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SHORT COMMUNICATION

Histone demethylases control root elongation in response to stress-signaling hormone abscisic acid

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ABSTRACT

Abscisic acid (ABA) plays critical roles during plant growth and development in response to various stresses. *Arabidopsis thaliana* histone demethylases JUMONJI-C DOMAIN-CONTAINING PROTEIN 30 (JMJ30) and JMJ32 control ABA-mediated growth arrest during the post-germination stage (2–3 days after germination). However, the roles of JMJ30 and JMJ32 in ABA responses at later stages of plant development remain largely unknown. Here, we show that JMJ30 and JMJ32 mediate ABA responses during root development. In the presence of ABA, *jmj30 jmj32* double mutants display longer primary roots than the wild type. Loss-of-function mutation in the *SNF1-RELATED PROTEIN KINASE 2.8* (*SnRK2.8*) gene also led to a longer primary root phenotype in response to ABA. Analysis of *JMJ30/JMJ32* and *SnRK2.8* expression suggested that they act in the same pathway to mediate ABA responses during root elongation at the seedling stage. Our findings highlight the importance of the JMJ30/JMJ32-SnRK2.8 module at two different developmental stages.

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External stress negatively impacts growth, development, and productivity of plants.[1] One major stress is categorized as abiotic or environmental stress, such as unfavorable atmosphere, chemical elements, sunlight/temperature, wind and water. Because plants are sessile organisms, they have developed various mechanisms to protect themselves against stresses. In the last two decades, much research has focused on understanding plant molecular frameworks toward improving crop yield even under stress conditions.[2-4]

Abscisic acid (ABA) is a key stress-signaling hormone.[5,6] In response to stress such as water deficit and high salt, not only the amount of ABA, but also ABA perception and response are modulated. Osmotic stress caused by drought and high salt triggers ABA biosynthesis, and the resulting ABA accumulates in the cytosol and binds to the ABA receptors PYRABACTIN RESISTANCE1 (PYR1)/PYR1-LIKE(PYL)/REGULATORY COMPONENTS OF ABA RECEPTORS (RCAR).[7,8] The activated ABA receptors bind to type 2C protein phosphatases (PP2Cs) like ABSCISIC ACID-INSENSITIVE1 (ABI1) or ABI2, inhibiting the catalytic activity of PP2C.[9] SNF1-RELATED PROTEIN KINASE2 (SnRK2) kinases are then released from PP2C-mediated inactivation and trigger gene expression through phosphorylation.[10-12] After reaching a certain threshold of ABA concentration or signaling, stomata are closed and gene expression is changed through *cis*-acting ABA-responsive elements (ABREs).[13] ABRE and a group of ABRE-binding transcription factors have pivotal roles in ABA-dependent gene expression. Although ABA-dependent gene induction is well characterized, how it is controlled at the levels of histone modification remains unclear.

Histones function both positively and negatively in the regulation of gene expression.[14] The N-terminal tail of histone H3 is modified post-translationally through acetylation, phosphorylation, methylation and ubiquitination.[15] Histone modification enzyme complexes catalize reversible lysine methylation central to epigenetic regulation by specifying when, where and which histone residues need to be modified. Despite their importance, the role of histone modification enzymes in ABA responses is not well characterized.

We recently reported that the histone demethylases JUMONJI-C DOMAIN-CONTAINING PROTEIN 30 (JMJ30) and JMJ32 control ABA-mediated growth arrest during the post-germination stage.[16] Under unfavorable environmental conditions, the B3 domain transcription factor ABSCISIC ACID INSENSITIVE3 (ABI3) is activated by ABA.[17] ABA-activated ABI3 promotes expression of *JMJ30*, presumably by direct binding via the evolutionally conserved RY motif.[16] JMJ30 and JMJ32 then remove repressive H3K27me3 marks at the *SnRK2.8* locus to activate its expression.[16] The upregulated *SnRK2.8* promotes ABA-dependent gene expression, which feeds forward to ABI3 activation.[16]

