Review

ABA perception and signalling

Agepati S. Raghavendra[1], Vijay K. Gonugunta[1], Alexander Christmann[2] and Erwin Grill[2]

[1] Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, India
[2] Lehrstuhl für Botanik, Technische Universität München, Emil-Ramann-Str. 4, D-85354 Freising, Germany

Plant productivity is continuously challenged by pathogen attack and abiotic stress such as drought and salt stress. The phytohormone abscisic acid (ABA) is a key endogenous messenger in plants' responses to such stresses and understanding ABA signalling is essential for improving plant performance in the future. Since the discovery of ABA as a leaf abscission- and seed dormancy-promoting sesquiterpenoid in the 1960s, our understanding of the action of the phytohormone ABA has come a long way. Recent breakthroughs in the field of ABA signalling now unfold a unique hormone perception mechanism where binding of ABA to the ABA receptors RCARs/PYR1/PYLs leads to inactivation of type 2C protein phosphatases such as ABI1 and ABI2. The protein phosphatases seem to function as coreceptors and their inactivation launches SNF1-type kinase action which targets ABA-dependent gene expression and ion channels.

Responses of ABA

Higher plants are sessile organisms that have evolved a high plasticity for adaptation to environmental challenges. Pathogens and abiotic stress such as drought and salt stress severely impact plant performance and productivity. The phytohormone ABA serves as an endogenous messenger in biotic and abiotic stress responses of plants [1–6]. Drought and high salinity result in strong increases of plant ABA levels, accompanied by a major change in gene expression and in adaptive physiological responses [7–11]. How environmental cues are perceived and integrated into alterations of physiologically active ABA levels is still largely a conundrum. A limiting water supply leads to an immediate hydraulic signal in plants that triggers ABA biosynthesis over long distances [8], whereas high humidity activates an ABA catabolising P450 enzyme within minutes of perceiving the stress condition [12]. Two recent publications unravelled the importance of transporter-driven uptake and export of ABA [13,14]. Upon perception of a stress signal ABA formation is induced primarily in vascular tissues and ABA is exported from the site of biosynthesis and uptake is stimulated into other cells by specific ATP-dependent transporters. The mechanism allows the rapid distribution of ABA into neighbouring tissues.

ABA is not only a stress signal but is also required to fine-tune growth and development under non-stress conditions. The physiological processes controlled under these conditions include the regulation of growth, stomatal aperture and hydraulic conductivity, as well as seed dormancy [15–17]. Stomatal closing is mediated by ABA-triggered changes of ion fluxes in guard cells [18–20]. Alteration of ABA sensitivity in a non-herbaceous plant revealed additional, less known functions [21]. ABA positively affected leaf size and bud dormancy of poplar (*Populus trichocarpa*) and negatively influenced the size of guard cells and internode length. Leaf size is regulated in concert with ethylene by a negative feedback of ABA on ethylene generation [22]. ABA also acts together with other phytohormones such as brassinosteroids, gibberellic acid and auxin in regulating plant growth and development [23–25].

An overwhelming number of signalling components that affect ABA-dependent stomatal closing and seed germination have been identified by forward and reverse genetic approaches [1,26,27]. However, the crosstalk between different phytohormone signalling pathways has frequently precluded a clear differentiation between primary and secondary ABA signalling components. The identification of a unique class of ABA receptors has now fundamentally changed this situation and laid the foundation for assembling the core signalling pathway.

Here, we highlight the recent discovery of RCAR/PYR1/PYL proteins (see Glossary) as ABA receptors, summarise the available crystal structure data which unravelled the

Glossary

ABF: ABA responsive element (ABRE) binding factors, a subfamily of bZIP transcription factor proteins, interact with the ABRE and promotes ABA-induced gene expression. Also named ABF (see below).
ABRE: ABA responsive promoter element, a conserved cis-element (c/tACGTggc), that allows the binding of AREB/ABF while promoting ABA-induced gene expression.
AREB: ABA-responsive element binding protein, binds to ABRE and modulates gene expression. Also named ABF (see above).
Bet V 1: birch pollen allergen, coded by a member of a large family of genes, similar to those of RCAR/PYR1/PYLs.
CIPKs: Calcineurin B-like Protein (CBL) Interacting Protein Kinases, a group of protein kinases that regulate ABA responses.
CPKs: Calcium-dependent Protein Kinases, positive regulators of ABA responses.
KAT: inwardly-rectifying (transporting) K^+ channel, essential for K^+ uptake during stomatal opening.
OST1: an ABA-activated protein kinase, homologue of SnRK2.2/SnRK2.3, that are positive regulators of ABA responses. The name is due to the physiological phenotype of OPEN STOMATA that is produced on the suppression of OST1 expression.
PP2Cs: protein phosphatases which fall under the category of type 2C, some members are negative regulators of ABA-induced responses.
RCARs/PYR1/PYLs: Regulatory Components of ABA Receptor/Pyrabactin Resistance Protein1/PYR-Like proteins; a family of START domain proteins; demonstrated to inhibit clade A PP2Cs, which are known to be negative regulators of ABA responses. These bind to ABA and facilitate the formation of a trimeric RCAR–ABA–PP2C complex which releases the negative regulation of ABA responses exerted by the PP2C.

Corresponding author: Grill, E. (erwin.grill@wzw.tum.de).

ABA binding mechanism and discuss the role of the 2C type protein phosphatases such as ABI1 in generating the high affinity ABA binding site. This paper emphasises how the core ABA signalling pathways controlling ion channels and gene expression are now unfolding as a result of ongoing efforts initiated by the identification of the ABA receptors.

Pyrabactin Resistance 1 and Regulatory Component of ABA Receptor 1

High affinity ABA binding proteins of *Arabidopsis* (*Arabidopsis thaliana*) have recently been identified by two research groups [28,29]. Sean Cutler's group characterised the synthetic chemical pyrabactin as a selective ABA agonist and identified *Arabidopsis* mutants insensitive to this growth regulator [28]. His group cloned the *Pyrabactin Resistance 1* (*PYR1*) locus and characterised PYR1 and several PYR1-related homologues of *Arabidopsis* (PYLs) as ABA-dependent inhibitors of Mg^{2+}- and Mn^{2+}-dependent serine/threonine phosphatases type 2C (PP2Cs, see Glossary). Prototypes of these PP2Cs are ABI1 (Abscisic Acid Insensitive 1) and its close homologue ABI2, which globally repress ABA responses and which have emerged as a focal point in the network of ABA signal transduction [30,31].

