and activation of the K^+ release channel GORK, resulting in anion and K^+ release from the guard cells.[13] This continuous process finally induces stomata closure.[12] Recent report showed that ABA receptor RCAR1-ABI1 coupled signaling regulates CPK21 to control anion channel SLAC1 and SLAH3.[13] Two other CDPK members, CPK3 and CPK6, were also shown to be involved in guard cell signaling in response to ABA. ABA and Ca^{2+} induce stomatal closure and stimulate the activity of slow-type anion channels, but the two effects are inhibited in *CPK3* and *CPK6* single or double mutants, by which mechanism CPK3 and CPK6 are positively involved in ABA-regulated stomatal movement.[14] Recently, CPK10 was shown to be a positive regulator of plant response to drought, which was evidenced by the findings that the loss-of-function mutant of the *CPK10* gene is hypersensitive to drought stress, while the *CPK10*-overexpression plants show enhanced tolerance to drought.[15] CPK10 interacts with a heat shock protein HSP1. The *HSP1* T-DNA insertion mutant *hsp1* shows similar phenotype with *cpk10* mutant under drought stress, suggesting that HSP1 functions directly downstream of CPK10.[15] We previously observed that the kinase activity of two homologous CDPKs in Arabidopsis, CPK4 and CPK11, is stimulated by ABA.[16] Loss-of-function mutants of *CPK4* and *CPK11*

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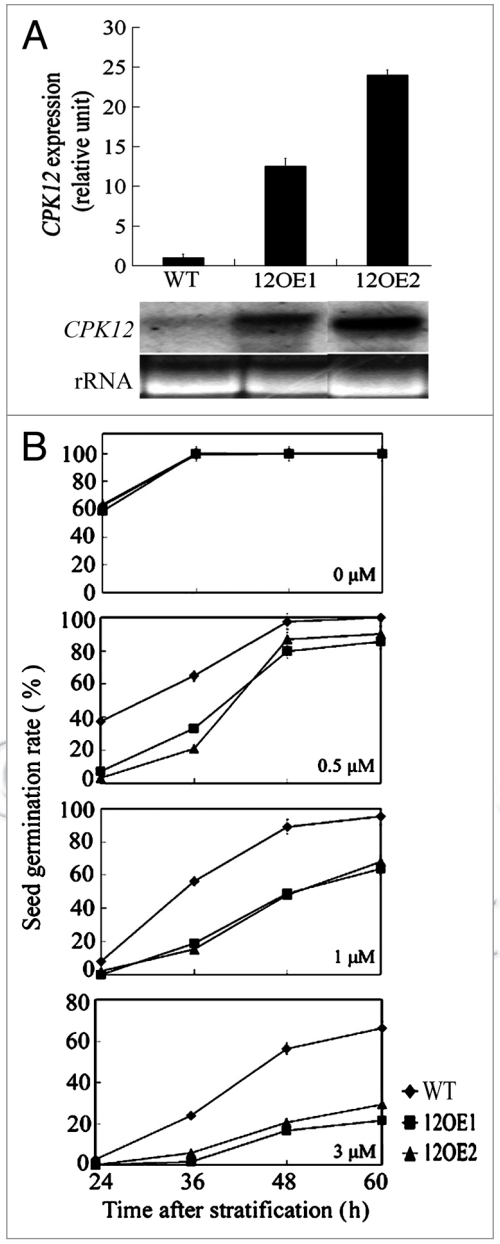

Figure 1. Overexpression of *CPK12* gene enhances the sensitivity of seed germination to ABA. (A) The mRNA amounts estimated by both real-time PCR (top columns; relative units, normalized relative to the mRNA level of the wild-type Col-5) and RNA gel blot (bottom, note that the RNA gel blot was performed in the same gel and the lane for 12OE2 was cut to be combined with two other lanes), in wild-type Col-5 (WT) and two different transgenic *CPK12* overexpression lines (indicated by 12OE1 and 12OE2). (B) Seed germination rates of the two *CPK12*-overexpression lines (12OE1 and 12OE2) and wild-type Col-5 plants in the MS media supplemented with different concentrations of (±)-ABA (0, 0.5, 1 and 3 μM). Germination was scored 24 h, 36 h, 48 h and 60 h after stratification. Values are the means ± SE from three independent experiments.

show ABA-insensitive phenotypes in seed germination, seedling growth and stomatal movement, and also exhibit salt insensitivity in seed germination and decreased tolerance of seedlings to salt stress, while *CPK4*- or *CPK11*-overexpressing plants show ABA hypersensitive phenotypes.[16] Both CPK4 and CPK11 phosphorylate two ABA-responsive transcription factors, ABF1 and ABF4, suggesting that the two kinases may regulate ABA signaling through these transcription factors.[16] These data show that CPK4 and CPK11 are two important positive regulators in CDPK/Ca^{2+}-mediated ABA signaling pathways. All these significant progresses deepen our understanding of the mechanisms of Ca^{2+} signaling in ABA signaling pathways.

