Comparative Analysis of the Conserved Functions of Arabidopsis DRL1 and Yeast KTI12

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Patterning of the polar axis during the early leaf developmental stage is established by cell-to-cell communication between the shoot apical meristem (SAM) and the leaf primordia. In a previous study, we showed that the DRL1 gene, which encodes a homolog of the Elongator-associated protein KTI12 of yeast, acts as a positive regulator of adaxial leaf patterning and shoot meristem activity. To determine the evolutionally conserved functions of DRL1, we performed a comparison of the deduced amino acid sequence of DRL1 and its yeast homolog, KTI12, and found that while overall homology was low, well-conserved domains were presented. DRL1 contained two conserved plant-specific domains. Expression of the DRL1 gene in a yeast KTI12-deficient yeast mutant suppressed the growth retardation phenotype, but did not rescue the caffeine sensitivity, indicating that the role of Arabidopsis Elongator-associated protein is partially conserved with yeast KTI12, but may have changed between yeast and plants in response to caffeine during the course of evolution. In addition, elevated expression of DRL1 gene triggered zymocin sensitivity, while overexpression of KTI12 maintained zymocin resistance, indicating that the function of Arabidopsis DRL1 may not overlap with yeast KTI12 with regards to toxin sensitivity. In this study, expression analysis showed that class-I KNOX genes were downregulated in the shoot apex, and that YAB and KAN were upregulated in leaves of the Arabidopsis drl1-101 mutant. Our results provide insight into the communication network between the SAM and leaf primordia required for the establishment of leaf polarity by mediating histone acetylation or through other mechanisms.

INTRODUCTION

Leaves are dedicated photosynthetic organs in plants, and diverse morphologies in various species allow them to inhabit a wide range of natural environments. Leaf organization is accomplished through serial processes according to specific temporal and spatial demands. Leaves originate postembryonically in a radial pattern from the periphery of the shoot apical meristem (SAM; Reddy, 2008), after losing their stem cell identity as meristem cells in response to a cell differentiation signal. Cells located in the peripheral regions of the SAM grow as leaf primordia (LP) and acquire leaf identity. During the early LP developmental stage, the LP establish three axes of leaf polarity: adaxial-abaxial, proximal-distal, and medial-lateral (Cho et al., 2007; Hasson et al., 2010). Adaxial-abaxial polarity is involved directly in the optimal formation of structures specific for the functions of the upper and lower surfaces of leaves. During the leaf maturation stage, the differentiation of vascular tissues also exhibits adaxial-abaxial polarity. Several studies have identified polarity determinant genes and revealed their cooperation and antagonism in the establishment of adaxial-abaxial polarity. Adaxial-determining homedomain-leucine zipper (HD-ZIPIII) genes, including PHABULOSA (PHB), PHAVOLUTA (PHV), and REVOLUTA (REV), and abaxial-determining genes of the KANADI (KAN) family in Arabidopsis (Arabidopsis thaliana), regulate each other by mutual inhibition of expression and activities (Izhaki and Bowman, 2007; Sarojam et al., 2010; Tsukaya, 2013). In addition, the YABBY (YAB) gene family integrates a genetic cascade for abaxialization by acting downstream of all abaxial polarity determinants (Eshed et al., 2001; 2004; Siegfried et al., 1999). MicroRNA miR165/166, which accumulates in the abaxial domain of the LP, regulates PHB/PHV transcript levels in the abaxial side (Kidner and Martienssen, 2004). In addition, trans-acting small interfering RNAs (ta-siRNAs) are also reported to downregulate the expression of members of the AUXIN RESPONSE FACTOR (ARF) gene family, ETTIN (ARF3) and ARF4, indicating that auxin signaling plays an important role in the patterning of leaf polarity (Chitwood et al., 2009; Griffiths-Jones et al., 2006; Hunter et al., 2006). The Elongator complex, which has histone acetyltransferase (HAT) activity and participates in several processes including RNA polymerase II (RNAPII) transcriptional elongation, tRNA anticodon modification and growth inhibition by the fungal tRNA zymocin toxin complex (Fichtner et al., 2002; Frohloff et al., 2001; Huang et al., 2005; Mehlgarten et al., 2010; Otero et al., 1999; Wittschieben et al., 1999), has been reported to regulate leaf polarity by indirectly repressing abaxial determining families and class-I KNOX family in Arabidopsis (Kojima et al., 2011; Neillissen et al., 2010). In addition, CENTER CITY (CCT)
and GRAND CENTRAL (GCT) are identified as the Arabidopsis orthologs of MEDIATOR12 (MED12) and MED13, respectively and are involved in the regulation of KAN expression independently of PHB in peripheral-abaxial identity (Gillmor et al., 2010). Collectively, these reports indicate that the patterning of leaf polarity is based on complicated communications between the SAM and the LP establishment of leaf axes plays an important role in overall leaf organization. Although the patterning of leaf polarity is based on cell position independently and are involved in the regulation of KTI12 during the early leaf developmental stage (Sussex, 1954), the mechanism that regulates this process turns out a little bit.