A yeast two-hybrid screen for regulators of ABI1 and ABI2 [29] was used to identify the Regulatory Component of ABA Receptor 1 (RCAR1), identical to PYL9 (Figure 1a), as an ABI1- and ABI2-interacting protein. RCAR1 expression enhanced ABA-dependent gene expression several-fold and antagonised the action of ABI1 and ABI2. RCAR1 emerged as a structural homologue of both potential phytohormone-binding proteins Bet V 1 (see Glossary) from birch (*Betula verrucosa*), proposed to bind brassinosteroids [32], and a cytokinin-binding protein of mung bean (*Vigna radiata*) [33]. Whereas RCAR1 did not bind brassinosteroids or cytokinins, binding studies with (*S*)-ABA (Figure 1b) yielded a dissociation constant of 0.7 μM for the physiologically active ABA by isothermal calorimetry indicating a strong RCAR1–ABA interaction. By contrast, *in vitro* analysis of purified RCAR1 and ABI2 revealed a selective and rapid inhibition of the protein phosphatase activity by (*S*)-ABA with a dissociation constant of 0.06 μM ABA, much lower than the value for RCAR alone suggesting that interaction of the two proteins provides the high affinity binding site required for ABA responses. The stereoisomers (*R*)-ABA and *trans*-ABA (Figure 1b) were more than 1000-fold less active in mediating ABI1 and ABI2 inhibition. Taken together, the birch Bet V1 homologues RCAR1, PYR1 and several PYL proteins of *Arabidopsis* bind ABA with high affinity and, as shown for RCAR1 and RCAR3, with stereoselectivity. In the presence of ABA these proteins inactivate certain PP2Cs such as ABI1, ABI2 and HAB1 (Hypersensitive to ABA 1).

Combinatorial interaction and PP2C regulation

The RCARs/PYR1/PYLs belong to the Bet V 1 superfamily of *Arabidopsis* and comprise a protein family with 14 members, which can be grouped into three subfamilies (Figure 1a). Members of all three subclades regulate ABI1, ABI2 or HAB1 in dependence of ABA. Analysis of RCAR1, 3, 8, 11 and 12 [28,29,34–36] and of RCAR6, 9 and 10 revealed an ABA-dependent inactivation of ABI1, ABI2 and/or HAB1. These and additional RCAR members physically interact with ABI1 [36]. The findings indicate that all RCAR family members are ABA binding proteins and

