Sumoylation of transcription factor MYB30 by the small ubiquitin-like modifier E3 ligase SIZ1 mediates abscisic acid response in *Arabidopsis thaliana*

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The phytohormone abscisic acid (ABA) plays an essential role in plant development and during the response of the plant to abiotic stress. In this study, we report that the R2R3-type transcription factor MYB30 is involved in the regulation of ABA signaling. *Arabidopsis* mutants lacking MYB30 are hypersensitive to ABA during germination and seedling growth. A K283R substitution in MYB30 blocks its SUMO E3 ligase SIZ1-mediated sumoylation in *Arabidopsis* protoplasts, indicating that MYB30 is sumoylated by SIZ1 and that K283 is the principal site for small ubiquitin-like modifier conjugation. Expression of *MYB30*^{K283R} in *myb30* partially rescues the mutant ABA-hypersensitive phenotype, but expression of wild-type *MYB30* complements the mutant phenotype. Overexpression of *MYB30* in wild-type results in an ABA-insensitive phenotype, whereas overexpression of *MYB30* in the *siz1* mutant does not alter *siz1* hypersensitivity to ABA. The *siz1-2 myb30-2* double-mutant exhibits greater ABA sensitivity than either single mutant, but a mutation in the SIZ1-sumoylated ABI5 transcription factor suppresses the ABA hypersensitivity of *myb30-2* to wild-type levels. Our results suggest that coordination of ABI5 and MYB30 sumoylation by SIZ1 may balance gene expression, which is required for regulation of ABA signaling during seed germination.

Abscisic acid (ABA) plays important roles throughout the life cycle of the plant, including during seed development and dormancy, seed germination, early seedling development, flowering, and in response to abiotic and biotic stress. Genetic studies have revealed that a number of loci are essential for ABA biosynthesis (1–5), catabolism (6, 7), and signal transduction (8, 9) in plants.

A number of studies have focused on understanding ABA signaling from perception to response. Recently, three groups of ABA receptors (ABAR/GUN5, GRGC/GTG, and PYR/PYL/RCAR) have been identified in *Arabidopsis* and shown to bind ABA with high affinity (10–14). The various functions and cellular locations of these ABA receptors point to the complexity of ABA signaling. For example, PYR/PYL/RCAR-modulated ABA signaling has been studied extensively and found to involve a double-negative regulatory system that includes the receptor, protein phosphatases (PP2C), and protein kinases (SnRK2) (15, 16).

A group of basic leucine zipper transcription factors, including ABI5, serve as the downstream targets of SnRK2 and phosphorylation is correlated with ABI5 transcriptional activity and protein stability (17–19). ABI5 undergoes 26S proteasome-mediated degradation and ABA stabilizes the ABI5 protein (17). Two ABI5 interacting proteins, AFP (ABI5-interacting protein) and KEG (KEEP ON GOING, a RING-finger ubiquitin E3 ligase) are required for ABI5 degradation (20, 21). *Arabidopsis* lacking either gene is hypersensitive to ABA and an *abi5* mutation suppresses the ABA sensitivity of both the *afp-1* and *keg* mutants (20, 21). Recently it has been shown that ABI5 is sumoylated by the small ubiquitin-like modifier (SUMO) E3 ligase SIZ1 at amino acid K391, and this sumoylation is also required for the stability of ABI5. Consistent with these data, the

siz1 mutant is hypersensitive to ABA and this phenotype is suppressed by the *abi5* mutation (22).

Sumoylation affects protein–protein interaction, protein subcellular localization, enzymatic activity, and stability, which in turn regulate multiple biological processes, including the cell cycle, DNA repair, and transcriptional activity (23, 24). In addition, sumoylation is important for hormonal and environmental responses in plants (25, 26). The identified SIZ1 targets are mostly transcription factors, such as PHR1, ICE1, FLD, and ABI5, which function in numerous biological pathways (22, 27–30).

In this study, the *myb30-2* transcription factor was identified in a mutant screen for components of ABA signaling in *Arabidopsis*. We show that *myb30-2* is hypersensitive to ABA during germination compared with wild type or *abi5-8*, and that sumoylation of MYB30 at amino acid K283 by SIZ1 is crucial for its function in ABA signaling. In addition, we found that MYB30 and ABI5 regulate largely different sets of genes and the *abi5-8 myb30-2* double-mutant exhibits wild-type–like ABA sensitivity. Our results indicate that MYB30 and ABI5 function in parallel pathways to regulate germination and that their SIZ1-dependent sumoylation results in coordinate regulation of germination in response to ABA.

