Plant Signalling from Genes to Biochemistry

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Before and beyond ABA: upstream sensing and internal signals that determine ABA accumulation and response under abiotic stress

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Abstract

Sensing and signalling events that detect abiotic stress-induced changes in plant water status and initiate downstream stress responses such as ABA (abscisic acid) accumulation and osmoregulation remain uncharacterized in plants. Although conclusive results are lacking, recent results from plants, and analogies to signalling in other organisms, suggest possible mechanisms for sensing altered water status and initial transduction of that signal. Internal signals that act downstream of ABA and modulate stress responses to reflect the type and severity of the stress and the metabolic status of the plant are also not well understood. Two specific types of signalling, sugar sensing and reactive oxygen signalling, are likely to be modulators of ABA response under stress. For both upstream sensing and signalling of plant water status as well as downstream modulation of ABA response, present results suggest several genetic strategies with high potential to increase our understanding of the molecular basis by which plants sense and respond to altered water status.

Abiotic stress can limit crop productivity and is a key determinant of the natural distribution of plant species. Abiotic stresses, such as low water potential and salt stress, that cause water loss or reduced water uptake also lead to accumulation of ABA (abscisic acid; [1–3]). Numerous studies have shown that ABA accumulation is a key factor in controlling downstream responses essential for adaptation to the stress. These responses include changes in stomatal conductance [4], growth [5], osmolyte accumulation [6,7] and gene expression [8]. Each of these responses is controlled by a complex web of signalling events. Understanding these signalling events is a prerequisite to manipulate plant stress responses in ways that can improve crop productivity under subopitimal conditions.

Conceptually, plant stress response can be divided into several phases of perception and signalling events (Figure 1). These events integrate to produce a stress response that is appropriate both to the external stress conditions and the internal physiological status of the plant. The starting point for any response to the environment is the perception of a signal that, regardless of the actual site or mechanism of perception, originates outside the plant tissue itself. This initial perception is connected to upstream signalling events that control many aspects of stress response including ABA accumulation. The accumulation of ABA then elicits another layer of perception and signalling that we have termed intermediate signalling. ABA response can also be influenced by a range of other signals that arise either from direct sensing of the stress or from stress-induced changes in metabolism or development. In the remainder of this review, we will discuss two relatively poorly understood aspects of stress signalling. The first is the external signal sensing and upstream signalling that precedes and controls ABA accumulation. We will further focus this discussion on the mechanisms of sensing and responding to low water potential. However, this discussion has relevance to other stresses, such as salinity, that

Key words: abiotic stress, abscisic acid (ABA), osmoregulation, reactive oxygen species (ROS), upstream sensing, upstream signalling.

Abbreviations used: ABA, abscisic acid; MAPK, mitogen-activated protein kinase; NCED, 9-*cis*-epoxycarotenoid dioxygenase; ROS , reactive oxygen species.

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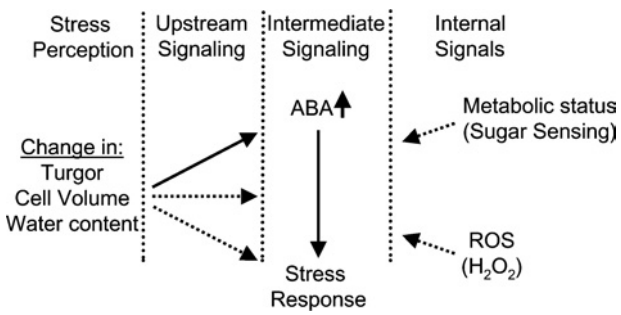
Figure 1 | Conceptual diagram of stress sensing and ABA signalling
Sensing of low water potential stress, such as soil drying or hyperosmolarity imposed using a non-ionic solute, occurs by an unknown mechanism and is followed by upstream signalling. Upstream signalling leads to ABA accumulation (solid line) and may also alter ABA response or induce certain stress responses independently of ABA (broken lines). ABA accumulation and perception of ABA induces intermediate signalling events that control downstream stress responses. ABA response can also be influenced by internal factors such as sugar sensing and ROS production.



can also cause a change in tissue water content. We will then discuss two specific internal signals, ROS (reactive oxygen species) production and sugar levels, which are possible links between cellular metabolic status and ABA-dependent stress responses.

