26. Komarova, Y. A., Akhmanova, A. S., Kojima, S., Galjart, N. & Borisy, G. G. Cytoplasmic linker proteins promote microtubule rescue *in vivo*. *J. Cell Biol.* **159,** 589–599 (2002).
27. Kirschner, M. & Mitchison, T. Beyond self-assembly: from microtubules to morphogenesis. *Cell* **45,** 329–342 (1986).
28. Moreno, S., Klar, A. & Nurse, P. Molecular analysis of the fission yeast *Schizosaccharomyces pombe*. *Methods Enzymol.* **194,** 795–823 (1991).
29. Bahler, J. *et al.* Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe*. *Yeast* **14,** 943–951 (1998).
30. Woods, A. *et al.* Definition of individual components within the cytoskeleton of *Trypanosoma brucei* by a library of monoclonal antibodies. *J. Cell Sci.* **93,** 491–500 (1989).

Supplementary Information accompanies the paper on **www.nature.com/nature**.

Acknowledgements We thank members of the Wellcome Trust Centre for Cell Biology for discussions, and R. Allshire, I. Davis, W. Earnshaw and H. Ohkura for critical reading of the manuscript. This work was supported by a Wellcome Trust Senior Research Fellowship to K.E.S. H.A.S. is currently a Caledonian Research Foundation Post-doctoral Fellow.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to K.E.S. (ken.sawin@ed.ac.uk).

Sphingolipid signalling in *Arabidopsis* guard cells involves heterotrimeric G proteins

Sylvie Coursol*†‡, Liu-Min Fan*†, Hervé Le Stunff‡§, Sarah Spiegel§, Simon Gilroy* & Sarah M. Assmann*

* *Department of Biology, Pennsylvania State University, 208 Mueller Laboratory, University Park, Pennsylvania 16802-5301, USA*
§ *Department of Biochemistry, Medical College of Virginia, Virginia Commonwealth University, 1101 East Marshall Street, Sanger Hall, Richmond, Virginia 2398-0614, USA*
† *These authors contributed equally to this work*

In animals, the sphingolipid metabolite sphingosine-1-phosphate (S1P) functions as both an intracellular messenger and an extracellular ligand for G-protein-coupled receptors of the S1P receptor family, regulating diverse biological processes ranging from cell proliferation to apoptosis[1–3]. Recently, it was discovered in plants that S1P is a signalling molecule involved in abscisic acid (ABA) regulation of guard cell turgor[4]. Here we report that the enzyme responsible for S1P production, sphingosine kinase (SphK), is activated by ABA in *Arabidopsis thaliana*, and is involved in both ABA inhibition of stomatal opening and promotion of stomatal closure. Consistent with this observation, inhibition of SphK attenuates ABA regulation of guard cell inward K^+ channels and slow anion channels, which are involved in the regulation of stomatal pore size. Surprisingly, S1P regulates stomatal apertures and guard cell ion channel activities in wild-type plants, but not in knockout lines of the sole prototypical heterotrimeric G-protein α-subunit gene, *GPA1* (refs 5–8). Our results implicate heterotrimeric G proteins as downstream elements in the S1P signalling pathway that mediates ABA regulation of stomatal function, and suggest that the interplay between S1P and heterotrimeric G proteins represents an evolutionarily conserved signalling mechanism.

In higher plants, stomata form pores on leaf surfaces that regulate both uptake of CO_2 for photosynthesis and loss of water vapour during transpiration. The plant hormone ABA regulates stomatal pore size by mediating turgor changes in the guard cell pair surrounding the pore. These changes involve alterations in ion channel activities, which represent the final effectors of these processes[9–11]. Recently, S1P was shown to stimulate stomatal closure and Ca^{2+} mobilization in *Commelina communis* guard cells[4]. In addition, ABA promotion of stomatal closure in this species was partially attenuated by DL-*threo*-dihydrosphingosine (DL-*threo*-DHS)[4], a competitive inhibitor of mammalian SphKs[12]. Nevertheless, little is known about the cellular mechanisms that regulate S1P action in plant cells[13,14].

