Abscisic Acid

Abscisic acid (ABA) is the major hormone that controls plants’ ability to survive in a harsh, changing environment. When challenged by water stress, plants increase their synthesis of ABA, which triggers responses including stomatal closure to reduce transpiration and expression of genes to produce osmoprotectants. Similar genes are ABA induced in maturing seeds and are thought to confer desiccation tolerance to the developing embryo. ABA also regulates seed dormancy and germination, contributes to developmental controls, and has a role in biotic stress responses. The ABA signaling pathway is conserved across all plants, including mosses, and is considered a very early adaptation to the terrestrial environment. Although one essential component of the ABA signaling pathway was identified more than 25 years ago, a key family of cytoplasmic ABA receptors was just identified in 2009. Research into the ABA signaling and response pathways is extremely vigorous in part because scientists are eager to expand plants’ stress tolerances in the face of global environmental changes.

ABA SYNTHESIS AND TRANSPORT

ABA was purified independently through studies of fruit abscission and bud dormancy and was named abscisin II and dormin. The name abscisic acid persisted, which is unfortunate because abscission is an indirect ABA response mediated by other hormones, whereas Dormin would have been a better name because ABA has a direct role in seed dormancy. Elucidation of the pathway for ABA synthesis has been ongoing since the 1960s, and since the 1980s has been augmented by molecular genetics approaches in Arabidopsis thaliana and other plants. The entire pathway was completely worked out at the beginning of the 21st century, more than 40 years after ABA was first purified. ABA research is conducted by countless laboratories around the world, and so many scientists have made critical contributions that it is difficult to single any out; however, Jan Zeevart’s contributions to elucidating ABA synthesis and metabolism should not be overlooked. Shortly before he died in 2009, he published a short biography that gives an engaging personal account of his work on ABA (Zeevart, 2009).

Synthesis

ABA is derived from the modification and cleavage of zeaxanthin, a 40-carbon carotenoid. Carotenoids are abundant pigments found in chloroplasts of photosynthetic tissues. Zeaxanthin epoxidase converts zeaxanthin to violaxanthin. This gene was first cloned from Nicotiana plumbaginifolia and referred to as NpABA2; in Arabidopsis, this gene is called ABA1. After structural modification, violaxanthin is converted to one of two 9'-cis-epoxycarotenoids, which in turn are substrates for the enzyme 9-cis-epoxycarotenoid dioxygenase (NCED). NCED cleaves the 40-carbon substrate into a 25-carbon by-product and the 15-carbon compound xanthoxin, which is subsequently converted to ABA-aldehyde and then ABA.

NCED genes were first identified from the Zea mays viviparous14 mutant, which has a higher rate of transpiration and reduced seed dormancy due to a lower ABA content. NCED gene expression is a major determinant of ABA accumulation. Expression is induced after a leaf is detached to initiate drying and coincides with the period of ABA synthesis during seed maturation. NCED is encoded by a multigene family whose members are differentially expressed. Some are expressed in roots, others in leaves (particularly in the stomatal guard cells and vascular parenchyma), and some are strongly induced by water stress. Loss of NCED gene function usually leads to reduced ABA levels, whereas overexpression is correlated with increased ABA levels, drought tolerance, and prolonged seed dormancy.

The 15-carbon product of NCED, xanthoxin, is converted to ABA via two enzymes. Xanthoxin is converted to abscisic aldehyde by ABA2, a short-chain dehydrogenase encoded by a single ABA2 gene in Arabidopsis. Loss-of-function aba2 mutants were identified as having a wilty phenotype caused by excessive transpiration, precocious germination due to a partial loss of seed dormancy, and the ability to germinate on inhibitory levels of salt or sucrose. Abscisic aldehyde is oxidized to ABA by an abscisic aldehyde oxidase (AAO). AAO requires a molybdenum cofactor; mutants unable to produce the cofactor, such as the tomato (Solanum lycopersicum) flacca mutant, also show an ABA-deficient wilty phenotype.

Inactivation and Conjugation

ABA accumulation is governed in part by structural modifications, including irreversible inactivation and reversible glucosylation. ABA can be inactivated by hydroxylation at the C-7’, C-8’, or the C-9’ positions; the C-8’ pathway is most prevalent. Although 8’-hydroxy ABA retains biological activity, it is rapidly converted to the inactive compounds phaseic acid and dihydrophaseic acid. This inactivation pathway is critical for the removal of ABA from drought-stressed plants upon rehydration as well as the elimination of ABA from seeds prior to germination. ABA 8’-hydroxylase is a cytochrome-P450 enzyme encoded by the four members of the CYP707A gene family in Arabidopsis. Inhibitors of this enzyme can confer some drought protection upon plants by maintaining elevated levels of ABA. One promising P450 inhibitor, uniconazole, also inhibits the action of other P450 enzymes, including those involved in
synthesis of gibberellins, so its application interferes with plant growth. However, it has been possible to make derivatives of uniconazole with increased specificity for ABA β-hydroxylase that do not interfere with gibberellin synthesis. These and similar studies are important in our quest for methods to promote drought tolerance in crop plants.

