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Insight

The turnover of ABI5 by scaffold proteins to attenuate ABA signaling

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Scaffold proteins are key elements in signaling pathways, helping other proteins to assemble in order to complete and enhance their function. General scaffold proteins promote the destabilization of transcription factors and/or connect them with repression complexes of gene expression, resulting in both cases in a negative regulation of their functions. Using a genetic and molecular approach, Li et al. (2024) reveal how the conserved scaffold protein Receptor for Activated C Kinase 1 (RACK1) negatively regulates abscisic acid (ABA) signaling through interaction with and inhibition of ABA-insensitive 5 (ABI5) during seed germination and early development.

As one of the most important phytohormones, ABA is crucial for plant development, including seed dormancy, seed germination, and seedling development (Chen et al., 2020). The ABI5 transcription factor is a key player in ABA-triggered processes (Lopez-Molina et al., 2001) and also acts as a molecular hub in the balance between early development and stress, integrating external and internal cues into cellular responses (Albertos et al., 2015). To coordinate these processes, several partners have been described to be involved in the control of the transcriptional activity and/or the protein stability of ABI5. This tight regulation encompasses different post-translational modifications (PTMs) that allow a specific and efficient control of ABI5, playing a significant role in the ABA signaling pathway. In this scenario, the

identification of scaffold proteins is especially interesting. The functional role of scaffold proteins enables recruitment from a huge variety of substrate adaptors, which in turn recognize specific proteins and direct them to ubiquitination and subsequent proteasomal degradation. In their study, Li et al. (2024) reveal that RACK1 recruits two CUL4-based E3 ligases (DWA1 and DWA2) which function together to mediate the turnover of ABI5, thereby efficiently turning down ABA signaling during germination and early seedling establishment.

ABI5 interactors in ABA signaling

Several studies have demonstrated that the activity of ABI5 can be transcriptionally and post-translationally regulated by the interplay with different partners. Seedling establishment requires ABI5 degradation via the ubiquitin–26S proteolytic pathway (Fig. 1). Many proteins serve as negative interactors, promoting ABI5 destabilization, such as CULLIN4 (CUL4)-based and KEEP ON GOING (KEG) E3 ligases (Liu and Stone, 2010) and DWD HYPERSENSITIVE TO ABA1 (DWA1) and 2 (Lee et al., 2010), or involved in ABI5 stabilization but in an inactive form, like the SMALL UBIQUITIN RELATED MODIFIER E3 ligase (SIZ1) (Miura et al., 2009). Another important checkpoint is the modulation of transcriptional activity to switch the downstream gene expression network on and off (Fig. 1). ABA induces ABI5 phosphorylation, promoting its function as a transcriptional activator through the binding to ABA-responsive elements (ABREs) and controlling ABA-inducible gene expression (Lopez-Molina et al., 2001). Turning off ABA responses also requires the capacity to inactivate the binding of ABI5 to the promoters of the downstream targets. Different kinases such as SnRK2 (Nakashima et al., 2009) and BIN2 (Hu and Yu, 2014), and PROTEIN

