Highlights:

The ABCG25 and ABCG40 ABA-transporters catalyze ATP-dependent efflux of ABA from vascular tissues and uptake by target tissues, respectively.

Regulation of ABI3 and ABI5 stability by the 26S proteasome plays an important role in ABA signaling during germination and early seedling growth.

AIP2, KEG, PRT6 and CUL4-based ubiquitin E3 ligases negatively regulate ABA signaling, whereas SDIR1 and RH2a are positive regulators.

The expression of ABFs/AREBs is regulated by WRKY transcription factors.
News on ABA transport, protein degradation and ABFs/WRKYs in ABA signaling

Regina Antoni, Lesia Rodriguez, Miguel Gonzalez-Guzman, Gaston A Pizzio and Pedro L Rodriguez

Addresses

Instituto de Biologia Molecular y Celular de Plantas, Consejo Superior de Investigaciones Cientificas-Universidad Politecnica de Valencia. Avd de los Naranjos, ES-46022 Valencia, Spain

Corresponding author: Rodriguez, Pedro L (prodriguez@ibmcp.upv.es)
The recent identification of abscisic acid (ABA) transporters provides an important insight into the delivery of ABA from the vascular system and its uptake by target cells. A putative connection with PYR/PYL receptors is envisaged, linking ABA uptake and intracellular perception by a fast and efficient mechanism. Downstream signaling of the core pathway involves regulation of ABA-responsive element binding factors (ABFs/AREBs) through phosphorylation, ubiquitination and sumoylation in the case of ABI5. Several E3 ligases appear to regulate ABA signaling either positively or negatively, although relatively few targets are known yet. ABFs/AREBs are themselves subjected to transcriptional regulation, and some transcription factors harboring the WRKY domain (WRKYs) appear to regulate their expression through W-box sequences present in the promoters of ABFs/AREBs.
Introduction

The phytohormone abscisic acid (ABA) represents a key signal to regulate plant growth and development as well as plant response to abiotic and biotic stress [1]. In the plant field, the pivotal role played by ABA to coordinate the plant adaptive response under drought stress and hence potential applications in agriculture have led to numerous studies focused on the elucidation of ABA perception and downstream signaling. Challenging our perspective as plant biologists, the discovery of ABA in humans and its prophylactic and therapeutic efficacy in mouse models of diabetes and atherosclerosis have further extended the interest in this animal/plant molecule [2*,3*]. In 2009, the plant family of PYR/PYL/RCAR ABA receptors was discovered and its connection with key elements of the pathway, i.e. PP2Cs and SnRK2s, was established (Figure 1). The module receptor-ABA-phosphatase controls phosphorylation signaling cascades in a ligand-dependent manner through regulation of ABA-activated SnRK2s and in concert with other kinases, e.g. calcium-dependent kinases (CPKs/CDPKs) (Figure 1). These findings have been extensively reviewed recently and they will not be the main topic of this review [1,4–10]. Instead, we will focus on other emerging aspects of the ABA pathway, such as the identification of ABA transporters, an update on the effect of protein degradation/stability in ABA signaling, the connection between ABFs/WRKYs transcription factors (TFs) as well as new reports on Mg-chelatase function.

Efflux and uptake of ABA

Since ABA biosynthesis occurs predominantly in vascular parenchyma cells and ABA has systemic effects, a requirement for efficient intercellular transport of ABA, beyond that of passive diffusion, had been envisaged [11–13]. For instance, ABA2, AAO3 and NCED3, key enzymes of the ABA biosynthetic pathway, are expressed in specific areas of vascular tissues, which suggested the existence of a transport system to deliver ABA to target tissues and cells [11–13]. In 2010, two ABA transporters were identified by genetic screenings [14**,15**]. A search for Arabidopsis ABA-hypersensitive mutants in germination and seedling growth led to the identification of the abcg25 mutant [14**]. The ABCG25 gene, which encodes an ATP-binding cassette (ABC) transporter, is expressed mainly in vascular tissues and the protein is localized at the plasma membrane (Figure 1). A transport assay with vesicle membranes obtained in transfected
insect cells indicated that ABCG25 might have ATP-dependent ABA-efflux activity in plant cells. Indeed, overexpression of ABCG25 in Arabidopsis led to reduced sensitivity to ABA-mediated inhibition of growth, probably because the cells remove ABA by active transport, and reduced water loss, probably because this transporter facilitates the delivery of ABA to guard cells.