Results

Genetic Screening for *Arabidopsis* Mutants Defective in Response to ABA. To identify components of ABA signaling in *Arabidopsis*, we screened a T-DNA (*Agrobacterium*-transferred DNA) insertion pool (Col-0 background) for changes in seed germination in the presence of exogenous ABA and identified the *1303H* mutant, which was hypersensitive to ABA (Fig. 1 A–C). Insertion of the T-DNA fragment in the mutant was detected (TAIL-PCR) in exon one of At3g28910, which encodes the MYB30 transcription factor that has previously been shown to be involved in plant defense responses (31–36) (Fig. 1D). As a result, *1303H* was renamed as *myb30-2*. The phenotype of *myb30-1* (SALK_122884), an allelic mutant obtained from the *Arabidopsis* Biological Resource Center (ABRC), was similar to that of *myb30-2* (Fig. 1D and Fig. S1). The expression of *MYB30* was abolished in both *myb30-1* and *myb30-2* (Fig. 1E). Germination and primary root growth of the *myb30* mutants was hypersensitive to ABA (0.1–0.5 μM), compared with these processes in wild-type (Fig. 1 A–C and Fig. S1). Cotyledon greenness of the *myb30* mutants was also sensitive to mannitol. Because there was no significant difference
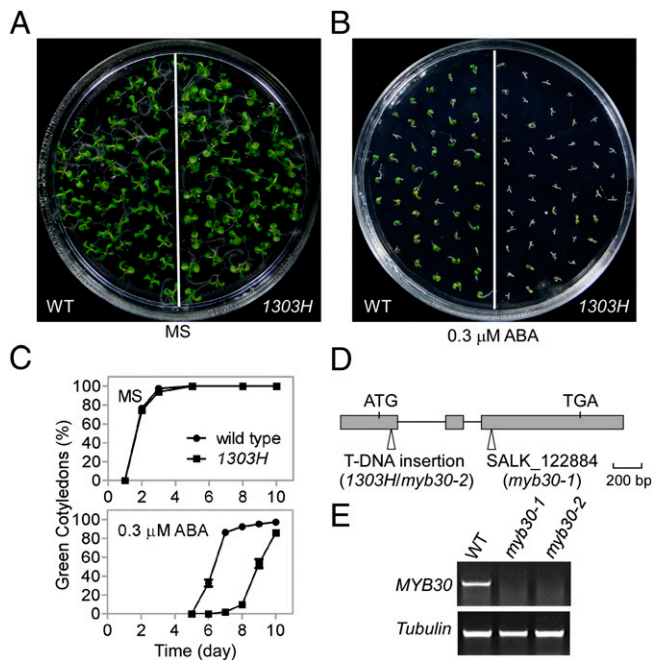
Fig. 1. *myb30* is hypersensitive to ABA. (*A* and *B*) ABA sensitivity of wild-type and the *1303H/myb30-2* mutant. Photographs were taken 8 d after sowing seeds on MS medium without (*A*) or with 0.3 μM ABA (*B*). (*C*) Germination (green cotyledons) frequencies were scored at the indicated times and represent an average of 100 seeds from three independent experiments ± SE. (*D*) The gene structure of At3g28910. Filled boxes indicate exons and the lines indicate introns. Sites of insertions in the *myb30-2* and *myb30-1* mutants are marked. (*E*) Expression of *MYB30* in wild-type, *myb30-1*, and *myb30-2* mutant seedlings was analyzed by RT-PCR with *Tubulin* included as a loading control.

in seedling growth and leaf water loss under osmotic/drought stress between the mutant and wild-type (Fig. S1), mutant sensitivity to mannitol postgermination provides additional evidence that MYB30 mainly functions in regulation of seed germination and very early seedling development.

