Forever young: how AHL15 delays developmental phase transitions to prevent ageing in plants

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Summary

Plants undergo specific developmental phase changes during their lifetime, such as the transition from juvenile seedlings to adult plants during vegetative development or the change from the vegetative to reproductive phase. Developmental phase transitions are tightly controlled processes that are regulated by several elements including age, phytohormones and environmental cues such as light and temperature. Each of these factors can affect the expression and activity of transcription factors (TFs), nuclear proteins that bind DNA to regulate the expression of genes. Many TFs are expressed during a specific developmental window and either trigger or inhibit developmental phase transitions by promoting or repressing the transcription of their target genes. Other types of proteins can also have an effect on developmental phase transitions, for example by altering phytohormone levels, transmitting environmental cues, or by interacting with DNA directly and modifying its structure. This latter process can influence developmental phase changes when the epigenetic code or the 3D organization of DNA is altered in a way that interferes with the ability of TFs to regulate the expression of their target genes. The research described in this thesis continues on the initial identification of the AT-HOOK MOTIF NUCLEAR LOCALIZED 15 (AHL15) protein as a general suppressor of developmental phase transitions in *Arabidopsis thaliana* (Arabidopsis), and focuses on its role in two different developmental phase transitions and on its molecular function as a transcriptional regulator.

Chapter 1 reviews the current knowledge on how developmental phase transitions are regulated in the model plant Arabidopsis, with an emphasis on the role of TFs in this process. We describe the life cycle of Arabidopsis and focus on several developmental transitions during the vegetative- and flowering stage (Figure 1). Vegetative Arabidopsis plants undergo a juvenile-to-adult transition also called vegetative phase change (VPC), which mainly affects leaf morphology. VPC is inhibited by juvenile-stage factors such as AHL15 and the microRNA miR156, which are both expressed at high level in young plants and repress expression of TFs encoded by the *SQUAMOSA PROMOTER BINDING-LIKE* (*SPL*) gene family respectively pre- and post-transcriptionally. With developmental time, the repression of *SPL* genes decreases, resulting in VPC and an adult morphology. Adult plants undergo the floral transition, which is responsive to several cues. Long days promote the floral transition in Arabidopsis by activation of the floral promoter *FLOWERING LOCUS T* (*FT*), and prolonged cold exposure (vernalization) silences the floral repressor *FLOWERING LOCUS C* (*FLC*). FT and FLC promote- and repress the expression of several flowering genes, respectively, and the floral transition

is induced when promoting cues are stronger than repressive cues. This leads to the formation of an inflorescence, whose architecture is determined by a balanced spatial expression pattern of several promoters- and repressors of

