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

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Chapter 1

General introduction

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Unlike animals, higher plants have the unique ability to continuously produce new tissues and organs in the apical and lateral direction throughout their life span via proliferation of pluripotent stem cells organized in niches called meristems. As sessile organisms, this allows plants to replace organs that are lost due to pathogen attack or herbivory, and also to adapt their architecture or developmental program in response to (adverse) abiotic environmental signals. The stem cells in meristems self-renew through asymmetric cell division, producing a daughter cell that maintains stem-cell identity and a second daughter cell that differentiates into a more specialized cell type (Soyars et al., 2016).

In angiosperm plants, two stem-cell niches are generated during embryogenesis: the shoot apical meristem (SAM) and root apical meristem (RAM). These apical meristems produce primary meristems such as the protoderm, which gives rise to the outside cell layer or epidermis of the plant; the procambium, which produces the xylem and phloem for water and nutrient transport, respectively; and the cambium, responsible for secondary growth controlling the thickness of stems and roots. All aboveground organs of the plant are produced by the SAM or by so-called axillary meristems (AMs) that arise in axils of leaves, whereas the RAM produces the cell layers of the main root, including the pericycle stem-cell layer, which produces the lateral roots, and thus determines the branching of the plant root system (Uchida and Torii, 2019).

During growth and development of the plant, the position of new organs or tissues within the plant body depends on the activity and communication between the different plant meristems. In this chapter, we review the current knowledge about the mechanisms controlling initiation, development, and activity of meristems.

The role of the shoot apical meristem in growth and development

The post-embryonic formation of all aerial plant parts depends on the activity of the SAM. The SAM in Arabidopsis is divided into four different zones: 1) the central zone (CZ), 2) the peripheral zone (PZ), 3) the rib meristem (RM) also referred to as organizing center (OC) and 4) the rib zone (RZ), which is located below the OC and produces the internal part of the stem (Fig. 1). The CZ consists of pluripotent stem cells that by continuous division maintain the stem cell niche, and displace daughter cells into the PZ, where the lateral organs such as leaves or flowers are formed. Slow stem cell divisions in the CZ also displace daughter cells downwards into the RM/OC. The OC controls stem-cell proliferation in the CZ and differentiation in the PZ and in this way maintains the function and organization of the SAM (Murray et al., 2012; Soyars et al., 2016).

Previous genetic studies have shown that the specification and maintenance of stem cells in the SAM is regulated by many factors, including transcriptional regulators, receptor kinases, miRNAs, small peptides, epigenetic marks, and plant hormones (Dodsworth, 2009; Bustamante et al., 2016; Somssich et al., 2016). *CLAVATA3 (CLV3)* and *WUSCHEL (WUS)*, are key factors that help the SAM to stay functional and organized. Arabidopsis plants mutated in *WUS* have no functional SAM. The spatial feedback loop between the WUS homeodomain transcription factor and the CLV3/CLV1 peptide ligand/receptor kinase maintains the cell identity in the OC. *WUS* is expressed in the OC, and can induce *CLV3* expression by moving to the CZ. In turn, the CLV3 peptide moves back to the OC where it

represses *WUS* expression by binding to the CVL1 receptor kinase (Somssich et al., 2016). Two main plant hormones, cytokinin and auxin, play an important role in the SAM regulatory network. Cytokinin (CK) is essential for continuous cell division in meristematic tissues, as it activates both *WUS* and *CLV3* expression, enabling *WUS* induction independent of the *CLV* pathway via the type-B ARABIDOPSIS RESPONSE REGULATOR transcription factors (type-B ARRs). Auxin, through its directional intercellular transport by the PIN FORMED (PIN) auxin efflux carriers, promotes cell differentiation and formation of lateral organs such as leaves and flowers (Reinhardt et al., 2000; Murray et al., 2012). Besides its role in organ initiation, auxin helps to maintain a balance between stem-cell activity and organ formation by regulating the CK signaling suppressing type-A ARRs, mediated by the AUXIN RESPONSE FACTOR (ARF) transcription factor MONOPTEROS (MP/ARF5). MP directly represses the expression of the type-A *ARR7* and *ARR15* genes, which subsequently enhances CK signaling (Zhao et al., 2010). At the same time, MP represses CK signaling by inducing the expression of the *ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6* (*AHP6*) gene (Besnard et al., 2014).

