The Plant Journal (2006) **47**, 776–787 doi: 10.1111/j.1365-313X.2006.02833.x

Mutation of SAD2, an importin β-domain protein in Arabidopsis, alters abscisic acid sensitivity

Paul E. Verslues[†], Yan Guo[†,‡], Chun-Hai Dong[§], Wujun Ma and Jian-Kang Zhu[*]
Institute for Integrative Genome Biology and Department of Botany and Plant Sciences, University of California, Riverside, CA 92521, USA

Received 9 March 2006; revised 17 May 2006; accepted 26 May 2006.
[*]For correspondence (fax +1 951 827 7115; e-mail jian-kang.zhu@ucr.edu).
[‡]Present address: National Institute of Biological Sciences, Beijing West Road 55, Life Science Park, Changping Dist. Beijing 102206, People's Republic of China.
[§]Present address: Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742, USA.
[†]The first two authors contributed equally to this work.

Summary

A number of protein and RNA-processing mutants have been shown to affect ABA sensitivity. A new mutant, *sad2-1*, was isolated from a T-DNA mutagenized population of *RD29A:LUC* plants and shown to have increased luminescence after ABA, salt, cold or polyethylene glycol treatments. Expression of several ABA- and stress-responsive genes was higher in the mutant than in the wild type. *sad2-1* also exhibited ABA hypersensitivity in seed germination and seedling growth. *SAD2* was found to encode an importin β-domain family protein likely to be involved in nuclear transport. *SAD2* was expressed at a low level in all tissues examined except flowers, but *SAD2* expression was not inducible by ABA or stress. Subcellular localization of GFP-tagged SAD2 showed a predominantly nuclear localization, consistent with a role for SAD2 in nuclear transport. Knockout of the closest importin β homolog of SAD2 in Arabidopsis did not duplicate the *sad2* phenotype, indicating that SAD2 plays a specific role in ABA signaling. Analysis of *RD29A:LUC* luminescence and ABA and stress sensitivity in double mutants of *sad2-1* and *sad1* or *abh1-7*, a newly isolated allele of ABH1 also in the *RD29A:LUC* background, suggested that SAD2 acts upstream of or has additive effects with these two genes. The results suggest a role for nuclear transport in ABA signal transduction, and the possible roles of SAD2 in relation to that of SAD1 and ABH1 are discussed.

Keywords: abscisic acid, SAD2, importin β, SAD1, ABH1, seed germination, abiotic stress.

Introduction

Abscisic acid (ABA) accumulates in response to abiotic stresses that lead to dehydration of plant tissue (Zeevaart, 1999). Abscisic acid is then a key inducer of downstream responses that lead to reduction of water loss, greater water uptake or tolerance of reduced tissue water content (Zhu, 2002). Abscisic acid also has roles in regulating a number of developmental processes such as flowering time and lateral root initiation (Brady *et al.*, 2003; Razem *et al.*, 2006). Although ABA signaling is incompletely understood, genetic studies have led to the discovery of several important signaling intermediates that mediate ABA-dependent changes in seed germination, stomatal conductance and expression of ABA-regulated genes.

Some of these ABA signaling intermediates, notably the ABI1 and ABI2 protein phosphatases and the ABI3, ABI4 and ABI5 transcription factors (Finkelstein *et al.*, 2002), the FRY1 inositol polyphosphate 1-phosphatase (Xiong *et al.*, 2001a) and the RLK1 receptor-like kinase (Osakabe *et al.*, 2005) are members of well-known classes of signaling molecules. Although the connections between these signaling molecules have yet to be elucidated, they may form part of a signal transduction chain that amplifies and transmits the ABA signal to downstream components. Other genetically identified modifiers of ABA response, however, do not fall into such well-studied classes of signaling molecules and instead indicate that processing or turnover of key mRNAs and proteins also plays a large role in controlling ABA response.

abh1 and *sad1* are hypersensitive to ABA in seed germination and either root growth or stomatal regulation and encode proteins involved in RNA metabolism. ABH1 encodes the large subunit (CPB80) of the mRNA 5′ cap-binding complex (Hugouvieux *et al.*, 2001, 2002). ABH1 interacts

with CPB20 to form a functional 5′ cap-binding complex and it has been shown that mutation of CPB20 also causes an ABA-hypersensitive phenotype similar to that of *abh1* (Papp *et al.*, 2004). SAD1 encodes a Sm-like protein possibly involved in RNA splicing, RNA export from the nucleus or RNA degradation (Xiong *et al.*, 2001b). Specific target mRNAs in an ABA response pathway for either SAD1 or ABH1 have not been conclusively identified. Because they affect aspects of general mRNA metabolism, it might be expected that either *abh1* or *sad1* would substantially alter the accumulation of a wide range of mRNAs. However, microarray analysis demonstrated that only 0.4% of the 8000 genes analyzed had a threefold or greater increase or decrease in mRNA level in *abh1* compared with wild type (Hugouvieux *et al.*, 2001). Thus ABH1, and most likely SAD1 as well, may be involved in the processing of only a small number of mRNAs or may affect a larger number of mRNAs in a more subtle manner, which has not yet been quantified. *abh1* and *sad1* do not appear to alter sensitivity to other hormones and are likely to affect ABA sensitivity and a relatively small number of other developmental processes, such as flowering (Bezerra *et al.*, 2004). In contrast, the *hyl1* mutation affects sensitivity to several hormones including ABA (Lu and Fedoroff, 2000). *HYL1* encodes a double-stranded RNA-binding protein important for miRNA biogenesis (Han *et al.*, 2004).

A connection between ABA, RNA processing and flowering has recently been shown by the demonstration that the nuclear RNA-binding protein FCA also has specific ABA-binding activity and acts as an ABA receptor (Razem *et al.*, 2006). Binding of ABA by FCA decreases the interaction of FCA with FY, an RNA 3′-end-processing factor that is also a known regulator of flowering. The blockage of FCA–FY interaction by ABA has two major effects: an increase in FLC which in turn delays flowering, and an increase in FCA itself through inhibition of an autoregulatory cleavage of the FCA mRNA by the FCA–FY complex. These data demonstrate a specific mechanism by which mRNA-processing functions as part of ABA signaling.

Protein modification and metabolism also affect the ABA response. The well-studied ABA-hypersensitive mutant *era1* encodes a protein farnesyl transferase and is likely to be involved in farnesylation of signaling proteins (Cutler *et al.*, 1996). In addition, mutation of the RPN10 subunit of the 26S proteosome also causes ABA hypersensitivity (Smalle *et al.*, 2003). RPN10 is unique in that a specific molecular target that may mediate ABA sensitivity has been identified: the ABI5 transcription factor is stabilized in the *rpn10-1* mutant (Smalle *et al.*, 2003). Whether or not this is the only ABA signaling molecule whose degradation is regulated by RPN10 is not known. Both *rpn10-1* and *era1* have other developmental and hormone sensitivity phenotypes (Smalle *et al.*, 2003; Yalovsky *et al.*, 2000), suggesting that they modify a number of signaling proteins. Degradation of ABI5 is also promoted by association with ABI5-BINDING PROTEIN1 (AFP1), a novel protein that may target ABI5 for ubiquitin-mediated degradation (Lopez-Molina *et al.*, 2003). AFP1 is induced by ABA and knockouts of AFP1 are hypersensitive to ABA (Lopez-Molina *et al.*, 2003).

A surprising aspect of these studies is the relative specificity and the degree to which these alterations in mRNA and protein metabolism affect ABA response. An as yet unanswered question is whether this is due to there being only a limited number of target mRNAs or proteins that are processed by ABH1, SAD1, ERA1 or RPN10, or whether ABA signaling is more dependent on correct RNA and protein processing than other hormone or developmental signaling pathways (Razem *et al.*, 2006). In either case, the phenotypes of these mutants raise the possibility that other processes related to RNA or protein processing may affect ABA signaling. In mammalian systems, it is well established that transport of signaling proteins between cellular compartments, especially the cytoplasm and nucleus, has a prominent role in signal transduction (Xu and Massague, 2004). An example of this is importin-mediated translocation of glucocorticoid receptor into the nucleus after hormone binding (Freedman and Yamamoto, 2004).

