RESEARCH PAPER

RACK1 is a negative regulator of ABA responses in Arabidopsis

Jianjun Guo1, Junbi Wang1,2, Li Xi1, Wei-Dong Huang2, Jiansheng Liang3 and Jin-Gui Chen1,*

1 Department of Botany, University of British Columbia, 6270 University Boulevard, Vancouver, BC, V6T 1Z4 Canada
2 College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China
3 College of Bioscience and Biotechnology, Yangzhou University, Yangzhou 225009, China

Received 24 April 2009; Revised 11 June 2009; Accepted 22 June 2009

Abstract

Receptor for Activated C Kinase 1 (RACK1) is viewed as a versatile scaffold protein in mammals. The protein sequence of RACK1 is highly conserved in eukaryotes. However, the function of RACK1 in plants remains poorly understood. Accumulating evidence suggested that RACK1 may be involved in hormone responses, but the precise role of RACK1 in any hormone signalling pathway remains elusive. Molecular and genetic evidence that Arabidopsis RACK1 is a negative regulator of ABA responses is provided here. It is shown that three RACK1 genes act redundantly to regulate ABA responses in seed germination, cotyledon greening and root growth, because rack1a single and double mutants are hypersensitive to ABA in each of these processes. On the other hand, plants overexpressing RACK1A displayed ABA insensitivity. Consistent with their proposed roles in seed germination and early seedling development, all three RACK1 genes were expressed in imbibed, germinating and germinated seeds. It was found that the ABA-responsive marker genes, RD29B and RAB18, were up-regulated in rack1a mutants. Furthermore, the expression of all three RACK1 genes themselves was down-regulated by ABA. Consistent with the view that RACK1 negatively regulates ABA responses, rack1a mutants lose water significantly more slowly from the rosettes and are hypersensitive to high concentrations of NaCl during seed germination. In addition, the expression of some putative RACK1-interacting, ABA-, or abiotic stress-regulated genes was mis-regulated in rack1a rack1b double mutants in response to ABA. Taken together, these findings provide compelling evidence that RACK1 is a critical, negative regulator of ABA responses.

Key words: ABA, drought, early seedling development, RACK1, salt, seed germination.

Introduction

Receptor for Activated C Kinase 1 (RACK1) was originally identified as a receptor for activated protein kinase C (PKC) in mammalian cells (Mochly-Rosen et al., 1991; Ron et al., 1994), but now it is viewed as a multi-functional protein that plays regulatory roles in diverse signal transduction pathways (reviewed by McCahill et al., 2002; Sklan et al., 2006). The protein sequences and the structure of RACK1 are highly conserved in plants (Chen et al., 2006; Guo et al., 2007; Ullah et al., 2008). However, the research on plant RACK1 lags behind its counterparts in mammals and yeasts, and the function of plant RACK1 remains poorly understood.

The first plant RACK1 was discovered as a G-protein β subunit-like protein in tobacco BY-2 cells (Ishida et al., 1993). Subsequently, the RACK1 gene was cloned from rice (Iwasaiki et al., 1995), alfalfa (McKann et al., 1997), rape (Kwak et al., 1997), and Arabidopsis (Vahlkamp and Palme, 1997). The amino acid sequence homologues of RACK1 was found in all the plant species examined (Chen et al., 2006; Guo et al., 2007). In earlier studies, the characterization of plant RACK1 was mainly concentrated on gene expression and induction studies (Ishida et al., 1993; McKann et al., 1997; Perennes et al., 1999). Recent genetic studies using the model plant Arabidopsis revealed that
RACK1 may have multiple functions in plants (Chen et al., 2006; Guo and Chen, 2008). Like their counterparts in mammals and yeasts, plant RACK1 proteins are also associated with ribosomes (Chang et al., 2005; Giavalisco et al., 2005). Nakashima et al. (2008) demonstrated that RACK1 proteins are key regulators of innate immunity in rice. Furthermore, rice RACK1 physically interacts with multiple proteins in the Rac1 immune complex (Nakashima et al., 2008), providing evidence that the scaffolding feature of RACK1 protein may be conserved in plants.

Accumulating evidence suggested that plant RACK1 may have a role in hormone responses. For example, the first plant RACK1 gene was discovered as an auxin-induced gene in tobacco BY-2 cells (Ishida et al., 1993). In these tobacco cells, salicylic acid (SA) can block the UV irradiation-induced RACK1 expression (Perennes et al., 1999). Interestingly, in alfalfa, RACK1 was induced by cytokinin, but not by auxin (McKhann et al., 1997). In rice, ABA can induce the protein expression of RACK1 in imbibed seeds (Komatsu et al., 2005), whereas in rice cell cultures, the expression of RACK1 can also be induced by methyl jasmonate, auxin, and ABA (Nakashima et al., 2008). In Arabidopsis, loss-of-function mutations in RACK1 conferred altered sensitivities to auxin, ABA, gibberellin, and brassinolide (Chen et al., 2006). Despite these findings, the exact role of RACK1 in any hormone response has not been well characterized. Arabidopsis RACK1 is defined here as a negative regulator of ABA responses.

Materials and methods

Plant materials and growth conditions

All mutants are in the Arabidopsis Columbia (Col-0) ecotype background. The rack1a-1 and rack1a-2 single mutants have been reported previously (Chen et al., 2006). The rack1b-1, rack1b-2, rack1c-1, and rack1c-2 single mutants, as well as rack1a-1 rack1b-2, rack1a-1 rack1c-1, and rack1b-2 rack1c-1 double mutants have been reported by Guo and Chen (2008).

For simplicity, the rack1a rack1b, rack1a rack1c, and rack1b rack1c double mutant nomenclature in this report refer specifically to the rack1a-1 rack1b-2, rack1a-1 rack1c-1 and rack1b-2 rack1c-1 mutants, respectively. Unless specified elsewhere, wild-type and mutant plants were grown under identical conditions with 14/10 h photoperiod at approximately 120 μmol m⁻² s⁻¹ at 23 °C.

Generation of RACK1A over-expression lines

The whole open reading frame of RACK1A (At1g18080) was amplified by PCR from a cDNA library made from seedlings grown in light for 10 d and cloned into the pENTR/D-TOPO vector (Invitrogen, Carlsbad, CA) and then subcloned into Gateway plant transformation destination vectors pB2GW7 (Karimi et al., 2002) by an LR recombination reaction. In this construct, the expression of RACK1A was driven by the 35S promoter of cauliflower mosaic virus. The 35S::RACK1A binary vector was transformed into Col-0 by Agrobacterium-mediated transformation (Clough and Bent, 1998).

The RACK1A protein level in 35S::RACK1A transgenic lines was examined by Western blot analysis using anti-RACK1A peptide antibodies (Chang et al., 2005). Goat-anti-rabbit immunoglobulin conjugated to horseradish peroxidase (Sigma-Aldrich Canada Ltd., Oakville, Ontario, Canada) was used as a secondary antibody. The blot was developed using the SuperSignal West Pico Chemiluminescent Substrate (PIERCE Biotechnology Inc., Rockford, Illinois).