ABA delivery from vascular tissues to the apoplast of guard cells might be connected with ABA uptake from the apoplast to the cytosol through another plasma membrane-localized transporter, ABCG40/PDR12 (Figure 1). ABCG40 was identified by direct screening for potential ABA transporters in the PDR-type subfamily of ABC transporters [15●●]. To this end, seed germination and stomatal response were analyzed in 13 out of 15 knockout mutants (abcg29-41), and as a result, the mutant abcg40 was identified as having marked differences with respect to wild type (wt). Stomata of abcg40 showed reduced stomatal closure and lower inhibition of stomatal opening in the presence of ABA, and therefore, abcg40 plants showed enhanced wilting under drought stress and reduced increase in leaf temperature in response to ABA. ABCG40 function is also required in cell types other than guard cells, although gene expression in guard cells was higher than in mesophyll cells. Thus, experiments conducted in rosette tissue also showed delayed and reduced expression of three ABA-responsive genes in abcg40. Results obtained with abcg40 seeds are more difficult to interpret, because although these seeds were less-sensitive to inhibition of germination mediated by exogenous ABA, they also showed faster germination on medium lacking ABA. Finally, biochemical experiments in the yeast heterologous system and tobacco cell suspensions showed that ABCG40 is a high-affinity ($K_m = 1 \mu M$) and specific uptake ABA transporter.

Although both transporters belong to the large ABC subfamily G, they are grouped in different branches because of an important structural difference, i.e. ABCG25 belongs to the branch of half-size transporters (AtABCG1–28) and ABCG40 to that of full-size transporters (AtABCG29–43) [16]. Since ABCG25 belongs to a large gene family, functional redundancy might explain why the abcg25 mutant does not show aerial phenotypes. However, ABCG40 also belongs to a gene family and, nevertheless, the stomatal response of abcg40 was notably affected. Since abcg40 also affects ABA-response of mesophyll cells, the authors could assess the contribution of ABCG40 to ABA uptake in Arabidopsis protoplasts, concluding that this gene product is
the major ABA importer in leaf-cell protoplasts. Moreover, an apparent paradox is now solved. The pH-dependent diffusion of undissociated ABA is a component of ABA uptake, which would be markedly reduced under drought stress that increases the pH of xylem sap [17]. The discovery of ABCG40 offers a reasonable alternative, under drought-stress less ABA would be nonspecifically trapped by passive diffusion in nontarget tissue and more ABA would be available for pH-independent uptake [15**].

**Protein degradation and transcriptional regulation**

The ubiquitin/26S proteasome pathway plays a key role in the perception and transmission of environmental and hormonal signals [18]. For instance, perception of auxins, jasmonates and gibberellins are closely linked to this pathway, and ethylene and ABA signaling also involve components of this protein degradation pathway [19]. Either negative or positive transcriptional regulators of these hormonal pathways are targets of the 26S proteasome, and therefore, inactivation of transcriptional repressors or ceasing degradation of activators, respectively, leads to hormone signaling. ABA signaling is affected in different mutants that show lesions either in a regulatory subunit of the 26S proteasome [20], different E3 ligases [21–24**,25**] or substrate receptors of E3 ligases [26**] (Figure 2). Additionally, sumoylation, which can act competitively on targets regulated by ubiquitination to regulate protein stability, also affects ABA signaling through negative regulation of ABI5 activity [27,28**]. Indeed, pioneering work on the regulation of ABI5 protein stability was crucial to link the 26S proteasome and ABA signaling [29].