In previous studies, it was reported that the Arabidopsis Deformed Roots and Leaves 1 (DRL1) gene encodes a homolog of Elongator-associated protein, yeast KILLER TOXIN INSENSITIVE2 (KTI12) protein, and acts as a positive regulator of adaxial leaf patterning and shoot meristem activity (Cho et al., 2007; Fichter et al., 2002; Frohloff et al., 2001; Nelissen et al., 2003). Although biochemical approaches revealed that KTI12 did not affect the HAT activity of Elongator, deletion of the KTI12 gene resulted in Elongator-linked phenotypes, indicating that KTI12 overlaps functionally with Elongator (Potrikus et al., 2005). In addition, KTI12 was shown by chromatin immunoprecipitation to bind chromatin throughout the genome. The yeast KTI12 gene was identified originally from Saccharomyces cerevisiae in a screen for resistance towards zymocin (Butler et al., 1994; Fichter and Schaffrath, 2002; Frohloff et al., 2001), a trRNase ribotoxin from Kluyveromyces lactis that inhibits the growth of sensitive yeast species (Jablonowski and Schaffrath, 2007; Jablonowski et al., 2008; Lu et al., 2005; Nandakumar et al., 2008). In addition to zymocin resistance, yeast strains deleted for the KTI12 gene exhibit hypersensitivity to temperature and caffeine (Fichter et al., 2002; Frohloff et al., 2001). Elongator (ELP) complex contains six subunit which form the core complex (ELP1-ELP3) and a second module (ELP4-ELP6; Winkler et al., 2001). In Arabidopsis, loss-of-function mutants of ELP showed pleiotropic effects including narrow leaves, disorganized SAM, short roots, high sensitivity to ABA and deficiencies in basal immunity (Chen et al., 2006; DeFraia et al., 2010; Nelissen et al., 2003; 2005; 2010; Zhou et al., 2009).

To further clarify the role of chromatin remodeling and transcription regulation during leaf development, we studied the structural and functional features of DRL1 involved in the patterning of leaf polarity. We analyzed evolutionally conserved functions of DRL1 and alterations in the SAM and leaves of the Arabidopsis drl1-101 mutant. Our comparative analysis of the function between Arabidopsis DRL1 and yeast KTI12 provides insight into the mechanism that regulates communication between the SAM and LP for proper establishment of leaf polarity.

**MATERIALS AND METHODS**

**Plant and yeast materials and growth conditions**

The drl1-101 was isolated from Ds-transposon insertion mutants in Arabidopsis Nossen-0 (No-0) background, as described by Cho et al. (2007). Seeds of plants were surface sterilized, germinated on Murashige and Skoog (MS) media and transferred to soil after 3 weeks. Plants were grown at 23°C under long-day light condition (50-100 µE/m²·s, 16 h light/8 h dark). All yeast strains are from Saccharomyces cerevisiae (Supplementary S1). KTI12-deficient mutant (YKL110C) and wild type (WT; BY4741) were used for thermosensitivity and caffeine sensitivity. KTI12-deficient mutant (LFY12) used for zymocin sensitivity assay was generated by interruption of KTI12 region by insertion of fragment from pYF6, YEP213 carrying the LEU2 (Butler et al., 1994). Yeast strains were routinely grown on standard rich and minimal growth media, YPD or SC media at 30°C (Sherman, 1991). To test complementation of thermosensitivity and caffeine sensitivity, yeast strains grew on media at 30°C or 39°C or on media with or without caffeine (Sigma, USA), respectively. For zymocin toxin sensitivity assay, WT (LS20) and kti12.1·LFY12 (LFY12) mutant were transformed with galactose inducible g-toxin gene expression vector, pHMS14 (Frohloff et al., 2001).