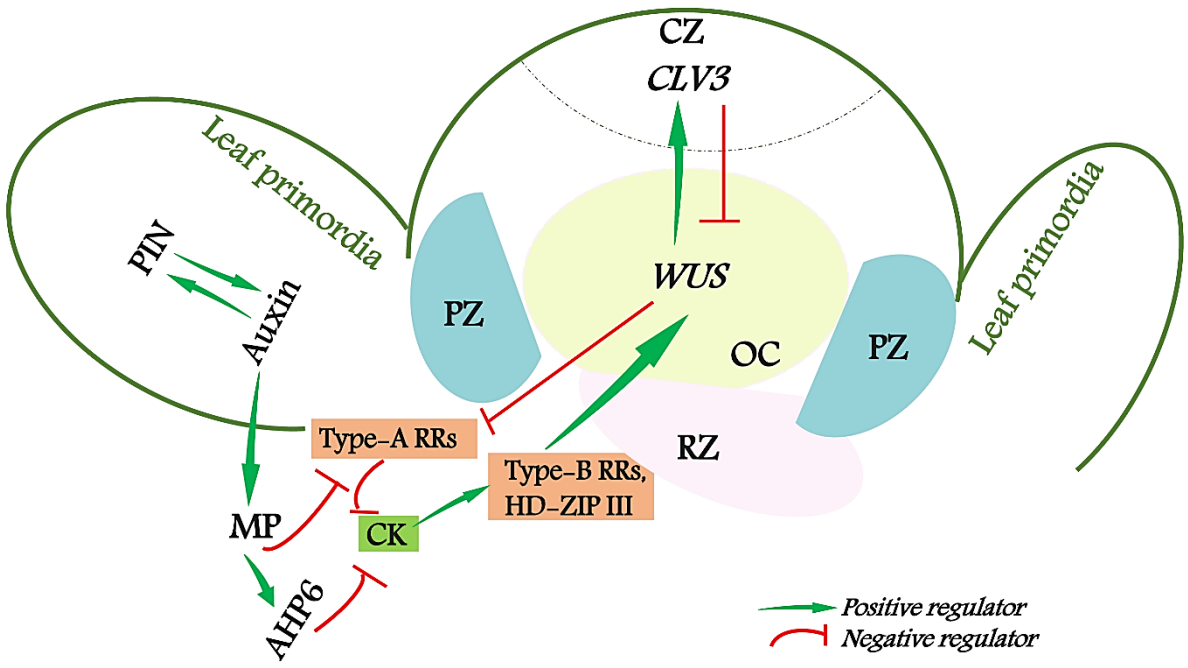

Figure 1. Main elements controlling shoot apical meristem (SAM) activity. The CLV3 peptide is expressed in the central zone (CZ) consisting of pluripotent stem cells. *WUS* is expressed in the organizing center (OC) and activates *CLV3* expression by moving to the CZ. CLV3 moves back to the OC, where negative feedback signaling by binding to the CLV1 receptor kinase represses *WUS* expression. The hormone cytokinin (CK) activates type-B ARRs, which by binding to the class III homeodomain–leucine zipper (HD-ZIP III) transcription factors promote *WUS* expression. WUS itself negatively regulates type-A ARRs (a negative regulator of CK signaling) in order to maintain CK signaling. Auxin in turn modulates stem-cell activity by repressing type-A ARR and inducing AHP6 expression through the ARF transcription factor MP/ARF5.

Post-embryonic shoot development in plants progresses through several distinct developmental phases, starting with the juvenile vegetative, and followed successively by the adult vegetative and the reproductive phases (Fig. 2). The transitions between these developmental phases are regulated mainly in the SAM, as in each phase the SAM produces new organs with different morphological features (Poethig, 2013). The SAM produces morphogenetic units called phytomers in the vegetative phase consisting of a stem, a node, and a leaf, with an axillary meristem (AM) in the axil of the leaf. AMs constitute another shoot meristem that can act as a SAM. They are thought to originate from the SAM and play an important role in secondary shoot production (branches) (Grbić and Bleecker, 2000; Wang et al., 2018). Upon floral transition, the SAM gradually matures into an inflorescence meristem (IM), initially producing phytomers with leaf-like bracts, and when fully matured phytomers with one or more flowers (Park et al., 2014; Wang et al., 2018). Upon successful fertilization, these flowers eventually develop into fruits with seeds.

