G Protein Regulation of Ion Channels and Abscisic Acid Signaling in Arabidopsis Guard Cells

Hemayet Ullah, *Howard University*
Xi-Qing Wang
Alan M Jones
Sarah M Assmann

Available at: https://works.bepress.com/hemayet-ullah/4/
**G Protein Regulation of Ion Channels and Abscisic Acid Signaling in Arabidopsis Guard Cells**

Xi-Qing Wang,1 Hemayet Ullah,2 Alan M. Jones,2 Sarah M. Assmann*1

The phytohormone abscisic acid (ABA) promotes plant water conservation by decreasing the apertures of stomatal pores in the epidermis through which water loss occurs. We found that Arabidopsis thaliana plants harboring transferred DNA insertion mutations in the sole prototypical heterotrimeric GTP-binding (G) protein α subunit gene, GPA1, lack both ABA inhibition of guard cell inward K⁺ channels and pH-independent ABA activation of anion channels. Stomatal opening in gpa1 plants is insensitive to inhibition by ABA, and the rate of water loss from gpa1 mutants is greater than that from wild-type plants. Manipulation of G protein status in guard cells may provide a mechanism for controlling plant water balance.

Heterotrimeric G proteins are key regulators of ion channels in animal cells (1, 2). Upon activation, the G protein α subunit (Go) binds GTP, resulting in separation of the α subunit from the βγ subunit pair (Gβγ). Go and Gβγ can both interact with downstream components of signaling pathways (2, 3). Among the important downstream effectors are K⁺ and Ca²⁺ channels, which are regulated by G proteins via both cytosolic signaling cascades and membrane-delimited pathways (1, 2). G protein–mediated ion-channel regulation is an integral component of vision, taste, smell, and hormonal signaling in mammalian systems (2–4).

In higher plants, guard cell ion-channel regulation controls stomatal apertures. Stomatal opening relies on increases in K⁺, Cl⁻, malate⁻, and sucrose in the guard cell symplast to drive water influx and cell swelling. These processes result in an outbowing of the guard cell pair and an increase in pore aperture. During stomatal opening, K⁺ uptake is mediated by inwardly rectifying K⁺ channels. During inhibition of stomatal opening by the plant hormone abscisic acid (ABA), these channels are inhibited (5–7). In guard cells, ABA activates phospholipases C and D (8–10) and can elevate cytosolic calcium levels via inositol 1,4,5-trisphosphate or other pathways (8, 11–14). Cytosolic Ca²⁺ elevation inhibits inwardly rectifying K⁺ channels (6, 7, 15) and activates slow anion channels that mediate Cl⁻ and malate⁻ efflux (15, 16). In mammalian systems, certain phospholipases C and D are regulated by heterotrimeric G proteins. In the Arabidopsis thaliana genome, there is only one prototypical Go gene, GPA1 (17, 18), and this gene is expressed in guard cells (Fig. 1A) (19). Thus, we hypothesized that GPA1 may regulate ion channels (20) and ABA response (21) in this cell type. We used two independent Arabidopsis lines harboring the recessive transferred DNA (T-DNA) knockout alleles gpa1-1 or gpa1-2 (22) to test these hypotheses.

Guard cells isolated from homozygous gpa1-1 and gpa1-2 plants (23, 24) failed to express full-length GPA1 transcripts (Fig. 1B), as expected (19, 22). In contrast to the response of wild-type plants, stomata of gpa1 mutant plants showed no inhibition of stomatal opening (25, 26) by ABA (Fig. 2A). We next used patch clamp techniques to test whether sensitivity of the inward K⁺ channels to inhibition by ABA had also been altered in the gpa1 mutants (27). Just as for stomatal opening, the inward K⁺ channels of the mutant plants were ABA insensitive (Fig. 2, B and C). Thus, ABA inhibition of inward K⁺ channels and stomatal opening require the presence of functional GPA1.

