Chapter

23

# *Abscisic Acid: A Seed Maturation and Antistress Signal*

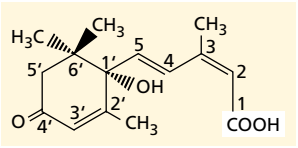
THE EXTENT AND TIMING OF PLANT GROWTH are controlled by the coordinated actions of positive and negative regulators. Some of the most obvious examples of regulated nongrowth are seed and bud dormancy, adaptive features that delay growth until environmental conditions are favorable. For many years, plant physiologists suspected that the phenomena of seed and bud dormancy were caused by inhibitory compounds, and they attempted to extract and isolate such compounds from a variety of plant tissues, especially dormant buds.

Early experiments used paper chromatography for the separation of plant extracts, as well as bioassays based on oat coleoptile growth. These early experiments led to the identification of a group of growth-inhibiting compounds, including a substance known as *dormin* purified from sycamore leaves collected in early autumn, when the trees were entering dormancy. Upon discovery that dormin was chemically identical to a substance that promotes the abscission of cotton fruits, *abscisin II*, the compound was renamed **abscisic acid** (**ABA**) (see Figure 23.1), to reflect its supposed involvement in the abscission process.

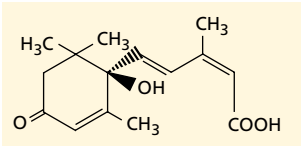
It is now known that ethylene is the hormone that triggers abscission and that ABA-induced abscission of cotton fruits is due to ABA's ability to stimulate ethylene production. As will be discussed in this chapter, ABA is now recognized as an important plant hormone in its own right. It inhibits growth and stomatal opening, particularly when the plant is under environmental stress. Another important function is its regulation of seed maturation and dormancy. In retrospect, *dormin* would have been a more appropriate name for this hormone, but the name *abscisic acid* is firmly entrenched in the literature.

## OCCURRENCE, CHEMICAL STRUCTURE, AND MEASUREMENT OF ABA

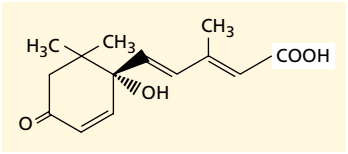
Abscisic acid has been found to be a ubiquitous plant hormone in vascular plants. It has been detected in mosses but appears to be absent in




**(*S*)-*cis*-ABA (naturally occurring active form)**




**(*R*)-*cis*-ABA (inactive in stomatal closure)**




**(*S*)-2-*trans*-ABA (inactive, but interconvertible with active [*cis*] form)**

**FIGURE 23.1** The chemical structures of the *S* (counterclockwise array) and *R* (clockwise array) forms of *cis*-ABA, and the (*S*)-2-*trans* form of ABA. The numbers in the diagram of (*S*)-*cis*-ABA identify the carbon atoms.

liverworts (see **Web Topic 23.1**). Several genera of fungi make ABA as a secondary metabolite (Milborrow 2001). Within the plant, ABA has been detected in every major organ or living tissue from the root cap to the apical bud. ABA is synthesized in almost all cells that contain chloroplasts or amyloplasts.

### The Chemical Structure of ABA Determines Its Physiological Activity

ABA is a 15-carbon compound that resembles the terminal portion of some carotenoid molecules (Figure 23.1). The orientation of the carboxyl group at carbon 2 determines the *cis* and *trans* isomers of ABA. Nearly all the naturally occurring ABA is in the *cis* form, and by convention the name *abscisic acid* refers to that isomer.

ABA also has an asymmetric carbon atom at position 1′ in the ring, resulting in the *S* and *R* (or + and –, respectively) enantiomers. The *S* enantiomer is the natural form; commercially available synthetic ABA is a mixture of approximately equal amounts of the *S* and *R* forms. The *S* enantiomer is the only one that is active in fast responses to ABA, such as stomatal closure. In long-term responses, such as seed maturation, both enantiomers are active. In contrast to the *cis* and *trans* isomers, the *S* and *R* forms cannot be interconverted in the plant tissue.

Studies of the structural requirements for biological activity of ABA have shown that almost any change in the molecule results in loss of activity (see **Web Topic 23.2**).

### ABA Is Assayed by Biological, Physical, and Chemical Methods

A variety of bioassays have been used for ABA, including inhibition of coleoptile growth, germination, or GA-induced α-amylase synthesis. Alternatively, promotion of stomatal closure and gene expression are examples of rapid inductive responses (see **Web Topic 23.3**).

Physical methods of detection are much more reliable than bioassays because of their specificity and suitability for quantitative analysis. The most widely used techniques are those based on gas chromatography or high-performance liquid chromatography (HPLC). Gas chromatography allows detection of as little as $10^{-13}$ g ABA, but it requires several preliminary purification steps, including thin-layer chromatography. Immunoassays are also highly sensitive and specific.

## BIOSYNTHESIS, METABOLISM, AND TRANSPORT OF ABA

As with the other hormones, the response to ABA depends on its concentration within the tissue and on the sensitivity of the tissue to the hormone. The processes of biosynthesis, catabolism, compartmentation, and transport all contribute to the concentration of active hormone in the tissue at any given stage of development. The complete biosynthetic pathway of ABA was elucidated with the aid of ABA-deficient mutants blocked at specific steps in the pathway.

### ABA Is Synthesized from a Carotenoid Intermediate

ABA biosynthesis takes place in chloroplasts and other plastids via the pathway depicted in Figure 23.2. Several ABA-deficient mutants have been identified with lesions at specific steps of the pathway. These mutants exhibit abnormal phenotypes that can be corrected by the application of exogenous ABA. For example, *flacca* (*flc*) and *sitiens* (*sit*) are "wilty mutants" of tomato in which the tendency of the leaves to wilt (due to an inability to close their stomata) can be prevented by the application of exogenous ABA. The *aba* mutants of *Arabidopsis* also exhibit a wilty phenotype. These and other mutants have been useful in elucidating the details of the pathway (Milborrow 2001).

The pathway begins with isopentenyl diphosphate (IPP), the biological isoprene unit, and leads to the synthesis of the $C_{40}$ xanthophyll (i.e., oxygenated carotenoid) **violaxanthin** (see Figure 23.2). Synthesis of violaxanthin is catalyzed by zeaxanthin epoxidase (ZEP), the enzyme encoded by the *ABA1* locus of *Arabidopsis*. This discovery provided conclusive evidence that ABA synthesis occurs via the "indirect" or carotenoid pathway, rather than as a small molecule. Maize mutants (*vp*) that are blocked at other steps in the carotenoid pathway also have reduced levels of ABA and exhibit **vivipary**—the precocious germination of seeds in the fruit while still attached to the plant (Figure 23.3). Vivipary is a feature of many ABA-deficient seeds.

Violaxanthin is converted to the $C_{40}$ compound **9′-*cis*-neoxanthin**, which is then cleaved to form the $C_{15}$ com-

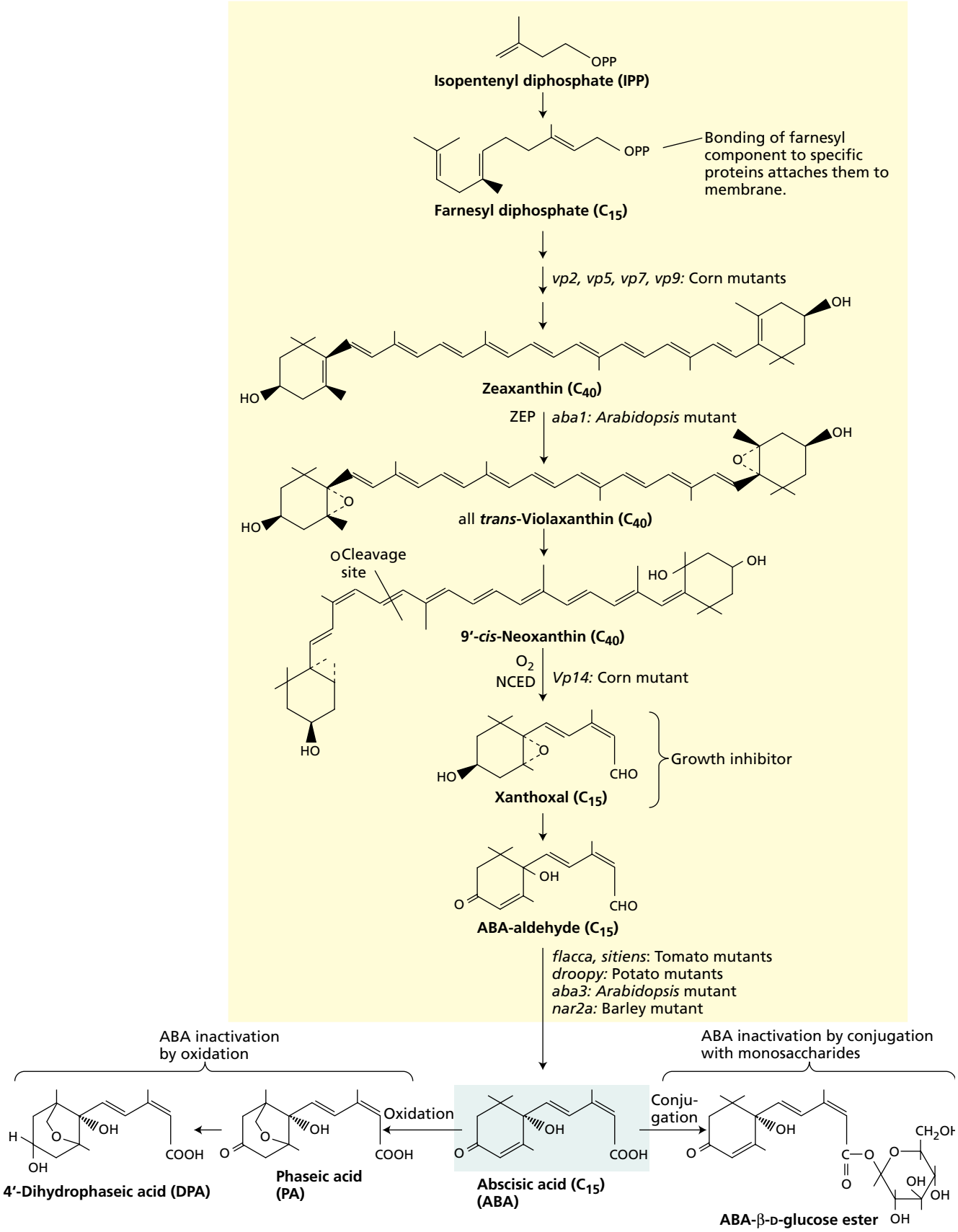



**FIGURE 23.2** ABA biosynthesis and metabolism. In higher plants, ABA is synthesized via the terpenoid pathway (see Chapter 13). Some ABA-deficient mutants that have been helpful in elucidating the pathway are shown at the steps at which they are blocked. The pathways for ABA catabolism include conjugation to form ABA-β-D-glucosyl ester or oxidation to form phaseic acid and then dihydrophaseic acid. ZEP = zeaxanthin epoxidase; NCED = 9-cis-epoxycarotenoids dioxygenase.

**FIGURE 23.3** Precocious germination in the ABA-deficient *vp14* mutant of maize. The VP14 protein catalyzes the cleavage of 9-*cis*-epoxycarotenoids to form xanthoxal, a precursor of ABA. (Courtesy of Bao Cai Tan and Don McCarty.)

pound **xanthoxal**, previously called *xanthoxin*, a neutral growth inhibitor that has physiological properties similar to those of ABA. The cleavage is catalyzed by **9-*cis*-epoxycarotenoid dioxygenase** (**NCED**), so named because it can cleave both 9-*cis*-violaxanthin and 9′-*cis*-neoxanthin.

Synthesis of NCED is rapidly induced by water stress, suggesting that the reaction it catalyzes is a key regulatory step for ABA synthesis. The enzyme is localized on the thylakoids, where the carotenoid substrate is located. Finally, xanthoxal is converted to ABA via oxidative steps involving the intermediate(s) **ABA-aldehyde** and/or possibly xanthoxic acid. This final step is catalyzed by a family of aldehyde oxidases that all require a molybdenum cofactor; the *aba3* mutants of *Arabidopsis* lack a functional molybdenum cofactor and are therefore unable to synthesize ABA.

