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Update on Signal Transduction

Genetic Analysis of Abscisic Acid Signal Transduction[1]

Sylvain Merlot and Jérôme Giraudat*

Institut des Sciences Végétales, Centre National de la Recherche Scientifique, 91198 Gif-sur-Yvette cedex, France

The scientific origins of ABA can be traced to several independent lines of investigation in the late 1940s (for review, see Addicott and Carns, 1983). In these studies, which in retrospect appear to have involved extracts that were rich in ABA, acidic inhibitors of coleoptile growth were detected that were also correlated with promoting activities on abscission and dormancy. It was only in the 1960s, however, that ABA was finally isolated and identified (for review, see Addicott and Carns, 1983). In 1963, Ohkuma and colleagues (Addicott and Carns, 1983) reported the crystallization of a substance that promoted abscission of young cotton fruits, which they called abscisin II. Shortly afterward, Wareing and colleagues purified from sycamore leaves a substance called dormin, which promoted bud dormancy (reviewed by Addicott and Carns, 1983). When dormin was chemically identified it was found to be identical to abscisin II. It was decided in 1967 to use ABA as the name, even though we now know that ethylene rather than ABA is actually the major factor causing organ abscission.

Since the 1960s significant progress has been made in understanding how ABA controls diverse, essential physiological processes such as seed development and germination, as well as plant adaptation to abiotic environmental stress (for review, see Zeevaart and Creelman, 1988; Giraudat et al., 1994). Many of the actions of ABA, in both seeds and vegetative tissues, involve modifications of gene expression at the transcriptional level. Multiple ABA-responsive genes have been isolated, and their analysis has provided insights into the biological function of the encoded proteins, as well as into the nature of the *cis-* and *trans*-acting factors involved in ABA responsiveness (for review, see Ingram and Bartels, 1996). A distinct type of ABA response is exemplified by the regulation of stomata. Stomatal aperture is defined by the turgor of the two surrounding guard cells. Guard cell volume is controlled osmotically mainly by large influxes (stomatal opening) or effluxes (stomatal closure) of K^+ and anions. A variety of single-cell techniques have established that ABA causes rapid (within minutes) alterations in the activity of K^+ and anionic channels in the plasma membrane of guard cells via pH- and Ca^{2+}- sensitive signaling cascades (for review, see Blatt and Thiel, 1993; Ward et al., 1995).

In recent years substantial progress toward understanding ABA action has come from combining classical genetics with modern methods for cloning the corresponding loci. In this *Update* we will illustrate how genetic analyses in model species such as *Arabidopsis thaliana* and maize (*Zea mays*) have shed new light on ABA biosynthesis, physiology, and signal transduction.

ABA BIOSYNTHESIS

ABA is a sesquiterpenoid with mevalonic acid as its precursor. The biosynthetic defects identified in a variety of ABA-deficient mutants, together with results of physicochemical studies, support the idea that higher plants synthesize ABA by the indirect (or C_{40}) pathway depicted in Figure 1 (for review, see Zeevaart and Creelman, 1988; Taylor, 1991). The maize *viviparous* (*vp*) mutants (*vp2*, *vp5*, *vp7*, and *vp9*) are blocked in the early steps of carotenoid biosynthesis. The Arabidopsis *aba1* (formerly called *aba*) and the *Nicotiana plumbaginifolia aba2* mutants are impaired in the epoxidation reactions converting zeaxanthin into violaxanthin (Koornneef et al., 1982; Marin et al., 1996). The Arabidopsis *aba2* mutant is impaired in the conversion of xanthoxin to ABA-aldehyde (Léon-Kloosterziel et al., 1996; Schwartz et al., 1997). Finally, multiple mutants are blocked in the final oxidation of ABA-aldehyde to ABA. This last step involves an enzyme that requires a molybdenum cofactor (Leydecker et al., 1995; Schwartz et al., 1997), and it is unclear whether any of the mutations affects the core enzyme directly.

