The GCR2 Gene Family Is Not Required for ABA Control of Seed Germination and Early Seedling Development in Arabidopsis

Jianjun Guo1, Qingning Zeng1,2, Mohammad Emami1, Brian E. Ellis2, Jin-Gui Chen1*

1 Department of Botany, University of British Columbia, Vancouver, British Columbia, Canada, 2 Michael Smith Laboratories, University of British Columbia, Vancouver, British Columbia, Canada

Abstract

Background: The plant hormone abscisic acid (ABA) regulates diverse processes of plant growth and development. It has recently been proposed that GCR2 functions as a G-protein-coupled receptor (GPCR) for ABA. However, the structural relationships and functionality of GCR2 have been challenged by several independent studies. A central question in this controversy is whether gcr2 mutants are insensitive to ABA, because gcr2 mutants were shown to display reduced sensitivity to ABA under one experimental condition (e.g. 22 °C, continuous white light with 150 μmol m⁻² s⁻¹) but were shown to display wild-type sensitivity under another slightly different condition (e.g. 23 °C, 14/10 hr photoperiod with 120 μmol m⁻² s⁻¹). It has been hypothesized that gcr2 appears only weakly insensitive to ABA because two other GCR2-like genes in Arabidopsis, GCL1 and GCL2, compensate for the loss of function of GCR2.

Principal Findings: In order to test this hypothesis, we isolated a putative loss-of-function allele of GCL2, and then generated all possible combinations of mutations in each member of the GCR2 gene family. We found that all double mutants, including gcr2 gcl1, gcr2 gcl2, gcl1 gcl2, as well as the gcr2 gcl1 gcl2 triple mutant displayed wild-type sensitivity to ABA in seed germination and early seedling development assays, demonstrating that the GCR2 gene family is not required for ABA responses in these processes.

Conclusion: These results provide compelling genetic evidence that GCR2 is unlikely to act as a receptor for ABA in the context of either seed germination or early seedling development.

Introduction

Abscisic acid (ABA) regulates diverse processes in plant growth and development, and is best known for its role in the inhibition of seed germination and early seedling development, and in the control of stomatal movement [1]. It has been demonstrated that the plant response to ABA requires some key signaling components, including protein kinases, protein phosphatases and transcription factors, and it has also been generally recognized that the ABA signal is perceived by multiple receptors, at the cell surface or inside the cells (recently reviewed in [2–5]). For example, FLOWERING TIME CONTROL PROTEIN A (FCA), a nuclear RNA-binding protein, has been proposed to be an ABA receptor controlling ABA-mediated RNA metabolism and flowering [6]. The H subunit of Mg-chelatase (CHLH), a chloroplast protein, has also been proposed to be an ABA receptor involved in mediating cellular responses to ABA during seed germination, post-germination growth and stomatal movement [7].

Recently, an additional receptor candidate, G-PROTEIN-COUPLED RECEPTOR 2 (GCR2), has been proposed to act as a plasma membrane receptor for ABA, controlling all the major responses mediated by ABA, including seed germination, early seedling development, stomatal movement and gene expression [8]. However, the structural relationships and functionality of GCR2 as an ABA-signaling G-protein-coupled receptor (GPCR) have been challenged by several independent studies [9–11]. While GCR2 was originally described as a classical GPCR [8], structural predictions from several robust transmembrane prediction systems subsequently showed that GCR2 was very unlikely to contain seven-transmembrane domains, a structural hallmark of any classical GPCRs. Instead, GCR2 was found to possess significant sequence and predicted structural similarity to homologs of bacterial lanthionine synthetase (LanC) in diverse species, suggesting that GCR2 may actually be a member of the LanC protein family [9,10]. Recent Fourier transform re-analysis has provided an explanation why GCR2 was erroneously predicted to be a GPCR [11]. In light of these discrepancies, it has been proposed that GCR2 may define a new type of ‘non-classical’ GPCR required for ABA perception [12].

We had previously reported that ger2 mutants do not display consistent ABA insensitivity under our experimental conditions (e.g. 23 °C, 14/10 hr photoperiod with 120 μmol m⁻² s⁻¹) [9]. However, since GCR2 is a member of small gene family, the possibility remained that ger2 mutants display little insensitivity to ABA because GCR2 shares partial functional redundancy with two
other GCR2-like genes in Arabidopsis, GCR2-LIKE 1 (GCL1) and GCR2-LIKE 2 (GCL2). In order to examine this possibility, we generated double and triple mutants between ger2, gcl1 and gcl2, and analyzed the sensitivity of these mutants to ABA in seed germination and early seedling development. Our results clearly demonstrate that none of the GCR2, GCL1 or GCL2 is required for ABA responses in seed germination or early seedling development, casting further doubt on the claim that GCR2 functions as an ABA receptor in these processes.