**Amino acid sequence alignment**

DRL1 and homologs nucleotide and amino acid sequences from various organisms were retrieved from NCBI GenBank database (http://www.ncbi.nlm.nih.gov/; www.ncbi.nlm.nih.gov) and amino acid sequences of DRL1 and homologs were aligned according to CLUSTALW multiple alignment program.

**Microscopic observation**

For anatomical analysis, samples were fixed in fixation solution under a vacuum, dehydrated by graded ethanol series and clarified, as described by Cho et al. (2007). Samples were observed on stereoscopic microscope (Leica M212.5, Leica, Germany) and light microscope (Axioskop2, Carl Zeiss, Germany). For scanning electron microscope, we followed the method described by Cho et al. (2007). Palisade cells localized between the midvein and in the margin of leaf blades were analyzed for measuring the cell number and size.

**Cloning of Arabidopsis and rice DRL1 and semi-quantitative RT-PCR**

Arabidopsis DRL1 gene and its rice ortholog (OsDRL1), Atfg13870 and Os11g0312782, respectively, were amplified from Arabidopsis and rice total mRNA using specific primer pairs and cloned, as described by Cho et al. (2007). Cloned DRL1 genes were transferred into yeast expression vector, pTD1 derived from pRS316 containing CEN6 replicon and ARS associated with histone 4 (ARSH4) (Ueda et al., 2001). KTI12 and DRL1 overexpression in multicopy for zymocin toxin sensitivity assay was achieved by YEpLac181 vector harboring KTI12 or DRL1 ORF with KTI12 promoter as described previously (Butler et al., 1994). Constructed plasmids were transformed into yeast by LiCl method or electroporation. Total RNA from the shoot apex of 7-day old seedling and leaves of 3-weeks old plants was isolated by RNasey mini kit (Qiagen, USA) and used as template for cDNA synthesis (TOYOBO, Japan). For semi-quantitative RT-PCR, PCR mixture containing 20 ng cDNA was reacted as follows: one cycle of 30 s at 95°C, followed by 30 cycles of 30 s at 95°C, 30 s at 53-58°C and 30 s at 72°C. The primer pair sequences of DRL1, SAM specific-, leaf polarity related-, cell proliferating regulatory genes, and β-TUBULIN 4 (TUB4) gene for a positive control are described in Supplementary Table S2.

**RESULTS**

Comparison of the deduced Arabidopsis DRL1 amino acid sequence with homologous sequences from yeast and other species

DRL1 was reported previously to encode a homolog of the yeast Elongator-associated protein, KTI12 (Cho et al., 2007; Nelissen et al., 2003). To examine the evolutionary history and structural features of the DRL1 protein, we aligned the Arabidopsis DRL1 sequence with the sequences of DRL1 homologs...
from various other species. Alignment of the amino acid sequences of DRL1 homologs from plants (Arabidopsis thaliana, Oryza sativa, Zea mays, and Glycine max), yeast (S. cerevisiae and Schizosaccharomyces pombe), protozoa (Dictyostelium discoideum), and animals (Danio rerio and Homo sapiens) revealed the presence of conserved domains including an ATP/GTP-binding motif in the N-terminus, two calmodulin (CaM)-binding motifs in the N- and C-terminal regions, and domains specific to plant species (plant-specific sequences I and II) (Fig. 1A). The Arabidopsis DRL1 amino acid sequence exhibited the highest similarity to the sequences of other plant DRL1 homologs with similarities of 58.55, 72.52, and 66.11% to the DRL1 homologs of O. sativa, G. max, and Z. mays, respectively, and the lowest similarity to the yeast DRL1 homologs from S. cerevisiae (28.15%; Fig. 1D). Based on an amino acid alignment and functional site prediction analyses, we identified a conserved sequence, KTQ(R)DVR(K) designated plant-specific sequence I, in the central region that could form a short α-helix and may have WD40 repeat-binding motif (Fig. 1B; Dinkel et al., 2013). We also identified a second invariant sequence, GQS(Y/T)SL designated plant-specific sequence II, in the C-terminal region that was conserved among dicot plants; could form a shorter α-helix than in the yeast proteins and may have NEK2 phosphorylation motif (Fig. 1C; Dinkel et al., 2013). Among the genes examined in this study, the H. sapiens DRL1 homolog encoded the longest amino acid sequence (Fig. 1A). A protein secondary structure prediction analysis of the full amino acid sequences of Arabidopsis (DRL1) and S. cerevisiae (KTI12) using the PHYRE2 program (www.sbg.bio.ic.ac.uk/phyre2) showed that the structure of both proteins was very similar (Supplementary Fig. S1). The DRL1 protein contained 13 α-helices and 6 β-sheets, while the KTI12 protein had 11 α-helices and 5 β-sheets (Supplementary Figs. S1A-S1D). Based on hydroxylation and transmembrane prediction analysis using TMpred and SPLIT programs, the DRL1 and KTI12 proteins appear to lack any transmembrane domains (Supplementary Figs. S1E and S1F).