MYB30 and ABI5 Regulate the Expression of Largely Different Sets of Genes. To determine if MYB30 functions in previously identified ABA signaling pathways, we crossed *myb30-2* to *abi1-3*, *abi2-2*, *abi3*, *abi4*, and *abi5-8* (all in Col-0) (37–41). Double-mutants of *abi1-3 myb30-2* and *abi2-2 myb30-2* exhibited enhanced ABA sensitivity relative to their single mutants, suggesting that MYB30 functions in parallel pathways with ABI1 or ABI2 during ABA signaling (Fig. S2). Double-mutants of *abi3 myb30-2* and *abi4 myb30-2* exhibited an ABA-insensitive phenotype similar to *abi3* and *abi4* single mutants (Fig. S2), suggesting that *abi3* and *abi4* are epistatic to *myb30*. However, double-mutants of *abi5-8 myb30-2* exhibited a wild-type–like phenotype during seed germination (Fig. 2*A*), suggesting that ABI5 and MYB30 may function in parallel pathways to coordinately regulate ABA signaling.

MYB30 has been shown to regulate defense and brassinosteroid (BR) responses by mediating the expression of genes involved in very long-chain fatty acid biosynthesis and the BES1 (bri1-Ethylmethane Sulphonate suppressor1) transcriptional activator in BR signaling, respectively (33, 34). To determine if MYB30 also regulates ABI5 target genes in response to ABA, expression of *EM1*, *EM6*, *RAB18*, *ADH*, and *RD29* was examined in *myb-30* mutants; no significant difference was observed between wild-type and *myb30*. Consistent with these results, the expression of *ABI5* did not change in *myb30*, and expression of *MYB30* was not affected by the *abi5* mutation (Fig. 2*B*). These

results suggest that ABI5 and MYB30 regulate different sets of genes and that ABI5 and MYB30 do not regulate each other.

The expression of *MYB30* was reduced by ABA treatment (Fig. 2*B*). ABA induces the expression of many plant genes associated with germination and dehydration responses (42). To identify ABA-responsive genes regulated by MYB30, wild-type and *myb30-2* mutant seedlings were treated with 100 μM ABA for 3 h, and ABA-responsive gene expression was examined by Solexa mRNA sequencing analysis. The expression of 120 vs. 41 genes was induced and reduced 1.5-fold in *myb30-2* compared with expression in wild-type (Table S1). Expression differences of four stress-responsive genes, *COR413*, *COR15B*, *LOX3*, and *BGL2*, were confirmed by real-time PCR (Fig. 2*B*). In the *myb30-2* mutant, the induction of *COR413* and *COR15B* was substantially lower and the induction of *LOX3* and *BGL2* was significantly higher than their expression in wild-type. The expression of *COR15B*, *LOX3*, and *BGL2* was regulated in the opposite manner by ABA in the *abi5-8* and *myb30-2* mutants (Fig. 2*B*). Consistent with the seed-germination phenotype in single and double *myb30* and *abi5* plants, the expression of *COR413*, *COR15B*, *LOX3*, and *BGL2* in response to ABA in the *abi5-8 myb30-2* double-mutant was similar to that in wild-type, suggesting that MYB30 and ABI5 may work in parallel to regulate the balance of gene expression during ABA signaling (Fig. 2*B*).

MYB30 Sumoylation at Residue K283 Is Crucial for the Response to ABA. Okada et al. (43) used SUMO E1 and E2 enzymes to reconstitute the *Arabidopsis* sumoylation cascade in *Escherichia coli* and showed that the K283 of MYB30 was the major sumoylation site (Fig. 3*A*). To determine if MYB30 is sumoylated in *Arabidopsis*, and if the target is K283, we generated
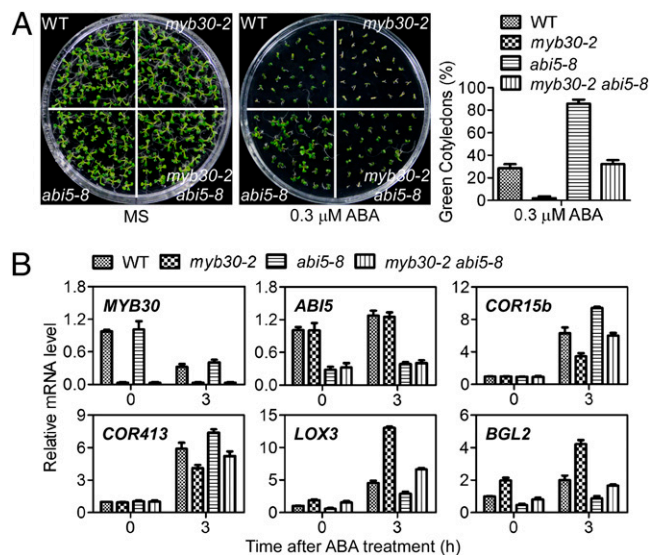

