New developments in abscisic acid perception and metabolism

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Abscisic acid is a powerful signaling molecule that accumulates in response to abiotic stress. However, no potential receptors that could perceive this increase in abscisic acid had been identified until recent reports of three abscisic acid binding proteins: the nuclear protein Flowering Time Control Locus A, the chloroplast protein Magnesium Protoporphyrin-IX Chelatase H subunit, and the membrane-associated protein G Protein Coupled Receptor 2. Abscisic acid metabolism also has a new and prominent component with the identification of a β-glucosidase capable of releasing biologically active abscisic acid from inactive abscisic acid-glucose ester in a stress-inducible manner. These observations refocus our attention on the metabolism underlying abscisic acid accumulation, sites of abscisic acid perception, and delivery of abscisic acid to those sites.

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Introduction

Abscisic acid (ABA) has key roles in regulating plant responses to abiotic stress and in controlling seed germination, growth, and stomatal aperture [1–3]. Physiological experiments with numerous plant species have indicated that accumulation of ABA above its basal level in unstressed plant tissues is required for its role in promoting abiotic stress resistance [2]. This ABA accumulation must then be followed by ABA perception and subsequent downstream signaling to activate ABA-regulated stress responses (Figure 1). While this general framework has been a part of our core knowledge of plant function for some time, there are still a number of important gaps in our knowledge of how ABA functions at the molecular and physiological levels (Box 1). In signaling downstream of ABA perception, some of the most intriguing recent results have involved the protein phosphatase 2Cs Abscisic Acid Insensitive 1 (ABI1) and ABI2 that have roles in many aspects of ABA signaling [1]. Particularly interesting is the recent observation that ABI2 interacts directly with Glutathione Peroxidase3, and this interaction affects the redox status and phosphatase activity of ABI2, providing a direct link between ABA signaling and redox status [4•]. Most relevant to this review is the interaction of ABI1 with phosphatidic acid to control stomatal regulation [5••], an observation that also links ABI1 to G protein signaling. Other recent data have highlighted the importance of SnRK2 kinases [6] and ubiquitination [7–9] in ABA signaling.

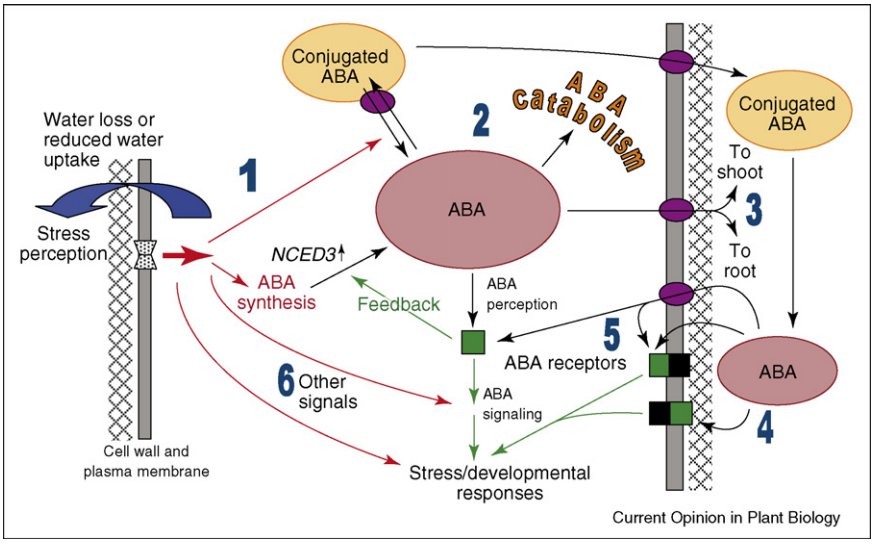
Before these signaling mechanisms downstream of ABA perception can be called into action, production, and delivery of biologically active ABA, and its initial perception by ABA receptors must occur. Although these aspects of ABA signaling have at times been less prominent in the molecular literature, several recent studies have brought these topics back to the fore of ABA research. Thus, the perception of ABA and control of ABA accumulation, particularly by deconjugation of inactive ABA, are the focus of this review.