Stress perception

The most common model of sensing external stimuli is that of a chemical ligand binding to a specific receptor. However, sensing of water status differs from sensing of other stimuli in that there is no chemical ligand that can be sensed. Therefore, other factors, such as changes in turgor, membrane strain, or molecular crowding are most probably the primary stimulus detected [9,10]. Although the identity of the primary stimulus and the proteins involved in detecting it are unknown, there are some relevant clues. Physiological experiments have implicated loss or reduction of turgor, presumably sensed as a mechanical change in cell shape or volume, as the main stimulus in eliciting ABA accumulation [11–13]. Hsiao [9] has presented a detailed argument that, over the range of water potential sensed by plants, physical properties such as water activity and structure change little, whereas turgor changes substantially and is most likely to be the initial stimulus. However, direct sensing of external osmolarity cannot be ruled out [14].

Thus far, our best molecular clues about how low water potential may be perceived come from what is known about osmosensing in yeast. SYNTHETIC LETHAL OF N-END RULE1 (*SLN1*), a two-component histidine kinase and one of the two known osmosensors in yeast, senses cellular turgor pressure [15,16] by an unknown mechanism. The other yeast osmosensing protein, SH3-DOMAIN OSMOSENSOR1 (SHO1), has a very similar function [16]. At least two of the *Arabidopsis* histidine kinase genes, ARABIDOPSIS THALIANA HISTIDINE KINASE1 (*ATHK1)* and CYTOKININ RESPONSE1 (*CRE1*), complement *sln1* deletion mutants of yeast [15,17] and *CRE1* can also respond to changes in turgor pressure when expressed in yeast [15]. Yet, these proteins have not been shown to function as osmosensors in plants.

Mammalian integrins are known to be involved in sensing mechanical stimuli [18], which in plants could be caused by a change in turgor. It has been argued that plant integrins could be involved in various types of mechano-sensing, including stress responses [19,20]. However, plant integrins, or the genes encoding them, have not yet been identified. Whatever the primary sensing mechanism, it must be linked to downstream molecules that transmit the signal.

Upstream signalling

In the case of low water potential, direct experiments demonstrating the involvement of specific signalling molecules are lacking. What information is available again comes largely through analogy to yeast signal transduction. Upon perception of water loss in yeast, both SLN1 and SHO1 activate a specific MAPK (mitogen-activated protein kinase) signalling cascade commonly referred to as the HOG pathway after HOG1, a MAPK that was the first component of the pathway to be discovered [21]. In plants, it has been observed that dehydration stress leads to increased gene expression of all the three (MAPK, MAPK kinase and MAPK kinase kinase) components of a typical MAPK signalling cascade [22,23] and these *Arabidopsis* kinases have been shown to interact in yeast in the correct sequence to produce a functional signalling cascade. It has also been observed that abiotic stresses, including dehydration, activate the protein phosphorylation activity of some *Arabidopsis* MAPKs [24].

In plants, however, the importance of a MAPK cascade in signalling changes in water status is uncertain for several reasons. As described above, the upstream sensors that could provide the input signal have not been identified. Likewise, MAPK signalling has not been shown to be necessary to activate any downstream stress responses in plants [2]. Also, with the present data, it is not possible to determine whether such a MAPK cascade is involved in the early signal transduction that is connected to the initial perception of the stress or is instead involved in downstream events, perhaps dependent on ABA accumulation. Gene expression of numerous other signal transduction-related proteins and activity of kinases and phosphatases have been shown to be altered by abiotic stress, but similar uncertainties exist for most, if not all, of these genes [2].