Fig. 2. MYB30 and ABI5 coordinately regulate ABA signaling. (*A*) The ABA sensitivity of *myb30-2* was suppressed by *abi5-8*. Photographs were taken 9 d after seeds were sown on MS medium without (*Left*) or with 0.3 μM ABA (*Right*). The mean germination values for wild-type and the *myb30-2*, *abi5* single, and *abi5* double-mutants in response to 0.3 μM ABA is shown in the graph to the right of the images. Green cotyledons were scored after 6 d of growth and represent an average of 100 seeds from at least three independent experiments ± SE. (*B*) Seven-day-old seedlings of wild-type, *myb30-2*, *abi5-8*, and *myb30-2 abi5-8* were treated without or with ABA (100 μM) for 3 h. Relative mRNA levels were determined by quantitative RT-PCR analysis. Transcript levels of *MYB30*, *ABI5*, *COR15b*, *COR413*, *LOX3*, and *BGL2* are illustrated. Three independent experiments were performed; data represent means ± SD.
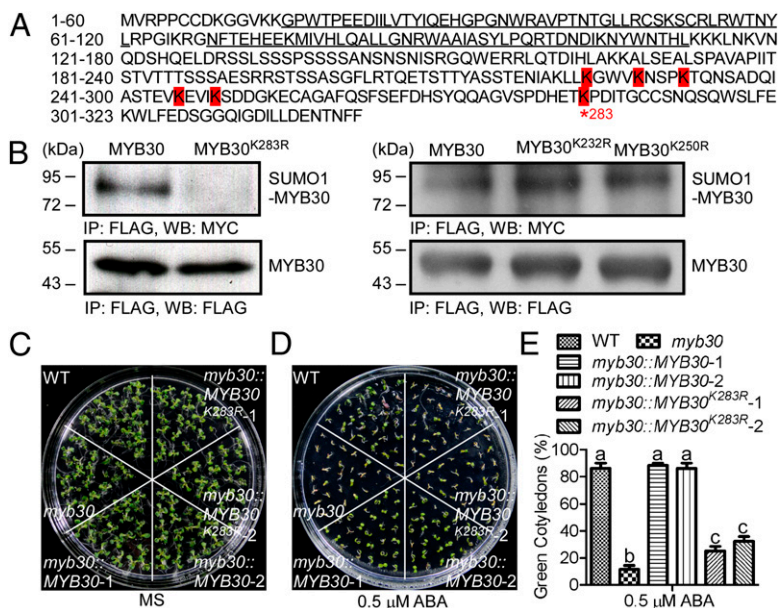
PLANT BIOLOGY

Fig. 3. MYB30 is sumoylated at residue K283. (*A*) Amino acids marked with red indicate predicted sumoylation sites in MYB30. K283, the major sumoylation site in vitro (43), is marked by an asterisk. The MYB DNA binding domain is underlined. (*B*) Amino acid K283 is the sumoylation site in vivo. Combinations of *Myc-SUMO1* and *Flag-MYB30*, *Flag-MYB30*^{K283R}, *Flag-MYB30*^{K232R}, or *Flag-MYB30*^{K250R} were coexpressed in *Arabidopsis* protoplasts. Soluble extracts from protoplasts were immunoprecipitated with anti-Flag antibody (IP: FLAG) and the immunoprecipitated products were detected with anti-Myc antibody to monitor SUMO-MYB30 conjugates (Output: MYC). Protein loading was detected with anti-Flag antibody (Input: FLAG). (*C* and *D*) MYB30^{K283} sumoylation is required for the response to ABA. Photographs were taken 10 d after seeds were sown on MS medium without (*C*) or with 0.5 μM ABA (*D*). (*E*) Germination frequencies were scored after 10 d of growth and represent an average of 100 seeds from three independent experiments ± SE. Based on a Student *t* test, genotypes with different letters are statistically different, *P* < 0.05.

