

Article Addendum

# A Novel Function of the 26S Proteasome in Repressing Class-1 *KNOX* Genes During Leaf Development

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## KEY WORDS

26S proteasome, *AE3/RPN8a*, class-I *KNOX* genes, leaf development

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Addendum to:

*The Proteolytic Function of the Arabidopsis 26S Proteasome is Required for Specifying Leaf Adaxial Identity*

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## ABSTRACT

Leaf organogenesis occurs within the peripheral zone of the shoot apical meristem (SAM). It has been known that several members of the class-1 *KNOTTED1*-like homeobox (*KNOX*) genes are expressed in the SAM, and their expression must be prevented during leaf primordium initiation and subsequent leaf development. A number of regulators that repress class-1 *KNOX* genes have been identified, and characterizations of these regulators greatly improved our knowledge of the genetic basis of leaf organogenesis. We have recently reported that the proteolytic function of the *Arabidopsis* 26S proteasome is involved in specifying leaf adaxial identity during leaf development, by characterizations of mutants defective in genes encoding several 26S proteasome subunits. Here we demonstrate that in addition to the role in leaf polarity establishment, the 26S proteasome also participates in repression of class-1 *KNOX* genes during leaf development. We show that loss of functions in *RPN8a* and *RPT2a*, two 26S proteasome subunit genes, resulted in leaves that produce ectopic outgrowths on the abaxial side of blades. These outgrowths were accompanied by the ectopic expression of several class-1 *KNOX* genes. These results indicate that the 26S proteasome is important in repressing class-1 *KNOX* genes and its function may be required until later leaf developmental stages.

Class-1 *KNOX* genes are members belonging to the super homeobox gene family, and are found in species throughout the plant kingdom.<sup>1</sup> The class-1 *KNOX* genes are expressed in the SAM for maintaining meristematic identity, whereas are downregulated at the position where the leaf initiates. It has been known that persistent repression of class-1 *KNOX* genes is required for subsequent leaf development.<sup>2,3</sup> In *Arabidopsis*, class-1 *KNOX* genes consists of four members: *SHOOT MERISTEMLESS (STM)*, *BREVIPEDICELLUS (BP)*, *KNAT2* and *KNAT6*. Ectopic expression of *BP* led to meristem- and organ-like structures forming on the leaf, or resulted in leaves with other phenotypic abnormalities.<sup>4-7</sup> To date, a number of genes have been found to play roles in repressing class-1 *KNOX* genes during leaf development. These include *ASYMMETRIC LEAVES1/2*,<sup>5,8,9</sup> *HIRA*,<sup>10</sup> *YABBY5*,<sup>6</sup> *BLADE-ON-PETIOLE1/2*,<sup>7,11,12</sup> *SERRATE (SE)* and *PICKLE (PKL)*,<sup>5</sup> *FERTILIZATION-INDEPENDENT ENDOSPERM (FIE)* and *CURLY LEAF (CLF)* complex,<sup>13</sup> and even posttranscriptional gene silencing components *RNA-DEPENDENT RNA POLYMERASE (RDR6)*<sup>14</sup> and *ARGONAUTE1 (AGO1)*.<sup>15</sup>

The regulated protein degradation by the ubiquitin/26S proteasome system is crucial for a wide range of developmental and physiological processes in eukaryotes.<sup>16</sup> Recently, we reported a role of the 26S proteasome in *Arabidopsis* leaf polarity formation.<sup>17</sup> We demonstrate here that during leaf development the 26S proteasome is also involved in repression of class-1 *KNOX* genes. In comparison to the wild-type leaves (Fig. 1A), the *ae3-1* mutant, which carries a lesion in the 26S proteasome *RPN8a* subunit,<sup>17</sup> produced narrow rosette leaves (Fig. 1B and C). In some rosette leaves and most cauline leaves, ectopic outgrowths formed on the abaxial side of leaf blade, associating with the midrib in the distal region (Fig. 1D and 1E). Some of these outgrowths could eventually form leaflets (Fig. 1E).<sup>17</sup> These abnormal leaf phenotypes can be observed in some other 26S proteasome subunit mutants, such as *hlr-2*, which harbors a defective *RPT2a* (Fig. 1F). These ectopic outgrowths indicate that *ae3-1* and *hlr-2* mutations may restore the meristematic capacity in the leaf.

