Ribosomal proteins promote leaf adaxial identity

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Establishing abaxial-adaxial polarity is central to leaf morphogenesis and function. Groups of genes that encode different components for leaf patterning have been identified in recent years. These include transcriptional factors, small RNAs, 26S proteasome and components required for post-transcriptional gene silencing and chromatin remodeling, showing a complex regulatory network and indicating that the regulation occurs at different levels. In this work, we report the identification and characterization of asymmetric leaves1/2 enhancer5 (ae5) and ae6 mutants. These two mutants had a phenotype of abnormal leaf patterning, with the abaxial mesophyll features appearing in the adaxial mesophyll domain, and double mutants ae5 as1/2 and ae6 as1/2 producing severely abaxialized leaves. AE5 and AE6 encode the ribosomal large subunit proteins RPL28A and RPL5A, respectively, and mutations in two other ribosomal protein genes, RPL5B and RPL24B, resulted in plant phenotypes similar to those of ae5 and ae6. Because these four ribosomal proteins are located in distinct sites in the ribosomal large subunit, we propose that the conserved translational function of the ribosome may be required for regulating key components during leaf patterning. Collectively, our data indicate that specific ribosome subunit-mediated translational control is essential in leaf polarity establishment.

KEY WORDS: Arabidopsis, ASYMMETRIC LEAVES1/2, Leaf development, Polarity establishment, Ribosomal proteins

INTRODUCTION

During leaf development, leaf primordia first initiate as a group of histologically uniform cells from the peripheral zone of the shoot apical meristem. Further development of the leaf primordia results in their patterning along three-dimensional axes: the adaxial-abaxial, proximodistal and mediolateral axes. A series of surgical experiments carried out by Sussex more than 50 years ago (Sussex, 1998; McConnell and Barton, 1998; McConnell et al., 2001; Emery et al., 2003; Zhong and Ye, 2004). Transcripts of these three genes are the targets of microRNA165 and 166 (mir165/166) (Rhoades et al., 2002; Tang et al., 2003). Two other genes, ASYMMETRIC LEAVES1 (AS1) and AS2, have also been considered to promote the adaxial identity of leaves by positively regulating PHB, PHV and REV (Lin et al., 2003; Xu et al., 2003). More recently, AS1/AS2 were found to repress mir165/166 so that PHB, PHV and REV transcripts may be stabilized (Li et al., 2005; Fu et al., 2007; Ueno et al., 2007). Recently, genes functioning in the trans-acting siRNA pathway have been uncovered that facilitate adaxial cell fate of leaves, including RNA-DEPENDENT RNA POLYMERASE6 (RDR6), SUPPRESSOR OF GENE SILENCING3 (SG3), ZIPPY (ZIP) and DICER-LIKE4 (DCL4) (Peragine et al., 2004; Li et al., 2005; Garcia et al., 2006; Xu et al., 2006).

In addition to the adaxial promoting components, several genes are required for leaf abaxial identity, including members of the KANADI (KAN) and YABBY (YAB) families (Sawa et al., 1999; Siegfried et al., 1999; Eshed et al., 2001; Kerstetter et al., 2001). KAN genes antagonize class III HD-ZIP genes (Emery et al., 2003), while the YABs are downstream genes of KAN (Eshed et al., 2004). Furthermore, two auxin response factor genes, AUXIN RESPONSE FACTOR3 (ARF3/ETT) and ARF4, promote abaxial identity (Pekker et al., 2005). ARF3 and ARF4 transcripts are the targets of a small RNA, termed tasiR-ARF (Allen et al., 2005). Finally, loss of function in 26S proteasome subunit genes results in plants with abaxialized leaves, suggesting that post-translational regulation is required for the normal leaf polarity (Huang et al., 2006).