Mutants of some proteolysis-related components have a pleiotropic effect including impaired ABA signaling. For instance, the rpn10 mutant, which is impaired in a subunit of the 19S regulatory particle of the 26S proteasome, is affected in a number of processes and it shows hypersensitivity to ABA in seed germination and root growth assays as well as stabilization of the short-lived ABI5 transcription factor [20]. Pleiotropic effects, including ABA hypersensitivity, were also found in the siz1 mutant, which was impaired in a SUMO E3 ligase. SIZ1 negatively regulates ABA signaling through sumoylation of ABI5, which inactivates the protein and prevents its proteasome-mediated degradation [28**]. ABI5 transcript accumulation, protein stability and protein phosphorylation are highly regulated by ABA [29]. In the absence
of ABA, ABI5 is degraded to allow germination and postgerminative growth, whereas ABA induces ABI5 stabilization, when applied between 48 and 60 h poststratification, to prevent early growth under osmotic stress conditions [29]. The RING E3 ligase KEG is required for ABI5 degradation under normal growth conditions and ABA causes ABI5 accumulation by promoting KEG degradation [22,26]. Phosphorylation of KEG is required for its ABA-induced degradation, which opens a possible link with the SnRK2s of the core ABA signaling pathway.

ABI5 seems to be a highly courted TF, since also CUL4-based E3 ligases regulate its stability through the proteins DWA1 and DWA2, which are the components of the ligase that mediate substrate recognition [30]. Finally, another element that regulates ABI5 protein levels is ABI five binding protein (AFP); however, its mechanism of action is not yet clear. AFP belongs to a small family of proteins, AFP1–4, that are able to interact with ABI5 [31,32]. Initially, it was proposed that AFP might promote ABI5 degradation by the 26S proteasome [31]; however, AFP is not an E3 ligase. Instead, a characteristic feature of AFP1–4 proteins is the presence of an ethylene-responsive element binding factor-associated amphiphilic repression (EAR) motif at the N-terminus. The EAR motif is a hallmark of transcriptional repressors such as AUX/IAA and NINJA proteins, which function as adaptor proteins to recruit the Groucho/Tup1-type co-repressor TOPELESS (TPL) [33]. Interaction of AFP2 and AFP3 with TPL has been observed by yeast two-hybrid assays, which suggests the tempting hypothesis that some AFP proteins and TPL (or TPL-related proteins) form a high-molecular mass complex, acting as transcriptional repressors of ABA signaling by blockade of ABI5 function [33].

ABI3 is another target of the 26S proteasome and the RING E3 ligase AIP2 is a negative regulator of ABA signaling that promotes ABI3 degradation [21,34]. Thus, during vegetative growth, ABA promotes ABI3 degradation through enhancement of AIP2 function [21]. Conversely, ABA promotes the accumulation of ABI3 during seed maturation and the time period when post-germination growth arrest occurs, via transcriptional and post-translational mechanisms. PRT6 (Proteolysis6) is another type of E3 ligase that negatively regulates seed sensitivity to ABA [24]. PRT6 is an N-recognin E3 ligase that recognizes amino-terminal destabilizing residues of proteins, targeting them for degradation at the 26S proteasome. Mutant prt6 seeds are very hypersensitive to ABA-mediated inhibition of seed germination and according to
genetic interactions with various *abi* mutants, it has been hypothesized that PRT6 might degrade a positive regulator of ABA signaling during seed after-ripening. The E3 ligases described up to now are genetically defined as negative regulators of ABA signaling. However, other E3 ligases, such as the RING finger E3 ligases SDIR1 (salt- and drought-induced ring finger1) and RHA2a (ring-H2), are genetically characterized as positive regulators because *sdirl* and *rha2a* mutants show reduced sensitivity to ABA in seed germination and early seedling growth assays, and in the case of *sdirl*, also reduced stomatal closure by ABA [23,25•]. Therefore, these ligases might be involved in the degradation of transcriptional repressors or negative regulators of ABA signaling.

**ABFs, WRKYs and Mg-chelatase in ABA signaling**

Different families of transcription factors regulate ABA signaling in a positive or negative manner [1]. Among the best known positive regulators of ABA signaling and key targets of SnRK2s are the bZIP-type ABFs/AREBs, which recognize the ABA-responsive elements in the promoters of ABA-inducible genes. A comprehensive analysis of the AREB1/ABF2, AREB2/ABF4 and ABF3 TFs has been performed through the generation of multiple combinations of mutants [35•]. During seed germination, none of the mutants showed different sensitivity to ABA compared to wt. However, vegetative responses to ABA were particularly impaired in the triple mutant *areb1 areb2 abf3*, as illustrated by its resistance to ABA-mediated inhibition of root growth and diminished expression of stress-responsive genes. Compared to this, the triple mutant only shows a modest increase in water-loss rate compared to wt, indicating that other targets of ABA-activated SnRK2s, different than bZIP-type AREB/ABFs, are mostly responsible for the regulation of stomatal aperture.