To examine whether these outgrowths were also accompanied by the ectopic expression of class-1 *KNOX* genes, we analyzed wild-type and mutant plants carrying a *BP::GUS* fusion. In wild-type plants (Fig. 1G), *GUS* staining was detected in the stem but not in the leaf blade. By contrast, most *ae3-1* leaves showed *GUS* staining in the midrib, although the intensity of *GUS* staining differed among mutant leaves (Fig. 1H–J). Interestingly, leaves

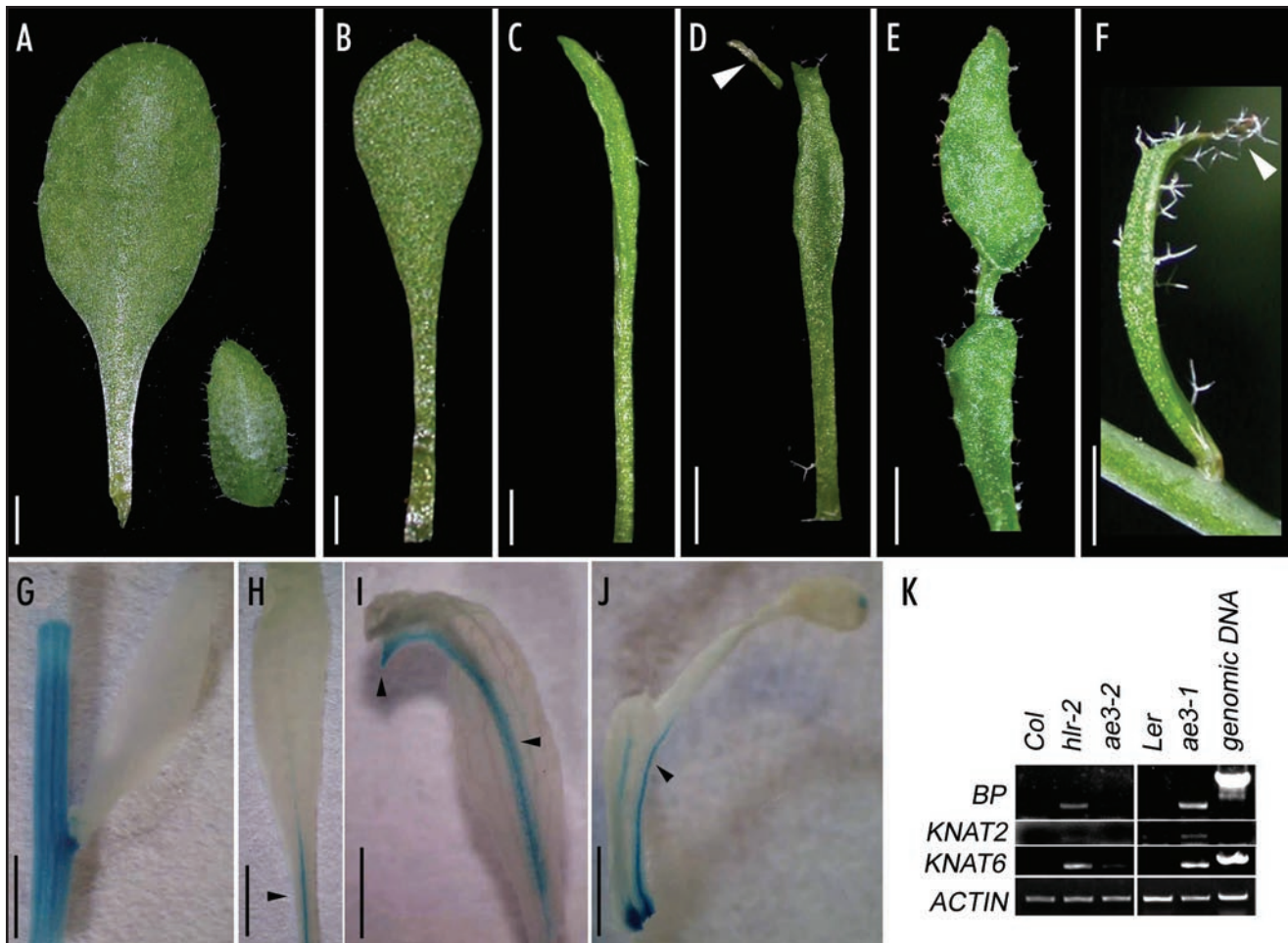


Figure 1. Altered leaf phenotypes and ectopic expression of class-1 *KNOX* genes in *ae3-1* and *hlr-2* plants. (A) A rosette leaf (left) and a cauline leaf (right) from a wild-type Landsberg *erecta* plant. (B–E) *ae3-1* leaves. Most rosette leaves of *ae3-1* became long and narrow (B), and some of them were very narrow (C). Ectopic outgrowths were formed on abaxial side of some rosette and cauline leaves, associating with the midrib in the distal region of blades. Arrowhead points to an outgrowth appearing on a rosette leaf (D). Some of the outgrowths extend their growth to form leaflet on cauline leaves (E). (F) An *hlr-2* cauline leaf with an ectopic outgrowth (arrowhead). (G–J) Analyses of GUS activity in leaves carrying a *BP::GUS* fusion in wild type (G) and *ae3-1* mutant plants (H–J). Note that leaves with outgrowths usually showed stronger GUS staining. (K) RT-PCR analyses of *BP*, *KNAT2* and *KNAT6* expression in *ae3-1*, *ae3-2* and *hlr-2* plants. Note that *ae3-1* (Ler) and *hlr-2* (Col) leaves used in the RT-PCR experiment had ectopic outgrowths, whereas the *ae3-2* (Col) had not such structures. Bars = 2 mm in (A–F), and 5 mm in (G–J).