Here, we implicate a new class of genes, the ribosomal large subunit protein encoding genes, in leaf polarity establishment. In animals, functions of the ribosomal proteins have been investigated extensively, and defects in these proteins can cause various kinds of diseases (Bilanges and Stokoe, 2007; Idol et al., 2007; Schepet et al., 2007) and development abnormalities (Oliver et al., 2004; Uechi et al., 2006; Marygold et al., 2007). Compared with those in animals, only a few ribosomal proteins in plants have been characterized. POINTED FIRST LEAVES1 (PFL1) and PFL2 encode the PRS18 and PRS13 ribosomal small subunit proteins, respectively, playing roles in leaf development (Van Lijsebettens et al., 1994; Ito et al., 2000). A semi-dominant mutation of the Arabidopsis MINUTE-LIKE1 gene, which encodes an additional ribosomal small subunit RPS5, led to early embryonic developmental defects by disrupting cell division (Weijers et al., 2001). Finally, loss of function in the SHORT VALVE1 (STV1) gene, which encodes a ribosomal large subunit protein RPL24B, resulted in the apical-basal patterning defect of gynoecium by influencing ARF3 translation (Nishimura et al., 2005). All these results indicate that ribosomal proteins are widely involved in different plant developmental processes.

In the present study, we report characterizations of two ribosomal large subunit genes, RPL28A and RPL5A, both of which play important roles in specifying leaf adaxial identity. Moreover, we found that other two ribosomal protein genes, STV1 and RPL5B, also have this function in leaf pattern formation, indicating that specific

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Accepted 30 January 2008
RESULTS Identification of new leaf polarity-controlling genes \textit{RPL28A} and \textit{RPL5A}

In a genetic screen for enhancers and suppressors of the \textit{as2-101} mutant, we identified two mutant plants showing similar enhanced \textit{as2} phenotypes. Further genetic analyses of these two mutants resulted in the isolation of two candidate single \textit{as2} enhancer mutants, designated \textit{ae5-1} and \textit{ae6-1} for \textit{asymmetric leaves enhancer}. Of these two mutants, \textit{ae5-1} had pale green leaves while \textit{ae6-1} showed normal plant phenotypes. The identified candidate \textit{ae5} and \textit{ae6} single mutants were then crossed back to \textit{as2-101}, respectively, and the plants with the similar enhanced \textit{as2} phenotypes were observed in both F2 progeny.

To identify the \textit{AE5} and \textit{AE6} genes, we carried out map-based cloning by crossing \textit{as5-1 as2-101} and \textit{as6-1 as2-101} to wild-type Col-0 plants. Using about 2000 recombinant chromosomes each, we mapped \textit{AE5} and \textit{AE6} to the lower arm of chromosome 2 and the upper arm of chromosome 3 in a less than 30 kb region, respectively (Fig. 1A,B). We sequenced coding regions of all putative genes in the \textit{ae5} region and identified a gene containing a G-to-A substitution in the second exon, resulting in a premature stop codon (Fig. 1A). This gene (At2g19730) encodes RPL28A, a protein in the large subunit of the ribosome. We then analyzed the \textit{ae6} mapping region and found another ribosomal protein coding gene (At3g25520), \textit{RPL5A} (Fig. 1B). Although sequencing of the \textit{RPL5A}-coding region did not show any change in the nucleotide sequence, the expression level of the \textit{RPL5A} gene was dramatically reduced (see Fig. S1 in the supplementary material). Subsequent analyses revealed that the 3' region of the gene was disrupted by an \textit{Arabidopsis} transposon \textit{Tag1} (Tsay et al., 1993), which may cause mRNA instability of this gene (see Fig. S1 in the supplementary material).

To further confirm by complementation that we had identified the correct genes, we transformed the \textit{ae5-1 as2-101} mutant with a complementation construct containing 1.3 kb of the \textit{RPL28A} gene plus 1.9 kb and 1.1 kb of its 5' and 3' regions (see Fig. S2A in the supplementary material). We also transformed the \textit{ae6-1 as2-101} mutant with a construct containing 1.8 kb of the \textit{RPL5A} gene together with 1.9 kb and 0.8 kb of the 5' and 3' regions (supplementary material Fig. S2B). A total of 20 and 25 transgenic lines, respectively, were obtained, of which all plants showed only \textit{as2-101} single mutant phenotypes (see Fig. S2C-F in the supplementary material), indicating that \textit{RPL28A} and \textit{RPL5A} indeed correspond to the enhanced \textit{as2} phenotypes in the \textit{ae5-1 as2-101} and \textit{ae6-1 as2-101} mutant plants.