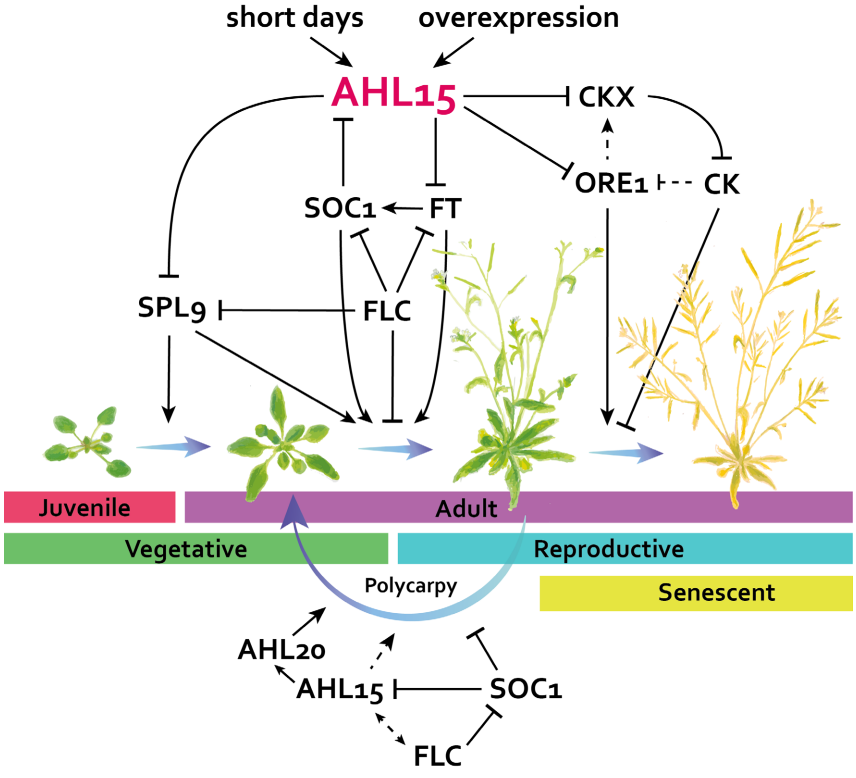

Figure 1: The effects of enhanced AHL15 expression on developmental phase transitions in monocarpic Arabidopsis. **Top:** AHL15 delays developmental phase transitions by repressing the expression of several genes when expressed at high levels. High AHL15 expression can be induced by short days or by overexpression in a transgenic plant. The juvenile-to-adult transition is promoted by SPL9, whose expression is repressed in AHL15 overexpression lines. Flowering is promoted by SOC1 and FT, which are both repressed by FLC. AHL15 inhibits flowering by repressing FT expression, and AHL15 expression during the flowering stage is repressed by SOC1. AHL15 also represses senescence via direct downregulation of ORE1 and CKX expression. ORE1 is a transcription factor that acts as a master regulator of senescence, and the senescence developmental program initiated by ORE1 induces transcription of CKX. CKX proteins inactivate cytokinins (CK), phytohormones that have an inhibitory effect on senescence. **Bottom:** Regulation of polycarpy by flowering time and AHL genes. FLC is a repressor of SOC1 and many other flowering-promoting genes, and helps to retain the vegetative state during the flowering stage. SOC1 levels show a strong negative correlation with polycarpic traits, indicating that it is a repressor of this phenotype. AHL15 is repressed by SOC1, and high AHL15 expression is associated with polycarpic traits. Its closest homolog AHL20 shows a significant correlation with polycarpic traits, suggesting that it is involved in polycarpic development. A positive correlation between AHL15 and FLC expression was also found, but the relation between these genes remains unclear.

flower development. Flowering also triggers senescence in rosette leaves, a process that helps relocate nutrients from old to young, developing tissues. Leaf senescence is regulated by several TFs of which *ETHYLENE INSENSITIVE 3* (*EIN3*), *ORESARA1* (*ORE1*) and *NAC-LIKE ACTIVATED BY AP3/PI* (*NAP*) act upstream of several genes involved in chlorophyll (Chl) catabolism as well other TFs that act further downstream in the senescence process. In addition, senescence is affected by phytohormones, of which ethylene is the best-known inducer of senescence whereas cytokinins (CKs) act as inhibitors. We also discuss the differences between plants that undergo complete senescence and death upon reproduction (monocarps) and plants that can resume their vegetative growth during- and after flowering and can flower again from these vegetative tissues (polycarps). We describe how FLC promotes polycarpic development in *Brassicaceae* species by repressing the floral transition in axillary meristems (AMs), and discuss the roles of *FRUITFULL* (*FUL*) and CKs in inflorescence arrest. At the end of **chapter 1**, we discuss what is currently known about the *AT-HOOK MOTIF NUCLEAR LOCALIZED* (*AHL*) gene family with a focus on *AHL15*, the gene on which the research in this thesis is focused, and describe its effects on plant development.

In **chapter 2**, we used a panel of Arabidopsis ecotypes that show the formation of aerial rosettes (ARs), a polycarpic trait that was observed in Arabidopsis lines overexpressing *AHL15*, to investigate the role of *AHL15* and several other genes in this phenotype. We quantified the AR phenotype and show that this phenotype is strongly variable and is positively correlated to the expression of *FLC* and *AHL20,* a close homolog of *AHL15.* We also show that the expression of *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*), a floral promoter that represses expression of *AHL15*, shows a strong negative correlation with the AR phenotype, and that the adult-state promoting gene *SPL15* shows a similar, but weaker trend. Finally, we show that silencing of *FLC* expression by vernalization results in a complete loss of the AR phenotype, demonstrating that FLC is a master regulator of AR production in these ecotypes (Figure 1). Our results show that a panel of phenotypically diverse ecotypes can be used to determine gene-to-phenotype relations, and that natural variation in the presumedly monocarpic species Arabidopsis can be used to study polycarpic development.

