Published in final edited form as:
Curr Biol. 2011 May 10; 21(9): R346–R355. doi:10.1016/j.cub.2011.03.015.

Evolution of Abscisic Acid Synthesis and Signaling Mechanisms

Felix Hauser[1], **Rainer Waadt**[1], and **Julian I. Schroeder**[*]
Division of Biological Sciences, Cell and Developmental Biology Section, University of California San Diego, 9500 Gilman Drive #0116, La Jolla, CA 92093-0116, USA.

Abstract

The plant hormone abscisic acid (ABA) mediates seed dormancy, controls seedling development and triggers tolerance to abiotic stresses, including drought. Core ABA signaling components consist of a recently identified group of ABA receptor proteins of the PYRABACTIN RESISTANCE (PYR)/REGULATORY COMPONENT OF ABA RECEPTOR (RCAR) family that act as negative regulators of members of the PROTEIN PHOSPHATASE 2C (PP2C) family. Inhibition of PP2C activity enables activation of SNF1-RELATED KINASE 2 (SnRK2) protein kinases, which target downstream components, including transcription factors, ion channels and NADPH oxidases. These and other components form a complex ABA signaling network. Here, an in depth analysis of the evolution of components in this ABA signaling network shows that (i) PYR/RCAR ABA receptor and ABF-type transcription factor families arose during land colonization of plants and are not found in algae and other species, (ii) ABA biosynthesis enzymes have evolved to plant- and fungal-specific forms, leading to different ABA synthesis pathways, (iii) existing stress signaling components, including PP2C phosphatases and SnRK kinases, were adapted for novel roles in this plant-specific network to respond to water limitation. In addition, evolutionarily conserved secondary structures in the PYR/RCAR ABA receptor family are visualized.

Introduction

Abscisic acid (ABA) is a stress-related signaling molecule reported in all kingdoms of life except in Archaea. Although well known and best studied in higher plants, in particular in *Arabidopsis thaliana*, there is evidence that the hormone is synthesized in plant-associated bacteria, plant pathogenic fungi, certain cyanobacteria, algae, lichens, protozoa, sponges and apparently even in human granulocytes [1–3]. In *Arabidopsis* and probably in all Embryophyta, the main abiotic factor leading to formation of ABA and thus triggering of signaling events is any form of limited cellular water availability. High ABA levels lead to preservation of seed dormancy [4], inhibition of germination and lateral root formation [5] and reduction of water transpiration through stomatal pores [6,7]. In lower photosynthetic species such as algae, cyanobacteria and lichen, drought or salt stress is also a factor that induces ABA synthesis but knowledge on signaling pathways is extremely limited [3].

[*]jischroeder@ucsd.edu.
[1]These authors contributed equally.

Supplemental Information
Supplemental Information includes one figure and four tables and can be found with this article online at doi:10.1016/j.cub.2011.03.015.

Factors affecting ABA levels in non-photosynthetic organisms described so far are heat (*Axinella polypoides*, *Homo sapiens* [1]) and nutrient limitation (*Apicomplexa* [2]).

Since the discovery of ABA in the 1960s [8] and the first identification of plant ABA pathway mutants in the 1980s [9], tremendous progress has been made in identifying mechanisms and genes involved in ABA metabolism, transport and signal transduction (reviewed in [10–12]).

Here we aim to place the various aspects of the plant ABA signaling network in an evolutionary context. In particular we try to shed light on the appearance of ABA signaling as part of diverse adaptations necessary to cope with environmental factors typical for the terrestrial habitat. For a more general detailed overview of ABA signaling in plants the reader may also consider other recent reviews [7,11–13].

Comprehensive Overview of Core Components and Modulators of ABA Signaling

ABA signaling can be divided into three different layers: (i) ABA metabolism and transport, (ii) ABA perception and signal transduction and (iii) ABA signal response and modulation (Figure 1). The onset of ABA signaling begins with ABA synthesis [10] and its long-distance transport [14,15]. ABA perception and signal transduction consists of so-called core signaling components, including PYRABACTIN RESISTANCE (PYR)/REGULATORY COMPONENT OF ABA RECEPTOR (RCAR) ABA receptors [16,17], group APROTEIN PHOSPHATASE 2Cs (PP2Cs) [18] and members of the SNF1-RELATED PROTEIN PROTEIN KINASE 2 (SnRK2) group of kinases [19–21]. PYR/RCAR–PP2C complex formation leads to inhibition of PP2C activity [16,17,22], thereby allowing activation of SnRK2s which target ion channels, NADPH oxidases and ABF/AREB/ABI5 type basic/region leucine zipper (bZIP) transcription factors (Figure 1) [23–26]. The complexity of ABA signaling in *Arabidopsis* is reflected by the number of PYR/RCARs (Figures 2 and 3) [16,17,27], 6–9 PP2Cs and 3 SnRK2s, which function in ABA signaling (see Supplemental Table S1 published with this article online for gene lists and respective references).