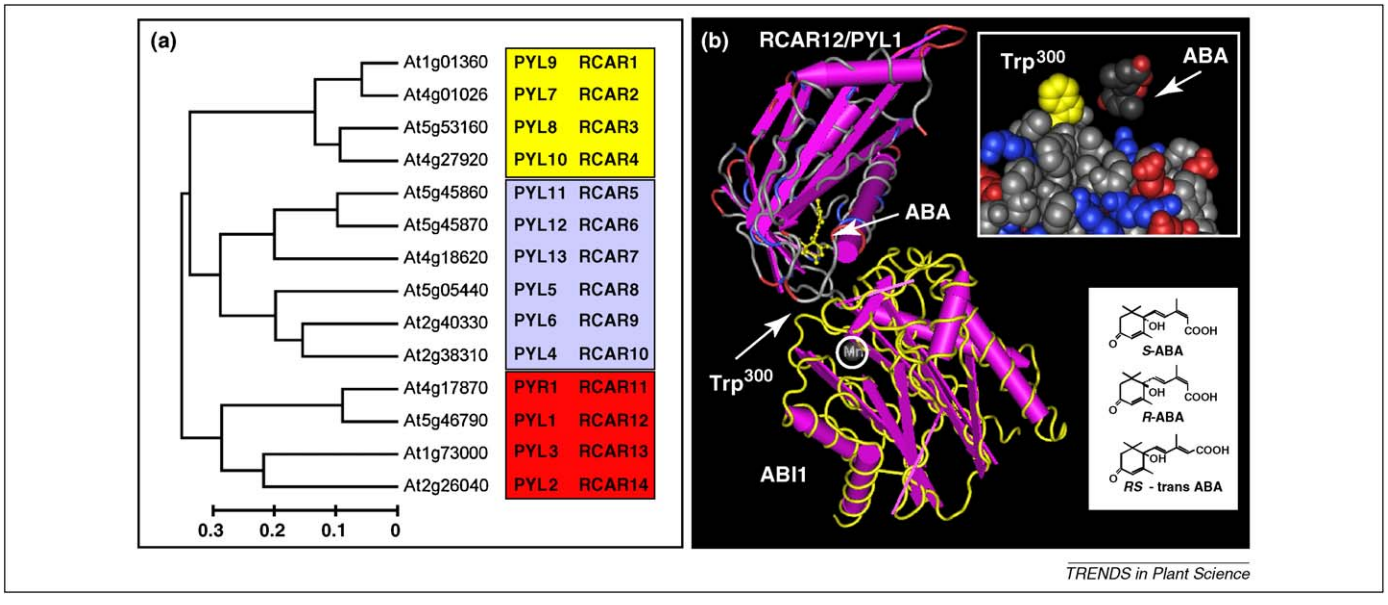

Figure 1. The ABA binding RCAR/PYR1/PYL proteins. **(a)** Phylogenetic tree of ABA binding proteins from *Arabidopsis*. The proteins can be grouped into three subfamilies I, II and III highlighted in yellow, blue and red, respectively. The RCAR and PYR1/PYL numbering is given as well as the gene numbers. **(b)** ABA binding by the heteromeric RCAR12/ABI1 complex based on the crystal structure provided in [48]. The ABI1 protein is highlighted by a yellow backbone indicating the peptide linkages. A short and long arrow denotes the RCAR-bound ABA molecule and Trp300 of ABI1, respectively. A white circle highlights a manganese ion bound to the active site of the PP2C. The RCAR protein obstructs the active site of the PP2C thereby inactivating ABI1. The secondary domains of α-helices and β-sheets are presented as pointed cylinders and flat arrows, respectively. Upper inset: space filling presentation of the ABI1 surface in the vicinity of ABA bound to RCAR (RCAR residues are not shown). The Trp^{300} (indicated in yellow) is close to the ABA molecule (oxygen atoms are shown in red) and interacts with ABA via a water molecule (not shown). Basic and acidic amino acid residues of ABI1 are denoted by blue and red, respectively. Lower inset: chemical structure of the physiologically active *(S)*-ABA as well as of the ABA isomers *(R)*-ABA, in which the orientation of the OH group in space is opposite to the orientation it holds in *(S)*-ABA, and *(R,S)*-trans-ABA.

that RCAR proteins can interact with and regulate the target PP2Cs in a combinatorial manner. All 14 RCARs were tested in protoplasts and with the exception of RCAR7/PYL13 all acted as positive regulators of ABA signalling [37]. There are approximately 80 PP2Cs in *Arabidopsis* [38] and six of the nine PP2Cs in clade A including ABI1, ABI2, HAB1 and HAB2 have been identified as negative regulators of ABA responses [39–45]. At this stage it is not clear whether all PP2Cs linked to ABA responses are regulated by RCARs or whether all RCAR members can regulate the same PP2C. If both assumptions are true, approximately 80 combinations (6 times 13 or 14) would be possible. These RCAR/PP2C complexes probably address different downstream signalling components and vary in their affinity to the hormone thus allowing for the adjustment of ABA signalling to strongly variable ABA levels.

The transcript levels of different RCARs and PP2Cs vary throughout development and in response to environmental challenge [28,29,34,35]. Different expression patterns of individual RCARs and PP2Cs are expected to reduce the numbers of combinatorial interactions in plant cells. In general, transcript levels of RCARs are down-regulated under stress conditions, whereas the abundance of PP2C transcripts is increased [34]. A concomitant change in RCAR and PP2C protein levels would result in an ABA desensitisation of the plants under abiotic stress, thus providing a mechanism for adjustment of ABA signalling to strongly increased ABA levels.

Crystal structures

X-ray diffractionation studies of PYR1 (RCAR11) in a complex with ABA [46,47] and trimeric complexes of ABA/ABI1/RCAR12 (PYL1) [48,49], as well as ABA/HAB1/RCAR14 (PYL2) [50] have elucidated the site of ABA binding and the steric mode of inhibition of protein phosphatase activity. The RCAR provides a cavity in the centre, encaged by seven β-sheets and two α-helical domains, which is similarly found in Bet V1 and related proteins and which functions as a ligand binding site. RCAR proteins thus have an open ligand binding pocket that is closed upon ABA binding by conformational change of two β-sheets engulfing the ABA molecule (Figure 1b), reminiscent of a gate/latch mechanism [50]. The ABA-induced conformational change facilitates the docking of RCAR to the catalytic site of the PP2C, thereby blocking substrate access to the phosphatase. A conserved tryptophan residue of the PP2C is involved in ABA binding by contacting ABA via a bound water molecule (Figure 1b). The occupation of the PP2C active site by RCAR in the trimeric receptor complex provides an explanation for the non-competitive inhibition of ABI1 and ABI2 mediated by ABA [29,34].

Receptors or coreceptors?

The question arises as to whether the ABA binding RCARs are ABA receptors or coreceptors forming a heteromeric receptor complex with a PP2C such as ABI1. The interaction between RCAR and PP2C generates the high affinity ABA binding site required for responses towards low nM ABA levels. The affinity of RCAR1 [29], RCAR3 [34] and RCAR8 [35] did not differ considerably, with K_d values for (*S*)-ABA of 0.7, 1.0 and 1.1 μM, respectively. By contrast, *(S)*-ABA binding to heteromeric receptor complexes revealed more than 10-fold lower K_d values of 64 nM for ABI2/RCAR1 and 38 nM for a truncated HAB1/RCAR8 [29,35]. Similarly, RCAR3 revealed half-maximal inhibition of ABI1 and ABI2 in the range of 15–40 nM [34]. Three observations are consistent with a coreceptor function of RCAR and PP2C: (i) the heteromeric complex provides the high affinity binding site for ABA which is of relevance at physiological ABA levels; (ii) the PP2C interacts with the RCAR-bound ABA molecule; and (iii) although the heteromeric receptor complex is also formed in the absence of ABA, the ligand promotes the assembly of or stabilises the holoreceptor, the heteromeric receptor complex. The conclusion of ligand-mediated receptor complex stabilisation is based on the observations of ABA-enhanced protein interaction of HAB1 and some RCARs in yeast (*Saccharomyces cerevisiae*) [28], by the stabilisation of PP2C inhibition in the presence of high ABA levels [29,34], and by co-immunoprecipitation studies [36]. Thus, experimental evidence supports a coreceptor function for both RCARs/PYR1/PYLs and PP2Cs.

Other ABA binding proteins

Until recently the identities of ABA receptors have remained either elusive or contested [51]. Reported ABA receptors include plastidic ABAR/CHLH/GUN5 [52,53] and plasma membrane-localised GCR2 [54] and GTG1/GTG2 [55] (Table 1). Analysis of ABA binding to these proteins employed radiolabelled assays, which are prone to artefacts [56,57]. Hence, validation of the results by a more robust ABA binding assay, such as ABA titration analysis by isothermal calorimetry is required to clarify ABA binding function. The identified components affect ABA responses and are thus likely to be involved in the network of hormonal responses. At this stage, however, it is unclear how the presumed ABA binding proteins feed into the molecular events governing the main ABA responses (i.e. regulation of germination, stomatal aperture and growth), all of which are controlled by RCAR/PYR1/PYL–PP2C complexes [29].

