Arabidopsis *ORGAN SIZE RELATED1* regulates organ growth and final organ size in orchestration with *ARGOS* and *ARL*

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Summary

- The growth of a plant organ to its characteristic size is regulated by an elaborate developmental program involving both internal and external signals. Here, we identify a novel Arabidopsis gene, *ORGAN SIZE RELATED1* (*OSR1*), that is involved in regulation of organ growth and overall organ size.
- A combination of genetic, cytological and molecular approaches was used to characterize the expression profile, subcellular localization and roles of *OSR1* during organ growth.
- Ectopic expression of *OSR1* in Arabidopsis resulted in enlarged organs, as a consequence of increases in both cell number and cell size. OSR1 shares a conserved OSR domain with ARGOS and ARGOS-LIKE (ARL), which is sufficient for their functions in promoting organ growth. *OSR1* is a plant hormone-responsive gene and appears to act redundantly with *ARGOS* and *ARL* during organ growth. The OSR proteins are localized to the endoplasmic reticulum.
- Our results suggest that three co-evolved members of the *OSR* family may act coordinately to orchestrate growth signals and cell proliferation and expansion, thereby affecting organ growth and final organ size.

Introduction

The architecture of a plant depends greatly on the number, size and shape of its organs, and organ size is undoubtedly of great importance to agronomic yield and biomass production in plants (Gonzalez *et al.*, 2009). The relative constancy of organ size within a given species, but its remarkable variance among species, suggests that the final size of an organ is defined by intrinsically developmental programs (Mizukami, 2001). Although the key pathways that limit organ growth in insects and mammals have been characterized (Dong *et al.*, 2007), the counterparts of many factors that determine organ size in insects and mammals are not found in plants, suggesting that the mechanism underlying organ size control in plants differs from that in animals. Given their sessile and post-embryonic organogenesis lifestyle, organ growth in plants is also greatly influenced by internal and environmental signals, including plant hormones, light, temperature and nutrients, and the final size of organs is largely influenced by the combined effect of these signals during organogenesis (Tsukaya, 2003, 2005).

In plants, the growth of an organ to its characteristic size occurs in two successive but overlapping phases: the cell proliferation phase, during which cell number increases dramatically, and the subsequent cell expansion phase, during which cells expand to appropriate sizes (Mizukami, 2001; Anastasiou & Lenhard, 2007). These two phases are referred to as 'growth by cell proliferation' and 'growth by cell expansion', respectively (Anastasiou & Lenhard, 2007). In Arabidopsis leaves, most cells begin to undergo cell expansion when cell proliferation arrests gradually from leaf tip to base; however, there are still some cells that maintain the meristematic competence to continue dividing and form the specific cell type within each cell layer (Donnelly *et al.*, 1999), suggesting that cell proliferation and expansion are coordinately controlled in development (Tsukaya & Beemster, 2006).

Recent genetic analyses have identified several factors that control organ size by regulating the cell proliferation and/or

cell expansion process in plants. Some of these factors have roles in positively regulating cell proliferation during organ growth, such as AINTEGUMENTA (ANT), ANGUST IFOLIA 3 (AN3), GROWTH-REGULATING FACTOR 5 (AtGRF5), JAGGED (JAG), STRUWWELPETER (SWP), SWELLMAP1 (SMP1) and KLUH (KLU). Ectopic expression of these genes in Arabidopsis prolongs the duration of cell proliferation, leading to the production of larger organs with more cells, while mutations in the genes encoding these factors reduce the duration of cell proliferation and thus result in smaller organs (Mizukami & Fischer, 2000; Autran *et al.*, 2002; Dinneny *et al.*, 2004; Ohno *et al.*, 2004; Clay & Nelson, 2005; Horiguchi *et al.*, 2005; Anastasiou *et al.*, 2007; Lee *et al.*, 2009). By contrast, some other factors, including AUXIN RESPONSE FACTOR2 (ARF2), BLADE ON PETIOLE1 (BOP1), PEAPOD1/2 (PPDs), BIG BROTHER (BB) and DA1, appear to restrict organ growth by limiting the period of proliferation, because loss of function in each of these genes results in enlarged organs as a result of the increased cell number (Ha *et al.*, 2003; Disch *et al.*, 2006; Schruff *et al.*, 2006; White, 2006; Li *et al.*, 2008). A general theme emerging from these studies is that the regulation of cell proliferation time, rather than cell division rate, is one of critical mechanisms for determining final organ size in plants (Anastasiou & Lenhard, 2007). However, a few factors that regulate organ growth by cell expansion have also been identified. For example, ROTUNDIFOLIA3 (ROT3) stimulates polar cell expansion in the longitudinal direction in Arabidopsis leaves (Kim *et al.*, 1998), and overexpression of AtGRF1/2 increases leaf size by generally increasing cell size (Kim *et al.*, 2003). BIGPETAL, a putative transcription factor in Arabidopsis, keeps petal size in check by limiting cell expansion (Szécsi *et al.*, 2006).

The Arabidopsis gene *ARGOS* was identified as an auxin-inducible gene that encodes a small plant-specific protein without a known functional domain. Overexpression of *ARGOS* in Arabidopsis dramatically increases lateral organ size through prolonged expression of the APETALA2 (AP2) transcription factor gene *ANT*, which affects cell proliferation by regulating the expression of *Cyclin D3;1* (*CycD3;1*) during organ growth (Krizek, 1999; Mizukami & Fischer, 2000; Hu *et al.*, 2003). In the Arabidopsis genome, there is a homologue of *ARGOS*, named *ARGOS-LIKE* (*ARL*), and it is interesting that *ARL* appears to regulate organ growth mainly by influencing cell expansion rather than cell proliferation (Hu *et al.*, 2006). A recent study revealed that there is a single-copy orthologue of *ARGOS* in the rice (*Oryza sativa*) genome, and detailed cellular analysis of transgenic Arabidopsis plants overexpressing *OsARGOS* indicated that *OsARGOS* affects organ growth by promoting both cell proliferation and cell expansion (Wang *et al.*, 2009). In addition, *ARGOS* and *OsARGOS* are induced by the phytohormones auxin and cytokinin, while *ARL* is induced by brassinosteroid (BR) (Hu *et al.*, 2003, 2006; Wang *et al.*, 2009), suggesting that ARGOS/ARL-modulated organ growth also involves environmental or growth cues during plant development.

To gain insights into ARGOS/ARL-regulated organ growth, we attempted to identify other factors involved in the ARGOS/ARL-related pathway. Here, we report a novel Arabidopsis gene, *ORGAN SIZE RELATED1* (*OSR1*), that regulates organ growth and final organ size by affecting both cell proliferation and expansion. OSR1 shares a small conserved OSR motif with ARGOS and ARL, which is required and sufficient for promoting organ growth. The three *OSR* genes show different responses to plant hormones and appear to be co-regulated during organ development. Our data suggest that co-evolved members of the OSR family may act coordinately in response to growth signals to regulate cell proliferation/expansion during plant organogenesis.

Materials and Methods

Plant materials and growth conditions

The *Arabidopsis thaliana* (L.) Heynh Columbia-0 accession was used in this study. Sterilized seeds were plated on 1/2 MS medium containing 1% sucrose and 0.8% agar, and vernalized at 4°C in darkness for 2–4 d. The plate was then transferred to a culture room at 22 ± 1°C with illumination of 80–90 μmol m^{-2} s^{-1} with a 16-h light : 8-h dark photoperiod for seed germination. For hormone treatment or root observation, seedlings were grown vertically for 7–12 d. For morphological analysis, 7-d-old seedlings were transferred to soil and grown in a growth room at 22 ± 1°C with the same illumination and photoperiod as in the culture room (Jing *et al.*, 2009).

Plant transformation

The 267-bp *OSR1* coding sequence was amplified by reverse transcription–polymerase chain reaction (RT-PCR) and cloned into pVIP96 for generation of the *35S-OSR1* construct (Hu *et al.*, 2003). To generate truncated *OSR* genes, cDNA fragments of *OSR1*, *ARGOS* or *ARL* with an added ATG and/or a stop codon were amplified and cloned into pVIP96. To monitor tissue-specific expression of *OSR1*, a 1.9-kb promoter region and the 433-bp 3′-untranslated region of *OSR1* were fused with the β-glucuronidase (*GUS*) gene in pBI101 to form the *proOSR1-GUS* construct. The coding sequence of *OSR1*, *ARGOS* or *ARL* was fused with the *GFP* gene and cloned into pVIP96 to form the *35S-OSR1-GFP*, *35S-ARGOS-GFP* or *35S-ARL-GFP* construct, respectively. All transgenic plants were generated by *Agrobacterium tumefaciens*-mediated transformation (Zhang *et al.*, 2006). Approximately 20 independently transgenic lines for each

construct were generated and at least three T3 homozygous lines with a single T-DNA insertion were used for detailed analyses.