**Fig. 1.** Amino acid sequence alignment and homology analysis of DRL1. (A) Amino acid sequence alignment. Conserved domains include an ATP/GTP-binding motif and two CaM-binding motifs (thin line), two plant-specific sequences (thick lines). The functions of the plant-specific motifs are unknown. Amino acid sequences of DRL1 and its homologs used in the alignment were retrieved from the NCBI GenBank database. The GenBank accession numbers of the amino acid sequences are NP_172840 (Arabidopsis thaliana), NP_001145516 (Zea mays), XP_003528855 (Glycine max), BAH-95225 (Oryza sativa), XP_643511 (Dictyostelium discoideum), NP_594556 (Schizosaccharomyces pombe), NP_012812 (Saccharomyces cerevisiae), NP_001119880 (Danio rerio), and NP_612425 (Homo sapiens). (B) A portion of the overall alignment containing plant-specific sequences I (box). (C) A portion of the overall alignment containing plant-specific sequences II (box). (D) Amino acid sequence homologies of DRL1 homologs from plants, animals, protozoa and yeasts to Arabidopsis DRL1.

**Complementation of the yeast kti12Δ mutant by the DRL1 protein**

In a previous study, we suggested that Arabidopsis DRL1 gene encodes a homolog of the yeast KTI12 gene, which interacts with the elongator complex (Cho et al., 2007; Fichtner et al., 2002; Frohloff et al., 2001; Nelissen et al., 2003). The KTI12 protein associates with Elongator, a six-subunit histone acetyltransferase complex with roles in RNAPII transcription and tRNA anticodon modification that are conserved among yeast and plant cells (Nelissen et al., 2003; 2005; 2010; Otero et al., 1999; Witschieneb et al., 1999). The yeast kti12Δ mutant exhibited a thermosensitive phenotype above 38°C and growth retardation caused by a delayed G1 phase in the cell cycle (Fichtner et al., 2002; Frohloff et al., 2001). To confirm that DRL1 might be related to yeast KTI12, we performed complementation tests using overexpression of Arabidopsis DRL1 and rice OsDRL1 genes in the yeast KTI12 deletion strain YKL110C (Fig. 2). The YKL110C strain grew normally at 30°C, but exhibited inhibited growth at 39°C, compared to WT BY4741 (Fig. 2). Growth at 39°C was restored in YKL110C harboring both DRL1 and OsDRL1 genes inserted into the yeast expression vector pTU1, indicating that the deletion of KTI12 was, at least...
A

BY4741
YKL110C
YKL110C-empty vector
YKL110C-DRL1
YKL110C-OsDRL1

30°C
39°C

B

0 2 4 6 8 10 12 14 16 18 20
Time (h)

0 2 4 6 8 10 12 14 16 18 20
Time (h)

Fig. 2. Complementation of thermosensitivity in yeast KTI12 mutants expressing plant DRL1 genes. (A) Thermosensitivity assay. ktt12Δ mutant (YKL110C) was transformed with pTU1 plasmid harboring Arabidopsis DRL1 and rice OsDRL1 genes. Empty pTU1 vector was used as a control. To test the thermosensitivity of yeast strains, serial dilutions of the yeast strains were replica-spotted and incubated on YPD media for 30 h at either permissive (30°C) or non-permissive (39°C) temperature. (B) Time-course experiment for quantitative analysis of yeast growth rate. To determine thermosensitivity, optical densities (ODs) of cell population were measured at 2-h intervals over 20 h at either permissive (30°C) or non-permissive (39°C) temperature, using a spectrophotometer at 600 nm.

partially rescued by expression of the plant DRL1 genes (Fig. 2A). Expression of DRL1 triggered higher extent of the restoration of yeast growth than expression of OsDRL1 (Fig. 2A). To confirm yeast growth rate in detail, time-course experiment for quantitative analysis was performed in liquid medium. During growth at 39°C, YKL110C harboring plant DRL1 genes showed delayed starting points of log phase, compare to WT BY4741 (Fig. 2B). YKL110C harboring plant DRL1 genes showed earlier starting of log phase than YKL110C (Fig. 2B), indicating that the function of plant DRL1 genes is partially conserved with that of yeast KTI12 gene.