35S:Myc-SUMO1, *35S:Flag-MYB30*, *35S:Flag-MYB30*^{K283R}, *35S:Flag-MYB30*^{K232R}, and *35S:Flag-MYB30*^{K250R} constructs. *Myc-SUMO1* plasmid was cotransformed with these *Flag-MYB30* plasmids individually into wild-type protoplasts. SUMO1 conjugation of MYB30 (Fig. 3*B*) was detected in protein extracts isolated from MYB30, MYB30^{K232R}, and MYB30^{K250R}, but not in MYB30^{K283R}. These results indicate that the sumoylation of MYB30 occurs in *Arabidopsis* at residue K283.

To examine if sumoylation of MYB30 is required for its function in ABA signaling, we transferred *35S:Flag-MYB30* or *35S:Flag-MYB30*^{K283R} plasmids into *myb30-2* plants. Two transgenic lines with equivalent expression of *MYB30*^{K283R} or *MYB30* (Fig. S3) were tested for ABA sensitivity. Expression of *MYB30* rescued the *myb30-2* ABA-sensitive phenotype; however, expression of an equivalent amount of *MYB30*^{K283R} only partially reduced *myb30-2* ABA sensitivity (Fig. 3 *C–E*), suggesting that sumoylation of K283 is critical for MYB30 function during ABA signaling.

SIZ1 Mediates the Sumoylation of MYB30. SIZ1 is a SUMO E3 ligase identified in *Arabidopsis* and shown to play an important role in responses to ABA through sumoylation of ABI5 (22). Our results showing that MYB30 is sumoylated in *Arabidopsis* and works in parallel with ABI5 suggested that MYB30 may also be a target for SIZ1. To test this hypothesis, *35S:Myc-SUMO1* and *35S:Flag-MYB30* or *35S:Flag-MYB30*^{K283R} were transferred into either wild-type or *siz1-2* protoplasts, and the sumoylation of MYB30 was monitored. Sumoylation was detected in wild-type but not in the *siz1-2* mutant, demonstrating that SIZ1 modulates the sumoylation of MYB30 (Fig. 4*A*).

To determine if SIZ1-dependent sumoylation of MYB30 is critical for the response of the plant to ABA, we generated the *siz1-2 myb30-2* double-mutant and expressed *Flag-MYB30* in wild-type and *siz1-2* plants. The double mutant exhibited enhanced ABA sensitivity compared with either single mutant (Fig.

4 *B* and *C* and Fig. S3). Overexpression of *MYB30* in wild-type resulted in transgenic plants that were insensitive to ABA during germination (Fig. 4*D*); however, overexpression of *MYB30* at a similar level in *siz1-2* (Fig. S3) did not affect the ABA-sensitive phenotype of the mutant (Fig. 4*D*). The ABA-sensitive phenotype of the *siz1* mutant may be because of the activation of ABI5 and inactivation of MYB30. In the *siz1-2 myb30-2* double-mutant, MBY30 is absent and ABI5 is activated; therefore, the double-mutant would be expected to be more sensitive to ABA than either single mutant.

