Guard cell actin reorganization has been observed in stomatal responses to a wide array of stimuli. However, how the guard cell signaling machinery regulates actin dynamics is poorly understood. Here, we report the identification of an allele of the Arabidopsis thaliana ACTIN-RELATED PROTEIN C2/DISTORTED TRICHOMES2 (ARP2/3) locus (encoding the ARP2/3 subunit of the ARP2/3 complex) designated high sugar response3 (hsr3). The hsr3 mutant showed increased transpirational water loss that was mainly due to a lesion in stomatal regulation. Stomatal bioassay analyses revealed that guard cell sensitivity to external stimuli, such as abscisic acid (ABA), CaCl₂, and light/dark transition, was reduced or abolished in hsr3. Analysis of a nonallelic mutant of the ARP2/3 complex suggested no pleiotropic effect of ARP2/3 beyond its function in the complex in regard to stomatal regulation. When treated with ABA, guard cell actin filaments underwent fast disruption in wild-type plants, whereas those in hsr3 remained largely bundled. The ABA insensitivity phenotype of hsr3 was rescued by cytochalasin D treatment, suggesting that the aberrant stomatal response was a consequence of bundled actin filaments. Our work indicates that regulation of actin reassembly through ARP2/3 complex activity is crucial for stomatal regulation.
Live cell imaging of actin filaments shows the dynamic turnover of F-actin in a stochastic manner (Staiger et al., 2009; Smertenko et al., 2010). Organization of the actin cytoskeleton is determined by the net effect of polymerization, depolymerization, severing, sliding, and bundling events, all of which have been imaged in planta (Staiger et al., 2009; Smertenko et al., 2010). These behaviors of actin filaments are regulated by a plethora of actin binding proteins (ABPs) that underpin the regulation of actin dynamics in response to intrinsic and extrinsic signals (Hussey et al., 2006; Staiger and Blanchoin, 2006). Considering the fundamental role of ABPs in coordinating actin dynamics and the importance of actin reorganization in stomatal regulation, it is reasonable to postulate that ABPs might play critical roles during guard cell stimulus-response coupling (Staiger, 2000). In this light, At-ADF1, a member of the actin depolymerizing factor (ADF) family, has been implicated in the control of stomatal aperture, which is substantiated by the constitutively closed stomata observed in an At-ADF1 overexpression line (Dong et al., 2001). In addition, the recently identified STOMATAL CLOSURE-RELATED ACTIN BINDING PROTEIN1 (SCAB1) also mediates stomatal closure and is an ABP that stabilizes actin filaments (Zhao et al., 2011). Unlike ADF, the SCAB1 family is specific to plants. Both increased and decreased activities of SCAB1 lead to stomatal insensitivity to ABA, suggesting that a fine balance of actin dynamicity is required for efficient guard cell regulation.

Here, using the hsr3 mutant, an allele of one of the subunits of the plant ARP2/3 complex, we provide evidence that the ARP2/3 complex finely regulates guard cell actin remodeling and stomatal movement in response to external signals. Based on our findings, we also suggest how actin can function as a hub in the guard cell signaling network.

RESULTS

Transpirational Water Loss Is Faster in hsr3 Compared with the Wild Type

The hsr3 mutant was recovered from a screen of M2 Arabidopsis seedlings showing high sugar response, and this mutation is inherited as a single recessive Mendelian locus (Baier et al., 2004). This mutant has a wilty appearance and exhibits a much

Figure 1. The hsr3 Mutant Shows Faster Transpirational Water Loss Than the Wild Type.

(A) Water loss from detached rosette leaves of 5-week-old wild-type (WT) and hsr3 plants. Data are means of six independent experiments ±sd (with four leaves from two plants in each experiment). Statistical analysis was performed by paired t test (P < 0.001).

(B) Stomatal density in wild-type and hsr3 plants. Data are means of stomatal density of 10 rosette leaves from 10 individual plants with ±se. Significant difference (*P < 0.05) was found between the wild type and hsr3 using the two-tailed t test.

