ARTICLES

The Mg-chelatase H subunit is an abscisic acid receptor

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Abscisic acid (ABA) is a vital phytohormone that regulates mainly stomatal aperture and seed development, but ABA receptors involved in these processes have yet to be determined. We previously identified from broad bean an ABA-binding protein (ABAR) potentially involved in stomatal signalling, the gene for which encodes the H subunit of Mg-chelatase (CHLH), which is a key component in both chlorophyll biosynthesis and plastid-to-nucleus signalling. Here we show that *Arabidopsis* ABAR/CHLH specifically binds ABA, and mediates ABA signalling as a positive regulator in seed germination, post-germination growth and stomatal movement, showing that ABAR/CHLH is an ABA receptor. We show also that ABAR/CHLH is a ubiquitous protein expressed in both green and non-green tissues, indicating that it might be able to perceive the ABA signal at the whole-plant level.

The phytohormone ABA has a vital function in plant adaptation to stressful environments by regulating stomatal aperture and the expression of stress-responsive genes, and in plant development such as seed maturation, germination and seedling growth[1–3]. Genetic approaches have permitted the characterization of numerous components involved in ABA signalling but have failed to identify ABA receptors[1–3]. Biochemical approaches provide another way to isolate ABA receptors by the identification of ABA-binding proteins that are putative ABA receptors[4–6]. Recently, the RNA-binding protein FCA, a homologue of an ABA-binding protein ABAP1 (ref. 6), was identified as an ABA receptor in the regulation of flowering time[7]. However, ABA receptors involved in seed development, seedling growth and stomatal movement remain elusive.

We previously reported an ABA-specific-binding protein from broad bean (*Vicia faba*) and found that this protein was potentially involved in ABA-induced stomatal signalling[5]. So we designated it ABAR (for putative abscisic acid receptor). On the basis of the sequencing information, we isolated from broad bean leaves a complementary DNA fragment encoding the carboxy-terminal half of about 770 amino acids of the putative H subunit (CHLH) of the magnesium-protoporphyrin-IX chelatase (Mg-chelatase). CHLH has been reported to have multiple functions in plant cells. In addition to its enzymatic functions as a subunit of the Mg-chelatase in producing photosynthetic apparatus[8], CHLH has a key function in mediating plastid-to-nucleus signalling[9–12]. We found that the yeast-expressed product of the cDNA fragment encoding the C-terminal portion of the broad bean ABAR or CHLH binds ABA specifically (data not shown). The information from *Vicia faba* led us to analyse the functions of ABAR in ABA signalling in *Arabidopsis thaliana*. Here we show that the *Arabidopsis* ABAR/CHLH (the *Arabidopsis* genomic locus tag for *CHLH* is At5g13630, and the GenBank accession numbers are AY070133, BT002311, NM_121366, Z68495 or AY078971) is an ABA receptor that regulates seed development, post-germination growth and stomatal aperture.

ABAR binds ABA specifically

The purified yeast-expressed *Arabidopsis* ABAR binds ABA as a saturable process (Fig. 1a). The ABAR protein possesses one binding