Molecular cloning of an ABA biosynthetic gene has been achieved only recently. The *N. plumbaginifolia ABA2* gene has been isolated by transposon-tagging and has been shown by mapping and functional complementation studies to represent the ortholog of the Arabidopsis *ABA1* locus (Marin et al., 1996). The ABA2 protein was shown by in vitro assays to be chloroplast-imported and to catalyze the conversion of zeaxanthin into antheraxanthin and, subsequently, violaxanthin. An intriguing observation is that in the absence of water stress, leaves of a presumably null *aba2* mutant allele still retain 23 to 48% of the wild-type ABA content (Marin et al., 1996). This clearly raises new questions as to the possible existence of secondary biosynthetic pathways and their physiological roles.

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* Corresponding author; e-mail jerome.giraudat@isv.cnrs-gif.fr; fax 33-1-6982-3695.

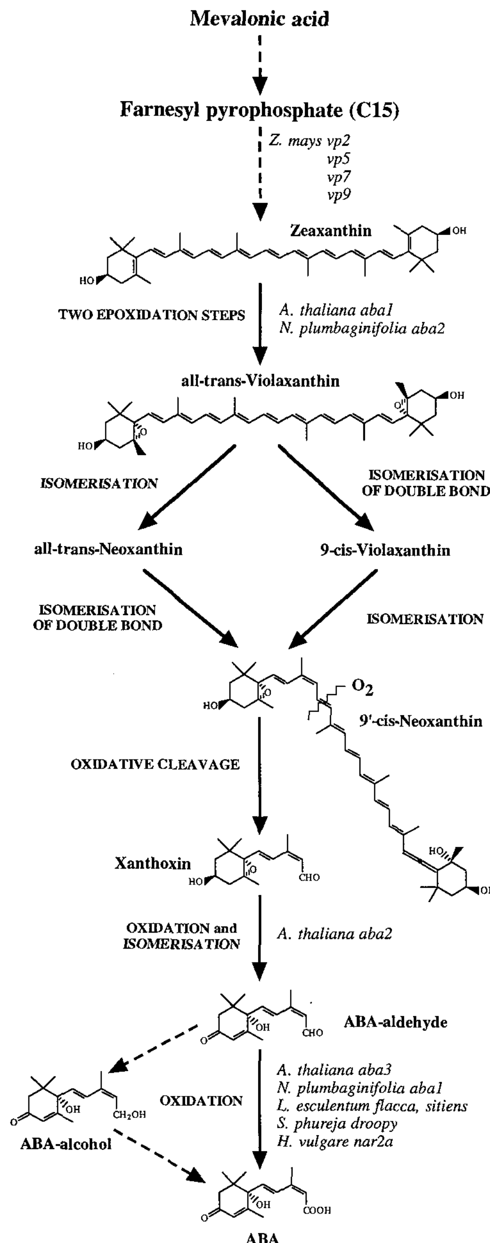

Figure 1. Schematic pathway of ABA biosynthesis in higher plants. The metabolic blocks in various ABA-deficient mutants are indicated. The minor shunt pathway that involves reduction of ABA-aldehyde to ABA-alcohol and oxidation of ABA-alcohol to ABA was uncovered by mutations such as *flacca* and *sitiens* in tomato (*Lycopersicon esculentum;* Taylor, 1991).

This pioneer work illustrates that cloned biosynthetic genes will yield important clues about the modes of regulation and the sites of ABA biosynthesis. These genes also represent potential tools for improving ABA-controlled traits in crop species.

ABA PHYSIOLOGY

During vegetative growth endogenous ABA levels increase when plants are exposed to conditions of water stress; ABA is an essential mediator in triggering plant responses to these adverse environmental stimuli (for review, see Zeevaart and Creelman, 1988). A striking phenotype displayed by most ABA-biosynthetic mutants is an increased tendency to wilt and/or enhance water loss from excised aerial parts, which is indicative of a defect in stomatal regulation (Koornneef et al., 1982; Léon-Kloosterziel et al., 1996; Marin et al., 1996). As mentioned above, a wealth of studies document that applied ABA inhibits stomatal opening and promotes stomatal closure (Blatt and Thiel, 1993; Ward et al., 1995). Altogether, these results support the idea that, during conditions of water stress, the increased ABA levels reduce water loss through transpiration by regulating stomatal aperture.