ABA activity is also governed by conjugation to glucose to form an ABA-glucosyl ester (ABA-GE) by an ABA glucosyltransferase. ABA-GE is inactive but readily reconverted to ABA by the action of a β-glucosidase. Loss of function of this glucosidase causes an ABA-deficient phenotype, revealing the importance of this sequestration pathway. Conversely, mutants in UDP glucosyltransferase, which cannot conjugate glucose to ABA, have an ABA-excess phenotype. ABA-GE, as an inactive and highly hydrophilic molecule, may be the preferred form of ABA for long distance transport.

**Long- and Short-Distance Movement of ABA**

Drought conditions are first experienced by the roots that are in direct contact with drying soil. Reduced soil water availability causes increased synthesis of ABA in the roots. Elevated ABA in the roots contributes to root growth under water stress conditions (see below). Furthermore, ABA synthesized in the root can be translocated through the xylem to the shoot and contribute to the shoot’s responses to drought. (Other translocated signals may also contribute to this response, including hydraulic signals, cytokinins, and pH changes.) Under drought stress conditions, the level of ABA that accumulates in the leaves is severalfold higher than that in the roots, in part because de novo ABA synthesis also occurs in the leaves. As yet the relative contributions of root-synthesized versus shoot-synthesized ABA in shoot responses are still being debated.

This signaling system from root to shoot is so sensitive that drying only a small portion of the root mass can promote stomatal closure, even when the leaf itself experiences no change in water availability. This effect can be exploited to reduce plant transpiration (and therefore the amount of water needed for irrigation) without affecting crop yields. Through irrigation techniques such as partial root zone drying or regulated deficit irrigation, root-initiated drought signals induce an increase in ABA levels in guard cells that reduce transpiration without causing other adverse drought effects like reduced growth or seed set.

Like auxin, ABA is a weak acid that is in a pH-dependent equilibrium between a charged (ABA⁺) and uncharged (ABAH) form. Although the uncharged form is thought to move freely across cell membranes, the anionic form presumably requires a transporter. AtABCG25 and AtABCG40 are members of the large and diverse ATP binding cassette (ABC) family of transporter proteins, subgroup G. ABCG25, which is primarily expressed in vascular tissue, appears to serve as an ABA efflux carrier. Overexpression of AtABCG25 reduces water loss from detached leaves. ABCG40 is expressed in guard cells and is also a specific transporter of ABA. Loss-of-function abcg40 mutants have guard cells with reduced sensitivity to ABA and are more susceptible to drought stress. Recently, two additional transporters have been implicated in ABA transport. DTX50 is a member of the DTX/MATE (Detoxification Efflux Carrier/Multidrug and Toxic compound Extrusion) family, and AIT1 (ABA-IMPORTING TRANSPORTER1) is a member of the NRT1/PTR (Nitrate transporter1/Peptide transporter) family, also known as NRT1.2, and proteins thought to transport ABA-GE have been identified in the vacuolar membrane.

Our understanding of the ABA synthesis, inactivation, conjugation, and transport pathways provides plant breeders with a sophisticated toolkit with which to develop plants that can produce the high food yields we need but require less of the increasingly limiting resource, fresh water. These efforts are complemented by similar efforts to breed plants that are optimized in their responses to ABA through studies of the ABA signaling and response pathways.

**ABA SIGNALING**

**ABA Receptors**

The main ABA receptors in plants are the PYRABACTIN RESISTANCE (PYR)/REGULATORY COMPONENT OF ABA RECEPTOR (RCAR) protein family. Their discovery was named by Science magazine as “one of the top breakthroughs of 2009.” Because of their importance, but in view of alternative potential ABA perception pathways, the PYR/RCAR pathway is termed the “core ABA signaling pathway.”

In Arabidopsis, there are 14 members of the PYR/RCAR gene family, and their expression patterns and binding affinities (which includes binding with ABA, binding with each other, and binding with other protein targets) affect their contributions to ABA signaling. When ABA binds to PYR/RCARs, the hormone moves into a pocket that is then enclosed by a receptor conformational change. The hormone + receptor complex binds to and inactivates negative regulators of downstream signaling, type 2C protein phosphatases (PP2C). PP2Cs were previously identified as critical regulators of ABA signaling, so the identification of this protein family truly makes a link between hormone and response. The ABA + PYR/RCAR complex downregulates PP2C activity through obstructing the phosphatase’s active site. The mode of action of the ABA + PYR/RCAR complex is similar to that of the gibberellin + GID1 complex in that the receptor + hormone complex increases the binding affinity of negative regulators, inactivating them. (In gibberellin signaling, the negative regulators are DELLA proteins.)

