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DRL1 Regulates Adaxial Leaf Patterning and Shoot Apical Meristem Activity in *Arabidopsis*

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Leaf shape is controlled early on by initiation at the shoot apical meristem (SAM), as well as by changes in the rates and planes of cell division and the polarity-dependent differentiation of leaf cells. To elucidate the regulation of this differentiation by signal(s) from the SAM, we screened for mutations in genes that might be involved in these early processes. A novel recessive mutant, *356-2* [identified as a new allele of the *deformed root and leaf1* (*drl1*) mutant], was isolated from a collection of *Ds* transposon insertion lines. The *356-2*/*drl1-101* mutant produces narrow, filamentous leaves and defective meristems. Its palisade cells have a spongy cell-like structure and are fewer in number, indicating that the leaves are abaxialized. Interestingly, some of those filament-like leaves have no vascular tissues inside their blades. *DRL1* encodes a protein similar to the yeast elongator-associated protein (EAP) KTI12. The amino acid sequence of DRL1 is universally conserved in prokaryotes and eukaryotes. These facts suggest that DRL1 might positively regulate leaf polarity and SAM activity by controlling cell proliferation and differentiation.

Keywords: adaxial patterning, *Arabidopsis thaliana*, *DRL1*, elongator-associated protein, leaf development, shoot apical meristem

The establishment and differentiation of lateral organs involves factors that are intrinsic to the primordia as well as interactions with the apical meristem from which they are derived. In *Arabidopsis*, the shoot apical meristem (SAM) is critical to the formation of the vegetative plant body. Leaves initiate post-embryonically at the periphery of the SAM in a radial pattern (Reinhardt et al., 2000). Repression of the Class-I *KNOX* gene *SHOOT MERISTEMLESS* (*STM*) and activation of the myb-domain transcription factor gene *ASYMMETRIC LEAVES1* (*AS1*) are essential for this initiation (Long et al., 1996; Byrne et al., 2000). Shortly afterward, the radially symmetrical primordia flatten in a plane parallel to the meristem periphery, and soon display dorsal-ventral or abaxial-adaxial ("abaxial", away from the meristem; "adaxial", adjacent to the meristem) polarity due to the asymmetrical distribution of cell types in the mature organ (Kim and Cho, 2006). The *BLADE-ON-PETIOLE1* gene of *Arabidopsis*, which encodes a BTB/POZ-domain protein, promotes proximodistal identity (Ha et al., 2004). Moreover, *PHABULOSA* (*PHB*), *PHAVOLUTA* (*PHV*), and *REVOLUTA* (*REV*), which encode Class-III homeodomain/Leu zipper (HD-ZIP III) transcription factors, regulate adaxial-abaxial polarity in *Arabidopsis* (McConnell et al., 2001; Otsuga et al., 2001). Semi-dominant gain-of-function mutations in *PHB* and *PHV* result in the formation of adaxialized leaves (McConnell et al., 2001). In addition, members of the gene families *YABBY* (*YAB*) and *KANADI* (*KAN*), which encode putative transcription factors and GARP transcription factors, respectively, regulate adaxial-abaxial polarity by specifying abaxial cell fate (Siegfried et al., 1999; Kerstetter et al., 2001; Emery et al., 2003). It has been suggested that miRNA controls leaf polarity by repressing those *PHB/PHV/REV* target genes (Kidner and Martienssen, 2004). For example, miRNA miR165/166, which accumulates in the abaxial domain of the primordium, might regulate the expression levels of *PHB/PHV* through the direct breakdown of their transcripts, affecting the formation and fate of adaxial cells (Kidner and Martienssen, 2004). This implies that the miRNA-mediated control of leaf morphogenesis plays important roles in the establishment of leaf polarity. Because of the development of adaxial-abaxial polarity in their primordia, leaves are dorsoventrally flattened. Thus, the pathways that control organ polarity are pivotal in regulating leaf shape and growth. Although many genes that regulate dorsoventral patterning have been identified, details of the mechanisms for genetic interactions between SAM activity and the differentiation of leaf organs remain unknown.

To elucidate the regulation of this differentiation by signals from the SAM, we isolated mutations in genes that might be involved in the early steps of leaf development. To this end, we screened a collection of *Arabidopsis thaliana Ds* transposon insertion lines. Here, we report the isolation of the novel recessive mutant *356-2*, a new *drl1* mutant allele, and the functioning of DRL1 in SAM activity and leaf development.

MATERIALS AND METHODS

Plant Materials and Growing Conditions

The *356-2/drl1-101* mutant allele was isolated from a

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Abbreviations: BAC, bacterial artificial chromosome; *Ds*, *Dissociation*; RT, reverse transcription; TAIL, thermal asymmetric interlaced

library of *Ds* transposon-containing *Arabidopsis* mutants (background ecotype Nossen-0) created using a local transposition system (Ito et al., 1999). Wild-type Nossen-0 (No-0) was used for backcrossing and for comparisons with mutant plants. A loss-of-function mutant allele (*drl1-102*) was obtained from ABRC (SALK_071178 insertion line). The *fil-1* and *fil-5 yab3-2* mutants used here for RT-PCR analysis are in the ecotype Landsberg *erecta* (*Ler*). For complementation analysis, transgenic plants were generated with a full-length *DRL11* (35S-*DRL1*) construct driven by the CaMV 35S promoter in the *drl1-101* mutant background. For GUS analysis, the *DRL1* promoter region (~1.2 kb) was PCR-amplified with the following primers: DRL1pro-for, 5′-GTCAAACGTTCGCTTGAAACCC-3′; and DRL1pro-rev, 5′-TGGTTTTTAAACACACTGCCTAC-3′. These were fused to the start codon of β-glucuronidase (GUS). Transgenic plants were generated by *Agrobacterium*-mediated transformation (Kim et al., 1999) and grown, in either MS-agar medium or small plastic pots containing vermiculite, at 22°C under continuous white fluorescent light (90.4~105.1 μmol m^{-2} s^{-1}).