A comprehensive expression study of JMJ genes in response to stress revealed that JMJ30 is upregulated by ABA during the vegetative stage in *Arabidopsis thaliana*.[18] However, the function of JMJ30 and JMJ32 in the ABA response during the vegetative stage remains unknown. To understand their roles, we performed phenotypic analysis using *jmj30 jmj32* double mutants in the absence and presence of ABA at the vegetative stage (Figure 1A–D). Three-day-old wild-type and *jmj30-2 jmj32-1* double mutant seedlings were transferred to half-strength MS plates with or without ABA. When grown and

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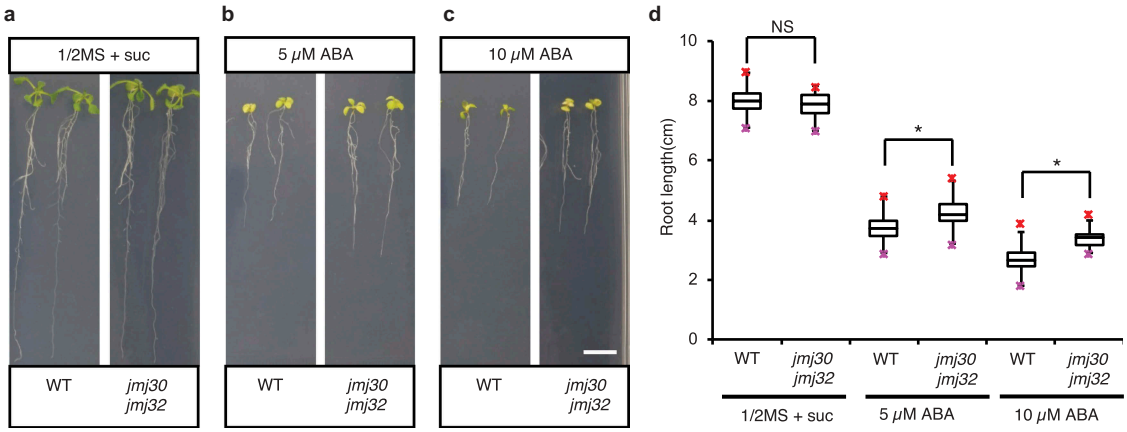

Figure 1. Root elongation in *jmj30 jmj32* double mutants is less sensitive to ABA. (A–C) Representative images of wild-type (WT) and *jmj30-2 jmj32-1* plants in the absence and presence of ABA. Wild-type and *jmj30-2 jmj32-1* seeds were sown on half-strength MS with 1% sucrose and stratified at 4°C for 3 days. Plants were grown under 24 h of light for 3 days and then transplanted onto half-strength MS plates with 1% sucrose supplemented with 0 μM ABA (A), 5 μM ABA (B), or 10 μM ABA (C) and grown vertically under 24 h of light for an additional 7 days. Bar = 1 cm. (D) Quantification of root length in wild-type and *jmj30-2 jmj32-1* plants shown in (A–C). Asterisks indicate significant differences based on two-tailed Student's *t*-test; $p < .01$; NS, nonsignificant. Values represent mean ± SD of 24 plants.

maintained on half-strength MS plates without ABA, wild-type and *jmj30-2 jmj32-1* plants showed no obvious phenotypic differences (Figure 1A); both displayed leaves of normal size and color and well-grown primary roots with many lateral roots (Figure 1A). No significant difference in primary root length was observed between the wild type and *jmj30-2 jmj32-1* without ABA ($p > .05$ by two-tailed Student's *t*-test) (Figure 1D). ABA-treated plants of both genotypes had smaller and paler leaves and shorter roots compared with control plants (Figure 1A–C). In the presence of 5 μM ABA, primary root length in the wild type was 3.7 ± 0.1 cm while roots of *jmj30-2 jmj32-1* plants were significantly longer at 4.2 ± 0.1 cm ($p < .01$ by two-tailed Student's *t*-test). Root elongation was inhibited more in the presence of 10 μM ABA than 5 μM ABA (Figure 1B, C); however, there were still significant differences in root length between the wild type and *jmj30-2 jmj32-1* ($p < .01$ by two-tailed Student's *t*-test) (Figure 1D). These results suggest that JMJ30 and JMJ32 are required for ABA-dependent inhibition of root growth during the vegetative stage.