Morphological and cytological analyses

The leaf and petal were used as representative organs to determine organ size and to assess the contributions of cell number and cell size. Fully expanded fifth leaves were excised and photographed, and then visualized under a microscope after clearing with chloral hydrate, as previously described (Jing *et al.*, 2009). The palisade cells at the central region of a half leaf beside the mid-vein were photographed to determine cell size and cell number per unit area. To determine petal size and to examine epidermal cells, fully expanded petals were excised and photographed, and then fixed in fresh Formaldehyde-Acetic Acid-Alcohol (FAA) solution and scanned with a scanning electron microscope (SEM; Hitachi S-4800; Hitachi, Tokyo, Japan). The areas of leaf blades, petals and cells were measured with IMAGE J software (http://rsbweb.nih.gov/ij/). The total number of palisade cells per leaf or epidermal cells per petal was calculated as the product of the leaf area or petal area and the cell number per unit area was determined using the microscope (Hu *et al.*, 2010).

Gene expression analysis

Total RNA was isolated with a guanidine thiocyanate extraction buffer, and RNA gel blots were performed as described previously (Hu *et al.*, 2000). Real-time quantitative RT-PCR (qRT-PCR) was carried out using a Rotor-Gene 3000 thermocycler (Corbett Research, Sydney, Australia) with the SYBR® Premix Ex Taq™ II kit (Takara, Dalian, China). The normalization and relative values of expression level for each gene were calculated from three biological replicates, as previously described (Hu *et al.*, 2010). The primers used were as follows: for *ANT*, 5′-AAGCACGGATTGGTAGAGTCG-3′ and 5′-GCATTTGTGCCACGGAACTTA-3′; for *CycD3;1*, 5′-GCAAGTTGATCCCTTTGACC-3′ and 5′-CAGCTTGGACTGTTCAACGA-3′; and for the internal control *ACTIN2*, 5′-GCTCCTCTTAACCCAAAGGC-3′ and 5′-CACACCATCACCAGAATCCAGC-3′. For the GUS staining assay, seedlings or organs of homozygous transgenic plants were incubated in a 50 mM sodium phosphate solution (pH 7.0) containing 5 mM $K_4Fe(CN)_6$, 5 mM $K_3Fe(CN)_6$, 0.1% Triton X-100, and 1 mM 5-bromo-4-chloro-3-indolyl-β-glucuronic acid (Gluc) at 37°C for several hours.

Protoplast preparation and co-transformation

The *OSR1*-, *ARGOS*- and *ARL-GFP* fusion genes cloned in the pUC vector were used for co-transformation with the plasmid containing an endoplasmic reticulum (ER)-localized chaperon-binding protein-RFP (Bip-RFP) (Kim *et al.*, 2001). Arabidopsis protoplasts were prepared and the fusion constructs were introduced into protoplasts by polyethylene glycol-mediated transformation according to the method described by Jin *et al.* (2001). The co-transformed protoplasts were incubated at 22°C in the dark for *c.* 12 h before visualization of GFP and RFP fluorescence under a confocal microscope.

Yeast two-hybrid (Y2H) assay

The coding regions of *OSR1*, *ARGOS* and *ARL* were cloned into pGADT7 and pGBKT7 to fuse with the activation domain (AD) and DNA-binding domain (BD), respectively. The constructs were then transformed into *Saccharomyces cerevisiae* strain AH109 (BD, Biosciences, Palo Alto, CA, USA) according to the manufacturer's protocol. Self-activation for each single construct was not detectable when yeast cells were cultured on SD selective medium (SD-His-Leu + 5 mM 3-AT or SD-His-Trp + 5 mM 3-AT). Co-transformation was conducted with each AD fusion construct and BD fusion construct, to test whether each OSR can interact with itself and other OSRs in yeast cells. The co-transformed yeast cells with *OsMADS7-AD* and *OsMADS13-BD* were used as a positive control (Cui *et al.*, 2010).

Phylogenetic tree construction

To elucidate the phylogenetic relationships among *OSR-like* genes, 39 predicted *OSR* homologues from 14 plant species were identified in the National Center for Biotechnology Information (NCBI) database and Phytozome (http://www.phytozome.net), and the coding sequences were used to construct a phylogenetic tree. All these *OSR-like* coding sequences were first aligned using MUSCLE 3.6 (Edgar, 2004), and local alignment was adjusted manually using GENEDOC (Nicholas *et al.*, 1997). PHYML version 2.4 was used to construct the phylogenetic tree and the parameters were set as described by Guindon & Gascuel (2003). The coding sequence of *OSR* homologue *Physcomitrella patens 169695* was used as an outgroup for the phylogenetic tree. The reliability of each interior branch of the tree was assessed by bootstrapping with 1000 replications.

Results

The expression profile of *OSR1* is similar to that of *ARGOS*

Our previous work showed that *ARGOS* and its homologue *ARL* regulate lateral organ growth mainly by affecting cell proliferation and cell expansion, respectively (Hu *et al.*,

2003, 2006). To further expand our knowledge of ARGOS/ARL-mediated organ development, we utilized the *Arabidopsis thaliana trans*-factor and *cis*-element prediction database (ATTED II) to identify the candidate genes that were likely to be co-expressed or co-regulated with *ARGOS/ARL* (http://atted.jp/). In this database, an Arabidopsis gene, *At2g41230*, was shown to be directly connected to both *ARGOS* and *ARL* in a co-expression network (Fig. 1a). We then compared the expression profiles of *At2g41230*, *ARGOS* and *ARL* during development using the AtGenExpress atlas, which comprises the expression profiles of 22 746 probe sets on an Affymetrix ATH1 array with triplicate expression estimates from 79 diverse development samples, ranging from embryogenesis to senescence and from roots to flowers (Schmid *et al.*, 2005). The expression profiles generated by the AtGenExpress atlas showed that the expression profiles of *At2g41230* and *ARGOS* were indeed comparable in most developing organs and stages, except in the apex and in flowers. The expression pattern and signal intensity, in contrast, were quite different between *At2g41230* and *ARL* (Supporting Information Fig. S1a). The expression profiles generated by Electronic Fluorescent Pictograph (e-FP) (Winter *et al.*, 2007) also suggested that *At2g41230* and *ARGOS* were expressed quite similarly in various organs (Fig. S1b).

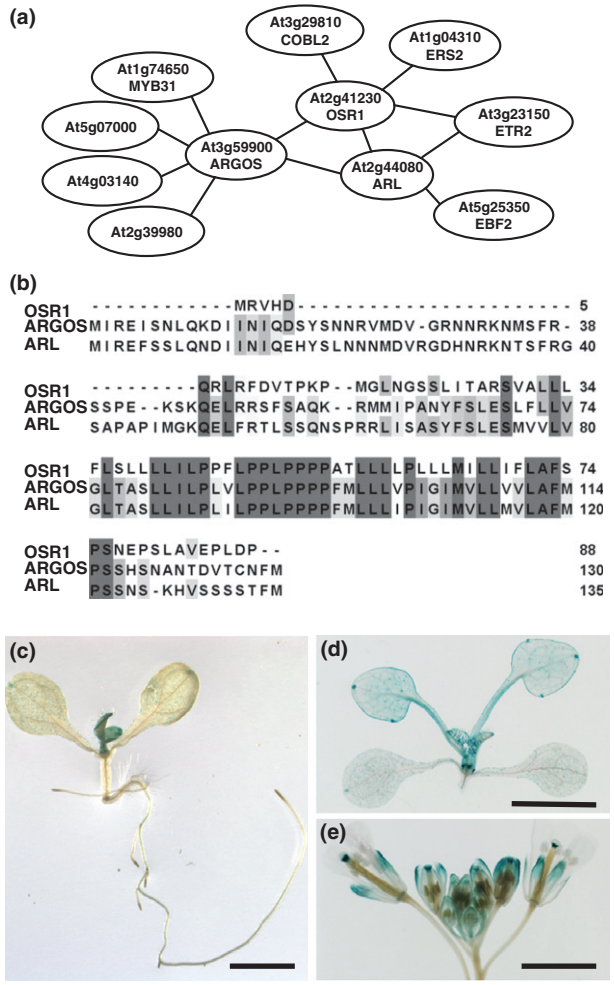

Fig. 1 *ORGAN SIZE RELATED1* (*OSR1*) expression and alignment of OSR1, ARGOS and ARGOS-LIKE (ARL). (a) Co-expression network of *OSR1*, *ARGOS* and *ARL* predicted using the *Arabidopsis thaliana trans*-factor and *cis*-element database (ATTED II) (http://atted.jp/). (b) Alignment of derived OSR1 with ARGOS and ARL. Shading types represent different levels of amino acid identity. (c–e) Tissue-specific expression of *OSR1*. GUS activity was assayed in transgenic plants harbouring *proOSR1-GUS*: (c) a 7-d-old seedling; (d) a 15-d-old plant; (e) flowers. Bars, 5 mm.

