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The role of the Arabidopsis AHL15/REJUVENATOR gene in developmental phase transitions

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Citation
Karami, O. (2017, September 5). *The role of the Arabidopsis AHL15/REJUVENATOR gene in developmental phase transitions*. Retrieved from https://hdl.handle.net/1887/54683

Version:	Not Applicable (or Unknown)
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Note: To cite this publication please use the final published version (if applicable).

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Issue Date: 2017-09-05

Summary

Just like other multicellular organisms, plants undergo several distinct developmental phase transitions, starting with embryogenesis, and subsequently progressing from the juvenile vegetative and the adult vegetative to the adult reproductive and finally to the gametophyte phase. Recent genetic and molecular biology studies have shown that the correct timing of plant developmental transitions is regulated by orchestration of gene expression in response to various environmental cues, triggering multiple regulatory pathways. Despite these shared developmental transitions, flowering plants display a wide range of life spans, varying from a few weeks for some annual species up to several thousand years for some perennial species, such as the sequoia trees. Related to their life span, plants have evolved two opposing growth habits. Many species are monocarpic, meaning that their life cycle is completed after flowering and producing offspring, even under optimal growth conditions. By contrast, polycarpic plants flower and reproduce more than once during their life history and are able to survive multiple successful offspring production events. The molecular basis of these two main growth habits in flowering plants is still largely unknown.

Developmental phase transitions have been shown to coincide with large scale remodeling of the chromatin structure. In plants, several candidate chromatin-remodelling proteins have been shown to play an important role in the regulation of plant developmental processes, among which the AT-HOOK MOTIF CONTAINING NUCLEAR LOCALIZED (AHL) proteins. The *Arabidopsis* genome encodes 29 AHL family members, containing either one or two AT-hook motifs and a PPC (Plant and Prokaryote Conserved) domain, that possibly act through remodeling of the chromatin structure. Molecular genetic studies in *Arabidopsis* have revealed that AHL proteins are involved in multiple aspects of pant growth and development, including flowering time, flower development, hypocotyl growth, and vascular tissue differentiation. The main objective of this PhD research was to understand the biological function of the *AHL15* gene and its homologs with a focus on yet unidentified roles in plant embryogenesis and -Ageing.

Chapter 1 reviews the current advances in understanding the molecular mechanisms regulating plant developmental phase transitions and the underlying differences between the mono-and polycarpic growth habit, with a focus on how the interaction between cellular factors and environmental cues mediate the plant developmental transitions and -life histories. In plants, embryogenesis usually takes place when haploid gametes meet to create a diploid zygote. However, somatic cells can be reprogrammed to totipotent embryonic cells that are able to form differentiated embryos in a process called somatic embryogenesis (SE). C**hapter 1** also discusses the involvement of plant hormones and some key transcription factors in the initiation of somatic embryos under *in vitro* conditions.

Chapter 2 focuses on the role of *AHL15* and its close homologs, *AHL19* and *AHL20* in SE and zygotic embryogenesis (ZE). In *Arabidopsis*, SE is usually induced by exogenous application of the synthetic auxin, 2,4-dichlorophenoxyacetic acid (2,4-D). Alternatively, SE can be induced by overexpression of certain transcription factor genes such as *BABY BOOM* (*BBM*). In this chapter, we show than *AHL15* overexpression induces SE on *Arabidopsis* seedlings in the absence of hormonal treatment. By contrast, *ahl15* loss-of-function mutants

show reduced somatic embryo induction in response to 2,4-D treatment or overexpression of the SE-inducing *BABY BOOM* (*BBM*) transcription factor. The *AHL15* gene is bound and transcriptionally-regulated by BBM during SE. During zygotic embryogenesis, *AHL15* is expressed in early embryos, where it is required for proper patterning and for development beyond the heart stage. Morphological and cellular analyses showed that a significant number of plants derived from *35S::AHL15* SEs are polyploid. Chromatin staining with fluorescent reporters suggested that AHL15 induces chromatin decondensation, which might lead to chromosome missegregation and thus to the occurrence of polyploid cells. Using centromere-specific markers, we demonstrated that polyploidisation is caused by endomitotic events, which specifically occur during the initiation of SE. Our findings indicate that *AHL15* is an important driver of plant cell totipotency acquisition, and based on our results, we propose that opening of the chromatin structure is required for the acquisition of embryonic competency in somatic plant cells.

In flowering plants, Ageing is defined by a series of developmental phase transitions that start with vegetative growth, followed by flowering and culminating in seed production. Tissue senescence and plant death follow seed production in monocarpic plants, while polycarpic plants prolong their life span by maintaining a number of vegetative axillary meristems, thereby allowing subsequent cycles of vegetative and reproductive development. **Chapter 3** describes the role of *AHL15* and its close homologs, *AHL19* and *AHL20*, in vegetative phase change and life history strategy. Here we show that the *AHL15* gene is a suppressor of developmental phase transitions. We therefore renamed *AHL15* to *REJUVENATOR* (*RJV*). Loss-of-function of *RJV* in *Arabidopsis* resulted in precocious appearance of adult vegetative traits and early flowering, whereas *RJV* overexpression prolonged the juvenile phase and delayed flowering in *Arabidopsis* and tobacco. We also show that RJV is a suppressor of axillary meristem maturation, with effects on plant shoot architecture and longevity. Expression of a dominant-negative *RJV-GUS* gene fusion accelerated axillary meristem maturation, whereas constitutive expression of *RJV* kept juvenile traits on axillary meristems during flowering and converted monocarpic *Arabidopsis* and tobacco plants into polycarpic plants with enhanced seed and biomass production. Our results show that *RJV* acts downstream of Ageing (*miR156*, *SPL*) and flowering (*SOC1*, *FUL*) genes as a molecular switch between monocarpic and polycarpic life history strategy.

In **Chapters 2 and 3**, we documented that overexpression of the *Arabidopsis* nuclear protein AHL15 leads to reprogramming of somatic cells to embryonic cells and to suppression of plant ageing. In **Chapter 4** we show that transient (4 hours) activation of overexpressed RJV-GR in *Arabidopsis* seedlings has long-term effects on plant development. RNA sequencing analysis detected an extensive reprogramming of the transcriptome 4 hours after RJV-GR activation, with respectively 540 and 1107 genes showing more than 2-fold up- and down-regulation. RJV seemed to act in a transcription level-dependent manner, activating predominantly low expressed genes and repressing mostly highly expressed genes. Rapid decondensation of heterochromatin was observed after RJV activation in leaf primordia and axillary meristems, indicating that the global reprogramming of the transcriptome by transient activation of this AT-Hook domain protein might at least in part be caused by extensive modulation of the chromatin configuration. Co-activated or co-repressed genes were often found to be physically linked in small chromosomal clusters, which is in

line with regulation at the chromatin level. More detailed analysis of down-regulated genes indicated that RJV represses plant ageing by targeting several components of the ageing pathway, including the *SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE* (*SPL*) genes, GA biosynthesis and photosynthesis-dependent sugar production. Our findings provide new insights in understanding plant age regulation, but further investigations are needed to test the relevance of these finding.