***Highlights**

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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

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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 μM) 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 TOPLESS (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 *sdir1* and *rha2a* mutants show reduced sensitivity to ABA in seed germination and early seedling growth assays, and in the case of *sdir1*, 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 $chlh^{P642L}$) [45] and *rtl1*(encoding $chlh^{L690F}$) [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 Ca^{++} 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. *sdir1* and *dwa1 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.

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●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.

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●This article should be compared with [40$^{\bullet\bullet}$] 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$^{\bullet\bullet}$]. Moreover, a *wrky18 wrky40 wrky60* triple mutant behaves as wt in this work, whereas it is ABA-hypersensitive in [40$^{\bullet}$].

40. Shang Y, Yan L, Liu ZQ, Cao Z, Mei C, Xin Q, Wu FQ, Wang XF, Du SY, Jiang T *et al.*: **The Mg-chelatase H subunit of Arabidopsis antagonizes a group of WRKY transcription repressors to relieve ABA-responsive genes of inhibition**. *Plant Cell* 2010, **22**:1909-1935.
●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$^{\bullet}$] shows they act as transcriptional activators.

41. Muller AH, Hansson M: **The barley magnesium chelatase 150-kd subunit is not an abscisic acid receptor**. *Plant Physiol* 2009, **150:**157-166.
●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.

42. Tsuzuki T, Takahashi K, Inoue SI, Okigaki Y, Tomiyama M, Hossain MA, Shimazaki KI, Murata Y, Kinoshita T: **Mg-chelatase H subunit affects ABA signaling in stomatal guard cells, but is not an ABA receptor in Arabidopsis thaliana**. *J.Plant Res.* 2011. DOI:10.1007/s10265-011-0426-x
●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 μM in the absence of the PP2C. Finally, 5 mM extracellular Ca^{++} restored ABA-induced stomatal closure of *rtl1*, which led the authors to suggest a role for CHLH in Ca^{++} mobilization from chloroplastic stores.

43. Zhang DP, Wu ZY, Li XY, Zhao ZX: **Purification and identification of a 42-kilodalton abscisic acid-specific-binding protein from epidermis of broad bean leaves**. *Plant Physiol* 2002, **128:**714-725.

44. Wu FQ, Xin Q, Cao Z, Liu ZQ, Du SY, Mei C, Zhao CX, Wang XF, Shang Y, Jiang T, Zhang XF, Yan L, Zhao R, Cui ZN, Liu R, Sun HL, Yang XL, Su Z, Zhang DP: **The magnesium-chelatase H subunit binds abscisic acid and functions in abscisic acid signaling: new evidence in Arabidopsis**. *Plant Physiol* 2009, **150:**1940-1954.
●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.

45. Shen YY, Wang XF, Wu FQ, Du SY, Cao Z, Shang Y, Wang XL, Peng CC, Yu XC, Zhu SY, Fan RC, Xu YH, Zhang DP: **The Mg-chelatase H subunit is an abscisic acid receptor**. *Nature* 2006, **443:**823-826.

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.

47. Perruc E, Kinoshita N, Lopez-Molina L: **The role of chromatin-remodeling factor PKL in balancing osmotic stress responses during Arabidopsis seed germination**. *Plant J* 2007, **52**:927-936.

48. Saez A, Rodrigues A, Santiago J, Rubio S, Rodriguez PL: **HAB1-SWI3B interaction reveals a link between abscisic acid signaling and putative SWI/SNF chromatin-remodeling complexes in Arabidopsis**. *Plant Cell* 2008, **20**:2972-2988.

49. Song CP, Agarwal M, Ohta M, Guo Y, Halfter U, Wang P, Zhu JK: **Role of an Arabidopsis AP2/EREBP-type transcriptional repressor in abscisic acid and drought stress responses**. *Plant Cell* 2005, **17**:2384-2396.

50. Sridha S, Wu K: **Identification of AtHD2C as a novel regulator of abscisic acid responses in Arabidopsis**. *Plant J* 2006, **46**:124-133.

51. Chen LT, Luo M, Wang YY, Wu K: **Involvement of Arabidopsis histone deacetylase HDA6 in ABA and salt stress response**. *J Exp Bot* 2010, **61**:3345-3353.

