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SHORT COMMUNICATION

Protein kinase CK2α subunits constitutively activate ABA signaling in *Arabidopsis*

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ABSTRACT

Protein kinase CK2 (formerly known as casein kinase II), a Ser/Thr protein kinase highly conserved in eukaryotes, is essential for cell survival by regulating a wide range of plant growth, development, and stress responses. A growing body of evidence has shown a link between CK2 and abscisic acid (ABA) signaling in response to abiotic stress. However, the roles of CK2 subunits in ABA signaling remain unclear in plants. Our recent work in *Arabidopsis thaliana* has revealed that CK2α and CK2β subunits inversely modulate ABA signal output. Here, we examine the roles of CK2αs, by assessing how CK2αs affect ABA signaling. Together with the previous findings, our mutant and transient expression analyses demonstrate that CK2αs positively modulate ABA signaling through the core ABA signaling pathway in the presence of ABA, though the positive effect of CK2αs are much smaller than that of core ABA signaling components in ABA response. In addtion, our current and previous findings also suggest that CK2αs play a role in maintaining constitutively active ABA signaling even in the absence of ABA independently of the core ABA signaling pathway. Thus, we found that CK2αs constitutively activate ABA signaling in the presence or absence of ABA in a different manner in *Arabidopsis* plants.

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Plants have evolved a wide spectrum of elaborate mechanisms to respond to changes in environment. The phytohormone abscisic acid (ABA) fulfills pivotal roles in plant development and stress responses.[1] Cellular dehydration during seed maturation or under stress conditions upregulates endogenous ABA levels, which activate many ABA-responsive genes with G-box-like *cis*-acting ABA-responsive elements (ABREs, PyACGTGG/TC) in their promoter regions.[1,2] In *Arabidopsis*, binding of the PYR/PYL/RCAR receptor proteins to ABA triggers the formation of ternary complexes with group-A protein phosphatase 2Cs (PP2Cs) and derepresses the subclass III SnRK2 protein kinases such as SRK2D/SnRK2.2, SRK2E/OST1/SnRK2.6, SRK2I/SnRK2.3.[3–7] Then, the SnRK2s phosphorylate basic leucine zipper (bZIP) transcription factors such as ABRE-binding protein/ABRE-binding factors (AREB/ABFs), which then activate the expression of ABA-responsive genes.[8–13] ABRE-dependent gene expression plays a major role in ABA-responsive gene expression as ABA signal output under dehydration stress.[14,15] A growing body of evidence suggests that protein kinase CK2, formerly known as casein kinase II, is involved in ABA signaling in response to abiotic stress in plants.[16,17]

CK2 is a Ser/Thr protein kinase highly conserved in eukaryotes, and is essential for cell survival. CK2 can function as a tetrameric holoenzyme comprised of two catalytic α and two regulatory β subunits or as each subunit with independent function.[18–20] The *Arabidopsis* genome encodes four α and four β subunits (CK2α1-4; CK2β1-4). CK2 regulates diverse aspects of plant growth, development, and abiotic stress responses.[16,17] Recently, we have demonstrated that CK2α and β subunits inversely modulate ABRE-dependent gene expression as ABA signal output in ABA signaling in *Arabidopsis* cells.[21] Our results indicate that CK2α1 and CK2α2 positively modulate ABRE-dependent gene expression, whereas CK2β1 negatively modulates ABRE-dependent gene expression mediated by AREB-SnRK2 pathway and by CK2αs.[21] However, recent biochemical analyses suggest that CK2 negatively regulates ABA signaling through facilitating both SnRK2 degradation and SnRK2 interaction with PP2C.[22] It thus remains unclear how CK2αs are involved in the positive modulation of ABA signal output. To explore this question, we used *RD29B* reporter plasmid,[23] which has been widely used as a representative reporter construct with ABA-responsive promoter to dissect ABA signaling in landmark studies.[8–10,24] We conducted transient expression analyses in *Arabidopsis* leaf mesophyll protoplasts using a β-glucuronidase (*GUS*) reporter gene under the control of ABRE *cis*-elements (*RD29B-GUS*) originated from the ABA-responsive RD29B promoter.[21] Transfection of CK2α1 or AREB1 induced a similar level of *RD29B-GUS* expression in the protoplasts without ABA treatment compared with the vector control (Figure 1). By contrast, the levels of *RD29B-GUS* expression induced by transfection of AREB1 were more than 7-fold higher than those of CK2α1 with ABA treatment (Figure 1). These