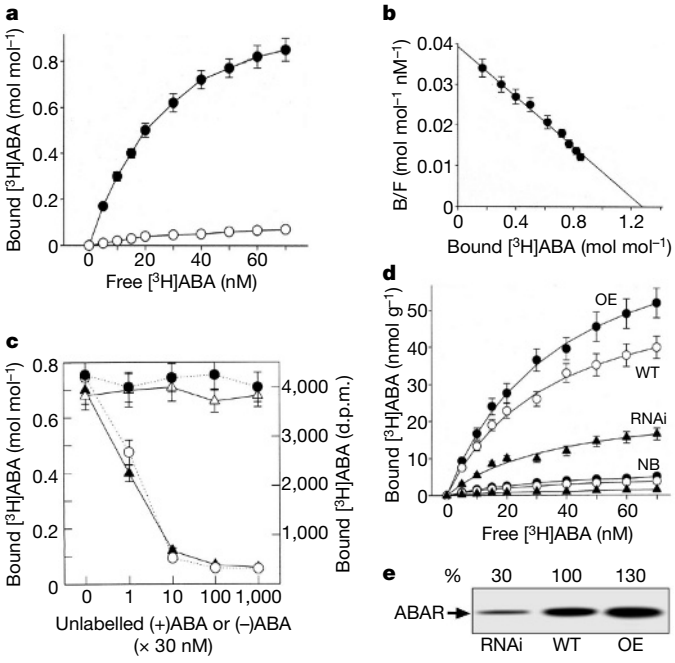

Figure 1 | ABAR binds ABA. **a**, Saturable ABA-specific binding (filled circles) to the pure yeast-expressed ABAR. Open circles, non-specific binding. **b**, Scatchard plot of binding data in **a**. B, [^{3}H]ABA bound; F, free [^{3}H]ABA. The parameters of the curve are $K_d = 32$ nM; $B_{max} = 1.28$ mol mol^{-1}; $R^2 = 0.98$. **c**, Displacement of [^{3}H](+)-ABA binding by (+)-ABA and (−)-ABA. Filled triangles, *in vitro* (+)-ABA binding (mol mol^{-1}); open triangles, *in vitro* (−)-ABA binding (mol mol^{-1}); filled circles, (−)-ABA pull-down assay (d.p.m.); open circles, (+)-ABA pull-down assay (d.p.m.). **d**, Saturable ABA-specific binding to the extracts of leaves of gl1 (WT), RNAi (line 12) and overexpressing (OE, line 1) lines. NB, non-specific binding. **e**, Immunosignal of ABAR protein in the extracts in **d** and the band intensities relative to the wild type. Each point in **a**–**d** is the mean ± s.d. ($n = 10$).

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site, as shown by a linear Scatchard plot and a maximum binding (B_{max}) of 1.28 mol ABA per mol of protein, and has a high binding affinity for ABA, as shown by its equilibrium dissociation constant (K_d) of 32 nM (Fig. 1b). Furthermore, the purified ABAR binds ABA in a highly stereospecific manner, which was revealed by the efficient displacement of [^{3}H](+)-ABA binding by the physiologically active form (+)-ABA but not by two inactive ABA isomers, (−)-ABA and *trans*-ABA, which are structurally similar to (+)-ABA (Fig. 1c; data for *trans*-ABA not shown).

We further analysed the ABA-binding ability of natural ABAR protein. The saturable process of ABA binding to the extracts of leaves was found (Fig. 1d). Upregulation of the ABAR level by overexpressing *ABAR* enhanced, but downregulation by RNA-mediated interference (RNAi) reduced, the B_{max} of ABA binding, whereas neither substantially changed K_d (from 35 to 38 nM) (Fig. 1d, e, and Supplementary Fig. 1), revealing that the changes in ABAR abundance alter the numbers of ABA-binding sites but do not modify the affinity. A pull-down assay with the ABAR-specific antiserum specifically co-precipitated the ABA-binding activities proportionally to the amounts of the ABAR protein (Supplementary Fig. 2), and the pulled-down ABA-binding activity was shown to be highly stereospecific for (+)-ABA (Fig. 1c; data for *trans*-ABA not shown). The data reveal that ABAR specifically binds ABA, and the binding meets primary criteria for an ABA receptor.

ABAR mediates ABA signalling as a positive regulator

To explore the functions of ABAR in ABA signalling, we generated *Arabidopsis* transgenic RNAi, antisense and overexpression lines. The plants underexpressing *ABAR* as a result of RNAi showed significant ABA-insensitive phenotypes in seed germination (Fig. 2a), post-germination growth arrest by ABA (Fig. 2b) and ABA-induced promotion of stomatal closure and inhibition of stomatal opening (Fig. 2c). In contrast, the plants overexpressing *ABAR* displayed the ABA-hypersensitive phenotypes (Fig. 2a–c) and were more resistant to dehydration from their leaves or whole plants, but the RNAi plants were more sensitive to dehydration (Fig. 2d, e). Overall, the ABAR levels were negatively correlated with the intensity of the ABA-insensitive phenotypes (Supplementary Fig. 3). The ABA concentrations did not change in the transgenic plants (in the range of 0.2 μg g^{-1} dry weight), showing that ABAR is not involved in ABA biosynthesis.