We used DL-*threo*-DHS and *N,N*-dimethylsphingosine (DMS), another potent inhibitor of mammalian SphKs[12], to investigate whether SphK might be involved in ABA-mediated changes in stomatal apertures in *A. thaliana*. DL-*threo*-DHS or DMS alone did not affect stomatal apertures (Fig. 1a, d). However, in epidermal peels that had been pre-treated with DL-*threo*-DHS or DMS, the effect of ABA on both stomatal opening and stomatal closure was significantly attenuated (Fig. 1a, d). Because inhibition of stomatal opening by ABA involves the inhibition of inwardly rectifying K^+ channels[10,11], we next assessed the effect of DMS on inward K^+ currents in *A. thaliana* guard cell protoplasts (GCPs)[6,15]. ABA inhibition of inward K^+ currents was abolished by pre-treating GCPs with DMS (Fig. 1b, c). Because ABA also promotes stomatal closure by activating slow anion channels[6,11,16], we next tested whether the effect of ABA on slow anion channels was altered by DMS. ABA activation of slow anion channel activity was partially inhibited by pre-treating GCPs with DMS (Fig. 1e, f). Together, these results suggest that ABA regulation of stomatal apertures and guard cell ion channel activities is transduced, at least in part, through SphK.

SphK activity has yet to be demonstrated in plants, although this enzyme has been characterized in animals[1] and yeast[17]. A related kinase activity able to phosphorylate D-*erythro*-dihydrosphingosine (D-*erythro*-DHS), which is structurally similar to D-*erythro*-sphingosine (Sph) except for the absence of the *trans*-4,5 double bond, has been reported in *Zea mays*[18]. A recombinant long-chain sphingoid base kinase (AtLCBK1) from *A. thaliana* was also shown to phosphorylate D-*erythro*-DHS[19]. Here, we show by direct assays of sphingoid base phosphorylation[20] that SphK activity is present in lysates prepared from leaves, mesophyll cell protoplasts (MCPs), or GCPs of *A. thaliana* (Fig. 2a). We found that Sph, a low abundance, naturally occurring sphingoid base isomer in *A. thaliana* leaves[21], was the best substrate among various exogenous sphingoid bases tested (Fig. 2b). Of the known inhibitors of mammalian SphKs, DL-*threo*-DHS was a substrate for *A. thaliana* SphK, whereas DMS was not (Fig. 2b). Furthermore, we found that DMS inhibited the activity of *A. thaliana* SphK in a concentration-dependent manner (Fig. 2c). Although a higher concentration of DMS was required for inhibition *in vitro*, such differences between the *in vitro* and *in vivo* concentrations are similar to those reported for other inhibitors used. For example, the phospholipase C (PLC) inhibitor U-73122 required an 80-fold higher concentration than was effective *in vivo* to inhibit guard cell PLC activity *in vitro*[22]. These results, together with those from Fig. 1, support the involvement of SphK in the regulation of guard cell responses to ABA.

We next tested the ability of ABA to modulate S1P levels *in vivo* by measuring the formation of [^{3}H]S1P from [^{3}H]Sph. Treatment of GCPs and MCPs with ABA resulted in a rapid and transient increase in [^{3}H]S1P formation, reaching a maximum after 2 min of ABA treatment (Fig. 2d, e). As expected, pre-treatment of MCPs with DMS blocked the ABA-induced increases in [^{3}H]S1P formation (data not shown). We next assayed SphK activity *in vitro* from protein extracts of MCPs that had been treated with ABA for various times *in vivo*. We found that stimulation of MCPs with ABA rapidly and transiently increased SphK activity (Fig. 2f), with a time course

‡ Present address: Station de Génétique Végétale, UMR 320 INRA/8120 CNRS/Université Paris-Sud/INA-PG, Ferme du Moulon, 91190 Gif-sur-Yvette, France (S.C.); and Laboratoire d'Activation Cellulaire et Transduction des Signaux, Institut de Biochimie et de Biophysique Moléculaire et Cellulaire, UMR 8619 CNRS, Université Paris-Sud, 91405 Orsay Cedex, France (H.L.S.).