The role of ABA in signaling stress conditions has also been extensively documented by molecular studies. The characterization of biosynthetic mutants has indeed demonstrated that endogenous ABA is required for the regulation of numerous genes by drought, salt, and (in fewer instances) cold (for review, see Giraudat et al., 1994; Ingram and Bartels, 1996; Shinozaki and Yamaguchi-Shinozaki, 1996). However, ABA is not the sole endogenous signal for adverse environmental conditions; these stresses also elicit ABA-independent pathways that are responsible for the induction of certain genes (for review, see Shinozaki and Yamaguchi-Shinozaki, 1996). Moreover, the characterization of ABA-biosynthetic mutants revealed that a given gene can be responsive to applied ABA and nevertheless be induced by stress in an ABA-independent manner (Gilmour and Thomashow, 1991).

During seed development ABA regulates several facets of middle and late embryogenesis that are essential for seed viability and germination. In mono- and dicotyledonous species, endogenous ABA content peaks during approximately the last two-thirds of seed development before returning to lower levels in the dry seed (for review, see Rock and Quatrano, 1995). Embryos of the ABA-biosynthetic *vp* mutants from maize are viviparous (for review, see McCarty, 1995), and seeds of the *aba* mutants from Arabidopsis (Koornneef et al., 1982; Karssen et al., 1983; Léon-Kloosterziel et al., 1996) and *N. plumbaginifolia* (Marin et al., 1996) fail to become dormant. These observations support the view that endogenous ABA inhibits precocious germination and promotes seed dormancy.

The contribution of ABA to the accumulation of protein reserves and to the acquisition of seed desiccation tolerance has been more controversial. Transcripts encoding either storage proteins or late-embryogenesis-abundant (LEA) proteins thought to participate in desiccation tolerance can be precociously induced by exogenous ABA in cultured embryos (for review, see Rock and Quatrano, 1995; Ingram and Bartels, 1996). However, although the maize *vp* and Arabidopsis *aba1* biosynthetic mutations significantly reduce the accumulation of several LEA mRNAs, in most cases the extent of this inhibition is not as pronounced as the reduction in ABA content (Paiva and Kriz, 1994; Parcy et al., 1994). Furthermore, these mutants do not display marked reductions in the abundance of storage protein mRNAs or in desiccation tolerance (Koornneef et al., 1989;

Paiva and Kriz, 1994; Parcy et al., 1994). The possibility that the residual ABA content in biosynthetic mutants may be sufficient to ensure near wild-type responses cannot be formally discarded. Alternatively, however, the mild phenotypes of the biosynthetic mutants may indicate that in the wild type, ABA acts in concert with additional developmental factors to control the accumulation of protein reserves and the acquisition of desiccation tolerance. Identifying these putative factors and deciphering their interactions with ABA should provide novel insights into the physiology of seed development.

ABA SIGNAL TRANSDUCTION

Unlike biosynthetic mutants, mutants that are altered in their responsiveness to ABA do not have reduced endogenous ABA content, and their phenotypes cannot be reversed to wild type by exogenous supply of ABA. Such response mutants are thus believed to unravel components of signal transduction cascades. ABA-response mutants have been described in several plant species (for review, see Giraudat et al., 1994; McCarty, 1995). Multiple mutations that either decrease or increase ABA sensitivity have been characterized in Arabidopsis (Table I). The five distinct ABA-insensitive (ABI) loci (*ABI1–ABI5*) were identified by selecting for seeds capable of germinating in the presence of inhibitory ABA concentrations (Koornneef et al., 1984; Finkelstein, 1994b). Conversely, mutations in the enhanced response to ABA (*ERA1*) locus confer to germinating seeds a hypersensitivity to applied ABA (Cutler et al., 1996).