Several small molecules act as intracellular messengers and transmit specific aspects of ABA signaling. For example, ABA causes the production of reactive oxygen species (ROS) that down-regulate the activity of the PP2C phosphatases [28] and activate Ca^{2+}-permeable channels (I_{Ca}) [29–31]. In addition, H_2O_2 also activates mitogen-activated protein kinases (MAPKs). MAPKs are inhibited by PP2Cs [18,32], phosphorylate ABI5 [33] and regulate S-type anion channel activity [34].

Phosphatidic acid, synthesized by phospholipase D [35], binds and inhibits protein phosphatase 1 (PP1) and PP2A phosphatases, which function in ABA and light signaling and interactions among these stimuli [36–38]. Additional enzymes involved in phospholipid metabolism also play a role in ABA signaling [39] (Figure 1).

Changes in cellular Ca^{2+} levels activate Ca^{2+}-DEPENDENT PROTEIN KINASES (CDPKs) [40] and CALCINEURIN B-LIKE PROTEIN (CBL) INTERACTING PROTEIN KINASES (CIPKs), the latter mediated through interaction with CBLs (reviewed in [41]). CIPKs (SnRK3s) appear to be involved in plant ion homeostasis and abiotic stress tolerance by regulating H^+, Na^+, Ca^{2+} and NO_3^- transporters and K^+ channels and interacting with transcription factors [41,42]. In addition, CIPKs physically interact with PP2Cs [43] but it still needs to be elucidated if CIPKs and PP2Cs can (de)phosphorylate each other or if both act together as a signaling module regulating target proteins such as K^+ channels (reviewed in [41]). CDPKs function in ABA-induced stomatal closing, and anion-channel and Ca^{2+}-

channel activation [44] and fulfill a dual function, as they directly phosphorylate PP2Cs and targets of SnRK2s, e.g. SLAC1, NADPH oxidases and ABFs [45–48]. The functional consequence of PP2C phosphorylation by CDPKs still needs to be clarified. Ca^{2+} in addition may function in parallel to ABA-induced transcriptional activation through calmodulin-binding transcription activators (CAMTAs) that can bind to ABA-regulated *cis*-acting elements (ABREs) [49].

ABA signaling affects developmental processes through the B3-type transcription factor ABI3 [50]. ABI3 recognizes ABA-responsive elements (ABREs) [51] and interacts with ABI5 [52]. ABI4, an AP2/ERF transcription factor, integrates ABA and sugar signaling [53,54] and controls ABI5 expression [55]. In contrast to ABF/AREB/ABI5, ABI4 and ABI3, which function early in the ABA signaling cascade, additional transcription factors (Figures 1 and 2, Supplemental Figure S1, Supplemental Table S1) may act as convergence points in the crosstalk between ABA and other plant hormone signaling networks, light signaling, plant defense and abiotic stress responses or developmental cues. They modulate the expression of ABA synthesis genes, PP2Cs and ABF/AREB/ABI5 or bind to ABRE elements (Figure 1). Thus, further work is needed to understand the architecture of the large transcriptional network in ABA signaling.

Post-translational protein modifications such as farnesylation [56,57], sumoylation [58] and ubiquitination [59] modulate ABA signaling, in some cases by targeting transcriptional regulators such as ABI3 and ABI5 (Figure 1). Furthermore, mRNA-binding proteins regulate transcript abundance of ABA synthesis genes and ABA signaling transducers [60,61]. Finally, chromatin remodeling factors could be targeted by PP2Cs [62].

Together, all of these ABA signaling components form a complex network that integrates and transduces ABA-mediated signals (Figure 1, Supplemental Table S1) that requires further characterization.

Key Components in ABA Signaling are Plant Specific

Overall sequence comparison of ABA signaling components as depicted in Figure 2 shows remarkable land-plant-specific conservation (see Supplemental Table S1 for a detailed list of proteins). Note that an interactive electronic version of Figure 2 displaying detailed information of all proteins is provided in Supplemental Figure S1. In particular, analyses show that ABA perception by PYR/RCAR family proteins and ABA-dependent transcriptional regulation first occurred in land plants (Figure 2, Supplemental Figure S1). Therefore, one can assume that within the plant kingdom ABA signaling evolved to its current stage as a consequence of the high selective pressure exerted by the temporal absence of water, for example, in coastal areas [63]. It is tempting to speculate that ABA signaling emerged as a more flexible, energy efficient way to produce osmo- and drought-protectant solutes than the continuous high production of these components observed in small ancient desiccation-tolerant plant species [63].

