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

Author: Karami, Omid
Title: The role of the Arabidopsis AHL15/REJUVENATOR gene in developmental phase transitions
Issue Date: 2017-09-05

Chapter 1

General introduction

The genetic basis of developmental phase transitions in plants

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Summary

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, they have evolved two opposing growth habits. Many species are monocarpic, as their life cycle is completed after flowering and producing offspring once, 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. All annual plants are monocarpic, but not all perennial plants are polycarpic. Some perennial plants grow for several years, but as they still die after flowering and seed set these plants are in fact monocarpic.

Both annual and perennial plants undergo several distinct developmental phases during their life history. Studies in annual and perennial species have shown that these developmental phase transitions are tightly linked to orchestration of gene expression in response to environmental cues such as light intensity and quality, day length, nutrient availability, and temperature. Recent advances in plant molecular biology have provided new insights in genetic pathways and molecular mechanisms that trigger or modulate developmental phase transitions in plants. In this chapter we will present and discuss our current knowledge these mechanisms with a focus on those pathways that distinguish monocarpic from the polycarpic life history strategy.

Introduction

Like all 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 the gametophyte phase (Fig. 1). During this last phase, the male and female gametophytes are produced, respectively pollen grains carrying two sperm cells and the embryo sac containing the egg cell and two polar nuclei. Fertilization of the egg cell by one of the sperm cells forms a diploid zygote, and fusion of the other sperm cell with the polar nuclei forms a triploid nucleus. The zygotic cell will undergo multiple rounds of cell division, eventually forming the embryo in which the basic body plan of a plant is laid down, comprising a root apical meristem (RAM), a shoot apical meristem (SAM), a hypocotyl, cotyledons and vascular tissue (Fig. 1). By simultaneous nuclear divisions of the triploid nucleus the endosperm is formed, which is important for seed growth as it generates space and is a source of hormones and nutrients for the growing embryo (Baroux et al., 2007; Locascio et al., 2014). In *Arabidopsis*, the nutrients in the endosperm eventually become absorbed by the cotyledons during seed maturation, whereas in other plants the endosperm is maintained as energy provider during seed germination and the initial development of the seedling (Sabelli and Larkins, 2009). Postembryonic development of the plant starts with the vegetative phase, during which the SAM produces leaves and side branches and the RAM allows the root to grow and to subsequently branch by forming lateral roots. The vegetative growth is considered into two distinct phases, the juvenile phase during which the plant is not competent to flower, and the adult phase in which plants have acquired the competence to flower (Huijser and Schmid, 2011). In some plants the transition between the juvenile and the adult phase, also referred to as the vegetative phase change, is marked by a distinguishable change in leaf morphology (heteroblasty) (Zotz et al., 2011). Upon acquisition of reproductive ability, the SAM becomes an inflorescence meristem that produces bract and flowers containing the reproductive organs. Below we will discuss the phases of plant development in more detail, with a focus on the changes that occur during the phase transitions (Bäurle and Dean, 2006; Huijser and Schmid, 2011; Poethig, 2013).

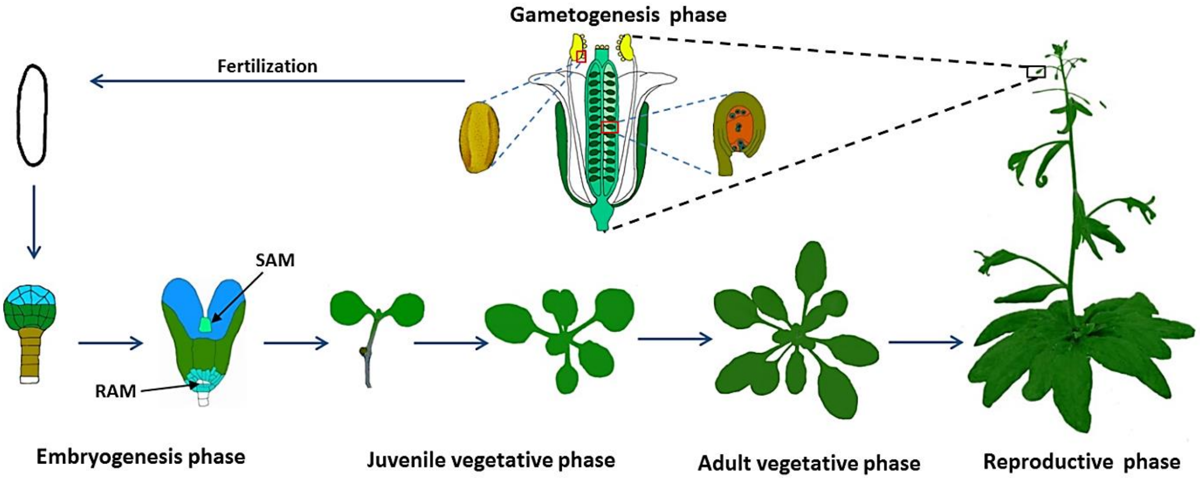

Figure 1. Developmental phase transitions during the life cycle of *Arabidopsis thaliana*. After fertilization, the basic body plan of the plant is laid down during embryogenesis, including the shoot and root apical meristem (resp. SAM and RAM). The subsequent germination of the seedling starts the vegetative phase, during which the SAM and RAM are responsible for organ formation, resulting in the shoot and root system. In the juvenile vegetative phase, plants are incompetent to flower, whereas plants in the adult vegetative phase have gained reproductive competency. *Arabidopsis* is a typical heteroblastic plant where juvenile and adult leaves show clear morphological differences. With the increasing number of leaves, the juvenile plant enters into the adult vegetative phase and acquires the competence to flower. During the change from the adult vegetative to the reproductive phase, the SAM becomes an inflorescence meristem that produces flowers and bracts instead of rosette leaves. As the flowers mature, the plant enters the gametogenesis phase, during which male and female gametes are formed within the flowers. The subsequent successful fusion of these gametes during fertilization starts the next life cycle with the development of the embryo from the zygote (for review see Bäurle and Dean, 2006; Huijser and Schmid, 2011; Poethig, 2013).

Embryogenesis

The first phase of a plant’s life starts with the fusion of the male and female gametes during fertilization to generate the zygote. This developmental switch, which is defined as gametophyte-to-zygotic transition, coincides with one of the most complex cellular reprogramming events, transforming the highly specialized meiotically programmed egg cell into a totipotent mitotically active embryonic cell (Pillot et al., 2010; She and Baroux, 2014). The gametophyte-to-zygotic transition has been shown to be accompanied by erasing and re-establishment of genomic imprinting (Raissig et al., 2013), by reprogramming of epigenetic information (Jullien, 2010; Wollmann, 2012) and by rapid removal and replacement of gametic Histon3.3 variants (Ingouff et al., 2010). However, how the zygotic cell acquires totipotency remains largely unknown.

Plant embryogenesis has been best-studied in the model dicot *Arabidopsis thaliana*. The first division of the zygote is asymmetric, giving rise to a smaller apical and a larger basal cell. By a subsequent series of symmetric and asymmetric cell divisions, which in *Arabidopsis* occur in an extremely ordered fashion compared to other plant species, the apical cell gives rise to the embryo proper that develops through a morphological series from globular, heart, and torpedo to the final bent cotyledon stage. In *Arabidopsis*, embryogenesis ends by accumulation of proteins, starch, and lipids in the cotyledons and eventually by desiccation of the embryo. In contrast to the apical cell, the basal cell only undergoes a limited number of symmetric cell divisions forming the suspensor, a row of cells that connects the embryo to the maternal tissue. At the early globular stage, the most apical suspensor cell is recruited to the basal side of the embryo proper to become the hypophysis, the founder cell of the RAM. Simultaneously, the SAM is established at the apical side of the embryo, and the subsequent initiation of the cotyledon primordia induces a change in embryo morphology from globular-shaped with radial symmetry to heart-shaped with bilateral symmetry (Jenik et al., 2007). The highly organized cell divisions, cell fate specification, and cell-cell communication that lead to apical-basal and radial patterning during plant embryogenesis are controlled by embryo-specific transcription factors, hormonal gradients, and signaling components (Lau et al., 2012; Hove et al., 2015). Below we will discuss the role of several key transcription factors and the plant hormones auxin and cytokinin.