Four of these Arabidopsis loci have already been cloned. As will be discussed in further detail below, the *ABI1* and *ABI2* genes encode homologous protein phosphatases (Leung et al., 1994, 1997; Meyer et al., 1994), and the *ABI3* gene is the ortholog of the maize *VP1* (Giraudat et al., 1992). Because available evidence indicates that the VP1 protein functions in ABA signaling as a transcriptional activator (McCarty et al., 1991; McCarty, 1995), ABI3 is by homology a putative transcription factor. The *ERA1* gene encodes the β-subunit of a farnesyl transferase that may function as a negative regulator of ABA signaling by modifying signal transduction proteins for membrane localization (Cutler et al., 1996).

Although many more loci probably still remain to be identified, the available Arabidopsis response mutants are useful to propose working models for the architecture of the ABA-signaling network at the whole plant level.

THE ABA-SIGNALING NETWORK IN ARABIDOPSIS

Each of the *abi1*, *abi2*, and *abi3* mutants exhibits, like ABA-deficient mutants, a marked reduction in seed dormancy (Koornneef et al., 1984; Ooms et al., 1993; Nambara et al., 1995). Conversely, seed dormancy is increased in *era1* mutants (Cutler et al., 1996). These observations are consistent with a role for these four loci in mediating the inhibitory effects of endogenous ABA on seed germination.

However, additional phenotypes have been observed in the *abi1* and *abi2* mutants and in the *abi3* mutants (Table I). The *abi1* and *abi2* mutations inhibit multiple actions of ABA in vegetative tissues. In particular, both mutants display abnormal stomatal regulation and defects in various ABA-mediated morphological and molecular responses to stress (Koornneef et al., 1984; Finkelstein and Somerville, 1990; Finkelstein, 1994a; Giraudat et al., 1994). In contrast, *abi3* mutations lead to numerous defects during embryo development. Seeds of the severe *abi3* mutants fail to degrade chlorophyll, do not acquire desiccation tolerance, and contain decreased levels of various storage protein and LEA mRNAs (Ooms et al., 1993; Parcy et al., 1994; Nambara et al., 1995).

A first possibility to account for these phenotypic differences is to hypothesize that, as shown in Figure 2A, *ABI3* acts in an ABA-signaling pathway distinct from *ABI1* and *ABI2* (Finkelstein and Somerville, 1990). Both pathways would impinge on seed germination and dormancy, but the *ABI3* pathway would have a major role in developing embryos, whereas the *ABI1*/*ABI2* pathway would act predominantly in vegetative tissues. Because the *abi4* and *abi5* mutations do not impair stomatal regulation but do inhibit the accumulation of the LEA transcript *AtEm6*, these loci have been proposed to act in the *ABI3* pathway (Finkelstein, 1994b). However, several of the additional seed phenotypes of severe *abi3* mutants are absent in *abi4* and *abi5*, which might be due to the leakiness of the available *abi4* and *abi5* mutant alleles. It is presently difficult to assign the *ERA1* locus to either one of the two pathways because the

Table I. *Summary of mutations affecting ABA signal transduction in Arabidopsis*
SD, Semidominant; R, recessive.

Mutation	Dominance[a]	Protein[b]	Physiological Processes Affected			
			Seed development	Dormancy, germination on ABA	Stomatal regulation	Responses to stress
abi1	SD	Protein phosphatase	No	Yes	Yes	Yes
abi2	SD	Protein phosphatase	No	Yes	Yes	Yes
abi3	R	Transcription factor	Yes	Yes	No	No
abi4	R	Unknown	Yes	Yes[c]	No	Unknown
abi5	R	Unknown	Yes	Yes[c]	No	Unknown
era1	R	Farnesyl transferase	Unknown	Yes	Unknown	Unknown

[a] Dominance of the mutant alleles over wild type. [b] Molecular function of the protein encoded by the locus. [c] It is presently unclear whether these mutations reduce seed dormancy.