Results

GCR2 homologs in Arabidopsis

The Arabidopsis genome contains two genes, GCL1 (gene locus At5g65280) and GCL2 (gene locus At2g20770), that encode proteins highly similar to GCR2 (Gao et al., 2007). Both GCR2 and GCL2 contain six exons and five introns with similar size distribution (Figure S1), while GCL1 contains five exons and four introns. Similar to GCR2, GCL1 and GCL2 are expressed in dry seeds and the expression of all three genes decreases once seeds have germinated [9]. The similarities in amino acid sequence and introns. Similar to GCR2, GCL1 and GCL2 are required for seed germination and early seedling development. Our results clearly demonstrate that none of the GCR2, GCL1 or GCL2 is required for ABA responses in seed germination or early seedling development, casting further doubt on the claim that GCR2 functions as an ABA receptor in these processes.

Mutant allele of GCL2

Within the GCR2 gene family, mutant alleles for only GCR2 and GCL1 have been reported previously [8,9]. We report here the isolation and characterization of a gcl2 mutant allele. By searching the SALK Institute sequence-indexed insertion mutant collection (http://signal.salk.edu/cgi-bin/tdnaexpress) and the GABI-KAT database (http://www.gabi-kat.de/), we identified one T-DNA insertion mutant of the GCL2 gene, GABI_115D02. This allele, which possesses a T-DNA insertion within the 3rd exon of the GCL2 gene, was designated as gcl2-1 (Figure 2A, Figure S2). RT-PCR analysis indicated that full-length GCL2 transcript is absent in gcl2-1, implying that this is likely a loss-of-function allele of GCL2 (Figure 2B). The gcl2-1 mutant does not display any obvious morphological phenotypes (Figure 2C).

Double and triple mutants between gcr2, gcl1 and gcl2

Because putative loss-of-function mutant alleles are now available for each member of GCR2 gene family, we generated all possible double and triple mutants between ger2, gcl1 and gcl2 in an effort to examine possible functional redundancy of these genes. We have previously reported the characterization of ger2 gcl1 double mutants [9], and here we report the other double mutants, including ger2 gcl2 and gcl1 gcl2, as well as the ger2 gcl1 gcl2 triple mutant. We found that neither the double mutants nor the triple mutant displayed any obvious morphological phenotypes under normal growth conditions (e.g. 14/10 hr photoperiod with approximately 120 μmol m⁻² s⁻¹ at 23°C) (Figure 3).

Subsequently, we used the simple, robust ABA-inhibition of seed germination and early seedling development assays to examine the ABA sensitivity of these mutants. First, we scored the percentage of seeds displaying radicle emergence in the presence of ABA as an accepted metric for the impact of these mutations on the ABA response during seed germination. We found that all single, double and triple mutants behaved like wild-type in this assay (Figure 4A). Next, we scored the percentage of green seedlings (seedlings with green cotyledons) when grown in the presence of ABA, as a measure of the impact of these mutations on the ABA response of seedlings during early development. As in the previous assay, we found that all single, double and triple mutants behaved like wild-type (Figure 4B). Finally, we used the ABA-inhibition of primary root growth assay to establish whether these mutants display insensitivity to ABA. In this assay, stratified seeds were imbedded on MS medium without ABA for 48 hr under normal germination conditions (23°C, 14/10 hr photoperiod at 120 μmol m⁻² s⁻¹). Then, germinated seeds with emerged radicles were transferred to MS medium containing ABA.

Figure 1. RT-PCR analysis of the expression of GCR2, GCL1 and GCL2 genes in various tissues and organs of young seedlings and mature plants. PCR was performed with 38 cycles. The expression of ACTIN2 was used as a control. doi:10.1371/journal.pone.0002982.g001
This assay allows us to specifically examine post-germination ABA sensitivity of these mutants. We found that all single, double and triple mutants again behaved like wild-type in this assay (Figure 4C).