Histological Analysis

For our anatomical analysis, samples were fixed in FAA solution under a vacuum (Kim et al., 1998). After dehydration through a graded ethanol series, they were embedded in Technovit 7100 resin (Kulze, Germany) and examined as described by Kim et al. (1998). Histological detection of GUS activity was performed using plants that had been grown on an MS medium according to the method of Kim et al. (1999). For SEM, the specimens were critical point-dried in liquid CO_2 after dehydration. The dried materials were mounted and coated with platinum-palladium in a sputter-coater, then examined with a scanning electron microscope (JSM-6700F; JEOL, Japan), using an accelerating voltage of 20 kV.

Cloning of *DRL1* and Semi-Quantitative RT-PCR

Genomic DNA fragments flanking the *Ds* of *drl1* were amplified by TAIL-PCR (Liu and Whittier, 1995), as described by Ito et al. (1999). A full-length coding sequence of DRL1 was isolated by RT-PCR, using specific primers -- DRL1-BamHI-for, 5′-CGGGATCCCGGCACCTAAAGCTTGAATTGA-3′; and DRL1-SacI-rev, 5′-CGAGCTCGAACACATCAATTTAGCCCCA-3′. Total RNA was purified from leaves according to the method of Kim et al. (1998). Semi-quantitative RT-PCR analysis was performed as described by Kim et al. (1998, 2002). The oligonucleotides DRL1-RT-for (5′-GCTGAGAAAGAATCTGAGAGG-3′) and DRL1-RT-rev (5′-CTCCAAACTCTCTGTTCAAG-3′) were used as primers for the RT-PCR of *DRL1*. Amplification of *TUB4* served as a positive control (Kim et al., 1998).

Phylogenetic Analysis

We produced a phylogenetic tree via PROTPARS, a maximum-parsimony algorithm that is included in the PHYLIP version 3.5 software package (Kim et al., 2002). The sequences conserved in DRL1 and its homologs were aligned according to the CLUSTALW program; these alignments were then refined manually with the MacClade program, as described by Kim et al. (2005). Topological robustness was assessed by bootstrap analysis with 100 replicates, using simple taxon addition. Several short sequences in the N- and C-terminal regions that could not be aligned unambiguously were excluded from this analysis.

RESULTS AND DISCUSSION

Isolation and Phenotype of the *356-2*/*drl1* Mutant

From a library of *Ds* transposon-containing *Arabidopsis* mutants (background ecotype Nossen-0), we isolated the mutant *356-2*, which forms abnormal leaves (Fig. 1). Seedlings of the subsequent generation segregated in a 1:3.45 ratio (11 with abnormal leaves and 38 with normal leaves),

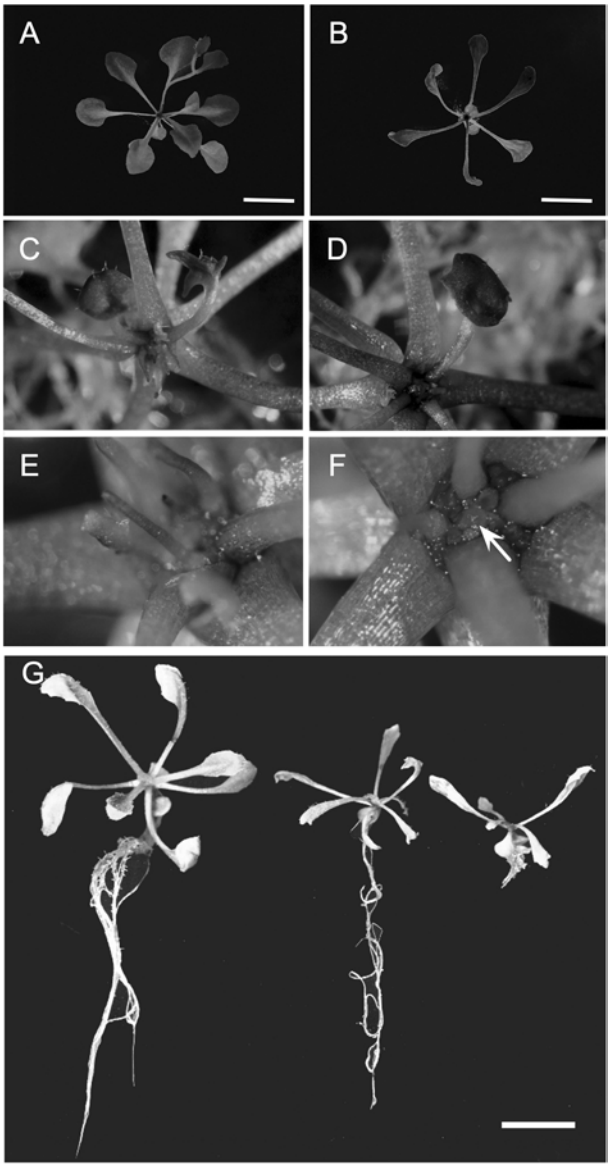

Figure 1. Gross morphology of 3-week-old plants from wild type (No-0) (**A**) and *drl1-101* mutant (**B**). Phenotype of trumpet-like leaves (**C**, **D**) and filamentous leaves (**E**). (**F**) Structure of SAM from 3-week-old *drl1-101* plants. (**G**) Leaf and root phenotypes of wild type (left) and *drl1-101* grown on MS media containing (right) or lacking (middle) 20 mg L^{-1} hygromycin. Bars = 1 cm.