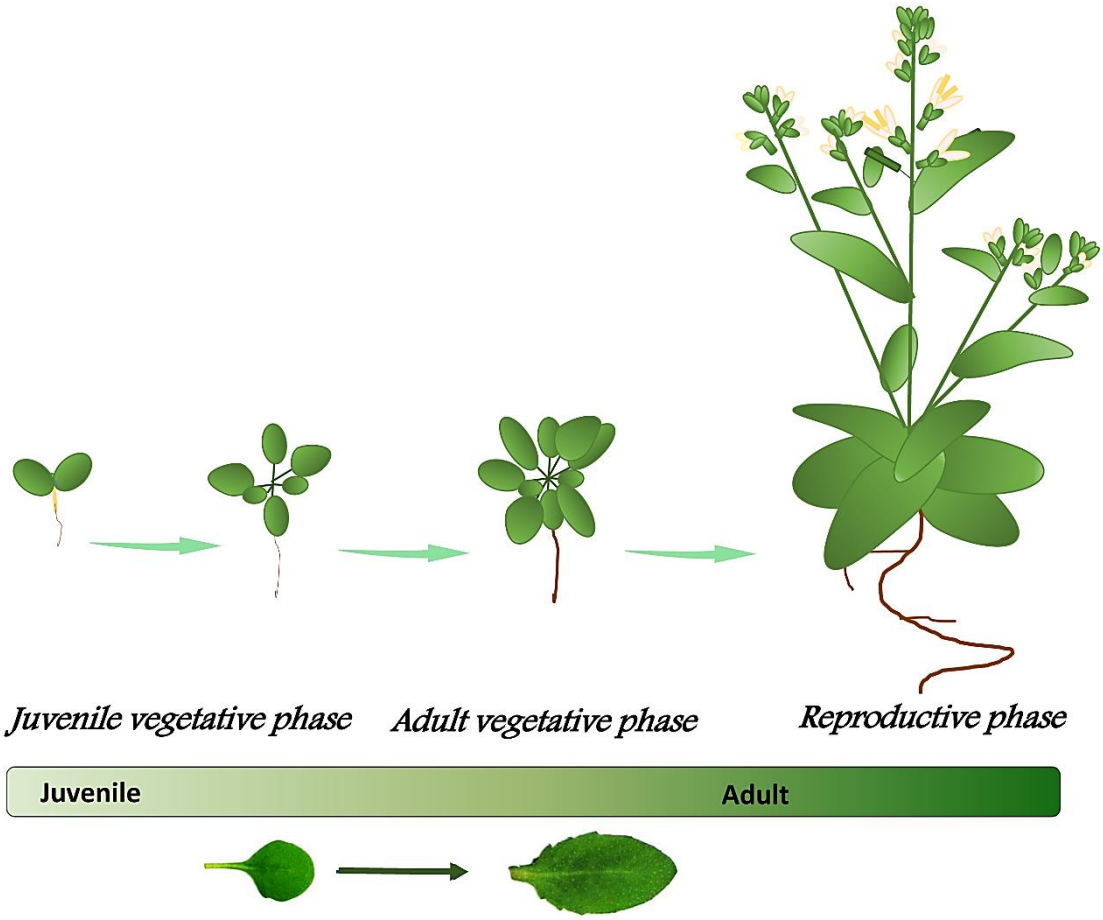

Figure 2. The two main developmental phase transitions in Arabidopsis. After germination, the seedling enters the vegetative phase, with the SAM and RAM responsible for organogenesis. The plant gradually goes through roughly three distinct developmental phases. The vegetative phase of growth is divided into a juvenile and an adult phase. Plant size and mass rapidly increase during the vegetative phase. Arabidopsis is a typical model plant in which, through leaf morphology, the juvenile phase is distinguishable from the adult phase (juvenile leaves are small and round in shape, with no trichomes on the abaxial side; in contrast, adult leaves are serrated and large, with trichomes produced on the abaxial side). In the juvenile vegetative phase, the plant is incompetent to flower, but during the adult vegetative phase the plant becomes flower competent and this starts its transition to the reproductive phase, in which the SAM becomes an IM giving rise to stems with bracts and flowers instead of leaves.

The juvenile-to-adult vegetative phase transition

In the juvenile vegetative phase, most plants are not competent to flower. Therefore, flowering requires the transition from juvenile to adult vegetative development, which is referred to as the vegetative phase change (VPC) (Poethig, 2013). The VPC is one of the most striking examples of a developmental switch that has a substantial impact on plant fitness and biomass (Demura and Ye, 2010). In many plants, the VPC coincides with distinguishable morphological changes such as changes in leaf shape or internode length, leaf position (phyllotaxis), the time interval between two consecutive (plastochron) leaf initiation events, or the appearance of trichomes on the abaxial leaf surface. These changes eventually result in the production of both juvenile and adult internodes or leaves on the same plant, which is known as heteroblasty (Huijser and Schmid, 2011; Poethig, 2013). In perennial woody plants, heteroblasty can be clearly observed, since these plants show a long juvenile period (Zotz et al., 2011; Huijser and Schmid, 2011). However, most of our knowledge on the molecular mechanisms governing this process is limited to Arabidopsis, since the majority of molecular genetic studies have focused on this annual model plant. In Arabidopsis, the VPC coincides with the production of larger and eventually serrated leaves that have a higher length:width ratio and develop trichomes on the abaxial leaf side (Telfer et al., 1997; Wu and Poethig, 2006; Poethig, 2013; Lee, 2000).

miRNAs, SPLs, and gibberellic acid regulate the juvenile-to-adult phase transition

The VPC is regulated by various endogenous factors that interact with environmental factors such as photoperiod, light intensity, and temperature. Studies in Arabidopsis have shown that two microRNAs (miR156 and miR157), a class of noncoding RNAs of 20–24 nucleotides involved in post-transcriptional regulation of gene expression, and their *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*SPL*) target genes play a crucial role in the VPC (Fig. 2) (Poethig, 1990; Lee, 2000; Huijser and Schmid, 2011; Poethig, 2013; Usami et al., 2009; He et al., 2018). Other studies have shown that miR156/157-regulated *SPL* genes are also drivers of the VPC in maize (Chuck et al., 2011) and a number of other herbaceous (Fu et al., 2012; Salinas et al., 2012; Shikata et al., 2012; Xie et al., 2012; Zhang et al., 2011) and woody plants (Wang et al., 2011).