Different rice and *Arabidopsis* WRKY TFs have been implicated in ABA signaling [36–38•,39•,40•]. Usually, WRKYs have been described as TFs inducible by pathogen infection or salicylic acid treatment, and indeed, a large number of pathogen-inducible genes contain W-box sequences that are recognized by WRKY proteins. Interestingly, ABA signaling genes as *ABF2, ABF4, ABI4* or *ABI5* contain W-box sequences in their promoter regions [38•,40•]. Thus, WRKY63 positively regulates expression of *ABF2* through binding to W-boxes of its promoter (Figure 3), but
intriguingly, wrky63 shows enhanced sensitivity to ABA during seed germination and seedling growth, whereas it is ABA-hyposensitive for stomatal closure [38*]. Using ChIP analysis, Shang et al. [40*] have shown that WRKY40 binds the promoters of _ABF2_, _ABF4_, _ABI4_ and _ABI5_, and for instance, represses _ABI5_ expression (Figure 3). Accordingly, the wrky40 mutant shows enhanced sensitivity to ABA-mediated inhibition of germination and early seedling growth. In agreement, Chen et al. [39*] obtained similar results during the characterization of wrky40. In contrast, conflicting results were obtained with respect to ABA sensitivity of wrky18 and wrky60 mutants, which are defined as positive regulators of ABA signaling [39*], whereas Shang et al. [40*] catalogued them as repressors. Finally, this article poses a model for Mg-chelatase H subunit (CHLH/ABAR)-mediated ABA signaling that involves recruitment of WRKY40 at the cytosol upon ABA perception by the cytosolic tail of CHLH [40*]. This model faces important criticisms since two groups have failed to show ABA binding by barley or Arabidopsis CHLH [41*, 42*], apparently the carboxylate group of ABA, which is required for bioactivity, is not required for ABA binding by CHLH [43, 44*] and finally, no alteration in regulation of stomatal aperture was reported in any of the single or combined wrky mutants [40*]. In spite of this controversy, it seems well supported that CHLH affects ABA signaling in stomatal guard cells, since impairment of its function in RNAi lines [45, 46**] or the missense mutants _cch_ (encoding chlhp642l) [45] and _rtl1_ (encoding chlhL690F) [42*] led to enhanced water-loss and lack of ABA-induced stomatal closing. Since another mutant impaired in a different subunit of Mg-chelatase, CHLI, shows impaired stomatal closure, it has been suggested that the Mg-chelatase complex as a whole plays an indirect role in ABA signaling, likely through regulation of Ca²⁺ mobilization from chloroplastic stores [42*]. Structural evidence supporting ABA-binding by CHLH would be a definitive answer to the above described controversy.
Conclusions

The recent identification of PYR/PYL intracellular ABA-receptors nicely matches with the discovery of an active transport system for ABA-uptake, which allows fast delivery of ABA to target cells for efficient inactivation of clade A PP2Cs through PYR/PYL receptors. It somehow seemed ABA signaling was inefficiently designed, spending so much investment on the core pathway, i.e. receptors-phosphatases-kinases, and depending exclusively on passive diffusion for intracellular ABA delivery. In addition to protein phosphorylation, regulation of protein stability by the 26S proteasome is an important mechanism for ABA signaling, particularly during germination and early seedling growth. Several E3 ligases are involved in this process, acting either positively or negatively. Additionally, a few E3 ligase mutants, e.g. sdirl and dwal dwa2, are also known to be affected in the regulation of stomatal aperture, and this phenotype can’t be explained with the reduced number of targets identified so far. Therefore, an important question for the future is the identification of additional targets of E3 ligases beyond of ABI3 and ABI5. Finally, transcriptional regulation of ABFs/AREBs by WRKYs is a novel finding in the complex regulation of gene expression in response to ABA.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

● of special interest

●● of outstanding interest


● The authors demonstrate the presence of free and conjugated ABA in human granulocytes and increase of free ABA after heat stress. ABA stimulates phagocytosis by human granulocytes, as well as ROS and NO production. ABA signaling involves activation of ADP-ribosyl cyclase, and a consequent increase of intracellular Ca²⁺.