Phylum-Specific ABA Biosynthesis Pathways

Two related ABA metabolism pathways have evolved in land plants and fungi (Figure 2, Supplemental Figure S1). The plastid-specific indirect pathway, which is characteristic of land plants, starts with the rate-limiting conversion of 9′-*cis* neo/violaxanthin to xanthoxin by 9′-*cis* epoxycarotenoid dioxigenases (NCED) [10]. Based on the presence of NCED homologs, 9′-*cis* neo/violaxanthin and other evidence [64], the moss *Physcomitrella patens* is currently the most ancient fully sequenced land plant containing this indirect ABA synthesis pathway. The indirect pathway probably evolved during land colonization [3,64] (Figure 2).

The direct cytosolic ABA biosynthesis pathway described for fungal pathogens (*Botryotinia fuckeliana*, *Botrytis cinerea*) [65] starts with farnesyldiphosphate, which is also an intermediate of the *cis* neo/violaxanthin biosynthesis pathway (for details see [3,10]). The near ubiquitous presence of the isoprenoid pathway [66] renders farnesyldiphosphate the core precursor of ABA biosynthesis in plants and fungi and possibly in other ABA-producing organisms (bacteria and algae [3], protozoa [2], sponges and human [1]).

The degradation of ABA is catalyzed by members of the ancient cytochrome P450 superfamily (*At*CYP707A; reviewed in [10,67]). Their role in hormone degradation and in particular the degradation of ABA emerged probably in a later phase of the land transition since the algae *Chlamydomonas reinhardtii* and the moss *Physcomitrella* do not encode orthologs of *At*CYP707A [67] (Figure 2 and Supplemental Figure S1).

ABA Perception by PYR/RCAR Family Proteins

In vascular plants, structural and molecular evidence shows that members of the PYR/RCAR family play a central role in ABA sensing (Figure 3) [16,17]. The members of this family belong to the Bet V I-like superfamiliy. Comparative analyses of different structures and sequences show that Bet V I-like proteins are present in all kingdoms of life (reviewed in [68]). Interestingly, this superfamily contains previously known hormone-binding proteins like PR10 (brassinosteroids [69]) and the cytokinin-specific binding protein CBP [70].

Overlaying PYR/RCAR protein structures with an alignment of 149 PYR/RCAR homologs used in the phylogenetic tree (Figure 3A) shows overall low conservation in the amino-terminal α1 helix of PYR/RCAR proteins (blue; <2%) and a 50% (white) or higher conservation (red; up to 100%) in the β-sheets and helices α2 and 3 (Figure 3A, center). ABA and PP2C binding residues, except for $PYR1_{I110}$ and $PYR1_{V163}$ (Figure 3C) and $PYL1_{D185}$ ($PYR1_{D155}$) (Figure 3B), are highly conserved in plant homologs [71–75]. Interestingly, key amino acids $PYR1_{K59}$ and $PYR1_{R116}$, crucial for ABA and PP2C binding [73] (Figure 3B,C), are found in most homologs. Overlaying the ABI1 structure with an alignment of homologs from 23 different plant species listed in Figure 2 shows conservation in the catalytic center. However, PYR/RCAR-interacting residues, including $ABI1_{W300}$, which is most important for the PYR/RCAR–ABA– ABI1 interaction [71,72,75], are not conserved in algae (Figure 3B).

Based on current genomic and functional data, *At*PYR/RCAR-related proteins are found only in land plants (Figure 2, Supplemental Figure S1). The earliest emergence of potential PYR/RCAR homologs can be observed at the evolutionary stage of *Marchantia polymorpha* (liverwort; *Mp*PYRL1; Figure 3, Supplemental Table S2), a species assumed to represent the basal lineage of the land plant phylum [63]. *At*PYR/RCARs and their homologs in other plant species can be grouped into three clades (I, II, III) [16] (Figure 3). In clades I–III, mono- and dicotyledonous-specific subclades are present. Clade III is subdivided into two subfamilies containing either *At*PYR1/PLY1 or *At*PYL2/3 (Figure 3A). So far, ABA binding and *in vivo* functions were described for members of all three clades (clade I: *At*PYL8/9; clade II: *At*PYL5; clade III: *At*PYR1 and *At*PYL1/2 [16,17,22,27,71–73,75]).