The publicly available cDNA of *At2g41230* is 749 bp long, and encodes a predicted novel protein of 88 amino acids (Fig. 1b). We named *At2g41230 ORGAN SIZE RELATED1* (*OSR1*). Surprisingly, a detailed protein alignment revealed that OSR1 appeared to contain a motif that is conserved in ARGOS and ARL (Fig. 1b).

To further determine the tissue-specific expression of *OSR1*, we generated transgenic Arabidopsis plants carrying an *OSR1* promoter::β-glucuronidase (*proOSR1-GUS*) fusion gene and examined GUS activities in transgenic plants. In 7-d-old seedlings, abundant GUS staining was detected in leaf primordia, and low GUS expression was detected in cotyledons (Fig. 1c). In expanding leaves, GUS staining was still detectable in the blade and the petiole, especially at the dispersed meristematic regions and the leaf margin (Fig. 1d). High GUS expression was also observed in floral organs (Fig. 1e).

Ectopic expression of *OSR1* increases final organ size predominantly by enhancing cell proliferation

To test whether *OSR1* has a function in the regulation of organ size, as does *ARGOS*, we generated transgenic *35S-OSR1* plants and examined their organ development. Compared with control lines, which contained an empty vector, all of 20 independently transgenic lines overexpressing *OSR1* displayed visibly enlarged aerial organs, including leaves, cotyledons, and floral organs (Fig. 2a,b, Table 1). As a result, the heights of transgenic plants were also increased (Table 1). RNA gel blot analysis of three independent homozygous T3 lines confirmed the high expression levels of the *OSR1* transgene in these transgenic plants (Fig. 2c). Detailed characterization of the fifth rosette leaf and the petal showed that, although the shape of both the leaf and the petal in *35S-OSR1* transgenic plants was not altered, the average areas of the leaf blade and the petal in *35S-OSR1* increased by *c.* 80% when compared with those in control plants (Fig. 2b,d). Moreover, overexpression of *OSR1* resulted in delayed flowering and longer roots (Table 1). These observations indicate that *OSR1* has a role in regulation of organ growth and final organ size.

Further comparison of the growth kinetics of the fifth leaves between control and *35S-OSR1* plants showed that

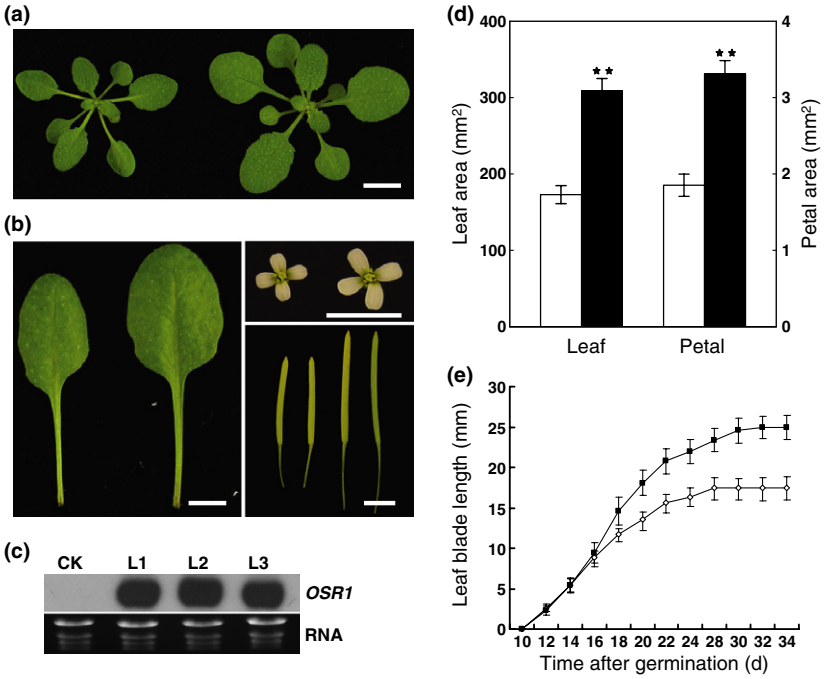

Fig. 2 Morphology of *35S-ORGAN SIZE RELATED1* (*OSR1*) transgenic plants. (a) Control (left) and *35S-OSR1* (right) 21-d-old transgenic plants. Bar, 1 cm. (b) Fully expanded fifth leaf (left panel), flower (right top panel) and silique (right bottom panel) in control (left) and *35S-OSR1* plants (right). Bars, 5 mm. (c) RNA blot analysis of the *OSR1* transgene in *35S-OSR1* plants. Total RNAs from a control line (CK) and three independent *35S-OSR1* lines (L1–L3) were assayed for RNA gel blot by *OSR1* coding region. (d) The areas of fully expanded fifth leaf blades and petals in control (CK; open bars) and *35S-OSR1* (closed bars) plants. At least six plants for each genotype were examined, and data are shown as mean ± SE; Student's *t*-test: **, $P < 0.01$. (e) Growth kinetics of the fifth leaves in CK (open symbols) and *35S-OSR1* (closed symbols) plants. The blade length was determined from at least10 leaves for each genotype after their emergence at 2-d intervals.

Table 1 Phenotype of *35S-ORGAN SIZE RELATED1* (*OSR1*) transgenic plants

Variable	Control	*35S-OSR1*
Cotyledon area (mm^2)	2.66 ± 0.37 (n = 10)	4.21 ± 0.48 (n = 10)**
Root length (mm)	24.4 ± 2.1 (n = 20)	30 ± 2.9 (n = 20)*
Flowering time (d)	28.1 ± 1.1 (n = 16)	34.3 ± 1.3 (n = 16)**
Silique length (mm)	12.6 ± 0.7 (n = 20)	16.3 ± 1.5 (n = 20)*
Plant height (cm)	32.6 ± 1.9 (n = 8)	42.1 ± 3.2 (n = 8)**

Student's *t*-test: *, $P < 0.05$; **, $P < 0.01$.

the leaf blades expanded at a similar rate from emergence to 5 d, and the size difference in the two genotypes was produced mainly afterwards (Fig. 2e), suggesting that the size increase in the *35S-OSR1* leaves may result from prolonged cell expansion and/or cell proliferation. To assess the contributions of cell proliferation and expansion to the increased organ size in *35S-OSR1* plants, we first visualized the palisade cells of fully expanded fifth leaves in the two genotypes. As shown in Fig. 3(a,b), the average size of palisade cells in *35S-OSR1* increased by *c.* 20% compared with that of controls, while the estimated palisade cell number per leaf increased by > 50%, demonstrating that the enlarged leaf in *35S-OSR1* is a result of increases in both cell number and size. Similarly, the increase in petal size of *35S-OSR1* could also be attributed to increases in both cell number and cell size, with cell number contributing *c.* 60% of the enlargement (Fig. 3c,d). To further substantiate the predominant role of *OSR1* in cell proliferation, we also introduced *proCycB1;1-Dbox-GUS* into *35S-OSR1* plants. As the CycB1;1-GUS reporter marks the cells at the G2-M phase (Criqui *et al.*, 2001), the enhanced GUS activities observed in young leaves and the root meristem region of *35S-OSR1* seedlings indicated the presence of more proliferating cells in the transgenic plants than in the control plants (Fig. S2). Therefore, we conclude that the size increase in *35S-OSR1* organs results from increases in both cell proliferation and cell expansion, with cell proliferation as the dominant factor.

