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Article Addendum

A model for the 26S proteasome and ribosome actions in leaf polarity formation

Qihua Ling,† Yao Yao† and Hai Huang*

National Laboratory of Plant Molecular Genetics; Shanghai Institute of Plant Physiology and Ecology; Shanghai Institute for Biological Sciences; Shanghai China

†These authors contributed equally to this work.

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Leaf morphogenesis requires the establishment of adaxial-abaxial polarity in emerging leaf primordia, and a number of genes participating in this process have been identified in recent years. We previously reported that the 26S proteasome is important in specifying the leaf adaxial fate. More recently, two papers from separate researches showed that several genes encoding ribosomal large subunit proteins also play an important role in leaf adaxial-abaxial patterning. Here we show that plants with a single mutation in the genes encoding either 26S proteasome subunits or ribosomal proteins shared similar abnormalities in some leaves, with an outgrowth formed on the distal part of the leaf abaxial side. Plants harboring these 26S proteasome or ribosome mutations in combination with an additional mutation *asymmetric leaves1* or *2 (as1 or as2)* demonstrated severely defective leaves, and the phenotypes of these double mutants were very similar. Because activities of the 26S proteasome and ribosome both affect the level of functional proteins, the recent findings suggest that a previously unrecognized regulation, the protein level regulation, is critical in normal leaf patterning. A regulatory model for the 26S proteasome and ribosome actions in leaf patterning is discussed.

Recently, several Arabidopsis genes encoding the 26S proteasome subunit and ribosomal proteins have been identified that play important roles in specifying leaf adaxial identity.[1-4] The 26S proteasome and ribosome are large protein or protein/rRNA complex, and mutations in different protein genes of each complex could result in plants with leaf adaxial-abaxial defects, whereas the phenotypes are relatively weak. To explore whether or how these two complexes may cooperate in controlling leaf patterning, we characterized genetically three genes among others of the complexes: *AE3*, *AE5* and *AE6*, which encode a 26S proteasome subunit RPN8A, and ribosomal proteins RPL28A and RPL5A, respectively. Compared with wild-type *Ler* plants (Fig. 1A), *ae3-1* (Fig. 1B), *ae5-1* (Fig. 1C) and *ae6-1* (Fig. 1D) did not exhibited strong leaf polarity defects,[1-3] but some leaves from each of these three mutants produced an outgrowth on the distal part of the adaxial leaf side (Fig. 1B–D, insets). In contrast to their single mutants and *as2-101* (Fig. 1E), double mutants containing *as2-101* or *as1-101* and *ae3-1*, *ae5-1* or *ae6-1* resulted in plants with severe but similar leaf phenotypes (Fig. 1F–H, for the *as1* combinations, data not shown). Briefly, most leaves were radially symmetric (Fig. 1F–H, arrowheads) and the remaining expanded lotus-like leaves had very rough adaxial surfaces (Fig. 1F–H, arrows).

It was previously proposed that several proteins from the 26S proteasome or ribosome complex demonstrated specific functions distinct from those of their complexes in protein degradation or translation.[5-8] However, mutations in different 26S proteasome or ribosomal protein genes examined all resulted in a similar leaf defect, albeit varying in severity, and double mutants with *as1* or *as2* all produced strong and very similar leaf phenotypes.[1,2,4] Based on these observations, it seems unlikely that the regulation of leaf patterning depends on functions of a particular protein of the complexes, but instead, the conserved functions of protein degradation or translation of the two complexes may be involved. How these two complexes function to determine leaf polarity is not yet clear. One possibility is that these two systems are required for an accurate balance in levels between the adaxial- and abaxial-promoting factors during leaf polarity formation. These factors include transcriptional factors and proteins required for small RNA biogenesis and action (reviewed in refs. 9 and 10), as two microRNAs, miR165 and miR166, and one trans-acting siRNA, tasiR-ARF, are important in leaf patterning.[11-14] It is known that some of the regulatory factors for leaf patterning act antagonistically, and exhibit complementary expression domains in multiple tissues. For example, adaxial-promoting genes *REV/PHB/PHV* antagonize abaxial-promoting ones *KAN1/KAN2/KAN3*[15,16] and tasiR-ARF which specifies the adaxial leaf fate antagonizes abaxial-promoters miR165/miR166.[17] On the other hand, the 26S proteasome and ribosome complexes are known to act selectively to process their targets,[18,19] and certain leaf patterning factors are likely to be the targets of these complexes. Therefore, a failure in degrading an abaxial-promoting factor (a loss of function in the 26S proteasome) and incapability in synthesizing its corresponding antagonistic

*Correspondence to: Hai Huang; Shanghai Institute of Plant Physiology and Ecology; Shanghai Institute for Biological Sciences; 300 Fenglin Road; Shanghai 200032 China; Tel.: +86.21.54924088; Fax: +86.21.54924015; Email: hhuang@sippe.ac.cn

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Figure 1. Mutant phenotypes suggest that the protein-level regulation is critical for normal leaf patterning. (A–E) Phenotypes of wild-type and single mutants. (A) wild-type *Ler*, (B) *ae3-1*, (C) *ae5-1*, (D) *ae6-1* and (E) *ae2-101*. Insets in (B–D) show cauline leaves with an ectopic outgrowth (arrowheads) on their distal part of the abaxial side. (F–H) Double mutant phenotypes of *ae3-1 as2-101* (F), *ae5-1 as2-101* (G) and *ae6-1 as2-101* (H). Arrowheads and arrows in (F–H) show the radially symmetric and lotus-like leaves with rough adaxial leaf surfaces, respectively. Bars = 5 mm in (A–H).

adaxial-promoting factor (a loss of function in ribosome) can result in the same consequence for leaf patterning. This model can explain why *ae3*, *ae5* and *ae6* single mutants share some similar abnormalities in the leaf and the severe leaf phenotypes of *ae3 as2*, *ae5 as2* and *ae6 as2* double mutants are very similar with each other.

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