The interaction between PYR/RCAR and PP2C proteins forms the basis for a technique to visualize ABA concentrations using a fluorescence resonance energy transfer technique: Transgenic plants can be generated that contain PYR1 and the PP2C ABI1 attached to different fluorescent proteins, and their interaction gives a particular emission spectrum when excited. The conformational change that occurs following the binding of ABA to PYR1 causes a reduction in the emission, which is proportional to the amount of ABA present.

The ABA response is also affected by the subcellular localization and stability of ABA receptors. CULLIN RING ligases are a class of E3 ubiquitin ligase that specifically targets certain proteins for degradation, including the PYL8 ABA receptor. This selective polyubiquitination is ABA sensitive. When ABA levels...
are low, PYL8 is preferentially degraded, and when ABA levels are elevated, it is not, thus providing a mechanism to amplify ABA responses but also to effectively shut them down when ABA levels diminish. Additionally, although their roles in ABA signaling have not been fully worked out, a family of CAR proteins (C2-domain ABA-related) has been identified that mediates the interaction of PYR/RCAR proteins and the plasma membrane and thus alters ABA sensitivity.

Several studies have suggested that additional proteins may serve as ABA receptors. There is some evidence that G-proteins play a role in ABA signaling and might act as ABA receptors, but there is uncertainty as to whether they specifically bind ABA. Mg-chelatase, which is located on the chloroplast membrane, is required for normal ABA response, but whether it is an ABA binding protein continues to be debated.

**Signaling Downstream of PYR/RCARs: PP2Cs and SnRKs**

Many of the downstream components of the ABA pathway were identified through genetic approaches. One of the first to be identified, in 1984, was ABA-INSENSITIVE1 (ABI1), which in 1994 was shown to encode a PP2C that negatively regulates ABA responses. However, until very recently, we did not know the identity of ABI1 targets nor how its activity is controlled by ABI. ABI1 is part of a large family of PP2Cs, six of which have a proven role in ABA signaling in Arabidopsis: ABI1, ABI2, HYPERSENSITIVE TO ABA1 (HAB1), HAB2, PP2CA, and ABA-INSENSITIVE1. These proteins are the critical integrators between ABA perception by the PYR/CAR proteins and ABA responses, at least in part through their control of the activity of a family of protein kinases that are positive effectors of ABA responses, the SNF1-related protein kinase 2s (SnRK2s).

SnRK2 protein kinases were first identified in wheat (*Triticum aestivum*) and broad bean (*Vicia faba*; a plant that is often used for studies of guard cells). PKABA1 (for Protein Kinase ABA-induced) was identified in 1999 as an ABA-induced gene in wheat seeds that transduces ABA’s inhibitory effects on gibberellin-inducible gene expression. AAPK (for ABA-Activated Protein Kinase) was identified in 1996 and subsequently shown to mediate V. faba guard cell ion channel response to ABA. The homologous *Arabidopsis* gene OPEN STOMATA1 (OST1) was identified through a thermal imaging strategy to find mutants with abnormal stomatal responses. As reported by Francis Darwin as early as 1904, the transpirational flow of water through stomata cools plants just as sweating cools us. Thus, plants with abnormally closed stomata are warmer than wild-type plants; conversely, abnormally open stomata lead to cooler plants.

OST1, PKABA1, and AAPK are all SnRK2s. SnRK2s are a subfamily of the large calcium-dependent protein kinase (CDPK)-SnRK superfamily. The *Arabidopsis* and rice (*Oryza sativa*) genomes each encode 10 SnRK2s, of which three have a confirmed role in ABA signaling: SnRK2.2, SnRK2.3, and SnRK2.6 (OST1) (also known as SRK2D, SRK2I, and SRK2E). These three SnRK2 proteins share a conserved regulatory domain at the C terminus through which inhibitory PP2Cs act. Physical binding between PP2Cs and SnRKs occurs, and the crystal structures of PYL-SnRK and PP2C-SnRK complexes have recently shown that SnRKs bind to PP2Cs in a similar way to the PYLs due to their similar domain structure. The kinases are activated by phosphorylation of conserved residues and inactivated by dephosphorylation by PP2Cs. In the absence of ABA, PP2Cs dephosphorylate SnRK2s and render them inactive. In the presence of ABA, the PYR/RCAR inactivation of PP2Cs allows SnRK2 activation and SnRK2’s phosphorylation of target proteins. In Arabidopsis, a triple mutant of the three ABA-regulated SnRK2s is strongly ABA insensitive, germinates precociously, and has a wilty phenotype. A decuple mutant in which all 10 SnRK2 genes are disrupted is similarly hypersensitive to osmotic stress, but viable. In abiotic stress situations, casein kinase 2 affects the turnover and stability of SnRKs, by phosphorylating them and inhibiting their function within the core ABA-signaling module, which strengthens their interaction with PP2Cs and targets SnRKs for degradation.