Generation of P_RACK1::GUS reporter lines

Previously, the genomic DNA 2740 bp upstream of the RACK1A start codon, 2215 bp upstream of the RACK1B start codon, and 1091 bp upstream of the RACK1C start codon were selected as putative promoter sequences for RACK1A, RACK1B, and RACK1C, respectively (Chen et al., 2006). In this study, shorter genomic DNA sequences were selected, the regions between each RACK1 gene and its nearest upstream gene. Specifically, the genomic DNA 1491 bp upstream of the RACK1A start codon, 682 bp upstream of the RACK1B start codon, and 371 bp upstream of the RACK1C start codon was amplified by PCR, respectively, and cloned into the PZP211 binary vector (Hajdukiewicz et al., 1994) upstream of the GUS gene that was ligated into the vector earlier. The binary vectors containing P_RACK1::GUS constructs were transformed into Col-0 by Agrobacterium-mediated transformation (Clough and Bent, 1998). GUS staining revealed no significant difference in expression patterns between this new set of promoter::GUS reporter lines and the lines generated earlier. These short genomic DNA sequences were also used for generating the GFP/CFP/YFP reporter lines described below. Furthermore, expression of RACK1-GFP/CFP/YFP fusion proteins under the control of these short genomic DNA sequences complemented rack1 mutants. Taken together, these results suggested that these short genomic DNA sequences probably contain most cis-acting regulatory elements that are required for the proper expression of RACK1 genes.

Generation of P_RACK1::RACK1-GFP/CFP/YFP reporter lines

The genomic DNA starting from the beginning of the promoter region (the same regions as used for the P_RACK1::GUS constructs) prior to the stop codon of each RACK1 gene was amplified by PCR and cloned into the Gateway entry vector using the pCR®8/GW/TOPO cloning kit (Invitrogen Inc.). The cloned fragments were then transferred into the Gateway compatible binary vectors pGW84, pGW84, and pGW94 (Nakagawa et al., 2007) by LR recombination reactions for constructing P_RACK1::RACK1A-GFP, P_RACK1B::RACK1B-CFP, and P_RACK1C::RACK1C-YFP, respectively. These vectors allow the fusion
of the fluorescent proteins to the C-terminal of RACK1 proteins. The binary vectors containing \( P_{RACK1}::RACK1-GFP/CFP/YFP \) constructs were transformed into Col-0, rack1a single mutant, and rack1a rack1b or rack1a rack1c double mutants by Agrobacterium-mediated transformation (Clough and Bent, 1998).

**ABA inhibition of seed germination and cotyledon greening assays**

Wild-type and mutant seeds from matched lots were surface-sterilized and sown on MS/G plates consisting of half-strength Murashige and Skoog (MS) basal medium supplemented with vitamins (Plantmedia, Dublin, Ohio), 1% (w/v) sucrose, 0.6% (w/v) phytoagar (Plantmedia), pH adjusted to 5.7 with 1 N KOH, and supplemented with different concentrations of ABA. Imbibed seeds were cold-treated at 4 °C in dark for 2 d, and then moved to 23 °C, with 14/10 h photoperiod (120 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)). Germination is defined as an obvious protrusion of the radicle through the seed coat. Green seedling is defined as the presence of two obviously green cotyledons.

**ABA inhibition of root growth assay**

For the root growth assay, sterilized seeds were sown on MS/G plates and cold-treated at 4 °C in dark for 2 d. The plates were then moved to germination conditions (23 °C, 14/10 h photoperiod at 120 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)) and placed vertically to allow the root to grow along the surface of the agar. Sixty hours later, the evenly-grown seedlings were transferred to MS/G plates supplemented with or without 5 \( \mu \text{M} \) ABA. The plates were placed under the same conditions with a vertical orientation for monitoring root growth. Seven days later, the length of the primary root was measured from each genotype.

**Water loss assay**

Water loss from the detached whole rosettes (with roots removed) of Col and rack1a single and double mutants was measured according to the method described by Tian et al. (2004) with minor modifications. Briefly, 20-d-old Col and rack1a mutants grown under short-day conditions (8/16 h photoperiod) were transferred from the growth chamber to the laboratory. The rosette from each plant was cut from its roots and weighed at different time points. The assay was performed at room temperature (~23 °C) under dim light conditions (6 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)) with 35% relative humidity. Three plants of each genotype were used and the water loss was calculated as the percentage of initial fresh weight at each time point.

**Salt stress germination assay**

Sterilized wild-type and rack1a mutant seeds were sown on MS/G plates supplemented with different concentrations of NaCl. Imbibed seeds were cold-treated at 4 °C in dark for 2 d, and then moved to 23 °C, with a 14/10 h photoperiod (120 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)). Germination is defined as an obvious protrusion of the radicle through the seed coat.

**RT-PCR and quantitative real-time RT-PCR**

For the analysis of transcripts of \( RACK1 \) genes in imbibed, germinating, and germinated seeds, the procedure of sampling has been described by Gao et al. (2007). For extracting total RNA from imbibed, germinating, and germinated seeds, hot borate RNA extraction method (Wilkins and Smart, 1996) was used. cDNA was synthesized using 1 \( \mu \text{g} \) total RNA by Oligo(dT)-primed reverse transcription using OMNIScript RT Kit (Qiagen, Mississauga, Ontario, Canada). Primers used for RT-PCR and quantitative RT-PCR are listed in Supplementary Table S1 at JXB online. The expression of \( ACTIN2 \) was used as a control.

For the ABA induction assay, 4.5-d-old light-grown seedlings of the wild type, the rack1a single mutant, and the rack1a rack1b and rack1a rack1c double mutants were used. Seedlings grown vertically on MS/G plates were moved to MS/G liquid medium without phytoagar and grown for another 2 h prior to ABA induction in an orbital shaking incubator. ABA was added at 10 \( \mu \text{M} \) (for \( RD29B \) and \( RAB18 \) induction) or 50 \( \mu \text{M} \) (for putative \( RACK1 \) interactors induction) for 2 h. Total RNA was isolated from ABA-treated and untreated whole seedlings using the RNeasy Plant Mini Kit (Qiagen). cDNA was synthesized as described above. Gene-specific primers (see Supplementary Table S1 at JXB online) for the ABA-responsive marker genes, \( RD29B \) and \( RAB18 \), and putative \( RACK1 \) interacting, ABA- or abiotic stress-regulated genes, At1g54510 (\( ATNEK1 \)), At2g35940 (\( EDA29 \)), At3g24080, At4g23570 (\( SGT1A \)), At4g27560, At5g03730 (\( CTR1 \)), At5g08415, and At5g08420 were used for quantitative RT-PCR analysis to compare the expression of these genes in the wild type and in rack1a mutants, with and without ABA treatment. The expression of \( ACTIN2 \) was used to normalize the expression of each gene. The quantitative real-time PCR was performed using the MJ MiniOpticon real-time PCR system (Bio-Rad, http://www.biorad.com) and IQ SYBR Green Supermix (Bio-Rad).