(C) Infrared thermography of wild-type and mutant plants. Four-week-old plants were imaged in high humidity (RH = 70%) for 40 min before a sudden drop in relative humidity to 35% was imposed. Following the humidity drop, the plants were imaged for 60 min at 1-min intervals. The experiment was repeated three times with the same conclusion, and a representative data set is shown here. Left panel, representative leaf thermal profiles of wild-type and hsr3 plants; right panel, a representative infrared image of wild-type and mutant plants taken at 30 min after the drop in humidity.
wider stomatal aperture in the dark than the wild type. It was hypothesized that the lesion in HSR3 caused aberrant stomatal behavior and therefore increased transpirational water loss. To establish a direct connection between the wilty phenotype and the effect on stomatal aperture, we performed a water loss assay. The data indicate that hsr3 had a significantly higher water loss ratio than the wild type (Figure 1A). As a higher stomatal density might also result in a constitutive acceleration of leaf water loss (Masle et al., 2005), we measured stomatal density. Interestingly, stomatal density in hsr3 was higher than that in the wild type (Figure 1B). To investigate whether the lesion affecting stomatal response, stomatal development, or both caused the increased transpirational water loss in hsr3 leaves, we surveyed leaf thermal profiles of wild-type and mutant plants using an infrared thermal imaging camera (Xie et al., 2006). The leaf thermal profile of hsr3 was comparable to that of the wild type at high relative humidity (70%), whereas the mutant exhibited a cooler phenotype in response to the low humidity challenge (Figure 1C). These results supported a responsive over a constitutive stomatal phenotype. Although it would seem likely that the wilty phenotype displayed by hsr3 is predominantly due to reduced stomatal sensitivity to closing signals, it is also possible that the increase in stomatal density contributes to the observed accelerated transpirational water loss.

**Stomatal Sensitivity to External Stimuli Is Attenuated or Abolished in hsr3 Compared with the Wild Type**

The observations described above prompted us to investigate whether HSR3 encoded a key guard cell signaling component involved in the regulation of stomatal movement. Therefore, stomatal responses to an array of well-established opening and closing signals were examined using a well-established stomatal bioassay (Webb and Hetherington, 1997). Since ABA is known to be involved in the stomatal response to low humidity (Xie et al., 2006), we first investigated ABA-promoted stomatal closure and ABA-inhibited stomatal opening. The hsr3 stomata were less open than the wild type after light treatment (see Supplemental Figure 1 online), and so two-way analysis of variance (ANOVA) (genotype × ABA interaction) was used to compare the ABA responsiveness in wild-type and mutant plants. In agreement with the cooler phenotype, upon a drop in relative humidity, the hsr3 mutant displayed hyposensitivity to ABA (Figures 2A and 2B). Extracellular Ca²⁺ promotes stomatal

---

**Figure 2. Stomata of hsr3 Plants Are Less Sensitive to External Stimuli.**

(A) Stomatal bioassays for ABA-induced closure. WT, the wild type.

(B) Stomatal bioassays for ABA-inhibited opening.

(C) Stomatal bioassays for Ca²⁺-induced closure.

Data in (A) to (C) are means of 120 stomatal aperture measurements from three replicates ±se. Statistical analyses were performed by two-way ANOVA (i.e., genotype × treatment interaction). P < 0.001 in all three assays.

(D) Stomatal sensitivity to light/dark transition in wild-type and hsr3 plants. Data show means of 120 stomatal aperture measurements from three replicates ±se. Statistical analysis was performed by two-tailed t test. **Significant difference (P < 0.01) between apertures in the same genotype before and after dark treatment.
closure by inducing increases in the cytoplasmic Ca\(^{2+}\) concentration (McAinsh et al., 1995; Allen et al., 2000). Stomatal bioassay analysis demonstrated that stomatal response to exogenous Ca\(^{2+}\) was dramatically reduced in the mutant (Figure 2C). Considering the absolute necessity of extracellular Ca\(^{2+}\) in dark-induced closure of stomata (Schwartz et al., 1988), we further checked whether the mutant displayed altered sensitivity to dark treatment. Strikingly, we found that the stomatal response to darkness in \textit{hsr3} was completely abolished (Figure 2D).