Collectively, the PYR/RCAR receptors, inhibitory PP2Cs, and activating SnRK2s form an integral ABA signaling module. Because each of the three components comprises a large number of proteins (14, 6, and 10, respectively, in Arabidopsis), an enormous number of combinations are possible. Preliminary results suggest that most of these combinations may form in vivo, and variation in the expression and affinity of PYR and PP2C family members in different cells allow for responses to ABA over a wide range of concentrations and tissue types. Ongoing studies are investigating associations between individual signaling proteins or protein combinations with specific downstream responses.

**Downstream Phosphorylation Targets**

Protein phosphorylation was identified early on as an essential part of ABA signaling. Some of the phosphorylated targets have been shown to be substrates for SnRK2s, but CDPKs also transduce the ABA response. In fact, some ABA-responsive proteins appear to be phosphorylated by both SnRK2s and CDPKs.

Upon release of inhibition by PP2Cs, SnRK2s become active and can autophosphorylate as well as phosphorylate other targets. Among the probable SnRK2 phosphorylation targets are several bZIP transcription factors, so named because they have a basic domain adjacent to a Leu zipper domain. Early studies of ABA-responsive genes (including rice response to ABA genes [Rab] and wheat embryo-specific [Em] genes) revealed a conserved promoter element called the ABA-responsive element (ABRE; DNA sequence ACGTGTC) that is one of the major cis-acting elements for ABA-responsive genes. The ABREs were used as probes to identify ABRE binding factors (ABFs) or ABA-responsive element binding factors (AREBs), which are bZIP transcription factors.

ABIs is another bZIP transcription factor that was identified genetically by its ABA-insensitive germination. In addition to being dephosphorylated by PP2C, ABIs can also be dephosphorylated in an ABA-dependent manner, by PROTEIN PHOSPHATASE6, which inhibits ABA responses. The interplay between different phosphatases in ABA-response pathways is currently little understood. Other members of this group,
including ABF2, ABF3, and ABF4, were identified biochemically or by their close sequence homology. ABF2, ABF3, and ABF4 appear to control most of the ABA-responsive genes, suggesting that these three are major ABA-responsive transcription factors. Selective fragments of these proteins or synthetic peptides were shown to be phosphorylated in vitro by several representative members of the SnRK2 family, which suggests that bZIP transcription factors are downstream targets of these kinases.

Some ABFs also appear to be phosphorylated by CDPKs, at least in vitro. CPKs are protein kinases whose activity is regulated by calcium binding. Like SnRK2s, CDPKs are a large protein family (34 in Arabidopsis), some of which have a confirmed role mediating ABA-induced transcription and other responses. Drought or other stress can lead to an increase in cytoplasmic calcium concentration \([\text{Ca}^{2+}]_{\text{cyt}}\), activating CDPK activity. CDPKs additionally have a role in guard cell responses to ABA, as described below.

SnRK2.6 (OST1) has recently been shown to phosphorylate the guard cell–localized anion channel SLAC1 (for SLOW ANION-ASSOCIATED CHANNEL1), a major ion channel responsible for stomatal closure in response to ABA. SLAC1 activity is reduced and ABA insensitive in loss-of-function cpk mutants and subject to CDPKs phosphorylation in vitro. SnRK2.6 also phosphorylates RBOHF, an enzyme that produces H$_2$O$_2$, which is a second messenger in guard cell signaling (see below). Thus, protein phosphorylation of diverse targets by SnRKs and CDPKs contributes to ABA-regulated transcription, ion channel activities, and second-messenger production. As these targets have all been identified only very recently, it is likely that other targets remain to be discovered.

ABA-Regulated Transcription

The ABF transcription factors described above are one of the major components of ABA-regulated transcription, but other transcription factors that function in ABA signaling have been identified. How the activity of these transcription factors is controlled is unknown; they are unlikely to be direct targets of the SnRKs because they lack the SnRK2 phosphorylation-site motifs. The seed-specific ABI3/VP1 transcription factor family was identified genetically. Arabidopsis abi3 mutants are nondormant, which suggests that they don’t sense endogenous ABA and germinate in the presence of exogenous ABA. Z. mays viviparous1 mutants have reduced seed dormancy and a tendency to germinate on the cob if the kernels are exposed to atmospheric humidity (viviparous means live birth). These proteins are characterized as B3 proteins because they have three basic domains and are closely related to other seed-specific transcription factors, including FUS3 and LEC2. They bind to the RY DNA element \([\text{CATGCA(TG)}]\) found in the promoters of many seed-specific genes.