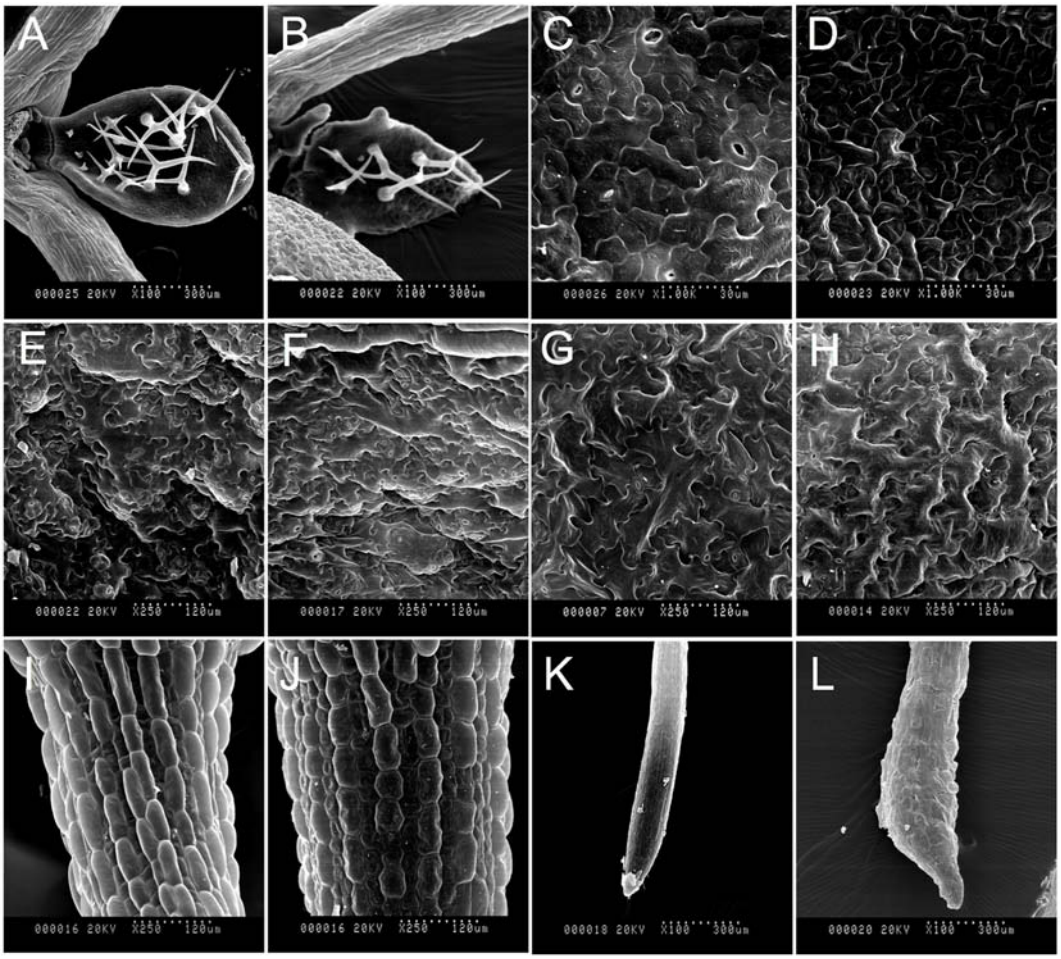

Figure 2. Scanning electron microscopy (SEM) analysis of wild type (No-0) (**A**, **C**, **E**, **G**, **I**, **K**) and *drl1-101* mutant (**B**, **D**, **F**, **H**, **J**, **L**) plants at 7 d old. (**A**, **B**) Phenotype of first leaf primordia. (**C**, **D**) Epidermal cells on adaxial side of first leaf blade. (**E**, **F**) Epidermal cells on abaxial side of first leaf blade. (**G**, **H**) Epidermal cells on adaxial side of cotyledon. (**I**, **J**) Middle part of hypocotyl. (**K**, **L**) Tip from primary root.

indicating that this is a nuclear recessive mutant. Its leaves were narrower than those of the wild type (No-0) at the fully expanded stage (Fig. 1A, B). The mutant also produced trumpet-like and filamentous leaves (Fig. 1C-F). This abnormal phenotype was more severe in young leaves than in old leaves (Fig. 1). Its hypocotyls also were shorter and wider than those of the wild type (Fig. 2I, J). This phenotype was caused by reduced cell elongation in length and increased elongation in width (Fig. 2I, J). However, the roots of the mutant were slightly shorter than those of the wild type when cultured on the MS medium (Fig. 1G); mutant root growth was significantly reduced when plants were cultured on an MS medium containing 10 μg mL^{-1} hygromycin (*Ds* insertion selective marker). This demonstrated a defect in the root apical meristems (Fig. 1G, 2K, L). Mutant leaves also showed abnormal patterns of trichome branching, with the first and second leaves having fewer trichomes than in the wild type (Fig. 2A, B). The *356-2* mutant also flowered somewhat later the wild type under long-day conditions (16 h light, 8 h dark). No significant differences were observed in other organs of the mutant. This *356-2* phenotype is very similar to that of *drl1* (Bancroft et al., 1993; Nelissen et al., 2003). Isolation of the affected gene (see below) also demonstrated that its flanking region is the same as the flanking sequence of the *drl1* mutation (GenBank S63816). Therefore, we named this mutant *drl1-101*. Our mutant was backcrossed with the wild type (No-0) and then characterized.