## ABA Concentrations in Tissues Are Highly Variable

ABA biosynthesis and concentrations can fluctuate dramatically in specific tissues during development or in response to changing environmental conditions. In developing seeds, for example, ABA levels can increase 100-fold within a few days and then decline to vanishingly low levels as maturation proceeds. Under conditions of water stress, ABA in the leaves can increase 50-fold within 4 to 8 hours. Upon rewatering, the ABA level declines to normal in the same amount of time.

Biosynthesis is not the only factor that regulates ABA concentrations in the tissue. As with other plant hormones, the concentration of free ABA in the cytosol is also regulated by degradation, compartmentation, conjugation, and transport. For example, cytosolic ABA increases during water stress as a result of synthesis in the leaf, redistribution within the mesophyll cell, import from the roots, and recirculation from other leaves. The concentration of ABA declines after rewatering because of degradation and export from the leaf, as well as a decrease in the rate of synthesis.

## ABA Can Be Inactivated by Oxidation or Conjugation

A major cause of the inactivation of free ABA is oxidation, yielding the unstable intermediate 6-hydroxymethyl ABA, which is rapidly converted to **phaseic acid** (**PA**) and **dihydrophaseic acid** (**DPA**) (see Figure 23.2). PA is usually inactive, or it exhibits greatly reduced activity, in bioassays. However, PA can induce stomatal closure in some species, and it is as active as ABA in inhibiting gibberellic acid–induced α-amylase production in barley aleurone layers. These effects suggest that PA may be able to bind to ABA receptors. In contrast to PA, DPA has no detectable activity in any of the bioassays tested.

Free ABA is also inactivated by covalent conjugation to another molecule, such as a monosaccharide. A common example of an ABA conjugate is **ABA-β-D-glucosyl ester** (**ABA-GE**). Conjugation not only renders ABA inactive as a hormone; it also alters its polarity and cellular distribution. Whereas free ABA is localized in the cytosol, ABA-GE accumulates in vacuoles and thus could theoretically serve as a storage form of the hormone.

Esterase enzymes in plant cells could release free ABA from the conjugated form. However, there is no evidence that ABA-GE hydrolysis contributes to the rapid increase in ABA in the leaf during water stress. When plants were subjected to a series of stress and rewatering cycles, the ABA-GE concentration increased steadily, suggesting that the conjugated form is not broken down during water stress.

## ABA Is Translocated in Vascular Tissue

ABA is transported by both the xylem and the phloem, but it is normally much more abundant in the phloem sap. When radioactive ABA is applied to a leaf, it is transported both up the stem and down toward the roots. Most of the radioactive ABA is found in the roots within 24 hours. Destruction of the phloem by a stem girdle prevents ABA accumulation in the roots, indicating that the hormone is transported in the phloem sap.

ABA synthesized in the roots can also be transported to the shoot via the xylem. Whereas the concentration of ABA in the xylem sap of well-watered sunflower plants is between 1.0 and 15.0 n*M*, the ABA concentration in water-stressed sunflower plants increases to as much as 3000 n*M* (3.0 μ*M* ) (Schurr et al. 1992). The magnitude of the stress-induced change in xylem ABA content varies widely among species, and it has been suggested that ABA also is transported in a conjugated form, then released by hydrolysis in leaves. However, the postulated hydrolases have yet to be identified.

As water stress begins, some of the ABA carried by the xylem stream is synthesized in roots that are in direct contact with the drying soil. Because this transport can occur before the low water potential of the soil causes any measurable change in the water status of the leaves, ABA is believed to be a root signal that helps reduce the transpiration rate by closing stomata in leaves (Davies and Zhang 1991).

Although a concentration of 3.0 μ*M* ABA in the apoplast is sufficient to close stomata, not all of the ABA in the xylem stream reaches the guard cells. Much of the ABA in the transpiration stream is taken up and metabolized by the mesophyll cells. During the early stages of water stress, however, the pH of the xylem sap becomes more alkaline, increasing from about pH 6.3 to about pH 7.2 (Wilkinson and Davies 1997).

The major control of ABA distribution among plant cell compartments follows the "anion trap" concept: The dissociated (anion) form of this weak acid accumulates in alkaline compartments and may be redistributed according to the steepness of the pH gradients across membranes. In addition to partitioning according to the relative pH of compartments, specific uptake carriers contribute to maintaining a low apoplastic ABA concentration in unstressed plants.

Stress-induced alkalinization of the apoplast favors formation of the dissociated form of abscisic acid, $ABA^-$, which does not readily cross membranes. Hence, less ABA enters the mesophyll cells, and more reaches the guard cells via the transpiration stream (Figure 23.4). Note that ABA is redistributed in the leaf in this way without any increase in the total ABA level. This increase in xylem sap pH may function as a root signal that promotes early closure of the stomata.

## DEVELOPMENTAL AND PHYSIOLOGICAL EFFECTS OF ABA

Abscisic acid plays primary regulatory roles in the initiation and maintenance of seed and bud dormancy and in the plant's response to stress, particularly water stress. In addition, ABA influences many other aspects of plant development by interacting, usually as an antagonist, with auxin, cytokinin, gibberellin, ethylene, and brassinosteroids. In this section we will explore the diverse physiological effects of ABA, beginning with its role in seed development.

### ABA Levels in Seeds Peak during Embryogenesis

Seed development can be divided into three phases of approximately equal duration:

1. During the first phase, which is characterized by cell divisions and tissue differentiation, the zygote undergoes embryogenesis and the endosperm tissue proliferates.
2. During the second phase, cell divisions cease and storage compounds accumulate.
3. In the final phase, the embryo becomes tolerant to desiccation, and the seed dehydrates, losing up to 90% of its water. As a consequence of dehydration, metabolism comes to a halt and the seed enters a **quiescent** ("resting") state. In contrast to dormant seeds, quiescent seeds will germinate upon rehydration.

The latter two phases result in the production of viable seeds with adequate resources to support germination and

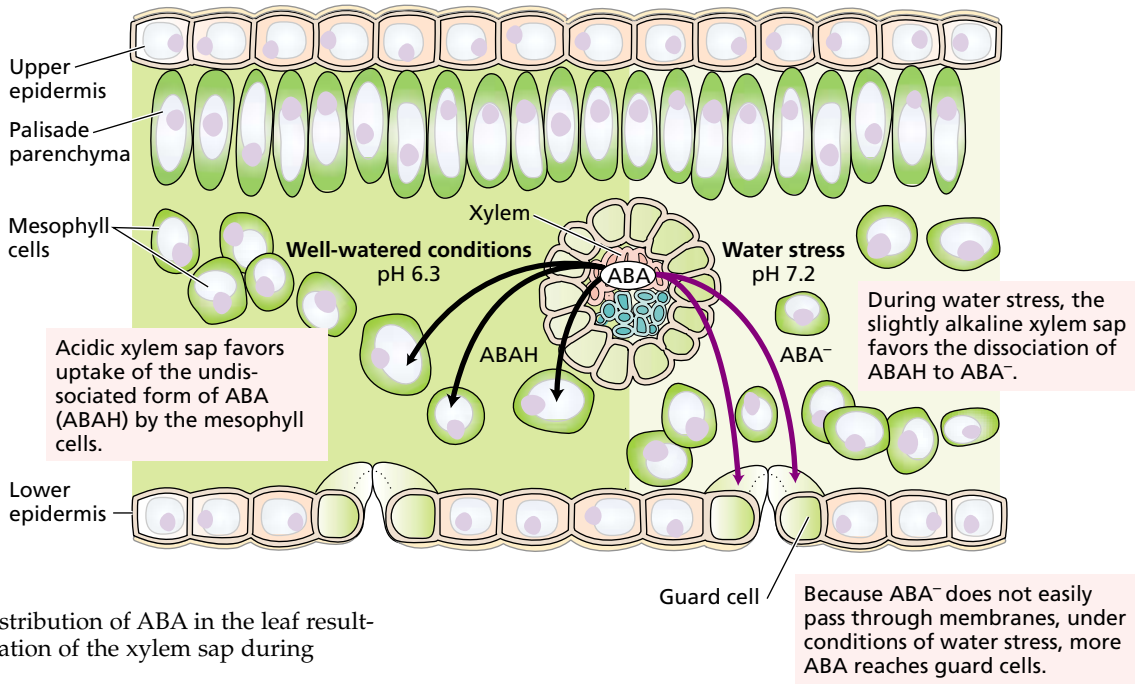



**FIGURE 23.4** Redistribution of ABA in the leaf resulting from alkalinization of the xylem sap during water stress.

the capacity to wait weeks to years before resuming growth. Typically, the ABA content of seeds is very low early in embryogenesis, reaches a maximum at about the halfway point, and then gradually falls to low levels as the seed reaches maturity. Thus there is a broad peak of ABA accumulation in the seed corresponding to mid- to late embryogenesis.

The hormonal balance of seeds is complicated by the fact that not all the tissues have the same genotype. The seed coat is derived from maternal tissues (see **Web Topic 1.2**); the zygote and endosperm are derived from both parents. Genetic studies with ABA-deficient mutants of *Arabidopsis* have shown that the zygotic genotype controls ABA synthesis in the embryo and endosperm and is essential to dormancy induction, whereas the maternal genotype controls the major, early peak of ABA accumulation and helps suppress vivipary in midembryogenesis (Raz et al. 2001).

### ABA Promotes Desiccation Tolerance in the Embryo

An important function of ABA in the developing seed is to promote the acquisition of desiccation tolerance. As will be described in Chapter 25 (on stress physiology), desiccation can severely damage membranes and other cellular constituents. During the mid- to late stages of seed development, specific mRNAs accumulate in embryos at the time of high levels of endogenous ABA. These mRNAs encode so-called **late-embryogenesis-abundant** (**LEA**) proteins thought to be involved in desiccation tolerance. Synthesis of many LEA proteins, or related family members, can be induced by ABA treatment of either young embryos or vegetative tissues. Thus the synthesis of most LEA proteins is under ABA control (see **Web Topic 23.4**).

### ABA Promotes the Accumulation of Seed Storage Protein during Embryogenesis

Storage compounds accumulate during mid- to late embryogenesis. Because ABA levels are still high, ABA could be affecting the translocation of sugars and amino acids, the synthesis of the reserve materials, or both.

Studies in mutants impaired in both ABA synthesis and response showed no effect of ABA on sugar translocation. In contrast, ABA has been shown to affect the amounts and composition of storage proteins. For example, exogenous ABA promotes accumulation of storage proteins in cultured embryos of many species, and some ABA-deficient or -insensitive mutants have reduced storage protein accumulation. However, storage protein synthesis is also reduced in other seed developmental mutants with normal ABA levels and responses, indicating that ABA is only one of several signals controlling the expression of storage protein genes during embryogenesis.

ABA not only regulates the accumulation of storage proteins during embryogenesis; it can also maintain the mature embryo in a dormant state until the environmental conditions are optimal for growth. Seed dormancy is an important factor in the adaptation of plants to unfavorable environments. As we will discuss in the next few sections, plants have evolved a variety of mechanisms, some of them involving ABA, that enable them to maintain their seeds in a dormant state.

### Seed Dormancy May Be Imposed by the Coat or the Embryo

During seed maturation, the embryo enters a quiescent phase in response to desiccation. Seed germination can be defined as the resumption of growth of the embryo of the mature seed; it depends on the same environmental conditions as vegetative growth does. Water and oxygen must be available, the temperature must be suitable, and there must be no inhibitory substances present.

In many cases a viable (living) seed will not germinate even if all the necessary environmental conditions for growth are satisfied. This phenomenon is termed **seed dormancy**. Seed dormancy introduces a temporal delay in the germination process that provides additional time for seed dispersal over greater geographic distances. It also maximizes seedling survival by preventing germination under unfavorable conditions. Two types of seed dormancy have been recognized: coat-imposed dormancy and embryo dormancy.