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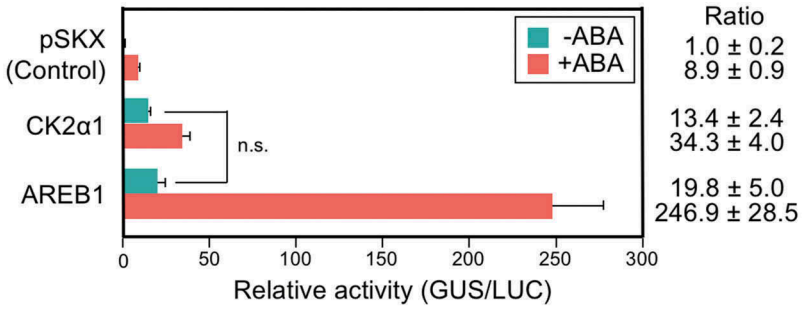

Figure 1. CK2α1 and AREB1 positively modulate ABRE-dependent gene expression in *Arabidopsis* leaf mesophyll protoplasts. Protoplasts were isolated from WT leaves. *RD29B-GUS* (5.0 μg per transfection) and *pBI35SΩ-ELUC* (1.0 μg per transfection) were used as the ABA-responsive reporter and internal control, respectively. The *pBI35S-AREB1* and *pSKX-CK2α1* plasmids were used as the effectors (2.5 μg per transfection), and *pSKX* was used for vector control. Values are shown as relative activity versus that of the control. After transfection, protoplasts were incubated for 14–18 h under dark conditions without ABA (blue bars) or with 2.0 μM ABA (orange bars). Bars indicate SD (n = 4). Experiments were performed three times, and a representative result is shown. n.s., not significant difference. More detailed methods were described in ref. 21.

data indicate that the effects of the addition of CK2α1 and AREB1 were similar in ABRE-dependent gene expression in the absence of exogenous ABA, whereas the effect of the addition of CK2α1 is much smaller than that of AREB1 in ABRE-dependent gene expression in the presence of exogenous ABA. Thus, we found that the positive effect of CK2α1 is much smaller than that of core ABA signaling components in ABRE-dependent gene expression in the presence of exogenous ABA.