The *drl1-101* Mutant Is Defective in the Dorsoventral Patterning of Leaves

Leaves 1 through 5 on the *drl1-101* mutant were narrower than those on the wild type (Fig. 1A, B), as was the primordium of the first mutant leaf (Fig. 2A, B), suggesting that this phenotype results from defective primordia. To dissect this morphological difference at the cellular level, we observed the palisade mesophyll cells of the first leaf using whole-mount preparations.

Anatomical differences in the abaxial-adaxial polarity of *Arabidopsis* leaves are evident shortly after leaf initiation (Kim and Cho, 2006). In the current study, the wild-type leaves had distinct, dense palisade mesophyll cells on the adaxial side (Fig. 3C). Sizes of the abaxial spongy mesophyll cells were more varied, and intercellular air spaces gradually became more prevalent in this region. The palisade layer in the wild-type leaf blades had regular-sized cells (Fig. 3C). In contrast, the *drl1-101* mutant leaf blades contain many

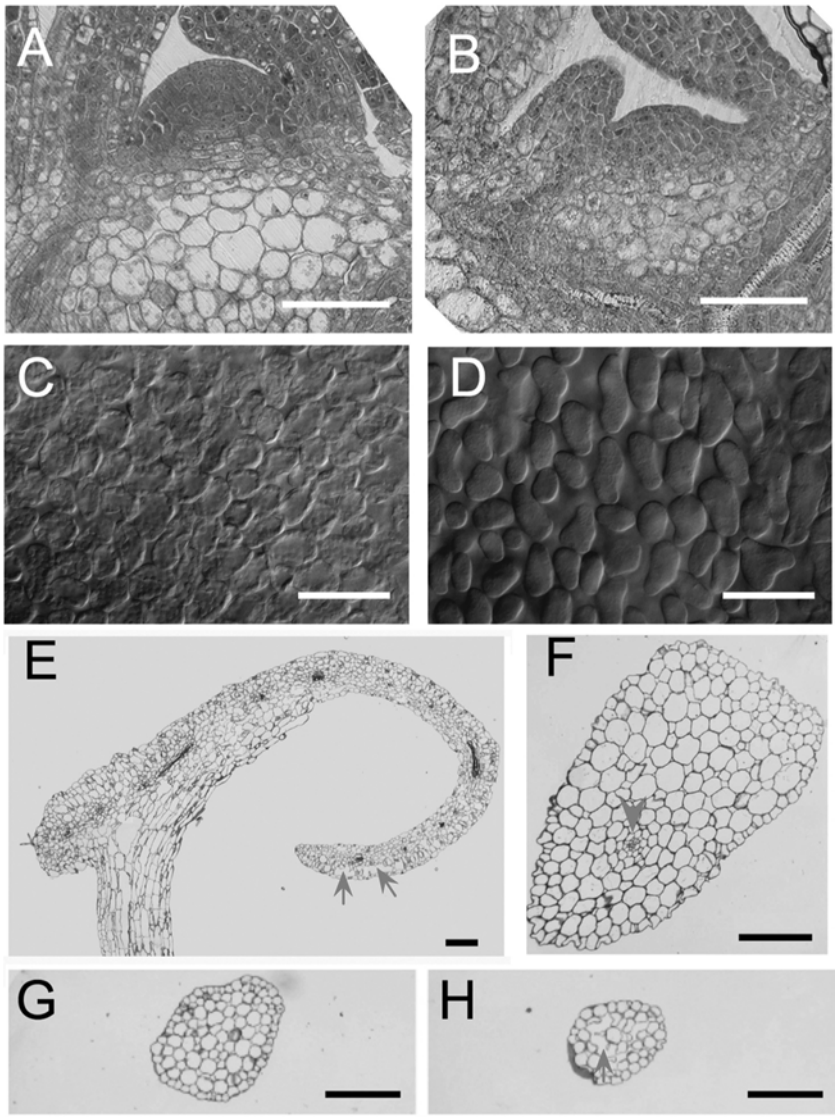

Figure 3. Anatomical analysis of wild type (No-0) and *drl1-101* mutant. Longitudinal sections of SAM from wild type (**A**) and *drl1-101* (**B**). Bars, 100 μm. Paradermal images of round palisade cells from wild type (**C**) and irregularly sized palisade cells from *drl1-101* (**D**). Transverse sections of trumpet-like leaves (**E**) and filamentous leaves (**F**-**H**) of *drl1-101*. Arrows indicate airspace normally found in spongy cells from wild type (**E**, **H**). Arrowhead indicates altered vascular tissues in filamentous leaf. Bars = 100 μm.

enlarged and irregularly sized palisade cells and intercellular spaces (Fig. 3D). Those leaves also contained fewer palisade cells than the wild type (Fig. 3C, D). This occurrence of larger but fewer leaf cells in the mutant strongly suggested that its cell division activity during leaf development is significantly altered.