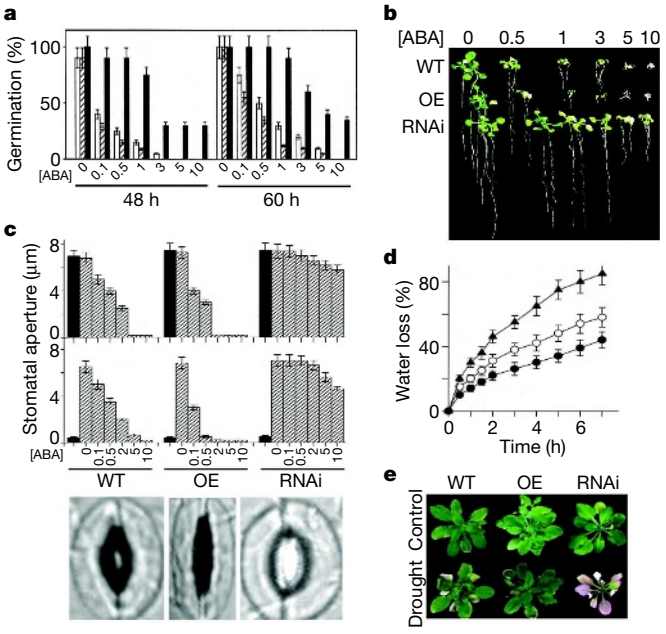

Figure 2 | Changes in *ABAR* expression alter plant sensitivity to ABA. **a**, **b**, Seed germination (**a**) and seedling (10 days in ABA) growth (**b**) of gl1 (WT, white columns in **a**), RNAi (black columns in **a**) and overexpressing (OE, hatched columns in **a**) lines in medium containing (±)-ABA. ABA concentrations are in μM. **c**, ABA-induced stomatal closure (top) and inhibition of opening (middle). Black columns, initial stomatal aperture; grey columns, apertures after ABA treatment. ABA concentrations are in μM. Bottom: stomatal apertures in the assay of inhibition of opening by 2 μM ABA. **d**, Water loss from detached leaves. Open circles, WT; filled circles, OE; triangles, RNAi. **e**, Plant status after drought treatment (controls were fully watered plants). The RNAi line 12 and OE line 1 were used. Error bars indicate s.d. ($n = 5$).

ABAR-mediated ABA signalling is a distinct process

Mg-chelatase, which is composed of three subunits, namely CHLD, CHLI and CHLH, catalyses the insertion of Mg^{2+} into protoporphyrin-IX (Proto) to form Mg-protoporphyrin-IX (MgProto), the first step unique to chlorophyll synthesis[8]. CHLH has a central function as a monomeric Proto-binding protein[8,15]. The *Arabidopsis genomes uncoupled 5* (*gun5*) mutant, resulting in a single amino acid Ala 990→Val mutation in CHLH, showed that CHLH is involved in plastid-to-nucleus retrograde signalling by controlling the metabolism of the tetrapyrrole signal MgProto or by sensing the signal[9–12]. We observed that treatment with exogenous ABA significantly decreased both the chlorophyll and Proto contents but stimulated *ABAR* expression and Mg-chelatase activity and enhanced the MgProto contents (Fig. 3a). This positive regulation of ABAR by ABA seems to support its function as an ABA sensor and indicates that ABA-induced chlorophyll decrease might not be attributable to the action of ABA on ABAR. However, as an ABA and Proto dual-ligand-binding protein, ABAR binds ABA independently of Proto (Supplementary Fig. 5), indicating that ABA signal perception might be distinct from Proto binding.

We observed, in a pharmaceutical assay using both norflurazon[16] (an inhibitor of the carotenoid biosynthetic enzyme phytoene desaturase that causes photo-oxidative damage to chloroplasts) and chloramphenicol (CP; an inhibitor of plastid translation), that an ABA-insensitive stomatal movement occurred in parallel with a decrease in ABAR levels (Supplementary Fig. 7), but no correlation of ABA-responsive stomatal movement with chlorophyll or MgProto contents was found (Supplementary Figs 6, 7). We also used a chemical-regulated inducible RNAi system[17] to investigate ABAR-mediated ABA signalling. After induction by 17β-oestradiol, a decrease in the ABAR levels was observed without an alteration in the chlorophyll and MgProto contents (Fig. 3b, and data not shown), and this decrease in ABAR levels induced a parallel insensitivity of stomatal movement to ABA (Fig. 3b). Using the protoplasts prepared from the inducible RNAi plants, we found that a decline of *ABAR* expression repressed the mRNA levels of *RD29A* (ref. 18), *MYB2* and *MYC2* (ref. 19)—genes that respond positively to ABA—but upregulated two negative regulators of ABA signalling, namely *ABI1* (refs 20–22) and *ABI2* (refs 22, 23) (Supplementary Fig. 8). These approaches provide additional, more direct, evidence for functions of ABAR as a positive regulator of ABA signalling independently of chlorophyll and MgProto.