**Results**

**RACK1 genes act redundantly to negatively regulate ABA responses during seed germination and early seedling development**

The *Arabidopsis* genome contains three \( RACK1 \) genes, designated as \( RACK1A \), \( RACK1B \), and \( RACK1C \), respectively, that encode three proteins with near 90% identity at the amino acid level (Chen et al., 2006). Previously, it was shown that three Arabidopsis \( RACK1 \) genes function in an unequally redundant manner to regulate rosette leaf production and root development (Guo and Chen, 2008). Because preliminary analysis using \( rack1a \) single mutants suggested that \( RACK1A \) also mediates the hormone...
response (Chen et al., 2006), it is likely that, similar to the situation in plant development, the three Arabidopsis RACK1 genes may also function redundantly to regulate hormone responses, although this has not been tested experimentally. Therefore, in this study, the specific focus was on the characterization of the role of the RACK1 genes in ABA responses.

Loss-of-function alleles of RACK1A, rack1a-1, and rack1c-2, are hypersensitive to ABA in seed germination and early seedling development (Chen et al., 2006). Although loss-of-function alleles of RACK1B (rack1b-1 and rack1b-2) and RACK1C (rack1c-1 and rack1c-2) displayed wild-type morphology (Guo and Chen, 2008), it had remained unknown if rack1b and rack1c mutants have altered sensitivity to ABA. In order to address this question, seeds of two independent loss-of-function alleles of each RACK1 gene were sown side-by-side on MS/G plates supplemented with different concentrations of ABA. Consistent with our previous findings (Chen et al., 2006), rack1a mutants displayed ABA hypersensitivity in the seed germination assay (Fig. 1A, B). However, rack1b and rack1c mutants exhibited wild-type sensitivity to ABA (Fig. 1A, B). These results suggested that among the three RACK1 genes, RACK1A is the prominent one that regulates the ABA response in seed germination.

Because it has previously been shown that loss-of-function mutations in RACK1B or RACK1C can enhance the developmental defects observed in rack1a mutants, it was necessary to examine if the ABA hypersensitivity of rack1a mutants in seed germination can also be enhanced by rack1b or rack1c mutations. Therefore, seeds of rack1a-1 rack1b-2, rack1a-1 rack1c-1, and rack1b-2 rack1c-1 double mutants (Guo and Chen, 2008) were sown side-by-side with rack1a single mutant seeds on ABA plates. It was found that, similar to the scenario of developmental traits (Guo and Chen, 2008), rack1a-1 rack1b-2 and rack1a-1 rack1c-1 double mutants displayed much stronger ABA hypersensitivity than rack1a-1 single mutants, whereas rack1b-2 rack1c-1 double mutants exhibited wild-type sensitivity to ABA in the seed germination assay (Fig. 1C, D).

![Fig. 1. ABA sensitivity of rack1 single and double mutants in the seed germination assay.](https://academic.oup.com/jxb/article-abstract/60/13/3819/530405)

Sterilized wild-type (Col) and rack1 single (A, B) and double mutant (C, D) seeds were sown on MS/G plates supplemented with different concentrations of ABA. The percentage of seeds with radicle emergence was scored 48 h (A, C) and 96 h (B, D) after the imbibed seeds were transferred from stratification conditions (4 °C, dark for 2 d) to germination conditions (23 °C, with 14/10 h photoperiod at 120 μmol m⁻² s⁻¹). Shown are the averages of three replicates ±SE.
Next, the percentage of seedlings with green cotyledons in the presence of ABA was scored to measure and compare the impact of these mutations on the ABA responsiveness of seedlings during early development. Without ABA treatment, almost all seeds of rack1 single and double mutants, similar to the wild type, could germinate and develop into green seedlings (Fig. 2). It was found that, similar to the situation of the seed germination assay, rack1a-1 rack1b-2 and rack1a-1 rack1c-1 double mutants displayed much stronger ABA hypersensitivity than rack1a-1 single mutants whereas rack1b-2 rack1c-1 double mutants exhibited wild-type sensitivity to ABA in the ABA inhibition of cotyledon greening assay (Fig. 2). These results suggested that RACK1 genes function redundantly to regulate ABA responses in seed germination and early seedling development, and supported the view that the RACK1A gene is the prominent member of the RACK1 gene family that regulates ABA responses in Arabidopsis.

The analysis of ABA sensitivity of rack1 single and double mutants was extended to post-germination root growth. Wild-type and mutant seeds were imbibed on MS/G medium without ABA for 60 h under normal germination conditions (23 °C, 14/10 h photoperiod at 120 μmol m⁻² s⁻¹). Germinated seeds with emerged radicles were then transferred to MS/G medium supplemented with ABA. This assay allows us specifically to examine the post-germination ABA sensitivity of these mutants. Because without ABA treatment, rack1a single mutants and rack1a rack1b and rack1a rack1c double mutants had shorter primary roots, compared with the wild-type (Guo and Chen, 2008), the percentage of root elongation inhibition (ABA treatment versus non-ABA treatment) was used to reflect the difference in ABA sensitivity in different genotypes more precisely. It was found that, as in the seed germination and cotyledon greening assays, rack1a-1 single mutants were hypersensitive to ABA, rack1a-1 rack1b-2 and rack1a-1 rack1c-1 double mutants displayed enhanced ABA hypersensitivity than rack1a single mutants, and rack1b-2 rack1c-1 double mutants exhibited wild-type sensitivity to ABA in this ABA inhibition of root growth assay (Fig. 3).

Collectively, these results suggested that the three RACK1 genes act redundantly to negatively regulate ABA responses in the ABA inhibition of seed germination, cotyledon greening, and root growth.

**Over-expression of RACK1A conferred ABA hyposensitivity**

To study the role of RACK1 genes in ABA responses further, transgenic lines were generated over-expressing RACK1A (35S::RACK1A), the prominent member of the RACK1 gene family. Western blot analysis indicated that the RACK1A protein level was elevated in three independent transgenic lines, designated as RACK1A Overexpressor.
lines 2-4, 5-6, and 9-6 (AOX2-4, AOX5-6, and AOX9-6), respectively (Fig. 4A). It was found that, consistent with the view that RACK1 functions as a negative regulator of the ABA response, these independent lines displayed a significantly reduced sensitivity to ABA in the root growth assay (Fig. 4B) and in the seed germination assay (see Supplementary Fig. S1 at JXB online).