To further characterize the polar nature of our *drl1-101* leaves, we used SEM to compare the adaxial and abaxial epidermal cells (Fig. 2A-H). Those on the adaxial sides were fewer in number and had a more complicated cell structure than the wild-type leaves (Fig. 2C, D). In contrast, the epidermal cells on the abaxial side of the *drl1-101* leaf blade appeared normal (Fig. 2E, F). Likewise, the epidermal cells on the adaxial sides of *drl1-101* cotyledons were unaltered (Fig. 2G, H), suggesting that the adaxial-abaxial patterning of cotyledons is not changed in the mutant.

The trumpet-shaped appearance of the mutant leaves resulted from the petiole being attached to the middle part of the blade (Fig. 1C, D). In transverse sections, this area was fused at the cellular level (Fig. 3E), and certain mesophyll cells in fused region had changed into petiole-like structures (Fig. 3E). Some palisade tissue also had a spongy cell-like structure (Fig. 3E).

Filamentous leaves of the *drl1-101* mutant were nearly radial at the initiation stage, but still displayed some polarity, as evidenced by the flat structure on the adaxial sides (Fig. 3F). Cells in the filamentous leaves were not fully differentiated (Fig. 3F-H).

In most leaf vascular bundles, xylem elements are closer to the adaxial leaf surface; in wild-type *Arabidopsis*, the phloem/xylem axis is perpendicular to the abaxial-adaxial leaf axis (Eshed et al., 2004). However, that vascular arrangement was not present in the filamentous mutant leaves (Fig. 3G, H). Similar reports have been made about the

abaxialized radial leaves of *phantastica* mutants from *Antirrhinum* (Waites et al., 1998) and the abaxialized cotyledons of a *phb phv rev* triple mutant (Emery et al., 2003). In addition, small and undifferentiated vascular tissues were present in the trumpet-like and filamentous leaf blades of our *drl1-101* mutant (Fig. 3E, F). Airspace also existed between the sponge cells of the blade (Fig. 3H). These results strongly suggest that the adaxial patterning of the *drl1-101* mutant is altered, and that it has an abaxial identity.

The Class-III HD-ZIP proteins and the *KAN* gene have been shown to be responsible for radial polarity in leaves (Siegfried et al., 1999; Eshed et al., 2001; Kerstetter et al., 2001) and stems (Emery et al., 2003). Based on their genetic and molecular data, the activities of members in these two families may be both mutual and antagonistic. For example, gain-of-function alleles in *KAN* result in the abaxialization of lateral organs and a loss of meristem development (Eshed et al., 2001; Kerstetter et al., 2001), similar to the *phb phb rev* loss-of-function phenotype. Our anatomical research revealed that the phenotype of *drl1-101* was similar to that of the *phb phb rev* loss-of-function mutant. In addition, the position and pattern of the vascular tissues were conspicuously altered in *drl1-101* (Fig. 3E-H).

Although organ growth may be abnormal in the *drl1* mutant (Nelissen et al., 2003), this phenomenon does not explain the adaxial patterning of its leaves. Overall, our anatomical examination strongly suggested that *DRL1* positively regulates adaxial patterning not only in the epidermal and mesophyll cells but also in vascular tissues of the blades, similar to the functioning of the Class-III HD-ZIP proteins and *KAN* in leaf development.

The *drl1-101* Mutant Shows Reduced Shoot Apical Meristem Activity

Early leaf development relies on the control of leaf initiation and formation in the flanking regions of the shoot apical meristem (SAM), as well as on changes in the rates and planes of division and the polarity-dependent differentiation of leaf cells (Steeves and Sussex, 1989). Therefore, the mechanism by which the SAM is maintained is essential to leaf formation. However, there is little evidence that the process of cell proliferation in the SAM determines the fate of these cells. To investigate the structure of the SAM and primordia at the stage of leaf formation, we observed the anatomy of longitudinal sections from 10-d-old seedlings. The SAM in the *drl1-101* mutant was reduced in size not only in its central zone but also in its rib zone (Fig. 3B). The mutant SAM also had fewer cells than did the wild type (Fig. 3A, B), suggesting that *DRL1* positively regulates shoot apical meristem activity. In addition, 3-week-old *drl1-101* plants had smaller SAMs (Fig. 1F).

In the wild-type SAM, the L1- and L2-layer cells divided anticlinally and ended up arranged perpendicularly to the shoot apex (Fig. 3A). In contrast, those of the mutant divided irregularly, resulting in altered formations (Fig. 3B). This suggested that the smaller SAM of the *drl1-101* mutant affects its leaf morphology, causing narrow, trumpet-like and filamentous leaves to develop.

Based on these observations, we can conclude that the *DRL1* gene is required for meristem activity, vascular tissue formation, and leaf polarity. Future genetics studies of the interactions between SAM-related genes (e.g., *STM*, *KNAT*,