Overexpression of *miR156* results in a prolonged juvenile phase, which has been observed in many plants including Arabidopsis (Schwab et al., 2005; Shikata et al., 2012; Fornara and Coupland, 2009; Wu et al., 2009b), rice (Xie et al., 2012), tomato (Zhang et al., 2011), and poplar (Wang et al., 2011). *miR156* differs from *miR157* in three nucleotides, but the overexpression phenotype of *miR157* is quite similar to that of *miR156* (Gandikota et al., 2007; Xu et al., 2016). The expression of the *miR156*/*miR157* genes is high at the young seedling stage, but gradually decreases as plant development progresses (He et al., 2018), leading to increased abundance of the SPL transcription factors (Fig. 3). Eleven of the seventeen *SPL* genes in Arabidopsis contain the *miR156/miR157* complementary sequence, but only six of those (*SPL2/9/10/11/13/15)* are involved in the VPC (Gandikota et al., 2007; Xu et al., 2016). miR156 and miR157 repress the *SPL* transcripts by direct binding, causing translational repression (He et al., 2018). In contrast to overexpression of *miR156* or *miR157*,

which reduces SPL abundance, SPL levels can be enhanced by introducing into the plant a miRNA-resistant gene version (*r-SPL*), in which the miR156/157 target site has been mutated. Alternatively, the same can be achieved by lowering miR156 abundance by overexpression of a miRNA target-site mimic (*35S:MIM156*) or by generating *mir156* loss-of-function mutations. In all cases, enhanced SPL abundance results in precocious appearance of adult traits and early flowering (Smith et al., 2009; Xu et al., 2016). Taken together, it can be concluded that miR156 and miR157 have a central role in the regulation of the VPC.

Together with a gradual decrease in *miR156*/*miR157* expression during shoot maturation, the expression of another miRNA gene, *miR172*, gradually increases (Fig. 3). The expression of *miR172* is low during the juvenile phase and increases during the VPC, since SPLs act as transcriptional activators of the *miR172* gene. 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., 2009a) (Fig. 3). In addition, SPLs promote other adult leaf traits such as elongation and serration independent of miR172. However, the genes that act downstream of SPLs and TOE1/TOE2 during the VPC have remained unidentified until now (Fig. 3).