***Coat-imposed dormancy.*** Dormancy imposed on the embryo by the seed coat and other enclosing tissues, such as endosperm, pericarp, or extrafloral organs, is known as **coat-imposed dormancy**. The embryos of such seeds will germinate readily in the presence of water and oxygen once the seed coat and other surrounding tissues have been either removed or damaged. There are five basic mechanisms of coat-imposed dormancy (Bewley and Black 1994):

1. *Prevention of water uptake*.
2. *Mechanical constraint*. The first visible sign of germination is typically the radicle breaking through the seed coat. In some cases, however, the seed coat may be too rigid for the radicle to penetrate. For the seeds to germinate, the endosperm cell walls must be weakened by the production of cell wall–degrading enzymes.
3. *Interference with gas exchange*. Lowered permeability of seed coats to oxygen suggests that the seed coat inhibits germination by limiting the oxygen supply to the embryo.
4. *Retention of inhibitors*. The seed coat may prevent the escape of inhibitors from the seed.
5. *Inhibitor production*. Seed coats and pericarps may contain relatively high concentrations of growth inhibitors, including ABA, that can suppress germination of the embryo.

***Embryo dormancy.*** The second type of seed dormancy is **embryo dormancy**, a dormancy that is intrinsic to the embryo and is not due to any influence of the seed coat or other surrounding tissues. In some cases, embryo dormancy can be relieved by amputation of the cotyledons. Species in which the cotyledons exert an inhibitory effect include European hazel (*Corylus avellana*) and European ash (*Fraxinus excelsior*).

A fascinating demonstration of the cotyledon's ability to inhibit growth is found in species (e.g., peach) in which the isolated dormant embryos germinate but grow extremely slowly to form a dwarf plant. If the cotyledons are removed at an early stage of development, however, the plant abruptly shifts to normal growth.

Embryo dormancy is thought to be due to the presence of inhibitors, especially ABA, as well as the absence of growth promoters, such as GA (gibberellic acid). The loss of embryo dormancy is often associated with a sharp drop in the ratio of ABA to GA.

***Primary versus secondary seed dormancy.*** Different types of seed dormancy also can be distinguished on the basis of the timing of dormancy onset rather than the cause of dormancy:

- Seeds that are released from the plant in a dormant state are said to exhibit **primary dormancy**.
- Seeds that are released from the plant in a nondormant state, but that become dormant if the conditions for germination are unfavorable, exhibit **secondary dormancy**. For example, seeds of *Avena sativa* (oat) can become dormant in the presence of temperatures higher than the maximum for germination, whereas seeds of *Phacelia dubia* (small-flower scorpionweed) become dormant at temperatures below the minimum for germination. The mechanisms of secondary dormancy are poorly understood.

## Environmental Factors Control the Release from Seed Dormancy

Various external factors release the seed from embryo dormancy, and dormant seeds typically respond to more than one of three factors:

1. *Afterripening*. Many seeds lose their dormancy when their moisture content is reduced to a certain level by drying—a phenomenon known as **afterripening**.
2. *Chilling*. Low temperature, or **chilling**, can release seeds from dormancy. Many seeds require a period of cold (0–10°C) while in a fully hydrated (imbibed) state in order to germinate.
3. *Light*. Many seeds have a light requirement for germination, which may involve only a brief exposure, as in the case of lettuce, an intermittent treatment (e.g., succulents of the genus *Kalanchoe*), or even a specific photoperiod involving short or long days.

For further information on environmental factors affecting seed dormancy, see **Web Topic 23.5**. For a discussion of seed longevity, see **Web Topic 23.6**.

## Seed Dormancy Is Controlled by the Ratio of ABA to GA

Mature seeds may be either dormant or nondormant, depending on the species. Nondormant seeds, such as pea, will germinate readily if provided with water only. Dormant seeds, on the other hand, fail to germinate in the presence of water, and instead require some additional treatment or condition. As we have seen, dormancy may arise from the rigidity or impermeability of the seed coat (coat-imposed dormancy) or from the persistence of the state of arrested development of the embryo. Examples of the latter include seeds that require afterripening, chilling, or light to germinate.

ABA mutants have been extremely useful in demonstrating the role of ABA in seed dormancy. Dormancy of *Arabidopsis* seeds can be overcome with a period of afterripening and/or cold treatment. ABA-deficient (*aba*) mutants of *Arabidopsis* have been shown to be nondormant at maturity. When reciprocal crosses between *aba* and wild-type plants were carried out, the seeds exhibited dormancy only when the embryo itself produced the ABA. Neither maternal nor exogenously applied ABA was effective in inducing dormancy in an *aba* embryo.

On the other hand, maternally derived ABA constitutes the major peak present in seeds and is required for other aspects of seed development—for example, helping suppress vivipary in midembryogenesis. Thus the two sources of ABA function in different developmental pathways. Dormancy is also greatly reduced in seeds from the ABA-insensitive mutants *abi1* (*ABA-insensitive1*), *abi2*, and *abi3*, even though these seeds contain higher ABA concentrations than those of the wild type throughout development, possibly reflecting feedback regulation of ABA metabolism. ABA-deficient tomato mutants seem to function in the same way, indicating that the phenomenon is probably a general one. However, other mutants with reduced dormancy, but normal ABA levels and sensitivity, point to additional regulators of dormancy.

Although the role of ABA in initiating and maintaining seed dormancy is well established, other hormones contribute to the overall effect. For example, in most plants the peak of ABA production in the seed coincides with a decline in the levels of IAA and GA.

An elegant demonstration of the importance of the ratio of ABA to GA in seeds was provided by the genetic screen that led to isolation of the first ABA-deficient mutants of *Arabidopsis* (Koornneef et al. 1982). Seeds of a GA-deficient mutant that could not germinate in the absence of exogenous GA were mutagenized and then grown in the greenhouse. The seeds produced by these mutagenized plants were then screened for **revertants**—that is, seeds that had regained their ability to germinate.

Revertants were isolated, and they turned out to be mutants of abscisic acid synthesis. The revertants germinated because dormancy had not been induced, so subsequent synthesis of GA was no longer required to overcome it. This study elegantly illustrates the general principle that the balance of plant hormones is often more critical than are their absolute concentrations in regulating development. However, ABA and GA exert their effects on seed dormancy at different times, so their antagonistic effects on dormancy do not necessarily reflect a direct interaction.

Recent genetic screens for suppressors of ABA insensitivity have identified additional antagonistic interactions between ABA and ethylene or brassinosteroid effects on germination. In addition, many new alleles of ABA-deficient or *ABA-insensitive4* (*abi4*) mutants have been identified in screens for altered sensitivity to sugar. These studies show that a complex regulatory web integrates hormonal and nutrient signaling.

## ABA Inhibits Precocious Germination and Vivipary

When immature embryos are removed from their seeds and placed in culture midway through development before the onset of dormancy, they germinate precociously—that is, without passing through the normal quiescent and/or dormant stage of development. ABA added to the culture medium inhibits precocious germination. This result, in combination with the fact that the level of endogenous ABA is high during mid- to late seed development, suggests that ABA is the natural constraint that keeps developing embryos in their embryogenic state.

Further evidence for the role of ABA in preventing precocious germination has been provided by genetic studies of vivipary. The tendency toward vivipary, also known as *preharvest sprouting*, is a varietal characteristic in grain crops that is favored by wet weather. In maize, several viviparous (*vp*) mutants have been selected in which the embryos germinate directly on the cob while still attached to the plant. Several of these mutants are ABA deficient (*vp2*, *vp5*, *vp7*, and *vp14*) (see Figure 23.3); one is ABA insensitive (*vp1*). Vivipary in the ABA-deficient mutants can be partially prevented by treatment with exogenous ABA. Vivipary in maize also requires synthesis of GA early in embryogenesis as a positive signal; double mutants deficient in both GA and ABA do not exhibit vivipary (White et al. 2000).

In contrast to the maize mutants, single-gene mutants of *Arabidopsis* (*aba1*, *aba3*, *abi1*, and *abi3*) fail to exhibit vivipary, although they are nondormant. The lack of vivipary might reflect a lack of moisture because such seeds will germinate within the fruits under conditions of high relative humidity. However, other *Arabidopsis* mutants with a normal ABA response and only moderately reduced ABA levels (e.g., *fusca3*, which belongs to a class of mutants[1] defective in regulating the transition from embryogenesis to germination) exhibit some vivipary even at low humidities. Furthermore, double mutants combining either defects in ABA biosynthesis or ABA response with the *fusca3* mutation have a high frequency of vivipary (Nambara et al. 2000), suggesting that redundant control mechanisms suppress vivipary in *Arabidopsis*.

[1] Named after the Latin term for the reddish brown color of the embryos.

## ABA Accumulates in Dormant Buds

In woody species, dormancy is an important adaptive feature in cold climates. When a tree is exposed to very low temperatures in winter, it protects its meristems with bud scales and temporarily stops bud growth. This response to low temperatures requires a sensory mechanism that detects the environmental changes (sensory signals), and a control system that transduces the sensory signals and triggers the developmental processes leading to bud dormancy.

ABA was originally suggested as the dormancy-inducing hormone because it accumulates in dormant buds and decreases after the tissue is exposed to low temperatures. However, later studies showed that the ABA content of buds does not always correlate with the degree of dormancy. As we saw in the case of seed dormancy, this apparent discrepancy could reflect interactions between ABA and other hormones as part of a process in which bud dormancy and growth are regulated by the balance between bud growth inhibitors, such as ABA, and growth-inducing substances, such as cytokinins and gibberellins.

Although much progress has been achieved in elucidating the role of ABA in seed dormancy by the use of ABA-deficient mutants, progress on the role of ABA in bud dormancy, which applies mainly to woody perennials, has lagged because of the lack of a convenient genetic system. This discrepancy illustrates the tremendous contribution that genetics and molecular biology have made to plant physiology, and it underscores the need for extending such approaches to woody species.

Analyses of traits such as dormancy are complicated by the fact that they are often controlled by the combined action of several genes, resulting in a gradation of phenotypes referred to as *quantitative traits*. Recent genetic mapping studies suggest that homologs of *ABI1* may regulate bud dormancy in poplar trees. For a description of such studies, see **Web Topic 23.7**.

## ABA Inhibits GA-Induced Enzyme Production

ABA inhibits the synthesis of hydrolytic enzymes that are essential for the breakdown of storage reserves in seeds. For example, GA stimulates the aleurone layer of cereal grains to produce α-amylase and other hydrolytic enzymes that break down stored resources in the endosperm during germination (see Chapter 20). ABA inhibits this GA-dependent enzyme synthesis by inhibiting the transcription of α-amylase mRNA. ABA exerts this inhibitory effect via at least two mechanisms:

1. VP1, a protein originally identified as an activator of ABA-induced gene expression, acts as a transcriptional repressor of some GA-regulated genes (Hoecker et al. 1995).
2. ABA represses the GA-induced expression of GA-MYB, a transcription factor that mediates the GA induction of α-amylase expression (Gomez-Cadenas et al. 2001).

## ABA Closes Stomata in Response to Water Stress

Elucidation of the roles of ABA in freezing, salt, and water stress (see Chapter 25) led to the characterization of ABA as a stress hormone. As noted earlier, ABA concentrations in leaves can increase up to 50 times under drought conditions—the most dramatic change in concentration reported for any hormone in response to an environmental signal. Redistribution or biosynthesis of ABA is very effective in causing stomatal closure, and its accumulation in stressed leaves plays an important role in the reduction of water loss by transpiration under water stress conditions (Figure 23.5).

Stomatal closing can also be caused by ABA synthesized in the roots and exported to the shoot. Mutants that lack the ability to produce ABA exhibit permanent wilting and are called *wilty* mutants because of their inability to close their stomata. Application of exogenous ABA to such mutants causes stomatal closure and a restoration of turgor pressure.

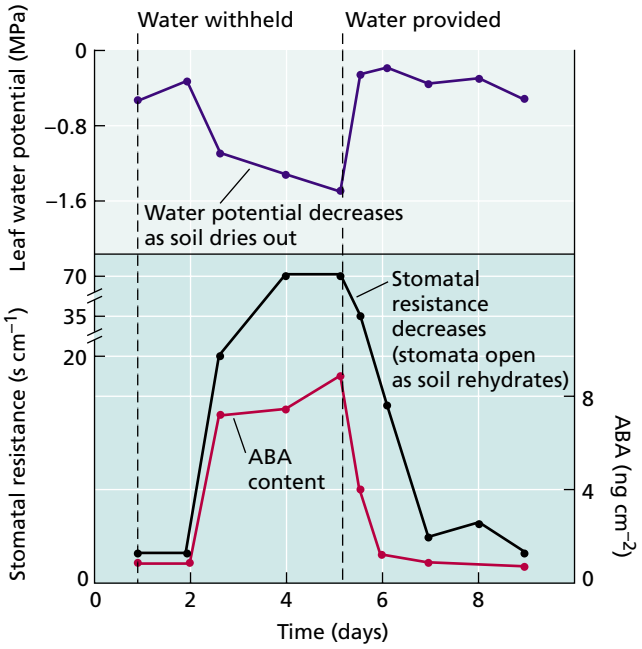



**FIGURE 23.5** Changes in water potential, stomatal resistance (the inverse of stomatal conductance), and ABA content in maize in response to water stress. As the soil dried out, the water potential of the leaf decreased, and the ABA content and stomatal resistance increased. The process was reversed by rewatering. (After Beardsell and Cohen 1975.)