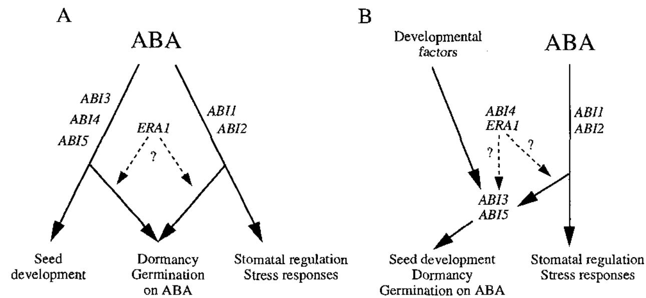

Figure 2. Hypothetical models explaining why various aspects of seed development are markedly altered in *abi3* (and to a certain extent *abi4* and *abi5*) mutants but not in *abi1* and *abi2*. Within each branch of the pathways, the loci are shown in an arbitrary order. A, The characteristic ABI3-dependent developmental processes are postulated to be under strict ABA control. The *ABI1* and *ABI2* loci would then act in a different ABA-signaling pathway from *ABI3*, *ABI4*, and *ABI5*. B, These developmental processes are postulated to be jointly regulated by ABA and by additional developmental factors, with both types of signals being mediated by ABI3 and possibly ABI5. The ABI1/ABI2 pathway may then mediate ABA signaling in seeds as in vegetative tissues, but only in seeds would this cascade recruit the seed-specific ABI3, ABI4, and ABI5 proteins. For the sake of clarity the ABI1/ABI2 branch is shown as a linear cascade in both models, but there is evidence that the ABI1 and ABI2 proteins have in fact partially redundant functions (Fig. 3).

distinguishing *abi* phenotypes have not yet been analyzed in *era1* mutants (Cutler et al., 1996).

In germination assays of double mutants, synergistic effects on ABA resistance were observed when *abi1* or *abi2* was combined with *abi3*, *abi4*, or *abi5* (Finkelstein and Somerville, 1990; Finkelstein, 1994b). These results have been proposed as evidence for the two-pathways model of Figure 2A. However, because of uncertainties about the exact severity of the mutations used in these studies, it appears difficult to discriminate whether the phenotypic enhancements resulted from genetic interactions between loci in two distinct pathways or in a common pathway (Guarente, 1993).

The model shown in Figure 2B provides an alternative to the two-pathways model of Figure 2A for explaining the phenotypic differences between *abi1*/*abi2* and *abi3* mutants. The two major features of this speculative model are that (a) the same ABI1-/ABI2- dependent cascade mediates ABA signaling in both seed and vegetative tissues, but this cascade recruits or interacts with the ABI3 protein only in seeds and (b) unlike ABI1 and ABI2, the role of ABI3 in seeds is not confined to ABA signaling. The first hypothesis is based on the following observations: The *ABI3* gene is exclusively expressed in seeds (Parcy et al., 1994); however, when it was ectopically expressed in the vegetative tissues of transgenic Arabidopsis plants, it genetically interacted with the endogenous *ABI1* in controlling diverse vegetative responses to ABA (Parcy and Giraudat, 1997). Preliminary evidence indicates that in nontransgenic seeds *ABI1* and *ABI2* act in the same ABA-signaling pathway as *ABI3* to control at least the accumulation of the storage protein *At2S3* and LEA *AtEm1* and *AtEm6* mRNAs. Expression of these mRNAs appears to be strictly ABI3-dependent because it is abolished in severe *abi3* mutants (Parcy et al., 1994). Nevertheless, abundance of the *AtEm6* mRNA is lower in *abi1 abi3-1* and *abi2 abi3-1* double mutants than in the leaky *abi3-1* single mutant (Finkelstein, 1993), and the *At2S3* and *AtEm1* mRNAs are decreased in *abi1* mutant seeds to levels intermediate between those in the wild type and those in the *aba1* biosynthetic mutant (Parcy and Giraudat, 1997).