Discussion

GCR2 has been proposed to represent a plasma membrane receptor for ABA mediating all major ABA responses [8,12], but studies from our laboratory and from others have challenged this model [9,10]. In order to bring more evidence to bear on the controversy surrounding this topic, in particular, on the question of possible functional redundancy of GCR2 and GCR2-like genes in ABA signalling, we report here the examination of the impact of all possible combinations of mutations in each member of GCR2 gene family on the ABA control of seed germination and early seedling development. Our results indicate that GCR2 is unlikely to be a functional ABA receptor mediating ABA responses in seed germination and early seedling development.
The GCR2 gene family is not required for ABA responses in seed germination and early seedling development

GCR2 belongs to a small gene family in Arabidopsis that consists of GCR2 and two additional genes, GCL1 and GCL2. The latter two genes encode proteins that are highly similar to GCR2 [9]. Because the gcr2 mutant displayed only slightly reduced sensitivity to ABA in seed germination and early seedling development assays under one experimental condition (e.g. 22°C, continuous white light with 150 μmol m⁻² s⁻¹) [8] while behaving like wild-type under another slightly different condition (e.g. 23°C, 14/10 hr photoperiod with 120 μmol m⁻² s⁻¹) [9], the ABA sensitivity of gcr2 mutants has been controversial. One hypothesis was that gcr2 mutants could have weak ABA phenotypes because GCR2 functions redundantly with GCL1 and GCL2 in ABA signalling. Similar scenarios have been documented for other plant hormone receptors. For example, although TIR1 functions as an auxin receptor [13,14], it contains single mutants display weak auxin phenotypes, whereas severe auxin phenotypes are observed in double, triple and quadruple mutants of the TIR1 gene family members [15]. Therefore, if GCR2 indeed functions as an ABA receptor and is functionally redundant with other members of the GCR2 gene family, one would expect to observe an enhanced ABA insensitivity in the corresponding double and triple mutants. To test this hypothesis, we generated all possible combinations of mutations in each member of the GCR2 gene family. By using the accepted and robust ABA-mediated inhibition of seed germination and early seedling development assays, we demonstrated that none of the gcr2, gcl1 and gcl2 single, double and triple mutants displayed ABA insensitivity (Figure 4). These results support our previous findings using gcr2 and gcl1 single and double mutants [9], and provide further evidence that the GCR2 gene family is not required for ABA responses in seed germination and early seedling development.

What is the function of the GCR2 gene family?

GCR2, GCL1 and GCL2 are all highly similar at the amino acid level to LanC-like (LANCL) proteins in mammals, and they possess all the structural features that categorize them as members of the LANCL protein family [16]. Prokaryotic LanC proteins are involved in the modification and transport of peptides by acting in concert with specific dehydratases to facilitate intramolecular conjugation of cysteine to serine or threonine residues, yielding macrocyclic thioether (lanthionine) products. These products display antimicrobial activity and are known as lantibiotics (reviewed in [17,18]). However, the presence of macrocyclic lanthionine-containing polypeptides similar to the prokaryotic lantibiotic structure has not yet been documented in higher eukaryotes. Therefore, the exact biological role of LANCL proteins is unclear. The gcr2, gcl1 and gcl2 single, double and triple mutants do not display any apparent morphological phenotypes (Figure 2, Figure 3), offering little clue to the exact biological role of GCR2 gene family in Arabidopsis. This lack of a marked deficiency phenotype implies that the GCR2 gene family may not be critical for plant development, but leaves open the possibility that these mutants may only exhibit phenotypes under certain conditions (e.g. under biotic and abiotic stresses).