Genetic studies have revealed that the WUSCHEL RELATED HOMEOBOX (WOX) transcription factor family (van Der Graaff et al., 2009) plays an important role in determining cell identity during early embryo patterning (Jenik et al., 2007; Breuninger et al., 2008; Ueda et al., 2011). The asymmetric division of the zygote is critical for the formation of the apical-basal body axis, and the *WOX2/8/9* genes that are co-expressed in the zygote play an important role in this first step. After this asymmetric division, *WOX2* expression becomes restricted to the apical cell lineage, while *WOX8/9* remain expressed in the basal cell lineage (Breuninger et al., 2008; Ueda et al., 2011). The *Arabidopsis wox2/8/9* mutants display an abnormal asymmetric division of the zygote and distorted embryo development (Breuninger et al., 2008; Ueda et al., 2011).

The establishment of the RAM by hypophysis specification during early embryogenesis is first determined by a transient overlap of auxin and cytokinin signaling, which then separates into a distal auxin domain and a proximal cytokinin domain (Efroni et al., 2016). The auxin domain is required for hypophysis fate, as mutants in genes that cause defects in auxin biosynthesis, transport, perception or response are all impaired in hypophysis division and formation (Jenik et al., 2007; Mo and Weijers, 2009; Wabnik et al., 2013).

The SAM and RAM are established during early embryogenesis by small populations of cells, called the organizing-centers. The earliest genes expressed in these shoot and root organizing-centers are respectively *WUSCHEL* (*WUS*) and *WOX5* (Mayer et al., 1998; Sarkar et al., 2007). Both WUS and WOX5 homeodomain-like transcription factors have been implicated in shoot and root stem-cell maintenance, respectively (Mayer et al., 1998; Haecker et al., 2004). In addition, serval members of the APETALA2/Ethylene Responsive Factor (AP2/ERF) transcription factor family, such as *BABY BOOM* (*BBM*) and the *PLETHORA* (*PLT*) genes *PLT1* and *PLT2*, *PLT3* have been shown to be required for root stem cell formation and embryo development (Galinha et al., 2007). These genes are expressed during early embryogenesis, where they are involved in maintaining cell division and preventing differentiation of embryogenic stem cells in a concentration-dependent manner (Boutilier et al., 2002; Galinha et al., 2007; Rybel et al., 2015). In *Arabidopsis*, mutations in these genes cause defects in root stem cell maintenance, leading to a severe rootless phenotype (Galinha et al., 2007).

The establishment of the protodermal cell layer during the transition from the 8- to the 16-cell embryo requires the expression of the homeodomain leucine zipper class IV (HD-ZIP IV) transcription factors ARABIDOPSIS THALIANA MERISTEM LAYER1 (ATML1) and its closest homologue PROTODERMAL FACTOR2 (PDF2) (Brambilla et al., 2014). The corresponding genes are specifically expressed in the protoderm and promote epidermal cell differentiation (Abe et al., 2003; Jenik et al., 2007; Takada and Jürgens, 2007). *atml1* and *pdf2* double mutants show severe embryo epidermal defects that lead to embryo lethality (Abe et al., 2003). *AtML1* expression is not restricted to early embryogenesis. The gene remains expressed in the developing epidermis of the embryo (Takada and Jürgens, 2007) and, subsequently, in the L1 layer of the SAM and leaf primordia (Takada et al., 2013). Both *AtML1* and *PDF2* maintain their expression in epidermal cells by binding to their own promoter (Takada and Jürgens, 2007).

Embryo morphogenesis and maturation is regulated by the B3 domain factors LEAFY COTYLEDON2 [LEC2], FUSCA3 [FUS3], and ABSCISIC ACID INSENSITIVE3 [ABI3]) and LEC1. B3 domain factors are related to the HAP3 subunit of the CCAAT binding factor family (Braybrook and Harada, 2008), and their corresponding genes are specifically expressed during embryogenesis (Santos-Mendoza et al., 2008). The *lec1*, *lec2*, and *fus3* loss-of-function mutants show defects in embryo identity, as embryos enter post-germination developmental programs such as the formation of trichomes on cotyledons. Moreover, *lec1* and *lec2* mutant embryos do not acquire dessication tolerance and have defects in accumulation of seed storage products (Santos-Mendoza et al., 2008). It has been shown that *LEC* genes control embryonic cell fate by modulating sucrose levels and auxin responses to promote cell division and embryonic maturation (Casson and Lindsey, 2006; Stone et al., 2008).

The plant hormone auxin contributes to a wide range of physiological and developmental processes (Teale et al., 2006; Vanneste and Friml, 2009), including most embryo pattern formation steps, such as embryonic axis formation, stem cell establishment, hypophysis establishment, and vascular patterning (Peer et al., 2011; Mo and Weijers, 2009; Wabnik et al., 2013; Hove et al., 2015). Auxin is not produced in all plant cells but after local biosynthesis in certain cells or tissues it is distributed to specific sites of the plant body by polar cell to cell transport by PIN-FORMED (PIN) auxin efflux carriers (Friml et al., 2003; Friml, 2010). PIN proteins determine the direction of polar auxin transport (PAT) through their asymmetric localization at the plasma membrane (Friml et al., 2003; Friml, 2010). In addition, the AUXIN1/LIKE-AUX1 (AUX/LAX) influx carriers are important drivers of PAT by mediating efficient uptake of auxin by cells and thus increasing the amount of auxin available for polar efflux by the PIN proteins (Bainbridge et al., 2008; Péret et al., 2012; Boot et al., 2016). The organized spatial expression and localization pattern of PIN and AUX/LAX carriers directs the differential accumulation of auxin in plant tissues (Kierzkowski et al., 2013). It is well established now that PAT plays a crucial role in embryo pattern formation, (Jenik et al., 2007; Mo and Weijers, 2009; Wabnik et al., 2013).

Detailed insight toward understanding of the molecular basis of auxin signaling in embryogenesis has revealed several molecular pathways of auxin action (Rybel et al., 2015; Mironova et al., 2017), but the main auxin signaling pathway is through the TRANSPORT INHIBITOR RESPONSE1/AUXIN SIGNALING F-BOX (TIR1/AFB) proteins (Smit and Weijers, 2015; Mironova et al., 2017). The TIR1/AFB F-box proteins control the activity of AUXIN RESPONSE FACTORs (ARFs) that bind to cis-regulatory auxin response elements in target gene promoters (Guilfoyle and Hagen, 2007; Boer et al., 2014). Under low auxin levels, Auxin/INDOLE-3-ACETIC ACID (Aux/IAA) proteins bind and repress the activity of ARF proteins (Guilfoyle and Hagen, 2007). When auxin levels are increased, the TIR1/AFBs use auxin as molecular glue to recruit the Aux/IAA proteins to the Skp1-Cullin-F-box (SCF) E3 ubiquitin ligase complex. Following their ubiquitination, Aux/IAA proteins are degraded by the 26S proteasome, thereby allowing ARFs to activate transcription of their target genes (Dharmasiri et al., 2005; Kepinski and Leyser, 2005; dos Santos Maraschin et al., 2009; Mironova et al., 2017). The auxin signal transduction through ARFs/Aux/IAAs has been shown to play an important role in embryonic vascular tissue formation and the establishment of the embryonic RAM (Hove et al., 2015; Rybel et al., 2015). Embryogenesis ends with maturation and desiccation of the embryo, a phase during which in dicot species the endosperm is consumed and used as energy source for the final growth and maturation of the embryo (Lopes and Larkins, 1993). The hormone abscisic acid (ABA) plays an important role in this final phase of embryogenesis and it keeps the embryo dormant in the desiccated seed, whereas antagonistically acting gibberellins (GAs) promote embryo germination and development into a seedling (Santos-Mendoza et al., 2008; Rajjou et al., 2012). Embryo maturation and seed germination are developmental phase transitions involving complex regulatory mechanisms that have been extensively reviewed elsewhere (Rajjou et al., 2012), but are beyond the scope of this Chapter and will therefore not be discussed here.