To understand the role of SnRK2.8 in ABA-mediated root elongation at the vegetative stage, we conducted phenotypic analyses of wild-type and *snrk2.8–1* plants. There was no significant difference in phenotype between wild-type and *snrk2.8–1* plants when grown and maintained on half-strength MS plates without ABA ($p > .05$ by two-tailed Student's *t*-test) (Figure 2A, C). When transferred onto 10 μM ABA plates, root growth was inhibited in both the wild type and *snrk2.8–1* (Figure 2B). However, the *snrk2.8–1* mutant was less sensitive to ABA, similar to *jmj30-2 jmj32-1* double mutants ($p < .01$ by two-tailed Student's *t*-test) (Figure 2C). These results suggest that SnRK2.8 is required for ABA-dependent inhibition of root growth during the vegetative stage.

To examine the relationship between JMJ30/JMJ32 and SnRK2.8 in response to ABA during the vegetative stage, we

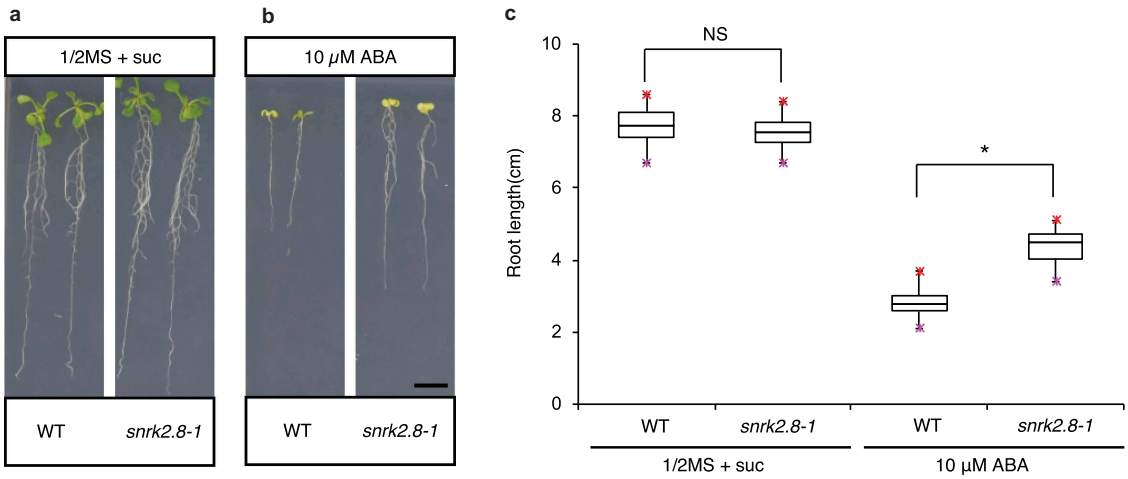

Figure 2. Root elongation in *snrk2.8* mutants is less sensitive to ABA. (A, B) Representative images of wild-type (WT) and *snrk2.8–1* plants in the absence and presence of ABA. Wild-type and *snrk2.8–1* seeds were sown on half-strength MS with 1% sucrose and stratified at 4°C for 3 days. Plants were grown under 24 h of light for 3 days and then transplanted onto half-strength MS plates with 1% sucrose supplemented with 0 μM ABA (A) or 10 μM ABA (B) and grown vertically under 24 h of light for an additional 7 days. Bar = 1 cm. (C) Quantification of root length in wild-type and *snrk2.8–1* plants shown in (A, B). Asterisk indicates significant difference based on two-tailed Student's *t*-test; $p < .01$; NS, non-significant. Values represent mean ± SD of 24 plants.