ABA perception inside and outside

The site of ABA perception and particularly the question of intracellular versus extracellular perception have intrigued plant biologists for many years. In the absence of a known receptor, a number of studies sought to test extracellular versus intracellular ABA perception [10–13]. The results showed that both intracellular and extracellular ABA perception could occur, leading to the hypothesis, which seems even more likely in light of recent data, of multiple sites of ABA perception. Other evidence in favor of multiple sites of ABA perception came from studies showing differences in the responses elicited by different ABA analogs [14•,15]. In addition, the multiple receptor hypothesis became more prominent as forward genetic screening failed to turn up a candidate ABA receptor. This difficulty in identifying ABA receptors led to a near stalemate in ABA receptor research for a number of years. That stalemate has now been broken by reports describing three ABA-binding proteins that have many of the characteristics of ABA receptors [16••,17••,18••].

The first two of these proteins to be reported, Flowering Time Control Locus A (FCA) and Magnesium Protoporphyrin-IX Chelatase H subunit (CHLH/GUN5/ABAR; referred to here as CHLH), established intracellular sites for ABA binding, and both are members of relatively

Figure 1



Conceptual diagram of stress perception and ABA metabolism, transport, and signaling. Large numbers indicate the six points outlined in Box 1. Red arrows indicate perception and signaling events upstream of ABA accumulation. Black arrows indicate ABA metabolism and transport processes. Green boxes and arrows represent ABA perception and downstream ABA-dependent signaling including feedback regulation by ABA. Purple ovals indicate possible sites where as yet unidentified ABA transporters could operate.

Box 1 Six short points likely to have a long impact on ABA research (numbers correspond to the numbers in Figure 1).

1. *Unknown upstream*. The initial events that are responsible for sensing abiotic stresses such as drought-associated water loss and that act upstream of and elicit ABA accumulation are not known. Also unclear is whether this initial stress perception is required to make the plant competent to respond to ABA (see #6).

2. *Tumultuous turnover*. Experiments have suggested a rapid turnover of ABA. It is now known that amount of active ABA can be regulated by synthesis, conjugation, and catabolism, but what is the relative importance of each of these processes and how are they regulated?

3. *Up and down*. The importance of root-to-shoot transport of ABA and/or ABA precursors/conjugates as well as the main site (root or shoot) of stress-induced changes in ABA metabolism remains unclear. Also, important is the question of whether ABA transporters play a role in distributing ABA within the plant or whether ABA distribution is predominantly controlled by pH gradients.

4. *In and out*. The ABA-binding proteins identified so far suggest that intracellular (nucleus and chloroplast) and extracellular ABA perception can both control many of the same phenotypes. Which sites (receptors) are most important for different ABA responses and how do they interact?

5. *Receptors revealed*. Three candidate receptors for ABA have been described, but it seems likely that there are more. Whether or not additional receptors will be found for intracellular ABA perception, extracellular perception, or both remains to be seen.

6. *Signaling and sensitivity*. A number of observations have indicated that exogenous ABA applied to unstressed plants does not elicit the same responses as stress-induced ABA accumulation. This could represent additional signaling mechanisms that act in parallel with ABA or an effect of stress that makes the plant competent to respond to ABA (increases the ABA sensitivity).

unexpected classes of proteins to be ABA receptors. FCA had been previously characterized as a nuclear RNA-binding protein that when complexed with FY, another floral regulator protein, caused cleavage of Flowering Locus C (FLC) RNA thereby blocking FLC expression and allowing flowering to occur [19]. Razem *et al.* [17••] found that binding of ABA to a site in the C-terminal portion of FCA disrupts the FCA–FY complex. This allows FLC mRNA to accumulate and delays flowering. These results were surprising partly because the mechanism of ABA signaling revealed apparently bypassed other ABA signal transduction components [20]. While this was unexpected, it did not overturn previous work on ABA signaling because it was also clear that FCA was a specific regulator of flowering and lateral root formation [17••]. Thus, the search was still on for a receptor that could regulate other key ABA-regulated events such as germination and stomatal movement.

Another equally unexpected ABA-binding protein emerged nearly simultaneously with FCA: The chloroplast protein CHLH also was shown to have specific ABA-binding activity [18••]. Plants with decreased CHLH expression had ABA-insensitive phenotypes in germination and stomatal closure while plants overexpressing CHLH were ABA hypersensitive in these responses [18••]. However, a mechanism by which CHLH could transmit the ABA signal has yet to be described. The significance of CHLH's location in the chloroplast is also unclear. The chloroplast is the site of ABA synthesis up to the point of the cleavage reaction catalyzed by the 9-*cis*-epoxycarotenoid dioxygenase (NCED) family of

enzymes [2]. Thus, the chloroplast would be a logical site of ABA perception for feedback regulation of ABA synthesis; however, CHLH itself is not involved in ABA synthesis, and such feedback regulation is unlikely to explain the phenotypes observed when CHLH expression was altered [18••]. Further work to establish the mechanism of CHLH function in ABA signaling is needed.