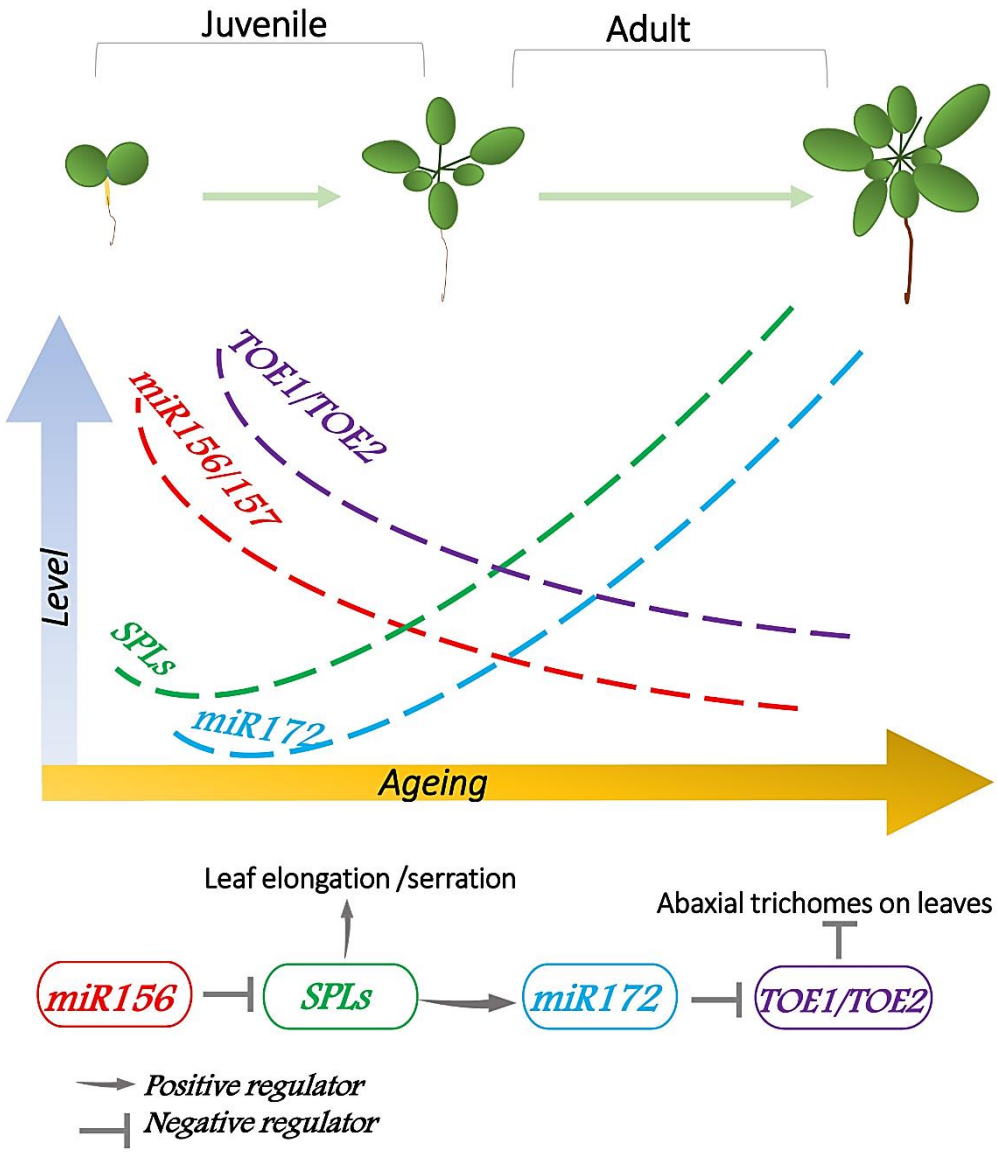

Figure 3. Regulation of the vegetative phase change in Arabidopsis. In early Arabidopsis development, levels of *miR156/157* (master regulator) are high in order to promote juvenile vegetative growth in seedlings. *miR156/157* levels (dotted red line) steadily decrease, allowing transcription factor production by *SPL* target genes (dotted green line) to increase gradually, and resulting in the appearance of adult leaf morphology. At the same time, the SPL transcription factors directly induce the expression of *miR172* (dotted blue line), which results in the suppression of the TOE1 and TOE2 transcription factors (dotted purple line), allowing trichome formation on the abaxial side of leaves.

Besides the miRNAs and their SPL targets, other endogenous factors such as the phytohormone gibberellic acid (GA) are involved in the VPC. In Arabidopsis, exogenous application of GA can accelerate the appearance of adult vegetative traits such as abaxial trichomes (Telfer et al., 1997; Park et al., 2017). In contrast, Arabidopsis GA-insensitive mutants or mutants deficient in GA biosynthesis show prolonged juvenile vegetative development. GA affects the adult phase by promoting the expression of some *SPL* genes, but no effect was observed on *miR156* expression (Fornara and Coupland, 2009; Galvão et al., 2012; Jung et al., 2012). GA therefore seems to promote SPL protein abundance, and as a consequence the VPC, in a miRNA-independent manner.

The vegetative-to-reproductive phase transition

The vegetative-to-reproductive phase transition involves the induction of flowering and plays a central role in the context of important agronomic traits such as plant biomass and seed- and fruit productivity. In fruit and seed crops, flowering should occur at the correct time when the environmental conditions are suitable to ensure maximal reproductive success (Jung and Müller, 2009). For the majority of plant species, transition to the reproductive phase or flowering occurs when they are in the adult phase. In these plants, flowering time largely depends on the duration of the juvenile phase. Some plants flower quickly after the transition to the adult phase, but other plants stay in the adult vegetative phase for a while before flowering.

The switch to the reproductive phase involves integrating multiple endogenous and environmental cues such as GA or the age pathway and photoperiod or vernalization, respectively (Fig. 4) (Amasino, 2010; Turnbull, 2011; Song et al., 2013). This involves a large number of genes that all act in a gene regulatory network (GRN) to coordinate the response to all these cues (Amasino, 2010; Turnbull, 2011; Song et al., 2013). In this GRN, *SUPPRESSOR OF CONSTANS1* (*SOC1*), *FLOWERING LOCUS T* (*FT*), *FLOWERING LOCUS C* (*FLC*), the *SPLs*, and *CONSTANS* (*CO*) are also known as floral pathway integrator genes, influencing flowering by integrating the different environmental and endogenous signaling pathways (Amasino and Michaels, 2010; Amasino, 2009; Andrés and Coupland, 2012; Hwang et al., 2019).