Four CK2α subunits in *Arabidopsis* function in a redundant manner to modulate ABA responses and other various developmental pathways.[25–27] In addition, *CK2α3* (At2g23080) is located adjacent to *CK2α4* (At2g23070) on the chromosome.[21] To analyze the role of CK2α *in planta*, we therefore generated CK2α4-RNAi plants in the *ck2α1 ck2α2 ck2α3* triple mutant background *(ck2α1/2/3-4i)*. Reverse transcription-PCR (RT-PCR) analysis of the *ck2α1/2/3-4i* plants confirmed that the expression of *CK2α1, CK2α2*, and *CK2α3* was completely interrupted by the T-DNA insertions and that *CK2α4* expression was slightly but significantly reduced compared with the WT plants (Figure 2A). A previous report indicated that under normal growth conditions, *ck2α1/2/3-4i* mutant plants display delayed flowering and decreased number of lateral roots in comparison with the wild type plants.[27] We compared the ABA sensitivity of *ck2α1/2/3-4i* mutant plants (Figure 2A) with that of *srk2d/e/i* (i.e. *snrk2.2/2.3/2.6*) triple mutant plants, which harbor triple knockout mutations of subclass III SnRK2s.[11,28] *ck2α1/2/3-4i* mutant plants display reduced sensitivity to ABA during germination compared with WT plants (Figure 2B and C) as reported previously,[27] indicating that CK2αs are positive modulators of ABA signaling during germination. Interestingly, compared between *ck2α1/2/3-4i* mutant and *srk2d/e/i* triple mutant plants, we found that the effect of CK2αs on ABA insensitivity during germination is much smaller than that of the subclass III SnRK2s (Figure 2B and C). These findings support the hypothesis that the positive effect of CK2αs are much smaller than that of core ABA signaling components in ABA responses in the presence of exogenous ABA. Collectively, together with the previous findings that CK2β1 suppressed CK2α-mediated ABRE-dependent gene expression[21] and that CK2αs positively modulate ABRE-dependent gene expression dependently of the core ABA signaling pathway in the presence of exogenous ABA,[21] these data suggest that CK2αs may be implicated in CK2-meidated fine-tuning ABA signaing through the core ABA signaling pathway under stress conditions.

Our transient expression analysis showed that the levels of *RD29B-GUS* expression induced by transfection of CK2α1 or AREB1 were more than 10-fold higher than those of the vector control without ABA treatment (Figure 1), indicating that unlike in the presence of exogenous ABA, CK2α1 as well as AREB1 activates ABRE-dependent gene expression in the absence of exogenous ABA. In fact, the expression levels of ABA-responsive marker genes were significantly reduced in *ck2α1/2/3-4i* mutant plants compared with WT plants under non-stressed conditions (Figure 2D), suggesting that CK2αs positively modulate ABA-responsive gene expression in the absence of exogenous ABA. Our previous data indicate that CK2αs activate ABRE-dependent gene expression independently of the core ABA signaling pathway in the absence of exogenous ABA.[21] In addition, our transient expression analyses in *areb1/2abf3* and *srk2d/e/i* mutant plants suggest that putative ABRE-binding transcription factors other than AREB/ABFs such as AREB1/ABF2, AREB2/ABF4, and ABF3, may regulate ABRE-dependent gene expression downstream of CK2α1 in the absence of exogenous ABA.[21] These collective findings support the view that CK2αs may be involved in constitutively maintaining ABA signaling even in the absence of exogenous ABA independently of the core ABA signaling pathway through putative ABRE-binding transcription factors other than the AREB/ABFs. Further research on the CK2-interacting transcription factors is required to reveal CK2-meidated signaling networks in ABA signaling in plants. Thus, our analyses define the role of CK2α in ABA signal output in the presence or absence of exogenous ABA. In summary, our data demonstrate that CK2α subunits constitutively activate ABA signaling in the presence or absence of ABA in a different manner in *Arabidopsis* plants.