In addition, the ktt12Δ mutant exhibited sensitivity to drugs including caffeine (Fichtner et al., 2002; Frohloff et al., 2001). We performed a complementation assay for caffeine sensitivity in YKL110C, with or without DRL1 expression. The YKL110C mutant was sensitive to caffeine (Figs. 3A, 3C) and DRL1 expression did not restore growth performance at 30°C or 39°C (Fig. 3A). To further confirm the caffeine sensitivity, we performed a time-course analysis of cell growth on media containing caffeine with different concentration. Expression of DRL1 restored the growth retardation for 9 h at 0 mM caffeine (Fig. 3C). However, expression of DRL1 did not restore the caffeine sensitivity when grown on 6 mM or 7.5 mM caffeine (Figs. 3B and 3C), indicating that the function of Arabidopsis DRL1 may not entirely overlap with yeast KTI12 in caffeine sensitivity.

Furthermore, the ktt12Δ mutant is known to exhibit zymocin toxin resistance (Fichtner et al., 2002; Frohloff et al., 2001). KTI12 overexpression also causes zymocin resistance, although the degree of zymocin resistance caused by elevated KTI12 gene expression is lower than that induced by deletion of KTI12 gene (Frohloff et al., 2001). We also performed an assay for zymocin resistance in WT and ktt12Δ mutant with or without DRL1 overexpression. Multiplicity DRL1 in combination with the GAL1-driven expression of the γ-toxin trRNase subunit from zymocin from vector pHMS14 in galactose medium failed to elicit zymocin resistance, while in multicycopy, the yeast KTI12

Fig. 3. Complementation of thermosensitivity and caffeine sensitivity of yeast KTI12 mutants expressing Arabidopsis DRL1. (A) Synchronized assay of thermo- and caffeine sensitivities. Serial dilution of yeast strains were replica spotted and incubated on combined conditions of temperature (30°C or 39°C) and growth media (YPD media without caffeine or with 6 mM caffeine) for 30 h. (B) Caffeine sensitivity assay. Serial dilution of yeast strains were replica-spotted and incubated on YPD media containing 0, 6, and 7.5 mM caffeine at 30°C for 30 h, respectively. (C) Growth curve of yeast strains on YPD media containing 0, 6, and 7.5 mM caffeine, respectively. To determine caffeine sensitivity, optical densities (ODs) of cell population were measured at 2-h intervals over 18 h using a spectrophotometer at 600 nm.
gene suppressed zymocin and triggered resistance to the trNase toxin (Fig. 4). This indicates that the function of Arabidopsis DRL1 may not overlap with yeast KTI12 in zymocin mediated growth inhibition.

Based on these results, DRL1 may have a biochemical function that is partially conserved with yeast KTI12, as well as a unique function that is specific to its plant context.

**Function of DRL1 during development in Arabidopsis**

Previously, we isolated the Arabidopsis drl1-101 mutant, which contains a Ds transposon in the C-terminal region of the DRL1 (At1g13870) gene, and performed genetic analysis demonstrating that the DRL1 gene is involved in leaf patterning and SAM formation (Cho et al., 2007). The drl1-101 mutant produced narrow leaves with a structure that was trumpet-like and filamentous (Cho et al., 2007). In this study, we found that the leaves of the drl1-101 mutant were serrated and diminished in size during the early stages of leaf development, compared to the WT (Figs. 5A and 5B). The palisade cells in the WT leaf blades exhibited an oval shape and were regular in size (Fig. 5C). In contrast, the palisade cells in the drl1-101 mutant were irregular sizes and reduced numbers accompanying an increase in the intracellular spaces (Fig. 5F). The number of cells in the drl1-101 mutant leaf blades was significantly reduced compared to that in WT leaf blades and resulted in 82% of WT (Fig. 5G).