ABA activation of slow anion channels (28–30) is thought to be another component of ABA inhibition of stomatal opening. Anion efflux occurring upon anion channel opening should depolarize the membrane and hinder K⁺ uptake. However, the hypothesis that G proteins regulate anion channels in plants had no experimental precedence, and few mammalian anion channels are G protein regulated. Accordingly, we tested whether ABA activation of slow anion channels was altered in the gpa1 mutants. We initially used an established protocol for recording the whole-cell anion channel response to ABA, which uses a cytosolic solution with moderate Ca²⁺ and strong pH buffering capacities (29, 31). We found that GPA1 does regulate plant anion channels. In both gpa1 mutants, ABA activation of anion channels was abolished (Fig. 3, A and B).

Activation of anion channels also promotes stomatal closure by mediating loss of anionic solutes and membrane depolarization that drives K⁺ efflux (28–30, 32, 33). Thus, we expected that ABA promotion of stomatal closure (34) would also be eliminated in gpa1 mutant plants, but it was not (Fig. 3C) (35). The uncoupling of ABA inhibition of stomatal opening (Fig. 2A) and ABA promotion of stomatal closure (Fig. 3C) demonstrates that these two effects are not simply the reverse of one another (28).

Consideration of the anion channel and stomatal closure experiments led to the interpretation that a parallel or compensatory pathway mediating ABA promotion of stomatal closure was present in the intact gpa1 guard cells (Fig. 3C) but was nonfunctional under the conditions of the patch clamp experiments (Fig. 3, A and B). One candidate member of such a pathway is cytosolic pH. Cytosolic pH was strongly buffered in the protocol of Fig. 3, A and B, yet ABA elevates cytosolic pH in intact guard cells (34, 36).

---

1Biology Department, Pennsylvania State University, 208 Mueller Laboratory, University Park, PA 16802–5301, USA.
2Department of Biology, University of North Carolina, Chapel Hill, NC 27599–3280, USA.
*To whom correspondence should be addressed. E-mail: sma3@psu.edu
Therefore, we next obtained electrophysiological measurements under weak cytosolic pH buffering (31) that would permit observation of anion channel regulation by ABA-induced alterations in pH. Normal ABA activation of anion channels was observed in all genotypes (Fig. 3, D and E), consistent with the observation of ABA-induced stomatal closure in epidermes of mutant plants (Fig. 3, F).

**Fig. 2.** Mutations *gpa1-1* and *gpa1-2* cause ABA insensitivity of stomatal opening and inward K+ channel regulation. (A) Comparison of ABA (20 μM) inhibition of stomatal opening in wild-type, *gpa1-1*, and *gpa1-2*. Error bars represent ±SE from three independent trials; n = 40 apertures per trial. Only wild-type plants showed significant ABA inhibition of stomatal opening (**P < 0.01, Student’s t test**). Photographs show representative stomata from the three genotypes after ABA treatment (open bars, -ABA; hatched bars, +ABA). (B) Whole-cell recordings of guard-cell inward K+ currents in the absence or presence of 50 μM ABA. K+ currents were recorded from a holding potential of −76 mV with voltage steps from −216 to +64 mV in +20-mV increments. ABA was added immediately after achieving the whole cell configuration. Recordings were obtained after 10 min. (C) Current/voltage relations (error bars represent ±SE) showed no ABA inhibition of inward K+ currents in *gpa1-1* and *gpa1-2* guard cells versus significant ABA inhibition in wild-type guard cells at voltages ≤−156 mV (**P < 0.01, Student’s t test**). Voltage protocols were as for (B). n = 9 to 15 cells.