conducted reverse-transcription quantitative polymerase chain reaction (RT-qPCR) analysis (Figure 3A–D). *JMJ30* is upregulated during the postgermination stage by ABI3 in response to ABA.[16] To understand *JMJ30* and *JMJ32* expression in response to ABA during the vegetative stage, we first examined *JMJ30* and *JMJ32* expression levels (Figure 3A, B). Consistent with previous observations, *JMJ30* was upregulated in response to ABA ($p < .01$ by two-tailed Student's *t*-test) (Figure 3A). Similar to the postgermination stage, upregulation of *JMJ32* expression was not observed (Figure 3B). To further confirm whether *JMJ30* upregulation in response to ABA is dependent on ABI3 function, we examined *ABI3* expression at the vegetative stage. Although we observed a significant difference in ABI5 expression in response to ABA, there was no difference in expression of the *ABI3* gene (Figure 3C, D). These data suggest that JMJ30 is upregulated by a factor other than ABI3 in vegetative stage, unlike in post-germination stage.

We next addressed the expression levels of *SnRK2.8* (Figure 3E). ABA-treated wild-type plants had more *SnRK2.8* transcripts than mock-treated wild-type plants ($p < .01$ by one-way ANOVA test) (WT with ABA vs. WT without ABA: $p < .01$ by post-hoc Tukey's HSD) (Figure 3E). In addition, *SnRK2.8* was not upregulated in the *jmj30-2 jmj32-1* background with or without ABA treatment (*jmj30-2 jmj32-1* with ABA vs. *jmj30-2 jmj32-1* without ABA: $p > .05$ by post-hoc Tukey's HSD) (Figure 3E). This result implies that *SnRK2.8* expression is controlled by JMJ30 in response to ABA during the vegetative phase.

We previously showed that the function of the JMJ30/JMJ32-SnRK2.8 module is dependent on the ABA-dependent transcription factor ABI3 during the postgermination stage. Here, we demonstrated the role of the JMJ30/JMJ32-SnRK2.8 module in response to ABA during root elongation at the vegetative stage. Although the function of the JMJ30/JMJ32-SnRK2.8 module in response to ABA is well conserved between the two different developmental stages, the upstream regulators are different. Thus, we conclude that an unknown factor(s) – X(s) – activates JMJ30 in response to ABA during root elongation at the vegetative stage. It will be interesting to identify such a factor in the future.

Materials and methods

Plant materials and growth conditions

All *Arabidopsis thaliana* lines used in this study were in the Columbia (Col-0) background. The *jmj30-2 jmj32-1* mutant was described previously.[19] The *snrk2.8-1* (SALK_073395) mutant was obtained from the Arabidopsis Biological Resource Center (ABRC). Prior to growth, genotypes were confirmed by PCR using Emerald Amp polymerase (Takara). Primers for genotyping were as follows: jmj30-2 genotyping-FW, CAAACTCTGCTGCAATCGATTTC; jmj30-2 genotyping-RV, GAAAATGTCACAAGCTCTTGCTTC; jmj32-1 genotyping-FW, GACTGAGAAAACCTGAACTCAGC; jmj32-1 genotyping-RV, GTCGTGTAAAGGACTGAAGGTTG; snrk2.8-1 genotyping-FW, CAAACCATGACACATCAGCAC; snrk2.8-1 genotyping-RV, AGGCTCCTGTTAATCACCAGG. All plants were grown at 22°C in a growth chamber under continuous light conditions after stratification at 4°C for 3 days.

Phenotypic and statistical analyses

Procedures for preparation of half-strength MS plates and seed surface sterilization were described previously.[16] For root elongation assays, sterilized wild-type, *jmj30-2 jmj32-1*, and *snrk2.8-1* seeds were placed on half-strength MS plates, stratified at 4°C for 3 days, and then placed in a growth chamber at 22°C under continuous light for 3 days. Three-day-old plants were transplanted onto half-strength MS plates with 1% sucrose supplemented with 0, 5, or 10 μM ABA and grown vertically under 24 h of light for an additional 7 days. Primary root length was measured, and statistical analyses were conducted using Microsoft Excel. Statistical significance was computed using a two-tailed Student's *t*-test.