Further assays were performed with a series of mutants defective in chlorophyll metabolism or plastid signalling. *hy1* (refs 24, 25) and *hy2* (ref. 26) mutants, containing lesions in haem oxygenase and phytochromobilin synthase genes, respectively, are alleles of two *gun* mutants, *gun2* and *gun3* respectively, that are defective in plastid signalling (refs 9, 10, 12). The *gun4* mutant has a lesion in a second

Proto-binding protein-encoding gene *GUN4* (refs 27, 28). *ch1* mutants contain lesions in the gene encoding chlorophyll *a* oxygenase[29]. *cch* is also a *gun* mutant and an allele of *gun5* but with a single nucleotide substitution at a different site, resulting in a single amino acid mutation Pro 642→Leu (ref. 9). These mutants have lower chlorophyll contents except *gun5*, which possesses a chlorophyll level comparable to that of its wild type (Col, Fig. 3c), and they have ABAR levels comparable to those of their wild-type plants except the *cch* mutant, which has a lower level (Fig. 3c). All the *gun* mutants *hy1*/*gun2*, *hy2*/*gun3*, *gun4*, *gun5* and *cch* have been shown to be involved in the same MgProto-triggered plastid-signalling pathway[11], but only the *cch* mutant had the ABA-insensitive phenotypes in germination (Fig. 3d), seedling growth (Fig. 3e) and ABA-induced stomatal movement (Fig. 3f). In all the mutants, no significant correlation of the chlorophyll levels with the ABA-responsive phenotypes was observed (Fig. 3c–f). The *cch* mutation, but not *gun5*, significantly decreased the ABA-binding activity of ABAR (Supplementary Fig. 9), which may explain the ABA-insensitive phenotypes in the *cch* mutant and wild-type phenotypes in the *gun5* mutant. Taken together, these data show clearly that ABAR is a positive regulator in ABA signal transduction involved in a signalling process that is distinct from chlorophyll metabolism and MgProto-mediated plastid retrograde signalling.