Here we describe *sad2-1*, a mutant in an importin β-domain protein that, like *sad1* (Xiong *et al.*, 2001b), has ABA- and stress-hypersensitive induction of a luciferase reporter under control of the RD29A promoter. *sad2-1* is also hypersensitive to ABA and stress in seed germination and seedling growth. The importin β domain is characteristic of proteins involved in nuclear transport; raising the possibility that SAD2 mediates nuclear transport of ABA signaling proteins. Knockout of another importin β protein highly homologous to SAD2 does not duplicate the *sad2* phenotype, indicating that this role in ABA signaling is a specific function of SAD2. Double mutant analysis of *sad2-1* with *sad1* or *abh1-7*, a newly isolated allele of *abh1*, indicates that SAD2 acts upstream of or has additive effects with SAD1 and ABH1. These results indicate that at least one importin β protein potentially involved in nuclear transport is also part of a chain of protein- and mRNA-processing events required for correct ABA signaling.

Results

Isolation of sad2-1 and abh1-7

sad2-1 (*super sensitive to ABA and drought2*) was isolated from a T-DNA mutagenized population of *RD29A:LUCIFERASE* (*RD29A:LUC*) plants in the C24 background. The T-DNA construct and mutagenesis procedures are described by Xiong *et al.* (2001a). Approximately 30 000 T-DNA lines, in pools of 500, were screened for altered luminescence following stress or ABA treatment using procedures similar to those described previously for a chemically mutagenized population of *RD29A:LUC* (Ishitani *et al.*, 1997). A group of

mutants that had increased stress- or ABA-responsive *RD29A:LUC* expression and ABA hypersensitivity in seed germination were isolated. One of these mutants, designated as *sad2-1*, is the focus of this study. *sad2-1* had significantly increased expression of *RD29A:LUC* after cold, polyethylene glycol (PEG) or NaCl treatment but not on normal media (Figure 1a,b). *RD29A:LUC* expression was also higher in *sad2-1* compared with wild type after treatment with exogenous ABA (Figure 1b,c).

Mutants with decreased stress- and ABA-responsive *RD29A:LUC* expression were also isolated, and one of these mutants was found to be allelic (see below) to the ABA-hypersensitive mutant *abh1*. This mutant was designated as *abh1-7* (*abh1-2*, *-3*, *-4*, *-5* and *-6* have been described by Bezerra *et al.*, 2004).

Gene expression changes in sad2-1

Increased accumulation of *LUC* mRNA in *sad2-1* after treatment with salt, ABA, cold or PEG was observed by RNA blot analysis (Figure 2a). This confirms that *sad2-1* affects mRNA expression from the *RD29A* promoter rather than the stability of the LUC protein. A RNA blot analysis also showed that mRNA levels of the endogenous *RD29A* gene were higher in *sad2-1* than in wild type after treatment with salt, osmotic stress or ABA (Figure 2a). This demonstrates that *sad2-1* affects expression of the endogenous *RD29A* gene in addition to the *RD29A:LUC* construct.

We also examined the expression of several other stress- and ABA-responsive genes using quantitative real-time PCR (Figure 2b). The ABA-responsive gene *RAB18* had increased expression in *sad2-1* after treatment with PEG, NaCl or ABA. This demonstrated that the effects of *sad2-1* were not limited to altered *RD29A* expression. Increased expression of another gene induced by ABA and stress, *COR15A*, was observed only after ABA treatment. In contrast, expression of *NCED3* was not altered in *sad2-1*. NCED3 is a key enzyme in ABA synthesis (Schwartz *et al.*, 2003) and is likely to be regulated in large part by signals upstream of ABA accumulation. This suggests that *sad2-1* affects responses down-

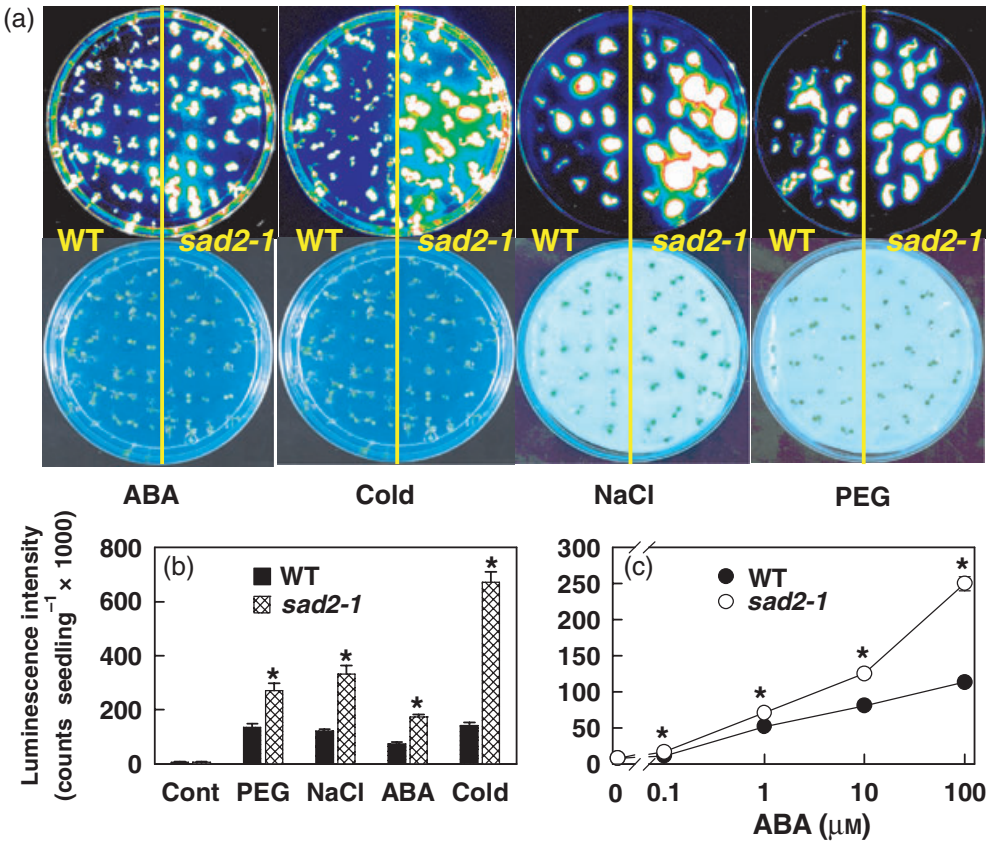

Figure 1. *RD29A:LUC* expression in *sad2-1* and wild-type seedlings.
(a) Luminescence intensity in 7-day-old wild-type and *sad2-1* seedlings after treatment with cold (0°C for 24 h), ABA (100 μM ABA for 3 h), NaCl (300 mM for 5 h) or PEG (30% for 3 h).
(b) Quantification of luminescence intensities of *sad2-1* and wild-type seedlings after each treatment in panel (a). Data are means ± SE (n = 6–25). Asterisks indicate a significant difference ($P \leq 0.002$) between mutant and wild type for that treatment.
(c) *RD29A:LUC* expression in *sad2-1* and wild-type seedlings 3 h after treatment with different concentrations of exogenous ABA. Data are means ± SE (n = 6). Asterisks indicate a significant difference ($P \leq 0.005$) between mutant and wild type for that treatment.