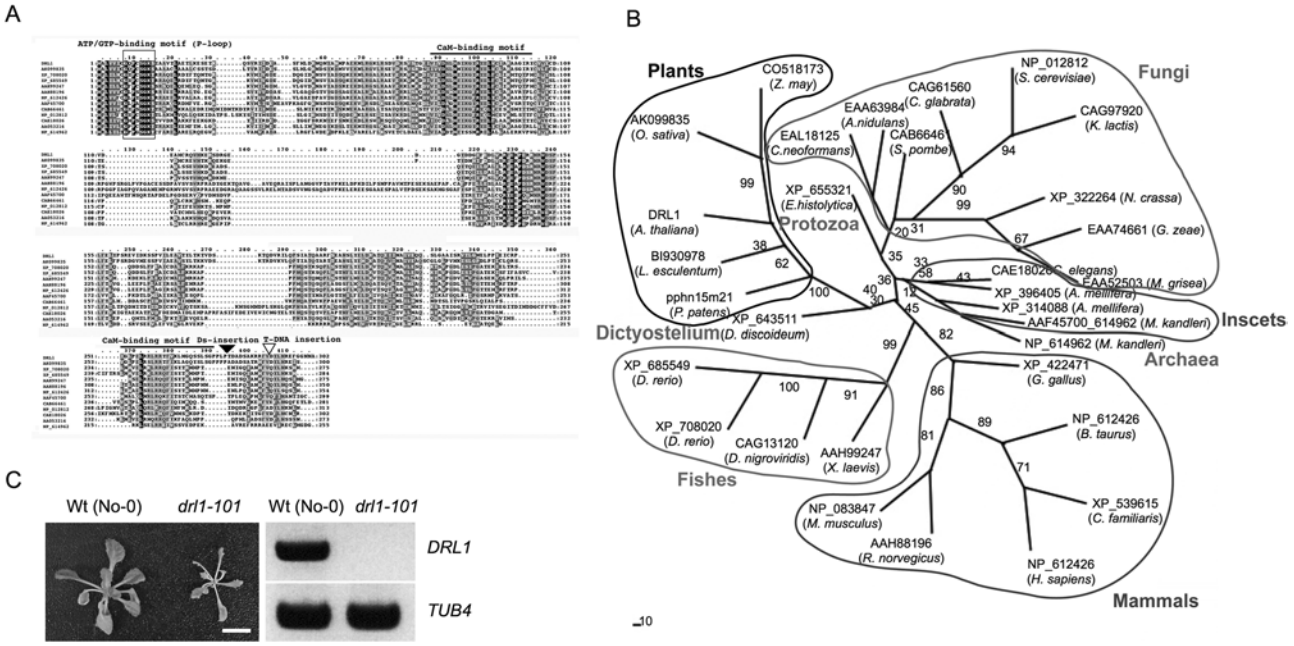

Figure 4. Alignment and phylogenetic tree of amino acid sequences for DRL1 and related proteins. (**A**) Two characteristic motifs: putative ATP/GTP binding motif near N termini (box), and two CaM-binding motifs (lines). Arrowheads indicate *Ds*-insertion site in *drl1-101* knockout mutant (black) and T-DNA insertion site in *drl1-102* knockout mutant (white). (**B**) Phylogenetic tree generated from deduced amino-acid sequences of DRL1 from *Arabidopsis*, plus homologs from 30 other organisms, including archaebacteria, protozoa, *Dictyostelium*, fish, insects, and mammals. Scale bar indicates 10 substitutions. Numbers at nodes indicate bootstrap values. (**C**) Expression level of *DRL1* mRNA in wild type (No-0) and *drl1-101* mutant, as determined by RT-PCR. Total RNA was isolated from aerial portions of 3-week-old plants; 0.5 μg was used per lane. Results from RT-PCR amplification of β-tubulin 4 (*TUB4*) mRNA are shown as control.

and *AS1*) and *DRL1* should reveal the mechanisms of the DRL1 pathway in SAM activity.

DRL1 Encodes a Protein That Associates with the Elongator Complex

TAIL-PCR analysis revealed that the transposed *Ds* element in *356-2* is positioned in the At1g13870 locus of the *Arabidopsis* BAC clone F16A4 of Chromosome 1. It is inserted in an exon of that locus (Fig. 4A), and an 8-bp duplicated sequence specific to the *Ac/Ds* insertion is present in the mutant (data not shown). To examine the expression level of the At1g13870 gene, we performed northern blot analysis with total RNA extracted from several wild-type tissues. No signals were detected (data not shown), indicating that this gene is expressed either at a very low level or in a restricted number of tissues. We also conducted RT-PCR analysis, using At1g13870-specific primers, to determine whether the At1g13870 gene was knocked out in the mutant. Because no band could be amplified (Fig. 4C), this confirmed the knock-out. However, a 1-kb cDNA of the At1g13870 locus was obtained using RT-PCR with total RNA isolated from 10-d-old seedlings.

To determine whether the leaf and SAM phenotypes of the *drl1-101* mutant are caused by this knockout, we generated transgenic *Arabidopsis* plants expressing the At1g13870 cDNA under the control of the cauliflower mosaic virus (CaMV) 35S promoter in the *drl1-101* mutant background. Independent transgenic lines displayed leaf structures similar to that of the wild-type phenotype (Fig. 5), indicating that the At1g13870 locus encodes the 356-2/DRL1 product. We also isolated a putative loss-of-function *DRL1* mutant (SALK_071178) created via T-DNA insertion, which was obtained from the *Arabidopsis* Biological Resource Center (ABRC). This line, which we designated *drl1-102*, also produced narrow leaves and defective meristems (data not shown), indicating that the At1g13870 locus encodes DRL1.

Comparison of the DNA sequences of *DRL1* and the cDNA clone revealed that the gene has a single exon. The *Ds* insertion in *356-2* is located at the C terminus (Fig. 4A). This gene encodes a predicted ORF of a 34-kDa protein with 302 amino acids (Fig. 4A). The predicted amino-acid sequence contains two characteristic motifs: 1) a putative ATP/GTP-binding motif near the N terminus and 2) two CaM-binding motifs near the N terminus and C terminus (Fig. 4A). Nelissen et al. (2003) have demonstrated *in vitro* that the C-terminal 100 amino acids of DRL1 can bind calmodulin in a calcium-dependent manner.