The various data summarized above are consistent with the same basic ABI1/ABI2 cascade acting in both seed and vegetative tissues but recruiting ABI3 only in seeds simply because the *ABI3* gene is not expressed in vegetative tissues. If this is the case, why do severe *abi3* mutants display additional seed phenotypes that are not observed in *abi1* and *abi2* mutants (Table I)? The model in Figure 2B postulates that the corresponding responses are under the joint control of ABA and additional developmental factors and that ABI3 mediates both types of signals. This interpretation is consistent with the following data: The characteristic phenotypes of severe *abi3* mutants are observed in *aba1 abi3-1* double mutants (which combine the biosynthetic *aba1* and the weak *abi3-1* mutations) but not in *aba1* single mutants (Koornneef et al., 1989). As discussed above (see "ABA Physiology"), this indicates that these ABI3-dependent responses may not be controlled by ABA alone.

That ABI3 action is not strictly confined to ABA signaling is further supported by evidence that VP1 (the maize homolog of ABI3) can regulate transcription independently of ABA (Vasil et al., 1995; Kao et al., 1996). The maize VP1 and Arabidopsis ABI3 putative transcription factors thus seem to contribute to and integrate ABA-dependent as well as ABA-independent developmental regulatory pathways in seed. This dual role of ABI3 would explain why seed processes that are jointly controlled by ABA and additional factors are more severely affected by *abi3* mutations (which inhibit both pathways) than by *aba1*, *abi1*, or *abi2* mutations (which only inhibit the ABA pathway). Like *ABI3*, the action of *ABI5* in seeds may not be strictly limited to ABA signaling, because abundance of the LEA *AtEm6* transcript is markedly more reduced in *abi5* (Finkelstein, 1994b) and severe *abi3* mutants (Parcy et al., 1994) than in the biosynthetic *aba1* mutant (Finkelstein, 1993; Parcy et al., 1994).

In summary, the remaining gaps in these tentative models point to the need for the identification of additional mutations inhibiting ABA signaling in seeds. Furthermore, the genetic interactions between loci may vary depending on the particular ABA response considered. The ABA-signaling network is thus likely to be more complex than any of the two simple models discussed above. In this regard, there is already evidence that the *ABI1*-/*ABI2*-dependent pathway is not a simple linear cascade as depicted in Figure 2.

THE ABI1 AND ABI2 PHOSPHATASES HAVE PARTIALLY REDUNDANT FUNCTIONS

The predicted ABI1 and ABI2 proteins display the same characteristic modular architecture. Their C-terminal domains share 86% amino acid identity and are homologous to protein Ser/Thr phosphatases of the 2C class (PP2C)

(Leung et al., 1994, 1997; Meyer et al., 1994). Phenotypic complementations of a yeast PP2C mutant and in vitro enzymatic assays using recombinant proteins support that both the ABI1 and ABI2 C-terminal domains do have PP2C catalytic activity (Bertauche et al., 1996; Leung et al., 1997). The N-terminal domains of ABI1 and ABI2 are less conserved (49% identity) and display no extensive similarity to other known proteins. It was initially hypothesized that the N-terminal domain of ABI1 might bind Ca^{2+} because this domain contains a local sequence motif that matches one of the consensus patterns proposed for EF-hand Ca^{2+}-binding sites (Leung et al., 1994; Meyer et al., 1994). Subsequent analyses, however, revealed that this sequence motif in ABI1 most likely corresponds to an analog rather than to a true EF-hand homolog (Bertauche et al., 1996).

The EMS-induced *abi1–1* and *abi2–1* mutants have both been isolated on the basis of their decreased sensitivity to ABA at the germination stage (Koornneef et al., 1984). Remarkably, these two mutants carry identical Gly-to-Asp substitutions at equivalent positions in the ABI1 (Gly-180) and ABI2 (Gly-168) phosphatase domains, respectively (Leung et al., 1994, 1997; Meyer et al., 1994). This single amino acid change markedly decreases the PP2C activity of the ABI1 and ABI2 proteins (Bertauche et al., 1996; Leung et al., 1997). By analogy to the human PP2Cα with a known structure (Das et al., 1996), the Gly- to- Asp substitutions probably disrupt the conformation of two neighboring invariant residues (Asp-177 and Gly-178 in ABI1 and Asp-165 and Gly-166 in ABI2), which are essential for metal coordination and phosphatase activity (Das et al., 1996; Leung et al., 1997).