## ABA Promotes Root Growth and Inhibits Shoot Growth at Low Water Potentials

ABA has different effects on the growth of roots and shoots, and the effects are strongly dependent on the water status of the plant. Figure 23.6 compares the growth of shoots and roots of maize seedlings grown under either abundant water conditions (high water potential) or dehydrating conditions (low water potential). Two types of seedlings were used: (1) wild-type seedlings with normal ABA levels and (2) an ABA-deficient, viviparous mutant.

When the water supply is ample (high water potential), shoot growth is greater in the wild-type plant (normal endogenous ABA levels) than in the ABA-deficient mutant. The reduced shoot growth in the ABA-deficient mutant could be due in part to excessive water loss from the leaves. In maize and tomato, however, the stunted shoot growth of ABA-deficient plants at high water potentials seems to be due to the overproduction of ethylene, which is normally inhibited by endogenous ABA (Sharp et al. 2000). This finding suggests that endogenous ABA promotes shoot growth in well-watered plants by suppressing ethylene production.

When water is limiting (i.e., at low water potentials), the opposite occurs: Shoot growth is greater in the ABA-deficient mutant than in the wild type. Thus, endogenous ABA acts as a signal to reduce shoot growth only under water stress conditions.

Now let's examine how ABA affects roots. When water is abundant, root growth is slightly greater in the wild type (normal endogenous ABA) than in the ABA-deficient mutant, similar to growth in shoots. Therefore, at high water potentials (when the total ABA levels are low), endogenous ABA exerts a slight positive effect on the growth of both roots and shoots.

Under dehydrating conditions, however, the growth of the roots is much higher in the wild type than in the ABA-deficient mutant, although growth is still inhibited relative to root growth of either genotype when water is abundant. In this case, endogenous ABA promotes root growth, apparently by inhibiting ethylene production during water stress (Spollen et al. 2000).

To summarize, under dehydrating conditons, when ABA levels are high, the endogenous hormone exerts a strong positive effect on root growth by suppressing ethylene production, and a slight negative effect on shoot growth. The overall effect is a dramatic increase in the root:shoot ratio at low water potentials (see Figure 23.6C), which, along with the effect of ABA on stomatal closure, helps the plant cope with water stress. For another example of the role of ABA in the response to dehydration, see **Web Essay 1**.

## ABA Promotes Leaf Senescence Independently of Ethylene

Abscisic acid was originally isolated as an abscission-causing factor. However, it has since become evident that ABA stimulates abscission of organs in only a few species and

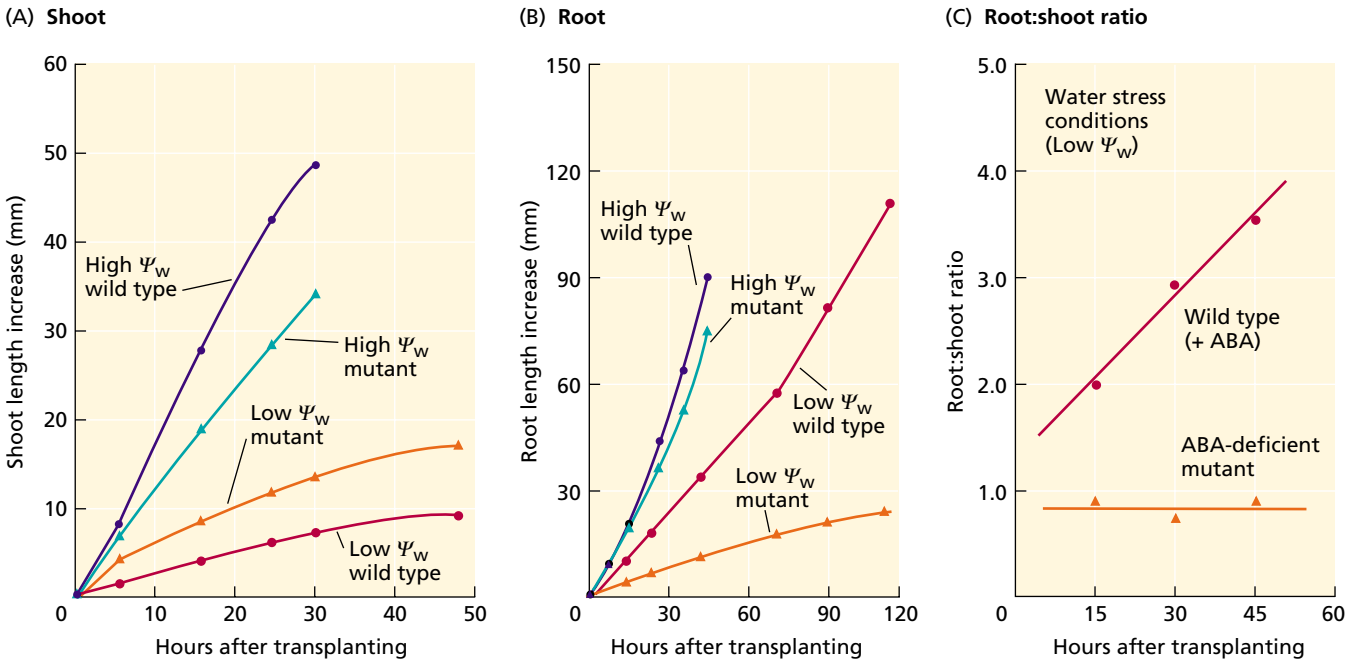



**FIGURE 23.6** Comparison of the growth of the shoots (A) and roots (B) of normal versus ABA-deficient (viviparous) maize plants growing in vermiculite maintained either at high water potential (–0.03 MPa) or at low water potential (–0.3 Mpa in A and –1.6 MPa in B). Water stress (low water potential) depresses the growth of both shoots and roots compared to the controls. (C) Note that under water stress conditions (low $\Psi_w$), the ratio of root growth to shoot growth is much higher when ABA is present (i.e., in the wild type) than when it is absent (in the mutant). (From Saab et al. 1990.)

that the primary hormone causing abscission is ethylene. On the other hand, ABA is clearly involved in leaf senescence, and through its promotion of senescence it might indirectly increase ethylene formation and stimulate abscission. (For more discussion on the relationship between ABA and ethylene, see **Web Topic 23.8**.)

Leaf senescence has been studied extensively, and the anatomical, physiological, and biochemical changes that take place during this process were described in Chapter 16. Leaf segments senesce faster in darkness than in light, and they turn yellow as a result of chlorophyll breakdown. In addition, the breakdown of proteins and nucleic acids is increased by the stimulation of several hydrolases. ABA greatly accelerates the senescence of both leaf segments and attached leaves.

## CELLULAR AND MOLECULAR MODES OF ABA ACTION

ABA is involved in short-term physiological effects (e.g., stomatal closure), as well as long-term developmental processes (e.g., seed maturation). Rapid physiological responses frequently involve alterations in the fluxes of ions across membranes and may involve some gene regulation as well, and long-term processes inevitably involve major changes in the pattern of gene expression.

Signal transduction pathways, which amplify the primary signal generated when the hormone binds to its receptor, are required for both the short-term and the long-term effects of ABA. Genetic studies have shown that many conserved signaling components regulate both short- and long-term responses, indicating that they share common signaling mechanisms. In this section we will describe what is known about the mechanism of ABA action at the cellular and molecular levels.

### ABA Is Perceived Both Extracellularly and Intracellularly

Although ABA has been shown to interact directly with phospholipids, it is widely assumed that the ABA receptor is a protein. To date, however, the protein receptor for ABA has not been identified. Experiments have been performed to determine whether the hormone must enter the cell to be effective, or whether it can act externally by binding to a receptor located on the outer surface of the plasma membrane. The results so far suggest multiple sites of perception.

Some experiments point to a receptor on the outer surface of the cell. For example, microinjected ABA fails to alter stomatal opening in the spiderwort *Commelina*, or to inhibit GA-induced α-amylase synthesis in barley aleurone protoplasts (Anderson et al. 1994; Gilroy and Jones 1994). Furthermore, impermeant ABA–protein conjugates have been shown to activate both ion channel activity and gene expression (Schultz and Quatrano 1997; Jeannette et al. 1999).

Other experiments, however, support an intracellular location for the ABA receptor:

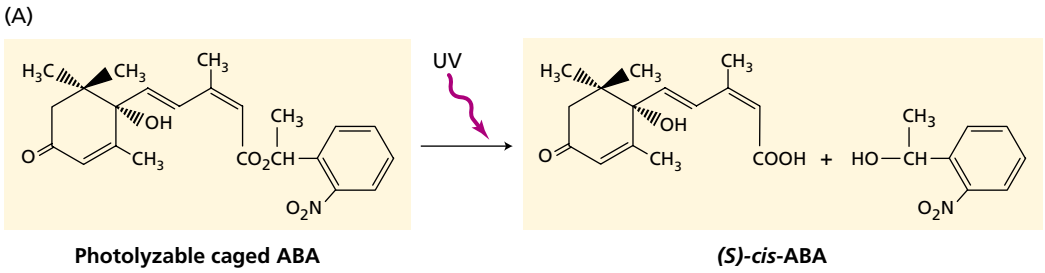



**FIGURE 23.7** Stomatal closure induced by UV photolysis of caged ABA in the guard cell cytoplasm. Single guard cells in stomatal complexes of *Commelina* were microinjected with caged ABA. (A) Photolysis reaction induced by UV irradiation. (B) The stomatal apertures recorded before and after a 30-second exposure of the cells to UV. (C, D) Light micrographs of the same stomatal complex in which the right-hand guard cell was loaded with the photolyzable cages ABA 10 minutes before UV photolysis (C) and 30 minutes after photolysis (D). (A and B from Allen et al. 1994; C and D courtesy of A. Allan, from Allan et al. 1994; © American Society of Plant Biologists, reprinted with permission.)

- Extracellular application of ABA was nearly twice as effective at inhibiting stomatal opening at pH 6.15, when it is fully protonated and readily taken up by guard cells, versus at pH 8, when it is largely dissociated to the anionic form that does not readily cross membranes (Anderson et al. 1994).
- ABA supplied directly and continuously to the cytosol via a patch pipette inhibited $K^+_{in}$ channels, which are required for stomatal opening (Schwartz et al. 1994).
- Microinjection of an inactive "caged" form of ABA into guard cells of *Commelina* resulted in stomatal closure after the stomata were treated briefly with UV irradiation to activate the hormone—that is, release it from its molecular cage (Figure 23.7) (Allan et al. 1994). Control guard cells injected with a nonphotolyzable form of the caged ABA did not close after UV irradiation.

Taken together, these results indicate that extracellular perception of ABA can prevent stomatal opening and regulate gene expression, and intracellular ABA can both induce stomatal closure and inhibit the $K^+_{in}$ current required for opening. Thus there appear to be both extracellular and intracellular ABA receptors. However, they have yet to be identified or localized.

## ABA Increases Cytosolic $Ca^{2+}$, Raises Cytosolic pH, and Depolarizes the Membrane

As discussed in Chapter 18, stomatal closure is driven by a reduction in guard cell turgor pressure caused by a massive long-term efflux of $K^+$ and anions from the cell. During the subsequent shrinkage of the cell due to water loss, the surface area of the plasma membrane may contract by as much as 50%. Where does the extra membrane go? The answer seems to be that it is taken up as small vesicles by endocytosis—a process that also involves reorganization of the actin cytoskeleton. However, the first changes detected after exposure of guard cells to ABA are transient membrane depolarization caused by the net influx of positive charge, and transient increases in the cytosolic calcium concentration (Figure 23.8).