Expression analysis

For ABA treatment, 4-day-old stratified plants grown on half-strength MS plates with 1% sucrose were treated with 10 μM ABA to induce rapid changes in gene expression. After 3 h of

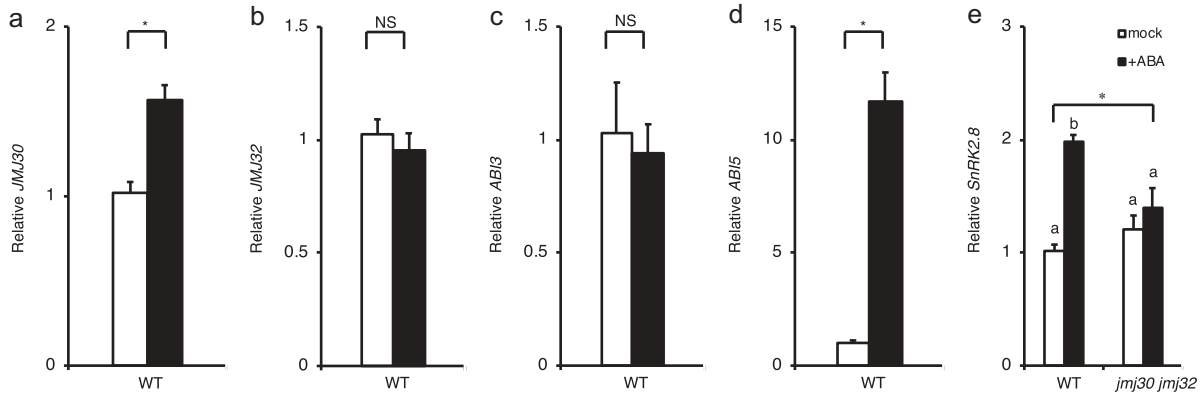

Figure 3. *JMJ30* and *SnRK2.8* expression is induced by ABA. (A–D) Expression of *JMJ30* (A), *JMJ32* (B), *ABI3* (C), and *ABI5* (D) in wild-type (WT) plants in response to 10 μM ABA. Results are from three independent experiments. Values represent mean ± SEM. Asterisks indicate significant differences based on two-tailed Student's *t*-test; $p < .01$; NS, nonsignificant. (E) Expression of *SnRK2.8* in wild-type and *jmj30-2 jmj32-1* plants in response to 10 μM ABA. Results are from three independent experiments. Values represent mean ± SEM. Asterisk indicates significant differences based on one-way ANOVA test; $p < .01$. Different letters indicate significant differences based on post-hoc Tukey's HSD test; $p < .01$.

treatment, seedlings were used for RNA extraction. RNA isolation and RT-qPCR methods followed a previously described protocol.[20] Three independent biological replicates were performed for qPCR analyses, and four technical replicates were conducted for each experiment. Statistical significance was computed using either one- way ANOVA test followed by post- hoc Tukey's HSD test or two- tailed Student's *t*-test for multiple- and single-paircomparisons, respectively. Primers for expression analyses were as follows: EIF4A1- FW, TCTTGGTGAAGCGTGATGAG; EIF4A1- AATCAACCTTACGCCTGGTG; JMJ30- FW GAATCACTTGGACTACCT CAATGC; JMJ30- RV, CATT GGAGACGATTTATT GGTCC; JMJ32- FW, GTTTCATTGTA CTGTCAAGGCTGG; JMJ32- RV, CATACTTGAT GTCAAACTGCA TGTC; ABI3- FW, ATGTATCTCC TCGAG AACAC; ABI3- RV, CCCTCGTATCAAATATTTG CC; ABI5- FW, ACCTAATCCAAACC CGAACC; ABI5- RV, TACCCTCCTCCTCCTGTCCT; SnRK2.8- FW, GTTGCCA ACCCT GAAAAGAG; SnRK2.8- RV, CCGAGCTTCTTCAA TGATCC.

Disclosure of potential conflicts of interest

No potential conflicts of interest are disclosed.

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