ABA signalling to ion channels

The discovery of RCAR/PP2Cs as ABA receptors has initiated a paradigm shift in our understanding of the molecular basis of ABA action and has paved the way to comprehending the main signalling events leading to ABA-responsive gene regulation and ion channel control. PP2C coreceptors interact with SNF1 (Sucrose-Nonfermenting Kinase1)-related protein kinases OST1/SnRK2.6/SnRK2E (see Glossary), SnRK2.2/SnRK2D and SnRK2.3/SnRK2I [58,59]. These protein kinases, which act as positive ABA key regulators, are structurally highly related and belong to the superfamily of sucrose-nonfermenting kinases (SNF) originally identified in yeast. PP2C coreceptor interaction with ABA-activated SnRK2s results in efficient inactivation of SnRK2s via dephosphorylation of multiple Ser/Thr residues in the activation loop. ABA perception by the RCAR/PYR1/PYL proteins suppresses PP2C-mediated dephosphorylation of the SnRKs and allows their activation [59].

Table 1. Reported ABA binding proteins

Reported ABA binding proteins	Localisation	Study	Dissociation constant -K_d (nM)	Comments	Refs
ABAR/CHLH/GUN5	Chloroplast and nucleus	Subunit of Mg-chelatase; ^{3}H-ABA binding, biochemistry and reverse genetics	32	Barley plants with mutated/disabled CHLH gene normal in their response to ABA; selective binding to ABA; link to ABA signal pathway unknown.	[52,53]
GCR2	Plasma membrane	G-protein-coupled receptor; ^{3}H-ABA binding, homology modelling and reverse genetics	20	GCR2 probably a plant homologue of bacterial lanthionine synthetase; binding experiments are questioned.	[54]
GTG1, GTG2	Plasma membrane	G-protein-coupled receptor-type G proteins; ^{3}H-ABA binding; double knockout mutant hypersensitive to ABA	36, 41	ABA response in mutants only partially impaired; mammalian homologue identified as an ion channel of the endoplasmic reticulum.	[55]
RCAR/PYR1/PYL	Nucleus and cytosol	Related to lipid binding START proteins; binding studies by isothermal calorimetry and NMR: triple and quadruple mutants ABA-insensitive	64 for RCAR1/ABI2, 38 for RCAR8/HAB1	Proteins inhibit negative key regulators of the ABA pathway, the PP2Cs ABI1, ABI2, HAB1, in the presence of ABA; selective ABA interaction at molecular level confirmed by the use of nonactive ABA stereoisomeres, reconstituted protein system, binding kinetics and mutagenesis.	[29,35]

Guard cells provide an attractive single cell system to study ABA responses [60]. The ABA signalling pathway controlling ion channels in stomata appears to be surprisingly short (Figure 2a). OST1 acts as positive regulator of stomatal closure [61]. It activates the anion channel SLAC1 (see Glossary) [62,63] and inhibits the cation channel KAT1 [64] (see Glossary) by phosphorylation. Both channels are reciprocally regulated by the ABA signalling pathway and by Ca^{2+} [20]. The Ca^{2+}-dependent regulation is probably provided by another SLAC1-stimulating protein kinase, the Ca^{2+}-dependent protein kinase CPK23 [65], and involves other related kinases such as CPK3 and CPK6 [66]. The ABA coreceptors ABI1 and the related PP2CA inhibit OST1-dependent SLAC1 activation