Regarding the transition from the vegetative to the reproductive phase in response to photoperiod, there are three main types of plants: i) short-day plants, in which a shorter photoperiod promotes flowering; ii) long-day plants, in which a long photoperiod promotes flowering; and iii) day-neutral plants, in which flowering does not depend on photoperiod (Song et al., 2013). Arabidopsis is a typical long-day plant with the B-box transcription factor CO at the core of the photoperiod pathway. Its abundance is up-regulated through light signaling in the leaf at the post-translational level (Fig. 4) (Turnbull, 2011; Song et al., 2013). CO performs its role by directly binding to the *FT* gene to activate its expression. The FT protein acts as a florigen signal that is transported from the leaf through the phloem to the SAM. In the SAM, FT interacts with the bZIP transcription factor FD. Together, FT and FD form a floral activator complex that activates the transcription of several flowering-promoting genes, including *SOC1* (Fig. 4), that switch the fate of the SAM to IM (Turnbull, 2011; Song et al., 2013; Jung et al., 2016; Abe et al., 2019).

Many plants in temperate climate regions have evolved to acquire reproductive activity by prolonged cold exposure through a process known as vernalization. A central node in the vernalization pathway is the MADS-box transcription factor FLC (Fig. 4), which acts as a potent repressor of flowering (Amasino and Michaels, 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 SAM (Fig. 4) (Deng et al., 2011; Matsoukas et al., 2012). Thus, FLC represses the flowering pathways in both leaf and meristem. Prolonged cold exposure leads to stable silencing of *FLC* expression by local chromatin modification and, subsequently, the induction of flowering (Kim and Sung, 2014). Besides chromatin modification, alternative splicing (Mahrez et al., 2016) and post-translational protein stabilization (Kwak et al., 2016) mechanisms have been shown to regulate FLC abundance at the molecular level.

Among flowering pathways, the GA pathway is an important endogenous pathway that controls the switch to the reproductive phase independent of the photoperiod (Yu et al., 2012; Davière and Achard, 2013). The control of flowering time by GA depends on GA biosynthesis, signaling, and degradation. GA-deficient mutants, either defective in GA biosynthesis or with higher GA degradation levels, show delayed flowering (Jung et al., 2012; Yu et al., 2012). DELLA proteins are central regulatory hubs in GA signaling-induced flowering. In this signal transduction, binding of GA to its receptor GIBBERELLIN INSENSITIVE DWARF1 (GID1) results in a conformational change that allows GID1 to bind to DELLA proteins and to form a GA–GID1–DELLA complex. The GA–GID1–DELLA complex subsequently promotes DELLA degradation by the 26S proteasome. (Silverstone and Pui, 1997; Griffiths et al., 2006; Sun, 2010; Galvão et al., 2012). GA signaling induces flowering mainly by promoting the expression of the *SPL* and *SOC1* genes (Fig. 4) (Jung et al., 2012, 2011; Fornara and Coupland, 2009).

In addition to the critical regulatory role of miR156/157 and miR172 in the VPC, these microRNAs also act as key players in the plant age-dependent floral pathway (Poethig, 2013). Also here, the age-related reduction in *miR156* expression leads to increasing levels of SPL transcription factors, which subsequently induce flowering by activating the transcription of *SOC1* and several other floral-promoting genes in the SAM (Fig. 4). 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 (Wang et al., 2011; Wu and Poethig, 2006; Andrés and Coupland, 2012; Wu et al., 2009b). Thus, miR156/157 and miR172 have opposite effects on flowering time, as overexpression of *miR156* delays flowering time, whereas overexpression of *miR172* accelerates flowering (Aukerman and Sakai, 2003; Huijser and Schmid, 2011; Poethig, 2013). Molecular genetic studies have shown that the ageing pathway is highly integrated into other flowering-time pathways (Wang, 2014).

APETALA1 (*AP1*) and *LEAFY* (*LFY*) are key floral-meristem identity genes that act downstream of the floral integrator SOC1 and eventually convert the vegetative SAM to an IM (Andrés and Coupland, 2012; Matsoukas et al., 2012; Blümel et al., 2015). The photoperiod-regulated FT–FD complex is directly involved in the transcriptional activation of *SOC1* in the SAM, and the central floral integrator SOC1 subsequently promotes flowering through activation of both floral-meristem genes *AP1* and *LFY* (Fig. 4) (Turnbull, 2011; Song et al., 2013).