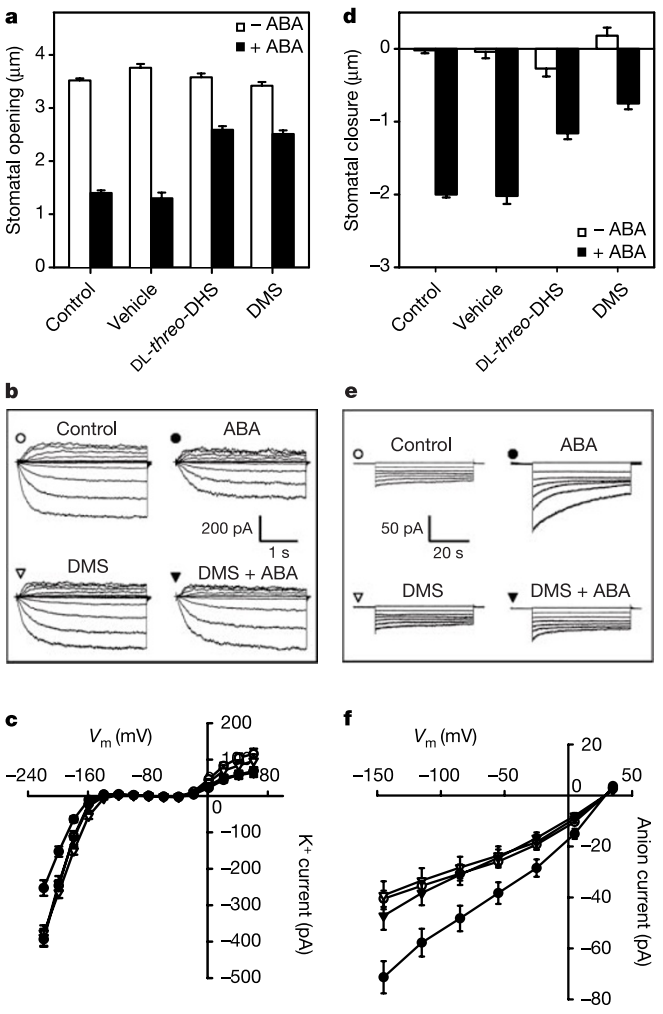

Figure 1 Effect of SphK inhibitors on ABA regulation of stomatal apertures and guard cell ion channel activities. **a**, Apertures after a 2.5 h pre-treatment in the dark in 20 μM DL-*threo*-DHS or 5 μM DMS (vehicle, dimethylsulphoxide; final concentration, ≤0.08%), and a further 2 h light treatment with or without 20 μM ABA. **b**, Representative whole-cell recordings of K^+ currents from wild-type GCPs pre-treated with 5 μM DMS for 30 min, and subsequently treated for 1.5 h with or without 50 μM ABA; 50 μM ABA was also included in bath solution. **c**, Current–voltage curves of time-activated whole-cell K^+ currents in wild-type GCPs; $n = 31$, 9, 21 and 16 cells for control, DMS, ABA and DMS + ABA treatments, respectively. **d**, Apertures after a 2.5 h light pre-treatment in 20 μM DL-*threo*-DHS or 5 μM DMS, and a further 3 h light treatment with or without 20 μM ABA. **e**, Representative whole-cell recordings of slow anion currents from wild-type GCPs treated as in **b**, except that 50 μM ABA was also included in the pipette solution[16] in ABA and DMS + ABA treatments. **f**, Current–voltage curves of steady-state whole-cell anion currents in wild-type GCPs; $n = 31$, 13, 24 and 14 cells for control, DMS, ABA and DMS + ABA treatments, respectively.