RT-PCR revealed that \textit{AE5} and \textit{AE6} were expressed in all plant tissues examined (Fig. 1C). In situ hybridization using the gene-specific sequence as probes showed that \textit{AE5} and \textit{AE6} transcripts were detected throughout the embryo (Fig. 1D,E) and leaf primordia (Fig. 1F,G) at earlier developmental stages. The same expression pattern was also detected in the reproductive organs, with the hybridization signals being throughout the inflorescence meristem, floral primordia and four types of young floral organs (Fig. 1H). The in situ hybridization experiments were performed with two types of negative controls: (1) sense probes (Fig. 1I); and (2) an insertional \textit{ae5-2} allele (Fig. 1J; for identification of \textit{ae5-2}, see Fig. S3 in the supplementary material). These results indicate that \textit{RPL28A} and \textit{RPL5A} are new regulators of leaf polarity despite their expression throughout leaf primordia.

Mutations of \textit{ae5} and \textit{ae6} affect leaf adaxial-abaxial polarity

To understand the functions of \textit{AE5} and \textit{AE6} in leaf development, we analyzed phenotypes of \textit{ae5/ae6 as1} and \textit{ae5/ae6 as2} mutants, as well as \textit{ae5} and \textit{ae6} single mutants. Compared with wild-type (Fig. 2A) and \textit{as2-101} (Fig. 2B) plants, both \textit{ae5-1 as2-101} (Fig. 2D) and \textit{ae6-1 as2-101} (Fig. 2E) displayed an increased number of lotus- and needle-like leaves (Table 1). Of these two double mutants, the \textit{ae5-1 as2-101} phenotypes appeared even more severe with almost all true leaves being needle-like in a proportion of the seedlings (Fig. 2D). Although most first two leaves in the \textit{ae6-1 as2-101} mutant were expanded, the adaxial surface of the leaves was rough (Fig. 2E). For the single mutants, \textit{ae6-1} had a phenotype similar to that of wild-type plants (Fig. 2G), whereas the early appearing leaves of \textit{ae5-1} were slightly longer and all leaves were pale green (Fig. 2F).
To characterize in more detail the developmental abnormalities of the ae5 and ae6 mutants, we analyzed phenotypes of two severe alleles, ae5-1 and ae6-2. Compared with the wild-type plants (Fig. 3A), although primordia of the first two rosette leaves in the mutants emerged normally (data not shown), subsequent leaf growth was delayed in ae5-1 (Fig. 3B) and ae6-2 (data not shown). In the first pair of fully expanded rosette leaves of wild-type plants, the lateral veins were evident only in the leaf blade (Fig. 3C,D). By contrast, vein branching in the ae5-1 (Fig. 3E, inset) and ae6-2 (Fig. 3F, inset) occurred more proximally in the petiole. In addition, the number of small veins that appear at later leaf developmental stages was reduced (Fig. 3E,F).

To determine the anatomical basis of the pale green leaves of ae5-1 and ae6-2 mutants, we analyzed their lamina structure by transverse sectioning. In wild-type Ler (Fig. 3G) and Col-0 (Fig. 3H) plants, four distinct cell types in the lamina of expanded leaves are recognizable along the adaxial-abaxial axis: adaxial epidermis, palisade mesophyll, spongy mesophyll, and abaxial epidermis. Of the two types of mesophyll cells, the adaxially located palisade cells are usually tightly arranged, whereas the abaxially positioned spongy cells are loosely arranged with intercellular spaces (Fig. 3G,H, arrows). However, in the ae5-1 and ae6-2 leaves, numerous intercellular spaces occurred in the adaxial palisade region (Fig. 3J, arrowheads), reflecting a disrupted leaf adaxial-abaxial axis. These results also suggest that the less tightly arranged palisade cells at least partially contribute to the pale green color of the ae5-1 and ae6-2 leaves.

Compared with the inflorescences in wild-type plants (Fig. 3K), those from some ae5-1 (Fig. 3L,M) and ae6-2 (data not shown) plants were abnormal. The inflorescence of some ae5-1 plants terminated early, by producing several secondary inflorescences (Fig. 3L). In wild-type plants, a secondary inflorescence is usually associated with a cauline leaf at its proximal end (Fig. 3K), whereas in ae5-1 plants a proportion of
secondary inflorescences lacked a subtending cauline leaf (Fig. 3M, arrowhead). Occasionally, some cauline leaves in ae5-1 (Fig. 3N) and ae6-2 (data not shown) formed ectopic outgrowths on their abaxial distal parts. All these results indicate that RPL28A and RPL5A are involved in multiple plant developmental processes.