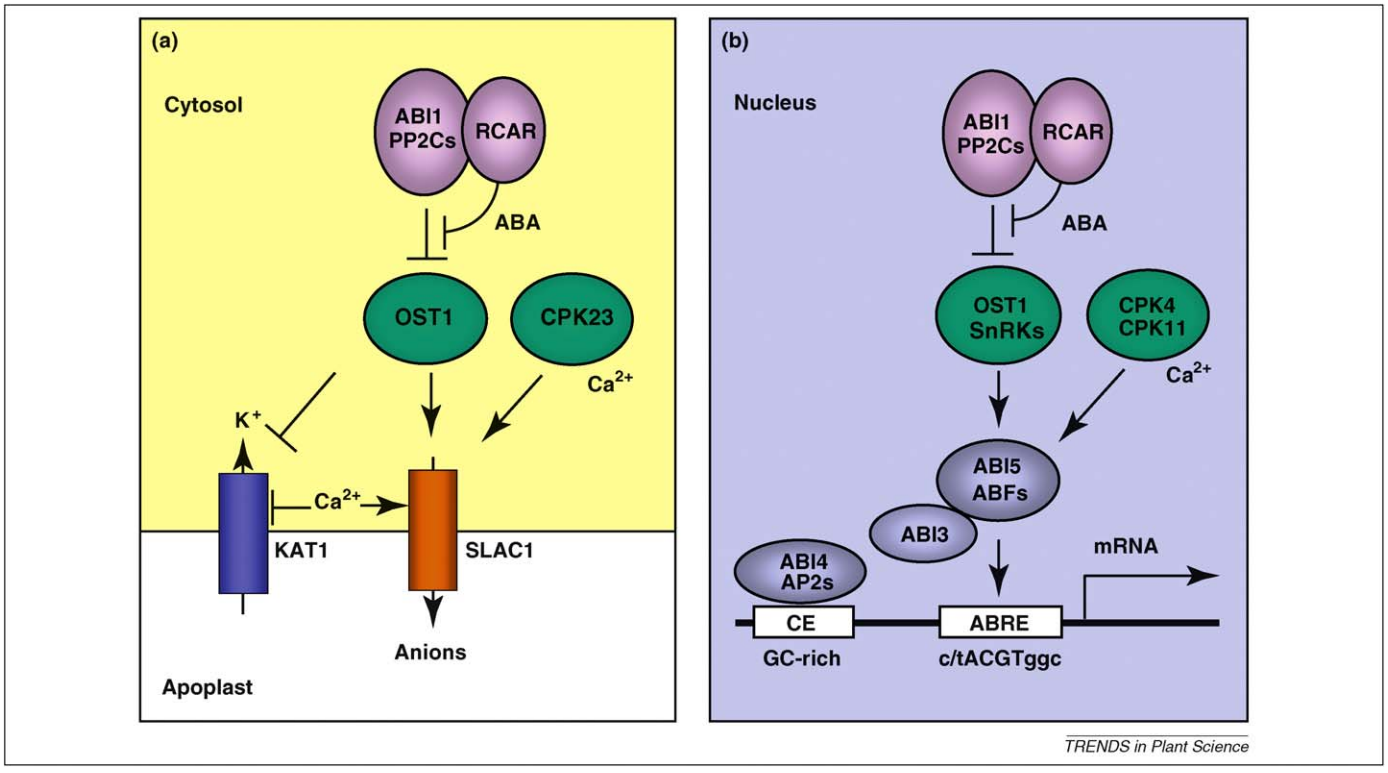

Figure 2. ABA signalling to ion channels and to the nucleus. The ABA receptor is formed by the heteromeric complex of a PP2C such as ABI1 and an ABA-binding RCAR member (both highlighted in pink). The receptor complex controls ABA signalling and is present in **(a)** the cytosol and **(b)** the nucleus. The phosphatase activity of the PP2C inhibits the action of the protein kinases (presented in green) OST1 and related SnRKs, and possibly of Ca^{2+}-dependent CPKs such as CPK23. In the presence of ABA, the phosphatase activity of the receptor is blocked. As a consequence, the protein kinases are released from inhibition and directly phosphorylate and regulate key targets of the ABA signalling pathway. In guard cells, key targets are the ion channels SLAC1 and KAT1, which are activated and inhibited by OST1 action, respectively. In the nucleus, key targets are the basic leucine zipper transcription factor ABI5 and related ABFs. Phosphorylated ABFs bind as dimers to the ABA-responsive cis-element (ABRE, see Glossary) and, in concert with other transcriptional regulators, provide the ABA-responsive transcription (components are presented in steel blue). ABI3 binds to ABI5 and enhances its action, whereas ABI4 and related AP2-type transcription factors target a GC-rich coupling element (CE) for optimal regulation of ABA-dependent gene expression.

via physical interaction [62,63]. ABA- and RCAR-mediated inactivation of the PP2C allows SLAC1 activation. It is tempting to speculate that control of ABA responsive ion channels is imposed by a preformed signalling complex consisting of an ABA receptor and an associated protein kinase. Such a model is consistent with the findings of plasma membrane-associated ABI1 [31,67], stable OST1–PP2C complex formation [63] and holoreceptor formation in the absence of elevated ABA levels in the cytosol [28,29,36]. OST1 also targets a plasma membrane-localised NADPH oxidase that generates H_2O_2 [68]. H_2O_2 increases mediate stomatal closure [69], probably by catalytic inactivation of ABI1 and ABI2, which are very sensitive to H_2O_2 and oxidation [70,71]. OST1-dependent H_2O_2 formation could initiate release of further active OST1 via PP2C inactivation in a positive feedback loop. Marked increases in H_2O_2 are induced in guard cells by exposure to methyl jasmonate, bicarbonate and elicitors, such as chitosan which is known to regulate stomatal aperture [72,73]. It is conceivable that the different signalling pathways target PP2Cs via the common secondary messenger H_2O_2.

Stomatal closure is initiated by the depolarisation of guard cells, which is triggered by anion release through SLAC1 [19,74]. Subsequently, the initial depolarisation activates outwardly-rectifying potassium channels. The loss of osmotically relevant ions then leads to water and turgor loss causing stomatal closing.

ABA pathway controlling gene transcription

Key transcriptional regulators of ABA-dependent gene expression are ABFs/AREBs (ABA-responsive Element Binding Factor/Protein, see Glossary), basic region/leucine zipper (bZIP)-type transcriptional regulators with ABI5 as a typical representative [75,76]. OST1 and the related SnRK2.2/SnRK2D and SnRK2.3/SnRK2I directly target ABF/AREBs in the nucleus (Figure 2b), and ABF2/AREB1 is phosphorylated *in vitro* by this class of ABA-activated protein kinases [37,77–79]. SnRK activation is promoted by ABA-mediated inactivation of the PP2Cs, which negatively regulate the protein kinases. Phosphorylation of ABI5 leads to its activation, whereas sumoylation antagonises ABI5 action [80]. The principle mode of SnRK and bZIP interaction has been pioneered by Walker-Simmons and coworkers in wheat (*Triticum aestivum*) [81]. ABF1 and ABF4/AREB2 are also phosphorylated by Ca^{2+}-dependent protein kinases CPK4 and CPK11 [82]. Other transcriptional regulators also contribute to ABA-specific transcription. ABI3, belonging to the B3 transcriptional regulators, binds to ABI5 and enhances its action. In addition, ABI4, an AP2-type transcription factor, and a number of additional transcription factors including MYC/MYB-type regulators act as positive ABA response regulators [83]. Finally, the homeodomain leucine zipper AtHB6 interacts with ABI1 and serves as a transcription factor to suppress ABA responses [84].

In their function as key regulators of ABA responses PP2Cs target a number of additional cellular components involved in abiotic stress responses. The interacting proteins comprise members of the SnRK3 class [85], the glutathione peroxidase as part of the redox homeostatic system [86] and fibrillin precursor, which is imported into plastids as a photosystem II protective and lipid-binding protein [30].

Perspectives

The principle pathways from ABA perception to ABA-dependent gene regulation and ion channel control are now elucidated. However, the intricacies and the orchestration of the numerous transcription factors involved remain to be fully characterised. In addition to SnRK2s as key regulators of ABA responses, a prominent function of Ca^{2+}-regulated protein kinases, CPKs and CIPKs (see Glossary) together with their regulatory Ca^{2+}-binding calcineurin B-like proteins, is emerging in regulating ion channels and targeting other ABA signalling components. The role and source of cytosolic Ca^{2+} increases in ABA responses is not fully understood. The generation and function of NAD-derived cADPR (cyclic ADP Ribose) as a second messenger in the ABA signalling cascade still remains a conundrum [87]. Regulation of ABA signalling implicates the control of physiologically active ABA. How ABA biosynthesis, transport, storage and turnover are regulated by environmental cues such as cold and drought is a major challenge that we need to understand. Furthermore, the molecular mechanisms of crosstalk between ABA and other phytohormone signalling pathways remain to be elucidated. Although many questions are still open, the current advances in ABA signalling in *Arabidopsis* pave the way to address the molecular events underlying stress responses in other plant species, with the prospect to improve the abiotic stress performance of crop plants.