The Role of Protein Phosphatases

PP2Cs are the best characterized protein phosphatases involved in ABA signaling. In eukaryotes PP2Cs inhibit stress-activated protein kinase cascades by dephosphorylating MAPKs and receptor-like kinases (reviewed in [18]). In *Arabidopsis*, which contains 10 groups and a total of 76 PP2Cs, only members of group A have been implicated in ABA signaling [18]. These are ABI1, ABI2, HAB1 (P2C–HA) and PP2CA (AHG3), which have

been shown to directly interact with PYR/RCAR ABA receptors [16,17,22,27]. HAB2, AHG1 and the recently identified *highly ABA-induced* HAI1, HAI2 (AIP1) and HAI3 also belong to the PP2C group A [18,21].

The variety of PP2C targets (Figure 1) may reflect a negative regulatory role of PP2Cs at different layers of ABA signaling. Recently, *Mp*ABI1 from *Marchantia polymorpha* (liverwort), has been identified as a negative regulator of ABA signaling [76]. Liverworts do not produce seeds, lateral roots and stomatal structures found in higher plants (for details on stomata evolution see [63] and references therein). However, ABA still plays a role in stress tolerance in this organism. Thus, *Mp*ABI1 seems so far the most ancient, characterized PP2C involved in ABA signaling. It is tempting to speculate that *Mp*PYRL1/ABI1 play a similar role in ABA signaling as described for their *Arabidopsis* homologs. In the next higher lineage of land plants, i.e. in the moss *Physcomitrella*, ectopic expression of the dominant *abi1–1* resulted in ABA hyposensitive phenotypes; e.g. decreased ABA-induced freezing tolerance and enhanced osmotic stress sensitivity [77]. Similarly, disruption of PpABI1A caused ABA hypersensitive phenotypes [77]. Overall, a total of 51 PP2C genes are encoded in the *Physcomitrella* genome, a number that is 1.5 times smaller than that in *Arabidopsis* [18,77]. *Physcomitrella* lacks group B and J members [77] and the group A of ABA-regulated PP2Cs contains only two genes compared to nine genes in *Arabidopsis*, suggesting that higher land plants increased the number of group A PP2Cs during evolution.

Roles of Protein Kinases

Protein kinases that function in ABA signaling belong to the SnRK2, SnRK3 (CIPK), CDPK (reviewed in [12,41]) and MAPK families [34,78]. With the exception of MAPKs [78], CDPK, SnRK2 and SnRK3 subfamilies of the SNF-1-like clade are mainly found in plants [13,40,41] (Supplemental Figure S1).

In *Arabidopsis* the SnRK2 subfamily consists of 10 members that have been categorized into three different subclasses (I, II and III) [79]. With the exception of SnRK2.9, all SnRK2s are activated by osmotic and salt stress [80]. Only SnRK2.2/3/6/7/8 are activated by ABA [19,80,81]. Among them, SnRK2.2/3/6, members of the subclass III, exhibit the strongest ABA activation [80]. The SnRK2 subfamily is conserved in land plants and their role in ABA signaling and osmotic stress responses have also been shown for members from pea, barley, maize and rice [79,82–84]. Similarly to *Arabidopsis*, rice harbors 10 and maize harbors 11 *SnRK2* genes [79,84]. These three species each encode 3 proteins that belong to the SnRK2.2/3/6 group. The rice and maize genes group together with SnRK2.6 (OST1), while *At*SnRK2.2 and *At*SnRK2.3 form a separate subgroup [84]. SnRK2s have also been identified in the (club) mosses *Selaginella moellendorffii* and *Physcomitrella* [13]. SnRK2s in algae, including *Chlamydomonas*, were classified as distinct in their sequences from higher plant SnRK2s [13]. SnRK2s appear to be land plant specific, with subclass III being the most ancient and subclass I the most recent form [13]. The carboxy-terminal region of subclass III SnRKs is necessary for the interaction with group A PP2Cs [20]. Sequence analyses of SnRK2.2/2.6 homologs from algae reveal that these kinases lack the PP2C–binding region or that they differ significantly from their *Arabidopsis* orthologs, supporting the hypothesis that SnRK2-mediated ABA signaling evolved in land plants.