OSR1 modulates cell proliferation through the expression of *ANT*

As *35S-OSR1* and *35S-ARGOS* plants are similar in terms of their organs being enlarged primarily through enhanced growth by cell proliferation, we suspected that *OSR1* modulates cell proliferation through the regulation of *ANT*, as does *ARGOS*. To test this hypothesis, we first compared the expression of *ANT* and *CycD3;1* in both expanding and

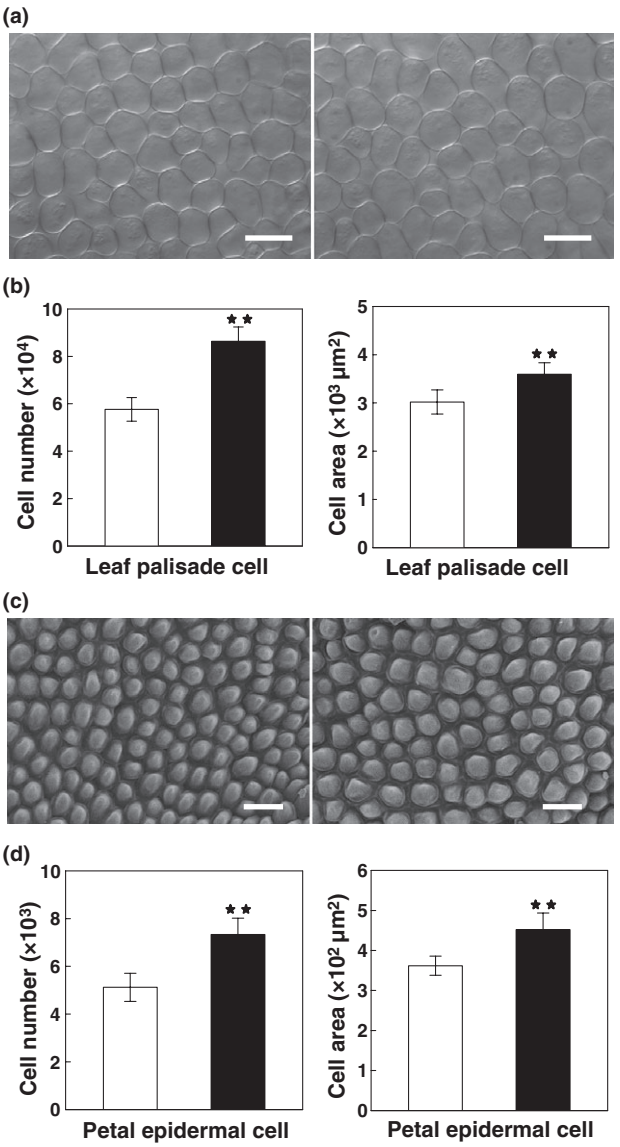

Fig. 3 Cytological characterization of *35S-ORGAN SIZE RELATED1* (*OSR1*) transgenic plants. (a) Palisade cells of the fully expanded fifth leaf in CK (left) and *35S-OSR1* (right) plants. Bars, 100 μm. (b) Estimated palisade cell number per leaf (left) and palisade cell area (right) in CK (open bars) and *35S-OSR1* (closed bars) plants. At least six fully expanded fifth blades from each genotype were photographed for determination of leaf area, and then cleared and visualized under a microscope to determine the palisade cell area and the estimated palisade cell number per leaf. Data are shown as mean ± SE; Student's *t*-test: **, $P < 0.01$. (c) The adaxial epidermal cells of petals in CK (left) and *35S-OSR1* (right) plants. Bars, 20 μm. (d) Estimated epidermal cell number per petal (left) and petal cell area (right) in CK (open bars) and *35S-OSR1* (closed bars) plants. The petals from four flowers in each genotype were used for determination of petal area, epidermal cell area and estimated cell number. Data represent mean ± SE; Student's *t*-test: **, $P < 0.01$.

expanded leaves between control and *35S-OSR1* plants. Similar to those observed in *35S-ARGOS* plants, the expression levels of *ANT* and *CycD3;1* in expanding leaves were comparable between the two genotypes. However, persistent expression of *ANT* and *CycD3;1* was detected even in leaves of 40-d-old *35S-OSR1* plants (Fig. 4a). This finding demonstrates that overexpression of *OSR1* prolonged the expression of *ANT* and *CycD3;1*, and thus the duration of cell proliferation, during leaf development. We then introduced the *ant-1* mutation into *35S-OSR1* transgenic plants, and, as expected, the *ant-1* mutation dramatically blocked leaf enlargement in *35S-OSR1* plants (Fig. 4b). Because ANT stimulates organ growth by promoting cell proliferation (Mizukami & Fischer, 2000), we reasoned that the slight size increase in *35S-OSR1/ant-1* leaves may be attributable to the effect of *OSR1* on cell expansion. To test this hypothesis, we carefully determined palisade cell size and the number of fully expanded fifth leaves in *ant-1* and *35S-OSR1/ant-1* plants, and found that the *ant-1* mutation indeed completely blocked the effect of *35S-OSR1* on cell proliferation, but not its effect on cell expansion (Fig. 4c,d), confirming that *OSR1*-mediated cell proliferation, but not cell expansion, occurs through the ANT pathway during organogenesis.

The OSR domain is sufficient to promote organ growth

As OSR1 shares a conserved domain with ARGOS and ARL, which comprises an identical LPPLPPPP motif and two putative transmembrane helices (Fig. 5a), we named it the Organ Size Related (OSR) domain. It is likely that the OSR domain is responsible for the function of the three members of the OSR family. To test whether this is the case, we generated transgenic plants overexpressing different truncated *OSR1* coding regions and examined their final leaf sizes. As shown in Fig. 5(b,c), transgenic plants harbouring a transgene that encodes a truncated OSR1 protein with an intact OSR domain or the OSR domain alone still exhibited enlarged organs, as did *35S-OSR1* plants. By contrast, overexpression of a transgene with a disrupted *OSR* domain could not recapitulate the organ phenotype of *35S-OSR1* plants. Consistently, overexpression of the OSR domain in ARGOS and ARL also resulted in the organ phenotypes of *35S-ARGOS* and *35S-ARL*, respectively (Fig. 5b,c). These results demonstrate that the OSR domain is essential and sufficient for promoting organ growth.

OSR proteins are ER-localized

To elucidate the biological roles of OSR proteins, we first visualized subcellular localization of the OSR1 protein in transgenic plants carrying *35S-OSR1-GFP*. The increased organ size in the transgenic plants indicated that the fusion gene was functional (Fig. S3a). The GFP florescence signals observed in root cells before and after plasmolysis suggested that OSR1 was localized in the cytoplasm (Fig. 6a). Because there are two predicted transmembrane