ae5 as2 and ae6 as2 double mutants produce severely abaxialized leaves
To further understand the roles of RPL28A and RPL5A in leaf patterning, we analyzed leaf phenotypes of ae5 as2 and ae6 as2 double mutants by SEM. The double mutant plants produced three types of leaves from weak to strong phenotypes: (1) expanded
leaves, which were rough on the adaxial lamina surface (Fig. 4A, arrow); (2) lotus-like leaves, in which the petiole was centrally attached underneath the blade (Fig. 4B); and (3) needle-like leaves (Fig. 4C). In wild-type plants, epidermal cells of the adaxial lamina surface are relatively large and of uniform size (Fig. 4D), whereas those of the abaxial side are smaller and mixed with some long and narrow cells (Fig. 4E, arrowheads). The epidermal cell patterns of the ae5-1 (see Fig. S4 in the supplementary material) and ae6-1 (data not shown) single mutant leaves were normal. However, the adaxial surface of the expanded leaves in ae5-1 as2-101 (Fig. 4A) and ae6-1 as2-101 (data not shown) was a mosaic containing some normal adaxial (Fig. 4F) and abnormal abaxial (Fig. 4G) epidermal cell patches. Most epidermal cells on the needle-like leaves were rectangular (Fig. 4H), except those on the apical part of the leaves, which showed partial differentiation with the abaxially featured long cells (Fig. 4I, arrowhead).

In the blade-petiole junction region of wild-type leaves, vascular bundles showed a pattern where xylem develops on the adaxial pole and phloem is located on the abaxial pole (Fig. 4J). In the expanded leaves of the weak allele ae6-1 as2-101, adaxial extension of the phloem was apparent (Fig. 4K). By contrast, in the lotus-like leaf of the more severe ae5-1 as2-101 allele, the vascular bundle structure comprised a concentric ring of phloem surrounding the least-developed xylem cells in the center (Fig. 4L). This is a typical phenotype of the abaxialized leaf, and this phenotype supports the idea that AS1/2 and RPL28A/5A are required for promoting leaf adaxial cell fate.

To obtain molecular evidence for the RPL28A and RPL5A function in specifying leaf polarity, we examined by in situ hybridization the expression pattern of two marker genes, FIL and REV. In wild-type plants, FIL is usually expressed on the abaxial side of leaves (Siegfried et al., 1999) (Fig. 5A), and this pattern was

### Table 1. Frequencies of lotus- and needle-like leaves in the mutant plants

<table>
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<tr>
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<th>First two leaves</th>
<th>Third to sixth leaves</th>
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<tr>
<td></td>
<td>Total leaves¹</td>
<td>L/N leaves (%)²</td>
</tr>
<tr>
<td>as2-101</td>
<td>235</td>
<td>19.5</td>
</tr>
<tr>
<td>ae5-1 as2-101</td>
<td>186</td>
<td>46.8</td>
</tr>
<tr>
<td>ae6-1 as2-101</td>
<td>202</td>
<td>23.8</td>
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*Plants were grown at 22°C.

¹Total leaves that were analyzed.

²Frequency is defined by the ratio of the number of lotus (L)- and needle (N)-like leaves to the total number of corresponding leaves analyzed.

