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 targeted 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 (Gα) binds GTP, resulting in separation of the α subunit from the βγ subunit pair (Gβγ). Gα 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⁻, malate²⁻, 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 ion channels that mediate Cl⁻ and malate²⁻ 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 Gα 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 inhibit 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).

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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

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**Fig. 1.** RT-PCR analysis of *GPA1* expression in guard cells of wild-type and *gpa1* mutant lines. (A) *GPA1* is more highly expressed in guard cell protoplasts (GCP) than in mesophyll cell protoplasts (MCP) of wild-type *Arabidopsis*. Different cycle numbers were performed as indicated to assess *GPA1* expression levels. Expected band sizes of 605 bp (*GPA1*) and 1 kb (Actin) were observed; the identity of *GPA1* products was confirmed by sequencing. (B) *GPA1* is expressed in wild-type guard cells and not in the guard cells of *gpa1* knockout mutants, as assessed by RT-PCR. *GPA1* primers (lanes 2, 4, and 6) bracketed the T-DNA insertion site; thus, no PCR product was expected from the *gpa1* lines. Actin bands indicate relative cDNA amounts.
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. 2).
Stomatal apertures in leaves taken directly from the growth environment (24) were ~20% greater in gpa1 mutant lines than in wild-type plants, suggesting that stomatal sensitivity to background levels of endogenous ABA is shifted in the gpa1 mutants. Water loss (37, 38) is greater from gpa1 leaves than from wild-type leaves (Fig. 4), confirming that elimination of GPA1 impacts water relations in planta. This information may contribute to efforts to engineer plants with improved stomatal regulation.

Previous pharmacological studies of guard cell function with G protein activators such as guanosine 5′-O-(3′-thiodiphosphate) (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
19. RNA was extracted from guard cell (purity ageat of 99.1%, guard cell protoplasts (mesophyll cell protoplasts) and mesophyll cell protoplast prepa-
20. Light-induced stomatal opening was assayed according to (29), except that after ABA application (20 μM), leaves were incubated for 3 hours before aperture measurement. For Fig. 3F, we assayed stomatal closure with the protocol of (34), although with minor modification. Leaves were incubated for 2 hours under 450 μmol m−2 s−1 of light, followed by the addition of 1 mM Na-butyrate and 20 μM ABA. Apertures were measured 2 hours later. The wild-type butyrate response [18] was saturated with 1 mM Na-butyrate.
23. On the basis of (37), 5 to 6 young leaves were excised from an individual plant, and their total fresh weight was measured during incubation in a chamber through which dry air was passed.
33. X.-Q. Wang, S. M. Assmann, unpublished observations.
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