ABA stimulates elevations in the concentration of cytosolic $Ca^{2+}$ by inducing both influx through plasma membrane channels and release of calcium into the cytosol from internal compartments, such as the central vacuole (Schroeder et al. 2001). Stimulation of influx occurs via a pathway that uses **reactive oxygen species** (**ROS**), such as hydrogen peroxide ($H_2O_2$) or superoxide ($O_2^{\bullet-}$), as secondary messengers leading to plasma membrane channel activation (Pei et al. 2000).

Calcium release from intracellular stores can be induced by a variety of second messengers, including inositol 1,4,5-trisphosphate ($IP_3$), cyclic ADP-ribose (cADPR), and self-

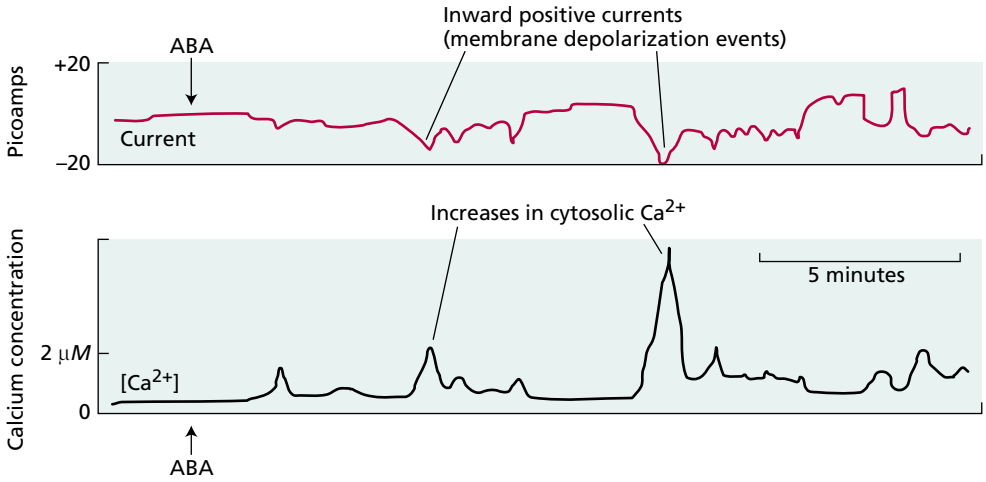



**FIGURE 23.8** Simultaneous measurements of ABA-induced inward positive currents and ABA-induced increases in cytosolic $Ca^{2+}$ concentrations in a guard cell of *Vicia faba* (broad bean). The current was measured by the patch clamp technique; calcium was measured by use of a fluorescent indicator dye. ABA was added to the system at the arrow in each case. (From Schroeder and Hagiwara 1990.)

amplifying (calcium-induced) $Ca^{2+}$ release. Recent studies have shown that ABA stimulates **nitric oxide** (**NO**) synthesis in guard cells, which induces stomatal closure in a cADPR-dependent manner, indicating that NO is an even earlier secondary messenger in this response pathway (Neill et al. 2002) (for background on NO, see Chapter 14 on the Web site).

The combination of calcium influx and the release of calcium from internal stores raises the cytosolic calcium concentration from 50 to 350 n*M* to as high as 1100 n*M* (1.1 m*M*) (Figure 23.9) (Mansfield and McAinsh, in Davies 1995). This increase is sufficient to cause stomatal closure, as demonstrated by the following experiment.

As in the experiment described earlier, calcium was microinjected into guard cells in a caged form that could be hydrolyzed by a pulse of UV light. This method allowed the investigators to control both the concentration of free calcium and the time of release to the cytosol. At cytosolic concentrations of 600 n*M* or more, release of calcium from its cage triggered stomatal closure (Gilroy et al. 1990). This level of intracellular calcium is well within the concentration range observed after ABA treatment.

In the preceding studies, intracellular free calcium was measured by the use of microinjected calcium-sensitive ratiometric fluorescent dyes[2], such as fura-2 or indo-1. However, microinjections of fluorescent dyes into single plant cells are difficult and often result in cell death. Success rates of viable injections into *Arabidopsis* guard cells can be less than 3%. In contrast, transgenic plants expressing the gene for the calcium indicator protein **yellow cameleon** make it possible to monitor several fluorescing cells in parallel, without the need for invasive injections (Allen et al. 1999b) (see **Web Topic 23.9**). Such studies have demonstrated that the cytosolic $Ca^{2+}$ concentration oscillates with distinct periodicities, depending on the signals received (Figure 23.10).

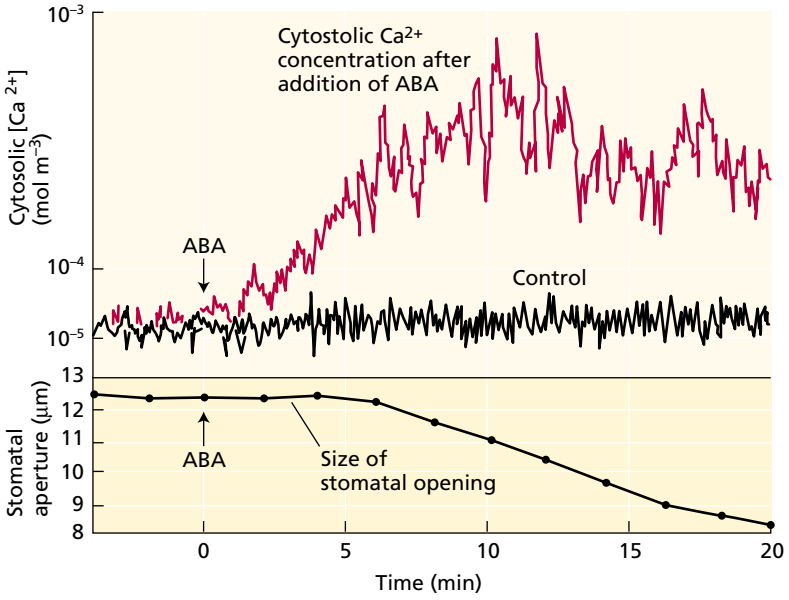



**FIGURE 23.9** Time course of the ABA-induced increase in guard cell cytosolic $Ca^{2+}$ concentration (upper panel) and ABA-induced stomatal aperture (lower panel). (From Mansfield and McAinsh 1995.)

[2] Ratiometric fluorescent dyes undergo a shift in their excitation and emission spectra when they bind calcium. On the basis of property, one can determine the intracellular concentrations of both forms of the dye (with and without bound calcium) by exciting them with the appropriate two wavelengths. The ratio of the two emissions provides a measure of the calcium concentration that is independent of dye concentration.

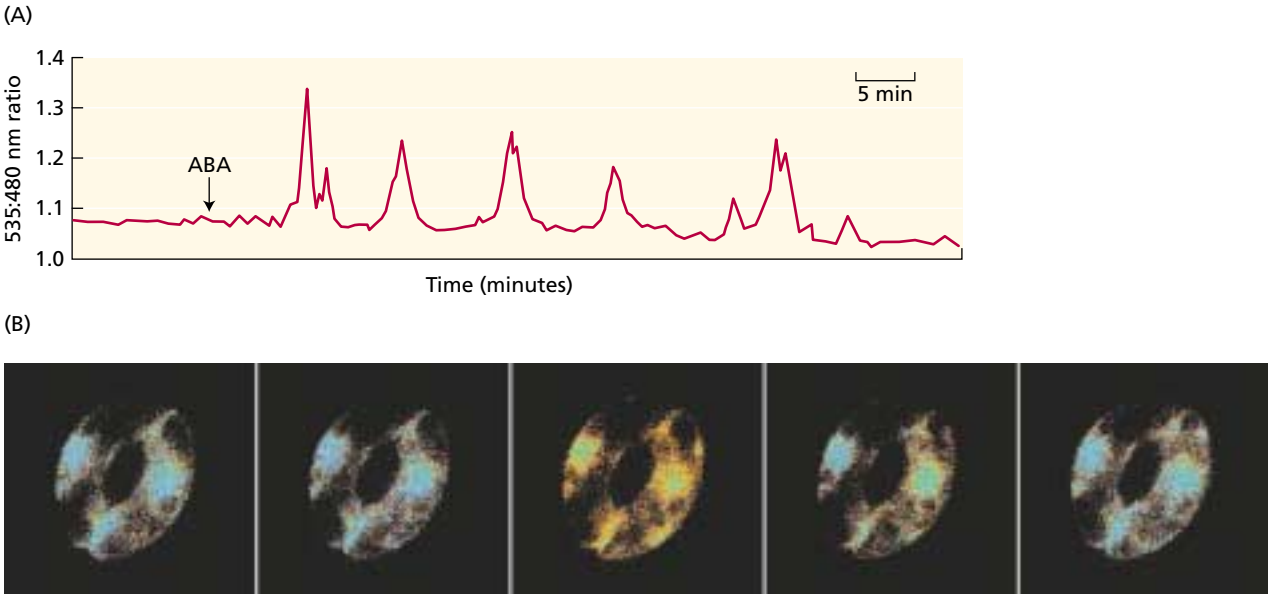



**FIGURE 23.10** ABA-induced calcium oscillations in *Arabidopsis* guard cells expressing yellow cameleon, a calcium indicator protein dye. (A) Oscillations elicited by ABA are indicated by increases in the ratio of flourescence emission at 535 and 480 nm. (B) Pseudo colored images of fluorescence in *Arabidopsis* guard cells, where blue, green, yellow and red represent increasing cytosolic calcium concentration. (From Schroeder et al. 2001.)

These results support the hypothesis that an increase in cytosolic calcium, partly derived from intracellular stores, is responsible for ABA-induced stomatal closure. However, the growth hormone auxin can induce stomatal opening, and this auxin-induced stomatal opening, like ABA-induced stomatal closure, is accompanied by *increases* in cytosolic calcium. This finding suggests that the detailed characteristics of the location and periodicity of $Ca^{2+}$ oscillations (the "$Ca^{2+}$ signature"), rather than the overall concentration of cytosolic calcium, determine the cellular response.

In addition to increasing the cytosolic calcium concentration, ABA causes an alkalinization of the cytosol from about pH 7.67 to pH 7.94. The increase in cytosolic pH has been shown to activate the $K^+$ efflux channels on the plasma membrane apparently by increasing the number of channels available for activation (see Chapter 6).

## ABA Activation of Slow Anion Channels Causes Long-Term Membrane Depolarization

The rapid, transient depolarizations induced by ABA are insufficient to open the $K^+$ efflux channels, which require long-term membrane depolarization in order to open. However, long-term depolarizations in response to ABA have been demonstrated. According to a widely accepted model, long-term membrane depolarization is triggered by two factors: (1) an ABA-induced transient depolarization of the plasma membrane, coupled with (2) an increase in cytosolic calcium. Both of these conditions are required to open calcium-activated slow (S-type) anion channels on the plasma membrane (Schroeder and Hagiwara 1990) (see Chapter 6). ABA has been shown to activate slow anion channels in guard cells (Grabov et al. 1997; Pei et al. 1997).

The prolonged opening of these slow anion channels permits large quantities of $Cl^-$ and $malate^{2-}$ ions to escape from the cell, moving down their electrochemical gradients. (The inside of the cell is negatively charged, thus pushing $Cl^-$ and $malate^{2-}$ out of the cell, and the outside has lower $Cl^-$ and $malate^{2-}$ concentrations than the interior.) The outward flow of negatively charged $Cl^-$ and $malate^{2-}$ ions generated in this way strongly depolarizes the membrane, triggering the voltage-gated $K^+$ efflux channels to open.

In support of this model, inhibitors that block slow anion channels, such as 5-nitro-2,3-phenylpropylaminobenzoic acid (NPPB), also block ABA-induced stomatal closing. Inhibitors of the rapid (R-type) anion channels, such as 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS), have no effect on ABA-induced stomatal closing (Schwartz et al. 1995).

Another factor that can contribute to membrane depolarization is inhibition of the plasma membrane $H^+$-ATPase. ABA inhibits blue light–stimulated proton pumping by guard cell protoplasts (Figure 23.11), consistent with the model that the depolarization of the plasma membrane by ABA is partially caused by a decrease in the activity of the plasma membrane $H^+$-ATPase. However, ABA does not inhibit the proton pump directly.