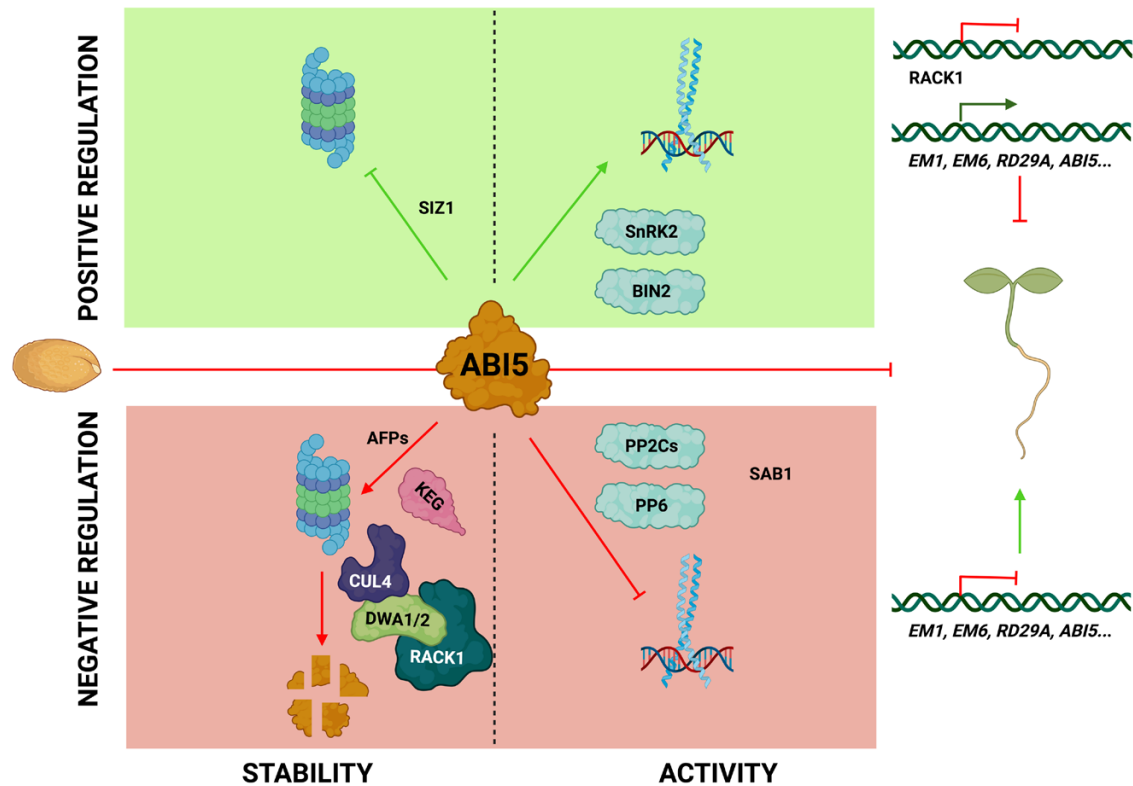


Fig. 1. ABI5 interactome based on the post-translational protein modifications. Different post-translational modifications may positively (green) or negatively (red) affect ABI5 protein stability (left) or transcriptional activity (right). Among the positive effectors, sumoylation by SUMO E3 ligases (SIZ1) has been reported, while ubiquitination by the corresponding E3 ubiquitin ligases (KEG, CUL4) promotes ABI5 destabilization. In addition, phosphorylation and dephosphorylation of ABI5 exerted by specific protein kinases and phosphatases is a well-known mechanism impacting on ABI5 functionality. SnRK2s promote extensive changes in gene expression through the control of the phosphorylation level. Moreover, BIN2 interacts physically with ABI5 to modulate the crosstalk between ABA and brassinosteroids through phosphorylation. In contrast, ABI5 is dephosphorylated by PROTEIN PHOSPHATASES type 2C (PP2Cs) and PROTEIN PHOSPHATASE6 (PP6), which inhibits its activity. Also, Sensitive to ABA 1 (SAB1) interacts with ABI5 and is involved in the reduction of its phosphorylation level affecting its protein stability (Ji *et al.*, 2019). Finally, members of the AFP-related Topless (TPL) co-repressors have the ability to physically associate with ABI5 and alter the transcriptional regulation of target genes. Created with BioRender.com

PHOSPHATASES type 2C (PP2Cs) (Lynch *et al.*, 2012) and PROTEIN PHOSPHATASE6 (PP6) (Dai *et al.*, 2013) have been described to regulate this step.

Many proteins involved in the transcriptional and post-translational regulation of ABI5 are related to the control of stability and/or activity, while other interactors that are becoming relevant have been described to modulate its function. These interacting partners serve as versatile scaffolds for the interactions between proteins and are promising targets for manipulation to improve agronomic traits related to the responses to changing environments or adjust the early seedling development under a variety of stress conditions. In this context, ABI Five binding Protein (AFP) facilitates ABI5 ubiquitin degradation, attenuating the ABA signal (Lopez-Molina *et al.*, 2003). INDUCER OF CBF EXPRESSION1 (ICE1) negatively affects the transcriptional activity of ABI5 to maintain the correct ABA signal during germination (Hu *et al.*, 2019). XPO1-interacting WD40 protein 1 (XIWI1) was also described to maintain the ABI5 stability in the nucleus (Xu *et al.*, 2019), acting as a positive switch in ABA responses. In the

current work by Li *et al.* (2024), the scaffold protein RACK1 has emerged as a novel regulator of ABI5, repressing its transcriptional activity and protein stability. Interestingly, RACK1 also interacts with DWA1 and 2, functioning together to promote ABI5 degradation.