A limiting factor in understanding upstream stress signalling is that molecular targets that are directly regulated by upstream signalling, as opposed to ABA-dependent and other downstream signalling, have not been conclusively identified. ABA accumulation itself is undoubtedly controlled by upstream events. However, there are several metabolic processes (e.g. ABA synthesis, ABA catabolism or ABA conjugation) that could control ABA accumulation. Within each of these

metabolic processes, there are several genes that may act as a rate-limiting factor. Also, these genes may be the subject of feedback regulation by ABA [3,25]. Despite this complexity, forward genetic analysis focused on genes involved in ABA metabolism is one of the most promising approaches to identify upstream stress signalling components. Of particular interest are the NCED (9-*cis*-epoxycarotenoid dioxygenase) genes. NCED catalyses the cleavage of the C_{25} carotenoids 9-*cis*-neoxanthin or 9-*cis*-violaxanthin to the C_{15} ABA precursor xanthoxin [26]. This reaction has been proposed to be rate limiting in ABA synthesis [26–29]. Among the nine NCED family genes in *Arabidopsis*, NCED3 is the most strongly induced by dehydration [28,30] and is a good candidate for direct regulation by upstream signalling.

In addition to ABA metabolism, other stress responses may be regulated directly by upstream signalling components in an ABA-independent manner. The recently isolated *Arabidopsis lwr2* mutant has decreased osmoregulatory solute accumulation in response to low water potential but is unaffected in transpirational water loss, ABA inhibition of seed germination and ABA-induced proline accumulation [31,32]. In the same set of studies, it was also observed that the ABA-deficient mutant *aba2-1* has wild-type levels of osmoregulatory solute accumulation in response to low water potential (P.E. Verslues and E.A. Bray, unpublished work; [31]). These results suggest that osmoregulatory solute accumulation may be an ABA-independent stress response controlled directly by upstream signalling components. LWR2 is probably one of these upstream signalling components. Molecular identification and characterization of the *LWR2* gene and gene product will be of great interest and is likely to increase further our understanding of osmoregulation and upstream stress signalling.

Modulation of the ABA signal

A number of studies at both the molecular and physiological levels have indicated that ABA accumulation is required, but is not sufficient, to elicit the response observed under stress conditions. This is true for growth responses of maize seedlings where endogenous ABA accumulation under low water potential stress and exogenous ABA applied to unstressed seedlings produces opposing effects on root and shoot growth [5,33]. It is also true for expression of the ABA-induced le25 gene [34] and proline accumulation (P.E. Verslues and E.A. Bray, unpublished work; [31]), where exogenous ABA application under unstressed conditions does not elicit the same level of response seen after low water potential treatment. Because all three of these stress responses have been shown to require ABA, a probable explanation is that other factors are also required to modulate or increase the plant's response to ABA under stress.

There are several possible candidates for modulators of ABA response. One possibility is that the upstream sensing and signalling components discussed above may interact with signalling downstream of ABA and amplify or modify the ABA signal. Another possibility is that internal signals reflecting metabolic or energy status modulate the responsiveness to ABA. This is logical as stress alters plant metabolism in a number of ways, from decreasing photosynthetic carbon uptake to altering ion transport to increasing compatible solute synthesis. The metabolic and energy status of the plant may influence the extent to which ABA-regulated stress responses such as altered growth or compatible solute accumulation can be supported. Recent evidence points to two particular factors, sugar sensing and reactive oxygen production, as potential links between metabolic status and ABA response.

Sugar sensing

Several authors have proposed that ABA is an important regulator of sugar sensing. This is based on the observation that several *Arabidopsis* mutants originally identified as being ABA-deficient (*aba1*, *aba2*, *aba3*) have also been found to be sugar insensitive [35,36]. In addition, the ABA-insensitive mutants *abi4* and *abi5* have also been found to have sugar insensitive seedling growth [35–37]. These authors have focused on the role of ABA in responding to 'sugar stress', a high level of sugar that blocks the growth of wild-type seedlings. However, the connection between ABA and sugar response found in these studies suggests that sugar sensing may modulate the effects of ABA under other types of stress.