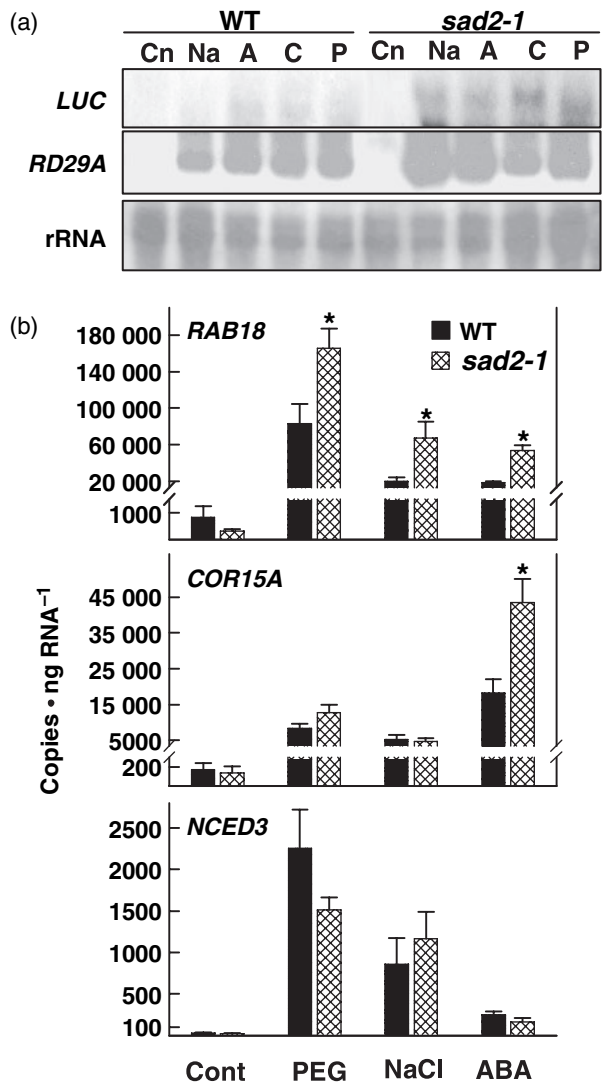

Figure 2. Gene expression in *sad2-1* and wild-type plants in response to stress or ABA treatment.
(a) RNA blot analysis of *RD29A:LUC* and endogenous *RD29A* expression in 7-day-old wild type and *sad2-1* seedlings. Treatments: Cn, control; Na, 300 mM NaCl for 5 h; A, 100 μM ABA for 3 h; C, 0°C for 24 h; P, 30% PEG for 5 h. The rRNA blot is shown as a loading control.
(b) Real-time PCR analysis of *RAB18*, *COR15A* and *NCED3* expression in 7-day-old wild-type and *sad2-1* seedlings. Treatments: Cont, control agar plate (−0.25 MPa); PEG, transfer to −1.2 MPa PEG-infused agar plate for 4 h; NaCl, transfer to plate containing 150 mM NaCl for 4 h; ABA, transfer to plate containing 100 μM ABA for 4 h. Data are means ± SE ($n = 3$ or 4). Asterisks indicate a significant difference ($P \leq 0.05$) between mutant and wild type for that treatment.

stream of ABA accumulation. We also used ABA radioimmunoassay (Bray and Beachy, 1985) to quantify seedling ABA content and found no significant difference in ABA content between wild-type and *sad2-1* seedlings under control conditions or after PEG treatment (data not shown). Thus, we focused further characterization of *sad2-1* on its increased sensitivity to ABA.

sad2-1 *is hypersensitive to ABA in seed germination and seedling growth*

sad2-1 was dramatically affected in the sensitivity of seed germination to exogenous ABA (Figure 3a). Low concentrations of ABA (0.1–0.5 μM) that had little or no effect on germination of the C24 wild type reduced *sad2-1* germination by approximately 50%. Increased ABA sensitivity was also seen at 1 and 1.5 μM ABA (Figure 3a). *sad2-1* was also hypersensitive to ABA in post-germination seedling growth. Ten-day-old wild-type seedlings showed little growth inhibition on media containing 0.4 μM exogenous ABA. In contrast, *sad2-1* seedlings had little shoot or root development on ABA-containing media (Figure 3b). We also observed that *sad2-1* plants flowered 4–5 days earlier than wild type (Figure 3c). This was the only morphological difference that we observed between *sad2-1* and wild type: rosette stage plants and mature plants (6 weeks after planting) were indistinguishable from wild type.

Another relevant question is whether *sad2-1* is also affected in its response to other hormones. We measured root elongation of wild type and *sad2-1* seedlings after transfer to media containing aminocyclopropane-1-carboxylic acid (ACC; 1, 2 or 4 μM), giberellic acid (GA; 100 μM), the cytokinin benzyl-adenine (BA; 0.1 μM), indole-3-acetic acid (IAA; 0.01 μM), epibrassinosteroid (EBR; 1.5 μM) and 2,4-dichlorophenoxyacetic acid (2,4-D; 0.01 μM). In all cases, the relative effect of the hormone treatment on root elongation was the same in wild type and *sad2-1* (data not shown). *sad2-1* was also not altered in its response to high levels of sugar (glucose, 1–6%). Together these data suggest that *sad2-1* is specific in its effect on the ABA response.

SAD2 *encodes an importin β-domain protein*

A 1 kb genomic DNA fragment flanking the T-DNA left border in *sad2-1* was obtained by plasmid rescue (Mathur *et al.*, 1998) and sequence analysis showed that the T-DNA was inserted in the seventh intron of *At2g31660*. Reverse transcriptase-PCR (Figure 3d) and RNA blot (data not shown) analysis confirmed that the *At2g31660* transcript is absent in *sad2-1*. To further confirm that the T-DNA insertion in *At2g31660* is the cause of the *sad2-1* phenotype, we isolated a 9309-bp genomic DNA fragment containing *At2g31660* by restriction digest of BAC clone T9H9. The fragment included 1033 bp upstream of the translation initiation codon and 1436 bp downstream of the stop codon. The fragment was cloned into the binary vector pCAMBIA1380 and used to transform *sad2-1* plants. Hygromycin-resistant seedlings had wild-type sensitivity of seedling growth to ABA and *RD29A:LUC* (Figure 3e). In agreement with this, *RD29A:LUC* expression of the hygromycin-resistant seedlings was decreased compared with untransformed *sad2-1* after treat-

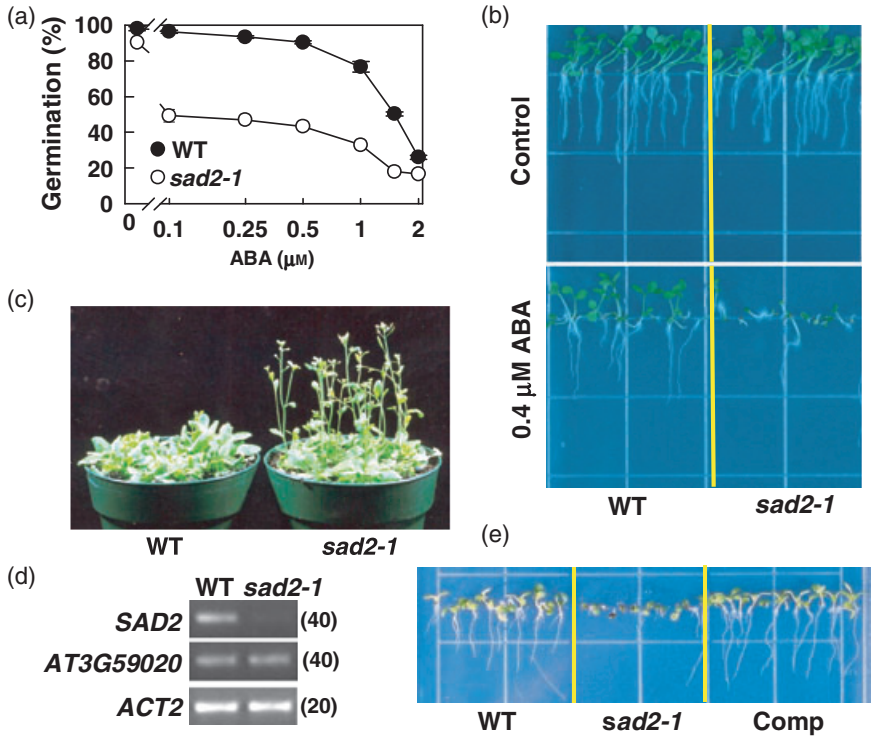

Figure 3. Abscisic acid-hypersensitive phenotype of *sad2-1* and identification of the *SAD2* gene.
(a) ABA sensitivity of seed germination in wild type and *sad2-1*. Germination (radicle emergence) was scored after 5 days of imbibition. Data are means ± SE ($n = 3$).
(b) ABA sensitivity of seedling growth in wild type and *sad2-1*. Photographs were taken after 9 days of growth on MS plates (3% sucrose) containing 0 or 0.4 μM ABA.
(c) Early flowering phenotype of *sad2-1*.
(d) Reverse transcriptase PCR analysis of *SAD2* and *At3g59020* expression in *sad2-1* and wild type. Total RNA was extracted from wild-type and *sad2-1* seedlings growing under control conditions. Reverse transcriptase PCR was performed as described in the methods using primers given in Table S1. Primers for *ACTIN2* (*ACT2*) were used as an amplification control. Numbers to the right are the number of PCR cycles performed for each gene. PCR reactions were repeated twice with essentially identical results each time.
(e) ABA sensitivity phenotype of wild type, *sad2-1* or complemented *sad2-1*. Complemented *sad2-1* seedlings are from homozygous T_3 plants of *sad2-1* transformed with wild-type *SAD2*. Photographs were taken after 10 days of growth on media containing 0.4 μM ABA.

ment with ABA, PEG, NaCl or cold (Figure S1). We also isolated homozygous plants of a SALK T-DNA insertion in *SAD2* (SALK_133577) and found that these plants were also hypersensitive to ABA (Figure S2). Together these data demonstrated that *At2g31660* is *SAD2*.