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Fig. 4. Aberrant adaxial-abaxial polarity in leaves of ae5-1 as2-101 and ae6-1 as2-101. (A-C) Three types of abnormal rosette leaves in ae5-1 as2-101. (A) An adaxial view of an expanded leaf showing a rough surface and some bumps (arrow). (B) A lotus-like leaf. (C) A needle-like leaf. (D-I) Analyses of the leaf epidermal patterns. (D,E) Epidermal cells on adaxial (D) and abaxial (E) surfaces of a wild-type leaf. (F,G) High magnification of epidermal cells in A. The same adaxial leaf side of ae5-1 as2-101 contained both adaxially (F) and abaxially (G) featured epidermal cells, which correspond to the boxed regions f and g in A, respectively. (H,J) High magnification of epidermal cells in C, corresponding to the boxed regions h and i, respectively. Arrowheads in E,G,I indicate long and narrow abaxially featured epidermal cells. (J-L) Transverse sections through the blade-petiole junction region. (J) A section from a wild-type Ler leaf. (K) A section from an expanded ae6-1 as2-101 leaf. (L) A section from an ae5-1 as2-101 lotus-like leaf. Arrowheads in J-L indicate phloem. ad, leaf adaxial side; ab, leaf abaxial side. Scale bars: 1 mm in A-C; 50 μm in D-L.
also observed in ae5-1 leaves (Fig. 5B). FIL expression appeared to be extended towards the adaxial side in some young leaves in as2-101 (Fig. 5C, arrowheads), and was further expanded throughout some needle-like leaf primordia of ae5-1 as2-101 (Fig. 5D, arrows; see Fig. S5 in the supplementary material). REV is known to be expressed in the adaxial domain of leaf primordia as well as in the vascular tissue of developing leaves (Eshed et al., 2001) (Fig. 5E). Although the REV expression pattern did not show obvious changes between the wild type (Fig. 5E), ae5-1 (Fig. 5F) and as2-101 (Fig. 5G), the normal REV expression pattern was not apparent in leaf
The reduction of the outgrowth numbers in the triple mutants was also accompanied by the impaired severity of outgrowth shapes (see Fig. S4 in the supplementary material). These results indicate that RPL28A and RPL5A may genetically interact with the KAN1/2 and ARF3/4 pathways to regulate leaf polarity.

Leaf polarity defects in stv1 as2 and rpl5b as2 double mutants

To determine whether other ribosomal subunit genes also participate in leaf patterning, we constructed stv1 as2 and rpl5b as2 double mutants. STV1 encodes a ribosomal large subunit protein RPL24B (Nishimura et al., 2005). Although stv1-1 (Fig. 7A) and rpl5b (Fig. 7E; for identification of rpl5b, see supplementary material Fig. S3) both produced pale green leaves, the epidermal cell patterns appeared normal (see Fig. S4 in the supplementary material). By contrast, stv1-1 as2-1 and rpl5b as2-1 double mutant plants demonstrated very severe phenotypes with most rosette leaves being needle-like (Fig. 7B,F). These needle-like leaves were covered with rectangular-shaped cells or abaxial-type cells (Fig. 7C,G, arrowheads). Similar to those in the ae5-1 as2-101 double mutant (Fig. 4L), the vascular pattern of these leaves indicated that they were abaxialized (Fig. 7D,H).

As RPL5B is a duplicated copy of RPL5A (AE6) in the Arabidopsis genome, we were interested in determining whether the ae6 rpl5b double mutant would more severely affect leaf polarity. Interestingly, the double heterozygote (ae6-2/+ rpl5b+/+) exhibited a phenotype similar to that of ae6-2 or rpl5b single mutant, with pale green leaves (see Fig. S6 in the supplementary material). However, plants with genotypes ae6-2/ae6-2 rpl5b/rpl5b, ae6-2/+ rpl5b/rpl5b, or ae6-2/ae6-2 rpl5b/+ were not found in the F2 progeny of the ae6-2 and rpl5b cross (see Fig. S6 in the supplementary material). Taken together, the enhanced as2 phenotypes by other ribosomal large subunit gene mutations indicate that the entire ribosomal activity may be required for normal leaf polarity establishment.

Subcellular localization of RPL28A and RPL5A

We next examined the subcellular localization of RPL28A and RPL5A by generating YELLOW FLUORESCENT PROTEIN (YFP)-RPL28A and -RPL5A fusions under the control of an

Fig. 7. Mutations in RPL24B and RPL5B enhance as2-1 polarity defects. (A) An stv1-1 seedling. (B) An stv1-1 as2-1 seedling. (C) SEM to examine epidermal cells on the top part of an stv1-1 as2-1 needle-like leaf. (D) Transverse section of an stv1-1 as2-1 needle-like leaf petiole. (E) An rpl5b seedling. (F) An rpl5b as2-1 seedling. (G) The top part of an rpl5b as2-1 needle-like leaf. (H) Transverse section of an rpl5b as2-1 needle-like leaf petiole. Arrowheads in C,G show the abaxially featured epidermal cells. Arrowheads in D,H indicate phloem. Scale bars: 0.5 cm in A,B,E,F; 50 μm in C,D,G,H.
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In addition, double mutants combining observed in leaves being pale green in color, and this type of leaves was also such defects in normal leaves or only showed very minor leaf polarity defects, while function in REV carrying a single mutation in the previously characterized leaf