The vegetative phase change

When seeds are germinated in the light, the seedling undergoes a developmental program referred to as photomorphogenesis, involving the production of chlorophyll and the onset of photosynthesis in the shoot part, the initiation of the first leaves by the SAM and the onset of root growth mediated by the RAM followed by the production of lateral roots (Weitbrecht et al., 2011). This first phase of post-embryonic plant development is referred to as the juvenile phase, and it is only after transition to the adult phase that the plant becomes competent to flower and to reproduce (Huijser and Schmid, 2011). The transition from juvenile-to-adult phase in plants is generally marked by morphological changes such as the position (phyllotaxis) and the timing (plastochron) between leaf initiation events, and the changes in leaf size and shape, trichome distribution and cell size, and internode length. This results in the appearance of both juvenile and adult leaves on the same plant, a situation also known as heteroblasty (Huijser and Schmid, 2011). Although a wide range of flowering plant species show morphological changes during their vegetative growth, the classical heteroblasty can be most clearly observed in perennial woody plants that have a long juvenile period. In these plants, the heteroblastic changes can be simply followed, but limited genetic and molecular resources and the long generation time of such plants has for a long time limited our understanding of the molecular basis of heteroblasty (Zotz et al., 2011; Huijser and Schmid, 2011). Recently, some progress has been made in poplar (Hsu et al., 2011; Wang et al., 2011), but most biological studies on heteroblasty have focused on model annuals that show clear heteroblastic changes during the vegetative phase change. In *Arabidopsis* grown under 16 hours photoperiod, the vegetative phase change typically occurs following the production of six to eight juvenile leaves. Juvenile leaves in *Arabidopsis* are small and round, and have smooth margins, long petioles and they do not carry trichomes on their abaxial side. In contrast, the adult leaves are bigger and have short petioles, smaller cells and elongated blades with serrated margins (Telfer et al., 1997). The juvenile leaves in maize are short, covered with wax, and lack epidermal hairs, whereas the adult leaves are long and narrow, lack wax and have epidermal hairs (Usami et al., 2009a).

Like for all developmental phase transitions, environmental cues have a high impact on the juvenile-to-adult phase transition. The photoperiod, light intensity, and ambient temperature have all been shown to influence the juvenile phase, but the photoperiod is the most important environmental cue that determines the timing of the juvenile-to-adult transition (Huijser and Schmid, 2011).

Compared to the adult-to-reproductive phase transition, which is the most relevant trait in crops, much less is known about the molecular mechanisms that mediate the juvenile-to-adult transition. However, recent progress in *Arabidopsis* has demonstrated that several microRNAs (miRNAs) play an important role in this phase transition (Huijser and Schmid, 2011; Poethig, 2013). Below the miRNA-based regulation will be discussed in more detail.

miRNAs and gibberellic acid regulate the juvenile-to-adult transition

MiRNAs are gene-encoded small RNA molecules of 20 to 24 nucleotides in length that by translation suppression of the mRNAs of their target genes play a critical regulatory role in various developmental aspects of eukaryotic organisms. Regulation of developmental phase changes by miRNAs was first discovered in *Caenorhabditis elegans*. Recent studies show that two miRNAs, miR156 and miR172, are involved in the juvenile-to-adult transition in *Arabidopsis* and other plant species (Fig. 2) (Wu and Poethig, 2006; Xie et al., 2006; Saeteurn K, 2007; Wang et al., 2011; Zhang et al., 2011; Fu et al., 2012; Xie et al., 2012; Poethig, 2013). *Arabidopsis* plants overexpressing miR156 show a prolonged juvenile phase (Wu and Poethig, 2006; Wu et al., 2009; Xu et al., 2016), whereas miR156 knockdown lines have a significantly shortened juvenile phase (Wu et al., 2009; Yang et al., 2013), suggesting that miR156 is a master regulator of the vegetative phase change in plants.

The regulation of the juvenile-to-adult transition by miR156 was shown to be mediated by translation suppression of the plant-specific SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) transcription factors (Cardon et al., 1999; Xie et al., 2006; Axtell et al., 2007; Preston and Hileman, 2013). In the juvenile shoot miR156 levels are high, resulting in low SPL levels, but the gradual down-regulation of miR156 expression during shoot development results in up-regulation of SPL expression, thereby inducing the juvenile to adult phase change (Wu and Poethig, 2006; Wu et al., 2009). Like *miR156* loss-of-function mutants, transgenic plants expressing a miR156-insensitive *SPL* gene display a short juvenile phase (Wang et al., 2008; Wu et al., 2009). In *Arabidopsis*, 11 of the 17 members of *SPL* gene family are targeted by miR156 (Gandikota et al., 2007), but only 6 of these genes (*SPL2/9/10/11/13/15*) contribute to the juvenile-to-adult transition. Consistent with the high degree of functional redundancy among the *Arabidopsis SPL* genes, loss-of-function mutations in single genes have no significant effect on the juvenile phase (Wu and Poethig, 2006; Wang et al., 2008; Usami et al., 2009; Wu et al., 2009). Only in *spl2/9/10//11/13/15* sextuple mutant plants prolonged juvenile phase phenotypes are observed, similar to those in plants overexpressing miR156 (Xu et al., 2016).

The gradual decrease of *miR156* expression during shoot maturation is accompanied by an SPL-induced gradual increase in *miR172* expression. miR172 promotes the development of trichomes on the abaxial side of leaves by repressing the expression of the APETALA2-LIKE (AP2-like) transcription factors TARGET OF EARLY ACTIVATION TAGGED1 (TOE1) and TOE2 (Aukerman and Sakai, 2003; Wu et al., 2009). In addition, SPLs promote the other adult leaf traits such as leaf elongation and leaf serration independent of miR172.

Besides miRNAs and SPL proteins, the phytohormone GA has a strong influence on the vegetative phase change in *Arabidopsis*. *Arabidopsis* mutants insensitive to GA or deficient in GA biosynthesis display a prolonged juvenile vegetative development (Telfer et al., 1997; Park et al., 2017). In contrast, exogenous application of GA results in precocious appearance of adult vegetative traits in particular trichome initiation in *Arabidopsis* (Telfer et al., 1997; Park et al., 2017). GA has no effect on *miR156* expression in *Arabidopsis*, but promotes the expression of some *SPL* genes in the adult phase (Wang et al., 2009a; Galvão et al., 2012; Jung et al., 2012; Yu et al., 2012). On the other hand, the exogenous application of GA has nearly the same effect on the vegetative phase change in wild-type plants and plants over-expressing miR156 (Yu et al., 2012). Thus, the promotion of the vegetative phase change by GA does not seem to be mediated by activation of *SPL* genes. In addition, the formation of trichomes in *Arabidopsis* on the abaxial side of adult leaves is independently promoted by GA and SPL proteins (Yu et al., 2012), suggesting that the GA and SPL synergistically promote the vegetative-phase transition in *Arabidopsis*.