There has been some ambiguity about the degree of dominance of the *abi1–1* and *abi2–1* mutations over their respective wild-type alleles (Koornneef et al., 1984; Finkelstein, 1994a). Recent observations, however, indicate that both mutations are fully dominant in reducing the ABA sensitivity of seed germination (Leung et al., 1997) and semidominant in altering stomatal regulation (Finkelstein, 1994a) and reducing the ABA sensitivity of root growth (Leung et al., 1997). Their (semi-)dominance is also supported by the ability of the mutant genes to induce mutant phenotypes in transgenic plants (Leung et al., 1994, 1997; Meyer et al., 1994; Armstrong et al., 1995). That *abi1–1* and *abi2–1* display a similar degree of dominance with respect to a given common phenotype is consistent with the finding that these mutations entail identical amino acid changes in homologous proteins.

How can these mutations behave (semi-)dominantly in planta if they reduce the phosphatase activity of the corresponding ABI protein? A first possibility could be that *abi1–1* and *abi2–1* are neomorphic gain-of-function mutations. In other words, the wild-type ABI1 and ABI2 proteins would not be involved in ABA signaling, but the mutant proteins would impair ABA action because they have gained the ability to bind a protein(s) that is not a substrate of the wild-type phosphatase. Although this scenario cannot be formally excluded, several observations tend to argue against it. In maize leaf protoplasts overexpression of the wild-type ABI1 phosphatase domain inhibits the activation by ABA of the barley (*Hordeum vulgare*) *HVA1* promoter (Sheen, 1996). Also, the aforementioned semidominant nature of the mutations with respect to root growth on ABA and stomatal regulation may suggest that the mutant abi1–1 and abi2–1 proteins compete with their wild-type counterparts in binding substrate(s) related to ABA signaling. Finally, expression of signaling elements is frequently up-regulated by the signal that they transduce, and the *ABI1* and *ABI2* mRNAs are both induced in response to ABA (Leung et al., 1997).

An alternative and more likely possibility would then be that *abi1–1* and *abi2–1* are dominant negative mutations. Action of the wild-type phosphatases would be required for ABA signaling and the mutant proteins would have (semi-)dominant effects because, possibly as a result of conformational alterations in their catalytic site, they form "poison complexes" with the endogenous substrate(s). Mutations leading to catalytically inactive protein phosphatases that nonetheless behave genetically and/or molecularly as dominant are known in other systems (Herbst et al., 1996). In this scenario, the dominance of the *abi1–1* and *abi2–1* mutations may further imply that the endogenous substrate(s) are not in vast excess (at least locally in the cell) compared with the ABI1 and ABI2 phosphatases.

Two sets of observations support that, as suggested by the above-mentioned biochemical characteristics, the ABI1 protein acts in planta in a phosphorylation-dependent signaling cascade(s). In guard cells the *abi1–1* mutant gene inhibits stomatal closure in response to ABA, and this effect can be partially suppressed by kinase antagonists (Armstrong et al., 1995). In maize leaf protoplasts the barley *HVA1* promoter can be activated by applied ABA or by overexpressing the catalytic domain of some protein kinases, and in both cases this activation can be decreased by overexpressing the wild-type ABI1 PP2C domain (Sheen, 1996). These two sets of data are in apparent contradiction with each other, because they suggest that ABI1 phosphatase activity is required for ABA promotion of stomatal closure but inhibits ABA activation of the *HVA1* promoter. This may reflect true differences between the ABA-signaling pathways involved in regulating ionic channels activity and gene expression, respectively. However, one should exercise caution in interpreting the above-mentioned preliminary data. In guard cells kinase antagonists may suppress the *abi1* mutant phenotype by more indirect means than by redressing the phosphorylation status of the ABI1 substrate(s). In maize protoplasts the activity of the overexpressed truncated ABI1 protein may no longer be subject to ABA modulation. Nevertheless, these initial studies clearly illustrate that combining genetics with single-cell analyses represents a promising approach for deciphering further the ABA-signaling cascades.