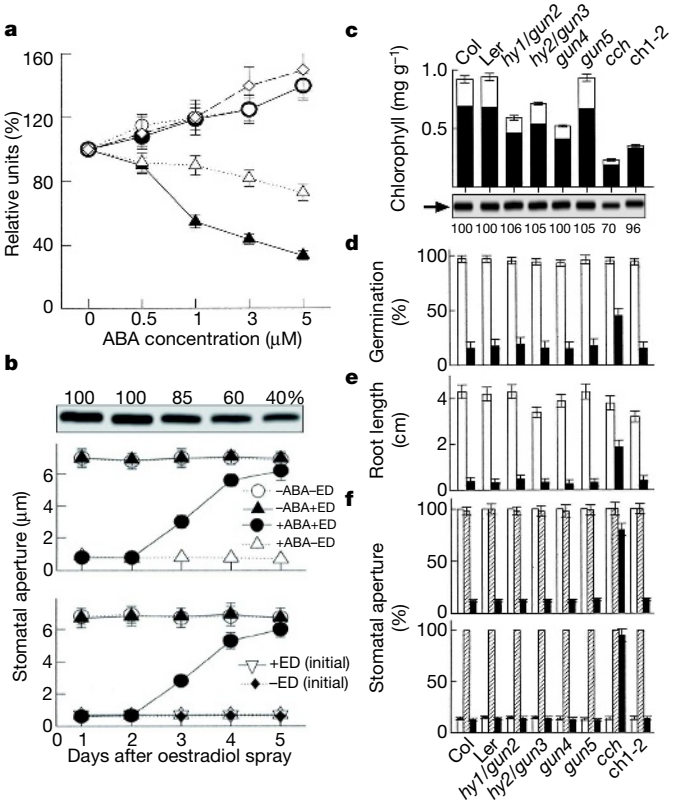

Figure 3 | ABAR-mediated ABA signalling is a distinct process. **a**, ABA treatment decreases the chlorophyll (filled triangles) and Proto (open triangles) levels, but enhances MgProto (filled circles) and ABAR protein (diamonds) levels and Mg-chelatase (open circles) activity in gl1 seedlings. **b**, Oestradiol (ED)-induced downregulation of *ABAR* expression (top panel; immunosignal and its relative intensity) in the inducible RNAi plants results in ABA-insensitive phenotypes in both stomatal closure (middle panel) and inhibition of stomatal opening (bottom panel) induced by 10 μM ABA. **c**, The ABAR level (indicated by arrow and relative band intensity) is reduced in *cch*, but not in the other mutants containing different levels of chlorophyll. Filled columns, chlorophyll *a*; open columns, chlorophyll *b*. **d**–**f**, *cch* is an ABA-insensitive mutant in germination (**d**, 1 μM ABA), seedling growth (**e**, root length of 7-day-old seedlings in 10 μM ABA) and stomatal movement (**f**, top, stomatal closure; bottom, stomatal opening; 10 μM ABA was used). Filled columns in **d** and **e**, with ABA; open columns, without ABA. Black columns in **f**, with ABA, 2 h; grey columns, without ABA, 2 h; white columns, without ABA, 0 h. Error bars indicate s.d. ($n = 5$).

ABAR is ubiquitous and regulates ABA-signalling genes

The *CHLH* expression was previously reported to be limited to the green tissues[8,10]. Available data at the Genevestigator site (http://www.genevestigator.ethz.ch) show the presence of *Arabidopsis CHLH* mRNA in seeds. We found that ABAR/CHLH is a protein that is expressed ubiquitously in the non-green tissues, including the roots (Fig. 4a). ABAR might therefore function at the whole-plant level.

We found that downregulation of *ABAR* expression by RNAi decreased the levels of the positive regulators of ABA signalling *RD29A* (ref. 18), *MYB2* (ref. 19), *MYC2* (ref. 19), *ABI4* (refs 30, 31), *ABI5* (refs 30, 32) and *OST1* (ref. 33), but enhanced the levels of three negative regulators, *ABI1* (refs 20–22), *ABI2* (refs 22, 23) and *CIPK15* (ref. 34) in leaves (Fig. 4b). These results are essentially consistent with those from the inducible RNAi protoplasts (Supplementary Fig. 8). The seed-specific ABA-signalling genes *ABI3* (refs 13, 14), *ABI4* (refs 30, 31) and *ABI5* (refs 30, 32) and their downstream genes *EM1* and *EM6*, which are both responsible for late embryogenesis[2,35], were all downregulated in the siliques of the RNAi plants (Fig. 4c). In most cases the *ABAR*-overexpressing plants regulated these genes in a manner contrary to the RNAi plants (Fig. 4b, c). The expression levels of these genes in the *gun5* mutant were similar to those in the wild-type Columbia (data not shown). The regulation of these ABA-signalling genes by ABAR supports the contention that ABAR is a positive regulator and indicates that it might function through various pathways.

Discussion

Previous studies have shown a multiplicity and complexity of ABA perception sites that may act at the outside or inside of cells to mediate different biological functions in plants[1–3,7]. We show that ABAR is an ABA receptor to perceive the ABA signal in seed germination, post-germination growth and stomatal movement, essentially from the following evidence: first, ABAR specifically binds ABA; second, transgenic downregulation of *ABAR* expression results in a decline in the number of ABA-binding sites and leads to ABA-insensitive phenotypes; third, *ABAR*-overexpressing plants have