52. Lee KH, Piao HL, Kim HY, Choi SM, Jiang F, Hartung W, Hwang I, Kwak JM, Lee IJ, Hwang I: **Activation of glucosidase via stress-induced polymerization rapidly increases active pools of abscisic acid**. *Cell* 2006, **126**:1109-1120.

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 in original format

Figure 1

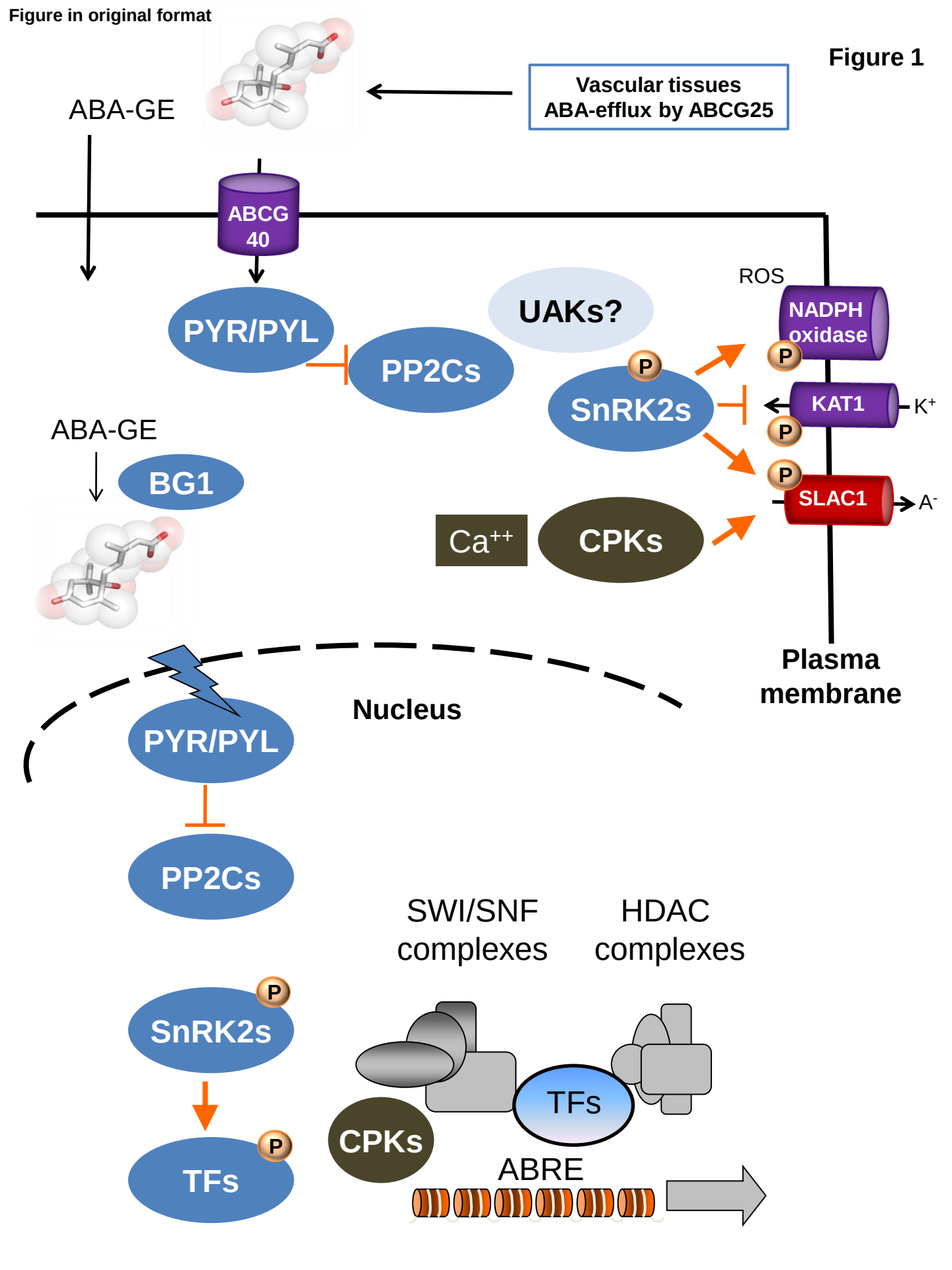

Figure 2

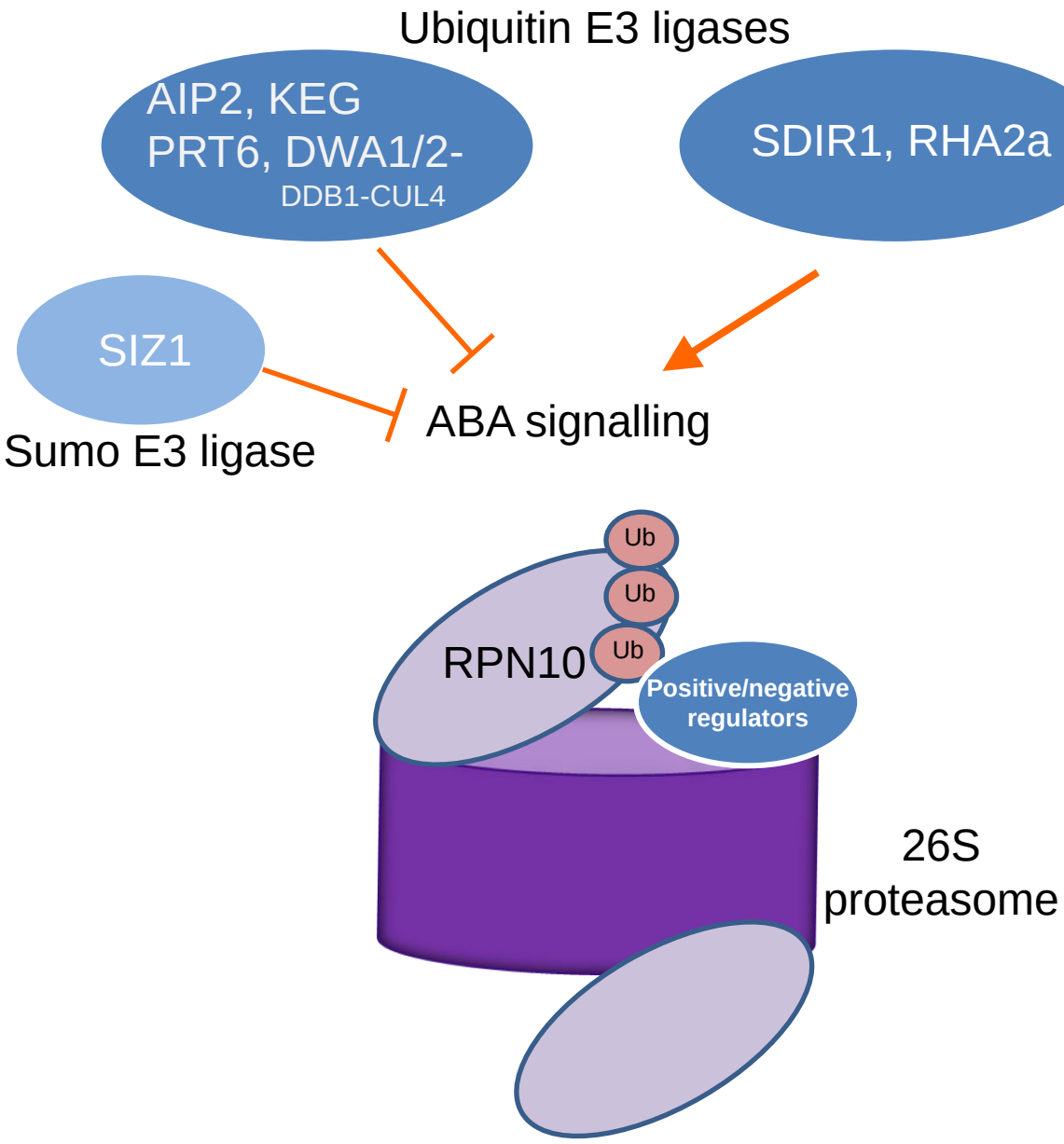
Ubiquitin E3 ligases
AIP2, KEG
PRT6, DWA1/2-
DDB1-CUL4
SDIR1, RHA2a
SIZ1
Sumo E3 ligase
ABA signalling
Ub
Ub
Ub
RPN10
Positive/negative
regulators
26S
proteasome

Figure 3