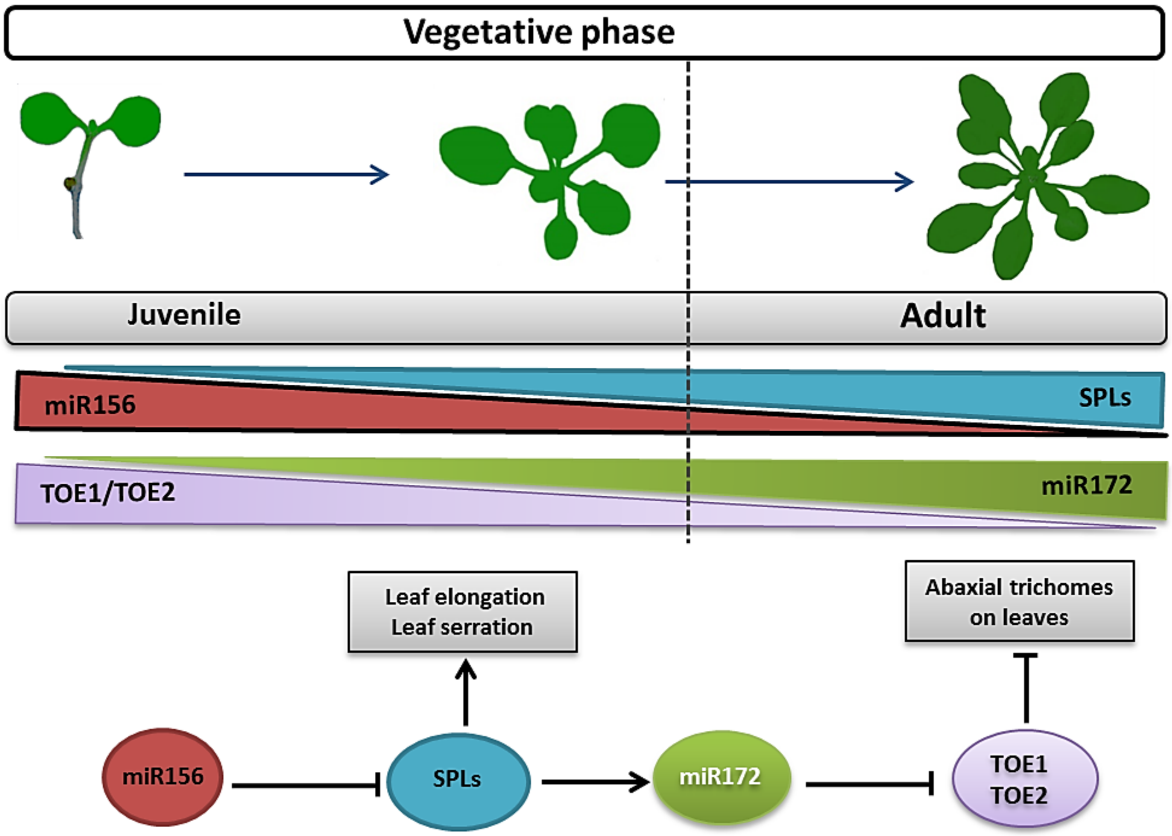

Figure 2. Regulation of the vegetative phase change in *Arabidopsis* by miR156 and miR172. miR156 is a master regulator of the vegetative phase change in plants. High expression of *miR156* maintains juvenility through translation suppression of *SPL* genes. A gradual decrease of the *miR156* transcription (brown bar) leads to enhanced production of SPLs proteins (turquoise bar), which promotes adult leaf morphology. SPLs directly induce *miR172* gene expression. Increased levels of miR172 (green bar) suppress the production of the TOE1 and TOE2 transcription factors (light purple bar), thereby allowing the development of trichomes on the abaxial side of leaves (for review see Huijser and Schmid, 2011; Poethig, 2013)

Vegetative-to-reproductive transition

The switch from vegetative growth to flowering is a major developmental transition in plants. For fruit and seed crops, the timing of vegetative-to-reproductive transition plays a crucial role in crop productivity, as flowering should take place in the correct season when the environmental conditions are suitable to ensure maximal reproductive success. Because of regional differences in the seasons, selection of the genotype with the correct timing for a region is part of the breeding process (Jung and Müller, 2009).

Acquisition of the reproductive competence in the SAM during vegetative growth is a key developmental switch in flowering. During the past two decades, genetic and physiological studies have led to the identification of a range of environmental cues that are involved in the acquisition of reproductive competence in plants (Amasino, 2010; Turnbull, 2011; Song et al., 2013). In *Arabidopsis*, four major flowering pathways have been identified: the photoperiod pathway, the vernalization pathway, the GA pathway, and the age pathway (Fig. 3) (Turnbull, 2011; Matsoukas et al., 2012; Song et al., 2013). A large number of genes acting within these pathways have been identified that either promote or inhibit flowering, and work in a complex genetic network. Central in this network are genes such as *CONSTANS* (*CO*), *FLOWERING LOCUS T* (*FT*), *SUPRESSOR OF CONSTANS 1* (*SOC1*), the *SPLs*, and *FLOWERING LOCUS C* (*FLC*) that are considered as floral integrators (Fig. 3), as they integrate the different environmental and endogenous signaling pathways that influence flowering (Amasino, 2010; Amasino and Michaels, 2010; Andrés and Coupland, 2012; Matsoukas et al., 2012).

In *Arabidopsis*, *CO* plays a key role in the photoperiod flowering pathway (Fig. 3), as its expression is up-regulated by light signaling in leaves (Turnbull, 2011; Song et al., 2013). CO promotes flowering by activation of *FT* transcription. *FT* encodes a florigen signal that is transported from the leaf through the phloem to the SAM. In the SAM, FT interacts with transcription factor FD, and the FT-FD complex activates the transcription of several flowering-promoting genes (Fig. 3) (Turnbull, 2011; Song et al., 2013).

A central node in the vernalization pathway is the MADS box transcription factor FLC (Fig. 3), which acts as a potent repressor of flowering (Amasino, 2010; Kim and Sung, 2014). FLC affects flowering by suppressing the *FT* and *SPL* genes in leaves and the *FD* and *SOC1* genes in the shoot apex (Deng et al., 2011; Matsoukas et al., 2012). Prolonged exposure to low temperatures leads to silencing of *FLC* expression by local chromatin modification and subsequently to induction of flowering (Kim and Sung, 2014).

The key components of the age pathway are miR156 and its *SPL* target genes (Matsoukas et al., 2012; Wang, 2014; Wang and Wang, 2015). Like for the vegetative phase change, the age-related reduction of *miR156* expression leads to increasing levels of SPL proteins, which subsequently induce flowering by activating the transcription of *SOC1* and several other floral-promoting genes in the shoot apex. Activation of *miR172* by SPL proteins in leaves leads to repression of a sub-family of *APATELA2* (AP2)-like target genes that are repressors of flowering (Aukerman and Sakai, 2003; Huijser and Schmid, 2011; Poethig, 2013). Molecular genetic studies have shown that the age pathway is highly integrated into other flowering time pathways (Wang, 2014).

The GA pathway is a photoperiod independent pathway that also plays an important role in the promotion of flowering through activation of the *SOC1* and *SPL* genes (Yu et al., 2012; Wang, 2014). Mutations that decrease the GA concentration or increase the degradation of GA delay flowering (Jung et al., 2012; Yu et al., 2012). The GA pathway is activated by GA signaling-induced degradation of the DELLA repressor proteins, (Sun, 2010; Davière and Achard, 2013).

Downstream of the floral integrators are the floral meristem identity genes, such as *APETALA1* (*AP1*) and *LEAFY* (*LFY*), that promote the transition of the vegetative to inflorescence meristem (Andrés and Coupland, 2012; Matsoukas et al., 2012; Blümel et al., 2015). The photoperiod-regulated FT-FD complex is directly involved transcriptional activation of *AP1*, whereas the central floral integrator SOC1 in the SAM promotes flowering through activation of both floral meristem genes *AP1* and *LFY* (Turnbull, 2011; Song et al., 2013).

The important advances in the understanding of the molecular control of reproductive development in *Arabidopsis* have subsequently facilitated the discovery of the similar mechanisms in other flowering plants.