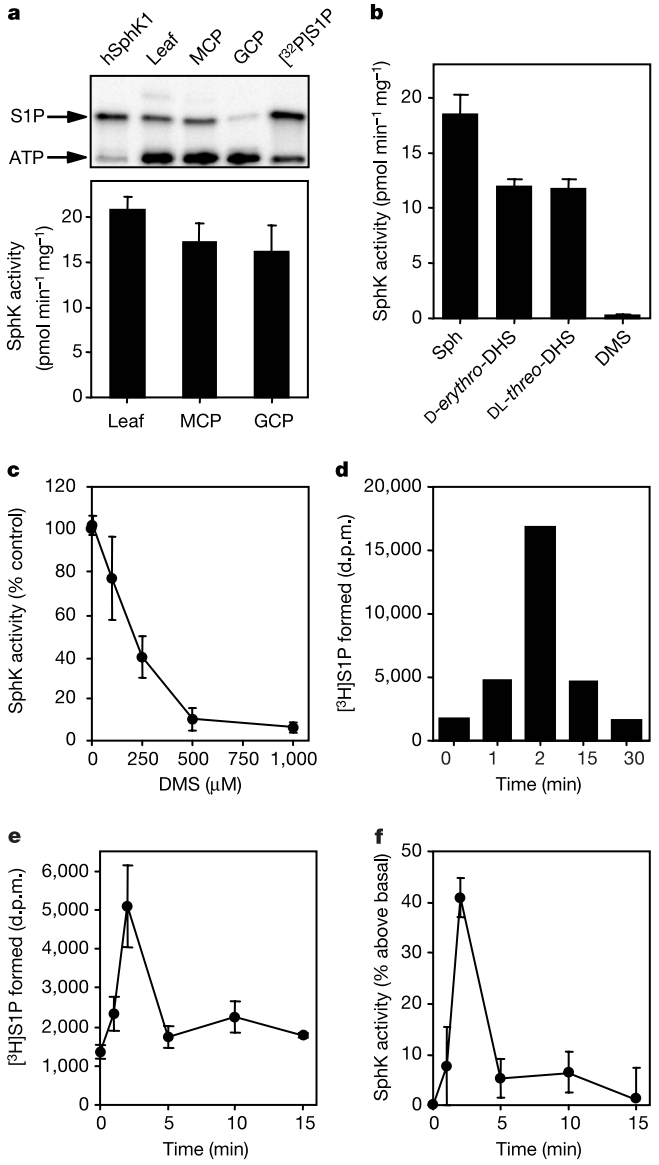

Figure 2 Effect of ABA on SphK activity. **a**, Thin layer chromatography (TLC) analysis of [^{32}P]S1P formed by lysates from leaves (25 μg), MCPs (25 μg), GCPs (3 μg) and recombinant human SphK1 (hSphK1) with 50 μM Sph as substrate (top panel). Without Sph added in the *in vitro* assay, no S1P was formed. The bottom panel shows the specific activity of SphK. **b**, Phosphorylation of various sphingoid bases (50 μM) by leaf lysate. **c**, Effect of various concentrations of DMS on SphK activity in leaf lysate with 5 μM Sph as substrate. **d**, Stimulation of [^{3}H]S1P formation *in vivo* by 50 μM ABA in GCPs. The figure shows data representative of one of three separate experiments. **e**, Stimulation of [^{3}H]S1P formation *in vivo* by 50 μM ABA in MCPs. Values are the mean ± s.e.m. from four independent experiments and data were compared using the Student's *t*-test at the 95% confidence level. **f**, SphK activity assayed *in vitro* from protein extracts of MCPs that had been treated with 50 μM ABA for various time *in vivo*.

similar to that of ABA-increased [^{3}H]S1P formation *in vivo* (Fig. 2d, e). Collectively, these results provide evidence that ABA increases the activity of SphK, leading to the production of S1P in plants. To investigate further the nature of the interaction between ABA and SphK, we assayed SphK stimulation by ABA in cell-free systems derived from leaves, MCPs or GCPs. We found that ABA increased the phosphorylation of both exogenously supplied Sph and an endogenous long-chain sphingoid base (see Supplementary Information). These results, together with those of Fig. 2, suggest that the ABA receptor, signal transduction system[9–11] and ABA-regulated SphK activity might exist in a tightly associated complex that survives the extraction process required for the *in vitro* assay.

In animal cells, an extracellular action of S1P by way of heterotrimeric G-protein-coupled receptors (GPCRs) for S1P is well established[1–3]. Recently, we used two independent *A. thaliana* transferred DNA (T-DNA) knockout lines, *gpa1-1* and *gpa1-2* (ref. 5), to provide evidence that GPA1 is involved in ABA signalling in *A. thaliana* guard cells[6]. Therefore, we examined whether S1P signals in guard cells can be transduced through GPA1, which in

turn regulates K^+ and slow anion channel activities and stomatal apertures[6]. We found that basal levels of SphK activity were similar in leaf and GCP lysates of wild-type and *gpa1* null mutant plants (data not shown). In wild-type plants, S1P inhibited the opening of closed stomata to a similar extent as ABA (Fig. 3a). In addition, S1P promoted the closure of opened stomata (Fig. 3d), in agreement with previous observations in *C. communis* guard cells[4]. By contrast, in *gpa1* null mutants, S1P neither inhibited stomatal opening nor promoted stomatal closure (Fig. 3a, d), although stomatal closure (Fig. 3d) in these genotypes occurs normally in response to ABA[6]. These results, together with those of Fig. 1, suggest that GPA1 functions downstream of SphK and S1P in ABA signal transduction pathways that lead to changes in stomatal apertures in *A. thaliana*. Studies using animal cells have shown that D-*erythro*-dihydro-sphingosine-1-phosphate (dihydro-S1P), which is structurally similar to S1P except for the absence of the *trans*-4,5 double bond, is a ligand for S1P receptors and activates the same downstream responses as S1P[23]. However, in contrast to S1P, dihydro-S1P neither promoted stomatal closure (Fig. 3d), as previously reported for *C. communis* guard cells[4], nor inhibited stomatal opening (Fig. 3a) in wild-type and *gpa1* null mutant plants.