We took several approaches to investigate the possible functions of the GCR2 gene family. Because GCR2 has been shown to physically bind to GPA1 [8] and GCL1 and GCL2 are highly similar to GCR2 at the amino acid level [9], we first examined a possible role for these proteins in G-protein signaling. First, we examined the possibility of GCL1 and GCL2 being classical GPCRs. Structural analysis in silico using the TMHMM2.0 (http://www.cbs.dtu.dk/services/TMHMM/) transmembrane segment prediction system predicted no transmembrane domains in GCL1 and GCL2 (Figure S3), similar to the situation for GCR2 [9]. This implies that the
GCR2 protein family members unlikely function as classical GPCRs, because they do not possess seven-transmembrane domains, a structural hallmark of any classical GPCRs. Second, because Liu et al. (2007) recently argued that GCR2 may define a new type of non-structural hallmark of any classical GPCRs. Second, because they do not possess seven-transmembrane domains, a G-protein-coupled signalling pathways, one would expect to observe shared phenotypes (morphological or conditional) between G-protein subunit mutants and GCR2/GCL2 mutants. One of the characteristic morphological traits of loss-of-function alleles of the G-protein subunit, one G-protein α subunit, one G-protein β subunit, and two G-protein γ subunit. We reasoned that, if GCR2 family proteins are important contributors to G-protein-coupled signalling pathways, one would expect to observe shared phenotypes (morphological or conditional) between G-protein subunit mutants and GCR2/GCL2 mutants. One of the characteristic morphological traits of loss-of-function alleles of the G-protein subunit, one G-protein α subunit, GPA1, and the sole Arabidopsis heterotrimeric G-protein β subunit, AGBl, is round shaped rosette leaves [23–25], but none of the gcr2, gcl1 and gcl2 single, double and triple mutants displayed such morphological defects (Figure 3). Another characteristic trait of gpa1 and agb1 mutants is the short hypocotyl and partially-opened hook phenotype in etiolated seedlings [23–25]. Again, none of the gcr2, gcl1 and gcl2 single, double and triple mutants displayed such phenotypes (Figure S4). Therefore, although GCR2 has been shown to physically interact with GPA1 [8], the exact role of GCR2 (and GCL1 and GCL2) in G-protein signaling remains unclear.

We explored indirectly the possibility that gcr2, gcl1 and gcl2 might possess conditional phenotypes by examining the stress-specific expression profile of GCR2, GCL1 and GCL2 genes in the Genevestigator Arabidopsis thaliana microarray database (https://www.genevestigator.ethz.ch/) [26]. We found that the expression of GCR2, GCL1 and GCL2 can be up- or down-regulated by certain stresses, but in general, the fold-change in transcript abundance was modest (Figure S5). Very few treatments can up- or down-regulate the transcription of GCR2, GCL1 or GCL2 more than two-fold. This indirect approach provided little information about specific biotic or abiotic conditions that would warrant testing the behaviour of gcr2 mutants directly.

Finally, we directly examined the sensitivity of gcr2 mutants to other plant hormones including auxin, cytokinin, gibberellin, ethylene and brassinosteroid, because these hormones regulate almost every aspect of plant growth and development. We used the root elongation inhibition assay for measuring the sensitivity of gcr2 mutants to auxin, cytokinin and brassinosteroid, used the hypocotyl elongation promotion assay for measuring the sensitivity to gibberellin, and used the triple response assay for measuring the sensitivity to ethylene. All gcr2, gcl1 and gcl2 single, double and triple mutants behaved like wild-type in all of these assays (Figure S6, Figure S7 and Figure S8).

In summary, our results from the examination of mutants carrying all possible combinations of mutations in each member of GCR2 gene family suggest that GCR2 is not required for the ABA control of seed germination and early seedling development, and do not support the hypothesis that GCR2 functions as a receptor of ABA in these processes. Because we have yet discovered any morphological or conditional phenotypes in gcr2 mutants, the exact role of GCR2 in plants remains unknown.

Materials and Methods

Plant materials and growth conditions

All mutants are in the Arabidopsis Columbia ecotopic background (Col-0). gcr2-4 and gcl1-1 single mutants have been described previously [9]. Plants were grown in 4 × 4 cm pots containing moistened Sunshine Mix #4 (Sun Gro Horticulture Canada Ltd., www.sungro.com) under 14/10 hr photoperiod with approximately 120 μmol m⁻² s⁻¹ at 23°C, unless specified elsewhere.

Isolation of T-DNA insertion mutant of GCL2

A T-DNA insertion mutant allele of GCL2 (At2g20770), GABI_115D02, was identified from the GABI-KAT (http://www.gabi-kat.de/) database. Mutants homozygous for the insertion locus were isolated by PCR genotyping using GCL2-specific primers (5'-ATGGCGGGGTGCGTTTCTTGATAC-G'₃ and 5'-GGCT-TCACTGAACATCATTG-3'). A T-DNA left border-specific primer (5'-ATATTGACCATCATGATGTC-3'). The insertion site was confirmed by sequencing. The T-DNA was found to insert in the 3' exon of the GCL2 gene. This mutant allele was designated as g2l-1.