**Fig. 3.** Mutants *gpa1-1* and *gpa1-2* exhibit complex ABA-induced stomatal closure and ABA regulation of anion currents. (A) ABA (50 μM) activation of anion currents in *gpa1-1* and *gpa1-2* guard cells under cytosolic conditions of moderate Ca2+ buffering and strong pH buffering (31). Slow anion channel currents were recorded from a holding potential of +30 mV with voltage steps from −145 to +35 mV in +30-mV increments. Currents were identified as slow anion currents by their kinetics and reversal potential (49). (B) Current-voltage relations as recorded in (A), showing that ABA failed to activate anion channel currents in *gpa1-1* and *gpa1-2* guard cells in the presence of strong cytosolic pH buffering [10 mM Hepes-tris (pH 7.5)]. Values are ±SE; n = 8 to 16 cells. Only wild-type cells showed significant ABA stimulation of anion current (P ≤ 0.01 at voltages ≤−25 mV). (C) All genotypes showed significant stomatal closure in response to 20 μM ABA (P ≤ 0.01, Student’s t test). Error bars represent ±SE from three independent trials; n = 40 per trial, (open bars, -ABA; hatched bars, +ABA). (D) ABA activation of anion channel currents with a pipette solution identical to that for (A) and (B) except for weak cytosolic pH buffering [0.1 mM Hepes-tris (pH 7.5)] (37). Voltage protocols were as for Fig. 3A. (E) Current-voltage relations (error bars represent ±SE) showed significant (P ≤ 0.01 at voltages ≤−25 mV) ABA activation of anion channel currents in all genotypes under these conditions. n = 6 to 9 cells. (F) Stomatal closure response to ABA in the presence of an intracellular pH clamp imposed by the membrane-permeant weak acid butyrate. Changes in stomatal aperture reflect final minus initial aperture. Error bars represent ±SE from three independent trials; n = 40 per trial. Butyrate significantly reversed ABA responses in all genotypes (P ≤ 0.01). The butyrate response was significantly greater in *gpa1* lines than in wild type (P ≤ 0.01) and did not differ significantly between *gpa1-1* and *gpa1-2*. Hatched bars, ABA; solid bars, ABA + 1 mM Na-butyrate.
on inward K can be clarified by the precision afforded by GPA1-2. Thus, the pH-independent pathway of guard cell function with G protein activation (13) may contribute to efforts to engineer plants for improved water relations in planta.

Previous pharmacological studies of guard cell function with G protein activators such as guanosine 5′-O-(3′-thiophosphate) (GTP-γ-S), and inactivators such as guanosine 5′-O-(2′-thiodiphosphate) (GDP-β-S) (18), suggested that active G proteins inactivate the inward K+ channels via both cytosolic and membrane-delimited pathways to inhibit stomatal opening (21, 39–41). Such data were challenged, however, by other pharmacological studies suggesting that G protein activation could stimulate stomatal opening (42) or had both stimulatory and inhibitory effects on inward K+ currents (43). These results can be clarified by the precision afforded by T-DNA mutagenesis (44), in which one specific protein is eliminated with retention of an otherwise wild-type genetic complement. Reverse genetics also has allowed us to directly test and support the hypothesis that ABA signaling in guard cells uses G protein activation (21). There is evidence that plant G proteins are involved in responses to light (45), pathogens (46), and several hormones (18, 47), including ABA as shown here. It will be of interest to unravel how plant G protein pathways can couple receptors with their cognate downstream effectors for such diverse and multiple signals, given that the Arabidopsis genome contains only GPA1 as a prototypical Gα subunit gene.

References and Notes
21. We used 4- to 6-week-old plants grown under short days. Seeds were germinated in a standard medium (25) in 7.5% (v/v) phytagel (Sigma), with kanamycin (50 mg/liter) for mutants. After 10 days, robust seedlings were transferred to Metromix 250 potting mixture (Scotts, Marysville, OH).
23. Light-induced stomatal opening was assayed according to (25). Normalization of aperture widths by age of initial fresh weight (Fig. 4) was performed using a 2-week-old wild-type curve (Scotts, Marysville, OH). Analysis confirmed that the wild-type curve differs significantly from the gpa1-1 and gpa1-2 responses at F = 0.01. Circles, wild type; squares gpa1-1; triangles, gpa1-2.

R E P O R T S

Fig. 4. Water loss is greater from gpa1 mutant leaves. Water loss is expressed as the percentage of initial fresh weight (37). Values are mean ± SE (error bars) of measurements with three individual plants per genotype. One of three independent trials is shown. Regression analysis confirmed that the wild-type curve differs significantly from the gpa1-1 and gpa1-2 responses at F = 0.01. Circles, wild type; squares gpa1-1; triangles, gpa1-2.