We next tested the ability of S1P to modulate guard cell ion channel activities in wild-type and *gpa1* null mutant plants. In mammalian cells, S1P activates muscarinic receptor-coupled inwardly rectifying K^+ channels, probably by way of the $S1P_3$ GPCR[24], and Ca^{2+}-dependent K^+ channels[25]. By contrast, our data show that S1P inhibits the inward K^+ channels in GCPs from wild-type plants. Importantly, this inhibitory effect is dependent on heterotrimeric G proteins, as evident from the lack of S1P inhibition of inward K^+ channels in *gpa1* null mutants (Fig. 3b, c). We next examined the effect of S1P on slow anion channels, which are activated by ABA, in part through heterotrimeric G proteins[6]. We found that S1P stimulated slow anion currents in guard cells of wild-type plants, but not in *gpa1* null mutant lines (Fig. 3e, f). This result is consistent with the absence of S1P stimulation of stomatal closure in these mutant lines (Fig. 3d). Together, these results highlight the central role of GPA1 in mediating the diametrically

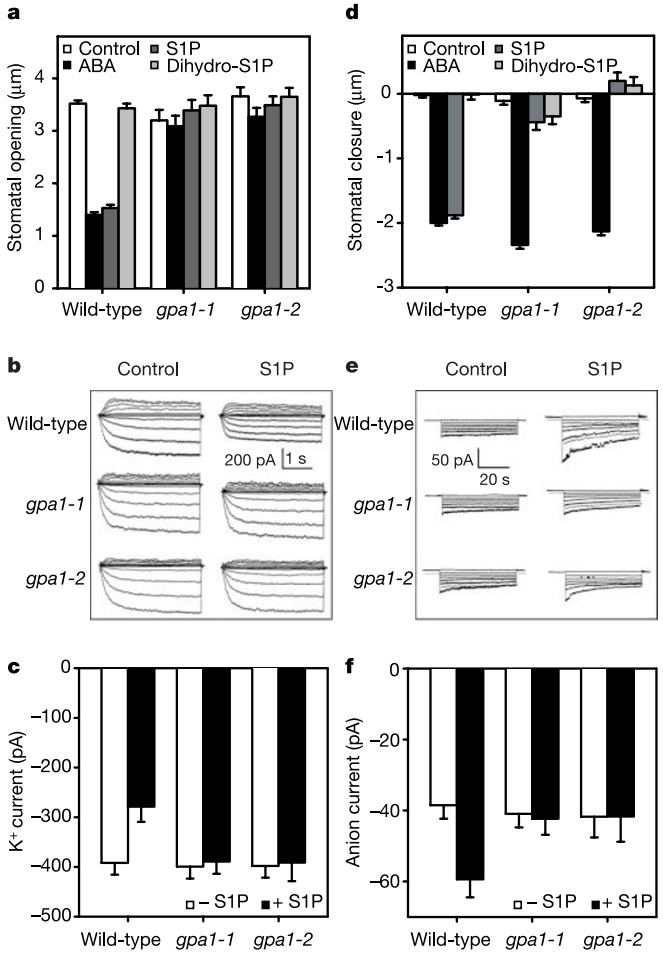

Figure 3 Effects of S1P on stomatal apertures and guard cell ion channel activities in wild-type and G-protein α-subunit (Gα) null mutant *gpa1-1* and *gpa1-2* plants. **a**, Apertures after a 2.5 h pre-treatment in the dark, and a further 2 h light treatment with or without 20 μM ABA, 10 μM S1P or 10 μM dihydro-S1P. **b**, Representative whole-cell recordings of K^+ currents from wild-type, *gpa1-1* and *gpa1-2* GCPs. Control recordings were obtained 10 min after achieving the whole-cell configuration. GCPs were subsequently challenged with 10 μM S1P, and recordings were acquired again after 20 min. **c**, Average time-activated whole-cell K^+ currents at −219 mV; $n = 18$, 10 and 7 cells for wild-type, *gpa1-1* and *gpa1-2*, respectively. **d**, Apertures after a 2.5 h light pre-treatment, and a further 3 h light treatment with or without 20 μM ABA, 10 μM S1P or 10 μM dihydro-S1P. **e**, Representative whole-cell recordings of slow anion currents from wild-type, *gpa1-1* and *gpa1-2* GCPs. GCPs were pre-treated with 10 μM S1P for 30 min; 10 μM S1P was also added to bath solution. **f**, Average steady-state whole-cell anion currents at −145 mV; $n = 25$, 24 and 23 cells, and $n = 29$, 29 and 23 cells for wild-type, *gpa1-1* and *gpa1-2* treated with or without S1P, respectively.