● Current prospects on the effect of ABA as stimulator of insulin release from human pancreatic cells and its use as an anti-diabetic drug.


● Identification of ABC25 as a plasma membrane ABA exporter. Expression of the gene was localized in vascular tissues, which suggest a role in ABA efflux at the site of ABA biosynthesis. abc25 seeds are ABA-hypersensitive; however abcg25 plants do not show aerial phenotypes. The ABA transport assay was performed with inside-out vesicles of transfected insect cells.

**Identification of ABCG40 as a plasma membrane ABA uptake transporter.** Mutant *abcg40* is defective in stomatal closure, expression of ABA-responsive genes and *abcg40* seeds are less sensitive to ABA-mediated inhibition of germination, indicating that active ABA uptake is required for efficient ABA signaling. Expression in yeast and tobacco cells as well as studies in *Arabidopsis* mesophyll cells were conducted to measure ABA uptake transporter activity.


17. Wilkinson S, Davies WJ: **Xylem sap pH increase: a drought signal received at the apoplastic face of the guard cell that involves the suppression of saturable abscisic acid uptake by the epidermal symplast.** *Plant Physiol* 1997, 113:559-573.


**This work provides evidence for the involvement of the N-end rule pathway of protein degradation in ABA signaling during seed germination and establishment. The authors identified prt6 as a mutant locus that, after-ripening, shows reduced germination and extreme ABA-hypersensitive inhibition of germination. PRT6 encodes an N-recognin E3 ligase that promotes proteolysis of proteins containing N-terminal basic amino acids. Epistasis analysis with abi3 and abi5 mutants suggest that PRT6 functions upstream of these TFs. PRT6 must promote degradation of a key positive regulator of ABA signaling during the after-ripening period, removing ABA sensitivity to promote germination. This gives a molecular explanation for the ABA desensitizing step that takes place during seed after-ripening.**


**This work and [23] identified E3 ligases that act as positive regulators of ABA signaling, and therefore, are supposed to degrade negative regulators of the pathway. Alternatively, these...**
ligases might activate positive regulators by monoubiquitination, which, in contrast to polyubiquitination, might lead to enhanced activity of the target. Whereas RHA2a only affects ABA signaling during germination and early seedling growth, SDI1 also affects the stomatal response. Target proteins of these ligases have not been identified yet.


This work extends the finding reported in [22]. KEG, the E3 ligase that ubiquitinates ABI5, is subjected to ABA-induced self-ubiquitination and proteasomal degradation. Phosphorylation of KEG, either by auto-phosphorylation or another staurosporine-sensitive kinase, is required for ABA-induced ubiquitination of KEG. How the ABA signal is transduced to KEG remains to be investigated.


Along with [27], this work provides evidence that sumoylation negatively regulates ABA signaling. The SUMO E3 ligase SIZ1 attenuates ABA signaling through transient inactivation of ABI5. siz1 mutants show enhanced ABA-mediated inhibition of seed germination and primary root growth as well as hyperinduction of ABA-responsive genes. ABI5 was less abundant in a siz1 mutant, suggesting that sumoylation prevents ABI5 protein degradation.


CULLIN-dependent E3 ligases had not been known to be involved in ABA signaling previously to this work. DWA1/2 mediate substrate recognition by CUL4-based E3 ligases, as F-box proteins do in CUL1-based E3 ligases (the well known SCF E3 ligases). Mutants dwa1 dwa2 are hypersensitive to ABA-mediated inhibition of germination, show higher levels of ABI5 and reduced water-loss compared to wt. A knockdown cul4 mutant is also hypersensitive to ABA. It is intriguing how KEG and DWA1/2 recognize the same ABI5 substrate and what targets of DWA1/2 proteins regulate water loss.


An indirect but important hint to elucidate the molecular function of AFP proteins in ABA signaling (see also [31,32]). This work reveals certain analogies between NINJA and AFP proteins, particularly their interaction with TOPELESS (TPL) through the EAR motif. Since NINJA acts as a transcriptional repressor of jasmonate signaling by connecting the co-repressor TPL to JAZ repressors, the authors suggest an analogous model for ABA signaling, where AFPs act as adaptor proteins to inhibit ABI5 function via TPL.