AP2-type transcription factors, first identified through the abi4 mutant, have diverse functions in ABA signaling, including effecting retrograde signaling between the chloroplast or mitochondria and the nucleus and integrating the ABA and sugar-signaling pathways in seeds. In Arabidopsis, ABI4 is one of the master regulators of ABA response, and the maize ortholog of ABI4 binds to a DNA element called the coupling element (CACCG) that is present in many ABA and sugar-regulated promoters.

The involvement of mitogen-activated protein kinases (MAPKs) in ABA signaling has been shown, for example, in the regulation of catalase for the generation of reactive oxygen species during stress signalling in guard cell closure. Recently, studies in Arabidopsis revealed that ABA can contribute to stress signaling by activating a complete MAPK pathway via transcriptional regulation of the first component of the pathway and that this pathway modulates ABA responses.

Microarray studies have revealed large numbers of genes that are regulated by ABA. Comparative studies between Arabidopsis and rice are helping to identify conserved promoter elements and transcription factors that control the ABA transcriptional response. A recent study identified more than 200 ABA-regulated transcription factors representing 20 protein families, all present within a single tissue type. Clearly, a major task will be to determine how these factors coordinate their actions to control precise temporal and cell type–specific expression of their target genes.

ABA IN WHOLE-PLANT PROCESSES

Guard Cell Responses

Francis Darwin (1898) asked, “Can we look at guard cells as sense-organs which, when the leaf is threatened by want of water, perceive the coming danger before the rest of the leaf? This idea is not wholly fanciful.” Few cells generate as much attention, passion, and interest as guard cells. Guard cells are extremely important because through modulating stomatal aperture they control the balance between gas exchange and transpirational water loss, one of the most essential issues for plant growth rate and stress tolerance. They provide us with one of the few opportunities to see plant movement. And as Darwin suggested, they function like sense organs, perceiving and responding to countless physical and chemical parameters.

When guard cells are exposed to ABA, they decrease in volume, close across the stomatal pore, and reduce the rate of water loss from the plant. The volume of the guard cells decreases because ions move outwards across the vacuolar and plasma membranes, and the net loss of ions causes water to move out of the cell by osmosis. The principal ions involved in guard cell movements are protons (H$^+$), potassium ions (K$^+$), and several anions, including chloride (Cl$^-$) and nitrate (NO$_3^-$). Malate (an organic acid) contributes to these cellular osmotic changes through transport across the membrane and by its reversible interconversion to starch (as a polymer, starch has a much lower osmotic effect than its constituent monomers). ABA promotes stomatal closure by activating anion and potassium channels on the vacuolar and plasma membranes. ABA also inhibits stomata from reopening by interfering with K$^+$ influx and downregulating a plasma membrane–localized H$^+$-ATPase.

When ABA is no longer present, the H$^+$-ATPase can be reactivated. Light also contributes to reactivation of the H$^+$-ATPase, which uses the energy of ATP to pump protons out of
the cell. As a consequence, the membrane potential of the cell becomes more negative (it becomes hyperpolarized), and the interior of the cell becomes alkalinized. Collectively, these effects contribute to the activation of $K^+$ influx channels. It is not known yet how anions are taken up into the cell again. Uptake of ions leads to water uptake and stomatal opening.

The signaling network by which ABA controls guard cell movements is under intense investigation, using techniques including measurements of ion currents into and out of whole cells or membrane patches and expression of ion channels in heterologous systems such as Xenopus laevis oocytes. Using these methods, the current through the membrane or a single channel can be recorded in the presence or absence of various second messengers, chemical inhibitors, or additional proteins. Collectively, these studies have revealed a complex regulatory network that includes the voltage and pH sensitivity of the ion channels themselves; second messengers, including cytosolic calcium ion ($[Ca^{2+}]_{cyt}$); and reactive oxygen species including hydrogen peroxide, nitric oxide, and protein kinases and phosphatases.

Cytosolic calcium ion was first identified as a second messenger in animal cells where it is an essential signal in the functions of muscle and nerve cells but also has a conserved role in egg activation following fertilization. In guard cells, ABA promotes an increase in $[Ca^{2+}]_{cyt}$ by activation of calcium channels at endo and plasma membranes, perhaps by changing the voltage sensitivity and kinetics of the channels. Elevated $[Ca^{2+}]_{cyt}$ levels activate anion channels and anion efflux. The calcium signal is thought to be transduced through calcium binding proteins, including CDPKs, and by potentiation of ion channels. As anions leave the cell, the membrane potential becomes less negative (depolarizes), which contributes to the activation of the voltage-sensitive $K^+$ efflux channel and provides a driving force for $K^+$ efflux. $K^+$ efflux channels are also strongly sensitive to pH; alkalinization of the cell is necessary and sufficient for the ABA-stimulated $K^+$ efflux. In addition to the short-term effects of elevated $[Ca^{2+}]_{cyt}$, long-term stomatal behavior may be programmed by $[Ca^{2+}]_{cyt}$ oscillations. Calcium oscillations have been observed in animal and plant cells, with the frequency of the oscillation conveying physiological information. The oscillations in guard cells are relatively slow and are thought to have a role in maintaining osmotic homeostasis as well as determining the extent and duration of stomatal closure.