ABI5 post-translational regulation in plants: what for?

ABI5 serves as a nexus in the crosstalk between external conditions, hormone responses, and signaling events. Thus, it is tightly regulated both by a high number of interacting partners and by different PTMs that ensure the correct molecular coordination to maintain the balance between stress conditions and growth. Upon exposure to abiotic stresses (such as salinity or drought), ABI5 proteasome-dependent turnover decreases, and its accumulation induces the expression of ABA-responsive genes (Uno *et al.*, 2000). This turnover is required for the inhibition of the growth and the activation of the stress tolerance response.

In this context, PTMs have been revealed to be key regulatory switches to modulate transcriptional activity and/or stability, that tailor the ABA response through modulating the status of ABI5 (Table 1). These PTMs include phosphorylation, carried out by kinases and reversed by the action of phosphatases, that finally coordinate the binding of ABI5 to specific promoters. Regarding the control of ABI5 stability, ubiquitination promotes its degradation, while sumoylation prohibits it. In addition, the specific S-nitrosylation of Cys153 also regulates ABI5, leading to its interaction with the E3 ubiquitin ligases KEG and CUL4. Through the regulation of the stability and activity of ABI5, PTMs turn the signaling events off and on to respond efficiently to internal and external challenges.

RACK1 scaffold protein in ABA signaling

Scaffold proteins of mitogen-activated protein kinase (MAPK) cascades, such as Ste5 in yeast and KSR (Kinase Suppressor of Ras 1) in mammals, have been studied in depth. Similarly, RACK1 proteins were identified as MAPK scaffold proteins in *Arabidopsis thaliana*. These proteins present seven WD-repeat domains that confer the scaffolding properties to interact with other signaling proteins (Ullah *et al.*, 2008; Adams *et al.*, 2011). Different RACK1 functions have been described in plants, such as responses to abiotic and biotic stresses and protein synthesis (Guo *et al.*, 2011). However, one of the more relevant roles of

RACK1 in plants is its involvement in hormone responses and, more specifically, in the ABA signaling pathway (Chen *et al.*, 2006; Guo and Chen, 2008).

There are three RACK1 proteins in *A. thaliana*, RACK1A, B, and C, and the three of them show unequal genetic redundancy, with RACK1A being indispensable (Guo and Chen, 2008). The loss-of-function *rack1a* single mutant as well as *rack1a/b* and *rack1a/c* double mutants are hypersensitive to ABA during seed germination, cotyledon greening, and root growth (Guo *et al.*, 2009, 2011). ABA-responsive marker genes (i.e. *RD29B* and *RAB18*) are up-regulated in *rack1a* mutants, correlating with the hypersensitive phenotype to this hormone. Contrarily, overexpressing *RACK1A* resulted in reduced ABA sensitivity. Together, these data support the role of RACK1 as a negative regulator of ABA signaling.

In their study, Li *et al.* (2024) demonstrated that RACK1 acts upstream of ABI5, a central hub of growth repression, and they observed the interaction between both proteins. Phosphorylation is key to ABI5 function, and Thr201 in ABI5 was identified as an important residue mediating the interaction between RACK1 and ABI5, as identified by bimolecular fluorescence complementation (BiFC) and co-immunoprecipitation (CoIP) assays using phosphomimic and phosphodead versions of ABI5. RACK1 was also observed to interact with DWA1 and DWA2, both having a role in ABI5 destabilization by the proteasome, confirming the key role of RACK1 in ABI5 degradation. However, this regulation occurs