The third reported ABA-binding protein brought a well-known class of signaling molecules into the group of proteins with potentially key roles in ABA perception. Plants contain a number of candidate G protein coupled receptors (GCRs) that have a characteristic seven membrane spanning domains. In the classical model of G protein signaling, ligand binding to the extracellular portion of the GCR activates it and causes dissociation of the intracellular Gα/Gβ/Gγ complex into Gα and Gβ/Gγ portions along with release of GDP and binding of GTP [21]. The dissociated Gα and Gβ/Gγ can then activate signal transduction by a number of mechanisms. Conclusively identifying candidate GCRs in Arabidopsis has been difficult and no ligand had been identified for any GCR until Lui *et al.* [16••] reported a candidate GCR, which they designated as GCR2, that had ABA-binding activity. The same study also provided evidence that GCR2 interacts with GPA1, the sole Arabidopsis Gα, and that binding of ABA to GCR2 causes it to disassociate from GPA1 as would be expected in the initiation of a classical G protein signaling cascade. Knockout or overexpression of GCR2 altered several ABA-dependent phenotypes [16••].

In spite of the data reported by Lui *et al.* [16••], questions about the role of GCR2 remain. Previous detailed bioinformatics analysis did not identify GCR2 as one of 394 Arabidopsis candidate seven transmembrane-domain GCRs [22], and the Arabidopsis genome annotation has identified GCR2 as being similar to the enzyme lanthionine synthetase. It is also of interest to note that, because GCR2 had a relatively high pH optimum of ABA binding (pH 7.5), its ABA-binding activity at typical apoplast pH values (pH 5.0–6.0 [23]) would be only approximately half of the maximal level. This point is of particular interest: Increased apoplastic pH has been proposed to be important in root-to-shoot stress signaling and would deprotonate ABA and thus inhibit its diffusion across the plasma membrane into the cell [23].

Despite these lingering questions, the potential involvement of GCRs in ABA perception and signaling is important not only because it potentially puts ABA perception in the context of a known signaling mechanism but also because it suggests a link to ABI1, a well-studied ABA-signaling factor. ABI1 binds phosphatidic acid produced by phospholipaseDα1 (PLDα1) and this binding tethers ABI1 to the membrane and promotes stomatal closure [5••]. The converse response, stomatal opening, is promoted by PLDα1 binding to GPA1 [5••,24]. Because of the broad effects of ABI1 and ABI2 on ABA signaling, it will be of interest to determine whether PLDα1 and phosphatidic acid also regulate other ABA responses, such as germination, which are affected in *abi1*, *gcr2*, and *gpa1* mutants [1,16••,25•]. Also of interest is whether other candidate GCRs bind ABA and participate in ABA signaling and whether other PP2Cs are involved in signaling through GPA1, as genetic data suggest they could be [26–28].

This flurry of activity in ABA receptor research raises the question of whether there are more candidate ABA receptors yet to be characterized. The answer to this question appears to be yes. The same studies using ABA anti-idiotypic antibodies that led to the eventual identification of FCA as an ABA receptor also identified another barley ABA-binding protein, ABAP1, whose molecular identity remains obscure [20,29]. Also, a number of other studies have identified ABA-binding activities in plant extracts [30–34], and it is unlikely that all of these activities can be explained by the three ABA-binding proteins characterized thus far. Additional data on what the ABA-binding motifs of FCA, CHLH, and GCR2 look like may be useful in searching for additional proteins with similar motifs.

Active ABA: regulation by metabolism, conjugation, and transport

For ABA receptors to function, there must first be an accumulation of biologically active ABA at the site of perception. Although ABA synthesis is required, whether or not it is the main factor in controlling how much ABA accumulates under stress is unclear. High levels of NCED3 overexpression led to only relatively small increases or no effect on ABA content under stress conditions [35,36•,37] but much larger increases in phaseic acid, the principle ABA catabolite [36•,37]. This is consistent with observations that exogenously applied ABA is rapidly catabolized [14•] and inhibitor and labeling studies that have also suggested a rapid turnover of ABA in both unstressed and dehydrated plant tissue [38,39,40•,41]. These observations establish both ABA synthesis and ABA catabolism as determinants of stress-induced ABA accumulation; however, the relative importance of these two processes in stress-induced ABA accumulation is unclear.