SAD2 is annotated as an importin β-2 subunit protein similar to importin 7 in vertebrates and RanBP7 (Ran binding protein 7) in *Drosophila melanogaster* (Merkle, 2003). Bollman *et al.* (2003) and Merkle (2003) have conducted extensive phylogenetic analysis of the 17 importin β proteins in Arabidopsis and showed that SAD2 is highly related (65% identical; Figure S3) to another importin β-domain protein encoded by *At3g59020*. Expression of this gene was unaffected in *sad2-1* (Figure 3d).

Expression and localization of SAD2

RNA blot analysis was performed to determine the tissue distribution and stress inducibility of *SAD2*. Low levels of *SAD2* transcripts were detected in roots, leaves, stem and siliques but not in flowers (Figure 4a). No induction of *SAD2* expression by salt, cold, ABA or dehydration treatment was observed (Figure 4b). The tissue distribution of *SAD2* expression was further examined by isolating a DNA fragment from the translational start codon to −1282 of *SAD2*, fusing it to the GUS reporter gene and generating transgenic plants. In agreement with the RNA blot results, GUS expression was detected in the entire seedling, root, leaf, stem and silique from 10 independent transgenic plants (Figure 4c and data not shown). In leaves, GUS staining was found in both epidermal cells and guard cells (Figure 4d).

We investigated the nuclear localization of SAD2 by transiently expressing an N-terminal fusion of GFP to SAD2 in leaf epidermal cells. SAD2-GFP was observed mostly in and around the nucleus with substantial amounts of SAD2-GFP also in the cytoplasm (Figure 4e). This is consistent with the expected function of SAD2 in nuclear transport (Goldfarb *et al.*, 2004; Merkle, 2003) although the exact function of SAD2 is not known.

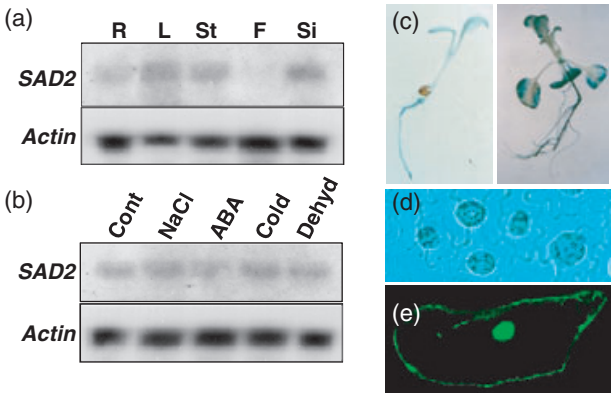

Figure 4. Expression and localization of SAD2.
(a) A RNA blot analysis of tissue-specific expression of *SAD2*. Tissues analyzed were root (R), leaf (L), stem (St), flower (F) and silique (Si). Thirty micrograms of total RNA was loaded per lane. Actin blot is shown as a loading control.
(b) A RNA blot analysis of stress and ABA inducibility of *SAD2* expression. Treatments were: Control (Cont); NaCl, 300 mM for 5 h; ABA, 100 μM for 3 h; cold, 0°C for 24 h; dehyd, dehydration to 70% of original fresh weight.
(c) Expression pattern of *SAD2*:GUS in seedlings.
(d) *SAD2*:GUS staining in leaf tissue showing expression of SAD2 in both epidermal and guard cells.
(e) Subcellular localization of GFP-tagged SAD2 after transient expression in leaf epidermal cells.

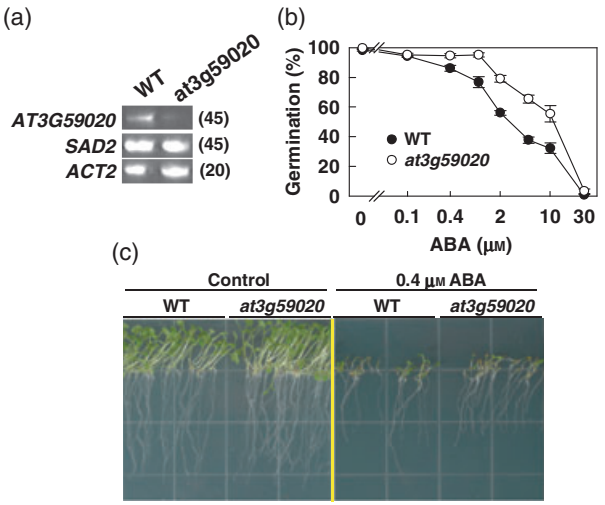

Figure 5. Abscisic acid sensitivity phenotype of a T-DNA knockout mutant of the SAD2 homolog AT3G59020.
(a) Reverse transcriptase PCR analysis of *At3g59020* and *SAD2* expression in the *at3g59020* T-DNA mutant and wild type. Total RNA was extracted from wild-type and *at3g59020* seedlings growing under control conditions. Reverse transcriptase PCR was performed as described in the methods using primers given in Table S1. Primers for *ACTIN2* (*ACT2*) were used as an amplification control. Numbers to the right are the number of PCR cycles performed for each gene. PCR reactions were repeated twice with essentially identical results each time.
(b) Abscisic acid sensitivity of seed germination in Col wild type and *atg359020*. Seeds were plated on half-strength MS media containing 0.5% sucrose and the indicated concentrations of ABA. Seed germination (scored by emergence of the radicle) was quantified 3 days after the end of seed stratification. Data are means ± SE ($n = 6$).
(c) Growth of Col wild type and *at3g59020* on control (0.5% sucrose) media or media containing 0.4 μM ABA. Photographs were taken 7 days after the end of seed stratification.

T-DNA knockout of the SAD2 homolog AT3G59020 does not mimic the SAD2 phenotype

At3g59020 encodes an importin β protein that shares the highest sequence homology to SAD2 out of the 17 importin β-domain proteins in Arabidopsis (Bollman *et al.*, 2003; Merkle, 2003; Figure S3). Because of this extensive homology, and because of the possibility that increased ABA sensitivity may be a general effect of altered nuclear transport, we investigated whether knockout of At3g59020 can also alter ABA sensitivity. Homozygous plants of a line containing a T-DNA insertion in the fourth exon of *At3g59020* (SALK_103333) were isolated by PCR screening. Reverse transcriptase PCR analysis showed that *At3g59020* is expressed at a low level in Col wild type and this expression was eliminated in the *at3g59020* T-DNA line (Figure 5a). Seed germination of *at3g59020* was less sensitive to ABA than that of wild type (Figure 5b). Note that the Col ecotype used in these experiments is itself less sensitive to ABA inhibition of seed germination than the C24 background of our *sad2-1* mutant (Figure 3). *at3g59020* did not substantially differ from wild type, however, in the effect of ABA (Figure 5c), PEG or NaCl stress (data not shown) on seedling growth. Thus, the *at3g59020* mutation does not duplicate the *sad2-1* phenotype. This suggests that *sad2-1* has a relatively strong and specific effect and raises the possibility that SAD2 could have a role in transport of ABA signaling intermediates.

Double mutant analysis of sad1/sad2-1 *and* sad2-1/abh1-7

From the same population of T-DNA-mutagenized *RD29A:LUC* we also isolated a T-DNA insertion in *ABH1* by thermal asymmetric interlaced (TAIL)-PCR. This allele was designated *abh1-7* and has an insertion in the ninth intron of *ABH1*. *abh1-7* exhibited enhanced ABA sensitivity (data not shown) similar to previous reports (Hugouvieux *et al.*, 2001, 2002; Papp *et al.*, 2004) but reduced *RD29A:LUC* expression (see below).