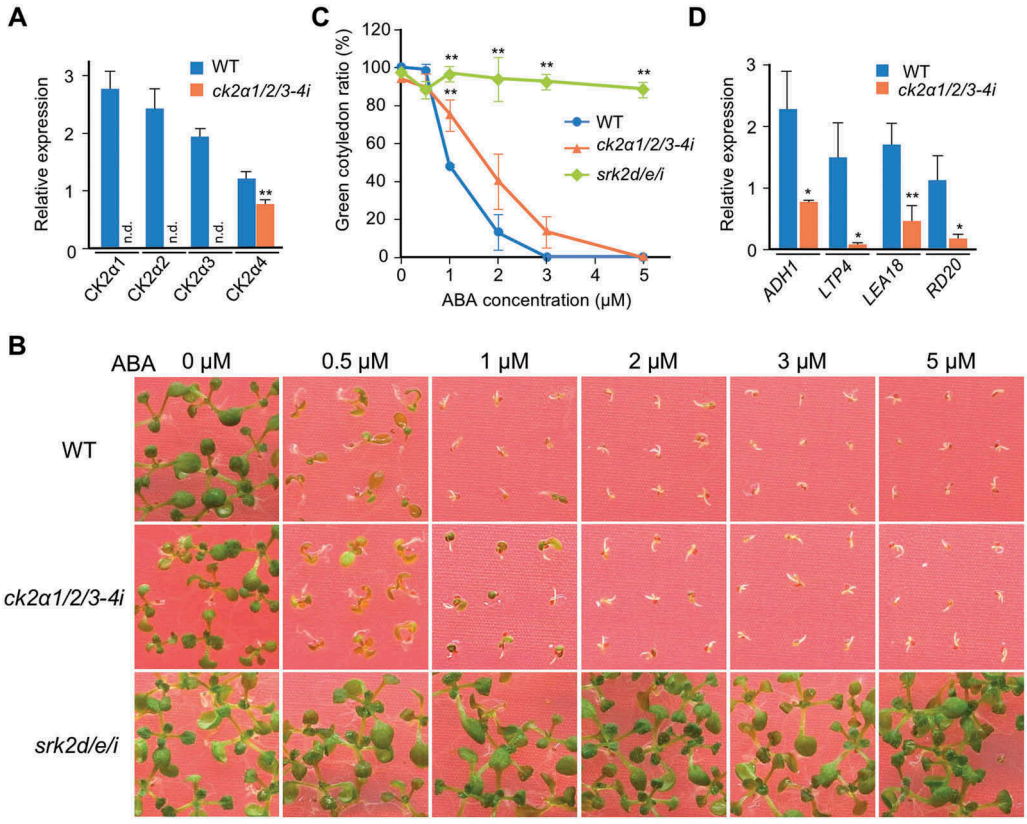

Figure 2. CK2αs positively modulate ABA signaling during seed germination and ABA-responsive marker genes even under non-stressed conditions. (A) relative expression levels of each *CK2α* gene in the WT and *CK2α4-RNAi in ck2a1/2/3* mutant (*ck2a1/2/3-4i*). The *ck2a1/2/3-4i* plant was generated by transformation of CK2α4-RNAi (N225788) construct, obtained from the Nottingham *Arabidopsis* stock centre (NASC), into *ck2a1 ck2a2 ck2a3* triple mutant plants (SALK_073328, SALK_129331, SALK_15200; obtained from the NASC) by *Agrobacterium*-mediated transfection. Total RNA was extracted from 8-day-old seedlings on GM agar plates[8] under constant light (40 ± 10 mol photons/m^2/s). *PP2Aa3* (*AT1g13320*) was used as an internal control. Data represent means and SDs of three biological replicates. n.d., not detected. RNA extraction and qRT PCR analysis were conducted as described in previously.[29] (B) *ck2a1/2/3-4i* mutant seedlings display enhanced tolerance to ABA-dependent inhibition of germination. Seedlings of *ck2a1/2/3-4i* mutant, *srk2d/e/i* triple mutant, and WT (CS60000) were grown on GM agar plates containing 0, 0.5, 1, 2, 3, and 5 μM ABA with 1% sucrose at 8 days after stratification under constant light. (C) ABA dose-response of cotyledon greening. Seedlings with green cotyledons were counted at 8 days after stratification under constant light with different ABA concentrations. Experiments were performed in triplicate on independent plates ($n = 25$ each). Bars indicate SD. $^{**}p < 0.01$, One way ANOVA test. (D) The expression levels of ABA-responsive marker genes were significantly reduced in *ck2a1/2/3-4i* mutant under non-stress conditions. Eight-day-old seedlings grown on the GM agar plates were used for the analysis under constant light conditions. *PP2Aa3* was used as an internal control. Data represent means and SDs of three biological replicates. $^{*}p < 0.05$, $^{**}p < 0.01$, Student's *t*-test.

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Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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