Table 1. Compilation of ABI5 post-translational regulation

PTM	Interactor	Effects	Phenotype	References
Phosphorylation	SnRK2s	Transcriptional activation and stabilization	Mutants are sensitive to desiccation, showed severe growth defects during seed development, a loss of dormancy level, and elevated seed ABA content.	Nakashima <i>et al.</i> (2009)
	BIN2	Transcriptional activation and stabilization	<i>BIN2</i> -overexpressing plants have an ABA-hypersensitive phenotype.	Hu and Yu (2014)
Dephosphorylation	PP2Cs	Transcriptional inactivation	Triple mutants manifest pleiotropic effects during the life cycle, related to the great reduced phosphorylation level of the bZIP transcription factor.	Lynch <i>et al.</i> (2012)
	PP6	Transcriptional inactivation and destabilization	Mutants exhibit an ABA hypersensitive phenotype.	Dai <i>et al.</i> (2013)
	SAB1	Inhibition of phosphorylation. Transcriptional inactivation and destabilization	Mutants are ABA hypersensitive at both the seed germination and post-germination stages.	Ji <i>et al.</i> (2019)
Ubiquitination	KEG	Degradation	Mutants show growth arrest and hypersensitivity to ABA and other ABA-related abiotic stresses.	Liu and Stone (2010)
	CUL4, DWA1, DWA2	Degradation	Mutants manifest delayed germination and ABA hypersensitive phenotypes.	Lee <i>et al.</i> (2010)
	AFP	Degradation	<i>afp</i> mutants are hypersensitive to ABA	Lopez-Molina <i>et al.</i> (2003)
Sumoylation	SIZ1	Inhibition of degradation	Mutants present ABA hypersensitivity for seed germination arrest and seedling primary root growth inhibition.	Miura <i>et al.</i> (2009)
S-nitrosylation	GSNO	Degradation	Germination promotion.	Albertos <i>et al.</i> (2015)