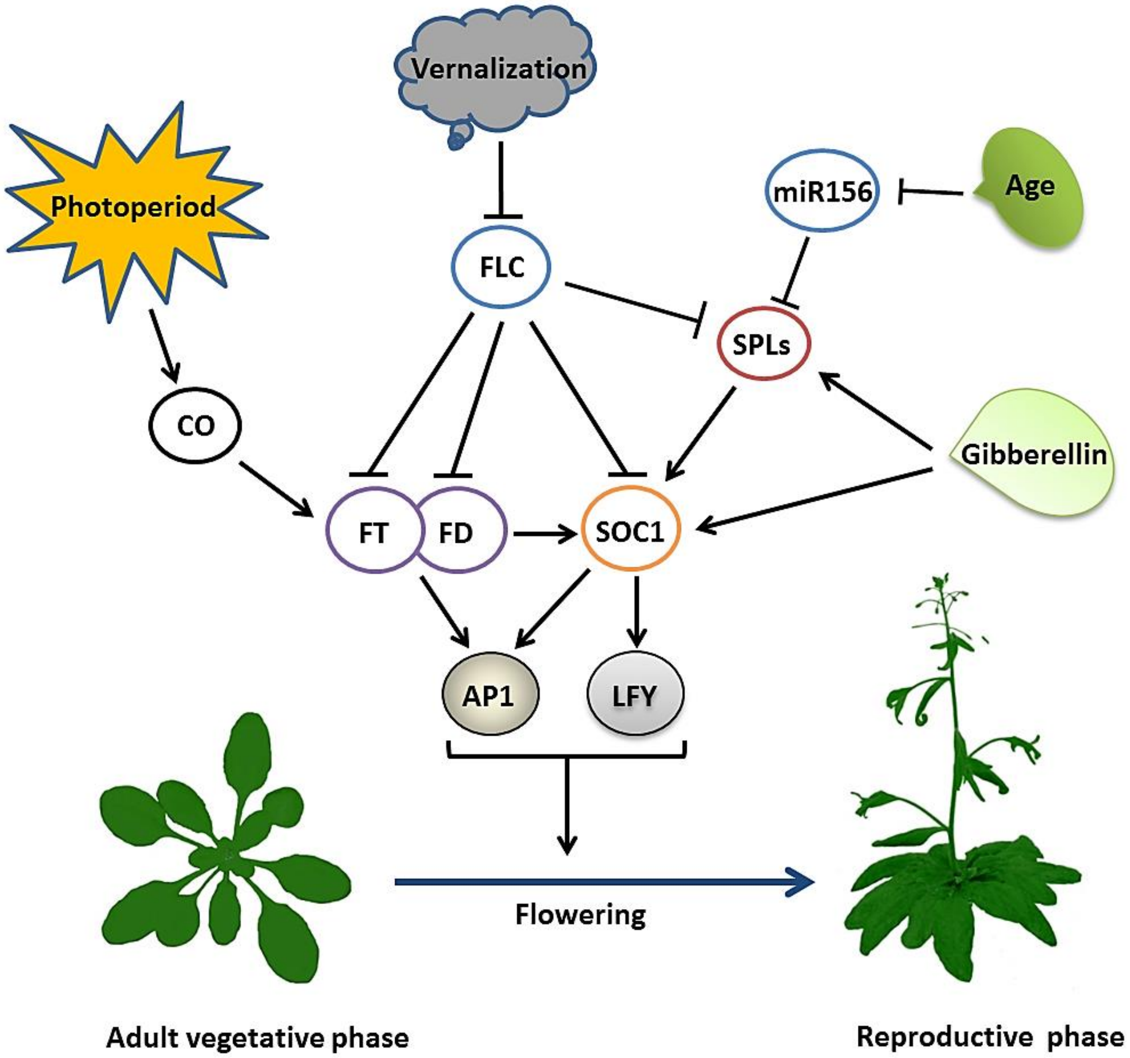

Figure 3. A simplified regulatory network of the different pathways controlling flowering in *Arabidopsis*. In the vernalization pathway cold treatment leads to stable repression of *FLC* transcription. The MADS box protein FLC determines the cold-period-dependent timing of flowering in *Arabidopsis* by repressing the expression of the floral integrator genes *FT*, *FD*, the *SPLs* and *SOC1*. *FT* expression is induced in the leaves by the photoperiod pathway through the accumulation of CO under long days. The FT protein subsequently travels to the SAM, where it physically interacts with FD to activate *SOC1* and *AP1*. In the age pathway, an age-dependent decline in the miR156 level allows an increased production of SPL proteins, which activate the transcription of *SOC1* and other floral integrators (not shown). The phytohormone GA independently promotes flowering through activation of *SOC1* (and *SPL* expression). The subsequent activation of the downstream floral meristem identity genes, such as *LFY* and *AP1*, completes the floral transition (for review see Turnbull, 2011; Andrés and Coupland, 2012; Song et al., 2013; Wang, 2014).

Mechanisms that differentiate between monocarpic or polycarpic plant growth habit

In annual or monocarpic plants, the whole plant body will senesce and die following a single reproductive phase, while polycarpic or perennial plants have more than one reproductive phase during their life history (Munné-Bosch, 2008; Thomas, 2013). For polycarpic plant growth it is critical that the plant maintains underground root stocks or axillary meristems in the vegetative state, allowing them to produce new shoots after seed set and during the next growing season (Munné-Bosch, 2008; Amasino, 2009).

The fact that monocarpic and polycarpic species occur within the same plant family implies that that the transition between polycarpic and monocarpic growth habit is based on relatively small genetic changes. In fact, the switch between poly- and monocarpy is considered as the most common growth form transition in angiosperms (Amasino, 2009). However, despite considerable interest in the molecular basis of these two main growth habits in flowering plants, only few genes have yet been identified that differentiate between the seed set-linked death in monocarpic plants and the survival of polycarpic plants even after multiple rounds of flowering and seed set.

In monocarpic plants, the vegetative development from axillary shoot meristems is suppressed (Amasino, 2009; Davies and Gan, 2012) and all energy is funneled toward reproductive activities (Thomas, 2013).The remobilization of nutrients from leaves to flowers and fruits is well-known as major cause of leaf senescence in monocarpic plants (Avila-Ospina et al., 2014; Distelfeld et al., 2014). Leaf senescence is an age-dependent mechanism that is directly connected to plant body senescence and death, and therefore this mechanism is likely to contribute to the diversity of plant growth habits among different plant species. It is a complex process that is affected by an extensive range of developmental and environmental signals (Fischer and Fischer, 2017). Recent molecular genetic studies have uncovered that the leaf senescence process strongly depends on a major reprogramming of gene expression (Pujol, 2015; Fischer and Fischer, 2017), involving transcription factors of the NAC-, WRKY-, C2H2- zinc finger-, AP2/EREBP, - and MYB-class (Schippers, 2015; Fischer and Fischer, 2017). In addition, histone- and DNA modifications have been implicated in the senescence process (Ay et al., 2014), and overexpression of an AT-hook protein was shown to delay leaf senescence in *Arabidopsis* (Lim et al., 2007). Plant hormones such as ethylene, jasmonic acid, salicylic acid, cytokinin and ABA have also been shown to play important roles in leaf senescence (Jibran et al., 2013; Fischer and Fischer, 2017). Of these hormones, cytokinin and ethylene seem most effective in controlling leaf senescence, as up-regulation of cytokinin signaling or impaired ethylene signaling was shown to delay leaf senescence (Jibran et al., 2013). Based on recent multi-omics approaches, a picture is now beginning to emerge on the leaf senescence programme that reveals clear cross-talk between the transcriptional regulatory networks and plant hormone signaling (Penfold and Buchanan-wollaston, 2014; Schippers, 2015; Kim et al., 2016). Nonetheless, many of the regulatory mechanisms remain elusive.