Many studies have shown that reactive oxygen species (ROS), such as hydrogen peroxide, and the reactive nitrogen species nitric oxide (NO) are also involved in transducing ABA signals in guard cells. An increase in accumulation of ROS can be observed following ABA treatment, and in the absence of ABA, introduction of ROS or NO promotes guard cell closure, demonstrating that they act downstream of ABA. $H_2O_2$ contributes to NO synthesis. NO is thought to act upstream of $[Ca^{2+}]_{cyt}$ and contribute to its increase in the cytoplasm, although it may also act through protein nitrosylation. ROS and NO are also involved in ABA-mediated inhibition of stomatal opening.

Protein kinases and phosphatases contribute to ABA signal transduction in guard cells. Their involvement was first demonstrated experimentally through the use of protein phosphatase and kinase inhibitors. Subsequently, potassium channels of the abi1 mutant were shown to be insensitive to ABA, suggesting that a protein kinase is required directly or indirectly for its activation. More recently, the identification of the SnRK2.6 protein kinase has revealed a direct role for phosphorylation on channel activity and sensitivity to $[Ca^{2+}]_{cyt}$.

Collectively, these studies have provided a fascinating glimpse into the interdependence of the signaling pathways acting in guard cells. They suggest a rigorous network by which the guard cells can maintain cellular homeostasis by extensive feedback mechanisms between components and yet maintain sensitivity and responsiveness. Although many of the participants in the network have been identified, the ways in which they coordinate and cooperate are still being elucidated, as are the functions of the PYR/RCARs in this network. The observation that guard cells display some heterogeneity in their ABA responses provides an additional area for further study: What is the molecular basis for this heterogeneity, and what, if any, selective advantage is conferred by it?

**ABA Effects on Root Growth**

Water stress and its concomitant ABA accumulation tend to promote root growth at the expense of shoot growth. (Because most plants get most of their water through their root system, this is a reasonable strategy!) In particular, ABA tends to promote the elongation of the primary root; it is thought that this response can help plants reach deep into the soil in search of water. The continued elongation of the primary root under water stress is ABA dependent and a consequence of several factors, including reduced expansion in the radial dimension, enhanced Pro accumulation at the root tip (to draw water into the cells and promote cell expansion), and enhanced cell wall loosening to contribute to cell expansion. Low levels of ABA are required for normal root development. The elevated levels of ABA that maintain root elongation under water stress actually inhibit root elongation under well-watered conditions. During water stress, ABA also limits ethylene production, which would otherwise interfere with root elongation.

In some plants, ABA or water stress limits the outgrowth of lateral roots. The lateral roots seem to enter a dormant state and resume outgrowth when ABA is removed. Arabidopsis and its close relatives can also initiate new drought-induced lateral roots that remain quiescent during drought stress but rapidly elongate and contribute to water uptake upon rehydration (drought rhizogenesis), a response that is drastically reduced in ABA-insensitive or synthesis mutants. Thus, at least in Arabidopsis, one of the plant’s responses to low water availability is to minimize the outgrowth of branch roots, maintain the elongation of the primary root, and remain primed for lateral root proliferation upon rewatering.

**Vegetative Dehydration Responses and Osmoprotectants**

Very few organisms or cells are desiccation tolerant. Cells are mostly water, removal of which can irreversibly denature proteins and disrupt membrane integrity, causing cell death.
Desiccation tolerant organisms include many bacteria, which can form extremely stable dehydrated spores, and several types of crustaceans and worms. (Brine shrimp of the genus Artemia are marketed as the pet sea monkey; the embryos are extremely desiccation tolerant and capable of prolonged storage at room temperature, followed by reanimation upon rehydration.) Most organisms use similar osmoprotectant strategies that include the accumulation of antioxidants, solutes (often sugars, particularly the disaccharides trehalose or sucrose, to contribute to cytoplasmic vitrification and protein and membrane stabilization), and chaperonin proteins, such heat shock proteins. There is considerable interest in understanding the mechanisms of cell desiccation tolerance, not only in plant cells but also in human cells. Methods are being developed through which human blood, sperm, and stem cells can be stored in a dry form, increasing their storage life and transportability. One of these methods involves the expression of an Artemia (sea monkey) heat shock protein, dramatically increasing the desiccation tolerance of the modified human cells.