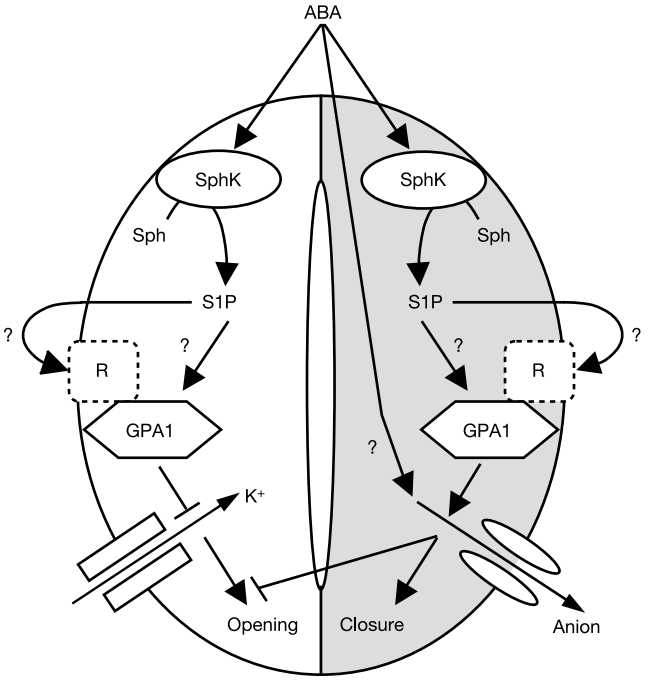

Figure 4 Model of ABA activation of S1P signalling in *A. thaliana* guard cells. The model integrates only the results presented here and previous data on *gpa1* knockout plants[6]. The other signalling elements known to operate in these cells[9–11] are omitted from this S1P-related model for the sake of clarity. S1P formed by ABA activation of SphK may function as a ligand for an unknown receptor (R), or may interact directly with GPA1 to inhibit and activate plasma membrane inwardly rectifying K^+ channels and slow anion channels, respectively, resulting in inhibition of stomatal opening and promotion of stomatal closure. Major points that remain to be clarified by further experiments are indicated by question marks. Because ABA activates stomatal closure and slow anion channels in *gpa1* knockout lines but S1P does not, the solid line is added to represent an S1P-independent mechanism of ABA action on stomatal closure, the precise nature of which requires further investigation. Possible interactions between the illustrated components and the diverse array of other intermediates known to be involved in guard cell ABA signalling[9–11] remain to be elucidated.

opposing effects of S1P on guard cell inward K^+ channels and slow anion channels.

In this study, we have provided a biochemical basis for how S1P is generated in guard cells in response to ABA, and have elucidated the underlying mechanisms whereby the S1P signal is transduced within the cellular context to mediate changes in guard cell turgor (Fig. 4). Future work may allow the GPA1-dependent pathways of S1P action (Fig. 4) to be linked with other known ABA signalling components, such as reactive oxygen species, cytosolic pH and levels of cytosolic Ca^{2+} (refs 9–11). Furthermore, it will be important to elucidate the molecular mechanisms by which S1P affects GPA1. In animal cells, S1P and dihydro-S1P act as extracellular ligands for the S1P receptor family[1,23]. In guard cells, S1P signal transduction by way of a GPA1-coupled S1P-like receptor is less likely, because dihydro-S1P did not affect stomatal responses (Fig. 3a, d). Moreover, the putative GPCR of *A. thaliana*, GCR1[7,8,26,27], bears no sequence homology to any of the conserved S1P receptors. Therefore, the S1P signal in guard cells may be transduced either through a structurally unique GPCR, by a direct interaction of S1P with GPA1, or through unidentified proteins that function to input signals to heterotrimeric G proteins independently of GPCRs. This latter possibility is lent credence by recent evidence in mammalian cells that AGS (activator of G-protein signalling) proteins can activate heterotrimeric-G-protein signalling pathways independently of GPCRs[7,8,28], and might help to explain the enigmatic intracellular action of S1P in mammalian cell growth, given that proteins serving as intracellular targets of S1P have not yet been identified[1,2,23]. Future work is likely to reveal novel mechanisms of S1P signalling through heterotrimeric G proteins in diverse eukaryotes. □

Methods

Preparation of GCPs and MCPs

GCPs were isolated as described[6,15]. MCPs were isolated by modification of a method described previously[15] (see Supplementary Methods).

Stomatal bioassay and electrophysiology

Stomatal aperture bioassays and whole-cell current recordings were conducted as described[6], with minor modifications (see Supplementary Methods).

Preparation of lysates from leaves, MCPs and GCPs

All procedures were conducted at 4 °C. Rosette leaves of 4- to 5-week-old plants[6,15] were homogenized with SphK buffer[20] containing 0.6% (w/v) insoluble polyvinylpyrrolidone (Sigma) and sand (− 50 +70 Mesh, Sigma). The homogenate was then centrifuged at 10,000*g* for 10 min. The supernatant was recovered, frozen with liquid N_2, and stored at −80 °C. Lysates from GCPs and MCPs were prepared by collecting and resuspending protoplasts in 400 µl of SphK buffer[20]. Protoplasts were homogenized and centrifuged at 10,000*g* for 10 min. The supernatants were then frozen with liquid N_2, and stored at −80 °C.