Generation of gcr2, gcl1 and gcl2 double and triple mutants

The gcr2-4 gcl1-1 double mutants have been described previously (Gao et al., 2007). The gcl1-1 gcl2-1 double mutant was generated by crossing gcr2-1 with gcl1-1 and isolated in the F2 progeny by PCR genotyping. The gcr2-4 gcl1-1 double mutant and gcr2-4 gcl1-1 gcl2-1 triple mutant were generated by crossing gcr2-1 single mutant with gcr2-4 gcl1-1 double mutant and isolated in the F2 progeny by PCR genotyping. All double and triple mutants were confirmed by subsequent RT-PCR analyses. For simplicity, the gcr2 gcl1, gcr2 gcl2 and gcl1 gcl2 nomenclatures in this report refer specifically to the gcr2-4 gcl1-1, gcr2-4 gcl2-1 and gcl1-1 gcl2-1 double mutants, respectively. The gcr2 gcl1 gcl2 nomenclature refers specifically to the gcr2-4 gcl1-1 gcl2-1 triple mutant.

ABA inhibition of seed germination and early seedling development assays

Wild-type and mutant seeds from matched lots were surface-sterilized with 80% ethanol for 2 min, followed by 50% bleach with 0.1% Tween-20 for 10 min, and washed with sterile, de-ionized water five times. Sterilized seeds were then directly sown on MS/G plates consisting of ½ Murashige & Skoog (MS) basal medium supplemented with vitamins (Plantmedia, www.plantmedia.com), 1% (w/v) sucrose, 0.6% (w/v) phytoagar (Plantmedia), pH adjusted to 5.7 with 1N KOH, and with or without ABA. Imibed seeds were cold-treated at 4°C in dark for 2 days, and then moved to 23°C, with 14/10 hr photoperiod (120 μmol m⁻² s⁻¹). The percentage of seed germination was scored every 12 hr, and was examined under a dissecting microscope. Germination is defined as an obvious protrusion of the radicle through the seed coat. Each experiment was conducted with three replicates.

For the ABA inhibition of early seedling development, two different assays were used. In the first assay, the percentage of seedlings with green cotyledons was scored from seeds germinated on MS/G plates containing 1.0 μM ABA for 11 days. In the second assay, the ABA inhibition of primary root growth was measured. Surface-sterilized wild-type and mutant seeds were imibed on MS/G medium and cold-treated at 4°C in dark for 2 days. Then, imibed seeds were placed under germination conditions (23°C, 14/10 hr photoperiod at 120 μmol m⁻² s⁻¹). Four-eight hours later, germinated seeds with emerged radicle were transferred to MS/G medium containing 2.0 μM ABA. The length of primary root was monitored every 24 hr. At least 10 seedlings were used for each genotype.

RT-PCR

RT-PCR was used to analyze the expression of GCR2, GCL1 and GCL2 in various tissues/organs. Total RNA was isolated from...
different parts of seedlings or mature plants using the TRIzol reagent (Invitrogen, www.invitrogen.com); cDNA was synthesized from 1 μg total RNA by oligo(dT)20-primed reverse transcription using THERMOSCRIPT RT (Invitrogen). GCR2-specific primers (5′-GGAAGATTTCGGAAGAAAGAACTGTG-3′), and 5′-CAGAGCTTGTGTTGGATCATTCATGTCG-3′, GCL1-specific primers (5′-ATGTTCACTCTGGTGTGAAGTTGCGAGA-3′, and 5′-TTATACTCCATACCAAGGA-3′), and GCL2-specific primers (5′-ATCGGAGGTGTCATCCAGAAG-3′ and 5′-TTAAAGCTGTAAGCCTGGAGAAG-3′) were used to amplify the transcript of these three genes. ACTIN2 (amplified by primers 5′-CCAGAAGGGATG-3′ and 5′-GAGGAGGCCTCGGTAGAG-3′) was used as a control in PCR reactions.

Supporting Information

**Figure S1** The gene structure of GCR2, GCL1 and GCL2. The genomic DNA sizes for GCR2, GCL1 and GCL2 are 1778 bp, 2181 bp and 1993 bp, respectively. The full-length CDs for GCR2, GCL1 and GCL2 are 1233 bp, 1302 bp and 1218 bp, respectively. The proteins encoded by GCR2, GCL1 and GCL2 are 1233 bp, 1302 bp and 1218 bp, respectively. The full-length CDs for GCR2, GCL1 and GCL2 are 2181 bp and 1993 bp, respectively. The gene structure of GCR2, GCL1 and GCL2. The transmembrane segment prediction for GCL1 (A) and GCL2 (B) was performed using the TMHMM2.0 (http://www.cbs.dtu.dk/services/TMHMM/).