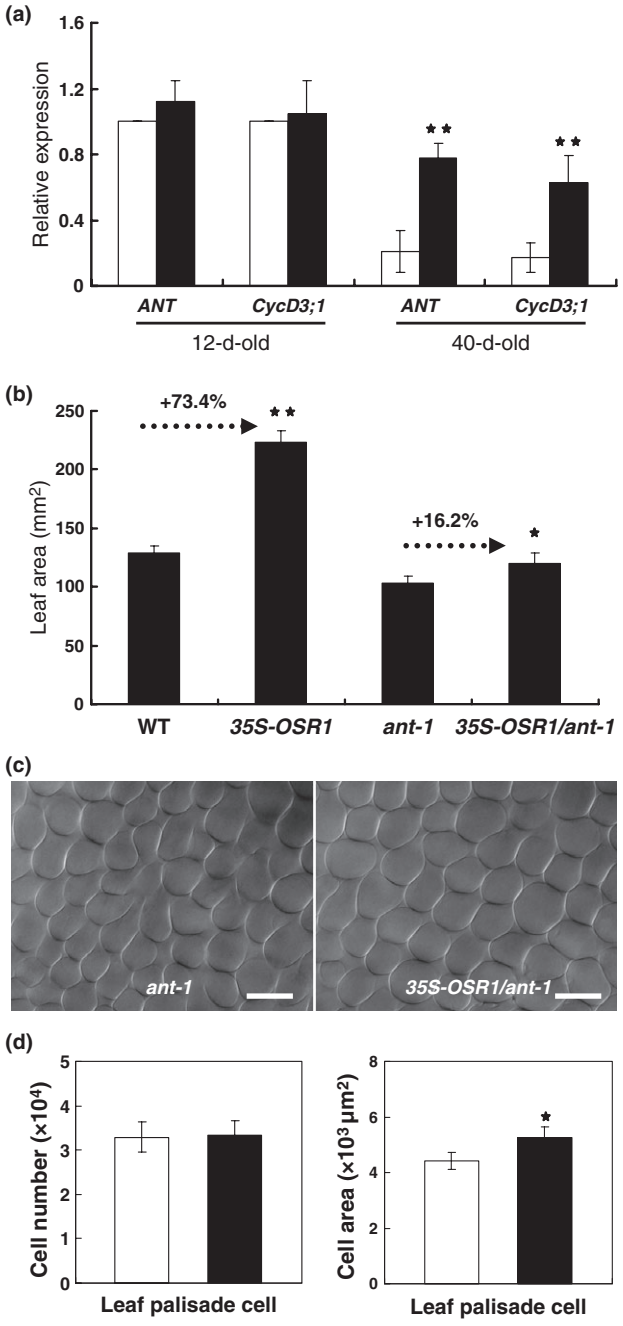

Fig. 4 ORGAN SIZE RELATED1 (OSR1)-mediated cell proliferation is AINTEGUMENTA (ANT)-dependent. (a) Prolonged expression of *ANT* and *CycD3;1* in *35S-OSR1* plants (control line (CK), open bars; *35S-OSR1*, closed bars). qRT-PCRs were performed with RNAs isolated from the leaves of 12-d-old (bars on left) and 40-d-old (bars on right) plants. Data are shown as mean ± SE for three biological replicates; Students' *t*-test: **, $P < 0.01$. (b) Blade areas of the fifth leaves in wild-type, *35S-OSR1*, *ant-1* and *35S-OSR1/ant-1* plants. Data are shown as mean ± SE for at least six blades in each genotype. Student's *t*-test: *, $P < 0.05$; **, $P < 0.01$. (c) Palisade cells of the fully expanded fifth leaf in *ant-1* and *35S-OSR1/ant-1* plants. Scale bars, 100 μm. (d) Palisade cell area and estimated palisade cell number per leaf in *ant-1* (open bars) and *35S-OSR1/ant-1* (closed bars) plants. Data are shown as mean ± SE for six blades in each genotype; Student's *t*-test: *, $P < 0.05$.

helices in OSR1, we suspected that they may be localized to the endomembrane system, probably to the ER. We then investigated whether the OSR1-GFP fusion protein co-localized with the ER marker protein Bip-RFP in protoplasts. As shown in Fig. 6(b), the green fluorescence signals of OSR1-GFP fusion proteins closely overlapped the red fluorescence signals of Bip-RFP, indicating that OSR1 is localized to the ER. Similarly, the other two OSR proteins, ARGOS and ARL, were also found to be ER-localized (Fig. S3a–e).

OSR1 regulates organ growth redundantly with *ARGOS* and *ARL*

To further investigate the relationships among *OSR1*, *ARGOS* and *ARL* in organogenesis, we obtained the T-DNA insertion mutant *osr1-1* (GABI_436G04) from Nottingham Arabidopsis Stock Centre (NASC) and *argos-1* (SAIL_896_G10) from Arabidopsis Biological Resource Center (ABRC), in which a T-DNA fragment was inserted into the *OSR1* coding region or the *ARGOS* 5′-untranslated region, respectively (Fig. S4a). RNA blot analysis revealed that *osr1-1* and *argos-1* were knock-out mutants (Fig. S4b). Neither *osr1-1* nor *argos-1* displayed an obvious reduction in organ size compared with wild-type plants. However, the *osr1-1 argos-1* double mutant displayed smaller organs, with a *c.* 12% reduction in the average blade area of the fully expanded fifth leaves compared with wild-type leaves (Fig. 7a,b), suggesting that *OSR1* and *ARGOS* are functionally redundant. As a mutant for the *ARL* gene is not available, we generated RNA-interfered *ARL* transgenic plants (*ARLi*) in the *osr1-1 argos-1* background, and found that underexpression of *ARL* resulted in just a slight further reduction in the average size of *osr1-1 argos-1* leaves (Fig. 7a,b, S4c). Nonetheless, reductions in palisade cell number and cell size were consistently observed in *osr1-1 argos-1* and *ARLi/ osr1-1 argos-1* plants (Fig. 7b). In addition, we also overexpressed *ARGOS* or *ARL* in *35S-OSR1* plants, and found that overexpression of neither *ARGOS* nor *ARL* further increased the size of *35S-OSR1* organs (Fig. S5a,b). Meanwhile, introduction of the *osr1-1* mutation into *35S-ARGOS* plants or the *argos-1* mutation into *35S-OSR1* plants did not attenuate organ enlargement in *35S-ARGOS* or *35S-OSR1* plants (Fig. S5c). These observations further support the hypothesis that these three genes are functionally redundant in organogenesis.

OSR1 is responsive to plant hormones and co-regulated with *ARGOS* and *ARL*

As *ARGOS* is induced by auxin and cytokinin and *ARL* by brassinosteroid (Hu *et al.*, 2003, 2006), we investigated whether *OSR1* is also responsive to plant hormones. qRT-PCR analysis in seedlings treated with various hormones

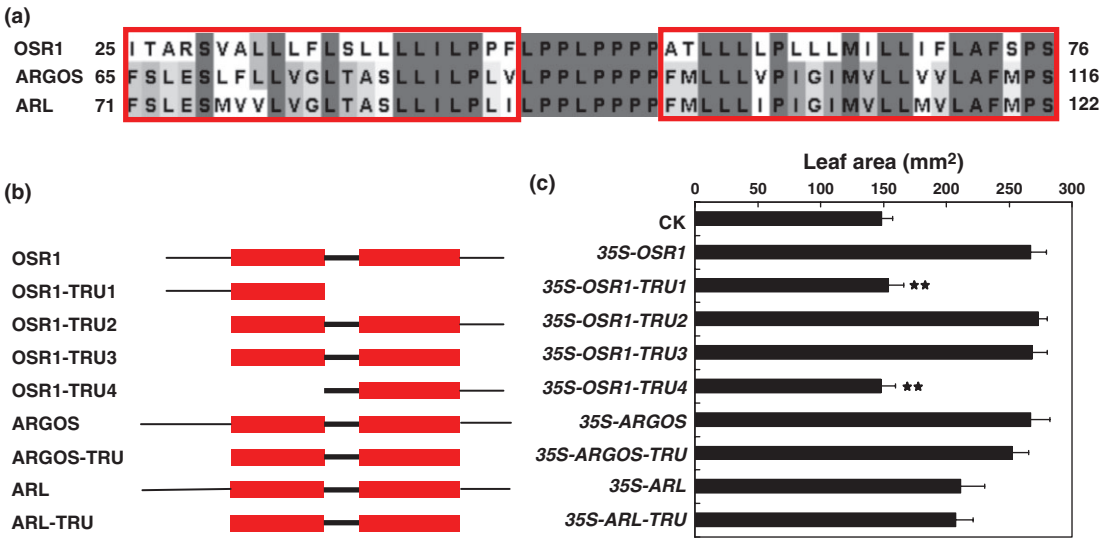

Fig. 5 The Organ Size Related (OSR) domain is functional in promoting organ growth. (a) The conserved OSR domain in OSR1, ARGOS and ARGOS-LIKE (ARL). Shading types represent different levels of amino acid identity, and two putative transmembrane helices are boxed. (b) Schematic illustration of the proteins encoded by *OSR1*, *ARGOS* and *ARL* and their truncated genes. The putative transmembrane helix is indicated as a red rectangle and the motif between two helices as a bold line. (c) Blade areas of the fifth leaves in transgenic plants overexpressing genes described in (b); data are shown as mean ± SE for at least eight plants in each genotype; Student's *t*-test: **, $P < 0.01$.

revealed that *OSR1* is induced by ethylene but repressed by ABA and epi-brassinolide (epi-BL) treatments (Fig. 8a), indicating that *OSR1* is also regulated by plant hormones, but the responses are different from those of *ARGOS* or *ARL*.

To further elucidate the regulation of *OSR* genes by plant hormones, we also identified the typical hormone-responsive elements in the *OSR* promoter regions, using the Plant *Cis*-acting Regulatory DNA Elements database (PLACE) (Higo *et al.*, 1999). As shown in Fig. 8(b), two ethylene-responsive elements of tomato *E4* (EREs), two ABA-responsive element-like (ABRE-like) elements and one GA-responsive element (GARE) were found in the *OSR1* promoter region. Within the *ARGOS* promoter, there were EREs, ABRE-like elements and GAREs as well as two typical auxin-responsive elements (AuxREs), which may confer the capacity for the induction of *ARGOS* by auxin, while a GARE and an ABRE-like element were identified in the *ARL* promoter region.

Because OSR1, ARGOS and ARL regulate organ growth in a redundant manner, we investigated whether they interact and whether their transcription is coordinated during organogenesis. We could not detect any *in vitro* interactions among them using the yeast two-hybrid assay (Fig. S6), suggesting that they may not function through homo- or heterodimerization. Interestingly, although we could not detect an obvious alteration of *ARGOS* and *ARL* mRNA levels in the *osr1-1* mutant, overexpression of *OSR1* resulted in suppression of *ARGOS* and *ARL* transcription (Fig. 8c). Similarly, overexpression of *ARGOS* also inhibited *ARL* expression (Fig. 8c). This finding suggests that the three *OSR* genes are co-regulated during organ development.