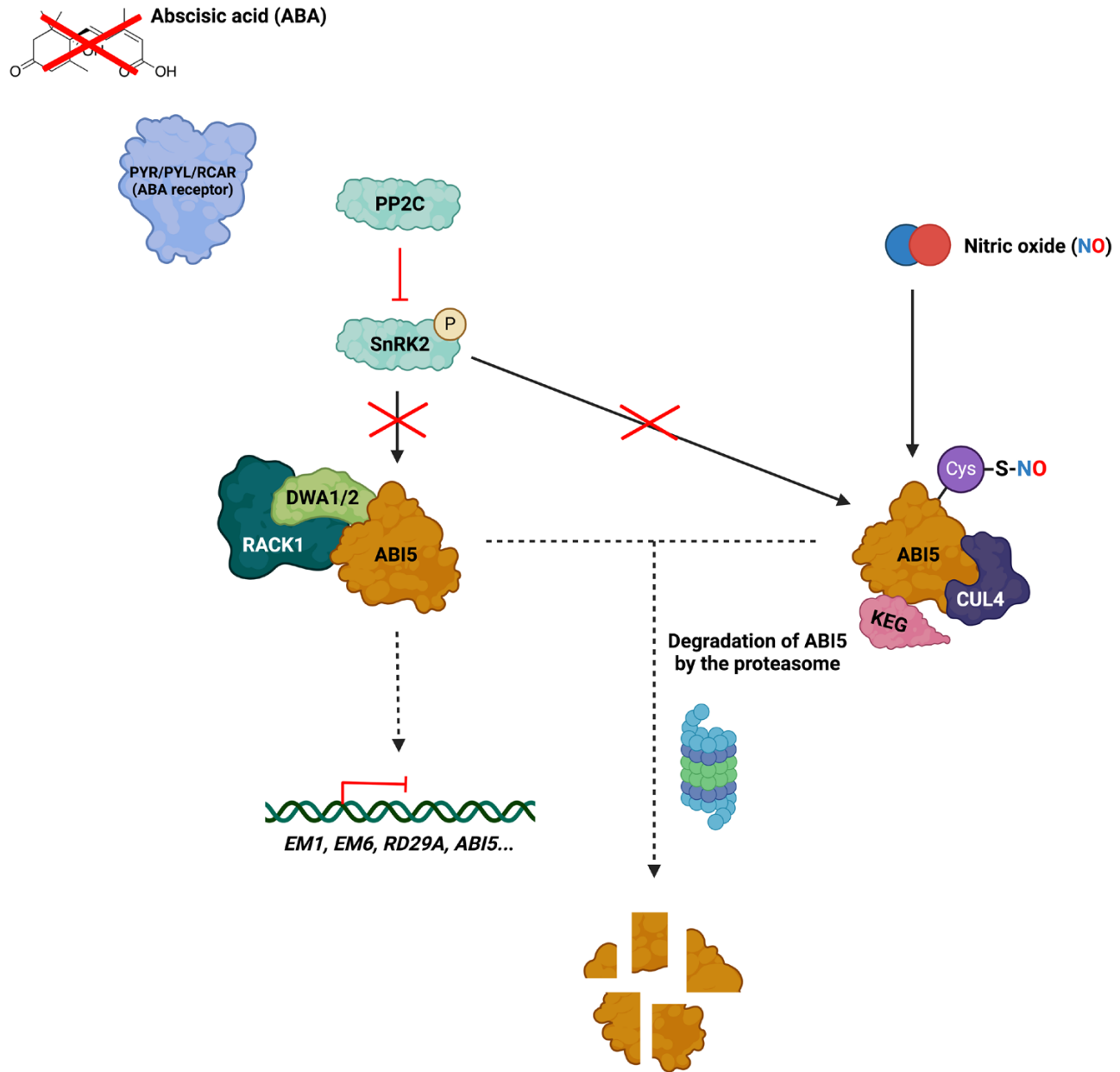


Fig. 2. Scaffold proteins and their postulated role in ABA signaling. RACK1 recruits two substrate receptors for CUL4-based E3 ligases (DWA1 and DWA2) which function together to mediate the turnover of ABI5, thereby efficiently turning down ABA signaling downstream of the module of PYR/PYL/RCAR receptors, the PP2C-negative regulators- and the SnRK2-positive partners. Similarly, alterations modulating the ABI5 redox status include S-nitrosylation of specific Cys residues that enables the interaction to the E3 ubiquitin ligases KEG and CUL4 to promote ABI5 destabilization. Created with BioRender.com

not only at the protein level but also at the transcriptional level, where a feedback regulatory loop includes RACK1 interaction with ABI5 to inhibit *ABI5* expression and, on the other hand, ABI5 also has a role in the repression of *RACK1* by direct binding to its promoter (Fig. 1). Indeed, transcription of *ABI5* and *RACK1* has an opposite pattern during seed germination, and in the presence of ABA *RACK1* expression is highly reduced. Li *et al.* (2024) were able to identify a region of the

RACK1 promoter containing G-box-like motifs that could be recognized by ABI5 in order to regulate its expression. This work, together with previous knowledge on the role of this scaffold protein in the ABA signaling pathway and, specifically, in how the complex of RACK1 and DWA1/2 regulates ABI5, provides a deeper understanding of one of the mechanisms through which this hormone regulates Arabidopsis seed germination (Fig. 2).

Stepping forward

Scaffold proteins can bind multiple signaling molecules and are able to recruit molecular hubs within specific subcellular domains or modulate the efficiency of signal transduction. In doing so, scaffolds organize plant signaling pathways and ensure an efficient and specific flow of information, serving as regulatory targets. This *modus operandi* provides specific checkpoints to tailor the transcriptional control of plant development and stress conditions that could be translated into crop improvement in the agricultural field. Understanding how and when scaffolds control all these pathways could be used to achieve increases in crop stress tolerance and consequently crop production. The molecular changes of the interaction dynamics under stress would result in the identification of useful targets that could be manipulated to increase plant resistance to different stressors.

Furthermore, knowledge of the key protein residues involved in the interaction of scaffolds with different targets and the hierarchy that governs the different PTMs, could result in the fine-tuning of protein regulation by new genomic techniques, undergoing an innovation that can drive more sustainable, competitive, and resilient agriculture. There is no doubt that this study is just a stepping stone towards more exciting research to come.

Conflict of interest

The authors have no conflicts to declare.

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