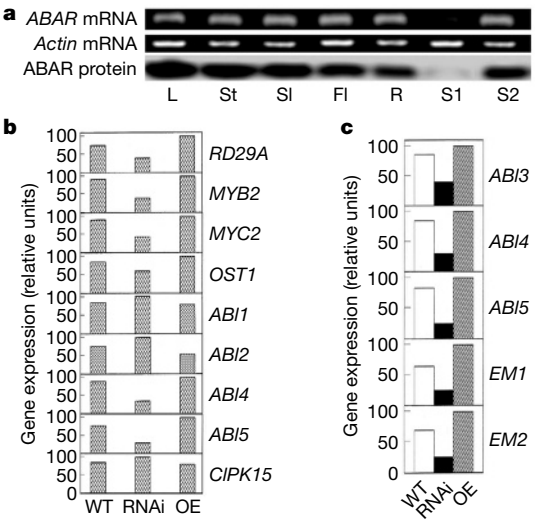

Figure 4 | Spatial expression of *ABAR* and alteration of ABA-signalling genes in transgenic plants. **a**, ABAR expression at the mRNA (actin mRNA as a loading control) and protein (immunodetected with anti-ABAR serum) levels in leaves (L), stems (St), siliques (Sl), flowers (Fl), roots (R), dry seeds (S1), and seeds kept at 20 °C for 24 h after stratification (S2). **b**, **c**, Real-time PCR analysis for expression of ABA-signalling genes in leaves (**b**) and green siliques (**c**) of gl1 (WT), RNAi (line 12) and overexpressing (OE line 1) plants.

ABA-hypersensitive phenotypes with an elevated number of ABA-binding sites; fourth, a loss-of-function mutation in *ABAR* results in an immature embryo; and last, a *cch* mutant that downregulates both *ABAR* expression and ABA-binding activity is an ABA-insensitive mutant like the post-transcriptional gene-silencing RNAi or antisense mutants. Thus, ABAR is a common key component in the chlorophyll biosynthetic process of chelating Mg^{2+} to Proto, plastid retrograde signalling to the nucleus and perception of the ABA signal. However, ABAR-mediated ABA signalling is distinct from other pathways like ABAR/GUN5-mediated plastid-to-nucleus signaling, which is independent of chlorophyll biosynthesis[9].

As a receptor, ABAR regulates a series of the components involved in the ABA signalling network (Fig. 4), but the downstream components interacting directly with ABAR will have to be identified in the future to explain how ABA signal perception by ABAR is relayed in cells. *ABAR* is a single-copy gene in the *Arabidopsis* genome, is highly conserved in plant species and even shares high sequence similarities to its homologues in bacteria. This evolutionary conservation indicates a possibly vital role for it in these organisms. Gaining a further insight into how ABAR works in this complex signalling network will be of great interest in understanding cell signalling in plants.

METHODS

Plant materials, generation of transgenic plants and growth conditions. *Arabidopsis thaliana* ecotype gl1 was used in the generation of transgenic plants. The constructs for creating transgenic *ABAR*-RNAi, *ABAR*-antisense and *ABAR*-overexpressing lines or chemically inducible RNAi lines are described in Supplementary Information. These constructs were introduced into the GV3101 strain of *Agrobacterium tumefaciens* and transformed into plants by floral infiltration. The homozygous T_3 seeds of the transgenic plants were used for analysis. For the inducible RNAi, ten putative transgenic lines were tested by northern and western blotting after application of 10 μM 17β-oestradiol (Sigma), and all ten lines showed a significant decline in *ABAR* transcript and product. The seeds of the *gun4-1*, *gun5-1* and *cch* mutants were a gift from J. Chory. The seeds of the mutants *hy1-1* (ABRC number CS67), *hy2-1* (CS68) and *ch1-2* (CS3362) were obtained from the Arabidopsis Biological Resource Center. Except for the mutants *hy1-1* and *hy2-1* with the ecotype Ler as background, all mutants were isolated from the ecotype Columbia. Plants were grown in a growth chamber at 20–21 °C on Murashige–Skoog medium at about 80 μmol photons $m^{-2}\,s^{-1}$, or in compost soil at about 120 μmol photons $m^{-2}\,s^{-1}$ over a 16-h photoperiod.

ABA binding assays. The ABA-binding activity of ABAR was assayed in accordance with previously described procedures[5], with modifications. The expression of the recombinant *ABAR* in yeast, purification of the expressed ABAR protein, and the detailed procedures of ABA-binding assays including *in vitro* binding and pull-down assays are described in Supplementary Information.