A triple areb1 areb2 abf3 mutant was generated and its ABA-response analyzed. Whereas ABA-sensitivity during seed germination was similar to wt, root growth was very resistant to ABA in the triple mutant. Water loss rate was slightly higher than wt, but the difference gradually disappeared. Instead, ABA-responsive gene expression was remarkably impaired in the triple mutant. Therefore, the enhanced drought sensitivity of the triple mutant is mostly due to impaired expression of stress-responsive genes than to altered transpiration rate.


The first report of a wrky mutant, namely wrky63/abo3, that shows altered ABA signaling both in seeds and stomata. Intriguingly, whereas wrky63/abo3 shows enhanced sensitivity of seed germination and root growth to ABA, the mutation impairs ABA-induced stomatal closure. WRKY63/ABO3 binds to the W-box sequences of the ABF2 promoter and positively regulates its expression.


This article should be compared with [40**] since both analyze the ABA sensitivity of wrky18, wrky40 and wrky60 mutants, and curiously, conflicting results are obtained. Thus, this work shows that wrky18 and wrky60 show reduced sensitivity to ABA in germination assays, whereas an opposite phenotype is found in [40**]. Moreover, a wrky18 wrky40 wrky60 triple mutant behaves as wt in this work, whereas it is ABA-hypersensitive in [40*].


This work shows that the magnesium chelatase H subunit CHLH/ABAR interacts through its cytosolic C terminus with a group of WRKY transcription factors, namely WRKY18, 40 and 60, and ABA enhances the interaction ABAR–WRKY40. wrky18, wrky40 and wrky60 knockout mutants show enhanced sensitivity to ABA during germination and early seedling growth, but similar sensitivity to wt in stomatal response to ABA. WRKY40 binds to the promoters of either ABA signaling, i.e. ABI4, ABI5 and ABF4, or ABA-responsive genes. Whereas this work classifies WRKY18 and 60 as repressors of ABA signaling, [39*] shows they act as transcriptional activators.


Barley Mg-chelatase mutants showed a wt response to ABA in post-germinative growth and stomatal closure. Recombinant protein, which was active in Mg-chelatase assays, did not bind
ABA. This conclusion was refuted in a recent study [44*], using an ABA-binding assay that covalently links ABA to a Sepharose column via the carboxylic group of the hormone.


- A genetic screening for mutants showing enhanced water-loss led to the identification of a rapid-transpiration mutant, named rtl1, which is ABA insensitive both for ABA-induced stomatal closure and inhibition of light-induced stomatal opening. Map-based cloning revealed a mutation in CHLH, L690F, as being responsible of the phenotype. The authors confirmed that both CHLH RNAi lines and the cch mutant (a missense mutation P642L) did not display ABA-induced stomatal closure. These results, together with [44*, 45], confirm CHLH affects ABA signaling in stomatal guard cells. Unfortunately, recombinant CHLH did not bind $^3$H-labeled ABA using similar conditions to those described in [44*], whereas PYR1 in the presence of ABI1 bound ABA. PYR1 alone did not bind ABA in this assay, likely because its Kd for ABA is $>$50 $\mu$M in the absence of the PP2C. Finally, 5 mM extracellular Ca$^{2+}$ restored ABA-induced stomatal closure of rtl1, which led the authors to suggest a role for CHLH in Ca$^{2+}$ mobilization from chloroplastic stores.


- The authors use ABA-affinity chromatography to detect ABA binding to Arabidopsis, barley and rice CHLH/ABAR. Additionally, they use $^3$H-labeled ABA to further confirm that the C-terminal part of ABAR (amino acid residues 631-999) binds ABA. Such portion of the protein might be an excellent starting point for co-crystallization with ABA and structural studies that would provide a definitive answer to the controversy with [41*, 42*] results. Finally, the authors identified two TILING abar alleles, abar-2 (encoding chlh$^{L348F}$) and abar-3 (chlh$^{S183F}$), which show opposite phenotypes in seed germination assays (the first one ABA-insensitive, the second one ABA-hypersensitive) but lack stomatal phenotypes.