With the isolation of *sad2-1* and *abh1-7*, we now had a collection of three mutants in the *RD29A:LUC* background involved in transport or RNA metabolism functions that affect ABA signaling. To better understand the interaction of these loci, we performed double mutant analysis of *sad1* and *sad2-1* and of *sad2-1* and *abh1-7*. *sad1/sad2-1* had higher *RD29A:LUC* expression than either *sad1* or *sad2-1* alone after treatment with ABA, cold or NaCl (Figure 6a). The data suggested an additive interaction between *sad1* and *sad2-1* on expression of *RD29A*. This is consistent with SAD1 and SAD2 acting in separate branches of stress and ABA

signaling that converge at the regulation of *RD29A*, or with SAD1 and SAD2 acting on the same signaling branch but having an additive effect on the signaling output. Growth of the *sad1*/*sad2-1* mutant under unstressed conditions was similar to that of *sad1* for either soil-grown plants (Figure 6a) or seedlings (Figures 6c, 7). In the sensitivity of seedling growth to ABA, *sad1*/*sad2-1* seedlings were more sensitive than *sad2-1* alone and of similar sensitivity or slightly more

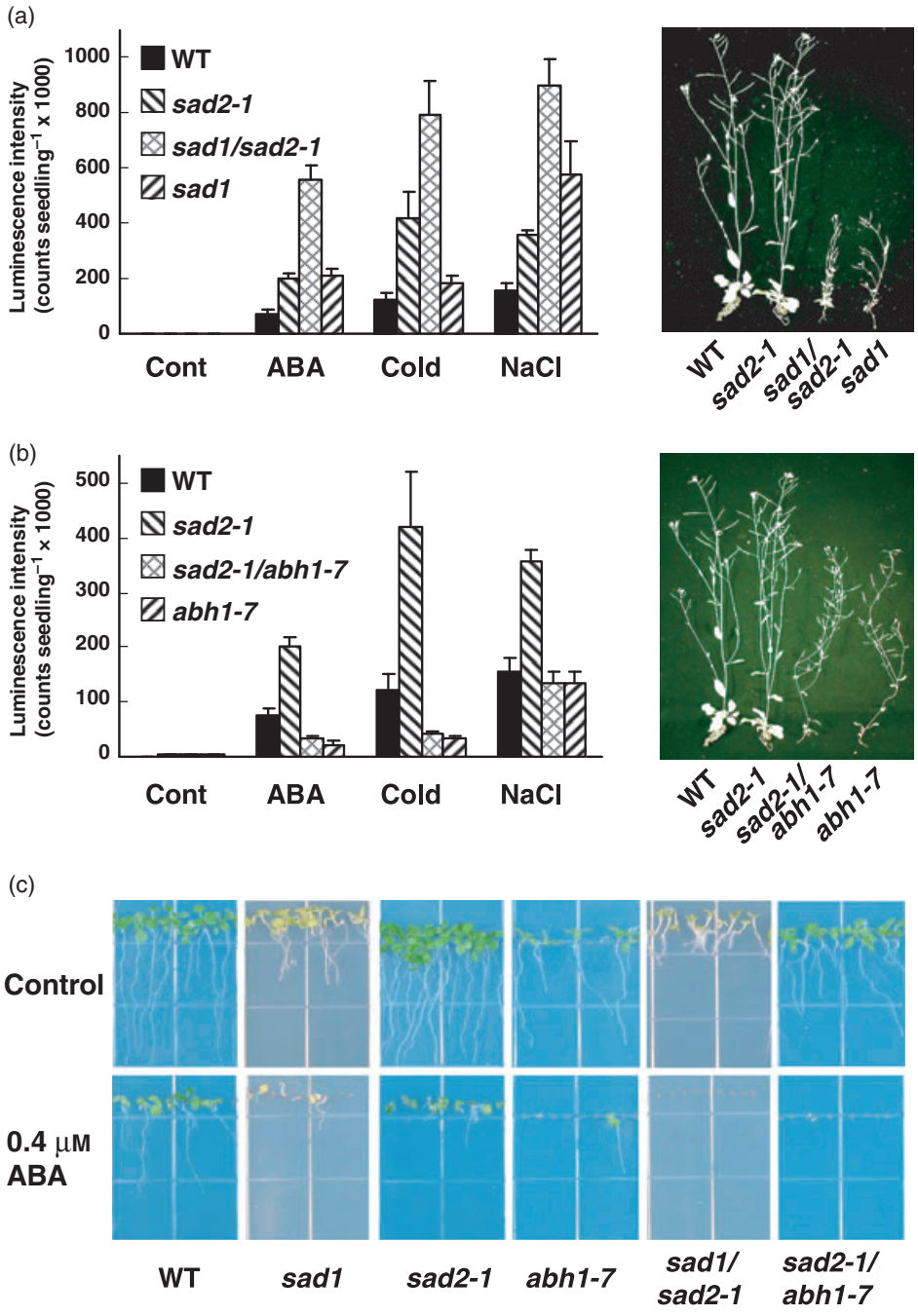

Figure 6. Analysis of *sad1/sad2-1* and *sad2-1/abh1-7* double mutants.
(a) Luminescence intensity after stress or ABA treatments of wild-type, *sad2-1*, *sad1*/*sad2-1* and *sad1* seedlings. Stress and ABA treatments are as described for Figure 1. Data are means ± SD (n = 4–6). Right panel: morphological phenotype of 6-week-old soil-grown plants of wild type, *sad2-1*, *sad1*/*sad2-1* and *sad1*.
(b) Luminescence intensity after stress or ABA treatments of wild type, *sad2-1*, *sad2-1/abh1-7* and *abh1-7* seedlings. Stress and ABA treatments are as described for Figure 1. Data are means ± SD (n = 4–6). Right panel: morphological phenotype of 6-week-old soil-grown plants of wild type, *sad2-1*, *sad2-1*/*abh1-7* and *abh1-7*.
(c) Abscisic acid sensitivity of wild type, *sad1*, *sad2-1*, *abh1-7* and double mutant seedlings. Photographs were taken after 10 days of growth on media containing 0 (control) or 0.4 μM ABA.

sensitive than *sad1* alone (Figure 6c). Seeds of the double mutant did not germinate and grow even after 20 days on 0.4 μM ABA (Figure 6c) whereas *sad1* did germinate and exhibited a low level of seedling growth. Likewise, when *sad1*/*sad2-1* seedlings were subjected to low water potential (using PEG-infused agar plates) or NaCl stress, the double mutant was more sensitive than *sad2-1* and of similar sensitivity or slightly more sensitive than *sad1* (Figure 7). The results suggest an epistatic effect of *sad1* over *sad2-1* on growth and ABA response or an additive effect, with *sad1* having the larger effect.

abh1-7 had reduced *RD29A:LUC* expression compared to wild type after treatment with ABA, cold or NaCl (Figure 6b). Although the underlying mechanisms are not known, ABH1 may have a different role in the regulation of *RD29A* expression than it does in seed germination and stomatal closure, where inactivation of ABH1 causes ABA hypersensitivity (Hugouvieux *et al.*, 2001). *sad2-1/abh1-2* also had reduced *RD29A:LUC* expression compared with wild type (Figure 6b). The decreased expression of *RD29A:LUC* in the double mutant is consistent with *abh1-7* acting downstream of *sad2-1*. Also, soil-grown plants of *sad2-1*/*abh1-7* were indistinguishable from *abh1-7* (Figure 6b). Like *sad2-1*, seedling growth of *abh1-7* was similar to wild type under unstressed conditions and only slightly more affected by low water potential or NaCl (Figure. 6c and 7). Seedling growth of *sad2-1/abh1-7* was more sensitive to ABA than that of the single mutant (Figure 6c), and the double mutant was also much more sensitive to low water potential or NaCl than either *sad2-1* or *abh1-7* (Figure 7). This last observation in particular suggests that the processes affected by SAD2 and ABH1 do not completely overlap, or that SAD2 and ABH1 both have a limited effect on the same signaling processes and these effects are additive in the double mutant.

Discussion

Our results show that knockout of SAD2, an importin β-domain protein, alters ABA sensitivity. The main phenotypes we observed in *sad2*, ABA hypersensitivity in seed germination, seedling growth and gene expression, are consistent with those observed in other mRNA and protein-processing mutants. Mutation of another highly homologous importin β-domain protein, AT3G59020, does not duplicate the *sad2* phenotype, thus suggesting that these effects on ABA sensitivity are specific to *sad2*. *sad2* also flowers earlier than wild type. This differentiates *sad2* from mutants like *abi2*, which affect ABA sensitivity in germination and growth but not flowering, or *fca* and *fy* which affect flowering but not ABA sensitivity of germination or growth (Razem *et al.*, 2006). From these data as well as double mutant analysis of *sad2-1* and two additional ABA sensitivity mutants, *sad1* and *abh1-7*, and other reports of the effects of protein and RNA processing on ABA sensitivity, we can begin to hypothesize about the function of SAD2 and how protein and RNA transport and metabolism may affect ABA sensitivity.