The establishment of adaxial-abaxial polarity of leaf blades requires communication with the SAM and is necessary for leaf growth. Morphological analysis of longitudinal sections from 10-day-old seedlings in a previous study showed that the SAM structure in the drl1-101 mutant was defective (Cho et al., 2007). To examine alterations in the drl1-101 SAM in detail, we observed the external shape and size of the SAM using scanning electron microscopy (SEM). The SEM analysis showed that the drl1-101 SAM was smaller in size compared to the WT and that the external shape was flatter than that of the WT (Figs. 5C and 5D).

To analyze effects of acquisition and maintenance of meristematic identity on leaf polarity patterning, we examined the expression levels of positive regulators of meristem identity acquisition and maintenance including the class-I KNOX genes KNATTED1 (KNAT1), KNAT2, and KNAT6, and the SHOOTMERICITLESS (STM) gene in WT and drl1-101 shoot apaxes. The expression levels of the class-I KNOX genes in the shoot apex were lower in drl1-101 than in the WT (Fig. 6A). In particular, STM expression was undetected in drl1-101 (Fig. 6A). Decreased class-I KNOX and STM expression may result in the smaller size and altered SAM structure in the drl1-101 mutant.

Because the drl1-101 mutant exhibited abaxialized leaves with altered cell differentiation, we examined the expression patterns of abaxial-determining genes in WT and mutant leaves. As noted above, the KAN and YAB gene families promote the abaxialization of leaves by repressing adaxial polarity determinants or integrating abaxial polarity determinants. The YAB gene family was expressed at high levels in drl1-101 mutant leaves relative to WT leaves (Fig. 6B). The YAB2 gene exhibited the strongest expression of the YAB gene family members in drl1-101 mutant leaves (Fig. 6B). The KAN1 gene was also expressed at high levels in the drl1-101 mutant relative to the WT (Fig. 6B). These results indicated that the altered phenotype of the drl1-101 leaf cells was caused by upregulated YAB and KAN gene expression, which regulates the abaxialization features of leaves. Taken together, these results indicate that defects in the establishment of adaxial-abaxial polarity might be coupled to genetic alteration within the meristem in the drl1-101 mutant.

Because the drl1-101 mutant exhibited altered cell number and size, we examined the expression patterns of cell cycle-related gene Cyclin D3;1 (CYCD3;1) and cell proliferation regulatory genes AINTEGUMENTA (ANT) and ARGOS in WT and drl1-101 mutant leaves. CYCD3;1 expression was increased, while ANT and ARGOS were not changed in the drl1-101 mutant relative to the WT (Fig. 6C). Despite the reduced cell number in drl1-101 mutant, the expression of cell cycle gene might be activated by the defects in DNA metabolism. Cycd3;1 directly regulates cell division from various external and internal signals, while ANT and ARGOS act upstream of cell division regulatory mechanisms (Dewitte et al., 2003; Hu et al., 2003; Mizukami and Fischer, 2000). Therefore, this result indicates that DRL1 might indirectly regulate cell division in the upstream of CycD3;1.

**DISCUSSION**

Genes homologous to Arabidopsis DRL1 and yeast KTI12 are present in the genomes of most organisms including fungi, fruit flies, human, and plants. However, their biological and genetic functions have not been studied in depth. More detailed studies on yeast KTI12 revealed that it acts as a regulator of HAT while associating with the elongator complex (Otero et al., 1999; Wittschieben et al., 1999). KTI12 is required for the HAT activity of Elongator complex in vivo. However, the specific role of

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**Fig. 4.** Effect of DRL1 overexpression on toxin resistance. KTI12 deletion mutant, LFY12 was generated by transformation with the pYF6 deletion construct. Yeast strains, WT (LS20) and kit12Δ mutant (LFY12) were transformed with GAL1-driven γ-toxin gene expression vector, pHMS14. LS20 transformants were transformed with YEpLac181 vector harboring KTI12 or DRL1 ORF with KTI12 promoter, respectively. Empty YEpLac181 vector was used as a control. Serial dilution of yeast strains were replica spotted and incubated on YPD media containing 2% glucose (inactivation of γ-toxin synthesis) or 2% galactose (activation of γ-toxin synthesis) for 30 h, respectively. Growth on galactose media means resistance towards toxin (Toxγ) and the inhibition of growth does sensitivity towards toxin (Toxγ).
KTI12 regulating Elongator HAT activity remains still unclear (Petrakis et al., 2005).

