Novel role of the AT-HOOK MOTIF NUCLEAR LOCALIZED 15 gene in Arabidopsis meristem activity and longevity

Rahimi, A.

Citation

Rahimi, A. (2020, September 1). *Novel role of the AT-HOOK MOTIF NUCLEAR LOCALIZED 15 gene in Arabidopsis meristem activity and longevity*. Retrieved from https://hdl.handle.net/1887/136334

Author: Rahimi, A.
Title: Novel role of the AT-HOOK MOTIF NUCLEAR LOCALIZED 15 gene in Arabidopsis meristem activity and longevity
Issue Date: 2020-09-01

Summary

Plant architecture has distinct forms in different plant species, but also within a species the final architecture of a plant is determined by its gradual development and changes therein induced by environmental conditions during the plant's life cycle. Higher plants have the ability to continually produce new organs and tissues during their lifetime. This unique ability is provided by pools of stem cells organized in so-called meristems. Primary meristems located at apices of the plant body are involved in elongation of the main plant axes, whereas secondary meristems in lateral positions play a role in branching and axis thickening. This meristem activity leads to the development of stems, leaves, branches, and inflorescences. **Chapter 1** of this thesis reviews current advances in our understanding of the molecular mechanisms that regulate plant meristems and their developmental phase transitions. Two main developmental phase transitions are distinguished during post-embryonic plant development. Upon germination, development starts with the juvenile seedling phase, and during the juvenile to adult vegetative phase transition (1) the plant acquires reproductive competence, allowing the plant to subsequently undergo the vegetative to reproductive phase transition (2), which ends with gametogenesis. The shoot apical meristem (SAM) is presented as the main regulator of these developmental phase changes. But also the importance of the shoot axillary meristems (AMs) located in axils of leaves is discussed, as AMs support the formation of secondary shoots (branches) and are important determinants of plant longevity. **Chapter 1** also discusses the procambium and cambium meristems, which continuously generate the major plant vascular tissues, xylem and phloem, via asymmetric periclinal cell divisions. The regulatory mechanisms and factors behind cambium meristem initiation and dynamics are shortly described. Following the reproductive phase and seed set, ageing monocarpic plants (flower only once) undergo senescence, which eventually leads to plant death. In contrast, polycarpic plants (flower more than once) maintain a number of vegetative AMs to support subsequent cycles of growth and to prolong their life span. The structure and function of the *Arabidopsis thaliana* (Arabidopsis) *AT-HOOK MOTIF NUCLEAR LOCALIZED* (*AHL*) gene family is introduced in **chapter 1**, and that of family member *AHL15* described and studied more in detail in **chapter 2**. *AHL15* in Arabidopsis encodes a nuclear protein containing a single DNA-binding AT-hook motif in the N-terminal part and plant- and prokaryote-conserved (PPC) domain at the C-terminus. The PPC domain contributes to physical interaction with other AHLs or nuclear proteins, whereas the AT-hook motif interacts with DNA at AT-rich stretches. In **chapter 2** we show a novel role for *AHL15* as a suppressor of AM maturation, as demonstrated by the reduced longevity phenotypes of single *ahl15* or multiple *ahl* loss-of-function mutants. In contrast, high expression of *AHL15* suppresses AM maturation, resulting in increased longevity in monocarpic Arabidopsis and *Nicotiana tabacum* (tobacco). Detailed analysis and observation showed that *AHL15* function is essential for suppression of AM maturation in the long living, polycarpic Arabidopsis *soc1 ful* double mutant. As expected, *AHL15* acts directly downstream of the flowering genes *SOC1* and *FUL* and in part by suppressing the biosynthesis of the growth- and ageing hormone gibberellic acid. We further compared the expression of the *AHL* gene family in Arabidopsis with that of its close polycarpic relative *Arabidopsis lyrata*. Surprisingly, the expression of several *AHL* genes (*AHL15*, *AHL17*, *AHL19*, *AHL20*, and *AHL27*) was significantly higher in rosette nodes of flowering *Arabidopsis lyrata* plants compared with seedlings, which was not the case in Arabidopsis, suggesting that differential regulation of *AHL* genes in AMs results in different life histories.

Chapter 3 discusses ageing in flowering plants, defined by different developmental phase transitions. The switch from the juvenile to the adult vegetative phase, also referred to as the vegetative phase change (VPC), is marked in Arabidopsis by changes in leaf morphology, with small, round juvenile leaves lacking abaxial trichomes turning into adult leaves that are elongated, serrated, and carry trichomes on the back side of the leaves. During the juvenile-to-adult transition, *miR156/157* expression gradually decreases, and that of their target *SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE* (*SPL*) genes increases to promote plant ageing. An *ahl15* loss-of-function mutant showed early VPC. In contrast, high expression of *AHL15* delayed VPC and flowering in Arabidopsis and tobacco. Expressing *AHL15* under control of a SAM-specific promoter revealed that AHL15 changes the VPC and flowering time by a direct effect on the SAM. AHL15 appeared to repress *SPL* expression independent of the miRNA pathway, and SPLs in turn suppress *AHL*s, suggesting a negative feedback loop between *AHL15* and *SPL*s. Overall, in **chapter 3** we identified a new branch of the ageing pathway in Arabidopsis, in which the longevity-promoting AHL15 protein represses ageing independent of *miR156/157*. **Chapter 4** of this thesis focuses on *AHL15*'s effect on Arabidopsis vascular cambium activity and secondary growth. Detailed analysis showed that in an *ahl15* loss-of-function mutant, interfascicular secondary growth is significantly reduced. In contrast, *AHL15* overexpression remarkably increases secondary xylem formation. A more detailed analysis revealed the unique expression pattern of *AHL15* in the Arabidopsis cambium ring. To confirm this pattern, we expressed *AHL15* under a cambium-specific promoter, *pPXY*, resulting in enhanced secondary-xylem formation in *pPXY:AHL15* stems compared to wild type stems. More interestingly, we confirmed that *AHL15* function is necessary for extensive wood formation in the *soc1ful* double mutant. As explained in **chapter 2,** *AHL15* is downstream of SOC1 and FUL. Close observations of the mechanisms for low or high secondary xylem in *ahl15* loss-of-function or *AHL15* overexpression lines, respectively, showed involvement of cytokinin, a central regulator of cambium activity and secondary growth. In our gene expression analysis, levels of the *IPT* cytokinin biosynthesis genes were low in *ahl15* and high in *pPXY:AHL15*, indicating that AHL15, by increasing cytokinin biosynthesis, enhances secondary xylem in Arabidopsis. Our findings support a novel cambium regulation pathway, in which *AHL15* acts downstream of SOC1 and FUL and upstream of *IPT* and *LOG* genes to enhance cambium activity and wood formation in the Arabidopsis stem. In addition, we show that the SOC1/FUL-AHL15-cytokinin pathway is also involved in the regulation of AM activity.