SIZ1 Stabilizes MYB30 During ABA Treatment. Sumoylation plays a role in protein–protein interaction, protein subcellular localization, enzymatic activity, and stability. To determine the biological function of MYB30 sumoylation, we expressed *35S:MYB30-GFP* or *35S:MYB30*^{K283R}-*GFP* in wild-type protoplasts. Both MYB30-GFP and MYB30^{K283R}-GFP localized to the nucleus (Fig. S4*A*). To examine if sumoylation changes MYB30 transcriptional activity, the *Arabidopsis* proteins were expressed in yeast, as previously reported (29). The MYB30^{K283R} mutation did not significantly affect MYB30 transcriptional activity in yeast (Fig. S4*B*). The *35S:Flag-MYB30* transgenic wild-type and *siz1-2* lines were then used to determine if sumoylation affects the stability of MYB30. The sumoylation of MYB30 was not significantly affected by ABA treatment (Fig. 5*A*); however, in the presence of ABA, MYB30 was less abundant in the *siz1-2* seedlings than in the wild-type seedlings (Fig. 5*B*). These data suggest that sumoylation by SIZ1 stabilizes MYB30. The expression of the *TAT3*, *LOX3*, *BGL2*, *bHLH*, *COR15b*, and *COR413* ABA responsive genes was rescued by expression *MYB30* but not by *MYB30*^{K283R} (Fig. 5*C*). These data, together with the results showing that *MYB30*^{K283R} partially reduced *myb30-2* ABA sensitivity, suggest that sumoylation of MYB30 by SIZ1 may also affect its transcriptional activity.
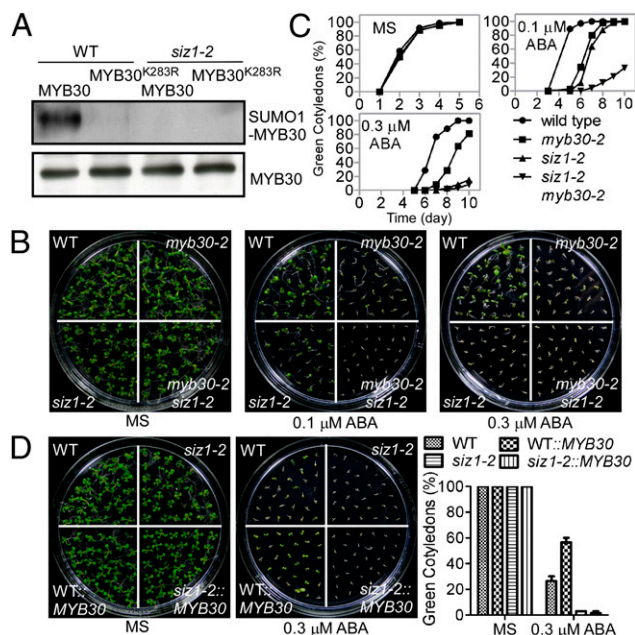
Fig. 4. SIZ1 mediates the sumoylation of MYB30. (*A*) SIZ1 mediates the sumoylation of MYB30 at K283. MYB30-SUMO1 conjugation was detected in wild-type but not in *siz1-2* protoplasts and mutation of MYB30 at K283 (MYB30^{K283R}) abolished this activity. (*B*) ABA phenotype of the *myb30-2 siz1-2* double-mutant. Seeds were sown on MS medium without (*Left*), or with 0.1 μM (*Center*) or 0.3 μM (*Right*) ABA and photographs were taken after 9, 7, or 9 d, respectively. (*C*) Green cotyledons were scored at the indicated times and represent an average of 100 seeds from at least three independent experiments ± SE. (*D*) SIZ1 regulates MYB30 function during ABA signaling. *Flag-MYB30* was expressed in wild-type and the *siz1-2* mutant and T$_4$ transgenic lines were used for phenotypic analyses. ABA sensitivity was monitored as described in (*B* and *C*). Seeds were sown on MS medium without (*Left*) or with 0.3 μM ABA (*Right*) and photographs were taken after 9 or 6 d, respectively. The mean germination values for wild-type, the *siz1* mutant, and wild-type and the *siz1* mutant overexpressing *MYB30* in response to 0.3 μM ABA is shown in the graph to the right of the images. Green cotyledons were scored after 9 (MS) or 6 (0.3 μM ABA) d of growth and represent an average of 100 seeds from at least three independent experiments ± SE.

Discussion

Results from our studies demonstrate that MYB30 is a component of ABA signaling in *Arabidopsis*. MYB30 is a R2R3-MYB transcription factor that has been shown to be a positive regulator of the hypersensitive response (HR), programmed cell death associated with disease resistance in plants, and of BR signaling. Overexpression of *MYB30* in *Arabidopsis* and tobacco increases resistance to different pathogens, whereas loss of *MYB30* function in *Arabidopsis* reduces HR-mediated cell death and pathogen resistance (31). MYB30 modulates HR and pathogen resistance via regulation of salicylic acid (SA) levels and SA-associated gene expression (32). In addition to its role in the HR, MYB30 functions to amplify BR signaling by directly interacting with BES1 and optimizing BES1 activity (34). Our results demonstrate that MYB30 is also involved in ABA signaling. Loss of *MYB30* function leads to hypersensitivity to ABA during germination. SIZ1 sumoylation of both MYB30 (at residue K283) and the ABI5 transcription factor leads to regulation of parallel pathways that coordinate germination in response to ABA.

Gene-expression analyses also suggest that MYB30 plays a role in ABA signaling in addition to its functions in HR and BR signaling. Eighteen genes were identified as possible targets of MYB30 during the HR response (33), 16 of which are also

regulated by ABA treatment. More than 100 genes were reduced at least twofold in the *myb30* mutant, with the expression of 13 changing significantly in response to BR (34) and 6 in response to ABA treatment. However, none of these genes changed expression in the *myb30* mutant compared with their expression in wild-type in response to ABA, suggesting that MYB30 regulates different target genes during HR, BR, and ABA signaling. The expression of *MYB30* is induced by bacterial pathogens (44) and Brassinolide (34) but reduced by ABA (Fig. 2*B*), providing additional evidence for MYB30 in these processes.