SphK assays

Aliquots of the MCP suspension (100 µl, approximately 7.5×10^5 protoplasts) were incubated at room temperature for 5 min, and treated with or without 50 µM ABA prepared as described[15]. After the appropriate incubation time, each sample was homogenized in a glass/Teflon homogenizer with 300 µl of SphK buffer[20], and centrifuged at 10,000*g* for 10 min. The supernatants were then frozen with liquid N_2, and stored at −80 °C. SphK activity was assayed by providing [γ-^{32}P]ATP and Sph (see Supplementary Methods) as substrates to the lysates, and measuring radiolabelled S1P formation[20]. Values are the mean ± s.e.m. from three to five independent experiments, and data were compared using the Student's *t*-test at the 95% confidence level.

Measurement of [^{3}H]S1P formation

Intracellular levels of [^{3}H]S1P were measured by modification of a method described previously[29]. Aliquots of MCP and GCP suspensions (200 µl, approximately 5×10^5 protoplasts) were incubated at 25 °C for 10 min, before the addition of 30 nM [^{3}H]Sph (20 Ci mmol^{-1}) and 0.2 mg ml^{-1} fatty-acid-free BSA for 1 min (approximately 300,000 d.p.m. per assay). Reactions were started by the addition of 50 µM ABA prepared as described[15]. After the appropriate incubation time, reactions were terminated by the addition of 500 µl of chloroform/methanol/concentrated HCl (100:200:1, v/v/v) and 40 µl of 2 M HCl. After vortexing, 250 µl of chloroform and 250 µl of 2 M KCl were added. The samples were mixed thoroughly, followed by centrifugation at 2,000*g* for 5 min. The resultant organic phases were collected, concentrated under a stream of N_2, and spotted onto Silica gel 60 thin-layer chromatography (TLC) plates. Samples were run in a 1-butanol/acetic acid/water (3:1:1, v/v/v) solvent system, and Sph and S1P identified with ninhydrin. After scraping off the spots corresponding to [^{3}H]S1P, radioactivity was measured by liquid scintillation counting (Optiphase Hisafe 3, Wallac).

Received 5 February; accepted 7 April 2003; doi:10.1038/nature01643.