In *Vicia faba* (broad bean), at least, the plasma membrane $H^+$-ATPase of the leaves is strongly inhibited by calcium. A calcium concentration of 0.3 $\mu M$ blocks 50% of the activity of $H^+$-ATPase, and 1 $\mu M$ calcium blocks the enzyme completely (Kinoshita et al. 1995). It appears that two fac-

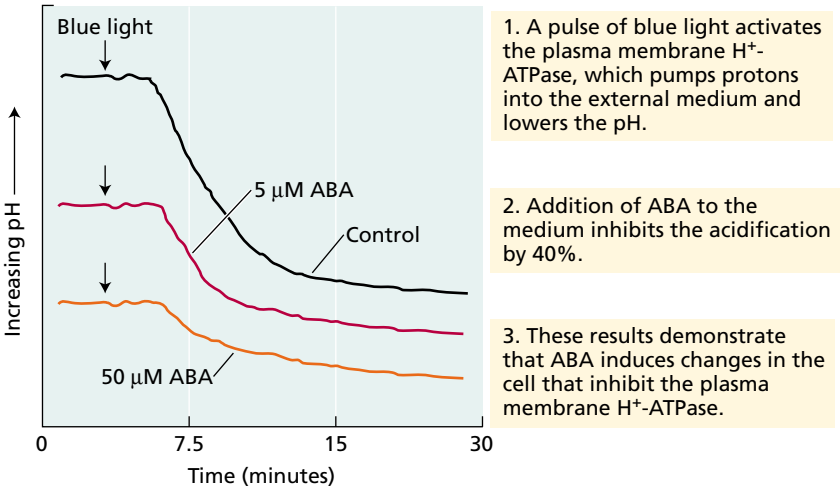



**FIGURE 23.11** ABA inhibition of blue light–stimulated proton pumping by guard cell protoplasts. A suspension of guard cell protoplasts was incubated under red-light irradiation, and the pH of the suspension medium was monitored with a pH electrode. The starting pH was the same in all cases (the curves are displaced for ease of viewing). (After Shimazaki et al. 1986.)

tors contribute to ABA inhibition of the plasma membrane proton pump: an increase in the cytosolic $Ca^{2+}$ concentration, and alkalinization of the cytosol.

In addition to causing stomatal closure, ABA prevents light-induced stomatal opening. In this case ABA acts by inhibiting the inward $K^+$ channels, which are open when the membrane is hyperpolarized by the proton pump (see Chapters 6 and 18). Inhibition of the inward $K^+$ channels is mediated by the ABA-induced increase in cytosolic calcium concentration. Thus calcium and pH affect guard cell plasma membrane channels in two ways:

1. They prevent stomatal opening by inhibiting inward $K^+$ channels and plasma membrane proton pumps.
2. They promote stomatal closing by activating outward anion channels, thus leading to activation of $K^+$ efflux channels.

## ABA Stimulates Phospholipid Metabolism

As discussed previously, much evidence supports a role for calcium both in the promotion of stomatal closing and in the inhibition of stomatal opening. According to the classic calcium-dependent signal transduction pathway of animal cells, $IP_3$ is released, along with diacylglycerol (DAG), when phospholipase C is activated by a G-protein in the plasma membrane (see Chapter 14 on the web site). Does ABA use the same pathway when it induces stomatal closure?

In agreement with this model, ABA has been shown to stimulate phosphoinositide metabolism in *Vicia faba* (broad bean) guard cells. To detect the effect of ABA on $IP_3$ release, it was necessary to include $Li^+$ in the incubation medium as an inhibitor of inositol phosphatase, which rapidly removes phosphate groups from $IP_3$. Under these conditions, a 90% ABA-induced increase in the level of $IP_3$ was measured within 10 seconds of hormone treatment (Lee et al. 1996). Recent studies in *Arabidopsis* using antisense DNA to block expression of an ABA-induced phospholipase C have shown that this enzyme is required for ABA effects on germination, growth, and gene expression (Sanchez and Chua 2001).

Heterotrimeric G-proteins may mediate the effects of ABA on stomatal movements. For example, in *Vicia faba* most studies have shown that G-protein activators, such as GTPγS, can inhibit the activity of the inward $K^+$ channels. Consistent with the inhibitor results, ABA failed to inhibit inward $K^+$ channels or light-induced stomatal opening in an *Arabidopsis* mutant with a defective Gα subunit (Wang et al. 2001). However, ABA still promoted stomatal closure in this mutant, indicating that inhibition of opening and promotion of closing take two distinct paths to the same end point—that is, closed stomata.

Other potential second messengers mediating the ABA response, such as phosphatidic acid and *myo*-inositol-hexaphosphate ($IP_6$) have been identified, but the relationship of these compounds to $IP_3$ and $Ca^{2+}$ signaling is not yet known.

All of these experiments indicate that stomatal guard cells respond to multiple signals, possibly involving multiple receptors and overlapping signal transduction pathways.

## Protein Kinases and Phosphatases Participate in ABA Action

Nearly all biological signaling systems involve protein phosphorylation and dephosphorylation reactions at some step in the pathway. Thus we can expect that signal transduction in guard cells, with their multiple sensory inputs, involves protein kinases and phosphatases. Artificially raising the ATP concentration inside guard cells by allowing the cytoplasm to equilibrate with the solution inside a patch pipette (see Chapter 6) strongly activates the slow anion channels.

This activation of the slow anion channels by ATP is abolished by the inclusion of protein kinase inhibitors in the patch pipette solution (Schmidt et al. 1995). Protein kinase inhibitors also block ABA-induced stomatal closing. In contrast, lowering the concentration of ATP in the cytosol inactivates the slow anion channels. Additional experiments confirm that this inactivation is due to the presence of protein phosphatases, which remove phosphate groups that are covalently attached to proteins. In

view of these results, it appears that protein phosphorylation and dephosphorylation play important roles in the ABA signal transduction pathway in guard cells.

There is now direct evidence for an ABA-activated protein kinase (AAPK) in *Vicia faba* guard cells (Li and Assmann 1996; Mori and Muto 1997). AAPK activity appears to be required for ABA activation of S-type anion currents and stomatal closing. This enzyme is an autophosphorylating protein kinase that either forms part of a $Ca^{2+}$-independent signal transduction pathway for ABA, or acts farther downstream of calcium-induced signaling events. (The presence of both $Ca^{2+}$-dependent and $Ca^{2+}$-independent pathways for ABA action will be discussed shortly.) In addition, two $Ca^{2+}$-dependent protein kinases, as well as MAP kinases, have been implicated in the ABA regulation of stomatal aperture.

The analysis of ABA-insensitive mutants has begun to help in the identification of genes coding for components of the signal transduction pathway. The *Arabidopsis abi1-1* and *abi2-1* mutations result in insensitivity to ABA in both seeds and adult plants. These *abi* mutants display phenotypes consistent with a defect in ABA signaling, including reduced seed dormancy, a tendency to wilt (due to improper regulation of stomatal aperture), and decreased expression of various ABA-inducible genes.

The defects in stomatal response include the ABA insensitivity of S-type anion channels—both inward and outward $K^+$ channels—and actin reorganization. Although nonresponsive to ABA, the mutant stomata will close when exposed to high external concentrations of $Ca^{2+}$, suggesting that they are defective in their ability to initiate $Ca^{2+}$ signaling. Consistent with this finding, ABA does not induce $Ca^{2+}$ oscillations in these mutants (Allen et al. 1999a).

## ABI Protein Phosphatases Are Negative Regulators of the ABA Response

The *Arabidopsis ABI1* and *ABI2* genes have been cloned and identified as encoding two closely related serine/threonine protein phosphatases. This finding suggests that ABI1 and ABI2 regulate the activity of target proteins by dephosphorylating specific serine or threonine residues, but none of their substrates have been definitively identified.

Because the *abi1-1* and *abi2-1* mutations result in decreased response to ABA, it was initially assumed that the wild-type genes *promote* the ABA response. However, the original mutations turned out to be dominant rather than recessive, and recent studies have shown that they act as "dominant negatives"; that is, one defective copy of the gene is sufficient to disrupt the ABA response by poisoning the activity of the functional gene products from the remaining wild-type allele.

Subsequently, recessive mutants of *ABI1* were obtained that exhibited a simple loss of *ABI1* activity. These recessive mutants of *ABI1* actually showed increased ABA sensitivity (Gosti et al. 1999). Furthermore, overproducing the wild-type gene products or their homologs (closely related proteins) by reintroducing the gene into plants, under control of a highly expressed promoter, confers *reduced* ABA sensitivity (Sheen 1998). Thus the wild-type function of these protein phosphatases is to inhibit the ABA response.

## ABA Signaling Also Involves $Ca^{2+}$-Independent Pathways

Although an ABA-induced increase in cytosolic calcium concentration is a key feature of the current model for ABA-induced guard cell closure, ABA is able to induce stomatal closure even in guard cells that show no increase in cytosolic calcium (Allan et al. 1994). In other words, ABA seems to be able to act via one or more calcium-independent pathways.

In addition to calcium, ABA can utilize cytosolic pH as a signaling intermediate. As previously discussed, a rise in cytosolic pH can lead to the activation of outward $K^+$ channels, and one effect of the *abi1* mutation is to render these $K^+$ channels insensitive to pH.

Such redundancy in the signal transduction pathways explains how guard cells are able to integrate a wide range of hormonal and environmental stimuli that affect stomatal aperture, and such redundancy is probably not unique to guard cells.

A simplified general model for ABA action in stomatal guard cells is shown in Figure 23.12. For clarity, only the cell surface receptors are shown.

## ABA Regulation of Gene Expression Is Mediated by Transcription Factors

Downstream of the early ABA signal transduction processes already discussed, ABA causes changes in gene expression. ABA has been shown to regulate the expression of numerous genes during seed maturation and under certain stress conditions, such as heat shock, adaptation to low temperatures, and salt tolerance (Rock 2000). The ABA- and stress-induced genes are presumed to contribute to adaptive aspects of induced tolerance (see Chapter 25). They include genes encoding proteases, chaperonins, proteins similar to LEA proteins, enzymes of sugar or other compatible solute[3] metabolism, ion and water channel proteins, enzymes that detoxify active oxygen species, and regulatory proteins such as transcription factors and protein kinases.

In a few cases, stimulation of transcription by ABA has been demonstrated directly. Gene activation by ABA is mediated by transcription factors. Four main classes of regulatory sequences conferring ABA inducibility have been identified, and proteins that bind to these sequences have

[3] An organic compound that can serve as a nontoxic, osmotically active solute in the cytosol; such compounds usually accumulate during water or salt stress (see Chapter 25).

1. ABA binds to its receptors.

2. ABA-binding induces the formation of reactive oxygen species, which activate plasma membrane $Ca^{2+}$ channels.

3. ABA increases the levels of cyclic ADP-ribose and $IP_3$, which activate additional calcium channels on the tonoplast.

4. The influx of calcium initiates intracellular calcium oscillations and promotes the further release of calcium from vacuoles.

5. The rise in intracellular calcium blocks $K^+_{in}$ channels.

6. The rise in intracellular calcium promotes the opening if $Cl^-_{out}$ (anion) channels on the plasma membrane, causing membrane depolarization.

7. The plasma membrane proton pump is inhibited by the ABA-induced increase in cytostolic calcium and a rise in intracellular pH, further depolarizing the membrane.

8. Membrane depolarization activates $K^+_{out}$ channels.

9. $K^+$ and anions to be released across the plasma membrane are first released from vacuoles into the cytosol.