Nuclear import proteins and their roles in signaling

The nuclear pore complex and nuclear import have been extensively studied in mammalian systems (Fahrenkrog *et al.*, 2004; Goldfarb *et al.*, 2004). Importin β was the first nuclear import receptor to be identified, and is a member of a large family of proteins sharing a characteristic N-terminal domain (Merkle, 2003). The classical model of importin β

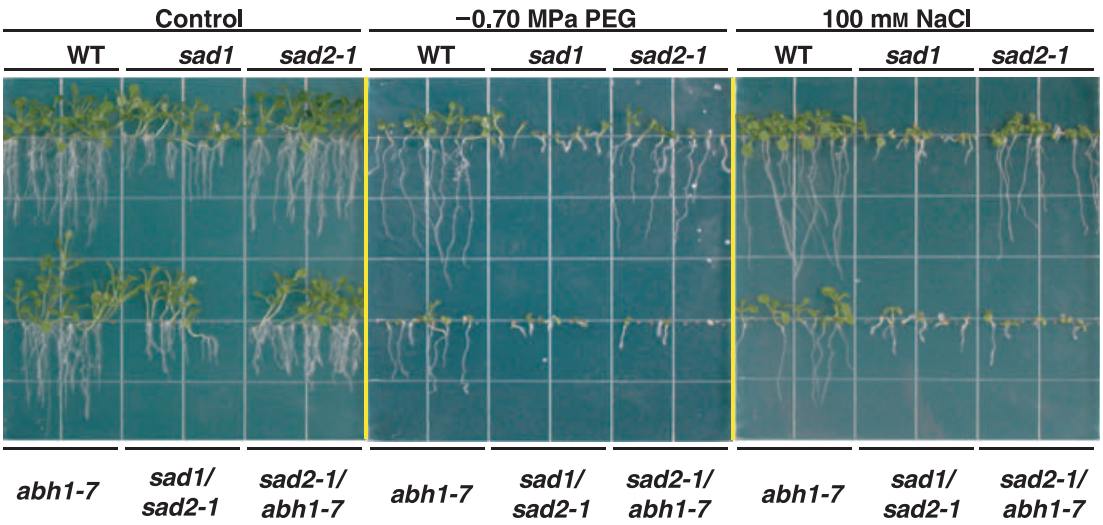

Figure 7. Stress sensitivity of wild type, *sad1*, *sad2-1*, *abh1-7* and double mutant seedlings.
Photographs were taken after 9 days of growth on control, low water potential (−0.7 MPa PEG) or salt (100 mM NaCl) media. All media contained 3% sucrose.

function is that importin β interacts, either directly or through the adaptor protein importin α, with cargo proteins containing a nuclear localization signal (NLS). Importin β then interacts with the nuclear pore complex and the cargo/importin α/importin β complex is translocated through the nuclear pore complex via a largely unknown mechanism (Goldfarb *et al.*, 2004; Merkle, 2003). Binding of RanGTP to importin α and importin β in the nucleoplasm causes releases of the cargo protein. The importins are then recycled to the cytoplasm and this is accompanied by RanGTP hydrolysis and release of RanGDP in the cytoplasm (Merkle, 2003). It is now known that proteins containing the importin β domain can function as either importins or transportins mediating nuclear protein import, or exportins which transport proteins out of the nucleus (Merkle, 2003).

Characterization of nuclear transport in plants has indicated that the major classes of proteins involved and their roles in transport are largely conserved compared to the mammalian model (Merkle, 2003). However, based on sequence comparison alone, it is not possible to establish clear functional orthologous relationships between importin β family proteins in Arabidopsis and similar proteins in yeast or human (Merkle, 2003). To date, few data on the function of specific transport proteins and their transported target proteins exist for plants. Thus, although SAD2 and At3g59020 are importin β family proteins, their actual role in transport and the cargo proteins that may be transported remain to be determined. SAD2 and At3g59020 are most similar to the human importin β family members RanBP7 and RanBP8 (Merkle, 2003). RanBP7 has been shown to mediate the import of ribosomal proteins (Jakel and Gorlich, 1998) and, in complex with importin β, histone H1 (Jakel *et al.*, 1999). RanBP8 forms heterodimers with importin β (Gorlich *et al.*, 1997) and is involved in the import of signal recognition particle19 (SRP19; Dean *et al.*, 2001).

Genetic analysis has established precedence for the involvement of plant nuclear transport proteins in development. The *HASTY* (*HST*) gene encodes an Arabidopsis exportin ortholog and an *hst* null mutant has several morphological phenotypes and an accelerated developmental program (Bollman *et al.*, 2003). In contrast, mutation of another Arabidopsis exportin, PAUSED (PSD), a ortholog of yeast exportin-t, causes delayed leaf initiation and transition to flowering (Hunter *et al.*, 2003). A *hst*/*psd* double mutant has a more severe phenotype than either single mutant (Hunter *et al.*, 2003), suggesting that these two exportins act on different pathways.

Possible roles of SAD1, SAD2 and ABH1 in ABA signaling

Possible positions of SAD1, SAD2 and ABH1 in ABA signaling are shown in Figure 8 based on the likely function of SAD2 in nuclear transport. Based on the increased ABA sensitivity of *sad2*, one strong possibility is that SAD2 is required for nuclear import of a negative regulator of ABA sensitivity (or nuclear export of a positive regulator). This may also require the participation of an importin α. In the double mutant analysis, the effects of *sad1* and *abh1-7* are epistatic to or additive with those of *sad2-1*. Thus, SAD1 and ABH1 may act downstream of SAD2 in the processing of mRNAs involved in ABA response. This could explain why *abh1-7* blocks the increased *RD29A:LUC* expression seen in *sad2-1*. However, the greater stress and ABA sensitivity of the *sad2-1/abh1-7* mutant suggests that the set of mRNAs affected by SAD2 and ABH1 may only partially overlap: more misregulated genes in the double mutant could lead to the greater stress sensitivity. There could also be an additive effect of the two mutants on the production of certain key mRNAs.

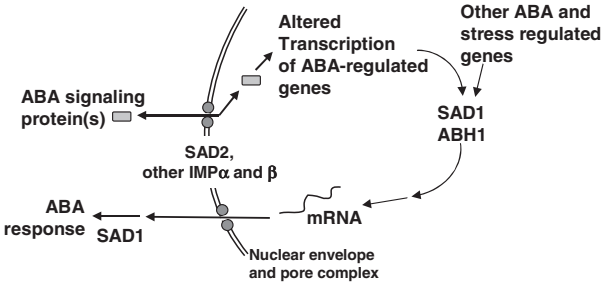

Figure 8. Model of the possible roles of SAD1, SAD2 and ABH1 in ABA signal transduction.
SAD2 may function in the nuclear localization of a negative regulator of ABA response (or nuclear export of a positive regulator) and could also function in export of other proteins or ABA-regulated RNAs. The process may also involve importin α and other importin β proteins, such as AT3G59020, that have more minor or more redundant roles. The transported regulator(s) may affect the expression of a set of ABA-regulated genes that also may require ABH1 and SAD1 for correct mRNA processing. Other ABA- or stress-inducible genes not affected by SAD2 may require ABH1 and/or SAD1 for correct expression. SAD1 may also act in the degradation of ABA- and stress-regulated mRNAs.

Similar reasoning can be applied to SAD1, except that the role of SAD1 (Figure 8) is more uncertain because of the many possible functions of SAD1. SAD1 may be involved in mRNA degradation or transport in addition to RNA processing (Xiong *et al.*, 2001b). The increased expression of *RD29A:LUC* in *sad1/sad2-1* again suggests that SAD1 and SAD2 either control different components of ABA signaling or have an additive effect on the production and translocation of the same signaling components.

The question of whether or not SAD2 and ABH1 (and SAD1) regulate the same genes is of particular interest because microarray analysis of ABA-induced changes in gene expression of both *abh1* (Hugouvieux *et al.*, 2001) and *sad2-1* (unpublished preliminary data of YG and J-KZ) found only a small number of genes with altered expression in either mutant. The only commonality we found between our data and the previously reported *abh1* microarray data was reduced expression of a homolog of the defense-related

protein thionin. We also found reduced expression of defensin genes and increased expression of two xyloglucan endotransglycosylase genes and a MYB-related transcription factor in *sad2-1* relative to wild type. Previous microarray analysis of *sad1* (Xiong *et al.*, 2001b) also revealed few changes in gene expression and we failed to observe any commonalities between the changes in gene expression in *sad1* and *sad2-1*. Additional microarray experiments, as well as other experiments that could identify the molecular targets of SAD2, SAD1 and ABH1, will be of future interest.