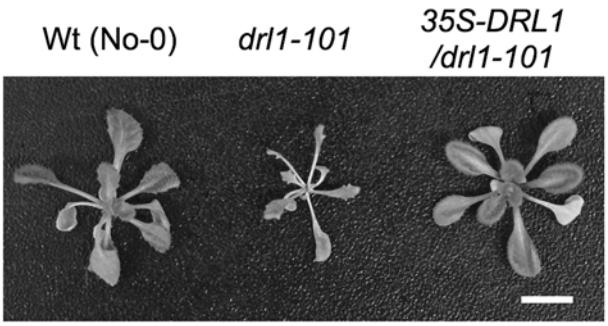

Figure 5. Complementation analysis for expression of *DRL1* under control of CaMV 35S promoter in *drl1-101* mutant. From left: rosette leaves from 15-d-old wild-type plants (No-0), *drl1-101* mutant plants, and transgenic plants expressing 35S::*DRL1* in *drl1-101* mutant background.

The *Arabidopsis* database indicated that *DRL1* is a single-copy gene in the genome. Its DRL1 protein is similar to the yeast KTI12 protein, which associates with the Elongator complex (Frohloff et al., 2001). KTI12 belongs to a family of chromatin-associated proteins that interact with that complex, a component of the elongating form of RNA polymerase II that also has histone acetyltransferase activity (Frohloff et al., 2001; Petrakis et al., 2005). DRL1 homologs are present in organisms ranging from archaebacteria to mammals, including *Methanopyrus kandleri, Schizosaccharomyces pombe*, *Caenorhabditis elegans*, *Drosophila melanogaster*, *Mus musculus*, and *Homo sapiens* (Fig. 4B). Our DRL1 protein exhibited 58% amino-acid identity to a rice protein (AK099835), 35% identity to a *Xenopus* protein (AAH99247), and 28% identity to the yeast KTI12 (NP_012812) (Fig. 4A). DRL1 and KTI12 each had a putative ATP/GTP binding motif near their N termini and two CaM-binding motifs -- one near their C termini and one in the middle of the protein (Fig. 4A). Their highly conserved sequences are indicated in Figure 4A.

To examine the evolutionary history of the *DRL1* genes, we compared the DRL1 sequence to sequences available for other KTI12 homologs. The phylogenetic tree revealed that DRL1 and the KTI12 homologs could be divided into several subfamilies (Fig. 4B), one each for fungal, plant, and animal proteins. This analysis also demonstrated that these amino acid sequences are evolutionally conserved from prokaryotes to eukaryotes. Moreover, DRL1 and the plant DRL1 homologs clearly form a monophyletic clade, suggesting the evolutionary conservation of DRL1 homologs in the genomes of all land plants (Fig. 4B).

DRL1 exhibits amino acid sequence similarity and shares common functional domains with the yeast KTI12/TOT4, which is an Elongator-associated protein (Fichtner et al., 2002). EAP plays an important role in the signaling toxicity of the *Kluyveromyces lactis* zymocin (Frohloff et al., 2001; Fichtner et al., 2002). Interestingly, Petrakis et al. (2005) have used chromatin immunoprecipitation to show that KTI12 is associated with chromatin throughout the genome, even in non-transcribed regions and in the absence of Elongator. The biochemical function of DRL1 related to chromatin remains unknown, but its elucidation will provide an important clue for understanding plant development.

Expression Patterns of the *DRL1* Gene

To further investigate the physiological roles of *DRL1*, we determined the expression patterns of this gene using promoter::*GUS* transgenic plants.

A *DRL1* promoter fragment (~1.2 kb) was generated by PCR and fused to the start codon of the GUS coding sequence. The $DRL1_{pro}$::*GUS* transgenes were expressed in various organs of adult transgenic plants, including leaf blades, floral organs, and root tips (Fig. 6A). GUS was strongly expressed in young leaf blades and the basal region of the SAM, but no expression was detected in older leaf