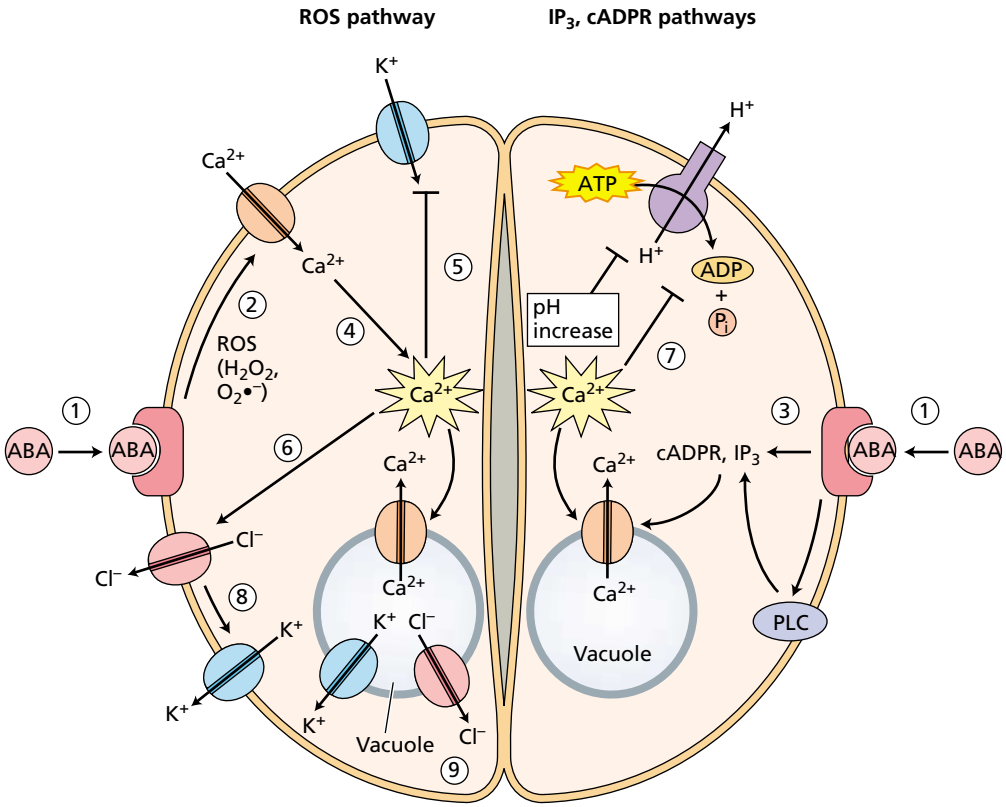



**FIGURE 23.12** Simplified model for ABA signaling in stomatal guard cells. The net effect is the loss of potassium and its anion ($Cl^-$ or malate$^{2-}$) from the cell. (R = receptor; ROS = reactive oxygen species; cADPR = cyclic ADP-ribose; G-protein = GTP-binding protein; PLC = phospholipiase C.)

been characterized (see **Web Topic 23.10**). Under stress conditions, induction of gene expression may be ABA dependent or ABA independent, and additional transcription factors have been identified that specifically mediate responses to cold, drought, or salt (see Chapter 25).

A few DNA elements have been identified that are involved in transcriptional repression by ABA. The best-characterized of these are the gibberellin response elements (GAREs) that mediate the gibberellin-inducible, ABA-repressible expression of the barley α-amylase gene (see Chapter 20).

Four transcription factors involved in ABA gene activation in maturing seeds have been identified by genetic means; mutations in the genes encoding any of these proteins reduce seed ABA responsiveness. The maize *VP1* (*VIVIPAROUS-1*) and *Arabidopsis ABI3* (*ABA-INSENSITIVE3*) genes encode highly similar proteins, and the *ABI4* and *ABI5* genes encode members of two other transcription factor families. *VP1/ABI3*, and *ABI4* are members of gene families found only in plants. In contrast, *ABI5* is a member of the basic leucine zipper (bZIP) family, whose members are present in all eukaryotes (Finkelstein and Lynch 2000).

Additional members of the *ABI5* subfamily have been identified by nongenetic means and are also correlated with ABA-, embryonic-, drought-, or salt stress–induced gene expression. Characterization of *vp1*, *abi4*, and *abi5* mutants has shown that each of these genes can either activate or repress transcription, depending on the target gene. Because the promoter of any given gene contains binding sites for a variety of regulators, it is likely that these transcription factors act in complexes made up of varying combinations of regulators, whose composition is determined by the combination of available regulators and binding sites.

To date, the protein ABI3/VP1 has been shown to interact physically with a variety of proteins, including ABI5 and its rice homolog (TRAB1). ABI5 also forms homodimers and heterodimers with other bZIP family members. There is additional evidence for indirect interactions that may be mediated by 14-3-3 proteins, a class of acidic proteins that dimerize and facilitate protein–protein interactions in a variety of signaling, transport, and enzymatic functions (**see**

Web Topic 23.11). These studies demonstrate the capacity for specific binding among a variety of transcription factors predicted to interact as components of regulatory complexes involved in ABA-induced gene expression.

### Other Negative Regulators of the ABA Response Have Been Identified

As described already, negative regulators of the ABA response (protein phosphatases) have been identified by isolation of dominant negative mutants such as *abi1* and *abi2* that result in ABA-insensitive phenotypes (analogous to the dominant negative effects of the ethylene receptor mutant *etr1*; see Chapter 22).

Other negative regulators have been identified through isolation of mutants exhibiting enhanced responses to ABA. Mutants showing increased sensitivity to ABA during germination include *era* (*e*nhanced *r*esponse to *A*BA) and *abh* (*AB*A *h*ypersensitive) (Cutler et al. 1996; Hugouvieux et al. in press). The *era* and *abh* mutants both confer ABA hypersensitivity in both stomatal closing and germination, making these mutants resistant to wilting and mildly drought tolerant.

***Farnesyl transferase.*** The *ERA1* gene was cloned, and its protein product was identified as a subunit of the enzyme farnesyl transferase. Farnesyl transferases catalyze attachment of the isoprenoid intermediate farnesyl diphosphate (see Chapter 13) to proteins that contain a specific signal sequence of amino acids. Many proteins that have been shown to participate in signal transduction are farnesylated. Farnesylated proteins are anchored to the membrane via hydrophobic interactions between the farnesyl group and the membrane lipids (see Figure 1.6). The identification of ERA1 as part of farnesyl transferase suggests that a protein that normally suppresses the ABA response requires farnesylation and is possibly anchored to the membrane.

***mRNA processing.*** *ABH1* encodes an mRNA 5′ cap–binding protein that may be involved in mRNA processing of negative regulators of ABA signaling. (Recall that eukaryotic messenger RNAs have a "cap" consisting of methylated guanosine at the 5′ end.) Comparison of transcript accumulation in wild-type and *abh1* plants showed a small number of misexpressed genes in the mutant, including some encoding possible signaling molecules.

***Ethylene insensitivity.*** *ERA3* was found to be allelic to a previously identified ethylene signaling locus, *ETHYLENE-INSENSITIVE 2* (*EIN2*) (Ghassemian et al. 2000) (see Chapter 22). In addition to displaying defects in ABA and ethylene responses, mutations in this gene result in defects in the responses to auxin, jasmonic acid, and stress. This gene encodes a membrane-bound protein that appears to represent a point of "cross-talk"—i.e., a common signaling intermediate—mediating the responses to many different signals.

***$IP_3$ catabolism.*** Other screens have identified ABA signaling mutants on the basis of incorrect expression of reporter genes controlled by ABA-responsive promoters. Although the defects in some of these mutants are limited to gene expression, others affect plant growth responses. One such mutant, termed *fiery* (*fry*) to reflect the intensity of light emission by its ABA/stress-responsive luciferase reporter, is also hypersensitive to ABA and stress inhibition of germination and growth. The *FIERY* gene encodes an enzyme required for $IP_3$ catabolism (Xiong et al. 2001). The mutant phenotype demonstrates that the ability to attenuate, as well as induce, stress signaling is important for successful induction of stress tolerance.

Similar to the signaling mechanisms documented for other plant hormones, ABA signaling involves the coordinated action of positive and negative regulators affecting processes as diverse as transcription, RNA processing, protein phosphorylation or farnesylation, and metabolism of secondary messengers. As the signaling components are identified, and often are found to function in responses to multiple signals, the next challenge is to determine how they can lead to ABA-specific responses.

## SUMMARY

Abscisic acid plays major roles in seed and bud dormancy, as well as responses to water stress. ABA is a 15-carbon terpenoid compound derived from the terminal portion of carotenoids. ABA in tissues can be measured by bioassays based on growth, germination, or stomatal closure. Gas chromatography, HPLC, and immunoassays are the most reliable and accurate methods available for measuring ABA levels.

ABA is produced by cleavage of a 40-carbon carotenoid precursor that is synthesized from isopentenyl diphosphate via the plastid terpenoid pathway. ABA is inactivated by both oxidative degradation and conjugation.

ABA is synthesized in almost all cells that contain plastids and is transported via both the xylem and the phloem. The level of ABA fluctuates dramatically in response to developmental and environmental changes. During seed maturation, ABA levels peak in mid- to late embryogenesis.

ABA is required for the development of desiccation tolerance in the developing embryo, the synthesis of storage proteins, and the acquisition of dormancy. Seed dormancy and germination are controlled by the ratio of ABA to GA, and ABA-deficient embryos may exhibit precocious germination and vivipary. ABA is also antagonized by ethylene and brassinosteroid promotion of germination. Although less is known about the role of ABA in buds, ABA is one of the inhibitors that accumulates in dormant buds.

During water stress, the ABA level of the leaf can increase 50-fold. In addition to closing stomata, ABA increases the hydraulic conductivity of the root and

increases the root:shoot ratio at low water potentials. ABA and an alkalinization of the xylem sap are thought to be two chemical signals that the root sends to the shoot as the soil dries. The increased pH of the xylem sap may allow more of the ABA of the leaf to be translocated to the stomata via the transpiration stream.

ABA exerts both short-term and long-term control over plant development. The long-term effects are mediated by ABA-induced gene expression. ABA stimulates the synthesis of many classes of proteins during seed development and during water stress, including the LEA family, proteases and chaperonins, ion and water channels, and enzymes catalyzing compatible solute metabolism or detoxification of active oxygen species. These proteins may protect membranes and other proteins from desiccation damage, or they may aid in recovery from the deleterious effects of stress. ABA response elements and several transcription factors that bind to them have been identified. ABA also suppresses GA-induced gene expression—for example, the synthesis of GA-MYB and α-amylase by barley aleurone layers.

There is evidence for both extracellular and intracellular ABA receptors in guard cells. ABA closes stomata by causing long-term depolarization of the guard cell plasma membrane. Depolarization is believed to be caused by an increase in cytosolic $Ca^{2+}$, as well as alkalinization of the cytosol. The increase in cytosolic calcium is due to a combination of calcium uptake and release of calcium from internal stores. This calcium increase leads to the opening of slow anion channels, which results in membrane depolarization. $IP_3$, $IP_6$, cADPR, PA, and reactive oxygen species all function as secondary messengers in ABA-treated guard cells, and G-proteins participate in the response. Outward $K^+$ channels open in response to membrane depolarization and to the rise in pH, bringing about massive $K^+$ efflux.

In general, the ABA response appears to be regulated by more than one signal transduction pathway, even within a single cell type. This redundancy is consistent with the ability of plant cells to respond to multiple sensory inputs. There is genetic evidence for cross-talk between ABA signaling and the signaling of all other major classes of phytohormones, as well as sugars.

## Web Material

### Web Topics

**23.1 The Structure of Lunularic Acid from Liverworts**
Although inactive in higher plants, lunularic acid appears to have a function similar to ABA in liverworts.

**23.2 Structural Requirements for Biological Activity of Abscisic Acid**
To be active as a hormone, ABA requires certain functional groups

**23.3 The Bioassay of ABA**
Several ABA-responding tissues have been used to detect and measure ABA.

**23.4 Proteins Required for Desiccation Tolerance**
ABA induces the synthesis of proteins that protect cells from damage due to dessication.

**23.5 Types of Seed Dormancy and the Roles of Environmental Factors**
This discussion expands on the various types of seed dormancy and describes how environmental factors affect seed dormancy.

**23.6 The Longevity of Seeds**
Under certain conditions, seeds can remain dormant for hundreds of years.

**23.7 Genetic Mapping of Dormancy: Quantitative Trait Locus (QTL) Scoring of Vegetative Dormancy Combined with a Candidate Gene Approach**
A genetic method for determining the number and chromosomal locations of genes affecting a quantitative trait affected by many unlinked genes is described.

**23.8 ABA-Induced Senescence and Ethylene**
Hormone-insensitive mutants have made it possible to distinguish the effects of ethylene from those of ABA on senescence.

**23.9 Yellow Cameleon: A Noninvasive Tool for Measuring Intracellular Calcium**
The features of the yellow cameleon protein that enable it to act as a reporter for calcium concentration are described.

**23.10 Promoter Elements That Regulate ABA Induction of Gene Expression**
A table of the different ABA response elements is presented.