Acknowledgments

E.G., V.K.G. and A.S.R. are supported by a joint DFG-DST (Deutsche Forschungsgemeinschaft-Department of Science and Technology) program (IND/FRG/DFG/P-18/2008). Work in the lab of A.S.R is supported by grants from the DST, New Delhi, India through a JC Bose National Fellowship Research Grant (SR/S2/JCB-06/2006). The financial support of the E.G. group by DFG GR-938/6, EU Marie-Curie-Program MEST-CT-2005-020232 and Fonds der Chemischen Industrie is gratefully acknowledged.

References

1 Christmann, A. *et al.* (2006) Integration of abscisic acid signalling into plant responses. *Plant Biol.* 8, 314–325
2 Melotto, M. *et al.* (2006) Plant stomata function in innate immunity against bacterial invasion. *Cell* 126, 969–980
3 Adie, B.A. *et al.* (2007) ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defenses in Arabidopsis. *Plant Cell* 19, 1665–1681
4 Hirayama, T. and Shinozaki, K. (2007) Perception and transduction of abscisic acid signals: keys to the function of the versatile plant hormone ABA. *Trends Plant Sci.* 12, 343–351
5 Galvez-Valdivieso, G. *et al.* (2009) The high light response in Arabidopsis involves ABA signaling between vascular and bundle sheath cells. *Plant Cell* 21, 2143–2162
6 Ton, J. *et al.* (2009) The multifaceted role of ABA in disease resistance. *Trends Plant Sci.* 14, 310–317
7 Priest, D.M. *et al.* (2006) Use of the glucosyltransferase UGT71B6 to disturb abscisic acid homeostasis in Arabidopsis thaliana. *Plant J.* 46, 492–502
8 Christmann, A. *et al.* (2007) A hydraulic signal in root-to-shoot signalling of water shortage. *Plant J.* 52, 167–174
9 Rabbani, M.A. *et al.* (2003) Monitoring expression profiles of rice genes under cold, drought, and high-salinity stresses and abscisic acid application using cDNA microarray and RNA gel-blot analyses. *Plant Physiol.* 133, 1755–1767