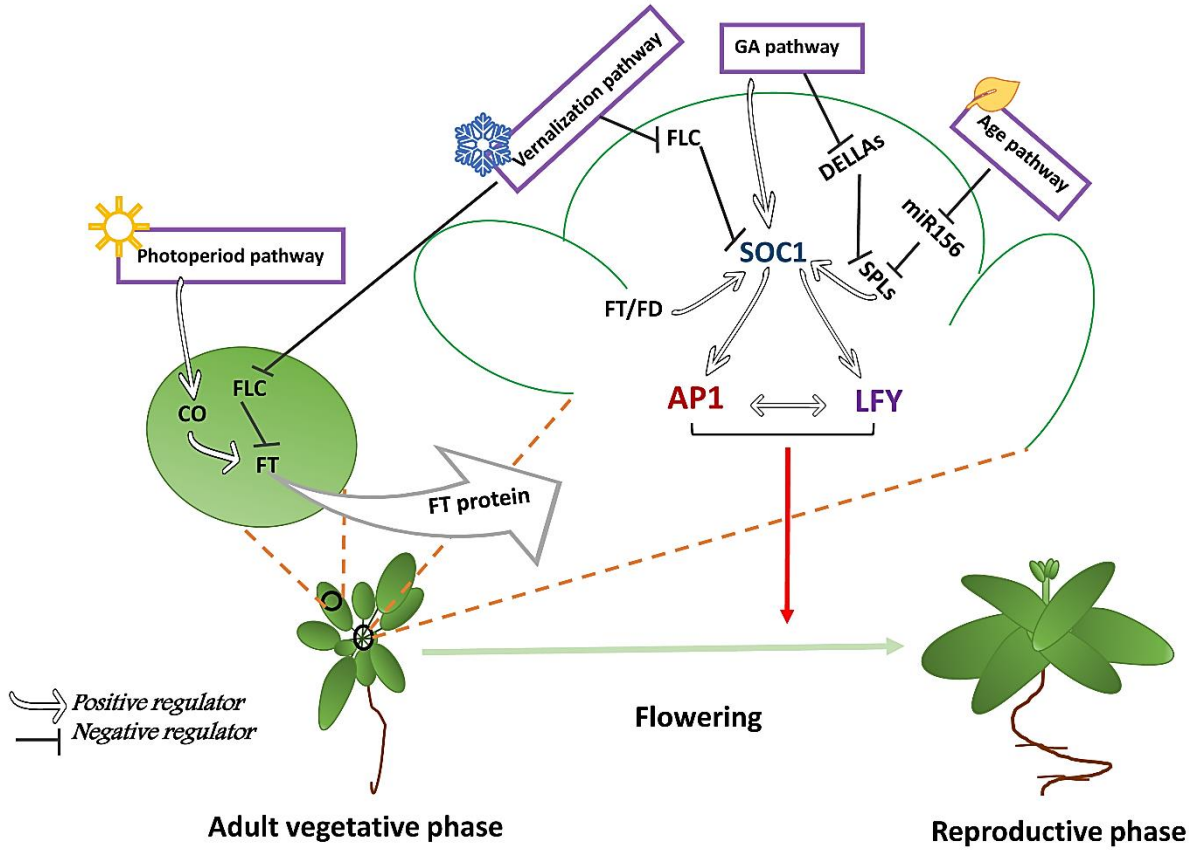

Figure 4. A simplified model of the gene regulatory network comprising the four pathways controlling flowering in Arabidopsis. In the photoperiod pathway, long days lead to CO accumulation in leaves, which induces FT expression. The FT protein travels through the phloem from the leaves to the SAM to interact with FD and activate *SOC1* and as a result subsequently *AP1* and *LFY*. In the vernalization pathway, cold treatment leads to stable repression of the *FLC* gene. The MADS-box protein FLC determines the cold-period-dependent timing of flowering by repression of the floral integrator genes *FT*, *FD* and *SOC1*. In the age-dependent pathway, a gradual reduction in *miR156* allows SPL abundance to increase, which activates *SOC1* and other floral integrators (not shown). Finally, the GA pathway promotes flowering independently by activation of *SOC1* and *SPL* genes. Subsequently, all four pathways help with transcriptional regulation of the floral integrators *FT* and *SOC1*, which promote *AP1* and *LFY* expression. *AP1* and *LFY* are required to complete the floral transition.

Axillary meristem formation and outgrowth

The ability to continue producing new organs throughout life is one of the unique post-embryonic features in plants. However, once the SAM is converted to IM, it will only produce a defined number of stems with flowers before its activity ceases, and its activity will be taken over by AMs that can act as SAM to establish secondary shoots (branches) (Pautler et al., 2013).

Genetic and physiological studies have identified several genes and plant hormones involved in AM formation (Scofield et al., 2018; Balkunde et al., 2017). Most of the key genes in regulating AM formation are transcription factors that were identified through phenotypic defects of loss-of-function mutants in the encoding genes. In Arabidopsis, the transcription factor *LATERAL SUPPRESSOR* (*LAS*) is required for AM formation, as shown by the *las* loss-of-function mutant that is no longer able to produce lateral shoots. *LAS* is florigenic, and

it migrates from the SAM toward the leaf axil (Greb et al., 2003). The putative transcription factors LAX PANICLE 1 (LAX1) and LAX PANICLE 2 (LAX2) interact with each other and are involved in AM formation in rice as *lax1* and *lax2* mutants both produce less AMs (Oikawa and Kyozuka, 2009; Tabuchi et al., 2011). Mutations in the *REVOLUTA* (*REV*) gene, encoding a class III homeodomain/leucine zipper TF (HD-ZIP III), reduce AM formation in Arabidopsis (Otsuga et al., 2001). The *REGULATOR OF AXILLARY MERISTEMS* (*RAX*) genes (*RAX1*, *RAX2*, and *RAX3*), encoding MYB-like transcription factors, are specifically expressed at the center of the leaf axil, and their loss of function affects AM formation in Arabidopsis and tomato (Keller et al., 2006). And lastly, cytokinin and auxin are two important plant hormones involved in AM formation (Wang et al., 2014a, 2014b).