Experimental procedures

Mutant isolation and genetic analysis

The T-DNA mutagenized population in the *RD29A:LUC* background (ecotype C24) has been previously described (Xiong *et al.*, 2001a). This population was screened for altered *RD29A-LUC* expression in response to low temperature, exogenous ABA or salt treatment as described previously (Ishitani *et al.*, 1997). Unmutagenized C24 *RD29A:LUC* is referred to as 'wild type' throughout this study. The T-DNA line SALK_103333 (Alonso *et al.*, 2003) was obtained from the Arabidopsis Biological Resource Center (ABRC) and plants homozygous for the T-DNA insertion obtained by PCR screening (primers listed in Table S1). Soil-grown plants were grown in Metro-Mix 350 (Scott-Sierra Horticultural Products, Marysville, OH, USA) in growth chambers with 16-h light at 22°C, 8-h dark at 18°C and 70% relative humidity.

sad2-1 and *abh1-7* mutant plants were backcrossed to wild type and F_1 and F_2 seedlings tested for ABA sensitivity, luminescence and bialaphos resistance. To obtain double mutants, *sad1*, *sad2-1* and *abh1-7* were crossed and the resulting F_2 progeny were used to identify homozygous double mutant lines.

Hormone and stress sensitivity assay

Mutant and wild-type seeds were surface sterilized, plated on Murashige and Skoog (MS) medium with 3% sucrose and 1.2% agar and stratified at 4°C for 3 days before transfer to 22°C under continuous light for germination and growth. For tests of the ABA sensitivity of germination and seedling growth, seeds were plated directly (before stratification) onto media containing the ABA concentrations indicated in the text or figures. For determination of the sensitivity of root elongation to hormone treatment seeds were germinated on hormone-free media, 4-day-old seedlings were transferred to hormone-containing media and root elongation was quantified 6 days later. Hormone treatments tested were: epibrassinosteroid (1.5 μM), gibberelic acid (100 μM), indol-3-acetic acid (0.01 μM), 2,4-dichlorophenoxyacetic acid (0.01 μM), 6-benzyladenine (0.1 μM) or aminocyclopropane-1-carboxylic acid (1–4 μM). No difference in root elongation between mutant and wild type was observed for any of these treatments.

For determination of low water potential or salt sensitivity, seedlings were plated directly (before stratification) onto salt-containing media (100 mM NaCl) or PEG-infused agar plates (−0.7 MPa) and seedling growth observed 7 or 9 days after the end of the stratification period. Polyethylene glycol-infused plates were prepared as described by Verslues and Bray (2004) with the modification that 0.5% or 3% sucrose was added to the media to be consistent with the other characterization of *sad2-1*.

Gene expression analysis

For RNA blot analysis, total RNA was extracted from wild-type roots, leaves, stems, siliques and flowers of Arabidopsis or 7-day-old wild-type and *sad2-1* seedlings treated with low temperature, ABA, NaCl, dehydration or PEG (concentrations used or duration of dehydration are described in the text or figure legends). Thirty micrograms of RNA per lane was used for RNA blot analysis. Blotting procedures and the *LUC*, *RD29A* and *ACTIN* probes were as previously described (Guo *et al.*, 2002). For *SAD2* RNA blots, the full-length cDNA was used as the probe.

For RT-PCR and real-time PCR analysis, total RNA was extracted using a RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). First strand cDNA was synthesized with 1 or 3 μg of total RNA (3 μg of RNA was used for RT-PCR of *SAD2* and *At3g59020* because of the low expression of these genes). Real-time PCR quantification of *RAB18*, *COR15* and *NCED3* expression was performed essentially as described by Tan *et al.* (2003) using a Sequence Detection System 7700 instrument (Applied Biosystems, Foster City, CA, USA). Quantification of copy number for each gene was done by amplifying a 400–600 bp portion of each gene containing the binding sites for the real-time PCR primers and Taq-man probe. The amplified DNA fragment was purified, quantified spectrophotometrically and diluted appropriately to generate a standard curve for calculation of the copy number of each transcript. Sequences of the oligonucleotides used for RT-PCR and real-time PCR analysis are given in Table S1.

Cloning and complementation of SAD2 and ABH1

Deoxyribonucleic acid flanking the left border of the inserted T-DNA in *sad2-1* plants was isolated by plasmid rescue (Mathur *et al.*, 1998) using *Bam*HI-digested genomic DNA. For *sad2-1* complementation, a 9302 bp DNA fragment of the *SAD2* gene including 1033 bp upstream of the translation initiation codon and 1436 bp downstream of the translation stop codon was digested from BAC clone T9H9 using *Eco*RI and *Spe*I. The *SAD2* gene fragment was cloned into the pCAMBIA 1380 vector and transformed into *sad2-1* plants using the floral dip method.

Deoxyribonucleic acid flanking the T-DNA left border in *abh1-7* plants was isolated by thermal asymmetric interlaced PCR as previously described (Zhu *et al.*, 2002).

SAD2:GUS *and* GFP-SAD2 *construction and analysis*

A SAD2:GUS construct was generated by PCR amplifying a 1282 bp fragment of the SAD2 promoter (primers sequences are given in Table S1) and cloning into the pCAMBIA 1391Z vector between the *Eco*RI and *Bam*HI sites. After transformation, 10 independent transgenic lines (T_2) were tested for GUS activity. Tissue was stained by incubating in GUS staining buffer overnight at 37°C followed by clearing with 70% ethanol.

An N-terminal fusion of GFP to *SAD2* was generated and used for transient expression following the procedures of Dong *et al.* (2001) with the modification that leaf epidermal cells were used for expression. GFP images were collected on a Leica SP2 confocal microscope.

Acknowledgements

We thank Rebecca Stevenson for assistance with the manuscript and ABRC for providing the *at3g59020* and *sad2-2* T-DNA lines. We also thank Liming Xiong (Danforth Plant Science Center, St Louis,

MO, USA) for assistance with the *sad2-2* T-DNA line. This work was funded by National Science Foundation grant IBN-0420152 and National Institutes of Health grant 5R01GM059138. PEV was supported in part by an NIH post-doctoral fellowship (1F32GM074445).

Supplementary Material

The following supplementary material is available for this article online:

Figure S1. *RD29A:LUC* expression in wild type, *sad2-1* and complemented *sad2-1*.

Figure S2. Phenotypic characterization of a second T-DNA allele of *sad2* (*sad2-2*).

Figure S3. Alignment of SAD2 and At3G59020.

Table S1 Oligonucleotides used in this study

This material is available as part of the online article from http://www.blackwell-synergy.com

References

Alonso, J.M., Stepanova, A.N., Leisse, T.J. *et al.* (2003) Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science*, **301**, 653–657.

Bezerra, I.C., Michaels, S.D., Schomburg, F.M. and Amasino, R.M. (2004) Lesions in the mRNA cap-binding gene ABA HYPERSENSITIVE 1 suppress FRIGIDA-mediated delayed flowering in Arabidopsis. *Plant J.* **40**, 112–119.

Bollman, K.M., Aukerman, M.J., Park, M.Y., Hunter, C., Berardini, T.Z. and Poethig, R.S. (2003) HASTY, the Arabidopsis ortholog of exportin 5/MSN5, regulates phase change and morphogenesis. *Development*, **130**, 1493–1504.

Brady, S.M., Sarkar, S.F., Bonetta, D. and McCourt, P. (2003) The ABSCISIC ACID INSENSITIVE 3 (ABI3) gene is modulated by farnesylation and is involved in auxin signaling and lateral root development in Arabidopsis. *Plant J.* **34**, 67–75.

Bray, E.A. and Beachy, R.N. (1985) Regulation by ABA of β-conglycinin expression in cultured developing soybean cotyledons. *Plant Physiol.* **79**, 746–750.

Cutler, S., Ghassemian, M., Bonetta, D., Cooney, S. and McCourt, P. (1996) A protein farnesyl transferase involved in abscisic acid signal transduction in Arabidopsis. *Science*, **273**, 1239–1241.