46. Legnaioli T, Cuevas J, Mas P: **TOC1 functions as a molecular switch connecting the circadian clock with plant responses to drought.** *EMBO J.* 2009, 28:3745-3757.

- TOC1 is a key component of the circadian clock that regulates ABA signaling. Thus, toc1-2 or TOC1 RNAi lines show enhanced sensitivity to ABA-induced stomatal closure and reduced water loss compared to wt, whereas stomata of TOC1 overexpressing plants (TOC1-ox) do not close properly in response to ABA. Mis-regulated genes in toc1-2 or TOC1-ox include different genes involved in ABA signaling, for instance CHLH/ABAR. Indeed, TOC1 binds to the promoter of CHLH/ABAR and represses its expression, which partially explains the enhanced water loss of TOC1-ox, since the authors confirm that CHLH/ABAR RNAI lines show higher water loss than wt plants. However, TOC1-ox show even higher water loss than CHLH/ABAR RNAI lines, which suggests TOC1 has additional targets in the ABA signaling pathway. For instance, ABI3 interacts with TOC1 [34], and it would be worthy to examine TOC1 binding to the promoters of key elements of the ABA core signaling pathway.


Figure legends.

**Figure 1.** A simplified model of the ABA pathway that integrates ABA transport and signaling. PYR/PYL/RCAR receptors perceive ABA intracellularly, either at cytosol or nucleus, and form stable ternary complexes with clade A PP2Cs. Thus, phosphatases are inactivated, which allows the activation of downstream targets of the PP2Cs, for instance SnRK2.2, 2.3 and 2.6/OST1. These kinases are either autophosphorylated or activated by putative upstream activating kinases (UAKs), leading to ABA-induced regulation of plasma membrane and nuclear targets, such as NADPH oxidase, KAT1, SLAC1 and ABFs/AREBs (reviewed in 1, 4-10). In addition to SnRK2s, the calcium-dependent protein kinases (CPKs) also regulate ion fluxes and transcriptional response to ABA, and for instance, the CPK and SnRK2 branches converge on the anion channel SLAC1. TFs are supposed to act in the context of chromatin and components of chromatin remodeling complexes, e.g. type SWI/SNF and histone deacetylases (HDAC), have been shown to regulate ABA signaling [47–51]. ABA and its glucose ester (ABA–GE) are subjected to intercellular and likely intracellular transport. The role of ABC transporters, ABCG25 and ABCG40, in ABA transport is highlighted and putatively connected with ABA perception. BG1 is an intracellular b-glucosidase localized to ER that releases ABA from ABA–GE stored in the vacuole or imported from the vascular system [52].
Figure 2. Ubiquitin and SUMO E3 ligases as regulators of ABA signaling. Whereas ubiquitin-modified proteins are targeted for degradation by the 26S proteasome, the fate of sumoylated proteins depends on the target. In the case of ABI5, sumoylation by SIZ1 protects it from proteasome degradation and maintains the TF in an inactive form. AIP2, KEG, PRT6 and DWA1/2-DDB1-CUL4 promote degradation of positive regulators of ABA signaling (ABI3 by AIP2, ABI5 by both KEG and DWA complex). Conversely, SDIR1 and RH2a are supposed to promote degradation of unidentified negative regulators. RPN10 is a regulatory subunit of the proteasome that mediates degradation of ABI5.

Figure 3. Transcriptional regulation of ABF2 and ABI5 expression by WRKY TFs. Several WRKYs have been involved in ABA signaling, namely WRKY2, WRKY18, WRKY40, WRKY60 and WRKY63. Binding to W-box sequences of ABF2 promoter by WRKY63 or ABF4, ABI4 and ABI5 promoters by WRKY40 has been demonstrated. WRKY63 activates expression of ABF2, whereas WRKY40 represses expression of ABI5.
Figure 1

ABA-GE

ABA-GE

ABC25

ABA-GE

Figure in original format
Ubiquitin E3 ligases

AIP2, KEG
PRT6, DWA1/2-DDB1-CUL4

SDIR1, RHA2a

SIZ1
Sumo E3 ligase

ABA signalling

Ubiquitin

Ub

Ub

Ub

RPN10

Positive/negative regulators

26S proteasome
Figure 3

Pro_{ABF2} → W-box → WRKY63

Pro_{ABI5} → W-box → WRKY40