Functional and gene-expression studies link SIZ1 to MYB30 during ABA signaling. SIZ1 stabilizes MYB30 upon ABA treatment and is required for the sumoylation of MYB30 at K283, which is critical for MYB30 function in response to ABA. Overexpression of *MYB30* in wild-type results in the transgenic plants that are insensitive to ABA during seed germination; however, this phenomenon is abolished in the *siz1* mutant, suggesting that the sumoylation of MYB30 regulates its activity in *Arabidopsis*, although it is likely not the case in yeast (Fig. S4*B*).

SIZ1 has been shown to modulate gene expression in response to HR, BR, and ABA signaling, as well as during the transition to flowering (22, 26, 45–48). For example, SIZ1 regulates systemic-acquired resistance through both NONEXPRESSOR OF PR1 (NPR1) gene-dependent and SA accumulation-dependent pathways (45). Results from the present studies provide additional support for a link between SIZ1 and ABA signaling. Expression of ABA-responsive genes displays similar patterns in the *siz1* and *myb30* mutants (Fig. S5); however, MYB30 mediates SA levels and SA-associated gene expression, most likely
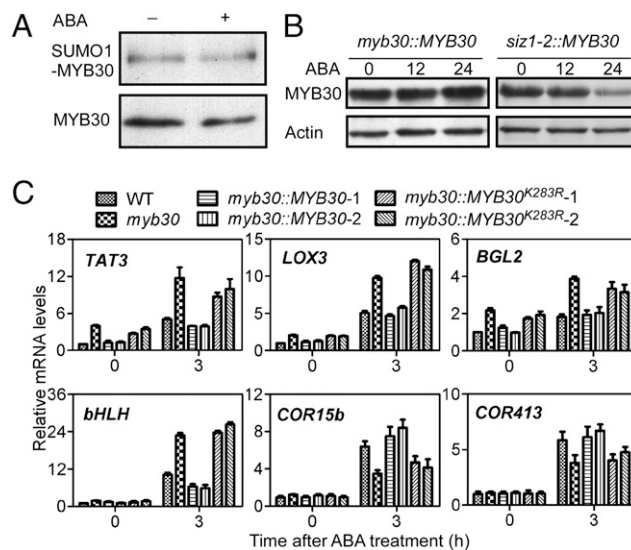

Fig. 5. MYB30^{K283} sumoylation is required for the response to ABA. (*A*) Sumoylation of MYB30 was not significantly affected by ABA. *Flag-MYB30* and *Myc-SUMO1* were coexpressed in wild-type protoplasts. For the ABA treatment, the protoplasts were incubated with 40 μM ABA for 1 h. Sumoylation was detected as described in Fig 3*B*. (*B*) ABA-induced MYB30 degradation in the *siz1-2* mutant. *Flag-MYB30* was expressed in wild-type and the *siz1-2* mutant. T$_4$ transgenic lines were used to test protein stability. Eight-day-old seedlings were treated without or with ABA (100 μM) for 12 or 24 h. MYB30 levels in wild-type or *siz1-2* seedlings were detected using immunoblots with anti-Flag antibody. Actin was used as a loading control. (*C*) Transcript abundance of *TAT3*, *LOX3*, *BGL2*, *bHLH*, *COR15b*, and *COR413* in different transgenic lines was determined by quantitative PCR using 1-wk-old seedlings without or with 100 μM ABA treatment for 3 h. mRNA levels are expressed relative to the value of wild-type seedlings before ABA treatment. Data represent means ± SD for three independent experiments.

independently of NPR1 (32), suggesting that MYB30 also regulates different sets of genes.

Several lines of evidence suggest that SIZ1 sumoylation of MYB30 and ABI5 regulates ABA signaling during germination. (*i*) Loss-of-function of the two transcription factors alters germination in response to ABA (Fig. 2*A*). (*ii*) The germination response of the *abi5 myb30* double-mutant restores wild-type responses to ABA during germination (Fig. 2*A*). (*iii*) The stability of the two transcription factors is likely regulated by SIZ1 sumoylation in response to ABA (22) (Fig. 5*B*).