Besides senescence, the phase identity of (axillary) shoot meristems plays an important role in determining the plant growth habit. The ability to maintain axillary meristems in the vegetative state after a successful round of offspring production is a key feature of polycarpic behavior. In annual monocarpic plants, the near simultaneous transition of all shoot meristems into the reproductive phase prevents vegetative development after seed set, and eventually leads to death of the plant body (Fig. 4). In contrast, many polycarpic plants prolong their life span by maintaining a number of axillary meristems in the vegetative phase (Fig. 4), thereby allowing subsequent cycles of reproductive development during the next growth season (Amasino, 2009). In some polycarpic plants, however, all axillary meristems do change to the reproductive phase, but vegetative development is maintained by the reversion of some inflorescence meristems to the vegetative state under specific environmental conditions (Tooke et al., 2005).

Consistent with the central position of the vegetative-to-reproductive phase transition in determining the life history of a plant, the key regulators of this phase transition play an important role in regulating the vegetative activity of axillary shoot meristems during the reproductive phase. Studies in Brassicaceae have recently started to reveal the genetic basis that differentiates between the monocarpic and polycarpic growth habit. The *Arabidopsis FLC* MADS box gene ortholog *PERPETUAL FLOWERING1* (*PEP1*) (Michaels and Amasino, 1999) controls the temperature-dependent switch from polycarpic to monocarpic growth in the conditionally polycarpic *Arabis alpine* and *Cardamine flexuosa* (Wang et al., 2009b; Zhou et al., 2013a). PEP1 blocks the vegetative to reproductive transition of meristems, leading to vegetative development, but low temperature-induced chromatin modifications (during winter) lead to repression of *PEP1* transcription and subsequently to flowering (in spring), explaining the temperature-dependent polycarpic life history of *A. alpine* and *C. flexuosa* (Wang et al., 2009b; Zhou et al., 2013a).

It has been shown that the *Arabidopsis* double mutant in the flowering genes *SOC1* and *FRUITFULL* (*FUL*) shows a perennial-like lifestyle (Melzer et al., 2008). Interestingly, the PEP1-induced polycarpic behavior of *A. alpine* and *C.* flexuosa was also shown to be caused by suppression of *AaSOC1* and *CfSOC1* expression (Bergonzi et al., 2013; Zhou et al., 2013a). This indicates that advances in the understanding of molecular mechanisms that control monocarpic life strategies can help to elucidate the molecular basis of the capacity of polycarpic plants to survive after multiple rounds of flowering.

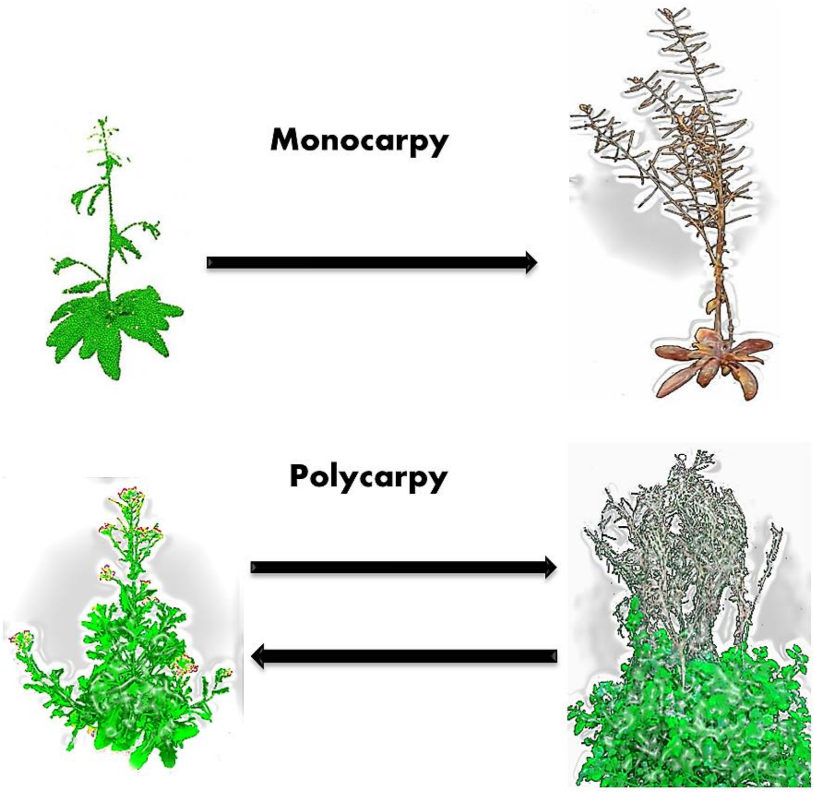

Figure 4. Schematic comparison of the monocarpic and polycarpic plant growth habit

Chromatin remodeling and AT-Hook motif nuclear proteins in plant developmental phase transitions

In the nucleus of multi-cellular organisms, the genomic DNA is packed into highly condensed chromatin, the complex of DNA with histone and non-histone proteins. The genomic studies in multicellular organisms have demonstrated that the structure and organization of chromatin determines the accessibility of the transcriptional machinery to the DNA, thereby dictating the transcriptome pattern (Ho and Crabtree, 2010). Similar to other multi-cellular organisms, remodeling of the chromatin structure plays a crucial role in temporal and spatial gene expression patterns during developmental processes in plants (Reyes, 2006; Exner and Hennig, 2008; Jarillo et al., 2009; Kaufmann et al., 2010). Large scale chromatin remodeling leading to global transcriptome reprogramming has been observed during embryonic-to-postembryonic and vegetative-to-reproductive phase transitions (Exner and Hennig, 2008; Holec and Berger, 2012; Han et al., 2015), thereby uncovering the role of chromatin remodeling in plant developmental phase transitions. During the past few years, molecular genetic studies have discovered histone modifications and DNA methylations play an important role in the regulation of developmental processes of plants (Reyes, 2006; Jarillo et al., 2009; Holec and Berger, 2012). Unlike animals, where the major epigenetic marks are established during embryo development, such epigenetic mechanisms operate throughout plant development (Jarillo et al., 2009). Therefore, the high level of developmental plasticity in plants is associated with differential regulation of epigenetic information. Recent studies have shown that the molecular memory of gene expression that is stored by epigenetic mechanisms is likely to be crucial for the plant growth behavior. For example, the expressions of the key floral transition genes including *FT*, *SOC1*, and *FLC*, are epigenetically regulated (Bratzel and Turck, 2015). A critical step for the perennial life history of *A. alpine* is the return of *PEP1* expression to its original levels a few weeks after the cold period, which is correlated with removal of the repressive trimethylation of histone H3 (H3K27me3) in the *PEP1* promotor (Wang et al., 2009b). Moreover, several other studies have shown an age-dependent increase in DNA methylation levels in plants, which might lead to repression of *miR156* expression (Hasbu et al., 2010; Saya et al., 2011; Huang et al., 2012; Yuan et al., 2014; Dubrovina and Kiselev, 2016).

Besides such epigenetic marks, many putative chromatin-remodelling proteins have been identified that are involved in plant developmental processes (Reyes, 2006; Jarillo et al., 2009; Holec and Berger, 2012). In many cases, however, the possible involvement of these proteins with remodeling of chromatin is only based on sequence homology with chromatin remodelers in other organisms.

A wide range of non-histone nuclear proteins, also known as high-mobility-group (HMG) proteins, have been found in eukaryotic cells to regulate functions of the complex eukaryotic genome (Bianchi and Agresti, 2005; Reeves, 2010). Three main families of HMG proteins have been identified, of which the HMGA proteins are considered as chromatin architectural factors involved in a diverse array of crucial cellular processes, including cell growth, differentiation, transformation, proliferation, death, and DNA replication and repair, chromatin remodeling, and gene transcription (Reeves, 2010; Sgarra et al., 2010; Ozturk et al., 2014). These proteins preferentially bind to the narrow minor groove of DNA at AT-rich stretches using a highly conserved small DNA-binding motif, called AT-hook (Aravind and Landsman, 1998). The AT-hook motif is not unique to HMGA proteins and has also been found in a large number of non-HMGA proteins, such as chromatin remodeling proteins, transcription factors, and ATP-dependent chromatin remodeling complexes (Aravind and Landsman, 1998;Cairns et al., 1999; Xiao et al., 2001; Singh et al., 2006; Su et al., 2006; Maffini et al., 2008; Lucas et al., 2009).