One of the developmental phase transitions that is inhibited by AHL15 is leaf senescence, a process in which Chl is broken down and results in visible changes in leaf color from green to yellow. In order to study this process more efficiently, we developed a method to quantify the Chl content of leaves based

on their visual appearance. In **chapter 3**, we describe a method for rapid, non-destructive Chl quantification based on digital images of plant leaves. We developed a plugin for the imageJ software that automatically detects leaves, and measures the pixel intensity in the red, green, and blue channels (RGB) in 8-bit digital images. These values can then be used to calculate several colorimetric visual indexes, of which the normalized Red value (R/(R+G+B)) is the most strongly correlated to leaf Chl content as determined by the traditional, labor-intensive method of Chl extraction. We applied this new image-based quantification method on a large panel of lettuce genotypes to measure leaf Chl content for a genome-wide association study (GWAS). With the data obtained in this way, we identified a significant peak around a locus involved in chloroplast development that was previously identified in a GWAS using a traditional Chl quantification method. Together, our data show that image-based Chl quantification is a suitable and more efficient alternative to the labor-intensive extraction-based Chl quantification, and can be applied in high-throughput systems.

We applied the protocol developed in **chapter 3** to measure the effect of *AHL15* on leaf senescence, which is described in **chapter 4**. We show that overexpression of *AHL15* delays leaf senescence, resulting in a stay-green phenotype, which was apparent during the plants life cycle as well as in dark-induced senescence in detached leaves. Loss-of-function *ahl15* plants showed a slight but significant acceleration of senescence, indicating that AHL15 has a repressive effect on senescence. We also investigated which genes act downstream of AHL15 during this process, and show that AHL15 directly represses the expression of the master regulator of senescence *ORESARA1* (*ORE1*). In addition, we show that AHL15 represses the expression of CK degrading enzymes of the CYTOKININ OXIDASE/DEHYDROGENASE (CKX) family, resulting in a delayed decrease of CK levels during dark-induced senescence. Thus, AHL15 represses senescence via two parallel pathways: by repressing *ORE1* expression, and by inhibiting CK degradation (Figure 1).

Finally, in **chapter 5**, we investigated where in the genome AHL15 binds, and how it regulates gene expression. We show that AHL15 binds closely upstream of the transcription start site (TSS) or just downstream of the transcription end site (TES) of genes, and is depleted in gene bodies. We show that constitutive *AHL15* overexpression results in stronger binding at native AHL15 binding sites and at ectopic sites, where it binds with a lower affinity than at its genuine native targets. In addition, we show that induction of nuclear localization of the AHL15-GR fusion protein by dexamethasone (DEX) results in

large transcriptional changes, of which the majority of genes is downregulated. Downregulated genes include floral promoters such as *FT* (Figure 1), explaining the delayed flowering phenotype of *AHL15*-overexpressing plants. Interestingly, AHL15 binding was higher near the TSS and TES of differentially expressed genes compared to genes whose expression was unaffected by DEX treatment. AHL15 peaks were most strongly enriched near the TSS of upregulated genes, and downregulated genes had the strongest AHL15 enrichment near the TES, suggesting that the location of AHL15 binding near a gene affects its expression. We also investigated the effect of AHL15 on chromatin accessibility with ATAC-seq in DEX-induced *p35S:AHL15-GR* plants, and surprisingly found that AHL15-GR activation did not affect chromatin accessibility. Comparison of AHL15 DNA binding sites with ATAC-seq data that shows where chromatin is accessible also revealed that AHL15 binds at poorly accessible regions that flank regions with high accessibility. In addition, AHL15 ChIP-seq peaks did not overlap with several epigenetic marks, but were located at the boundaries of regions enriched in such marks. Together, this indicates that AHL15 and likely also other AHL family proteins have an affinity for regions with reduced accessibility and that are poorly covered by most epigenetic marks.. Finally, we show that AHL15 binds in the same regions as the high-mobility group A (HMGA) protein GH1-HMGA2/HON5, which has several AT-Hook DNA-binding domains. GH1-HMGA2/HON5 was previously shown to interfere with the formation of gene loops, which are necessary for efficient transcription of the gene they encompass. The overlap in DNA binding sites suggests that like GH1-HMGA2/HON5, AHL15 can affect the 3D organization of DNA which can influence gene expression, explaining why *AHL15* overexpression affects plant development so strongly.

In conclusion, our genome-wide analyses reveal that AHL15 acts as a transcriptional regulator that binds to DNA with little sequence specificity, but with a preference for regions depleted of epigenetic signatures and that this preference overlaps with that of another type of chromatin architecture-modifying protein class, suggesting that the large-scale changes in gene expression induced by AHL15 stem from changes in chromatin architecture. By combining this information with phenotypic and gene expression data, we show that AHL15 directly represses the expression of several TFs involved in developmental processes, and thereby inhibits developmental phase changes associated with ageing.