Expression of RACK1 in imbibed, germinating, and germinated seeds

Because our genetic analyses demonstrated that RACK1 genes regulate seed germination and early seedling development, it was necessary to examine whether the expression of RACK1 genes is correlated with seed germination and early seedling development. Three different assays were used to examine the expression of the RACK1 genes. First, RT-PCR was used to examine the presence of RACK1 transcript in imbibed, germinating, and germinated seeds. It was found that the transcripts of the RACK1A gene could clearly be detected during the whole process of seed germination (Fig. 5A). The transcripts of RACK1B and RACK1C appeared to be only weakly expressed in imbibed seeds under stratification conditions, but were readily detectable in germinating and germinated seeds (Fig. 5A).

In the second assay, the promoter activity of each of the three RACK1 genes was examined using the RACK1::GUS (P_{RACK1}::GUS) reporter lines. Consistent with the RT-PCR results, the promoters of the RACK1 genes were active in imbibed, germinating, and germinated seeds (Fig. 5B). The GUS staining was readily detected in the cotyledons of seeds 24 h after imbibition, and in the cotyledons and radicles of seeds 48 h after imbibition (under stratification conditions, 4 °C, dark). One day after the imbibed seeds had been transferred from stratification conditions to germination conditions (23 °C, 14/10 h photoperiod, 120 μmol m⁻² s⁻¹), RACK1 promoters were active in the protruding radicles of germinating seeds. Another day later, when radicle protrusion through seed
coats was apparent in most seeds, the GUS staining appeared to be stronger in roots (particularly, in the root apical meristem) than in shoots (Fig. 5B), consistent with a role of \( \text{RACK1} \) in root development (Guo and Chen, 2008). In the third assay, \( \text{RACK1} \) protein expression was examined during seed germination and early seedling development. Fusion proteins between \( \text{RACK1} \) and green, cyan or yellow fluorescent protein (GFP, CFP or YFP) were generated. Specifically, \( \text{RACK1A} \) promoter::\( \text{RACK1A-GFP} \) (\( \text{PRACK1A::RACK1A-GFP} \)), \( \text{RACK1B} \) promoter::\( \text{RACK1B-CFP} \) (\( \text{PRACK1B::RACK1B-CFP} \)), and \( \text{RACK1C} \) promoter::\( \text{RACK1C-YFP} \) (\( \text{PRACK1C::RACK1C-YFP} \)) lines were generated, in which the expression of the fusion proteins was driven by the native promoters of \( \text{RACK1A} \), \( \text{RACK1B} \), and \( \text{RACK1C} \), respectively. Because among \( \text{rack1} \) mutants, only \( \text{rack1a} \) single mutants (but not \( \text{rack1b} \) or \( \text{rack1c} \) single mutants) and \( \text{rack1a rack1b} \) and \( \text{rack1a rack1c} \) double mutants displayed morphological and ABA phenotypes, the functionalities of these fusion proteins were tested by transforming the related constructs into \( \text{rack1a} \) single mutants (for \( \text{PRACK1A::RACK1A-GFP} \)), \( \text{rack1a rack1b} \) (for \( \text{PRACK1B::RACK1B-CFP} \)) or \( \text{rack1a rack1c} \) (for \( \text{PRACK1C::RACK1C-YFP} \)) double mutants. In each case, it was found that the fusion proteins could function equivalently to the corresponding wild-type form of \( \text{RACK1} \) protein (data not shown). By using these reporter lines, it was found that the GFP/CFP/YFP fluorescence could be...
detected in the imbibed, germinating, and germinated seeds of \( P_{RACK1A}::RACK1A-GFP \), \( P_{RACK1B}::RACK1B-CFP \), and \( P_{RACK1C}::RACK1C-YFP \) lines (Fig. 6). Similar to the situation of \( P_{RACK1}::GUS \) reporter lines, RACK1-GFP/CFP/YFP proteins were expressed strongly in the protruding radicles of germinating seeds and the root apical meristem of germinated seeds. Taken together, the expression of \( RACK1 \) genes, the activity of the \( RACK1 \) promoter, and the expression of \( RACK1 \) proteins in imbibed, germinating, and germinated seeds are consistent with their proposed roles in seed germination and early seedling development.

ABA marker genes, RD29B and RAB18, were up-regulated in rack1a mutants

To get an insight into the role of \( RACK1 \) in ABA responses, it was necessary to investigate if \( RACK1 \) is involved in the regulation of ABA-induced gene expression in young seedlings. The expression of two well-known ABA marker genes, \( \text{RESPONSIVE TO DESSICATION29B (RD29B)} \) and \( \text{RESPONSIVE TO ABA18 (RAB18)} \) whose expressions are under direct regulation through ABA-responsive elements (ABRE) (Yamaguchi-Shinozaki and Shinozaki, 1994; Mantyla et al., 1995; Uno et al., 2000; Umezawa et al., 2006) was chosen for examination. Quantitative RT-PCR analysis revealed that without ABA induction, the transcript levels of RD29B and RAB18 were up-regulated 2–5-fold in the rack1a single mutant and rack1a rack1b and rack1a rack1c double mutants (Fig. 7). Upon ABA treatment, the transcripts of RD29B and RAB18 were dramatically increased in both wild-type and in rack1a single and double mutants (Fig. 7). Although the differences in the transcript levels of RD29B and RAB18 between wild-type and rack1a mutants with ABA treatment was not as large as those without ABA treatment, the rack1a rack1b and rack1a rack1c double mutants accumulated more RD29B and RAB18 transcripts than the wild-type in response to ABA (approximately 50% increase) (Fig. 7). These results support the view that \( RACK1 \) negatively regulates ABA responses.

The transcription of three \( RACK1 \) genes was down-regulated by ABA

Our genetic and molecular analyses demonstrated that \( RACK1 \) negatively regulates ABA responses in the ABA-mediated inhibition of seed germination, cotyledon greening and root growth, and ABA-induced gene expression. Because the expression of some negative regulators of ABA signalling, such as Rop 10 (Zheng et al., 2002), is also negatively regulated by ABA, the possibility whether the

---

**Fig. 6.** Analysis of \( RACK1 \) protein expression in imbibed, germinating and germinated seeds using \( P_{RACK1}::RACK1-GFP/CFP/YFP \) reporter lines. GFP/CFP/YFP fluorescence was examined in seeds placed under stratification conditions for 24 h and 48 h, and in seeds that had been placed in stratification conditions for 48 h and subsequently transferred to germination conditions for 24 h and 48 h, respectively.
expression of RACK1 genes themselves may be regulated by ABA was examined. Interestingly, it was found that the transcription of all three RACK1 genes was significantly down-regulated by ABA treatment in young seedlings (Fig. 8), in contrast to that in rice cell cultures or in imbibed rice seeds where RACK1A transcripts or RACK1A protein were shown to be up-regulated by ABA (Komatsu et al., 2005; Nakashima et al., 2008).