10 Seki, M. *et al.* (2002) Monitoring the expression pattern of around 7,000 Arabidopsis genes under ABA treatments using a full-length cDNA microarray. *Funct. Integr. Genomics* 2, 282–291
11 Zeller, G. *et al.* (2009) Stress-induced changes in the Arabidopsis thaliana transcriptome analyzed using whole-genome tiling arrays. *Plant J.* 58, 1068–1082
12 Okamoto, M. *et al.* (2009) High humidity induces abscisic acid 8′-hydroxylase in stomata and vasculature to regulate local and systemic abscisic acid responses in Arabidopsis. *Plant Physiol.* 149, 825–834
13 Kang, J. *et al.* (2010) PDR-type ABC transporter mediates cellular uptake of the phytohormone abscisic acid. *Proc. Natl. Acad. Sci. U. S. A.* 107, 2355–2360
14 Kuromori, T. *et al.* (2010) ABC transporter AtABCG25 is involved in abscisic acid transport and responses. *Proc. Natl. Acad. Sci. U. S. A.* 107, 2361–2366
15 Leung, J. and Giraudat, J. (1998) Abscisic acid signal transduction. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49, 199–222
16 Parent, B. *et al.* (2009) Drought and abscisic acid effects on aquaporin content translate into changes in hydraulic conductivity and leaf growth rate: a trans-scale approach. *Plant Physiol.* 149, 2000–2012
17 Finkelstein, R.R. *et al.* (2002) Abscisic acid signaling in seeds and seedlings. *Plant Cell* 14 (Suppl.), S15–S45
18 Levchenko, V. *et al.* (2005) Cytosolic abscisic acid activates guard cell anion channels without preceding Ca^{2+} signals. *Proc. Natl. Acad. Sci. U. S. A.* 102, 4203–4208
19 Vahisalu, T. *et al.* (2008) SLAC1 is required for plant guard cell S-type anion channel function in stomatal signalling. *Nature* 452, 487–491
20 Siegel, R.S. *et al.* (2009) Calcium elevation-dependent and attenuated resting calcium-dependent abscisic acid induction of stomatal closure and abscisic acid-induced enhancement of calcium sensitivities of S-type anion and inward-rectifying K channels in Arabidopsis guard cells. *Plant J.* 59, 207–220
21 Arend, M. *et al.* (2009) Expression of the Arabidopsis mutant ABI1 gene alters abscisic acid sensitivity, stomatal development, and growth morphology in gray poplars. *Plant Physiol.* 151, 2110–2119
22 LeNoble, M.E. *et al.* (2004) Maintenance of shoot growth by endogenous ABA: genetic assessment of the involvement of ethylene suppression. *J. Exp. Bot.* 55, 237–245
23 De Smet, I. *et al.* (2003) An abscisic acid-sensitive checkpoint in lateral root development of Arabidopsis. *Plant J.* 33, 543–555
24 Zhang, S. *et al.* (2009) The primary signaling outputs of brassinosteroids are regulated by abscisic acid signaling. *Proc. Natl. Acad. Sci. U. S. A.* 106, 4543–4548
25 Achard, P. *et al.* (2006) Integration of plant responses to environmentally activated phytohormonal signals. *Science* 311, 91–94
26 Jammes, F. *et al.* (2009) MAP kinases MPK9 and MPK12 are preferentially expressed in guard cells and positively regulate ROS-mediated ABA signaling. *Proc. Natl. Acad. Sci. U. S. A.* 106, 20520–20525
27 Zhao, Z. *et al.* (2008) Functional proteomics of Arabidopsis thaliana guard cells uncovers new stomatal signaling pathways. *Plant Cell* 20, 3210–3226
28 Park, S.Y. *et al.* (2009) Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science* 324, 1068–1071
29 Ma, Y. *et al.* (2009) Regulators of PP2C phosphatase activity function as abscisic acid sensors. *Science* 324, 1064–1068
30 Yang, Y. *et al.* (2006) Fibrillin expression is regulated by abscisic acid response regulators and is involved in abscisic acid-mediated photoprotection. *Proc. Natl. Acad. Sci. U. S. A.* 103, 6061–6066
31 Moes, D. *et al.* (2008) Nuclear localization of the mutant protein phosphatase abi1 is required for insensitivity towards ABA responses in Arabidopsis. *Plant J.* 54, 806–819
32 Markovic-Housley, Z. *et al.* (2003) Crystal structure of a hypoallergenic isoform of the major birch pollen allergen Bet v 1 and its likely biological function as a plant steroid carrier. *J. Mol. Biol.* 325, 123–133
33 Pasternak, O. *et al.* (2006) Crystal structure of Vigna radiata cytokinin-specific binding protein in complex with zeatin. *Plant Cell* 18, 2622–2634
34 Szostkiewicz, I. *et al.* (2010) Closely related receptor complexes differ in their ABA selectivity and sensitivity. *Plant J.* 61, 25–35
35 Santiago, J. *et al.* (2009) Modulation of drought resistance by the abscisic acid receptor PYL5 through inhibition of clade A PP2Cs. *Plant J.* 60, 575–588
36 Nishimura, N. *et al.* (2009) PYR/PYL/RCAR family members are major in-vivo ABI1 protein phosphatase 2C-interacting proteins in Arabidopsis. *Plant J.* 61, 290–299
37 Fujii, H. *et al.* (2009) In vitro reconstitution of an abscisic acid signalling pathway. *Nature* 462, 660–664
38 Schweighofer, A. *et al.* (2004) Plant PP2C phosphatases: emerging functions in stress signaling. *Trends Plant. Sci.* 9, 236–243
39 Merlot, S. *et al.* (2001) The ABI1 and ABI2 protein phosphatases 2C act in a negative feedback regulatory loop of the abscisic acid signalling pathway. *Plant J.* 25, 295–303
40 Yoshida, R. *et al.* (2006) The regulatory domain of SRK2E/OST1/SnRK2.6 interacts with ABI1 and integrates abscisic acid (ABA) and osmotic stress signals controlling stomatal closure in Arabidopsis. *J. Biol. Chem.* 281, 5310–5318
41 Kuhn, J.M. *et al.* (2006) The protein phosphatase AtPP2CA negatively regulates abscisic acid signal transduction in Arabidopsis, and effects of abh1 on AtPP2CA mRNA. *Plant Physiol.* 140, 127–139
42 Saez, A. *et al.* (2006) Enhancement of abscisic acid sensitivity and reduction of water consumption in *Arabidopsis* by combined inactivation of the protein phosphatases type 2C ABI1 and HAB1. *Plant Physiol.* 141, 1389–1399
43 Robert, N. *et al.* (2006) A hypermorphic mutation in the protein phosphatase 2C HAB1 strongly affects ABA signaling in Arabidopsis. *FEBS Lett.* 580, 4691–4696
44 Nishimura, N. *et al.* (2007) ABA-Hypersensitive Germination1 encodes a protein phosphatase 2C, an essential component of abscisic acid signaling in Arabidopsis seed. *Plant J.* 50, 935–949
45 Rubio, S. *et al.* (2009) Triple loss of function of protein phosphatases type 2C leads to partial constitutive response to endogenous abscisic acid. *Plant Physiol.* 150, 1345–1355
46 Nishimura, N. *et al.* (2009) Structural mechanism of abscisic acid binding and signaling by dimeric PYR1. *Science* 326, 1373–1379
47 Santiago, J. *et al.* (2009) The abscisic acid receptor PYR1 in complex with abscisic acid. *Nature* 462, 665–668
48 Miyazono, K. *et al.* (2009) Structural basis of abscisic acid signalling. *Nature* 462, 609–614
49 Yin, P. *et al.* (2009) Structural insights into the mechanism of abscisic acid signaling by PYL proteins. *Nat. Struct. Mol. Biol.* 16, 1230–1236
50 Melcher, K. *et al.* (2009) A gate-latch-lock mechanism for hormone signalling by abscisic acid receptors. *Nature* 462, 602–608
51 McCourt, P. and Creelman, R. (2008) The ABA receptors – we report you decide. *Curr. Opin. Plant Biol.* 11, 474–478
52 Shen, Y.Y. *et al.* (2006) The Mg-chelatase H subunit is an abscisic acid receptor. *Nature* 443, 823–826
53 Wu, F.Q. *et al.* (2009) The magnesium-chelatase H subunit binds abscisic acid and functions in abscisic acid signaling: new evidence in Arabidopsis. *Plant Physiol.* 150, 1940–1954
54 Liu, X. *et al.* (2007) A G protein-coupled receptor is a plasma membrane receptor for the plant hormone abscisic acid. *Science* 315, 1712–1716
55 Pandey, S. *et al.* (2009) Two novel GPCR-type G proteins are abscisic acid receptors in Arabidopsis. *Cell* 136, 136–148
56 Risk, J.M. *et al.* (2009) Reevaluation of abscisic acid-binding assays shows that G-Protein-Coupled Receptor2 does not bind abscisic Acid. *Plant Physiol.* 150, 6–11
57 Risk, J.M. *et al.* (2008) FCA does not bind abscisic acid. *Nature* 456, E5–E6
58 Vlad, F. *et al.* (2009) Protein phosphatases 2C regulate the activation of the Snf1-related kinase OST1 by abscisic acid in Arabidopsis. *Plant Cell* 21, 3170–3184
59 Umezawa, T. *et al.* (2009) Type 2C protein phosphatases directly regulate abscisic acid-activated protein kinases in Arabidopsis. *Proc. Natl. Acad. Sci. U. S. A.* 106, 17588–17593
60 Sirichandra, C. *et al.* (2009) The guard cell as a single-cell model towards understanding drought tolerance and abscisic acid action. *J. Exp. Bot.* 60, 1439–1463
61 Mustilli, A.C. *et al.* (2002) Arabidopsis OST1 protein kinase mediates the regulation of stomatal aperture by abscisic acid and acts upstream of reactive oxygen species production. *Plant Cell* 14, 3089–3099