Phylogeny of *OSR-like* genes

Our finding suggests that the Arabidopsis *OSR* genes belong to a small family that functions in coordination of growth signals and development. To expand our knowledge of the evolutionary relationships among *OSR* genes, we performed a BLAST search with OSR domain in the databases and identified 39 predicted *OSR* homologues in 14 plant species. Phylogenetic analysis of these *OSR* homologues revealed that a single-copy *OSR* gene exists in the *Physcomitrella patens* genome, and multiple duplication events have occurred during the evolution of plant species (Fig. S7). The four Arabidopsis *OSR* homologues were probably generated through three gene duplication events. One gene duplication event happened before the divergence of core eudicots, giving rise to *ARGOS/ARL* and *OSR1*/At2g41225. Two recent independent gene duplications produced *ARGOS* and *ARL*, as well as *OSR1* and *At2g41225* (Fig. S7). *At2g41225* is located alongside *OSR1* in the genome, and appears to encode a protein containing an incomplete OSR domain (data not shown). In addition, although multiple *OSR* homologues were identified in grass species, such as *Oryza sativa*, *Sorghum bicolor* and *Zea mays*, they obviously experienced different evolutionary histories from Arabidopsis *OSR* genes (Fig. S7).

Discussion

A number of genes involved in organ size control in plants have been identified, and these genes are referred to as 'intrinsic yield genes' (IYGs) because of their potential to

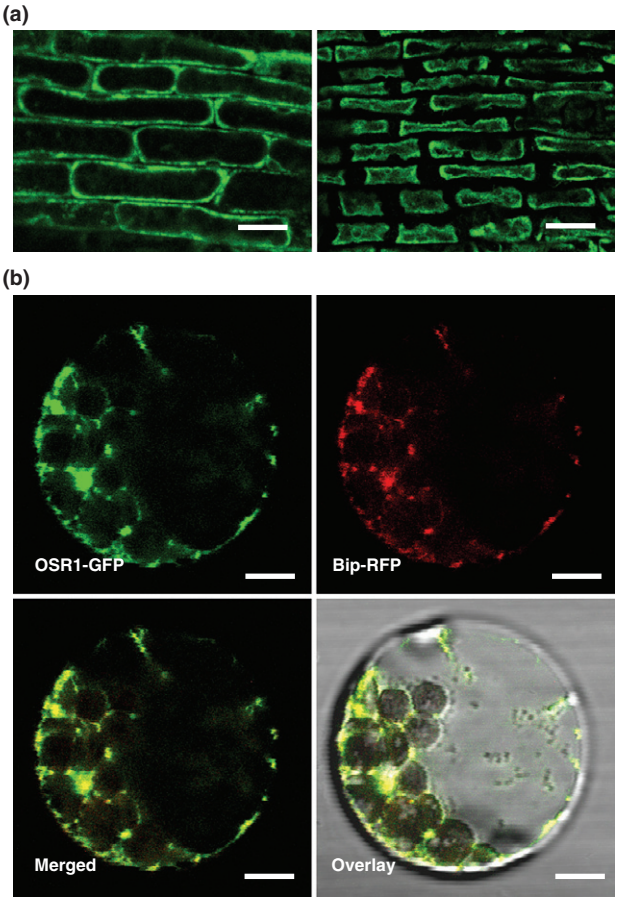

Fig. 6 ORGAN SIZE RELATED1 (OSR1) is localized to the endoplasmic reticulum. (a) GFP fluorescence was visualized with the root cells of 7-d-old seedlings carrying *35S-OSR1-GFP* before (left) and after (right) plasmolysis with 20% sucrose. Bars, 5 μm. (b) Protoplasts co-transformed with *OSR1-GFP* and *Bip-RFP*, a fusion gene encoding an ER marker protein. The merged image shows the overlap of green and red fluorescent signals, and the overlay represents the merged GFP and RFP signals overlaid with a bright field. Bars, 10 μm.

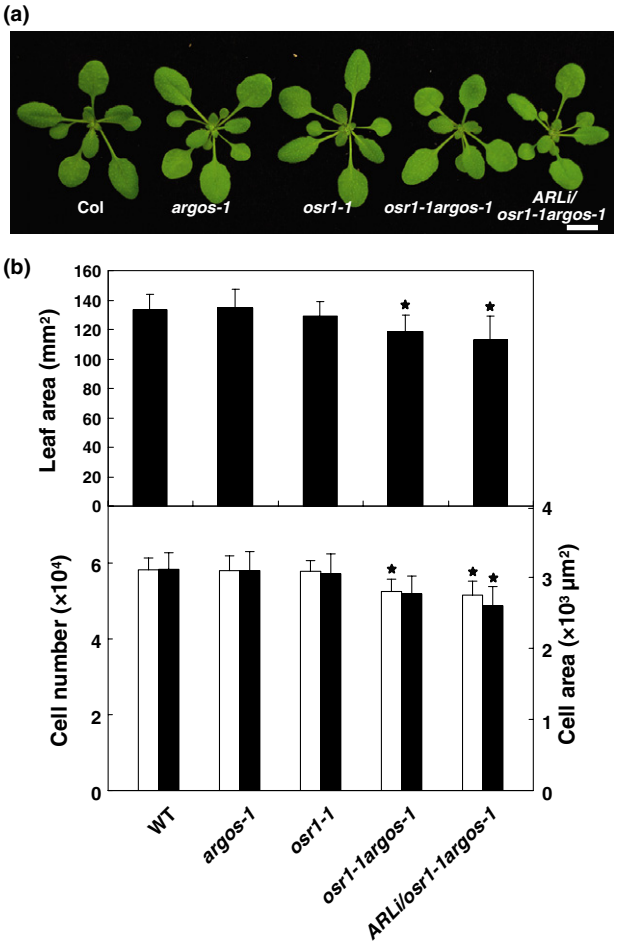

Fig. 7 *ORGAN SIZE RELATED1* (*OSR1*), *ARGOS* and *ARGOS-LIKE* (*ARL*) act redundantly. (a) Thirty-day-old plants of wild type (WT), *argos-1*, *osr1-1*, *osr1-1 argos-1* and *ARLi/osr1-1 argos-1* (from left to right). Bar, 1 cm. (b) Blade areas (upper panel), the palisade cell area (closed bars) and estimated palisade cell number (open bars) per fifth leaf (lower panel) of WT, *argos-1*, *osr1-1*, *osr1-1 argos-1* and *ARLi/osr1-1 argos-1* (from left to right). Data are shown as mean ± SE for at least six blades in each genotype; Student's *t*-test: *, $P < 0.05$.

boost the biomass and yield of agronomic plants (Gonzalez *et al.*, 2009; Krizek, 2009). However, most of these genes appear to regulate organ growth through independent pathways (Busov *et al.*, 2008; Gonzalez *et al.*, 2009), suggesting that the molecular mechanisms of organ size control in plants are more complicated than those in animals. Here, we identified the *OSR1* gene, which regulates organ growth by affecting both cell proliferation and cell expansion, and functions through prolonged expression of *ANT*, as does *ARGOS*. OSR1 shares low amino acid homology with ARGOS and ARL, but they do contain a relatively conserved OSR domain that is sufficient to promote organ growth. Although the molecular and biochemical mechanisms are still unclear, our characterization of *OSR1*, together with the previous work on *ARGOS* and *ARL*, reveals a small family of proteins that influence organ growth and final organ size by regulating cell proliferation and/or cell expansion.