rack1 mutants display reduced water loss

Our genetic and molecular analyses suggested that RACK1 genes are negative regulators of ABA responses. Additional evidence was sought to support this conclusion. Because ABA is a critical regulator of stomatal movements (opening and closure), it was examined if rack1 mutants may display alternations in water loss from rosettes. The water loss from the detached whole rosette (with root removed) of rack1 single mutant and rack1a rack1b and rack1a rack1c double mutants was measured and compared with the wild type. It was found that the rack1a single mutant and rack1a rack1b and rack1a rack1c double mutants lost water significantly slower than the wild type (Fig. 9), whereas the RACK1A over-expressors lost water significantly faster than the wild type (see Supplementary Fig. S2 at JXB online). These results implied that rack1 mutants may have extensive stomatal closure, probably because of their hypersensitivity to ABA, although this has not been experimentally tested.
rack1 mutants display hypersensitivity to salt during seed germination

Both drought and salt stress signal transduction pathways involve osmotic homeostasis and ABA plays an important role in some of these processes (reviewed by Zhu, 2002; Xiong et al., 2002). For example, many studies have observed that mutants with altered ABA sensitivity are affected in germination on salt-containing media. Therefore, it was necessary to extend our analysis of rack1a mutant to the salt stress response. The sensitivity of the rack1a single mutant and rack1a rack1b and rack1a rack1c double mutants to different concentrations of NaCl during seed germination was examined. It was found that the rack1a single mutant and the rack1a rack1b and rack1a rack1c double mutants displayed hypersensitivity to NaCl (Fig. 10), consistent with the view that these mutants are hyper-sensitive to ABA. As expected, RACK1A over-expressors displayed hyposensitivity to NaCl (see Supplementary Fig. S3 at JXB online).

RACK1 interaction network

Our genetic and molecular characterization provided strong evidence that RACK1 regulates ABA responses. In an attempt to understand the molecular mechanism by which RACK1 regulates ABA responses, proteins that may interact with RACK1 were sought. The RACK1 interaction network was generated using the BAR Arabidopsis Interactions Viewer (Geisler-Lee et al., 2007). This tool predicts interactome of protein of interest in Arabidopsis. Only RACK1A and RACK1C are present in the BAR Arabidopsis Interactions Viewer database (http://bar.utoronto.ca/interactions/cgi-bin/arabidopsis_interactions_viewer.cgi). The database predicts 53 potential interactors for RACK1A (see Supplementary Table S2 at JXB online) and 68 potential interactors for RACK1C (see Supplementary Table S3 at JXB online). Among the 121 interactors indentified, 35 proteins interact with both RACK1A and RACK1C, whereas RACK1A has 18 unique interactors and RACK1C has 33 (Supplementary Table S2 and Supplementary Table S3 at JXB online).

To examine the possibility that RACK1 may work together with these potential RACK1 interactors regulating ABA responses, these 86 interactors identified through the BAR Arabidopsis Interactions Viewer were searched against the Genevestigator (Zimmermann et al., 2004; https://www.genevestigator.com/gv/index.jsp) and the available literatures for their potential roles in ABA or abiotic stress responses. A total of eight genes was identified from these 86 candidates. These genes are briefly summarized here. At1g54510 (ATNEK1) encodes a member of the NIMA-related serine/threonine kinases that have been linked to cell-cycle regulation in fungi and mammals, and was shown to be up-regulated by ABA (Nishimura et al., 2007). At2g35940 (EDA29, embryo sac development arrest 29) encodes a putative homeodomain transcription factor and was identified as an ABA-induced gene (Hoth et al., 2002). At3g24080 was identified as a salt-induced gene in a differential subtraction screening (Gong et al., 2001) and was shown to be up-regulated by ABA according to Genevestigator. At4g23570 (SGT1A, suppressor of G2 allele of skp1) is involved in plant disease resistance (Austin et al., 2002; Nakashima et al., 2008) and its rice orthologue physically interacts with RACK1 protein (Nakashima et al., 2008). SGT1 is slightly down-regulated by ABA according to Genevestigator. At4g27560 encodes a putative glycosyltransferase and was shown to be a salt-induced gene (Gong et al., 2001) and was shown to be up-regulated by ABA according to Genevestigator. At5g08415 encodes a putative glycosyltransferase and was shown to be a salt-induced gene (Gong et al., 2001) and was shown to be down-regulated by ABA according to Genevestigator. At5g08420 encodes an RNA-binding protein and is shown to be up-regulated by ABA according to Genevestigator.
Then, the ABA induction of these eight genes was examined in rack1a rack1b double mutant seedlings and compared with that in the wild type. Among these eight genes, the transcript levels of two genes, At1g54510 (ATNEK1) and At3g24080, were reduced in response to ABA in the rack1a rack1b mutant background, compared with the wild type (Fig. 11). The transcript levels of five genes, At2g35940 (EDA29), At4g23570 (SGT1A), At5g03730 (CTR1), At5g08415, and At5g08420 were increased in response to ABA in the rack1 rack1b mutant background (Fig. 11). One gene, At4g27560, responded to ABA similarly in the wild type and in rack1a-1 rack1b-2 mutant background (Fig. 11). The alternation of ABA responses of these genes in the rack1a rack1b mutant background implied that RACK1 may be involved in the ABA signalling route towards induction of these genes.

**Discussion**

Accumulating evidence suggested that RACK1 regulates plant development and that RACK1 may be involved in hormonal responses in plants. However, the role of RACK1 in any hormone signalling pathways has not been defined.

---

**Fig. 11.** Quantitative RT-PCR analysis of the expression of selected putative RACK1 interactors in response to ABA. The transcript levels of At1g54510 (ATNEK1), At2g35940 (EDA29), At3g24080, At4g23570 (SGT1A), At4g27560, At5g03730 (CTR1), At5g08415, and At5g08420 in wild-type (Col) and rack1a rack1b double mutants with ABA treatment (50 μM for 2 h), compared with no ABA treatment, were analysed by quantitative RT-PCR. The expression of ACTIN2 was used as control. The transcript level of each gene was normalized against that in Col without ABA treatment, with the value of the first biological replicate set as 1. Shown are the mean values of three biological replicates ± SE.
prior to this study. Six lines of evidence directly or indirectly support the conclusion that Arabidopsis RACK1s are negative regulators of ABA responses. (i) rack1a single mutants and rack1a rack1b and rack1a rack1c double mutants were hypersensitive to ABA in the ABA inhibition of seed germination, cotyledon greening, and root growth. (ii) Over-expression of RACK1A conferred ABA hyposensitivity. (iii) The expression of ABA marker genes, RD29B and RAB18, was up-regulated in the young seedlings of rack1a single mutants and rack1a rack1b and rack1a rack1c double mutants. (iv) The expression of three RACK1 genes was down-regulated by ABA. (v) rack1a single mutants and rack1a rack1b and rack1arack1c double mutants lose water from detached rosettes significantly slower than wild-type plants. (vi) rack1a single mutants and rack1a rack1b and rack1a rack1c double mutants were hypersensitive to NaCl during seed germination.