Some desiccation tolerance is critical for plants, and methods to avoid death by desiccation evolved early in land plant evolution. Many nonvascular plants can survive desiccation, whereas this ability has been lost from the vegetative tissues of most vascular plants. There are a few exceptions though. Some lycopsids (e.g., Selaginella tamarisci) and angiosperms (e.g., Craterostigma plantagineum) are extremely desiccation tolerant and are commonly referred to as resurrection plants. Their ability to withstand rapid and extreme desiccation has a high cost because maintaining high levels of osmoprotectants causes the plants to be small and slow growing; these are not good candidates for food plants. However, they are excellent experimental organisms through which to identify strategies for improving drought tolerance in crops, and extensive studies of their protein and metabolic profiles are being performed. Just as sea monkeys can enhance human cell tolerances, so the resurrection plants can enhance plant tolerances.

As in other organisms, desiccation of plant tissues induces the expression of proteins that preserve membrane and protein structural integrity, including heat shock proteins and late embryo abundant (LEA) proteins such as dehydrins. Others are involved in the biosynthesis of compatible solutes, such as Gly betaine, trehalose, and Pro, or regulate the control of ion channels and water channels (aquaporins). Still others protect the cell against ROS that accumulate during dehydration. The signaling pathway controlling expression of these genes includes an ABA-dependent branch and an ABA-independent branch that may be activated by the physical changes that accompany water loss. Many of the desiccation-induced proteins are encoded by genes with ABRE elements in their promoters, and many have been shown to be direct targets of the ABA-regulated transcription factors.

Fruit Ripening

Ethylene regulates the ripening of climacteric fruits such as tomato. ABA is also considered to be a positive regulator in both climacteric and nonclimacteric fruits, although it plays a greater role in nonclimacteric fruits. A peak of ABA synthesis, which often precedes the peak in ethylene synthesis, occurs during ripening or the onset of ripening, and exogenous ABA application can promote ripening in several different species. In tomato, the zinc-finger transcription factor ZFP2 represses ABA synthesis. Overexpression or downregulation of ZFP2 leads to delayed or accelerated fruit ripening, respectively, which provides an additional tool for fine-tuning the timing of fruit production.

Seed Development

Seeds are amazing evolutionary innovations that contribute to the predominance of angiosperms and gymnosperms. The development of most seeds consists of morphogenesis, maturation, and desiccation. Morphogenesis is the period during which the single-celled zygote develops its axes and rudimentary body plan. Maturation is the period during which the nutrients required for seedling establishment are mobilized into the seed and stored in the endosperm or cotyledons; at this point, many seeds are green and soft, like edible fresh peas or soybeans. Desiccation is preceded by the accumulation of osmoprotectants and dehydrins, including LEA proteins. With desiccation the seeds become dormant to resume their development later when growing conditions are optimal.

ABA is an extremely important regulator of seed development. Initially, maternally synthesized ABA contributes to the accumulation of nutrient stores, but another wave of ABA derived from the embryo activates seed-specific transcription factors to induce expression of LEAs and accumulation of osmoprotectants similar to those that accumulate in vegetative tissues under water stress. Seeds that are unable to make ABA do not desiccate or become dormant (for example, the viviparous mutants). Most seeds can remain dormant for a very long time. ABA levels usually decrease after primary dormancy is established; this period is called after-ripening. The seeds remain responsive to their external environment, and under adverse conditions can increase ABA synthesis to maintain dormancy. Factors that determine when dormancy ends and germination begins are complex and include a suite of environmental factors, including temperature, light, and soil moisture, the extent to which ABA levels have decreased (dictated in part by time), and levels of the germination-promoting gibberellin hormones. Prior to germination, expression of the ABA-degrading enzyme encoded by CYP707A increases, removing residual ABA and allowing gibberellin responses to take over.

ABA and Biotic Stress Responses

ABA contributes both positively and negatively to disease resistance in multiple ways. Some pathogens gain entry to the plant through stomatal pores. Frequently, plants respond to the presence of pathogens by closing their stomata, via the ABA synthesis and signaling pathway. In the typical arms race fashion, some bacteria have developed mechanisms to interfere with this ABA response, which causes stomata to reopen. Some bacteria produce coronatine, a bacterial phytotoxin that acts antagonistically to ABA. Bacterial proteins are also implicated in...
the control of the guard cell H^+-ATPase that must be downregulated for stomatal closure. Plant mutants with increased activity of this H^+-ATPase have ABA-insensitive stomata and are more susceptible to pathogens.