CPK12 may Function as an Antagonist to Positive Roles of Other CDPK Members in ABA Signaling

In a cell signaling pathway involving reversible protein phosphorylation, protein kinase-mediated signaling process by phosphorylation is believed to be terminated or balanced by a de-phosphorylation event that is mediated by a protein phosphatase. Whereas many members of CDPK family have been identified as positive players in ABA signaling, our knowledge about protein phosphatases that antagonize CDPKs in ABA signaling has been limited. Interestingly, we observed that CPK12, a homolog of CPK4 and CPK11, functions as a negative regulator of ABA signaling,[17] suggesting that one CDPK member-triggered ABA signaling event may be balanced by another CDPK member, and that different members of CDPK family may constitute thus a regulation loop by functioning positively and negatively in ABA signal transduction. This mechanism may provide cells with diversity of tactics to balance or terminate ABA signaling when stressful conditions are well passed. Also, a CDPK-signaling balancer with another CDPK member may be useful in cell signaling in response to Ca^{2+}, as a CDPK-mediated process may be terminated by a Ca^{2+} sensor in the presence of Ca^{2+} without Ca^{2+} signal receding.

Both Up- and Downregulation of *CPK12* Expression Result in ABA Hypersensitivity

We previously observed that the *CPK12*-RNAi mutants were hypersensitive to ABA in seed germination and post germination seedling growth.[17] To investigate whether upregulation of *CPK12* gene alters ABA signaling, we generated *CPK12*-overexpression lines under the control of the cauliflower mosaic virus (CaMV) 35S promoter (**Fig. 1A**). The seeds of the *CPK12*-overexpressors germinated normally as the wild-type seeds did in the ABA-free medium (0 μM ABA), but in the media supplemented with different concentrations of (±)-ABA, their germination rate was significantly more inhibited by ABA than that of the wild-type seeds (**Fig. 1B**). In seedling growth, there was no difference between *CPK12*-overexpression plants and wild-type seedlings on ABA-free medium (**Fig. 2**; 0 μM ABA). However, on the medium containing 0.3 μM and 0.5 μM (±)-ABA, the growth of the *CPK12*-overexpression seedlings was more inhibited than that of the wild-type seedlings (**Fig. 2**; 0.3 μM and 0.5 μM ABA). We screened more than ten *CPK12*-overexpression lines, and all the lines showed ABA hypersensitivity in seed germination and post-germination growth. Thus, the *CPK12*-overexpressors showed unexpectedly

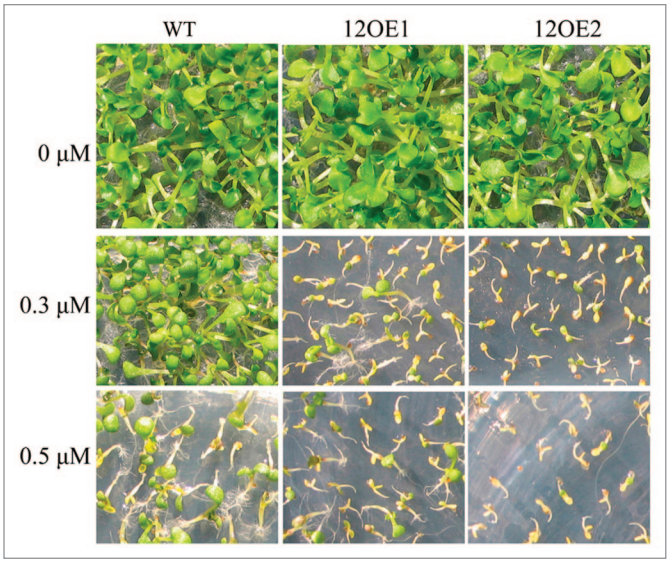

Figure 2. Overexpression of *CPK12* gene enhances the sensitivity of post-germination growth to ABA. Seeds of the two *CPK12*-overexpression lines (12OE1 and 12OE2) and wild-type Col-5 plants were directly planted in the ABA-free (0 μM) medium and the media containing 0.3 μM or 0.5 μM (±)-ABA, and the post-germination growth was investigated 15 d after stratification. The experiments were repeated five times with the same results.

the same phenotypes as those of *CPK12*-RNAi lines.[17] These findings indicate a high complexity of the CPK12-mediated ABA signal transduction.

How does CPK12 Work as a Balancer in ABA Signaling?

CPK12 phosphorylates two ABA responsive transcription factor ABF1 and ABF4, and interacts with, phosphorylates, and stimulates a type 2C protein phosphatase ABI2.[17] ABF1 and ABF4 are members of ABA-responsive, basic leucine zipper transcription factors positively involved in ABA signaling transduction,[18,19] but ABI2 is an important member of the clade A type 2C protein phosphatases negatively involved in ABA signaling.[20,21] CPK12 may use these important downstream targets that function distinctly in ABA signaling. The CPK12-induced stimulation of phosphatase activity of ABI2, which inhibits downstream positive ABA-signaling regulators SnRK2s,[21] may be abolished when CPK12 is downregulated, while the CPK12-induced inhibition of ABF1 and ABF4 transcription factors may be relieved when CPK12 is upregulated. Consequently, ABA signaling may be improved in both cases, which may partly explain the ABA hypersensitive phenotypes with both the *CPK12* overexpressors (**Figs. 1 and 2**) and RNAi lines.[17] However, whether and how CPK12 can function to selectively act on its targets needs further studies in the future. In addition, signaling components upstream of CPK12 and other identified ABA signaling CDPK members remain to be identified in the future, though several functional substrates have been identified. Identification of additional CDPK members and their downstream substrates and elucidation of the mechanisms underlying these CDPKs in ABA signaling pathways will greatly improve our understanding of plant signaling in response to environmental challenges.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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