Our discovery of RACK1 as a negative regulator of ABA signalling in seed germination and early seedling development expanded the long list of negative regulators of ABA signalling. ABA INSENSITIVE 1 (ABI1) and ABI2, both protein phosphatase 2Cs (PP2Cs), are among the first negative regulators of ABA signalling identified through genetic screens (reviewed by Finkelstein et al., 2002; Himmelbach et al., 2003; Hirayama and Shinozaki, 2007; McCourt and Creelman, 2008; Wasilewska et al., 2008). Subsequently, other PP2Cs, including ABA HYPERSENSITIVE GERMINATION1 (AHG1) (Nishimura et al., 2004, 2007) and AHG3/AtpPP2CA (Nishimura et al., 2004; Yoshida et al., 2006), HYPERSENSITIVE TO ABA 1 (HAB1) (Saez et al., 2004, 2006) and HAB2 (Saez et al., 2004; Yoshida et al., 2006) have also been demonstrated as negative regulators of ABA signalling in seed germination and early seedling development (reviewed by Hirayama and Shinozaki, 2007). Many other negative regulators of ABA signalling in seed germination and early seedling development have been discovered through reverse genetics or gene expression studies (Finkelstein et al., 2002). For example, through reverse genetics, the heterotrimeric G-proteins are proposed to be negative regulators of ABA signalling in seed germination and early seedling development (reviewed by Perfus-Barbeoch et al., 2004; Assmann, 2005; Chen, 2008), because the loss-of-function alleles of Arabidopsis heterotrimeric G-protein α (Gα) and β (Gβ) subunits are hypersensitive to ABA in these processes (Ullah et al., 2002; Pandey et al., 2006). Similarly, a putative G-protein-coupled receptor (GPCR) in Arabidopsis, GCR1, is a negative regulator of ABA signalling in seed germination and early seedling development (Pandey and Assmann, 2004; Pandey et al., 2006). A small GTPase, Rop10, was also characterized as a negative regulator of ABA signalling in Arabidopsis (Zheng et al., 2002).

Genetic screens have also yielded many other critical components of ABA signalling, including ABI3, ABI4, and ABI5 (reviewed by Finkelstein et al., 2002; Himmelbach et al., 2003; McCourt and Creelman, 2008). ABI3 is a B3 domain transcription factor. ABI4 is an APETALA2 domain transcription factor. ABI5 is a basic leucine zipper transcription factor. It is believed that these transcription factors mediate ABA responses by controlling ABA-induced gene expression. Although at least three different types of proteins have been proposed as ABA receptors in the last three years, including FLOWERING TIME CONTROL PROTEIN A (FCA) (Razem et al., 2006), a nuclear RNA-binding protein, the H subunit of Mg-chelatase (CHLH) (Shen et al., 2006), a chloroplast protein, and G-Protein-Coupled Receptor 2 (GCR2), a proposed seven-transmembrane GPCR (Liu et al., 2007), as reviewed by McCourt and Creelman (2008), none of these proposed ABA receptors appeared to function as the major receptor mediating ABA signalling in seed germination and early seedling development. Furthermore, the structure and functionality of GCR2 have been challenged by other studies (Gao et al., 2007; Johnston et al., 2007; Guo et al., 2008; Illingworth et al., 2008; Risk et al., 2009). Subsequent studies also do not support the claim that FCA is an ABA receptor (Jang et al., 2008; Razem et al., 2008; Risk et al., 2008). At the cell surface, there are a few candidate ABA receptors perceiving the ABA signal. For example, a leucine-rich repeat (LRR) receptor-like kinase 1, RPK1, has been shown to function as a positive regulator of ABA signalling in seed germination, early seedling growth, stomatal closure, and ABA-induced gene expression in Arabidopsis (Osakabe et al., 2005). Recently, the Arabidopsis A4 subfamily of lectin receptor kinases, LeaRks, has been shown to function as negative regulators of the ABA response in seed germination (Xin et al., 2008). However, the ability of these candidate receptors to bind ABA has not been established. In 2009, two new types of ABA receptors have been proposed, including two novel GPCR-type G-proteins, GTG1 and GTG2 (Pandey et al., 2009), and the PYR/PYL (RCAR) family of START proteins (Ma et al., 2009; Park et al., 2009), which has led to new discussions on ABA receptors (Pennisi, 2009).

As discussed above, molecular and genetic studies have already identified a rich collection of signalling components, positive or negative regulators, involved or required in ABA signalling (reviewed by Finkelstein et al., 2002; Himmelbach et al., 2003; Hirayama and Shinozaki, 2007; McCourt and Creelman, 2008; Wasilewska et al., 2008). However, it remains elusive how these signalling components are coordinated to regulate ABA responses. The identification of RACK1 proteins, whose mammalian counterparts function as multi-functional scaffold proteins, as redundant, negative regulators of ABA responses may help to provide new insights into the complex ABA signalling networks. In a preliminary analysis, a RACK1 interaction network was generated through a bioinformatics approach (see Supplementary Table S2 and Supplementary S3 at JXB online). The transcription of a total of eight genes among those 86 candidate interactors has been shown to be regulated by ABA or abiotic stresses. It was found that ABA induction of seven of these eight ABA-regulated genes was altered in the rack1a rack1b double mutant background, implying that RACK1 may be involved in the ABA signalling route towards the induction of these genes. One of the well
characterized proteins in our list is CTR1 which has been shown to be a negative regulator of ethylene signalling (Kieber et al., 1993). CTR1 was down-regulated by ABA in the wild type whereas it was up-regulated in the rack1a rack1b double mutant background (Fig. 11). Because ethylene is considered to be a negative regulator of ABA signalling in seed germination and a positive regulator of ABA signalling in root growth (Gazzarrini and McCourt, 2001; Wang et al., 2007), it raises the possibility that RACK1 may serve as a nexus for these two hormone signalling pathways. This will be investigated further in future studies. On the other hand, the relationship between RACK1 and other known components in the ABA signalling pathway is unknown. The next major challenge is to position RACK1 precisely in the intricate ABA signal transduction network.

In summary, it is demonstrated that rack1a single and double mutants are hypersensitive to ABA in the ABA inhibition of seed germination, cotyledon greening, and root growth whereas over-expression of RACK1A confers ABA hyposensitivity. It was shown that the expression of ABA-responsive marker genes, RD29B and RAB18, are up-regulated in rack1a mutants and the RACK1 genes are down-regulated by ABA. Consistent with ABA hyposensitivity, rack1a mutants lose water significantly more slowly from the rosettes and are hypersensitive to high concentrations of NaCl during seed germination. Furthermore, the expression of some known ABA- or abiotic stress-regulated genes which encode putative RACK1 interactors was altered in the rack1a rack1b mutant background, in response to ABA. Collectively, these results have defined RACK1s as critical regulators of ABA signalling. Because RACK1 functions as a scaffold protein in mammalian cells, our work may help provide new insights into the complex ABA signalling network.