In plants, organ growth is largely influenced by intrinsic and environmental signals, including plant hormones. Most plant hormones, such as auxin, cytokinin, BR and ethylene, have been demonstrated to play an important role in regulating cell proliferation and/or cell expansion (Tsukaya, 2002b, 2006). However, how these hormonal signals are integrated into the developmental program remains largely unknown. Although our observations indicate that the three *OSR* genes are functionally redundant, their transcription is regulated by different plant hormones. *ARGOS* is strongly induced by auxin and cytokinin, and the induction depends on AUXIN-RESISTANT1 (AXR1) (Hu *et al.*, 2003). *ARL*, in contrast, is induced by BR, and this induction relies on the BR recep-

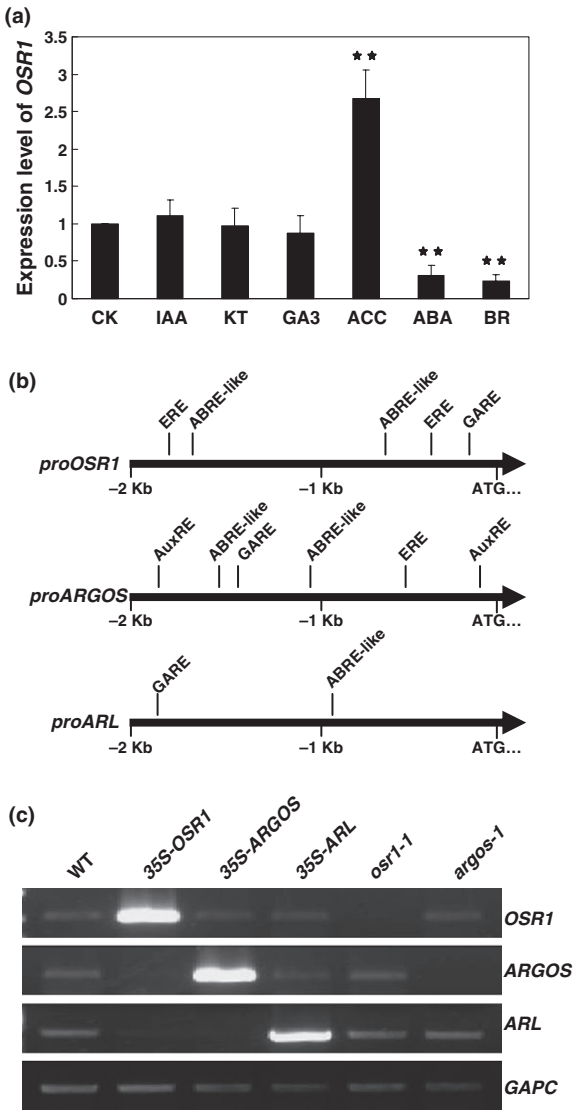

Fig. 8 Hormonal responses of *ORGAN SIZE RELATED1* (*OSR1*) and the co-regulation of *OSR* genes. (a) Transcriptional regulation of *OSR1* by plant hormones. qRT-PCR was performed with 12-d-old wild-type (WT) seedlings treated with 5 μM IAA, 5 μM kinetin (KT), 100 μM GA_3, 10 μM ACC, 2 μM ABA, 1 μM epi-brassinolide (epi-BL) or water (CK), respectively. Data are shown as mean ± SE for three biological replicates; Student's *t*-test: **, $P < 0.01$. (b) Hormone-responsive elements identified in *OSR1*, *ARGOS* and *ARL* promoter regions. ABRE-like, abscisic acid-responsive element-like; AuxRE, auxin-responsive element; ERE, ethylene-responsive element of tomato *E4*; GARE, GA-responsive element. (c) Expression of *OSR1*, *ARGOS* and *ARL* in WT, *35S-OSR1*, *35S-ARGOS*, *35S-ARL*, *osr1-1* and *argos-1* plants. Note the decreased levels of *ARGOS* and *ARL* mRNA in *35S-OSR1* plants and of *ARL* mRNA in *35S-ARGOS* plants.

tor BRI1 (Hu *et al.*, 2006). Furthermore, *OSR1* is up-regulated by ethylene but repressed by ABA and BR. These findings suggest that these plant hormones regulate organogenesis by modulating the expression of different *OSR* genes that perform similar molecular functions.

During organogenesis, growth of an organ to its characteristic size involves two overlapping and coordinated processes; that is, cell proliferation and cell expansion. For example, cell expansion and cell proliferation occur concurrently in a growing Arabidopsis leaf (Donnelly *et al.*, 1999). However, cell division and cell expansion are generally considered to be separately controlled (Neufeld *et al.*, 1998). Indeed, most identified factors involved in plant organ size control have been found to affect either cell proliferation or cell expansion; only a few factors, such as ARF2, ERBB-3 Binding Protein 1 (EBP1) and HERCULES1 (HRC1), have an impact on both cell proliferation and cell expansion (Horváth *et al.*, 2006; Century *et al.*, 2008; Gonzalez *et al.*, 2009). Most importantly, a compensatory mechanism between cell expansion and cell proliferation has often been found in studies of plant organ development (Tsukaya, 2002a). Although the *OSR* genes function in a redundant way at the organ level, the cellular mechanisms underlying such functions are apparently different among the three genes. *ARGOS* mainly affects the duration of cell proliferation (Hu *et al.*, 2003), whereas *ARL* mainly influences cell expansion (Hu *et al.*, 2006). *OSR1* predominantly regulates cell proliferation but also affects expansion. In addition, overexpression of *OSR1* or *ARGOS* suppresses the expression of *ARGOS* and/or *ARL*, suggesting that these genes are co-regulated in plants. Therefore, our results, together with the recent work on *OsARGOS* (Wang *et al.*, 2009), suggest that the members of the OSR family may be co-evolved factors that are involved in the coordination of cell proliferation and expansion in plant development.

Taken together, our results define a plant-specific pathway by which the co-evolved members of the OSR family integrate plant growth signals into the regulation of cell proliferation and expansion during organ growth (Fig. S8). This pathway, presumably, acts together with other identified or as yet unknown pathways, to control organ development and determine overall organ size in plants. However, the molecular mechanism by which *OSR* genes regulate cell proliferation and/or cell expansion remains unclear. OSR proteins are ER-localized, and they must function together with other molecules to regulate the expression of *ANT* and/or other genes that affect cell division and expansion machineries. Thus, identification of the proteins interacting with OSR will further our understanding of how cell proliferation and cell expansion are coordinated in plant growth and development. Finally, the increase in biomass in *OSR1*-overexpressing plants suggests that *OSR1* or its orthologues may be potential targets for genetic engineering to increase crop yields.

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References

Anastasiou E, Kenz S, Gerstung M, MacLean D, Timmer J, Fleck C, Lenhard M. 2007. Control of plant organ size by *KLUH/ CYP78A5*-dependent intercellular signaling. *Developmental Cell* **13**: 843–856.

Anastasiou E, Lenhard M. 2007. Growing up to one's standard. *Current Opinion in Plant Biology* **10**: 63–69.

Autran D, Jonak C, Belcram K, Beemster GT, Kronenberger J, Grandjean O, Inzé D, Traas J. 2002. Cell numbers and leaf development in Arabidopsis: a functional analysis of the *STRUWWELPETER* gene. *The EMBO Journal* **21**: 6036–6049.

Busov VB, Brunner AM, Strauss SH. 2008. Genes for control of plant stature and form. *New Phytologist* **177**: 589–607.

Century K, Reuber TL, Ratcliffe OJ. 2008. Regulating the regulators: the future prospects for transcription-factor-based agricultural biotechnology products. *Plant Physiology* **147**: 20–29.

Clay NK, Nelson T. 2005. The recessive epigenetic swellmap mutation affects the expression of two step II splicing factors required for the transcription of the cell proliferation gene *STRUWWELPETER* and for the timing of cell cycle arrest in the *Arabidopsis* leaf. *Plant Cell* **17**: 1994–2008.

Criqui MC, Weingartner M, Capron A, Parmentier Y, Shen WH, Heberle-Bors E, Bögre L, Genschik P. 2001. Sub-cellular localisation of GFP-tagged tobacco mitotic cyclins during the cell cycle and after spindle checkpoint activation. *Plant Journal* **28**: 569–581.

Cui R, Han J, Zhao S, Su K, Wu F, Du X, Xu Q, Chong K, Theißen G, Meng Z. 2010. Functional conservation and diversification of class E floral homeotic genes in rice (*Oryza sativa*). *Plant Journal* **61**: 767–781.

Dinneny JR, Yadegari R, Fischer RL, Yanofsky MF, Weigel D. 2004. The role of *JAGGED* in shaping lateral organs. *Development* **131**: 1101–1110.

Disch S, Anastasiou E, Sharma VK, Laux T, Fletcher JC, Lenhard M. 2006. The E3 ubiquitin ligase BIG BROTHER controls *Arabidopsis* organ size in a dosage-dependent manner. *Current Biology* **16**: 272–279.

Dong J, Feldmann G, Huang J, Wu S, Zhang N, Comerford SA, Gayyed MF, Anders RA, Maitra A, Pan D. 2007. Elucidation of a universal size-control mechanism in *Drosophila* and mammals. *Cell* **130**: 1120–1133.

Donnelly PM, Bonetta D, Tsukaya H, Dengler RE, Dengler NG. 1999. Cell cycling and cell enlargement in developing leaves of Arabidopsis. *Developmental Biology* **215**: 407–419.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**: 1792–1797.

Gonzalez N, Beemster GT, Inzé D. 2009. David and Goliath: what can the tiny weed Arabidopsis teach us to improve biomass production in crops? *Current Opinion in Plant Biology* **12**: 157–164.

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52**: 696–704.