On the basis of phenotypic analyses of loss-of-function mutants and the ability of CIPKs to interact with PP2Cs, CIPK1/3/8/14/15/20/23/24 were implicated in ABA signaling [41,43]. The CIPK gene family is also found in algae but not in *Chlamydomonas*. Protozoans (unicellular eukaryotes: *Naegleria gruberi* and *Trichomonas vaginalis*) harbor single members of CIPK proteins [41]. One region in CIPKs has been identified to mediate interaction with the PP2C ABI2 and designated as the PPI motif [43]. The PPI motif seemed

to emerge in algae, as it is found in *Chlorella variabilis* but not in *Ostreococcus lucimarinus*, suggesting that the CIPK–PP2C interaction might have already existed in at least some algae.

CDPKs have been identified in land plants, algae and protozoans, such as *Plasmodium falciparum*, but not in yeast and nematodes [40] (Supplemental Figure S1). Thus, CDPKs might exist only in plants and protozoans [40]. The CDPKs that are involved in ABA signaling are CPK3/4/6/11/32 [44,45,47]. CPK4 and CPK11 are closely related genes and their function may partially overlap because both phosphorylate the transcription factors ABF1 and ABF4 [47]. CPK3/6 and CPK23 regulate Ca^{2+}-permeable channels and S-type anion channel currents [44,48]. Recently, it has been shown that ABI1 can be phosphorylated by CPK23 *in vitro* and that the presence of ABI1 stimulated CPK23 autophosphorylation [48]. These *in vitro* findings suggest that CDPKs target core ABA signaling components. Whether the CPK23–ABI1 interaction also exists *in planta* needs to be analyzed. Research in potato showed that *St*CDPK5 positively regulated ROS production by phosphorylating NADPH oxidases in response to pathogens [46]. NADPH oxidases function in ABA signal transduction [30] and are also targeted by the OST1 kinase [26].

MAPKs are abundant in all eukaryotes (Figure 2, Supplemental Figure S1). MAPKs phosphorylate a wide range of target proteins, including other kinases and/or transcription factors [78]. Diverse stimuli, including pathogens, abiotic stress, H_2O_2, ABA and phytohormones activate MAPKs (reviewed in [78]). A role for MAPKs in ABA signaling has been shown for MPK1/2/3/6/9/12 [34,78]. Interestingly, MPK6 activity is inhibited by physical interaction with the PP2C ABI1 [32]. In contrast to CIPKs, which may directly interact with the active center of ABI2 [43], MPK6 interacted with the amino terminus of ABI1 (aa 1–93) [32], suggesting a different mechanism for interaction. A negative regulation of MAPK pathways by PP2Cs is well conserved in yeast and mammals and a MAPK interaction motif in group A PP2Cs has been identified at least in HAB2 and *At*PP2CA but not in ABI1 (reviewed in [18]).

Taken together, protein kinases except SnRK2s existed before the land colonization by plants. In addition, none of these protein kinase families function specifically in ABA signaling. Even the strongly ABA-activated kinase SnRK2.6 (OST1) could be activated by osmotic stress in an ABA-independent manner [20]. Considering the related phosphorylation preferences of SnRKs and CDPKs [85], one can speculate that ABA-specific signaling may have evolved from overlapping stress signaling functionality.

Membrane Proteins

Functions in ABA signaling of transmembrane G-protein coupled receptors and associated heterotrimeric GTP-binding proteins have been found for *Arabidopsis* and animals [86,87] (Figure 1). These advances in plasma membrane-associated ABA signaling have been reviewed and presented in detail elsewhere and the reader is referred to these articles [1,11,86–88] (Figure 1 and Supplemental Figure S1; see also genes listed in Supplemental Table S1).

K^+ fluxes across cell membranes, which regulate stomatal aperture, can be mediated by voltage-dependent K^+ channels [89]. These channels share topological similarities with Shaker-type channels in *Drosophila melanogaster* and mammals [90]. The domain architecture of plant K^+ channels, however, differs from their animal counterparts. Cytosolic Ca^{2+}, PP2C phosphatases, SnRK2s and CIPKs regulate K^+ channel activity [25,91–93]. Plant shaker channels are conserved among mono- and dicots [94]. While *Physcomitrella* contains one homolog that does not belong to any of the plant Shaker channel groups [94],

this family of proteins is absent in unicellular green algae such as *Chlamydomonas* [90] (Figure 2, Supplemental Figure S1).