For ABA, and for other hormones, there is another factor: conjugation. ABA can be conjugated to glucose (as well as other sugars) to form ABA-glucose ester (ABA-GE) that is biologically inactive [42]. However, the significance of ABA conjugation and whether or not it is a readily reversible modification or a 'dead end' that permanently removes ABA from the biologically active hormone pool had not been determined. While ABA glycosyltrans-

ferases have been described [36•,43–45], until recently we still lacked molecular knowledge of ABA glucosidases that could release ABA-GE back into the pool of biologically active ABA. That situation has now changed with the identification of *Arabidopsis thaliana* β-glucosidase1 (AtBG1).

AtBG1 was first identified as a gene upregulated by salt stress and subsequent molecular characterization demonstrated that AtBG1 could hydrolyze ABA-glucose ester [46••]. *atbg1* plants exhibited a number of phenotypes consistent with ABA deficiency and had decreased seed ABA levels and a slightly decreased level of dehydration-induced ABA accumulation [46••]. Conversely, plants that overexpressed ATBG1 had increased levels of dehydration-induced ABA accumulation. These results show that ABA conjugation and deconjugation are dynamic processes and have a significant role in controlling levels of biologically active ABA both under unstressed conditions and under stress when the amount of free ABA increases dramatically.

Dehydration treatment caused ATBG1 to polymerize (molecular weights consistent with ATBG1 10-mers were observed) and this polymerization increased the specific activity of ATBG1 in ABA-GE hydrolysis [46••]. Thus, despite the original observation that ATBG1 gene expression is increased by stress, the key factor in upregulating its activity under stress is polymerization. Another interesting finding was that ATBG1 is an endoplasmic reticulum-localized protein. By contrast, ABA-GE is found mostly in the vacuole and intercellular space [47]. To bridge this spatial separation between ABA-GE and ATBG1, the existence of a transport system for ABA-GE was proposed [46••]. Since no transporter for ABA, ABA-GE, or other ABA metabolites or precursors has yet been identified, this is a significant question for future experiments.

The transport question is also significant at the whole plant level where transport of ABA [48] or of ABA-GE with subsequent release of free ABA in the leaf [47,49] have been proposed as root-to-shoot signals. ABA-GE has been found in xylem sap of both stressed and unstressed plants and a salt-induced extracellular glucosidase activity capable of hydrolyzing ABA-GE has also been observed in leaves [47,49,50]. Interestingly, no difference in extracellular ABA-GE hydrolyzing activity was found in ATBG1 knockout and overexpression plants [46••]. Thus, the extracellular hydrolysis of ABA-GE must be performed by another enzyme. Identification of this enzyme would allow the importance of extracellular localized ABA-GE to be determined. It should also be noted that hypotheses about root-to-shoot ABA transport, which are often mentioned in the physiological literature, must consider whether ABA synthesis capacity of roots, which is more likely to be substrate limited than that of shoots, is sufficient to influence shoot ABA content [51].

New and old questions about ABA

The identification of ABA receptors and ATBG1 as well as other enzymes involved in ABA metabolism herald a renewed emphasis on events at or upstream of the level of ABA perception (Figure 1). These new observations suggest a number of areas likely to be of importance in future ABA research (Box 1). Perhaps the biggest gap in our knowledge is in the sensing and signaling events that lie upstream of stress-induced ABA accumulation. For example, how is the polymerization of ATBG1 induced? Such events must surely be connected to the initial perception of stress, particularly dehydration of plant tissue. Such upstream signaling can induce some stress responses independently of ABA, induce ABA synthesis, and increase the plants response to ABA [52–54]; however, the nature of this initial signal and its perception are unknown. Also, the question of ABA and ABA-GE transport both within the cell and from root to shoot has also been raised anew by the description of ATBG1 and our increased understanding of where and how ABA is perceived. A number of studies have described specific, saturable uptake of ABA by various plant tissues and provided strong evidence for the existence of ABA transporters [55–62]. These data have not yet been followed up by the identification of an ABA transporter. Thus, much of our new information is leading us back to some old questions.

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