\textbf{hsr3 Is an Allele of the Gene That Encodes the ARPC2 Subunit of the ARP2/3 Complex}

To determine the identity of the gene responsible for the \textit{hsr3} phenotype, a map-based cloning approach was adopted. The mutation was mapped to the protein-coding gene At1g30825 (K. Thodey, K. Sorefan, F. de Jong, C. Smith, F. Corke, D.C. Logan, D.B. Szymanski, and M.W. Bevan, unpublished data), which was previously shown to encode the ARPC2 subunit of the \textit{Arabidopsis} ARP2/3 complex (El-Din El-Assal et al., 2004). The \textit{hsr3} mutant was found to be an allele of this gene, which carries a C-to-T transition in the 6th exon and introduces a nonsense mutation (Q216-*). It was reported that a mutation in ARPC2 caused a distorted trichome phenotype (El-Din El-Assal et al., 2004). In agreement with this, the \textit{hsr3} mutant had abnormal trichomes (Figure 3A), which further supported the conclusion that \textit{hsr3} is allelic to \textit{arpc2}. To provide direct evidence that the stomatal phenotype in \textit{hsr3} was caused by the genetic lesion in \textit{ARPC2}, stomatal bioassay analyses were conducted on

\textbf{Figure 3.} \textit{hsr3} Is an Allele of the ARPC2 Locus.

(A) Scanning electron microscopy images of trichomes in wild-type (left) and \textit{hsr3} (right) plants. Trichomes were normally branched in wild-type plants but distorted in \textit{hsr3} plants. Bars = 200 \textmu m.

(B) Stomatal bioassays for ABA-induced closure. Stomatal bioassay data demonstrate that wild-type \textit{Hsr3} (\textit{gHsr3}) restores wild-type ABA sensitivity (ABA-induced closure) in \textit{hsr3} guard cells. Data are means of 120 stomatal aperture measurements from three replicates \pm SE. Statistical analyses were performed by two-way ANOVA (i.e., genotype \times ABA interaction) \(P > 0.05\) between the wild type and \textit{hsr3}/\textit{gHsr3}; \(P < 0.001\) between the wild type and \textit{hsr3}. WT, the wild type.

\textbf{Figure 4.} Mutants in Different Subunits of the ARP2/3 Complex Show a Similar Stomatal Phenotype.

Stomatal bioassay data suggest that \textit{hsr3} and \textit{arp2-1}, two nonallelic mutants of the ARP2/3 complex, have a comparable stomatal response to external stimuli (hypersensitivity to Ca\(^{2+}\)-induced closure). Data are means of 120 stomatal aperture measurements from three replicates \pm SE. Statistical analyses were performed by two-way ANOVA (i.e., genotype \times Ca\(^{2+}\) interaction). \(P < 0.001\) between wild-type (WT) and mutant lines.
The hsr3 and arp2-1 Mutants Show a Comparable Stomatal Response to CaCl$_2$

The ARP2/3 complex consists of seven subunits, and homologs for all these subunits have been identified in Arabidopsis (Li et al., 2003; Goley and Welch, 2006; Staiger and Blanchon, 2006). If the function of ARPC2 in regulating stomatal movement was based upon the activity of the ARP2/3 complex, it was expected that mutations in other subunits of this complex would display a similar phenotype. To investigate this hypothesis, we performed a stomatal bioassay using wild-type, hsr3, and actin related protein2-1 (arp2-1) arp2-1 plants (Li et al., 2003). The results revealed that both hsr3 and arp2-1 stomata were less sensitive to CaCl$_2$-induced closure (Figure 4), suggesting no pleiotropic effect of ARPC2 beyond its function within the ARP2/3 complex in regard to stomatal regulation.