Voltage-dependent anion channels that mediate ABA-induced stomatal closure have been defined as slow-(S-type; SLAC) and rapid-activated (R-type; QUAC) channels [91,95]. SLAC1 functions in stomatal closure in response to diverse stimuli, including ABA, Ca^{2+}, CO_2, ROS and ozone [96,97]. SLAC1 is a distant homolog of bacterial (TehA) and fungal (Mae1) C_4-dicarboxylate transporters [96,97]. In contrast to TehA and Mae1, SLAC1 contains an amino-terminal extension that is required for the phosphorylation-dependent channel activation [24,48,98,99]. The *Arabidopsis* genome encodes four SLAC1 homologs [90,96,97]. Two of them were able to functionally complement *slac1*, suggesting functional conservation [96]. Close homologs were also identified in rice, poplar and the unicellular green algae *Chlamydomonas* [90,97] (Figure 2, Supplemental Figure S1).

Due to the reduced R-type anion channel activity in *Atalmt12* in response to malate, *At*ALMT12 (QUAC1) was suggested to represent an R-type channel [100]. *Atalmt12* exhibited reduced ABA-, Ca^{2+}-, CO_2- and dark-induced stomatal closure [100,101]. First identified in wheat [102], aluminium-activated malate transporters (ALMT) share a typical architecture consisting of the uncharacterized protein family five domain (UPF0005) and 5–7 transmembrane domains [103]. Although the UPF0005 domain is found in viruses and all kingdoms of life, the ALMT architecture is found only in land plants and not in the unicellular green algae *Chlamydomonas* [90,103] (Figure 2, Supplemental Figure S1).

NADPH oxidases (NOX), which produce extracellular ROS, are widely distributed in eukaryotic organisms [104] (Figure 2, Supplemental Figure S1). During evolution, NOX diverged to five subtypes differing in their amino-terminal extension [104]. The prototypical plant-specific subtype designated as respiratory burst oxidase homolog (Rboh) contains two amino-terminally located Ca^{2+}-binding EF hands [104]. Specific NOX subtypes in animals (NOX5 and DUOX) harbor four EF hand domains or two EF hand domains and a peroxidase-like domain [104]. Recently, the structural characterization of rice *Os*RbohB revealed a four EF-hand fold similar to calcineurin B and therefore overall a NOX5 architecture [105]. Rbohs are activated by Ca^{2+} and phosphorylation [104,106] and function in ABA-induced activation of Ca^{2+} channels and induction of stomatal closure [30]. One OST1-dependent phosphorylation site modulating RbohF activity (S174) [26] is conserved in all ten *Arabidopsis* Rbohs and in RbohF orthologs from land plants, including the moss *Physcomitrella*.

ATP-binding cassette (ABC) proteins constitute a large, diverse and ubiquitous superfamily (Figure 2, Supplemental Figure S1) with more than 120 members each in *Arabidopsis* and rice [107]. ABC proteins transport hormones, lipids, metals, secondary metabolites and xenobiotics [107]. ABA transporters belong to the G subfamily (WBC and PDR subfamilies). *At*ABCG25 exports ABA from vascular tissues [15] whereas *At*ABCG40 was reported to import ABA into guard cells [14].

Taken together, ion channels and transporters have served as a powerful platform and as rapid signaling targets to identify genes and mechanisms that mediate early rapid ABA signal transduction [7].

Transcriptional Regulators

Many ABA-induced genes contain a conserved ABRE in their promoter regions (reviewed in [108]). Transcription factors identified as being able to bind ABREs were designated as ABF/AREBs (reviewed in [108]). In *Arabidopsis* these transcriptional activators belong to the group A subfamily of bZIP transcription factors [109]. Another group A bZIP

transcription factor that was identified in a forward genetic screen for ABA-resistant germination is ABI5 [110]. The bZIP transcription factors regulate different processes in plants, including pathogen defense, light and stress signaling and are conserved in eukaryotes [109,111]. Compared to the 75 members in *Arabidopsis*, the human genome harbors 56 genes, *Drosophila* 27 and yeast 14 (reviewed in [111]). These transcription factors seem to have emerged in protozoans (unicellular eukaryotes). The protozoan *Giardia lamblia* contains just one bZIP transcription factor. However, another protozoan, *Dictyostelium discoideum*, harbors 19 members (reviewed in [111]). Our search for ABF/AREB/ABI5 orthologs in non-land plant species did not result in any identified protein with an ABF/AREB-type protein architecture (Figure 2, Supplemental Figure S1), underlining the lineage-specific evolution of bZIP proteins in plants and animals. Interestingly, we could identify ABF/AREBs in *Selaginella*, but not in *Physcomitrella* and *Marchantia polymorpha*, suggesting that ABF/AREBs evolved in mosses. ABRE *cis*-acting elements are also plant specific [111]. The high diversity of these transcription factors is reflected by sequence differences between ABF/AREB/ABI5 orthologs from *Arabidopsis* and other di- or monocotyledonous species (Figure 2, Supplemental Figure S1). However, the regulation of ABF/AREBs by SnRK2s and their function in ABA signaling is conserved in rice, wheat and barley [112–114].