Other assays. Screening of T-DNA insertion knockout mutants in the *ABAR* gene, phenotype analysis, RNA gel blotting, reverse transcriptase-mediated PCR, real-time PCR, production of anti-ABAR serum, immunoblotting and immunolabelling, chlorophyll and porphyrin measurements, assays of the effects of ABA on ABAR expression and Mg-chelatase activity, and induction of RNAi are described in Supplementary Information.

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1. Leung, J. & Giraudat, J. Abscisic acid signal transduction. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **49**, 199–222 (1998).
2. Finkelstein, R. R., Gampala, S. S. & Rock, C. D. Abscisic acid signaling in seeds and seedlings. *Plant Cell* **14**, S15–S45 (2002).
3. Himmelbach, A., Yang, Y. & Grill, E. Relay and control of abscisic acid signaling. *Curr. Opin. Plant Biol.* **6**, 470–479 (2003).
4. Hornberg, C. & Weiler, E. W. High affinity binding sites for abscisic acid at the plasmalemma of *Vicia faba* guard cells. *Nature* **310**, 321–324 (1984).
5. Zhang, D. P., Wu, Z. Y., Li, X. Y. & Zhao, Z. X. Purification and identification of a 42-kilodalton abscisic acid-specific-binding protein from epidermis of broad bean leaves. *Plant Physiol.* **128**, 714–725 (2002).
6. Razem, F. A., Luo, M., Liu, J. H., Abrams, S. R. & Hill, R. D. Purification and characterization of a barley aleurone abscisic acid-binding protein. *J. Biol. Chem.* **279**, 9922–9929 (2004).
7. Razem, F. A., El-Kereamy, A., Abrams, S. R. & Hill, R. D. The RNA-binding protein FCA is an abscisic acid receptor. *Nature* **439**, 290–294 (2006).
8. Walker, C. J. & Willows, R. D. Mechanism and regulation of Mg-chelatase. *Biochem. J.* **327**, 321–333 (1997).
9. Mochizuki, N., Brusslan, J. A., Larkin, R., Nagatani, N. & Chory, J. *Arabidopsis genomes uncoupled 5* (*GUN5*) mutant reveals the involvement of Mg-chelatase H subunit in plastid-to-nucleus signal transduction. *Proc. Natl Acad. Sci. USA* **98**, 2053–2058 (2001).
10. Surpin, M., Larkin, R. M. & Chory, J. Signal transduction between the chloroplasts and the nucleus. *Plant Cell* **14**, S327–S338 (2002).
11. Strand, Å., Asami, T., Alonso, J., Ecker, J. R. & Chory, J. Chloroplast to nucleus communication triggered by accumulation of Mg-protoporphyrinIX. *Nature* **421**, 79–83 (2003).
12. Nott, A., Jung, H. S., Koussevitzky, S. & Chory, J. Plastid-to-nucleus retrograde signaling. *Annu. Rev. Plant Biol.* **57**, 730–759 (2006).
13. Giraudat, J. *et al.* Isolation of the *Arabidopsis ABI3* gene by positional cloning. *Plant Cell* **4**, 1251–1261 (1992).
14. Nambara, E., Naito, S. & McCourt, P. A mutant of *Arabidopsis* which is defective in seed development and storage protein accumulation is a new *abi3* allele. *Plant J.* **2**, 435–441 (1992).
15. Karger, G. A., Reid, J. D. & Hunter, C. N. Characterization of the binding of deuteroporphyrin IX to the magnesium chelatase H subunit and spectroscopic properties of the complex. *Biochemistry* **40**, 9291–9299 (2001).
16. Chamovitz, D., Pecker, I. & Hirschberg, J. The molecular basis of resistance to the herbicide norflurazon. *Plant Mol. Biol.* **16**, 967–974 (1991).
17. Guo, H. S., Fei, J. F., Xie, Q. & Chua, N. H. A chemical-regulated inducible RNAi system in plants. *Plant J.* **34**, 383–392 (2003).
18. Yamaguchi-Shinozaki, K. & Shinozaki, K. A novel *cis*-acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low-temperature, or high-salt stress. *Plant Cell* **6**, 251–264 (1994).