ARPC2 Is Expressed in Arabidopsis Guard Cells

Disruption of ARPC2 activity led to stomatal hyposensitivity to physiological and environmental stimuli. Accordingly, it was expected that ARPC2 would be expressed in guard cells. To confirm this, RT-PCR using RNA extracted from guard cell protoplasts and entire rosette leaves was performed. It was found that ARPC2 was expressed in both guard cells and whole leaves (Figure 5). Isolated guard cell protoplasts (see Supplemental Figure 2A online) were checked using a hemocytometer, and the purity was over 99% when calculated on a cell number basis. CARBONIC ANHYDRASE1 (CA1; At3g01500), which has a relatively lower expression level in guard cells than in mesophyll cells, served as an additional control for the purity of guard cell protoplasts (Pandey et al., 2002; Hu et al., 2010). Transcripts of CA1 were not detectable in guard cells with the tested PCR cycle, while transcripts of GC1 (a guard cell–specific gene) were detected under the same amplification conditions (see Supplemental Figure 2B online). We also performed in silico analysis using the publicly available guard cell–specific microarray data (Yang et al., 2008) and found that genes encoding subunits of the ARP2/3 complex (except ARPC4, which has no probe on the ATH1 Chip) were expressed in guard cells (see Supplemental Figure 2C online).

ABA-Induced Guard Cell Actin Dynamics Are Aberrant in hsr3

Our stomatal bioassay analyses revealed that hsr3 stomata opened less than Columbia-0 (Col-0) upon light treatment and were less sensitive than Col-0 to ABA in ABA-promoted stomatal closure (Figure 2; see Supplemental Figure 1 online), while its parental line (A3L3) showed a comparable stomatal response to Col-0 (see Supplemental Figure 3 online). Interestingly, wild-type plants pretreated with phalloidin phenocopy the hsr3 mutant (Kim et al., 1995). Therefore, we speculated that the hsr3 mutation might cause stabilization of actin, which was known to impede stimulus-induced stomatal movement (Gao et al., 2008). Interestingly, another allele of ARPC2 (dis2-1) has been reported to have more dense populations of actin bundles in the trichome cytosol compared with the wild type (El-Din El-Assal et al., 2004). To test whether hsr3 impacted actin stabilization, we investigated ABA-induced guard cell actin disassembly as a model. To visualize actin dynamics in guard cells, we crossed an actin-specific green fluorescent protein (GFP) reporter (Voigt et al., 2005) into the hsr3 mutant (see Supplemental Figure 4 online). For quantitative analysis of guard cell actin rearrangement in response to ABA, we classified actin organization into three distinct patterns: (1) sparse, transversely oriented actin filaments in integral or fragmented forms; (2) numerous actin filaments most of which are in transverse directions; (3) meshwork-like or spot-like actin filaments lacking any regular orientation (Figure 6A; see Supplemental Figure 5 online). Wild-type and hsr3 guard cells that had not been treated with ABA showed similar actin organizational patterns with type 1 actin dominating the cell population (Figures 6B and 6C). The percentage of type 1 actin decreased dramatically in guard cells of both wild-type (from 85.4 to 23.5%) and hsr3 (from 87.5 to 38.1%) plants upon ABA treatment (Figures 6B and 6C). However, the wild-type guard cells with type 3 actin comprised 57.9% of the population treated with ABA, whereas only 21.4% of those hsr3 guard cells treated with ABA showed type 3 actin filaments (Figures 6B and 6C). In fact, most of the hsr3 guard cells (78.6%) retained transversely oriented actin filaments (type 1+type 2) in response to ABA (Figures 6B and 6C). Taken together, our results suggest that the change of actin filament configuration upon ABA treatment is disrupted by the hsr3 mutation.

Cytochalasin D Restores Wild-Type Sensitivity to ABA in hsr3 Stomata

Based on the above results, we proposed that the mutant stomatal phenotypes might be a result of the aberrant guard cell actin dynamics brought about by the hsr3 mutation. A pharmacological approach using actin-modifying agents was adopted to investigate this further. Treatment with CD is able to depolymerize actin filaments and accelerate stomatal movements in response to light and ABA (Gao et al., 2008). We found that...
pretreatment with 10 μM CD had no effect on steady state stomatal aperture under light or ABA treatment in the wild type (Figure 7). However, pretreatment of hsr3 guard cells with the same amount of CD restored wild-type stomatal sensitivity to light and 1 μM ABA (Figure 7). Therefore, we concluded that the impeded actin disorganization in hsr3 guard cells confers hypo-sensitivity of stomata to ABA.