Supplementary data

Supplementary data are available at JXB online.

Supplementary Fig. S1. ABA sensitivity of RACK1A over-expression lines in the seed germination assay.

Supplementary Fig. S2. Water loss assay of RACK1A over-expression lines.

Supplementary Fig. S3. Salt stress sensitivity of RACK1A over-expression lines during seed germination.

Supplementary Table S1. Primers used in this study.

Supplementary Table S2. RACK1A-interacting proteins identified by using the BAR Arabidopsis Interactions Viewer.

Supplementary Table S3. RACK1C-interacting proteins identified by using the BAR Arabidopsis Interactions Viewer.

Acknowledgements

We thank Dr Julia Bailey-Serres (University of California, Riverside) for providing anti-RACK1A peptide antibodies.

This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada (grant No. RGPIN311651-05), the Canada Foundation for Innovation (grant No. 10496), and the National Natural Science Foundation of China (grant No. 30528023). JW and LX are supported by scholarships from the China Scholarship Council.

References

Assmann SM. 2005. G protein signalling in the regulation of Arabidopsis seed germination. Science STKE 2005, cm11.

Austin MJ, Muskett P, Kahn K, Feys BJ, Jones JDG, Parker JE. 2002. Regulatory role of SGT1 in early R gene-mediated plant defenses. Science 295, 2077–2080.

Chang IF, Szick-Miranda K, Pan S, Bailey-Serres J. 2005. Proteome characterization of evolutionarily conserved and variable proteins of Arabidopsis cytosolic ribosomes. Plant Physiology 137, 848–862.

Chen JG. 2008. Heterotrimeric G proteins in plant development. Frontiers in Bioscience 13, 3321–3333.

Chen JG, Ullah H, Temple B, Liang J, Guo J, Alonso JM, Ecker JR, Jones AM. 2006. RACK1 mediates multiple hormone responsiveness and developmental processes in Arabidopsis. Journal of Experimental Botany 57, 2697–2708.

Clough SJ, Bent AF. 1998. Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. The Plant Journal 16, 735–743.

Finkielstein RR, Gampala SS, Rock CD. 2002. Abscisic acid signalling in seeds and seedlings. The Plant Cell 14, S15–S45.

Gao Y, Zeng Q, Guo J, Cheng J, Ellis BE, Chen JG. 2007. Genetic characterization reveals no role for the reported ABA receptor, GCR2, in ABA control of seed germination and early seedling development in Arabidopsis. The Plant Journal 52, 1001–1013.

Gazzarrini S, McCourt P. 2001. Genetic interactions between ABA, ethylene and sugar signalling pathways. Current Opinion in Plant Biology 4, 387–391.

Geisler-Lee J, O’Toole N, Ammar R, Provart NJ, Millar AH, Geisler M. 2007. A predicted interactome for Arabidopsis. Plant Physiology 145, 317–329.

Giavalisco P, Wilson D, Kreitler T, Lehrach H, Klose J, Gobom J, Fucini P. 2005. High heterogeneity within the ribosomal proteins of the Arabidopsis thaliana 80S ribosome. Plant Molecular Biology 57, 577–591.

Gong Z, Koiwa H, Cushman MA, et al. 2001. Genes that are uniquely stress regulated in salt overly sensitive (sos) mutants. Plant Physiology 126, 363–375.

Guo J, Chen JG. 2008. RACK1 genes regulate plant development with unequal genetic redundancy in Arabidopsis. BMC Plant Biology 8, 108.

Guo J, Liang J, Chen JG. 2007. RACK1: a versatile scaffold protein in plants? International Journal of Plant Developmental Biology 1, 95–105.
Guo J, Zeng Q, Emami M, Ellis BE, Chen JG. 2008. The GCR2 gene family is not required for ABA control of seed germination and early seedling development in Arabidopsis. PLoS ONE 3, e2982.

Hajdukiewicz P, Svab Z, Maliga P. 1994. The small, versatile pPZP family of Agrobacterium binary vectors for plant transformation. Plant Molecular Biology 25, 989–994.

Himmelbach A, Yang Y, Grill E. 2003. Relay and control of abscisic acid signalling. Current Opinion in Plant Biology 6, 470–479.

Hirayama T, Shinozaki K. 2007. Perception and transduction of abscisic acid signals: keys to the function of the versatile plant hormone ABA. Trends in Plant Science 12, 343–351.

Hoth S, Morgante M, Sanchez JP, Hanafey MK, Tingey SV, Chua NH. 2002. Genome-wide gene expression profiling in Arabidopsis thaliana reveals new targets of abscisic acid and largely impaired gene regulation in the abi1-1 mutant. Journal of Cell Science 115, 4891–4900.

Illingworth CJ, Parkes KE, Snell CR, Mullineaux PM, Reynolds CA. 2008. Criteria for confirming sequence periodicity identified by Fourier transform analysis: application to GCR2, a candidate plant GPCR? Biophysical Chemistry 133, 28–35.

Ishida S, Takahashi Y, Nagata T. 1993. Isolation of cDNA of an auxin-regulated gene encoding a G-protein β-subunit-like protein from tobacco BY-2-cells. Proceedings of the National Academy of Sciences, USA 90, 11152–11156.

Iwasaki Y, Komano M, Ishikawa A, Sasaki T, Asahi T. 1995. Molecular cloning and characterization of cDNA for a rice protein that contains seven repetitive segments of the Trp-Asp forty-amino-acid repeat (WD-40 repeat). Plant and Cell Physiology 36, 505–510.

Jang YH, Lee JH, Kim JK. 2008. Abscisic acid does not disrupt either the Arabidopsis FCA-FY interaction or its rice counterpart in vitro. Plant and Cell Physiology 49, 1898–1901.

Johnston CA, Temple BR, Chen JG, Gao Y, Moriyama EN, Jones AM, Siderovski DP, Willard FS. 2007. Comment on 'A G protein-coupled receptor is a plasma membrane receptor for the plant hormone abscisic acid'. Science 318, 914c.

Karimi M, Inze D, Depicker A. 2002. GATEWAY vectors for Agrobacterium mediated plant transformation. Trends in Plant Science 7, 193–195.

Kieber JJ, Rothenberg M, Roman G, Feldmann KA, Ecker JR. 1993. CTR1, a negative regulator of the ethylene response pathway in Arabidopsis, encodes a member of the raf family of protein kinases. Cell 72, 427–441.

Komatsu S, Abbasi F, Kobori E, Fujisawa Y, Kato H, Iwasaki Y. 2005. Proteomic analysis of rice embryo: an approach for investigating G protein-regulated proteins. Proteomics 5, 3932–3941.