with the outgrowth usually showed stronger GUS staining (Fig. 1I and J) than those that had no ectopic outgrowth (Fig. 1H). We also performed reverse transcription-polymerase chain reaction (RT-PCR) to analyze *BP*, *KNAT2* and *KNAT6* expression in wild-type and mutant leaves. Strikingly, *BP*, *KNAT2* and *KNAT6* transcripts were present in *ae3-1* and *hlr-2* leaves that carried outgrowths, whereas were absent in wild-type leaves or nearly undetectable in the mutant leaves that had no ectopic outgrowth (Fig. 1K). These results suggest that the outgrowths on the *ae3-1* and *hlr-2* leaves may be caused by the ectopic expression of class-1 *KNOX* genes.

In *Arabidopsis*, genes that repress class-1 *KNOX* members can be grouped into two general types, based on the ectopic expression of *KNOX* genes in the single gene mutation or in the combined gene mutations.<sup>15</sup> The first category (type I) includes *AS1/2*, *BOP1/2*, *FILAMENTOUS FLOWER*, *YABBY3*, *AGO1*, *FIE* and *CLF*. The mis-expression of all or some class I *KNOX* genes can be detected in the leaves of plants with these gene mutations. The second category (type II) comprises *SE*, *PKL* and *RDR6*. The class I *KNOX* genes are normally repressed in the *se*, *pk1*, *rdr6* single mutant plant leaves, but

are expressed more robustly in the *se as1(2)*, *pk1 as1(2)* and *rdr6 as1(2)* double mutant leaves.<sup>5,14</sup> Our results showed that the 26S proteasome subunit genes belong to the type I *KNOX* repressor category.

Taken together, our data reveal that the 26S protein degradation machinery is important in leaf developmental regulation, not only for leaf polarity establishment but also for class-1 *KNOX* gene repression. It would be of great interest to identify in the future the targets of the 26S proteasome, and to uncover whether the targets share functions in regulating both *KNOX* and polarity genes.

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