One example of this connection between ABA, stress and sugar sensing is low water potential-induced proline accumulation. Proline accumulation at low water potential requires ABA ([38]; P.E. Verslues and E.A Bray, unpublished work) but the response can be enhanced by increased sugar supply or light [39,40]. The sugar- and ABA-insensitive mutant *abi4* has a higher level of low water potential-induced proline accumulation in the absence of external sugars but not in the presence of 3% (w/v) sucrose (P.E. Verslues and E.A. Bray, unpublished work). This suggests that there is a specific signalling link between sugar sensing and ABA-induced proline accumulation, and ABI4 is one of the mediators of this link. Thus the level of proline accumulation observed is determined by an interaction of external signals (water loss caused by low water potential), intermediate signalling (ABA accumulation and response) and internal signals (sugar sensing). Such interactive effects have been observed in a number of other physiological studies; the challenge now is to find the molecular basis for these interactions.

Reactive oxygen

Several types of plant stress are known to lead to increased levels of ROS in plant tissue [41–43], and ROS, along with ABA accumulation, has been proposed to be a key component of 'cross tolerance' to multiple types of stress [42]. Reactive oxygen can be generated by a range of metabolic sources, including chloroplast electron transport, mitochondrial respiration, and peroxisomal lipid and photorespiratory metabolism [42,43]. High levels of ROS lead to cellular damage and are involved in programmed cell death in both mammalian and plant cells [43]. Plant cells are able to tolerate

significant levels of ROS production and contain high levels of antioxidants, such as ascorbate and glutathione, to prevent uncontrolled ROS accumulation [42].

Increasing evidence suggests that ROS can have effects on signalling and metabolism at levels well below that required to cause general cellular damage. Thus even relatively small changes in the production of ROS, or the antioxidants needed to control ROS levels, can have significant effects on signal transduction. In addition, there is evidence that the specific chemical identity of the ROS or antioxidant molecules involved and the site of ROS production can determine the specificity of the response [43–45]. Links between ROS and hormone signalling have been proposed [42,46]. There is specific genetic evidence that ROS generated by NADPH oxidases act downstream of ABA in mediating stomatal closure [47]. Changes in ROS production or scavenging may also be involved in signalling a variety of other hormone and stress responses [41,48–50]. The link between ROS and ABA signalling is strongest for the control of stomatal aperture. However, ROS also affects root growth [47] and germination (P.E. Verslues, Y.-S. Kim and J.-K. Zhu, unpublished work).

Several lines of experiments will be needed to obtain a better understanding of the ABA–ROS interaction. The genes that are responsible for ROS production under stress will need to be identified. This will lead to a better understanding of which metabolic processes are the source of ROS and could lead to the discovery of new signalling functions for well known metabolic enzymes. Also, it will be important to extend investigation of the ABA–ROS interaction to additional ABA-regulated traits such as changes in growth under stress, specific changes in gene expression and ABA-induced proline accumulation. This latter trait is particularly intriguing because, although proline accumulation is ABA-regulated, one of the proposed functions of proline or proline metabolism under stress is ROS scavenging [51] or regulation of cellular redox status [52].

Future prospects

Many of the pieces are now in place to advance our understanding of upstream stress signalling and the interaction of ABA with other signals. Genes likely to be directly regulated by upstream signalling, such as *Arabidopsis NCED3* and other ABA metabolism genes, have now been identified. This means that forward genetic screens using promoter/reporter constructs, such as those previously used to identify factors regulating *RD29A* or *CBF3* [53,54], can now be targeted more effectively to identify upstream sensing and signalling components. The genetic resources available in *Arabidopsis* are useful in another regard: the availability of mutants impaired in one part of stress response make possible strategies that allow normally linked parts of the stress response to be studied independently of one another. Examples are the use of ABA-deficient mutants to identify stress responses that can be induced independently of ABA accumulation, or the use of lines impaired in ROS production or scavenging to investigate ABA response in the presence of altered ROS production.

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