DRL1 homologs exhibited 28-77% amino acid sequence homology with that of Arabidopsis DRL1 (Figs. 1A and 1D). The existence of only a single gene copy of DRL1 homologs in the genomes of all organisms may suggest that no gene duplication occurred during evolution and that the function of DRL1 homologs may be highly conserved (Cho et al., 2007). In this study, we showed that DRL1 proteins have structurally conserved domains with other species as well as plant-specific sequences (Fig. 1A), which suggest existence of differences in the evolution of conserved biochemical functions specific to plants. Expression of Arabidopsis and rice DRL1 genes in the yeast kti12Δ mutant complemented growth retardation at 39°C, indicating that this function of yeast KTI12 appears to be conserved in and rescued by plant DRL1 genes (Fig. 2). In contrast, KTI genes conferred resistance to zymocin, which affects RNAPII-dependent gene transcription in yeast and also targets tRNAs for anticond cleavage (Frohloff et al., 2001; Jablonowski and Schaffrath, 2007; Jablonowska et al., 2006; Lu et al., 2005). Mutations in KTI genes including KTI12 cause caffeine sensitivity as well as zymocin resistance (Fichtner et al., 2002; Frohloff et al., 2001). In this study, DRL1 expression in the yeast kti12Δ mutant did not reveal the suppression of the caffeine sensitive phenotype (Fig. 3). In addition, despite elevation of DRL1 expression in multicopy in a yeast WT background, DRL1 failed to suppress the zymocin sensitive phenotype (Fig. 4). This is in contrast to yeast KTI12, which when overexpressed, suppresses zymocin action (Butler et al., 1994; Fichtner et al., 2002; Frohloff et al., 2001). In addition, Arabidopsis ELP1 encoding a homolog of yeast Elongator subunit ELPI/KTI17 complemented zymocin resistance, but not caffeine sensitivity, in the yeast elp1 mutant (Chen et al., 2006). Thus, the failure of plant ELP1 and DRL1 to rescue the caffeine sensitivity in yeast mutants strongly suggests that the role of Elongator and DRL1 in response to caffeine may have changed between yeast and plants during the course of evolution.

On the other hand, exchangeable Elongator function in tRNA modification between plant and yeast indicated that remarkable structural conservation remained in animals, fungi and plants (Chen et al., 2009; Huang et al., 2005; Mehlgarten et al., 2010). Despite structural conservation, Elongator function may differ in unicellular and multicellular organisms, based on the functional characterization of Elongator mutants from yeast and plants (Nelissen et al., 2005). Four components of the Elongator complex, ELOGATA1 (ELO1), ELO2, ELO3, and DRL1/ELO4, have been identified as a HAT and have shown to influence transcription factors and RNAPII preinitiation complex in response to developmental and environmental cues (Kornberg, 2005; Poss et al., 2013).

A crucial question is why many Elongator and Mediator mutants have defective leaf phenotype and how Elongator and Mediator affects leaf morphology? Perhaps the most controversial answer is the dependent processes on specificity of HAT and/or tRNA modification activity. Actually, Elongator is known to be multifunctional protein involved in biotic and abiotic stresses as well as in leaf morphogenesis (Chen et al., 2006; DeFaria et al., 2010; Nelissen et al., 2003; 2005; 2010; Zhou et al., 2009). How they control specifically to leaf polarity? In this study, we showed that the DRL1 gene regulates the expression
histone acetylation is involved in the regulation of leaf initiation and control of meristem activity (Shen and Xu, 2009). In addition, patterns of meristem-controlling transcription factors and the due to its involvement in the maintenance of stable expression leaf patterning, flowering, gametogenesis and embryogenesis, on plant developmental processes including organ formation, cell differentiation. Chromatin remodeling has a critical influence and maintenance of pluripotent cells in the SAM by preventing RNAPII and Elongator induces cell differentiation through the and establishment of leaf polarity (Chua et al., 2005; Ishiba-

Our future studies will address how chromatin remodeling by mediated histone acetylation or through other mechanisms. results of previous studies are consistent with our speculation Jones et al., 2006; Huang et al., 2006; Yao et al., 2008). The subunit, function in the identification of leaf polarity (Griffiths-

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