**DISCUSSION**

**The ARP2/3 Complex Regulates Stomatal Movement**

Dynamic stimulus-induced actin reorganizations facilitate stomatal opening and closure (Kim et al., 1995; MacRobbie and Kurup, 2007; Gao et al., 2008). Previously identified actin regulators function via association with downstream effectors instead of directly binding to actin per se (Lemichez et al., 2001; Choi et al., 2008). In this study, we characterized stomatal behavior in the hsr3 mutant that carries a lesion in ARPC2. Arabidopsis ARPC2 encodes a putative ortholog of the yeast ARPC2 component of the ARP2/3 complex. Arabidopsis ARPC2 can substitute for the function of Saccharomyces cerevisiae ARPC2, while yeast two-hybrid analysis showed that Arabidopsis ARPC2 interacts with Arabidopsis ARPC4 (El-Din El-Assal et al., 2004). This suggested that ARPC2 participates in an Arabidopsis ARP2/3 complex. Phenotypic similarity between the prospective Arabidopsis ARP2/3 complex subunits and the homologs of ARP2/3 complex regulators has provided strong genetic evidence for the concept of an Arabidopsis complex (reviewed in Deeks and Hussey, 2005). More recently, hemagglutinin-tagged ARPC4
The working concentration for CD is 10 μM, and the final concentration of DMSO in each treatment was 0.1% (v/v). Data show means of 120 stomatal aperture measurements from three replicates ± SE. Statistical analyses were performed by two-tailed t test. **Significant difference (P < 0.01) when compared with the wild type (WT) pretreated with DMSO and then subjected to the same ABA treatment conditions.

Models for ARP2/3 Complex-Mediated Stomatal Movement

The rescue of the ARP2/3 complex phenotype by CD shows that ABA-induced guard cell closure requires radial-cable structures to be removed or reorganized. The assembly of finer unbundled structures associated with partially and fully closed apertures is not critical for the closure process. The stable open-aperture cable configuration is behaving as a cytoskeletal brake to ABA-mediated guard cell closure. The hsr3 mutation is rescued by catastrophic drug-induced depolymerization, suggesting that the ARP2/3 complex contributes to ABA-induced rearrangement or disassembly of obstructive F-actin.

The ARP2/3 complex can participate in mechanisms to exert force on actin filaments and microtubules during reconstructions of the cytoskeleton. Actin filaments generated by cortical ARP2/3 complexes maneuver the mammalian meiotic spindle (Yi et al., 2011). In Arabidopsis roots, the ARP2/3 complex is required for light-induced actin cable alignment in rapidly expanding cells (Dyachok et al., 2011), suggesting analogous functions for the plant ARP2/3 complex in exerting force upon stable cytoskeletal structures. The dispersal of heavily bundled actin to finer filaments could be enhanced by ARP2/3 complex-driven mechanical separation. Alternatively, the complex might aid reconfiguration through a reverse funneling effect. The funneling hypothesis states that excess F-actin barbed ends become capped in a stochastic manner to raise monomer concentration and drive rapid polymerization from new barbed ends generated by ARP2/3 complexes or other nucleation factors (Carlier and Pantaloni, 1997; Akin and Mullins, 2008). In the case of guard cell closure, mass activation of ARP2/3 complexes by ABA signaling could drop the monomer concentration and enter actin cables into a state of depolymerization. Removal of the functional ARP2/3 complex in hsr3 mutants would maintain monomer concentration during the early stages of reconfiguration and stabilize the inhibitory cables. Testing this hypothesis is beyond the scope of this investigation.