In this paper, we showed that genes encoding ribosomal proteins specifying leaf adaxial identity

Ribosomal proteins are new regulators in specifying leaf adaxial identity

In this paper, we showed that genes encoding ribosomal proteins play important roles in specifying the leaf adaxial identity. Plants carrying a single mutation in the previously characterized leaf adaxial-promoting genes usually result in mild abnormalities in the leaf adaxial-abaxial polarity. For example, plants with loss of function in REV, PHB, PHV, RDR6, SGS3 and ZIP either had normal leaves or only showed very minor leaf polarity defects, while such defects in as1/as2 single mutants with the wild-type ERECTA were also subtle. Although single mutations in some 26S proteasome subunit genes can lead to the production of needle-like leaves, which is considered to be a strong defect in leaf adaxial-abaxial polarity, the frequency of such leaves is very low (Huang et al., 2006). Unlike other leaf adaxial-promoting factors, the severe ae5 and ae6 alleles exhibited a defective phenotype with almost all leaves being pale green in color, and this type of leaves was also observed in stvl-1 and rpl5b single mutants. This phenotype was caused, at least in part, by the ectopic intercellular spaces, which should be normally located in the abaxial spongy mesophyll layer of the wild-type leaves but are present in the adaxial palisade layer of the mutant ones. In addition, double mutants combining as1/as2 with ribosomal protein gene mutations can cause severe defects in the adaxial-abaxial leaf polarity. Our data strongly suggest that ribosomal proteins are a new type of regulator in leaf polarity establishment.

The translational function of the ribosome is required for leaf polarity establishment

The ribosome is composed of two subunits, a large subunit and a small subunit, both of which are formed by ribosomal proteins and rRNAs. The Arabidopsis genome contains 227 genes encoding 80 ribosomal proteins, 48 and 32 of which are on the large and small subunits, respectively (Carroll et al., 2007). By interacting with different rRNAs at specific regions, they form different active sites required for the ribosomal function (Ramakrishnan, 2002). In animals, ribosomal proteins are involved in either specific or nonspecific developmental processes (Bilanges and Stokoe, 2007; Ishijima et al., 1998; Idol et al., 2007; Marygold et al., 2007; Oliver et al., 2004; Uechi et al., 2006). For example, knockout of some ribosomal protein genes in zebrafish resulted in a spectrum of developmental defects with varying degrees of abnormality in the brain, body trunk, eyes and ears (Uechi et al., 2006). In Drosophila, 88 genes that encode cytoplasmic ribosomal proteins were characterized, 64 of them were found to correspond to the similar dominant ‘Minute’ phenotypes (Marygold et al., 2007). Some ribosomal proteins or protein complexes with specific functions were also documented: RPL22 and RPL7 function in transcription repression (Ni et al., 2006); RPL5, RPL11 and RPL23 act in inhibition of protein ubiquitination and degradation (Arva et al., 2005; Dai and Lu, 2004; Dai et al., 2004); and RPL11 is involved in reduction of histone H4 acetylation (Dai et al., 2007).

The functions of four Arabidopsis ribosomal proteins have been characterized and the corresponding mutants pfl1/rps18 (Van Lijssebettens et al., 1994), pfl2/rps13 (Ito et al., 2000), aml1/rps5 (Weijers et al., 2001) and stvl/rpl24 (Nishimura et al., 2005) all exhibited pleiotropic developmental defects. However, it was also observed that a loss of functions in different ribosomal protein genes affecting specific developmental processes. For example, STV1 (RPL24) regulates apical-basal patterning of the gynoecium (Nishimura et al., 2005). We propose that regulation of the leaf adaxial-abaxial polarity requires a conserved translational function of the Arabidopsis ribosome. First, although RPL28A, RPL5A, RPL5B and RPL24B are all large subunit proteins, according to data from yeast (Spahn et al., 2001) and bacteria (Korostelev et al., 2006; Bilanges and Stokoe, 2007; Selmer et al., 2006), these four proteins are located in three distinct sites on the large subunit, whereas plants with mutations in each of these genes suffer similar adaxial-abaxial polarity defects. Second, pfl2/rps13 and pfl1/rps18 also display either pale green leaves (Van Lijssebettens et al., 1994) or the defective mesophyll pattern (Ito et al., 2000), similar to that in the large subunit mutants ae5, ae6, stvl and rbl5b. Finally, the double heterozygous plant (ae6/rpl5b/) exhibited the pale-green leaf phenotype (see Fig. S6 in the supplementary material). These data suggest that a partial loss of the ribosome function may cause some similar leaf polarity defects. As the ribosome is known to be the conserved machinery of translation in all organisms, the regulation of leaf polarity at the translational level must be very important.