Kwang JM, Kim SA, Lee SK, Oh SA, Byoun CH, Han JK, Nam HG. 1997. Insulin-induced maturation of Xanopus oocytes is inhibited by microinjection of a Brassica napus cDNA clone with high similarity to a mammalian receptor for activated protein kinase C. Planta 201, 245–251.

Liu X, Yue Y, Li B, Nie Y, Li W, Wu WH, Ma L. 2007. A G protein-coupled receptor is a plasma membrane receptor for the plant hormone abscisic acid. Science 315, 1712–1716.

Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, Christmann A, Grill E. 2009. Regulators of PP2C phosphatase activity function as abscisic acid sensors. Science 324, 1064–1068.

Mantyla E, Lang V, Palva ET. 1995. Role of abscisic acid in drought-induced freezing tolerance, cold acclimation, and accumulation of LTI78 and RAB18 proteins in Arabidopsis thaliana. Plant Physiology 107, 141–148.

Mccahill A, Warwicker J, Bolger GB, Houslay MD, Yarwood SJ. 2002. The RACK1 scaffold protein: a dynamic cog in cell response mechanisms. Molecular Pharmacology 62, 1261–1273.

McCourt P, Creelman R. 2008. The ABA receptors: we report you decide. Current Opinion in Plant Biology 11, 474–478.

McKhanh HL, Frugier F, Petrovics G, de la Peña T, Jurkevitch E, Brown S, Kondorosi E, Kondorosi A, Crespi M. 1997. Cloning of a WD-repeat-containing gene from alfalfa (Medicago sativa): a role in hormone-mediated cell division? Plant Molecular Biology 34, 771–780.

Mochly-Rosen D, Kanher H, Lopez J. 1991. Identification of intracellular receptor proteins for activated protein kinase C. Proceedings of the National Academy of Sciences, USA 88, 3997–4000.

Nakagawa T, Kurose T, Hino T, Tanaka K, Kawamukai M, Niwa Y, Toyooka K, Matsuoka K, Jinbo T, Kimura T. 2007. Development of series of gateway binary vectors, pGWBS, for realizing efficient construction of fusion genes for plant transformation. Journal of Bioscience and Bioengineering 104, 34–41.

Nakashima A, Chen L, Thao NP, Fujiwara M, Wong HL, Kuwano M, Umemura K, Shirasu K, Kawasaki T, Shimamoto K. 2008. RACK1 functions in rice innate immunity by interacting with the Rac1 immune complex. The Plant Cell 20, 2265–2279.

Nishimura N, Yoshida T, Kitahata N, Asami T, Shinozaki K, Hirayama T. 2007. ABA-Hypersensitive Germination1 encodes a protein phosphatase 2C, an essential component of abscisic acid signalling in Arabidopsis seed. The Plant Journal 50, 935–949.

Nishimura N, Yoshida T, Murayama M, Asami T, Shinozaki K, Hirayama T. 2004. Isolation and characterization of novel mutants affecting the abscisic acid sensitivity of Arabidopsis germination and seedling growth. Plant and Cell Physiology 45, 1485–1499.

Osakabe Y, Maruyama K, Seki M, Satou M, Shinozaki K, Yamaguchi-Shinozaki K. 2005. Leucine-rich repeat receptor-like kinase1 is a key membrane-bound regulator of abscisic acid early signalling in Arabidopsis. The Plant Cell 17, 1105–1119.

Pandey S, Assmann SM. 2004. The Arabidopsis putative G protein-coupled receptor GCR1 interacts with the G protein α subunit GPA1 and regulates abscisic acid signalling. The Plant Cell 16, 1616–1632.

Pandey S, Chen JG, Jones AM, Assmann SM. 2006. G-protein complex mutants are hypersensitive to abscisic acid regulation of germination and postgermination development. Plant Physiology 141, 243–256.

Pandey S, Nelson DC, Assmann SM. 2009. Two novel GPCR-type G proteins are abscisic acid receptors in Arabidopsis. Cell 136, 136–148.

Park SY, Fung P, Nishimura N, et al. 2009. Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. Science 324, 1068–1071.
Pennisi E. 2009. Stressed out over a stress hormone. Science 324, 1012–1013.

Perennes C, Glab N, Guglieni B, Doutriaux MP, Phan TH, Planchais S, Bergounioux C. 1999. Is arcA3 a possible mediator in the signal transduction pathway during agonist cell cycle arrest by salicylic acid and UV irradiation? Journal of Cell Science 112, 1181–1190.

Perfus-Barbeoch L, Jones AM, Assmann SM. 2004. Plant heterotrimeric G protein function: insights from Arabidopsis and rice mutants. Current Opinion in Plant Biology 7, 719–731.

Razem FA, El-Kereamy A, Abrams SR, Hill RD. 2006. The RNA-binding protein FCA is an abscisic acid receptor. Nature 439, 290–294.

Razem FA, El-Kereamy A, Abrams SR, Hill RD. 2008. Retraction. The RNA-binding protein FCA is an abscisic acid receptor. Nature 456, 842.

Risk JM, Day CL, Macknight RC. 2009. Reevaluation of abscisic acid-binding assays shows that G-Protein-Coupled Receptor2 does not bind abscisic acid. Plant Physiology 150, 6–11.

Risk JM, Macknight RC, Day CL. 2008. FCA does not bind abscisic acid. Nature 456, E5–E6.

Ron D, Chen CH, Caldwell J, Jamieson L, Orr E, Mochly-Rosen D. 1994. Cloning of an intracellular receptor for protein kinase C: a homolog of the β-subunit of G-proteins. Proceedings of the National Academy of Sciences, USA 91, 839–843.

Saez A, Apostolova N, Gonzalez-Guzman M, Gonzalez-Garcia MP, Nicolas C, Lorenzo O, Rodriguez PL. 2004. Gain-of-function and loss-of-function phenotypes of the protein phosphatase 2C HAB1 reveal its role as a negative regulator of abscisic acid signalling. The Plant Journal 37, 354–369.

Saez A, Robert N, Maktabi MH, Schroeder JI, Serrano R, Rodriguez PL. 2006. Enhancement of abscisic acid sensitivity and reduction of water consumption in Arabidopsis by combined inactivation of the protein phosphatases type 2C ABI1 and HAB1. Plant Physiology 141, 1389–1399.

Shen YY, Wang XF, Wu FQ, et al. 2006. The Mg-chelatase H subunit is an abscisic acid receptor. Nature 443, 823–826.

Sklan EH, Podoly E, Soreq H. 2006. RACK1 has the nerve to act: structure meets function in the nervous system. Progress in Neurobiology 78, 117–134.

Tian L, DellaPenna D, Zeevaart JAD. 2004. Effect of hydroxylated carotenoid deficiency on ABA accumulation in Arabidopsis. Physiologia Plantarum 122, 312–340.

Ullah H, Chen JG, Wang S, Jones AM. 2002. Role of a heterotrimeric G protein in regulation of Arabidopsis seed germination. Plant Physiology 129, 897–907.