APETALA2 (AP2)/ethylene responsive factor (ERF) domain transcription factors belong to a large plant-specific multi-gene family and have been divided into four subfamilies (AP2, DREB, ERF and RAV) [115]. The *AP2/ERF* gene family size radiated along with land colonization and harbors 147 members in *Arabidopsis*, compared to 13–14 in unicellular green algae (*Chlorella* sp. and *Chlamydomonas*) and 57 in the clubmoss *Selaginella* (reviewed in [115]). ABI4 is a single member of the A-3 subgroup of the DREB subfamily [115] and provides a link between ABA and sugar signaling [53,54]. Single ABI4 orthologs from maize and rice have been characterized, showing high homology to *Arabidopsis* ABI4 in the AP2 domain but high diversity outside this domain [116,117] (Figure 2, Supplemental Figure S1). *Zm*ABI4 from maize could only partially rescue the *Atabi4-1* phenotype [116].

B3 domain transcription factors control embryo maturation and the transition to dormancy and are mainly involved in hormone signaling pathways [118,119]. Five major classes of genes contain the B3 domain, including the ABI3/Viviparous1 (VP1) family [119]. The ABI3 family in *Arabidopsis* consists of ABI3, FUS3 and LEC2, and these proteins have partially overlapping roles in mid-to-late embryo development (reviewed in [118]). B3 domain transcription factors emerged in algae (*Chlamydomonas, Volvox carteri*) with a single member that is more similar to the ABI3 group [119] (Figure 2, Supplemental Figure S1). Compared to the other B3 domain transcription factor families, the ABI3 family is relatively small with five members in the moss *Physcomitrella* and rice and two in poplar [119]. The evolutionary role of ABI3 in ABA signaling is reflected by its physical interaction with ABI5 [52]. The *Physcomitrella Pp*ABI3 is capable of interacting with wheat *Hv*ABI5, although to a lesser extent than *At*ABI3, suggesting conservation of this interaction [120]. *Pp*ABI3 mediates ABA-dependent dessication tolerance in *Physcomitrella* [121] but did not fully complement the *Atabi3–6* mutant, suggesting functional diversification [120]. This is also supported by differential ABA dependencies of the transcriptional activation of reporter gene constructs by different ABI3 orthologs [51,120].

Transcription factor families involved in ABA signaling have been identified in land plants, algae and other eukaryotic kingdoms (Figure 2, Supplemental Figure S1). While CAMTAs, which can bind to ABA-responsive elements [49], also exist in animals, the ABF/AREB subfamily of bZIP transcription factors is specific for land plants (Figure 2, Supplemental Figure S1). Note that ABF/AREBs are direct targets of core ABA signaling components (Figure 1). Plant transcription factor families radiated during the evolution of higher plants

[115], suggesting a co-evolution of these transcription factors with land colonization by plants.

Perspectives

The presence of ABA biosynthesis in various prokaryotes and eukaryotes as well as its role in stress-related signaling indicates that ABA is a very ancient molecule. ABA signaling in its current form today is the result of divergent evolution originating from an ancient repertoire of proteins, chemical ABA precursors and other small signaling molecules present in early unicellular eukaryotes. Evolutionary processes as part of dynamic adaptations lead to the occupation of novel ecological niches and to the development of specific signaling mechanisms.

The PYR/RCAR–PP2C–dependent ABA signal transduction network found in *Arabidopsis* is one example of land plant specific adaption (Figures 1 and 2). In contrast to ethylene and auxin signaling components, which partially exist in algae [122,123], sequence similarities suggest that the ABA signaling network described for *Arabidopsis* is conserved only in land plants (Figures 2 and 3, Supplemental Figure S1). Genome sequences and molecular data from species adapted to special environments, such as the salt cress *Thellungiella halophila*, and from more ancient species like liverworts (*Marchantia polymorpha*) are needed to identify species-specific adaptations in an evolutionary context.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Shintaro Munemasa for critical discussions. We apologize to the authors whose relevant contributions and original articles could not be cited or discussed due to space limitations. This research was supported by NIH (GM060396-ES010337) and NSF (MCB0918220) and the Division of Chemical, Geo and Biosciences at the Office of Energy Biosciences of the Department of Energy (DE-FG02-03ER15449) grants (J.I.S.), by a SNF fellowship (to F.H.) and by a Feodor Lynen-fellowship from the Alexander von Humboldt-Foundation (to R.W.).