In addition, our results suggest that MYB30 and ABI5 regulate parallel pathways during germination in response to ABA. The two transcription factors regulate different sets of genes in response to SIZ1 sumoylation (22) (Fig. S5) and different subgroups of ABA-responsive genes (Table S1). Taken together, our results suggest that regulation of ABI5 and MYB30 by SIZ1 sumoylation could serve to balance gene expression in response to ABA (Fig. S6).

Materials and Methods

Plant Growth and ABA Treatment. *Arabidopsis* seeds were sown on Murashige and Skoog (MS) medium with 0.3% agar, kept in the dark for 3 d at 4 °C to break dormancy, and then grown in a growth chamber at 23 °C in constant light. To monitor ABA sensitivity, seeds were sown on MS medium or MS medium containing the indicated concentrations of ABA (Sigma). Germination frequencies were obtained by scoring green cotyledons for an average of 100 seeds from three independent experiments ± SE. Stable WT:: MYB30 and *siz1-2::MYB30* T$_4$ lines were used to monitor ABA-induced MYB30 degradation. Eight-day-old seedlings were treated with water (0 h) or ABA for 12 or 24 h. Seedlings were homogenized on ices in extraction buffer [10 mM Tris (pH 7.5), 0.5% Nonidet P-40, 2 mM EDTA, 150 mM NaCl, 1 mM PMSF, and 1% (vol/vol) protease inhibitor mixture (Sigma)]. Extracts were centrifuged at 12,000 × *g* for 15 min at 4 °C and the resulting supernatant was used to detect MYB30 levels in wild-type or *siz1-2* seedlings by immunoblot analysis.

The *Arabidopsis siz1-2* mutant was kindly provided by Paul M. Hasegawa (Purdue University, West Lafayette, IN). The *myb30-1* (SALK_122884), *abi1-3* (SALK_076309), *abi2-2* (SALK_015166), *abi3* (SALK_138922), *abi4* (SALK_080095), and *abi5-8* (SALK_013163) mutants were obtained from the ABRC.

Transgenic Plants. The *MYB30* or *MYB30^{K283R}* coding region was cloned into the pCAMBIA1307-Flag binary vector driven by a cauliflower mosaic virus 35S promoter. *MYB30* or *MYB30* (*K283R*) overexpressing transgenic plants (in the Col-0, *myb30-2* or *siz1-2* backgrounds) were generated using *Agrobacterium*-mediated floral transformation. RT-PCR and immunoblot analysis were used to detect the abundance of the transgenes and proteins. Stable WT::*MYB30*, *siz1-2::MYB30*, *myb30-2::MYB30* and *myb30-2::MYB30^{K283R}* T$_4$ lines were used for phenotypic analyses.

Quantitative RT-PCR Analysis. Seven-day-old seedlings grown on MS plates were used for ABA treatments. The seedlings were harvested for RNA extraction after treatment with 100 μM ABA for 3 h. Total RNA isolation, reverse transcription and quantitative real-time PCR amplification were as described (27, 29). The primer pairs used are shown in Table S2. The value of wild-type without ABA treatment (0 h) was normalized to 1 with differences calculated as relative expression.

In Vivo SUMO Conjugation Assays. For transient expression of *MYB30* and *SUMO1* in *Arabidopsis* protoplasts, the *SUMO1* coding region was amplified and inserted into the pCAMBIA1205-Myc plasmid to produce the Myc-SUMO1 fusion protein.

The *35S:Flag-MYB30* and *35S:Myc-SUMO1* were coexpressed in wild-type or *siz1-2* protoplasts by polyethylene glycol-mediated transformation (22). After a 20-h incubation at 23 °C, the protoplasts were harvested and lysed in 1 mL extraction buffer. For ABA treatment, protoplasts were incubated with ABA (at a final concentration of 40 μM) at 23 °C for 1 h (22).

Fifteen microliters of anti-Flag agarose conjugate (Sigma) were incubated with the extract supernatant for 2 h at 4 °C. After washing five times in 1 mL of extraction buffer, proteins were detected by immunoblot analysis (49). Anti-Myc (Sigma) antibody was used to detect the Flag-MYB30-Myc-SUMO1 conjugation. Anti-Flag (Sigma) antibody was used to detect the abundance of Flag-MYB30 as a control.

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