19. Abe, H. *et al.* *Arabidopsis* AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcription activators in abscisic acid signaling. *Plant Cell* **15**, 63–78 (2003).
20. Leung, J. *et al.* *Arabidopsis* ABA response gene *ABI1*: features of a calcium-modulated protein phosphatase. *Science* **264**, 1448–1452 (1994).
21. Meyer, K., Leube, M. P. & Grill, E. A protein phosphatase 2C involved in ABA signal transduction in *Arabidopsis thaliana*. *Science* **264**, 1452–1455 (1994).
22. Gosti, F. *et al.* ABI1 protein phosphatase 2C is a negative regulator of abscisic acid signaling. *Plant Cell* **11**, 1897–1909 (1999).
23. Leung, J., Merlot, S. & Giraudat, J. The *Arabidopsis ABSCISIC ACID-INSENSITIVE2* (*ABI2*) and *ABI1* encode homologous protein phosphatase 2C involved in abscisic acid signal transduction. *Plant Cell* **9**, 759–771 (1997).
24. Davies, S., Kurepa, J. & Vierstra, R. D. The *Arabidopsis thaliana HY1* locus, required for phytochrome-chromophore biosynthesis, encodes a protein related to heme oxygenases. *Proc. Natl Acad. Sci. USA* **96**, 6541–6546 (1999).
25. Muramoto, T., Kohchi, T., Yokota, A., Hwang, I. & Goodman, H. M. The *Arabidopsis* photomorphogenic mutant *hy1* is deficient in phytochrome chromophore biosynthesis as a result of a mutation in a plastid heme oxygenase. *Plant Cell* **11**, 335–347 (1999).
26. Kohchi, T. *et al.* The *Arabidopsis HY2* gene encodes phytochromobilin synthase, a ferredoxin-dependent biliverdin reductase. *Plant Cell* **13**, 425–436 (2001).
27. Larkin, R. M., Alonso, J. M., Ecker, J. R. & Chory, J. GUN4, a regulator of chlorophyll synthesis and intracellular signaling. *Science* **299**, 902–906 (2003).
28. Verdecia, M. A. *et al.* Structure of the Mg-chelatase cofactor GUN4 reveals a novel hand-shaped fold for porphyrin binding. *PLoS Biol.* **3**, 777–789 (2005).
29. Espineda, C. E., Linford, A. S., Devine, D. & Brusslan, J. A. The *AtCAO* gene, encoding chlorophyll *a* oxygenase, is required for chlorophyll *b* synthesis in *Arabidopsis thaliana*. *Proc. Natl Acad. Sci. USA* **96**, 10507–10511 (1999).
30. Finkelstein, R. R. Mutations at two new *Arabidopsis* ABA response loci are similar to the *abi3* mutations. *Plant J.* **5**, 765–771 (1994).
31. Finkelstein, R. R., Wang, M. L., Lynch, T. J., Rao, S. & Goodman, H. M. The *Arabidopsis* abscisic acid response locus *ABI4* encodes an APETALA2 domain protein. *Plant Cell* **10**, 1043–1054 (1998).
32. Finkelstein, R. R. & Lynch, T. J. The *Arabidopsis* abscisic acid response gene *ABI5* encodes a basic leucine zipper transcription factor. *Plant Cell* **12**, 599–609 (2000).
33. Mustilli, A. C., Merlot, S., Vavasseur, A., Fenzi, F. & Giraudat, J. *Arabidopsis* OST1 protein kinase mediates the regulation of stomatal aperture by abscisic acid and acts upstream of reactive oxygen species production. *Plant Cell* **14**, 3089–3099 (2002).
34. Guo, Y. *et al.* A calcium sensor and its interacting protein kinase are global regulators of abscisic acid signaling in *Arabidopsis*. *Dev. Cell* **3**, 233–244 (2002).
35. Manfre, A. J., Lanni, L. M. & Marcotte, W. R. Jr. The *Arabidopsis* group 1 LATE EMBRYOGENESIS ABUNDANT protein ATEM6 is required for normal seed development. *Plant Physiol.* **140**, 140–149 (2006).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Information The sequence of the cDNA encoding part of CHLH is deposited in GenBank under accession number DQ376081. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to D.P.Z. (zhangdp@sohu.net).