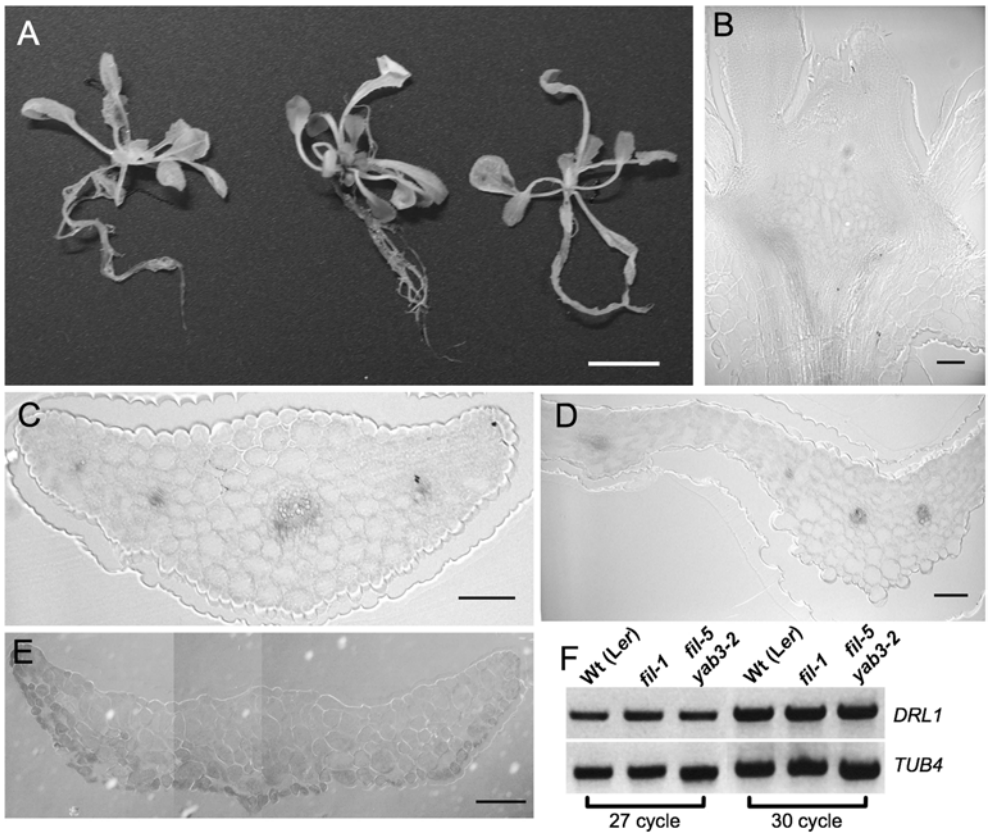

Figure 6. Expression analysis of *DRLpro::GUS* plants, *drl1-101* plants expressing *FILpro::GUS*, and *DRL1* mRNA in wild type and several mutants. (**A**) Left, 23-d-old wild-type plant (Col-0); middle and right, *DRLpro::GUS* transgenic plants. (**B**) Longitudinal section of SAM from 10-d-old *DRLpro::GUS* transgenic seedling. Transverse section of young (**C**) and old (**D**) leaf blades from *DRLpro::GUS* transgenic plant. (**E**) Expression pattern of *FILpro::GUS* in *drl1-101* mutant. Bars = 0.1 mm. (**F**) Semi-quantitative RT-PCR was carried out with *DRL1*-specific primers in wild type (Ler), *fil-1* mutant, and *fil-5 yab3-2* double mutant.

blades (Fig. 6A). GUS staining was observed in all parts of the leaves, particularly in their veins (Fig. 6D, E). Consistent with these observations, microarray analysis data compiled in Genevestigator (https://www.genevestigator.ethz.ch/) have indicated that *DRL1* is expressed widely in various organs. Taken together, these results suggest that *DRL11* functions in various *Arabidopsis* tissues, especially the leaf vascular bundles and meristems, where it appears to be involved in the early stages of organ differentiation.

The *YAB* family of abaxially expressed genes, which encode putative transcription factors with high-mobility-group and zinc-finger domains, have been shown to promote abaxial cell fates in lateral organs and meristem development (Eshed et al., 1999; Siegfried et al., 1999; Kumaran et al., 2002). Our *drl1-101* mutant had abaxialized leaves and a defective SAM, suggesting that *DRL1* is involved in regulating adaxial-abaxial patterning. To examine whether this gene is active in establishing such polarity, we studied its expression in the *yab* mutant. Because *FILAMENTOUS FLOWER* (*FIL*)/*YAB1*, a member of the *YAB* gene family, is thought to act redundantly with *YAB2* and *YAB3*, we also monitored *DRL1* expression in the *fil-5 yab3-2* double mutant. Expression was normal in the *fil-1* and *fil-5 yab3-2* mutants (Fig. 6F), suggesting that DRL1 is not regulated by the abaxially restricted expression of the *YAB* family (Siegfried et al., 1999).

To investigate the expression pattern of an abaxial marker, we introduced a FIL_{pro}*::GUS* gene into *drl1-101*. In transverse sections from young narrow leaves of the F2 FIL_{pro}*::GUS/drl1-101* plants, GUS was detected on the abaxial sides (Fig. 6E), a pattern similar to that in the parental FIL_{pro}*::GUS* marker lines (data not shown). Taken together, our results demonstrate that expression of the *YAB* family of abaxial-regulating genes is not responsible for adaxial fate determination in which DRL1 is involved.

PHB/PHV/REV genes possibly control the adaxial patterning of leaves by specifying adaxial cell fate (McConnell et al., 2001). In contrast, members of the gene families *YAB* and *KAN* regulate adaxial-abaxial polarity by specifying abaxial cell fate (Siegfried et al., 1999; Kerstetter et al., 2001; Emery et al., 2003). Our results indicated that *DRL1* is expressed on both the adaxial and abaxial sides of the leaf primordia. Thus, *DRL1* may regulate *PHB/PHV/REV* expression on the adaxial side and/or *KAN* expression on the abaxial side. Although many genes that modulate dorsoventral patterning have been identified, the detailed mechanisms for genetic interactions between SAM activity and differentiation of leaf organs remain unknown. Kim et al. (2007) have demonstrated that *ATHB23*, a Class-I HD-ZIP gene, has important roles in SAM activity and adaxial-abaxial leaf polarity under the control of the *PHB* gene. Therefore, DRL1 may regulate these Class-I and Class-III HD-ZIP genes during early leaf development.

Further elucidation of the target processes for these transcription factors will allow us to understand the role of temporal and spatial coordination of differential growth, as well as the link with hormonal regulation, during the formation of adaxial–abaxial polarity in early leaf development and SAM activity.

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