The regulation of stretch-activated calcium ion channels by the actin cytoskeleton offers another alternative scenario for ARP2/3 complex function during guard cell closure. These mechanosensitive channels are present in guard cells and respond to changes in tension of the plasma membrane (Zhang et al., 2007). Stabilization of F-actin by phalloidin inhibits the channels while CD enhances their activation (Zhang et al., 2007).
Stable F-actin could therefore act as a feedback mechanism to suppress stretch activation under stable high turgor (open aperture) conditions. The ARP2/3 complex and its activators in plants are associated with the plasma membrane (Dyachok et al., 2008; Kotchoni et al., 2009; Dyachok et al., 2011), suggesting that active ARP2/3 complexes can contribute to the F-actin network in proximity to stretched activated channels. Disruption of this subpopulation of cortical F-actin in the hsr3 mutant therefore has the potential to perturb second messenger signaling during the closure process. CD treatment, however, is nonselective and will remove both endoplasmic cassettes and ARP2/3 complex-generated cortical structures. Considering the importance of Ca²⁺ influx across the plasma membrane in guard cell ABA signaling, it might be that stimulus-induced elevation of [Ca²⁺]cyt is aberrant in hsr3 mutants. However, this possibility requires detailed investigation in the future. Other possibilities are that the genetic lesion in HSR3 might disrupt ABA-induced vacuolar responses, such as ion release (MacRobbie, 1998; MacRobbie and Kurup, 2007) and tonoplast structure alteration (Gao et al., 2005; Higaki et al., 2006), or endocytic cycling of critical factors at the plasma membrane, such as POTASSIUM CHANNEL IN ARABIDOPSIS THALIANA1 (Sutter et al. 2007).

In conclusion, our data suggest that the ARP2/3 complex is a critical component of the guard cell signaling network and ensures an optimal stomatal aperture under certain environmental conditions by finely regulating actin dynamics. hsr3 is an important tool to elucidate the molecular events mediated by actin in guard cell signaling and illuminate new targets for engineering improved water use efficiency in plants.

METHODS

Plant Material

The Arabidopsis thaliana ecotype used in this study was Col-0. The A3L3 line, the hsr3 mutant, and the hsr3 complementation line were from Mike Bevan (John Innes Centre, Norwich, UK). Plants constitutively expressing GFP-FABD2 (Ketelaar et al., 2004) were from Patrick Hussey (University of Durham, Durham, UK). The arp2-1 mutant was kindly provided by Zhenbiao Yang (University of California, Riverside, CA).

Measurements of Water Loss, Stomatal Density, and Leaf Thermal Profile

To monitor water loss, four fully expanded rosette leaves at the principal growth stage 5.10 (Boyce et al., 2001) were detached from the plants and weighed immediately on aluminum foil. The detached leaves were then incubated on the foil on a laboratory bench (RH = 35%) and weighed at designated time intervals. To determine stomatal density, 10 negative abaxial epidermis impressions were made for 100 fully expanded rosette leaves from 10 individual plants (stage 5.10) as described (Gray et al., 2005). The stomata of three distinct leaf areas (base, middle, and tip) within a fixed size (0.16 mm²) were counted for each negative impression, and stomatal density (number of stomata/0.16 mm²) was averaged for the 10 impressions. The thermal imaging experiments were performed as previously described (Xie et al., 2006). Five-week-old wild-type and mutant plants were placed in an acrylic horticultural propagator (William Sankey Products) and viewed under a thermal imaging camera (model SC1000; Flir Systems). To induce stomatal closure, plants were challenged with a drop in relative humidity (from ~70 to ~35%) by removing the lid of the propagator as described in Xie et al., 2006), and thermal images were recorded automatically at 1-min intervals over a 100-min experimental period that consisted of 40 min before the RH drop and 60 min after.

Measurements of Stomatinal Apertures

All stomatal bioassays were performed as described (Webb and Hetherington, 1997) with some modification. Plants were grown in a Microclimate growth chamber (Snijders Scientific) with a 22°C (day)/20°C (night) temperature cycle and a 10/-14-h light/dark regime for 5 weeks. To examine stomatal opening, abaxial epidermal strips of the youngest, fully expanded rosette leaves were carefully peeled and floated on 10 mM MES/KOH, pH 6.5, buffer to close stomata. The peels were subsequently transferred to the opening buffer (10 mM MES/KOH, pH 6.15, and 50 mM KCl) with or without ABA and incubated for the indicated times under PPF conditions. The first strand cDNA was generated by use of Superscript II reverse transcriptase (Invitrogen) according to the manufacturer’s instructions. For each sample, 0.15 µg of total RNA was reverse transcribed to produce first strand cDNA. The amplification products were visualized in 0.8% (w/v) agarose gels by ethidium bromide staining. Primers used are listed in Supplemental Table 1 online.