1. Spiegel, S. & Milstien, S. Sphingosine-1-phosphate, a key cell signaling molecule. *J. Biol. Chem.* **277,** 25851–25854 (2002).
2. Pyne, S. & Pyne, N. J. Sphingosine 1-phosphate signalling and termination at lipid phosphate receptors. *Biochim. Biophys. Acta* **1582,** 121–131 (2002).
3. Hla, T., Lee, M.-J., Ancellin, N., Paik, J. H. & Kluck, M. J. Lysophospholipids—receptor revelations. *Science* **294,** 1875–1878 (2001).
4. Ng, C. K.-Y., Carr, K., McAinsh, M. R., Powell, B. & Hetherington, A. M. Drought-induced guard cell signal transduction involves sphingosine-1-phosphate. *Nature* **410,** 596–599 (2001).
5. Ullah, H. *et al.* Modulation of cell proliferation by heterotrimeric G protein in *Arabidopsis*. *Science* **292,** 2066–2069 (2001).
6. Wang, X.-Q., Ullah, H., Jones, A. M. & Assmann, S. M. G protein regulation of ion channels and abscisic acid signaling in *Arabidopsis* guard cells. *Science* **292,** 2070–2072 (2001).
7. Jones, A. M. G-protein-coupled signaling in *Arabidopsis*. *Curr. Opin. Plant Biol.* **5,** 402–407 (2002).
8. Assmann, S. M. Heterotrimeric and unconventional GTP binding proteins in plant cell signaling. *Plant Cell* **14,** S355–S373 (2002).
9. Assmann, S. M. & Wang, X.-Q. From milliseconds to millions of years: guard cells and environmental responses. *Curr. Opin. Plant. Biol.* **4,** 421–428 (2001).
10. Blatt, M. R. Cellular signaling and volume control in stomatal movements in plants. *Annu. Rev. Cell Dev. Biol.* **16,** 221–241 (2000).
11. Schroeder, J. I., Kwak, J. M. & Allen, G. J. Guard cell abscisic acid signalling and engineering drought hardiness in plants. *Nature* **410,** 327–330 (2001).
12. Kohama, T. *et al.* Molecular cloning and functional characterization of murine sphingosine kinase. *J. Biol. Chem.* **273,** 23722–23728 (1998).
13. Brownlee, C. Intracellular signalling: sphingosine-1-phosphate branches out. *Curr. Biol.* **11,** R535–R538 (2001).
14. Ng, C. K.-Y. & Hetherington, A. M. Sphingolipid-mediated signalling in plants. *Ann. Bot. (Lond.)* **88,** 957–965 (2001).
15. Pandey, S., Wang, X.-Q., Coursol, S. & Assmann, S. M. Preparation and applications of *Arabidopsis thaliana* guard cell protoplasts. *New Phytol.* **153,** 517–526 (2002).
16. Pei, Z.-M., Kuchitsu, K., Ward, J. M., Schwarz, M. & Schroeder, J. I. Differential abscisic acid regulation of guard cell slow anion channels in *Arabidopsis* wild-type and *abi1* and *abi2* mutants. *Plant Cell* **9,** 409–423 (1997).
17. Dickson, R. C. & Lester, R. L. Sphingolipid functions in *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta* **1583,** 13–25 (2002).
18. Crowther, G. J. & Lynch, D. V. Characterization of sphinganine kinase activity in corn shoot microsomes. *Arch. Biochem. Biophys.* **337,** 284–290 (1997).
19. Nishiura, H., Tamura, K., Morimoto, Y. & Imai, H. Characterization of sphingolipid long-chain base kinase in *Arabidopsis thaliana*. *Biochem. Soc. Trans.* **28,** 747–748 (2000).
20. Olivera, A., Kohama, T., Tu, Z., Milstien, S. & Spiegel, S. Purification and characterization of rat kidney sphingosine kinase. *J. Biol. Chem.* **273,** 12576–12583 (1998).
21. Sperling, P., Zähringer, U. & Heinz, E. A sphingolipid desaturase from higher plants. Identification of a new cytochrome b_5 fusion protein. *J. Biol. Chem.* **273,** 28590–28596 (1998).
22. Staxén, I. *et al.* Abscisic acid induces oscillations in guard-cell cytosolic free calcium that involve phosphoinositide-specific phospholipase C. *Proc. Natl Acad. Sci. USA* **96,** 1779–1784 (1999).
23. van Brocklyn, J. R. *et al.* Dual actions of sphingosine-1-phosphate: extracellular through the G_i-coupled receptor Edg-1 and intracellular to regulate proliferation and survival. *J. Cell Biol.* **142,** 229–240 (1998).
24. Himmel, H. M. *et al.* Evidence for EDG-3 receptor-mediated activation of $I(K_{Ach})$ by sphingosine-1-phosphate in human atrial cardiomyocytes. *Mol. Pharmacol.* **58,** 449–454 (2000).
25. Schilling, T. *et al.* Lysophospholipids induce membrane hyperpolarization in microglia by activation of IKCA1 Ca^{2+}-dependent K^+ channels. *Neuroscience* **109,** 827–835 (2002).
26. Joseffson, L. G. & Rask, L. Cloning of a putative G-protein-coupled receptor from *Arabidopsis thaliana*. *Eur. J. Biochem.* **249,** 415–420 (1997).
27. Colucci, G., Apone, F., Alyeshmerni, N., Chalmers, D. & Chrispeels, M. J. *GCR1*, the putative *Arabidopsis* G protein-coupled receptor gene is cell cycle-regulated, and its overexpression abolishes seed dormancy and shortens time to flowering. *Proc. Natl Acad. Sci. USA* **99,** 4736–4741 (2002).
28. Cismowski, M. J., Takesono, A., Bernard, M. L., Duzic, E. & Lanier, S. M. Receptor-independent activators of heterotrimeric G-proteins. *Life Sci.* **68,** 2301–2308 (2001).
29. Meyer zu Heringdorf, D. *et al.* Sphingosine kinase-mediated Ca^{2+} signalling by G-protein-coupled receptors. *EMBO J.* **17,** 2830–2837 (1998).

Supplementary Information accompanies the paper on **www.nature.com/nature**.

Acknowledgements We thank J. P. Hobson for the recombinant purified hSphK1, X.-Q. Wang, A. Olivera and J.-N. Pierre for technical advice, J. Coursol for statistical analyses, and T. Jacob and C. K.-Y. Ng for critically reading the manuscript. This work was supported by grants from the United States Department of Agriculture (USDA) and the National Science Foundation to S.M.A., from the USDA to S.G., and from the National Institutes of Health to S.S.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to S.M.A. (sma3@psu.edu).