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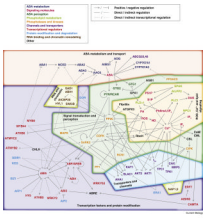

Figure 1. The ABA signaling network derived and inferred from curated literature listed in Supplemental Table S1

The network is divided into six main functional categories: ABA metabolism and transport (red); perception and signal transduction (dark green); ROS, Ca^{2+} and lipid signaling (orange); transporters and channels (blue); transcription factors and protein modification (purple); and RNA processing and chromatin remodeling (light green). ABA signaling nodes are given by their protein or molecule names and colored according to their role in ABA metabolism (dark blue); ABA perception (dark green); signaling molecules (magenta); phospholipid metabolism (light green); phosphatases and kinases (orange); channels and transporters (purple); transcriptional regulators (red); protein modification and degradation (light blue); RNA binding and chromatin remodeling (brown) and others (black). For more detailed information about the ABA signaling components please refer to the text and Supplemental Table S1 and references therein. Connections represent positive (arrow) and negative (block) regulation or currently unknown (line). Regulations are direct (bold line), indirect (faint line) or transcriptional (dashed line).

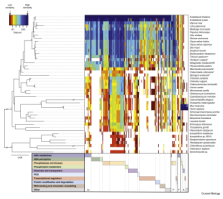

Figure 2. Similarity heatmap of proteins involved in ABA metabolism and signaling mechanisms

An interactive version of this figure displaying details of all proteins investigated is provided as Supplemental Figure S1. The color key (top left) represents the similarity to the closest match and ranges from dark red (low similarity) to blue (high similarity). White areas represent no hit below the e-value threshold (<10 E −10) applied. Note the absence or low similarity of many ABA signaling proteins at the transition from algae to land plants. The coloring in the heatmap is scaled column-wise based on bit values from protein blasts against genome (all) and nucleotide/EST $^{(+)}$ databases listed in Supplemental Table S3. Homologs of *Arabidopsis* (At), other plant (P), fungal (F) and animal (A) proteins are ordered according to their functional category as in Figure 1. Tools used for data analyses are listed in Supplemental Table S4. The displayed guiding tree (left) used for ordering the organisms was generated with 16S/18S rRNA sequences. Nodes labeled with an asterisk have bootstrap support greater than 95%. Scale bar indicates 0.09 substitutions per site.

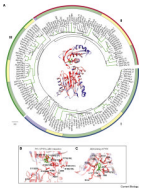

Figure 3. Conservation of land plant PYR/RCAR ABA receptors

(A, center) Structure of PYL1 (upper part) in complex with ABI1 (lower part) (PDB: 3KDJ [75]) colored according to percent conservation in an alignment of 23 ABI1 and 149 PYR/RCAR plant homologs. The color of secondary structures changes gradually from blue (amino acid conservation ≤2%) via white (~50%) to dark red (100%). The bound ABA molecule is colored green. A maximum likelihood phylogenetic tree of PYR/RCAR homologs (PYRL) in sequenced plant genomes encircles the central PYL1–ABI1 structure. The phylogenetic tree is divided into three (I, II, III) clades according to [16] with *Arabidopsis* PYR/RCARs labeled red. A putative protein from *Marchantia polymorpha* (liverwort, *Mp*PYRL1) probably representing the earliest identified homolog at the transition to land plants is labeled blue. Green, yellow and purple bars indicate PYRL proteins in dicots, monocots and lower plants, respectively. The underlying protein alignment and phylogenetic tree was evaluated using programs and web resources listed in Supplemental Tables S3 and S4. Branches with bootstrap confidence ≥95% are highlighted in green (100 bootstraps). The tree was rooted using *Mesorhizobium loti* mlr1698 as an out-group [68]. Scale bar indicates 0.2 substitutions per site. Accessions of *Arabidopsis* PYR/RCARs and their homologs are given in Supplemental Table S2. (B) Detailed view of the molecular contacts between PYL1 (upper part) and ABI1 (lower part) (PDB: 3KDJ [75]). Relevant amino acids and positions are given either for PYL1 or for PYR1 (parentheses). (C) Conservation of the PYR1 ABA binding pocket (PDB: 3K3K [73]). Coloring scheme in panel B and C is as described for panel A. Molecular graphics images were produced using tools described in Supplemental Table S4.