

A 67-Amino-acid Protein Regulates Leaf Development by Binding to Class III HD-ZIP Transcription Factors in *Arabidopsis* 

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To investigate leaf development in Arabidopsis, an activation tagging mutant pool was generated and screened. A mutant showing aberrant leaf development, designated B9, was chosen for further characterization. B9 leaves were small and downward-curled. Some B9 plants had cup-shaped leaves or pin-like structures. These plants finally stopped producing leaves, suggesting that shoot apical meristem is arrested. TAIL-PCR and RT-PCR analysis revealed that at3g52770 (B9) is activated. This gene encodes a small protein with 67 amino acids. Arabidopsis has B9-like (B9L) gene and two more genes containing high homology to B9, comprising B9 family. Electron microscopic observation of B9 leaves revealed that the adaxial side was partially abaxialized. Expression level of class III HD-ZIP transcription factors regulating adaxial identity of leaf was not changed but YAB3 expression was increased. To confirm B9 phenotypes B9 or B9L was overexpressed. The transgenic plants showed the similar phenotypes to B9 mutant. Especially almost all of B9L OX plants showed meristem arrest, resulting in plants without leaves. These phenotypes are very similar to miR166 OX plants and class III HD-ZIP multiple mutants. Furthermore sequence alignment showed that B9 and B9L have homology with class III HD-ZIP transcription factors. All proteins have coiled-coil region, suggesting that they can interact. Yeast two hybrid and GST-pull down assays showed that they could interact. Therefore we suggest that B9 and B9L are novel negative regulators of class III HD-ZIP transcription factors, thereby regulating meristem function and radial patterning in Arabidopsis.