**23.11 The Two-Hybrid System**
The GAL4 transcription factor can be used to detect protein-protein interactions in yeast.

### Web Essay

**23.1 Heterophylly in Aquatic Plants**
Abscisic acid induces aerial-type leaf morphology in many aquatic plants.

## Chapter References


Allan, A. C., Fricker, M. D., Ward, J. L., Beale, M. H., and Trewavas, A. J. (1994) Two transduction pathways mediate rapid effects of abscisic acid in *Commelina* guard cells. *Plant Cell* 6: 1319–1328.

Allen, G. J., Kuchitsu, K., Chu, S. P., Murata, Y., and Schroeder, J. I. (1999a) *Arabidopsis* abi1-1 and abi2-1 phosphatase mutations reduce abscisic acid-induced cytoplasmic calcium rises in guard cells. *Plant Cell* 11: 1785–1798.

Allen, G. J., Kwak, J. M., Chu, S. P., Llopis, J., Tsien, R. Y., Harper, J. F., and Schroeder, J. I. (1999b) Cameleon calcium indicator reports cytoplasmic calcium dynamics in *Arabidopsis* guard cells. *Plant J.* 19: 735–747.

Anderson, B. E., Ward, J. M., and Schroeder, J. I. (1994) Evidence for an extracellular reception site for abscisic acid in *Commelina* guard cells. *Plant Physiol.* 104: 1177–1183.

Beardsell, M. F., and Cohen, D. (1975) Relationships between leaf water status, abscisic acid levels, and stomatal resistance in maize and sorghum. *Plant Physiol.* 56: 207–212.

Bewley, J. D., and Black, M. (1994) *Seeds: Physiology of Development and Germination*, 2nd ed. Plenum, New York.

Cutler, S., Ghassemian, M., Bonetta, D., Cooney, S., and McCourt, P. (1996) A protein farnesyl transferase involved in abscisic acid signal transduction in *Arabidopsis*. *Science* 273: 1239–1241.

Davies, P. J., ed. (1995) *Plant Hormones: Physiology, Biochemistry and Molecular Biology*, 2nd ed. Kluwer, Dordrecht, Netherlands.

Davies, W. J., and Zhang, J. (1991) Root signals and the regulation of growth and development of plants in drying soil. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 42: 55–76.

Finkelstein, R. R., and Lynch, T. J. (2000) The *Arabidopsis* abscisic acid response gene ABI5 encodes a basic leucine zipper transcription factor. *Plant Cell* 12: 599–609.

Finkelstein, R. R., Wang, M. L., Lynch, T. J., Rao, S., and Goodman, H. M. (1998) The *Arabidopsis* abscisic acid response locus ABI4 encodes an APETALA2 domain protein. *Plant Cell* 10: 1043–1054.

Ghassemian, M., Nambara, E., Cutler, S., Kawaide, H., Kamiya, Y., and McCourt, P. (2000) Regulation of abscisic acid signaling by the ethylene response pathway in *Arabidopsis*. *Plant Cell* 12: 1117–1126.

Gilroy, S., and Jones, R. L. (1994) Perception of gibberellin and abscisic acid at the external face of the plasma membrane of barley (*Hordeum vulgare* L.) aleurone protoplasts. *Plant Physiol.* 104: 1185–1192.

Gilroy, S., Read, N. D., and Trewavas, A. J. (1990) Elevation of cytoplasmic calcium by caged calcium or caged inositol trisphosphate initiates stomatal closure. *Nature* 343: 769–771.

Gomez-Cadenas, A., Zentella, R., Walker-Simmons, M. K., and Ho, T.-H. D. (2001) Gibberellin/abscisic acid antagonism in barley aleurone cells: Site of action of the protein kinase PKABA1 in relation to gibberellin signaling molecules. *Plant Cell* 13: 667–679.

Gosti, F., Beaudoin, N., Serizet, C., Webb, A. A. R., Vartanian, N., and Giraudat, J. (1999) ABI1 protein phosphatase 2C is a negative regulator of abscisic acid signaling. *Plant Cell* 11: 1897–1909.

Grabov, A., Leung, J., Giraudat, J., and Blatt, M. (1997) Alteration of anion channel kinetics in wild-type and *abi1-1* transgenic *Nicotiana benthamiana* guard cells by abscisic acid. *Plant J.* 12: 203–213.

Hoecker, U., Vasil, I. K., and McCarty, D. R. (1995) Integrated control of seed maturation and germination programs by activator and repressor functions of Viviparous-1 of maize. *Genes Dev.* 9: 2459–2469.

Hugouvieux, V., Kwak, J. M., and Schroeder, J. I. (In press) A mRNA cap binding protein, ABH1, modulates early abscisic acid signal transduction in *Arabidopsis*. *Cell*.

Jeannette, E., Rona, J.-P., Bardat, F., Cornel, D., Sotta, B., and Miginiac, E. (1999) Induction of *RAB18* gene expression and activation of $K^+$ outward rectifying channels depend on an extracellular perception of ABA in *Arabidopsis thaliana* suspension cells. *Plant J.* 18: 13–22.

Kinoshita, T., Nishimura, M., and Shimazaki, K.-I. (1995) Cytosolic concentration of $Ca^{2+}$ regulates the plasma membrane $H^+$-ATPase in guard cells of fava bean. *Plant Cell* 7: 1333–1342.

Koornneef, M., Jorna, M. L., Brinkhorst-van der Swan, D. L. C., and Karssen, C. M. (1982) The isolation of abscisic acid (ABA) deficient mutants by selection of induced revertants in non-germinating gibberellin sensitive lines of *Arabidopsis thaliana* L. *Heynh*. *Theor. Appl. Genet.* 61: 385–393.

Lee, Y., Choi, Y. B., Suh, S., Lee, J., Assmann, S. M., Joe, C. O., Kelleher, J. F., and Crain, R. C. (1996) Abscisic acid-induced phosphoinositide turnover in guard cell protoplasts of *Vicia faba*. *Plant Physiol.* 110: 987–996.

Li, J., and Assmann, S. M. (1996) An abscisic acid-activated and calcium-independent protein kinase from guard cells of fava bean. *Plant Cell* 8: 2359–2368.

Mansfield, T. A., and McAinsh, M. R. (1995) Hormones as regulators of water balance. In *Plant Hormones: Physiology, Biochemistry and Molecular Biology*, 2nd ed., P. J. Davies, ed., Kluwer, Dordrecht, Netherlands, pp. 598–616.

Milborrow, B. V. (2001) The pathway of biosynthesis of abscisic acid in vascular plants: A review of the present state of knowledge of ABA biosynthesis. *J. Exp. Bot.* 52: 1145–1164.

Mori, I. C., and Muto, S. (1997) Abscisic acid activates a 48-kilodalton protein kinase in guard cell protoplasts. *Plant Physiol.* 113: 833–839.

Nambara, E., Hayama, R., Tsuchiya, Y., Nishimura, M., Kawaide, H., Kamiya, Y., and Naito, S. (2000) The role of *abi3* and *fus3* loci in *Arabidopsis thaliana* on phase transition from late embryo development to germination. *Dev. Biol.* 220: 412–423.

Neill, S. J., Desikan, R., Clarke, A., and Hancock, J. T. (2002) Nitric oxide is a novel component of abscisic acid signaling in stomatal guard cells. *Plant Physiol.* 128: 13–16.

Pei, Z.-M., Kuchitsu, K., Ward, J. M., Schwarz, M., and Schroeder, J. I. (1997) Differential abscisic acid regulation of guard cell slow anion channels in *Arabidopsis* wild-type and abi1 and abi2 mutants. *Plant Cell* 9: 409–423.

Pei, Z. M., Murata, Y., Benning, G., Thomine, S., Klusener, B., Allen, G. J., Grill, E., and Schroeder, J. I. (2000) Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. *Nature* 406: 731–734.

Raz, V., Bergervoet, J. H. W., and Koornneef, M. (2001) Sequential steps for developmental arrest in *Arabidopsis* seeds. *Development* 128: 243–252.

Rock, C. D. (2000) Pathways to abscisic acid-regulated gene expression. *New Phytol.* 148: 357–396.

Saab, I. N., Sharp, R. E., Pritchard, J., and Voetberg, G. S. (1990) Increased endogenous abscisic acid maintains primary root growth and inhibits shoot growth of maize seedlings at low water potentials. *Plant Physiol.* 93: 1329–1336.

Sanchez, J.-P., and Chua, N.-H. (2001) *Arabidopsis* PLC1 is required for secondary responses to abscisic acid signals. *Plant Cell* 13: 1143–1154.

Schmidt, C., Schelle, I., Liao, Y.-J., and Schroeder, J. I. (1995) Strong regulation of slow anion channels and abscisic acid signaling in guard cells by phosphorylation and dephosphorylation events. *Proc. Natl. Acad. Sci. USA* 92: 9535–9539.

Schroeder, J. I., and Hagiwara, S. (1990) Repetitive increases in cystolic $Ca^{2+}$ of guard cells by abscisic acid activation of nonselective $Ca^{2+}$ permeable channels. *Proc. Natl. Acad. Sci. USA* 87: 9305–9309.

Schroeder, J. I., Allen, G. J., Hugouvieux, V., Kwak, J. M., and Waner, D. (2001) Guard cell signal transduction. *Annu. Rev. Plant Phys. Plant Mol. Biol.* 52: 627–658.

Schultz, T. F., and Quatrano, R. S. (1997) Evidence for surface perception of abscisic acid by rice suspension cells as assayed by Em gene expression. *Plant Sci.* 130: 63–71.

Schurr, U., Gollan, T., and Schulze, E.-D. (1992) Stomatal response to drying soil in relation to changes in the xylem sap composition of *Helianthus annuus*. II. Stomatal sensitivity to abscisic acid imported from the xylem sap. *Plant Cell Environ.* 15: 561–567.

Schwartz, A., Ilan, N., Schwartz, M., Scheaffer, J., Assmann, S. M., and Schroeder, J. I. (1995) Anion-channel blockers inhibit S-type anion channels and abscisic acid responses in guard cells. *Plant Physiol.* 109: 651–658.

Schwartz, A., Wu, W.-H., Tucker, E. B., and Assmann, S. M. (1994) Inhibition of inward $K^+$ channels and stomatal response by abscisic acid: An intracellular locus of phytohormone action. *Proc. Natl. Acad. Sci. USA* 91: 4019–4023.

Sharp, R. E., LeNoble, M. E., Else, M. A., Thorne, E. T., and Gherardi, F. (2000) Endogenous ABA maintains shoot growth in tomato independently of effects on plant water balance: Evidence for an interaction with ethylene. *J. Exp. Bot.* 51: 1575–1584.

Sheen, J. (1998) Mutational analysis of protein phosphatase 2C involved in abscisic acid signal transduction in higher plants. *Proc. Natl. Acad. Sci. USA* 95: 975–980.

Shimazaki, K., Iino, M., and Zeiger, E. (1986) Blue light–dependent proton extrusion by guard cell protoplasts of *Vicia faba*. *Nature* 319: 324–326.

Spollen, W. G., LeNoble, M. E., Samuels, T. D., Bernstein, N., and Sharp, R. E. (2000) Abscisic acid accumulation maintains maize primary root elongation at low water potentials by restricting ethylene production. *Plant Physiol.* 122: 967–976.

Wang, X.-Q., Ullah, H., Jones, A. M., and Assmann, S. M. (2001) G protein regulation of ion channels and abscisic acid signaling in *Arabidopsis* guard cells. *Science* 292: 2070–2072.

White, C. N., Proebsting, W. M., Hedden, P., and Rivin, C.J. (2000) Gibberellins and seed development in maize. I. Evidence that gibberellin/abscisic acid balance governs germination versus maturation pathways. *Plant Physiol.* 122: 1081–1088.

Wilkinson, S., and Davies, W. J. (1997) Xylem sap pH increase: A drought signal received at the apoplastic face of the guard cell that involves the suppression of saturable abscisic acid uptake by the epidermal symplast. *Plant Physiol.* 113: 559–573.

Xiong, L., Lee, H., Ishitani, M., Zhang, C., and Zhu, J.-K. (2001) *FIERY1* encoding an inositol polyphosphate 1-phosphatase is a negative regulator of abscisic acid and stress signaling in *Arabidopsis*. *Genes Dev.* 15: 1971–1984.