By contrast, during later stages of pathogen attack, some pathogens manipulate the ABA synthesis and response pathways to increase ABA responses, increasing the plant’s susceptibility to the pathogen. Negative interactions have been identified between ABA and hormones involved in defense response signaling, which may direct plant resources into either abiotic or biotic stress responses. Drought-stressed plants are highly susceptible to pathogen attack not only because of their weakened physiological state but also because through elevated ABA levels they specifically downregulate some defense responses. These observations highlight the high level of importance plants place upon avoiding desiccation; it is better to ignore a pathogen and deal with the water stress problems when simultaneously challenged.

Interactions between ABA and Other Hormones and Signals

As well as having individual roles in plant development, all hormones function in networks with other hormone signalling pathways. For ABA, some of these interactions are known, such as the opposing roles of ABA and gibberellin in seed dormancy and germination. Ethylene also negatively regulates ABA biosynthesis during seed dormancy and nitric oxide-induced ABA inactivation. It is also known that some of the effects of ABA such as root growth inhibition occur via the positive regulation of ethylene biosynthesis.

Several genes within the ABA-signaling pathway act as nodes of crosstalk with other hormones. For example, auxin activates ABI3 transcription, and brassinosteroids negatively regulate ABI3 and ABI5 epigenetically. Cytokinins also promote the degradation of ABI3 and ABI5 proteins. Systemic acquired accumulation to abiotic stress such as heat stress requires an autopropagating wave of reactive oxygen species that spreads from the site of exposure and interacts with stress-specific signals such as ABA accumulation. These are just a few of the additional layers of complexity that modulate ABA responses and integrate them into environmental and developmental responses and that are an exciting part of ongoing ABA research.

Progress toward Drought-Tolerant Plants

There is no question that we face an uncertain future as the human population continues to grow. The ongoing efforts of plant breeders have given us high-yielding plant varieties that produce fantastic yields but that also demand a constant supply of fresh water to maintain their productivity. Most plants must open their stomata during daylight hours to take up CO2 for photosynthesis, resulting in water loss through transpiration. When the soil water level decreases, plants either close their stomata and stop photosynthesis or wilt. Ongoing water stress leads to a suppression of shoot growth and the initiation of vegetative dehydration responses, which can dramatically decrease the rate of plant growth and ultimate seed yields. As global food requirements grow, so too do the demands for fresh irrigation water. It is critical that we develop plants that can provide us with food even when we give them less water; we need more crop per drop.

Most of our crop plants have been bred to produce high yields under well-watered conditions, and many are less drought tolerant than their wild relatives. Efforts to identify drought tolerance loci and introduce them into crop varieties through conventional breeding approaches are underway. One approach is to engineer ABA receptors so that they can be specifically activated by agrochemicals. Other approaches involve directly introducing genes that contribute to ABA synthesis or signaling or desiccation responses, using constitutive or inducible promoters (see Yang et al. [2010] for a thorough review of this topic). Some varieties produced through these efforts have enhanced drought tolerance, although so far none have been widely tested under real field conditions. Food security, ensuring that sufficient food is produced globally and locally, is one of the main driving forces behind plant research. Developing drought-tolerant crop plants is a high-priority goal for plant breeders and plant biotechnology companies.

CONCLUSIONS AND FUTURE DIRECTIONS

Cellular desiccation tolerance mediated by ABA synthesis and signaling was essential for land plant success. As plants grew in complexity, these pathways increased in sophistication, although the signaling module consisting of PYR/RCAR receptors, PP2C protein phosphatases and SnRK2 protein kinases has been highly conserved. Ongoing studies include investigations into the mechanisms that control where ABA is synthesized and the relative contributions of locally synthesized versus transported ABA in leaves and roots. What roles do other putative ABA binding proteins and ABA receptors have? We need to unravel the functions of the diverse isoforms of each of the core signaling components, further examining the targets of protein phosphorylation by the SnRK2 and CDPK kinases. How do the calcium-dependent and -independent pathways integrate? We don’t yet know how the ABA-responsive transcription factors function collectively, nor how the specific suite of activated transcription factors correlates with gene expression in a cell-specific way. Downstream, how do the gene targets contribute to the plant’s ability to survive and reproduce in the dry terrestrial environment?

How are ABA and non-ABA desiccation responses integrated? How are CO2 and light stimuli integrated with ABA in determining stomatal aperture? How does ABA promote increases in [Ca^{2+}]_{cyt}, ROS, and NO, and how do these second messengers mediate the activities of vacuolar and plasma membrane ion channels? How do sugar, gibberellins, and light signaling integrate with ABA into seed maturation, dormancy, and germination responses? Last but certainly not least, how can plant breeders use our understanding of ABA signaling to produce drought-tolerant plants without sacrificing yields or pathogen resistance?

More than 100 years ago, Francis Darwin was captivated by the movement of guard cells; we anticipate that this little hormone will continue to fascinate and excite plant biologists for many years to come.
RECOMMENDED READING

(This is a representative list of sources to help the reader access a huge body of literature. We apologize in advance to those whose work is not included.)

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