The *Arabidopsis thaliana* genome encodes 29 AT-HOOK MOTIF CONTAINING NUCLEAR LOCALIZED (AHL) proteins that have either one or two AT-Hook DNA binding domains (Fujimoto et al., 2004; Matsushita et al., 2007; Ng et al., 2009; Zhao et al., 2013). AHL proteins also contain a Plant and Prokaryote Conserved (PPC) domain that is involved in the physical interaction between AHL proteins or with histones or other nuclear transcription factors (Fujimoto et al., 2004; Matsushita et al., 2007; Street et al., 2008; Zhao et al., 2013). *AHL* gene families seem to be land plant-specific, as they have been found in early diverging land plants such as *Physcomitrella patens* and *Selaginella moellendorffii*, but not in water dwelling uni- or multicellular algae (Gallavotti et al., 2011; Zhao et al., 2014).

Molecular genetic studies in *Arabidopsis* have revealed that AHL proteins are involved in multiple aspects of plant growth and development, including flowering time (Weigel et al., 2000; Street et al., 2008; Xiao et al., 2009; Jin et al., 2011), flower development (Ng et al., 2009; Gallavotti et al., 2011; Jin et al., 2011), hypocotyl growth (Street et al., 2008; Xiao et al., 2009; Zhao et al., 2013), vascular tissue differentiation (Zhou et al., 2013b), and hormonal response (Matsushita et al., 2007; Ng et al., 2009). Consistent with the lack of phenotypes in the single or double mutants of *Arabidopsis AHL* genes, a high degree of functional redundancy has been suggested among these genes, and therefore most information on the function *AHL* genes has come from phenotypic changes induced by overexpression of these genes in *Arabidopsis* (Street et al., 2008; Xiao et al., 2009; Zhao et al., 2013).

Based on the detection of epigenetic modifications around the DNA-binding sites of *AHL22*, *AHL16*, and *AHL21* (Ng et al., 2009; Yun et al., 2012; Xu et al., 2013), AHL proteins have been considered to act through chromatin modification. In addition, AHL proteins have been shown to preferably bind to Matrix Attachment Regions (MARs) (Fujimoto et al., 2004; Lim et al., 2007; Ng et al., 2009; Xu et al., 2013). MARs are AT-rich short DNA sequences (about 200-300 bp) that bind to the nuclear matrix and organize the chromatin into distinct loop domains (Heng et al., 2004; Girod et al., 2007; Chavali et al., 2011; Wilson and Coverley, 2013). In animals, several MAR-binding proteins have been identified (Wang et al., 2010), among which the AT-hook motif-containing special AT-rich sequence-binding protein 1 (SATB1) that is implicated in several cellular processes such as gene expression regulation, chromatin organization, and histone modification (Yasui et al., 2002; Cai et al., 2003; Kumar et al., 2007; Han et al., 2008; Kohwi-Shigematsu et al., 2012). However, the exact molecular mode of plant AHL proteins in the regulation of plant developmental aspects remains unknown yet.

Somatic embryogenesis: reversing a developmental phase transition

The ability of a plant cell to acquire totipotency and enter the embryogenesis programme is not restricted to the zygotic cell, as in specific (apomictic) plant species embryos are derived from diploid ovule cells or from unreduced gametophytes by asexual reproduction (without fertilization) (Ozias-Akins, 2006). In many flowering plants, however, vegetative somatic cells can be reprogrammed to a pluripotent state under appropriate *in vitro* conditions. This developmental pathway, which involves reversion of the transition from the embryonic to post-embryonic phase, is called somatic embryogenesis (SE) and considerably resembles ZE. Similar to ZE, distinct morphological and physiological stages can be recognized during SE, such as the transition from globular to cotyledon stages and finally the accumulation/storage of nutrients in the maturing embryo required for subsequent germination and seedling development (Arnold et al., 2002). Because of these similarities, SE is considered as a more easily accessible model system to study the biochemical and molecular processes in ZE (Zimmerman, 1993; Mordhorst et al., 1998).

More than 50 years ago, the induction of somatic embryos from differentiated plant cells was first demonstrated in carrot cell suspension cultures (Steward et al., 1958). Since then, SE has been reported in a wide range of dicot and monocot plant species. Besides providing an excellent tool for a better mechanistic understanding of embryogenesis and totipotency in plants, SE has offered great potential for applications in plant biotechnology and plant breeding, including genetic transformation, somatic hybridization, clonal propagation, synthetic seed technologies, cryopreservation, and somaclonal variation (Imin et al., 2005; Wang et al., 2012).

SE is typically achieved by exogenous application of plant hormones, and in 65% of the recent protocols the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D), a synthetic analog of the natural auxin IAA, has been employed for SE induction. Therefore, auxin is considered as a key trigger of SE in most plants (Stone et al., 2008; Elhiti et al., 2013a; Wójcikowska et al., 2013), which is in line with its crucial role in the regulation of ZE (Friml et al., 2003; Jenik and Barton, 2005; Jenik et al., 2007; Lau et al., 2012; Robert et al., 2013). In line with the important role of PIN proteins in establishing of polar auxin transport-mediated patterning of the early ZE (Friml et al., 2003), PIN-driven auxin gradients have also been shown to be important during SE, not for the initiation of SE, but rather for the later development of somatic embryos (Philipsen, 2017).

Upregulation of several ARF genes, *ARF5/6/8/16/17*, that either promote or inhibit of auxin-responsive genes (Guilfoyle and Hagen, 2007; Boer et al., 2014) has been reported in embryogenic cultures of *Arabidopsis* (Gliwicka et al., 2013) and other plants (Shealy et al., 2003; Legrand et al., 2007; Singla et al., 2007; Sharma et al., 2008). Recently, mutations in the *ARF6* and *ARF8* genes were shown to arrest SE in *Arabidopsis* (Su et al., 2016). These data indicate that ARFs are required for SE. Other studies have shown that members of the *YUCCA* (*YUC*) gene family, encoding enzymes in the key tryptophan-dependent auxin biosynthesis pathway, are important for SE (Zhao, 2012). *YUC1/4/6/10* genes are upregulated (Bai et al., 2013; Elhiti et al., 2013a) and auxin biosynthesis is enhanced in 2,4-D-induced embryogenic cultures (Jiménez, 2005; Elhiti et al., 2013a). Moreover, quadruple mutants in the *YUC1/2/4/6* genes cause defects in SE (Bai et al., 2013), indicating that IAA biosynthesis is required for SE and suggesting that not the exogenously applied 2,4-D but rather the endogenous YUC-mediated IAA biosynthesis provides the auxin signal required for proper SE.

The effectiveness of 2,4-D in inducing SE has been attributed on the one hand to its stability as auxin analog. On the other hand, however, at the high concentrations used during SE induction, 2,4-D might rather act as a stress-inducing herbicide, and the involvement of this stress in acquiring embryonic cell identity has been proposed as well. In several studies indicate that 2,4-D indeed induces many stress-related genes during 2,4-D-induced SE (Zavattieri et al., 2010; Gliwicka et al., 2013; Fehér, 2015). Consistent with the observed acquisition of embryogenic ability in somatic cells following stress treatments, such as heavy metal-, ultraviolet radiation-, osmotic-, temperature- or hypoxia stress in many plant species (Zavattieri et al., 2010), the stressor effect of 2,4-D may be more important than its auxin activity in the induction of SE.