**Figure S2** T-DNA insertion site in gcl2-1 mutant. Genomic DNA from mutant plants homozygous for the gcr2-1 locus was used to amplify DNA fragments in PCR reactions. GCL2 gene-specific forward or reverse primers and the T-DNA left border (LB) primer were used in PCR reactions. The T-DNA insertion site was confirmed by sequencing. In gcr2-1 allele, a tandem T-DNA with two outward facing LB was inserted in the 3rd exon of the GCL2 gene. In this allele, the T-DNA insertion resulted in the deletion of 48 bp from the 3rd exon of GCL2 gene. Lower case letters indicate 5′- or 3′-UTR regions or introns. Positions of the T-DNA left border (LB) are shown.

**Figure S3** Transmembrane domain predictions for GCL1 and GCL2. The transmembrane segment prediction for GCL1 (A) and GCL2 (B) was performed using the TMHMM2.0 (http://www.cbs.dtu.dk/services/TMHMM/).

**Figure S4** Etiolated seedlings of gcr2, gcl1 and gcl2 single, double and triple mutants. Shown are 2.5 d-old seedlings grown under darkness. Shown are 3 d-old, dark-grown seedlings in the presence of 10 μM ACC. (B) The hook region of wild-type Col (left) and gcr2 gcl1 gcl2 triple mutant (right) in the presence of 10 μM ACC.

**Figure S5** The in silico analysis of the expression of GCR2 (At1g52920), GCL1 (At5g65280), and GCL2 (At2g20770) in response to various treatments. Data were imported from Genevestigator Arabidopsis thaliana microarray database (https://www.genevestigator.ethz.ch/). Only treatments that induce > 2.0-fold change in the transcript level of GCR2, GCL1 or GCL2 are shown. Number of chips used in each treatment is indicated in parentheses. Ratios of treatment to non-treatment control are scaled with different colors.

**Figure S6** Sensitivities of gcr2, gcl1 and gcl2 single, double and triple mutants to auxin, cytokinin and brassinosteroid in the root elongation inhibition assays. Wild-type and mutant seeds were germinated on MS/G medium without hormones. Three days later, seedlings were transferred to Petri-dishes containing MS/G medium and individual hormone, and the Petri-dishes were placed vertically to monitor primary root growth. The length of primary root was measured five days later. Shown are the averages of at least 10 seedlings±S.E. (A) Sensitivities to auxin. Synthetic auxin, 1-naphthaleneacetic acid (NAA), was used at 0.3 μM. (B) Sensitivities to cytokinin. Synthetic cytokinin, 6-benzylaminopurine (6-BA), was used at 1.0 μM. (C) Sensitivities to brassinosteroid. 24-epibassinolide (24-epiBL) was used at 0.1 μM.

**Figure S7** Sensitivities of gcr2, gcl1 and gcl2 single, double and triple mutants to gibberellin in the hypocotyl elongation assay. Surface-sterilized wild-type and mutant seeds were directly sown on MS/G medium with or without 10 μM GA3 and cold-treated at 4°C in dark for 2 days. Then, imbibed seeds were transferred to growth conditions (25°C, 14/10 hr photoperiod at 120 μmol m-2 s-1). Four days later, the length of hypocotyl was measured. Shown are the averages of at least 15 seedlings±S.E.

Acknowledgments

We thank the Nottingham Arabidopsis Stock Centre and the GABI-KAT for providing the gil2-1 mutant.

**Author Contributions**

Conceived and designed the experiments: BEE JGC. Performed the experiments: JG QZ ME. Analyzed the data: JG QZ ME. Wrote the paper: BEE JGC.

References

1. Finkelstein RR, Rock CD (2002) Abscisic acid biosynthesis and response. In: Somerville CR, Meyerowitz EM, eds. The Arabidopsis Book. Rockville: American Society of Plant Biologists, doi: 10.1199/tab.0058, www.aspb.org/publications/arabidopsis/.