The *abi1–1* and *abi2–1* mutants display several common phenotypes, including decreased sensitivity to the inhibition by ABA of seed germination and seedling growth, altered stomatal regulation, and decreased responsiveness of certain transcripts to applied ABA (Koornneef et al., 1984; Finkelstein and Somerville, 1990; Finkelstein, 1994a; Gosti et al., 1995). Several of these phenotypes are not only

qualitatively but also quantitatively very similar (Leung et al., 1997), suggesting that the homologous ABI1 and ABI2 proteins perform overlapping functions in controlling the ABA responses delineated by the common mutant phenotypes. Such a scheme is consistent with the observation that in germination assays the *abi1–1 abi2–1* double mutant is no more resistant to ABA than the parental single mutants (Finkelstein and Somerville, 1990). Finally, this functional redundancy would explain why recessive alleles have not been identified at the *ABI1* and *ABI2* loci on the basis of seed germination on ABA.

The roles of the ABI1 and ABI2 proteins are unlikely to be completely redundant. Several responses triggered by water stress, such as drought rhizogenesis (Vartanian et al., 1994), and accumulations of the *ATHB7* (Söderman et al., 1996), *RAB18*, and *AtDi21* (Gosti et al., 1995) transcripts are inhibited in *abi1–1* but not in *abi2–1*. Conversely, inductions of the *ADH* transcript by water stress and by exogenous ABA are substantially more affected in *abi2–1* than in *abi1–1* (de Bruxelles et al., 1996).

As summarized in Figure 3, evidence indicates that the homologous ABI1 and ABI2 phosphatases act as redundant signaling elements for certain ABA responses but that each of these proteins has additional specific roles in mediating other ABA actions. An intriguing (and testable) possibility could be that, depending on the cellular and physiological context, the ABI phosphatases bind to either common or distinct regulatory proteins (via their more divergent N-terminal domains) and/or substrates.

PERSPECTIVES

In recent years the combined use of classical and molecular genetic approaches has generated a wealth of new data concerning ABA biosynthesis, physiology, and signal transduction. It is becoming clear that ABA biosynthesis and signal transduction are not simple linear pathways but rather make up ramified and redundant branches. The information and tools gathered so far will permit the design of novel, and possibly more targeted, mutant screens to fill some of the multiple remaining gaps in our understanding of these processes. It is also important to keep in mind that in the plant ABA often acts in conjunction with other hormones (such as GAs) or stimuli. The corresponding cross-talk elements may be unraveled by characterizing mutations that simultaneously affect distinct signal transduction pathways.

The genetic approach has already unraveled several key elements of the ABA-signaling network, although their interrelationships remain somewhat unclear. Genes that were originally identified from mutational analysis can be used for biochemical and molecular approaches. For example, several attempts are now being made to identify proteins that directly interact with the already characterized VP1 and ABI proteins. Exciting developments are also expected from the emerging combinations of genetics with the powerful single-cell biophysical and microinjection techniques.

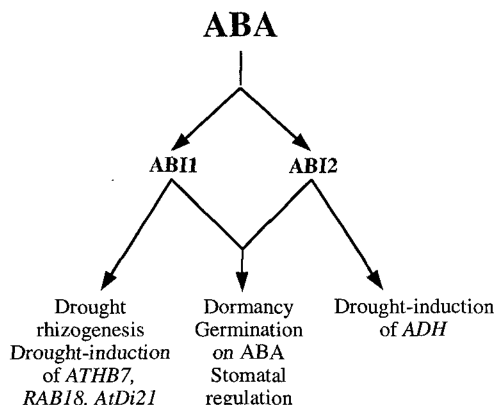

Figure 3. Schematic model of the partially redundant roles of ABI1 and ABI2 in ABA signaling.

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