With respect to importance of SE for plant breeding and propagation, many attempts have been made to understand the molecular basis of this phenomena in different plant species such as *Arabidopsis*, carrot, alfalfa, and conifers (Rose and Nolan, 2006; Namasivayam, 2007; Yang and Zhang, 2010a; Elhiti et al., 2013b; Smertenko and Bozhkov, 2014). So far, many genes have been identified that are involved in SE (Imin et al., 2008; Lucau-Danila et al., 2010; Yang and Zhang, 2010; Elhiti et al., 2013b; Gliwicka et al., 2013; Nic-Can et al., 2013; Smertenko and Bozhkov, 2014). Most of these genes have been identified as being up-regulated during SE, but their exact function in acquisition of embryogenic ability and embryo development has not been determined yet. Some of these genes, however, encode transcription factors that have been found to promote SE in the absence of exogenous 2,4-D or other plant growth regulators. For genes such as *BBM/PLT4* (Boutilier et al., 2002), *WUS* (Zuo et al., 2002), *(LEC1* (Lotan et al., 1998), and *LEC2* (Stone et al., 2001) it was shown that overexpression of a single gene can induce the pluripotent state in plant cells leading to SE. In addition, they also appeared to be key regulators of ZE.

The *BBM/PLT4* gene, for example is expressed during early stages of ZE in *Arabidopsis* (Boutilier et al., 2002; Galinha et al., 2007). *BBM/PLT4* overexpression is sufficient to induce SE from vegetative organs in *Arabidopsis* (Boutilier et al., 2002), tobacco (Srinivasan et al., 2007), chinese white poplar (Deng et al., 2009), and sweet pepper (Heidmann et al., 2011) in the absence of exogenous auxin. It was also found that overexpression of the *BBM* ortholog from Glycine max (*GmBBM1*) or of a close homolog of *BBM/PLT4* in *Arabidopsis*, *PLT5*, induce embryonic callus on *Arabidopsis* seedlings (El Ouakfaoui et al., 2010; Tsuwamoto et al., 2010), suggesting that the BBM function is probably conserved among family members and throughout higher plants. Up-regulation of two auxin biosynthesis genes, *YUC3* and *YUC8,* in *Arabidopsis* seedlings overexpressing BBM (Horstman, 2015) indicates that, similar to 2,4-D-induced SE, auxin biosynthesis is also important for BBM-induced SE.

The *WUS* transcription factor is well-known for its role in maintaining the pool of stem cells in the SAM (Mayer et al., 1998). Ectopic expression of *WUS* promotes SE in *Arabidopsis* root tissues without hormonal treatment (Zuo et al., 2002). *WUS* overexpression was also reported to significantly increase the SE efficiency in *Coffea canephora* (Arroyo-Herreraet al., 2008), whereas *WUS* knockdown in *Brassica napus* results in a reduced SE efficiency (Elhiti et al., 2010). Maintenance of cells in a relatively undifferentiated state by

WUS (Mayer et al., 1998) and the prevention of cell differentiation by *BBM* (Srinivasan et al., 2007; Horstman et al., 2015) indicates that repression of cellular differentiation might be essential for SE initiation.

Despite the fact that the *LEC1* and *LEC2* genes control distinct aspects of ZE such as embryo growth, embryo organ identity, and seed maturation (Stone et al., 2001; Braybrook and Harada, 2008), the SE induction by their overexpression in *Arabidopsis* indicated that these genes also promote embryonic cell identity (Lotan et al., 1998; Stone et al., 2001). Both *LEC* genes have been reported to be significantly up-regulated during 2,4-D-induced SE in *Arabidopsis* (Ledwoń and Gaj, 2011) and *Picea abies* (Uddenberg et al., 2011), whereas *Arabidopsis lec* mutants are impaired in 2,4-D-induced SE (Gaj et al., 2005).Moreover, the improved SE by ectopic expression of *LEC1* and *LEC2* in tobacco (Guo et al., 2013) and cacao (Zhang et al., 2014) shows the ability of these genes to promote SE in other plant species. The *LEC* genes seem to play a role in maintaining embryonic identity in somatic cells.

Recent studies have shown that *LEC2*-induced SE is accompanied by elevation of endogenous auxin levels in embryonic tissues. Like for BBM overexpression, several member of the *YUC* gene family, were shown to be significantly upregulated in *Arabidopsis* seedling-overexpressing *LEC2*. The rapid activation of *YUC2*, and *YUC4* expression by LEC2 (within 1 h after induction of LEC2 activity) suggests that LEC2 is a master regulator of auxin biosynthesis during SE-induction (Stone et al., 2008). In animals, the essential role of chromatin modification in the pluripotent stem cells establishment has been demonstrated (Gaspar-Maia et al., 2011; Apostolou and Hochedlinger, 2013; Buganim et al., 2013). The current genetic studies in plants also have indicated that the SE processes is accompanied by global modification of chromatin (Nic-Can et al., 2013; Fehér, 2015). Moreover, the SE potential of several *Arabidopsis* tissues was enhanced by down regulation of the H3K27me3 activity of Polycomb repressive complex 2 (PRC2) (Mozgova et al., 2017). However, the detailed contribution of chromatin modifications in SE is largely unknown.

Although several studies have predicted possible molecular pathways controlling SE (Radoeva and Weijers, 2014; Smertenko and Bozhkov, 2014), SE is a complex phenomenon and the exact molecular mechanisms or signaling pathways that lead to the induction of SE are one of the challenges to be solved with modern molecular biology.

Outline of the thesis

This thesis describes the functional analysis of the *AHL15* gene encoding an AT-Hook motif protein that was originally identified by a yeast one-hybrid screen. Functional analysis of this gene revealed that overexpression leads to the formation of somatic embryos on *Arabidopsis* seedlings in the absence of hormone treatment (van der Zaal and Hooykaas, 2004).

In **Chapter 2**, we focused on the initiation of SE by *AHL15* overexpression on *Arabidopsis* seedlings and the role of this gene in zygotic embryogenesis (ZE). *ahl15* loss-of-function mutants showed reduced somatic embryo induction in response to 2,4-D treatment or overexpression of the SE-inducing BBM transcription factor. The *AHL15* gene appeared to be bound and transcriptionally-regulated by BBM during SE. During zygotic embryogenesis *AHL15* was found to be 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* somatic embryos were 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 was caused by endomitotic events that specifically occurred 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 suggest that opening of the chromatin structure is required for the acquisition of embryonic competency in somatic plant cells.

More detailed analyses revealed that AHL15 is not specifically involved in the embryogenesis program, but that, surprisingly, the protein rather is involved in post embryonic development. The research described in **Chapter 3** focused on the role of *AHL15* and its close homologs, *AHL19* and *AHL20,* in the vegetative phase change, flowering and plant longevity. Because plants overexpressing *AHL15* reverse adult meristems to a juvenile state, AHL15 was named REJUVENATOR (RJV). In this chapter we showed that the AHL15/RJV is a suppressor of developmental phase changes. Loss of *RJV* gene function accelerated plant aging, whereas *RJV* overexpression converted monocarpic *Arabidopsis thaliana* and *Nicotiana tabacum* plants into polycarpic plants with enhanced seed and biomass production. Our results show that *AHL15/RJV* acts downstream of aging (*miR156*, *SPL*) and flowering (*SOC1*, *FUL*) genes as a molecular switch between monocarpic and polycarpic life history strategy. *RJV* expression acts as a molecular switch for life history strategies in plants, and can be used as a breeding tool to promote sustainable plant production by converting annual crops into perennial plants.

In **Chapter 4** we analysed our observation that transient (4 hours) activation of overexpressed AHL15-GR in *Arabidopsis* seedlings leads to long term effects on plant development. RNA sequencing analysis detected an extensive reprogramming of the transcriptome 4 hours after AHL15-GR activation. AHL15 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 AHL15 activation in leaf primordia and axillary meristems, indicating that the global reprogramming of the transcriptome by AHL15 might at least in part be caused by extensive modulation of the chromatin configuration. Co-activated or co-repressed genes were often 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 AHL15 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.

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