2. Hirayama T, Shinozaki K (2007) Perception and transduction of abscisic acid. In: Finkelstein RR, Rock CD (eds) Abscisic acid biosynthesis and response. In: Somerville CR, Meyerowitz EM, eds. The Arabidopsis Book. Rockville: American Society of Plant Biologists, doi: 10.1199/tab.0058, www.aspb.org/publications/arabidopsis/.

3. Verslues PE, Zhu JK (2007) New developments in abscisic acid perception and metabolism. Curr Opin Plant Biol 10: 447–452.

4. Wang XF, Zhang DP (2008) Abscisic acid receptors: multiple signal-perception sites. Annu Bot 101: 311–317.

5. Wasielska A, Vlad F, Sirichandra C, Redko Y, Jammes F, Valon C, Frey NF, Leung J (2008) An update on abscisic acid signaling in plants and more ... Mol Plant 1: 198–217.

6. Raem AM, El-Kereamy A, Abrams SR, Hill RD (2006) The RNA-binding protein FCA is an abscisic acid receptor. Nature 439: 290–294.

7. Shen YX, Wang XF, Wu FQ, Du SY, Cao Z, Zhang Y, Wang XL, Peng CC, Yu XG, Zhu SY, Fan RC, Xu YH, Zhang DP (2006) The Mg-chelatase H subunit is an abscisic acid receptor. Nature 443: 823–826.

8. 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.
9. 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. Plant J 52: 1001–1013.

10. Johnston CA, Temple BR, Chen JG, Gao Y, Moeykens 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.

11. 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? Biophys Chem 133: 28–35.

12. Liu X, Yue Y, Li W, Ma L (2007) Response to comment on “A G Protein-Coupled Receptor Is a Plasma Membrane Receptor for the Plant Hormone Abscisic Acid”. Science 318: 914d.

13. Dharmasiri N, Dharmasiri S, Estelle M (2005) The F-box protein TIR1 is an auxin receptor. Nature 435: 441–445.

14. Kepinski S, Leyser O (2005) The Arabidopsis F-box protein TIR1 is an auxin receptor. Nature 435: 446–451.

15. Dharmasiri N, Dharmasiri S, Weijers D, Lechner E, Yamada M, Hobbie L, Ehrismann JS, Jurgens G, Estelle M (2005) Plant development is regulated by a family of auxin receptor F-box proteins. Dev Cell 9: 109–119.

16. Chen JG, Ellis BE (2008) GCR2 is a new member of the eukaryotic lanthionine synthetase component C-like protein family. Plant Signaling Behavior 3: 307–310.

17. Sahl HG, Bierbaum G (1998) Lantibiotics: biosynthesis and biological activities of uniquely modified peptides from gram-positive bacteria. Annu Rev Microbiol 52: 41–79.

18. Chatterjee C, Paul M, Xie L, van der Donk WA (2005) Biosynthesis and mode of action of lantibiotics. Chem Rev 105: 633–684.

19. Assmann SM (2002) Heterotrimeric and unconventional GTP binding proteins in plant cell signaling. Plant Cell (Suppl) 14: S353–S373.

20. Perius-Backbosch L, Jones AM, Assmann SM (2004) Plant heterotrimeric G protein function: insights from Arabidopsis and rice mutants. Curr Opin Plant Biol 7: 719–731.

21. Jones AM, Assmann SM (2004) Plants: the latest model system for G-protein research. EMBO Rep 5: 572–578.

22. Temple BRS, Jones AM (2007) The plant heterotrimeric G-protein complex. Annu Rev Plant Biol 58: 249–266.

23. Ullah H, Chen JG, Young JC, Im KH, Sussman MR, Jones AM (2001) Modulation of cell proliferation by heterotrimeric G protein in Arabidopsis. Science 292: 2966–2969.

24. Ullah H, Chen JG, Temple B, Boyes DC, Alonso JM, Davis KR, Ecker JR, Jones AM (2003) The β-subunit of the Arabidopsis G protein negatively regulates auxin-induced cell division and affects multiple developmental processes. Plant Cell 15: 393–409.

25. Chen JG, Gao Y, Jones AM (2006) Differential roles of Arabidopsis heterotrimeric G-protein subunits in modulating cell division in roots. Plant Physiol 141: 887–897.

26. Zimmermann P, Hirsch-Hoffmann M, Hennig L, Gruissem W (2004) GENEVESTIGATOR. Arabidopsis microarray database and analysis toolbox. Plant Physiol 136: 2621–2632.