Ha CM, Kim GT, Kim BC, Jun JH, Soh MS, Ueno Y, Machida Y, Tsukaya H, Nam HG. 2003. The *BLADE-ON-PETIOLE 1* gene controls leaf pattern formation through the modulation of meristematic activity in Arabidopsis. *Development* **130**: 161–172.

Higo K, Ugawa Y, Iwamoto M, Korenaga T. 1999. Plant *cis*-acting regulatory DNA elements (PLACE) database: 1999. *Nucleic Acids Research* **27**: 297–300.

Horiguchi G, Kim GT, Tsukaya H. 2005. The transcription factor AtGRF5 and the transcription coactivator AN3 regulate cell proliferation in leaf primordia of *Arabidopsis thaliana*. *Plant Journal* **43**: 68–78.

Horváth BM, Magyar Z, Zhang Y, Hamburger AW, Bakó L, Visser RG, Bachem CW, Bögre L. 2006. EBP1 regulates organ size through cell growth and proliferation in plants. *The EMBO Journal* **25**: 4909–4920.

Hu Y, Bao F, Li J. 2000. Promotive effect of brassinosteroids on cell division involves a distinct *CycD3*-induction pathway in Arabidopsis. *Plant Journal* **24**: 693–701.

Hu Y, Poh HM, Chua NH. 2006. The Arabidopsis *ARGOS-LIKE* gene regulates cell expansion during organ growth. *Plant Journal* **47**: 1–9.

Hu Y, Xie Q, Chua NH. 2003. The Arabidopsis auxin-inducible gene *ARGOS* controls lateral organ size. *Plant Cell* **15**: 1951–1961.

Hu Z, Qin Z, Wang M, Xu C, Feng G, Liu J, Meng Z, Hu Y. 2010. The Arabidopsis SMO2, a homologue of yeast TRM112, modulates progression of cell division during organ growth. *Plant Journal* **61**: 600–610.

Jin JB, Kim YA, Kim SJ, Lee SH, Kim DH, Cheong GW, Hwang I. 2001. A new dynamin-like protein, ADL6, is involved in trafficking from the *trans*-Golgi network to the central vacuole in Arabidopsis. *Plant Cell* **13**: 1511–1526.

Jing Y, Cui D, Bao F, Hu Z, Qin Z, Hu Y. 2009. Tryptophan deficiency affects organ growth by retarding cell expansion in Arabidopsis. *Plant Journal* **57**: 511–521.

Kim DH, Eu YJ, Yoo CM, Kim YW, Pih KT, Jin JB, Kim SJ, Stenmark H, Hwang I. 2001. Trafficking of phosphatidylinositol 3-phosphate from the *trans*-Golgi network to the lumen of the central vacuole in plant cells. *Plant Cell* **13**: 287–301.

Kim GT, Tsukaya H, Uchimiya H. 1998. The *ROTUNDIFOLIA3* gene of Arabidopsis thaliana encodes a new member of the cytochrome P-450 family that is required for the regulated polar elongation of leaf cells. *Genes & Development* **12**: 2381–2391.

Kim JH, Choi D, Kende H. 2003. The AtGRF family of putative transcription factors is involved in leaf and cotyledon growth in Arabidopsis. *Plant Journal* **36**: 94–104.

Krizek BA. 1999. Ectopic expression of *AINTEGUMENTA* in Arabidopsis plants results in increased growth of floral organs. *Developmental Genetics* **25**: 224–236.

Krizek BA. 2009. Making bigger plants: key regulators of final organ size. *Current Opinion in Plant Biology* **12**: 17–22.

Lee BH, Ko JH, Lee S, Lee Y, Pak JH, Kim JH. 2009. The Arabidopsis *GRF-INTERACTING FACTOR* gene family performs an overlapping function in determining organ size as well as multiple developmental properties. *Plant Physiology* **151**: 655–668.

Li Y, Zheng L, Corke F, Smith C, Bevan MW. 2008. Control of final seed and organ size by the *DA1* gene family in *Arabidopsis thaliana*. *Genes & Development* **22**: 1331–1336.

Mizukami Y. 2001. A matter of size: developmental control of organ size in plants. *Current Opinion in Plant Biology* **4**: 533–539.

Mizukami Y, Fischer RL. 2000. Plant organ size control: *AINTEGUMENTA* regulates growth and cell numbers during organogenesis. *Proceedings of the National Academy of Sciences, USA* **97**: 942–947.

Neufeld TP, de la Cruz AF, Johnston LA, Edgar BA. 1998. Coordination of growth and cell division in the *Drosophila* wing. *Cell* **93**: 1183–1193.

Nicholas KB, Nicholas HB Jr, Deerfield DW II. 1997. GeneDoc: analysis and visualization of genetic variation. *Embnew News* **4**: 1–4.
Ohno CK, Reddy GV, Heisler MG, Meyerowitz EM. 2004. The Arabidopsis *JAGGED* gene encodes a zinc finger protein that promotes leaf tissue development. *Development* **131**: 1111–1122.
Schmid M, Davison TS, Henz SR, Pape UJ, Demar M, Vingron M, Schölkopf B, Weigel D, Lohmann JU. 2005. A gene expression map of *Arabidopsis thaliana* development. *Nature Genetics* **37**: 501–506.
Schruff MC, Spielman M, Tiwari S, Adams S, Fenby N, Scott RJ. 2006. The *AUXIN RESPONSE FACTOR 2* gene of Arabidopsis links auxin signalling, cell division, and the size of seeds and other organs. *Development* **133**: 251–261.
Szécsi J, Joly C, Bordji K, Varaud E, Cock JM, Dumas C, Bendahmane M. 2006. BIGPETALp, a bHLH transcription factor is involved in the control of *Arabidopsis* petal size. *The EMBO Journal* **25**: 3912–3920.
Tsukaya H. 2002a. Interpretation of mutants in leaf morphology: genetic evidence for a compensatory system in leaf morphogenesis that provides a new link between cell and organismal theories. *International Review of Cytology* **217**: 1–39.
Tsukaya H. 2002b. Leaf development. In: Somerville CR, Meyerowitz EM eds. *The Arabidopsis book*. Rockville, MD, USA: American Society of Plant Biologists: e0072. doi:10.1199/tab.0072, URL: http://www.bioone.org/doi/pdf/10.1199/tab.0072.
Tsukaya H. 2003. Organ shape and size: a lesson from studies of leaf morphogenesis. *Current Opinion in Plant Biology* **6**: 57–62.
Tsukaya H. 2005. Leaf shape: genetic controls and environmental factors. *The International Journal of Developmental Biology* **49**: 547–555.
Tsukaya H. 2006. Mechanism of leaf-shape determination. *Annual Review of Plant Biology* **57**: 477–496.
Tsukaya H, Beemster GT. 2006. Genetics, cell cycle and cell expansion in organogenesis in plants. *Journal of Plant Research* **119**: 1–4.
Wang B, Sang Y, Song J, Gao XQ, Zhang X. 2009. Expression of a rice *OsARGOS* gene in Arabidopsis promotes cell division and expansion and increases organ size. *Journal of Genetics and Genomics* **36**: 31–40.
White DW. 2006. *PEAPOD* regulates lamina size and curvature in Arabidopsis. *Proceedings of the National Academy of Sciences, USA* **103**: 13238–13243.
Winter D, Vinegar B, Nahal H, Ammar R, Wilson GV, Provart NJ. 2007. An ''Electronic Fluorescent Pictograph'' browser for exploring and analyzing large-scale biological data sets. *PLoS ONE* **2**: e718.
Zhang X, Henriques R, Lin SS, Niu QW, Chua NH. 2006. *Agrobacterium*-mediated transformation of *Arabidopsis thaliana* using the floral dip method. *Nature Protocols* **1**: 641–646.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Expression profiles of *ORGAN SIZE RELATED1* (*OSR1*), *ARGOS* and *ARGOS-LIKE* (*ARL*).

Fig. S2 *CycB1;1-GUS* expression in *35S-OSR1* organs.

Fig. S3 Subcellular localization of ARGOS and ARGOS-LIKE (ARL).

Fig. S4 Molecular characterization of *organ size related1-1* (*osr1-1*), *argos-1* and *35S-ARLi* transgenic plants.

Fig. S5 Genetic redundancy of *ORGAN SIZE RELATED1* (*OSR1*), *ARGOS* and *ARGOS-LIKE* (*ARL*).

Fig. S6 Yeast two-hybrid assays for members of the ORGAN SIZE RELATED (OSR) family.

Fig. S7 Phylogenetic tree of the predicted *ORGAN SIZE RELATED* (*OSR*) homologues.

Fig. S8 A proposed model for roles of *ORGAN SIZE RELATED* (*OSR*) genes in organogenesis.

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