Dean, K.A., von Ahsen, O., Gorlich, D. and Fried, H.M. (2001) Signal recognition particle protein 19 is imported into the nucleus by importin 8 (RanBP8) and transportin. *J. Cell Sci.* **114**, 3479–3485.

Dong, C.H., Kost, B., Xia, G.X. and Chua, N.H. (2001) Molecular identification and characterization of the Arabidopsis AtADF1, AtADF5 and AtADF6 genes. *Plant Mol. Biol.* **45**, 517–527.

Fahrenkrog, B., Koser, J. and Aebi, U. (2004) The nuclear pore complex: a jack of all trades? *Trends Biochem. Sci.* **29**, 175–182.

Finkelstein, R.R., Gampala, S.S.L. and Rock, C.D. (2002) Abscisic acid signaling in seeds and seedlings. *Plant Cell*, **14**, S15–S45.

Freedman, N.D. and Yamamoto, K.R. (2004) Importin 7 and importin alpha/Importin beta are nuclear import receptors for the glucocorticoid receptor. *Mol. Biol. Cell* **15**, 2276–2286.

Goldfarb, D.S., Corbett, A.H., Mason, D.A., Harreman, M.T. and Adam, S.A. (2004) Importin alpha: a multipurpose nuclear-transport receptor. *Trends Cell Biol.* **14**, 505–514.

Gorlich, D., Dabrowski, M., Bischoff, F.R., Kutay, U., Bork, P., Hartmann, E., Prehn, S. and Izaurralde, E. (1997) A novel class of RanGTP binding proteins. *J. Cell Biol.* **138**, 65–80.

Guo, Y., Xiong, L.M., Ishitani, M. and Zhu, J.-K. (2002) An Arabidopsis mutation in translation elongation factor 2 causes superinduction of CBF/DREB1 transcription factor genes but blocks the induction of their downstream targets under low temperatures. *Proc. Natl Acad. Sci. USA*, **99**, 7786–7791.

Han, M.H., Goud, S., Song, L. and Fedoroff, N. (2004) The Arabidopsis double-stranded RNA-binding protein HYL1 plays a role in microRNA-mediated gene regulation. *Proc. Natl Acad. Sci. USA*, **101**, 1093–1098.

Hugouvieux, V., Kwak, J.M. and Schroeder, J.I. (2001) An mRNA cap binding protein, ABH1, modulates early abscisic acid signal transduction in Arabidopsis. *Cell*, **106**, 477–487.

Hugouvieux, V., Murata, Y., Young, J.J., Kwak, J.M., Mackesy, D.Z. and Schroeder, J.I. (2002) Localization, ion channel regulation, and genetic interactions during abscisic acid signaling of the nuclear mRNA cap-binding protein, ABH1. *Plant Physiol.* **130**, 1276–1287.

Hunter, C.A., Aukerman, M.J., Sun, H., Fokina, M. and Poethig, R.S. (2003) PAUSED encodes the Arabidopsis exportin-t ortholog. *Plant Physiol.* **132**, 2135–2143.

Ishitani, M., Xiong, L., Stevenson, B. and Zhu, J. (1997) Genetic analysis of osmotic and cold stress signal transduction in *Arabidopsis*: interactions and convergence of abscisic acid-dependent and abscisic acid-independent pathways. *Plant Cell*, **9**, 1935–1949.

Jakel, S. and Gorlich, D. (1998) Importin beta, transportin, RanBP5 and RanBP7 mediate nuclear import of ribosomal proteins in mammalian cells. *EMBO J.* **17**, 4491–4502.

Jakel, S., Albig, W., Kutay, U., Bischoff, F.R., Schwamborn, K., Doenecke, D. and Gorlich, D. (1999) The importin beta/importin 7 heterodimer is a functional nuclear import receptor for histone H1. *EMBO J.* **18**, 2411–2423.

Lopez-Molina, L., Mongrand, S., Kinoshita, N. and Chua, N.H. (2003) AFP is a novel negative regulator of ABA signaling that promotes ABI5 protein degradation. *Genes Dev.* **14**, 410–418.

Lu, C. and Fedoroff, N. (2000) A mutation in the arabidopsis HYL1 gene encoding a dsRNA binding protein affects responses to abscisic acid, auxin, and cytokinin. *Plant Cell*, **12**, 2351–2365.

Mathur, J., Szabados, L., Schaefer, S., Grunenberg, B., Lossow, A., Jonas-Straube, E., Schell, J., Koncz, C. and Koncz-Kalman, Z. (1998) Gene identification with sequenced T-DNA tags generated by transformation of Arabidopsis cell suspension. *Plant J.* **13**, 707–716.

Merkle, T. (2003) Nucleo-cytoplasmic partitioning of proteins in plants: implications for the regulation of environmental and developmental signalling. *Curr. Genet.* **44**, 231–260.

Osakabe, Y., Maruyama, K., Seki, M., Satou, M., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2005) Leucine-rich repeat receptor-like kinase1 is a key membrane-bound regulator of abscisic acid early signaling in Arabidopsis. *Plant Cell*, **17**, 1105–1119.

Papp, I., Mur, L.A., Dalmadi, A., Dulai, S. and Koncz, C. (2004) A mutation in the Cap Binding Protein 20 gene confers drought tolerance to Arabidopsis. *Plant Mol. Biol.* **55**, 679–686.

Razem, F.A., El-Kereamy, A., Abrams, S.R. and Hill, R.D. (2006) The RNA-binding protein FCA is an abscisic acid receptor. *Nature*, **439**, 290–294.

Schwartz, S.H., Qin, X.Q. and Zeevaart, J.A.D. (2003) Elucidation of the indirect pathway of abscisic acid biosynthesis by mutants, genes, and enzymes. *Plant Physiol.* **131**, 1591–1601.

Smalle, J., Kurepa, J., Yang, P.Z., Emborg, T.J., Babiychuk, E., Kushnir, S. and Vierstra, R.D. (2003) The pleiotropic role of the 26S proteasome subunit RPN10 in Arabidopsis growth and development supports a substrate-specific function in abscisic acid signaling. *Plant Cell*, **15**, 965–980.

Tan, B.C., Joseph, L.M., Deng, W.T., Liu, L.J., Li, Q.B., Cline, K. and McCarty, D.R. (2003) Molecular characterization of the *Arabid-*

opsis 9-*cis* epoxycarotenoid dioxygenase gene family. *Plant J.* **35**, 45–56.

Verslues, P.E. and Bray, E.A. (2004) *LWR1* and *LWR2* are required for osmoregulation and osmotic adjustment in Arabidopsis. *Plant Physiol.* **136**, 2831–2842.

Xiong, L.M., Lee, B.H., Ishitani, M., Lee, H., Zhang, C.Q. and Zhu, J.-K. (2001a) FIERY1 encoding an inositol polyphosphate 1-phosphatase is a negative regulator of abscisic acid and stress signaling in Arabidopsis. *Genes Dev.* **15**, 1971–1984.

Xiong, L.M., Gong, Z.Z., Rock, C.D., Subramanian, S., Guo, Y., Xu, W.Y., Galbraith, D. and Zhu, J.-K. (2001b) Modulation of abscisic acid signal transduction and biosynthesis by an Sm-like protein in Arabidopsis. *Dev. Cell* **1**, 771–781.

Xu, L. and Massague, J. (2004) Nucleocytoplasmic shuttling of signal transducers. *Nat. Rev. Mol. Cell Biol.* **5**, 209–219.

Yalovsky, S., Kulukian, A., Rodriguez-Concepcion, M., Young, C.A. and Gruissem, W. (2000) Functional requirement of plant farnesyltransferase during development in Arabidopsis. *Plant Cell*, **12**, 1267–1278.

Zeevaart, J.A.D. (1999) Abscisic acid metabolism and its regulation. In *Biochemistry and Molecular Biology of Plant Hormones* (Hall, M. and Libbenga, K. eds). Amsterdam: Elsevier Science B.V., pp. 189–207.

Zhu, J.-K. (2002) Salt and drought stress signal transduction in plants. *Ann. Rev. Plant Biol.* **53**, 247–273.

Zhu, J., Gong, Z., Zhang, C., Song, C.-P., Damsz, B., Inan, G., Koiwa, H., Zhu, J.-K., Hasegawa, P.M. and Bressan, R.A. (2002) OSM1/SYP61: a syntaxin protein in Arabidopsis controls abscisic acid-mediated and non-abscisic acid-mediated responses to abiotic stress. *Plant Cell*, **14**, 3009–3028.