Microscopy

Transgenic plants harboring 3SS:GFP-FABD2 (Ketelaar et al., 2004; Voigt et al., 2005) in the wild-type and hsr3 background were used for visualization of the actin cytoskeleton in guard cells of mature leaves from 3-week-old plants. Detached whole leaves were incubated under opening conditions (PPFD = 120 µmol m⁻² s⁻¹, 10 mM MES/KOH, pH 6.15, and 50 mM KCl) for 3 h and subsequently treated with 5 µM ABA or mock solutions for 0.5 h. The abaxial epidermis of the leaves was used to observe guard cell filamentous actin. All images were collected using the LSM 510 Meta confocal microscope (Zeiss) equipped with a 488-nm argon ion laser as excitation source and a 505- to 550-nm band-pass filter. To observe trichome morphology, the first pairs of true leaves of 10-d-old seedlings were incubated in the fixative solution (2.7% glutaraldehyde + 0.1 M sodium cacodylate, pH 7.2) overnight at 4°C and subsequently subjected to a dehydration process with ethanol. They were then subject to a critical point drying process and coated with gold using a sputter coater. The coated samples were viewed in a scanning electron microscope (Cambridge Stereoscan S4).

Accession Numbers

Sequence data from this article can be found in the Arabidopsis Genome Initiative or GenBank/EMBL databases under the following accession numbers: At3g27000 (ARP2), At1g13180 (ARP0), At2g30910 (ARP1A), At2g31300 (ARP1B), At1g30825 (ARP2), At1g60430 (ARP3), At1g14147 (ARP4), and At1g01710 (ARP5).
Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure 1. Light-Induced Stomatal Opening Is Inhibited in hsr3.

Supplemental Figure 2. Expression of Genes in Purified Guard Cell Protoplasts.

Supplemental Figure 3. Stomatal Responses to External Stimuli in Col-0 Are Comparable to Those in A3L3 and GFP-FABD2 Lines.

Supplemental Figure 4. Identification of F3 Plants Homozygous at Both the hsr3 Mutation and the GFP-FABD2 Insertion Loci.

Supplemental Figure 5. Representative Confocal Images of Guard Cells Showing Three Types of Actin Organization.

Supplemental Table 1. Primers Used in the Gene Expression Analysis.

ACKNOWLEDGMENTS

We thank Bob Porter for technical assistance in scanning electron microscopy. We also acknowledge the assistance of the UK Biotechnology and Biological Science Research Council (BB/J002364/1) and the Overseas Research Students Awards Scheme for providing research funding.

AUTHOR CONTRIBUTIONS

K.J. participated in writing the article, designed and performed the experiments, and collected and interpreted the data. K.S. cloned HSR3 and made the native gene-complementation line (hsr3/gHSR3). M.J.D. participated in writing the article and performed confocal microscopy to visualize dynamic actin organization in guard cells. M.W.B. managed mutant screening and characterization work. P.J.H. participated in writing the article and performed confocal microscopy to analyze the data. A.M.H. participated in writing the article, designed and performed the experiments, and collected and interpreted the data.

Received January 29, 2012; revised March 29, 2012; accepted April 16, 2012; published May 8, 2012.

REFERENCES


The ARP2/3 Complex Mediates Guard Cell Actin Reorganization and Stomatal Movement in Arabidopsis
Kun Jiang, Karim Sorefan, Michael J. Deeks, Michael W. Bevan, Patrick J. Hussey and Alistair M. Hetherington

Plant Cell; originally published online May 8, 2012;
DOI 10.1105/tpc.112.096263

This information is current as of December 22, 2017