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microRNA-directed Cleavage of ATHB-15 mRNA Regulates Vascular Development in Inflorescence Stems

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Vascular system is an elaborated network of conducting tissues that interconnects all plant organs and transports water, minerals, organic compounds, and signaling molecules throughout the plant body. It consists of two conducting tissues, xylem and phloem, and procambial/cambial cells. Vascular development is initiated by the formation of provascular cells that subsequently develop into procambium, from which both conducting tissues are eventually differentiated. In older plant parts, vascular tissues can be developed through the activity of a secondary meristem, called vascular cambium. Plant growth hormones, such as auxin and brassinosteroids, also play regulatory roles in vascular tissue differentiation. It is now generally accepted that a unified molecular mechanism modulates temporal and spatial development of vascular tissues, although vascular patterns and organizations are diverse in different plant species. However, the molecular components and schemes that direct vascular development are poorly understood. Recent application of molecular genetic tools, mainly established in Arabidopsis, greatly accelerated the identification of the genes involved in vascular development and the elucidation of regulatory mechanisms at the molecular level. A set of class III homeodomain-leucine zipper (HD-ZIP III) proteins has been suggested to regulate vascular development, based on the gene expression patterns and phenotypic characterizations of mutant plants. In Arabidopsis, the class III HD-ZIP gene family include five members; ATHB-15, ATHB-8, PHAVOLUTA (PHV), PHABULOSA (PHB), and REVOLUTA (REV). PHV, PHB, and REV are expressed in vascular tissues, apical and floral meristems, and adaxial domains of lateral organs, whereas ATHB-15 and ATHB-8 are predominantly expressed in vascular tissues, suggesting that the two genes may have important roles in vascular development. It has been reported that ATHB-8 is induced by auxin and promotes procambial and cambial cell differentiation into xylem tissues. However, a loss-of-function *athb-8* mutant does not show any defects in vascular pattern and development, indicating that ATHB-8 is not essential for vascular development. Although ATHB-15 has not been systematically examined yet, the exclusive expression of ATHB-15 and ZEH13, an ATHB-15 gene homologue from *Zinnia elegans*, suggests a role for ATHB-15 in vascular development. Interestingly, a microRNA (miRNA) binding sequence has been recently predicted in ATHB-15 mRNA. It is therefore envisioned that a mechanism governed by miRNA binding might direct vascular development through ATHB-15. miRNAs are small noncoding RNA molecules that regulate target genes either by mRNA cleavage or by translational repression (Kidner and Martienssen 2003; Bartel 2004). They exert the regulatory role through complementary base pairing to target mRNAs. A handful of miRNAs and their target genes have been characterized in plant developmental processes, including flowering time control, floral development, leaf polarity, and leaf morphogenesis. However, in most cases, only the target genes have been examined without functional characterization of relevant miRNA mutants, resulting in ambiguous functional relationship between miRNAs and their target genes. In this work we isolated an Arabidopsis mutant in which miRNA166a is activated and demonstrated that miRNA166a-mediated ATHB-15 mRNA cleavage is a principal mechanism for the regulation of vascular development. In the gain-of-function miRNA166a mutant, the ATHB-15 transcript level was drastically reduced, and the vascular system was evidently altered with expanded xylem tissue and interfascicular region, indicating that vascular cell differentiation is promoted. Arabidopsis plants with reduced ATHB-15 expression also showed a similar phenotype, but it was reversed in transgenic plants overexpressing a miRNA166a-resistant ATHB-15. miRNA166a mediates ATHB-15 mRNA cleavage in standard wheat germ extracts and in Arabidopsis. Furthermore, the miRNA166a complementary sequences are highly conserved in mRNAs of ATHB-15 and its gene homologues from rice, maize, *Zinnia*, and moss. It is therefore likely that ATHB-15 is regulated by miRNA166a through mRNA cleavage in all vascular plants. Altogether, we propose a model for the regulation of vascular development in which miRNA166a-mediated regulation of ATHB-15 is a primary molecular device for the proper maintenance of vascular system, which is further modulated by ATHB-8 through both the posttranscriptional regulation by miRNA166a and the transcriptional regulation by auxin. Other HD-ZIP III genes seem to play additional roles in fine regulation of vascular development. PHV, PHB, and REV regulate organ polarity by specifying adaxial (xylem) and abaxial (phloem) cell fates and ascertain vascular patterning. Furthermore, the conserved basic architecture of vascular systems and HD-ZIP III genes in dicots, monocots, and moss suggest that the miRNA166-mediated regulation of vascular development may be a general rule in all vascular plants.