Roles of the ribosome in the regulatory network in leaf patterning

The molecular mechanism of how ribosomal proteins regulate leaf polarity-controlling genes is not yet clear. One possibility is that the ribosome may genetically promote the HD-ZIP III mediated

Fig. 8. Subcellular localization of YFP-RPL28A and YFP-RPL5A in the Arabidopsis root. (A-C) YFP-RPL28A was detected in both nuclei and cytoplasm. (D-I) Signals of YFP-RPL28A (D-F) and YFP-RPL5A (G-I) were found in both nucleolus and nucleoplasm. (A,D,G) YFP fluorescence; (B,E,H) bright-field differential interference contrast images; (C,F,I) merged views. Scale bars: 20 μm in A-C; 5 μm in D-I.

estradiol-inducible promoter. These two constructs were introduced into wild-type Ler, ae5-1 as2-101 and ae6-1 as2-101 mutant plants, respectively, and the double mutant phenotypes could be rescued (data not shown), indicating that YFP-RPL28A and YFP-RPL5A are biologically active. In the root tissue of wild-type Ler plants, YFP-RPL28A (Fig. 8A-C) and YFP-RPL5A (data not shown) were both located in the nuclei and cytoplasm. In addition to the strong YFP signals in the nucleolus, YFP-RPL28A (Fig. 8D-F) and YFP-RPL5A (Fig. 8G-I) were also present in the nucleoplasm. These results indicate that the ribosomal large subunit proteins in plants possess a similar subcellular localization pattern to those in animals (Claussen et al., 1999).
pathway in the adaxial domain of leaves. We have two lines of evidence to support this idea: (1) ae5-1 can enhance a weak rev allele, rev-9; and (2) accumulation of REV transcripts were reduced in ae5 as2 and ae6 as2 double mutants. In addition, AS1/AS2 are known to regulate PHB, PHV and REV positively in leaves (Lin et al., 2003; Fu et al., 2007; Ueno et al., 2007), and as1/as2 phenotypes could be enhanced in the ribosomal protein gene mutation backgrounds.

Another possibility is that the ribosome may genetically repress ARF3/4, KAN or their downstream genes. This hypothesis is supported by the observation that ae5 ett arf4 and ae6 ett arf4 triple mutants dramatically suppress an ett arf4 phenotype in which outgrowths are formed on the abaxial leaf side. In addition, ae5 kan1 kan2 also exhibited the reduction of the outgrowth numbers. It was proposed that juxtaposition of adaxial and abaxial domain is required for lamina outgrowth (Waints and Hudson, 1995), and adaxial and abaxial characteristics on the same abaxial side of the kan1 kan2 leaves might cause the formation of ectopic outgrowths (Eshed et al., 2004). Based on this proposal, it is possible that the balanced juxtaposition of the adaxial and abaxial cell patches on the same leaf side may be crucial for the outgrowth formation. The addition of ae5 or ae6 mutation in the ett arf4 and kan1 kan2 backgrounds may alter the original juxtaposition balance by promoting abaxial leaf characteristics, and therefore repressed the outgrowth phenotype.

We thank J. L. Bowman, K. Okada, P. Zambryski and the ABRC (Ohio State University, Columbus, OH, USA) for seeds of mutants rev-9, kan-1/2-kan1-2 kan2-1/4, rev-1 et-1, ett-3, aos-2 (SALK_138179), aos-6 (SALK_089790), rpl5b (SALK_010121) and arf2-2 (SALK_070506). We thank N. Chua for plant expression vector pER8, X. Gao for SEM, and X. Gao for plant sample preparation and sectioning. This research was supported by grants from the Chinese National Scientific Foundation (30630041 and 30721061) and Chinese Academy of Sciences (KSCX2-YW-N01-6) to H.H.

Supplementary material
Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/135/7/1235/D1

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