62 Geiger, D. *et al.* (2009) Activity of guard cell anion channel SLAC1 is controlled by drought-stress signaling kinase-phosphatase pair. *Proc. Natl. Acad. Sci. U. S. A.* 106, 21425–21430
63 Lee, S.C. *et al.* (2009) A protein kinase-phosphatase pair interacts with an ion channel to regulate ABA signaling in plant guard cells. *Proc. Natl. Acad. Sci. U. S. A.* 106, 21419–21424
64 Sato, A. *et al.* (2009) Threonine at position 306 of the KAT1 potassium channel is essential for channel activity and is a target site for ABA-activated SnRK2/OST1/SnRK2.6 protein kinase. *Biochem. J.* 424, 439–448
65 Geiger, D. *et al.* (2010) Guard cell anion channel SLAC1 is regulated by CDPK protein kinases with distinct Ca2+ affinities. *Proc. Natl. Acad. Sci. U. S. A.* 107, 8023–8028
66 Mori, I.C. *et al.* (2006) CDPKs CPK6 and CPK3 function in ABA regulation of guard cell S-type anion- and Ca(2+)-permeable channels and stomatal closure. *PLoS Biol.* 4, e327
67 Zhang, W. *et al.* (2004) Phospholipase D alpha 1-derived phosphatidic acid interacts with ABI1 phosphatase 2C and regulates abscisic acid signaling. *Proc. Natl. Acad. Sci. U. S. A.* 101, 9508–9513
68 Sirichandra, C. *et al.* (2009) Phosphorylation of the Arabidopsis AtrbohF NADPH oxidase by OST1 protein kinase. *FEBS Lett.* 583, 2982–2986
69 Pei, Z.M. *et al.* (2000) Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. *Nature* 406, 731–734
70 Meinhard, M. *et al.* (2002) The sensitivity of ABI2 to hydrogen peroxide links the abscisic acid-response regulator to redox signalling. *Planta* 214, 775–782
71 Meinhard, M. and Grill, E. (2001) Hydrogen peroxide is a regulator of ABI1, a protein phosphatase 2C from Arabidopsis. *FEBS Lett.* 508, 443–446
72 Kolla, V.A. *et al.* (2007) Hydrogen peroxide production is an early event during bicarbonate induced stomatal closure in abaxial epidermis of Arabidopsis. *Planta* 225, 1421–1429
73 Srivastava, N. *et al.* (2009) Nitric oxide production occurs downstream of reactive oxygen species in guard cells during stomatal closure induced by chitosan in abaxial epidermis of Pisum sativum. *Planta* 229, 757–765
74 Negi, J. *et al.* (2008) CO2 regulator SLAC1 and its homologues are essential for anion homeostasis in plant cells. *Nature* 452, 483–486
75 Finkelstein, R. *et al.* (2005) Redundant and distinct functions of the ABA response loci ABA-INSENSITIVE(ABI)5 and ABRE-BINDING FACTOR (ABF)3. *Plant Mol. Biol.* 59, 253–267
76 Choi, H.I. *et al.* (2005) Arabidopsis calcium-dependent protein kinase AtCPK32 interacts with ABF4, a transcriptional regulator of abscisic acid-responsive gene expression, and modulates its activity. *Plant Physiol.* 139, 1750–1761
77 Fujii, H. *et al.* (2007) Identification of two protein kinases required for abscisic acid regulation of seed germination, root growth, and gene expression in Arabidopsis. *Plant Cell* 19, 485–494
78 Yoshida, T. *et al.* (2010) AREB1, AREB2, and ABF3 are master transcription factors that cooperatively regulate ABRE-dependent ABA signaling involved in drought stress tolerance and require ABA for full activation. *Plant J* 61, 672–685
79 Fujita, Y. *et al.* (2009) Three SnRK2 protein kinases are the main positive regulators of abscisic acid signaling in response to water stress in Arabidopsis. *Plant Cell Physiol.* 50, 2123–2132
80 Miura, K. *et al.* (2009) Sumoylation of ABI5 by the Arabidopsis SUMO E3 ligase SIZ1 negatively regulates abscisic acid signaling. *Proc. Natl. Acad. Sci. U. S. A.* 106, 5418–5423
81 Johnson, R.R. *et al.* (2002) The abscisic acid-responsive kinase PKABA1 interacts with a seed-specific abscisic acid response element-binding factor, TaABF, and phosphorylates TaABF peptide sequences. *Plant Physiol.* 130, 837–846
82 Zhu, S.Y. *et al.* (2007) Two calcium-dependent protein kinases, CPK4 and CPK11, regulate abscisic acid signal transduction in Arabidopsis. *Plant Cell* 19, 3019–3036
83 Yamaguchi-Shinozaki, K. and Shinozaki, K. (2006) Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annu. Rev. Plant Biol.* 57, 781–803
84 Himmelbach, A. *et al.* (2002) Homeodomain protein ATHB6 is a target of the protein phosphatase ABI1 and regulates hormone responses in Arabidopsis. *EMBO J.* 21, 3029–3038
85 Ohta, M. *et al.* (2003) A novel domain in the protein kinase SOS2 mediates interaction with the protein phosphatase 2C ABI2. *Proc. Natl. Acad. Sci. U. S. A.* 100, 11771–11776
86 Miao, Y. *et al.* (2006) An Arabidopsis glutathione peroxidase functions as both a redox transducer and a scavenger in abscisic acid and drought stress responses. *Plant Cell* 18, 2749–2766
87 Wu, Y. *et al.* (2003) The *abi1-1* mutation blocks ABA signaling downstream of cADPR action. *Plant J.* 34, 307–315