The AMs can undergo immediate outgrowth or initially develop into dormant axillary buds. The latter may then grow out, leading to the formation of lateral shoots. In Arabidopsis and many other plants, plant shape and biomass are highly influenced by axillary bud outgrowth (referred to as branching) during plant growth and development. Branching is considered one of the most important agronomic traits. The identification of genes controlling branching has provided tools for genetic manipulation to improve plant architecture and enhance yield in crops.

In the last decade, significant progress has been made in understanding the regulation of bud outgrowth and branching. Whether buds are dormant or growth out is influenced by a wide range of internal factors and environmental cues such as light quality, shoot apex damage, and availability of nutrients (Kim et al., 2010; Djennane et al., 2014; Rameau et al., 2015).

Plant hormones are the most important internal factors that regulate shoot branching. Extensive studies have been performed on understanding hormone-regulated bud outgrowth. Auxin was the first plant hormone that has been shown to suppress the outgrowth of axillary buds. Classical decapitation experiments have shown that the SAM inhibits bud outgrowth, since its removal allows the lateral buds to grow out to secondary shoots. Apical dominance can be recovered, however, by applying auxin to the decapitated shoot, showing that auxin provided by the shoot tip suppresses bud outgrowth and promotes apical dominance (De Smet and Jürgens, 2007; Domagalska and Leyser, 2011; Waldie and Leyser, 2018) (Fig. 5). Unlike auxin, cytokinin directly promotes axillary bud outgrowth (Fig. 5). It has been shown that either overexpression of genes encoding enzymes mediating cytokinin biosynthesis *ISOPENTENYLTRANSFERASE* (*IPTs*) or exogenous cytokinin application triggers bud outgrowth (Ferguson and Beveridge, 2009; Domagalska and Leyser, 2011; Waldie and Leyser, 2018). Strigolactones (SLs) are a recently discovered class of plant hormones with a specific role in bud outgrowth. SLs inhibit bud outgrowth and branching (Fig. 5) in Arabidopsis and other species (rice, pea, tomato, and petunia). It was shown that the *MORE AXILLARY GROWTH MAX* genes (*MAX1*, *3*, *4*, *5*) in Arabidopsis and *MAX* orthologs *RAMOSUS* (*RMS*) *1, 2, 3, 5, 6* in pea, *DWARF* (*D*) *10, 17, 27* in rice and *DECREASED APICAL DOMINANCE* (*DAD*) *1*, *2*, *3* in petunia are involved in SL biosynthesis, and that mutants in these genes showed increased shoot branching compared to the wild type (Johnson et al., 2006). Auxin stimulates SL biosynthesis by up-regulation of the responsible genes (Foo et al., 2005). Therefore, SL biosynthesis may be part of the apical-dominance-promoting and bud outgrowth-inhibiting auxin pathway (Fig. 5). Furthermore, SL regulates

shoot branching by rapidly modulating auxin transport. (Shinohara et al., 2013). Two other phytohormones, GA and ABA, are considered bud-outgrowth inhibitors (Rameau et al., 2015; Gonzalez-Grandio et al., 2017; Yao and Finlayson, 2015). BRC1, by promoting the expression of *HOMEOBOX PROTEIN* (*HB*) *21*, *40*, and *53* mediates ABA accumulation and Several other genes have been identified to be involved in shoot branching. Among these, *TEOSINTE BRANCHED 1* (*TB1*) is known as a key gene that controls branching in maize. TB1 acts as a bud-outgrowth repressor, as the maize *tb1* mutant shows high tiller and aerial branch production. Mutation in the *TB1* orthologues *OsTB1* in rice or *BRANCHED 1* (*BRC1*) in Arabidopsis and pea also increase shoot branching (Aguilar-Martínez et al., 2007; Braun et al., 2012; Martín-Trillo et al., 2011; Minakuchi et al., 2010). The *BRC1/TB1* genes encode a protein belonging to the TCP transcription-factor family, which is specifically expressed in axillary buds and required for bud-outgrowth inhibition and branch repression (Gonzalez-Grandio et al., 2017). BRC1 is a key target of hormonal signaling (Fig. 5) (Dun et al., 2012). Overall, all of the factors mentioned above together control bud outgrowth with BRC1 at a central position (Fig. 5) (Seale et al., 2017).

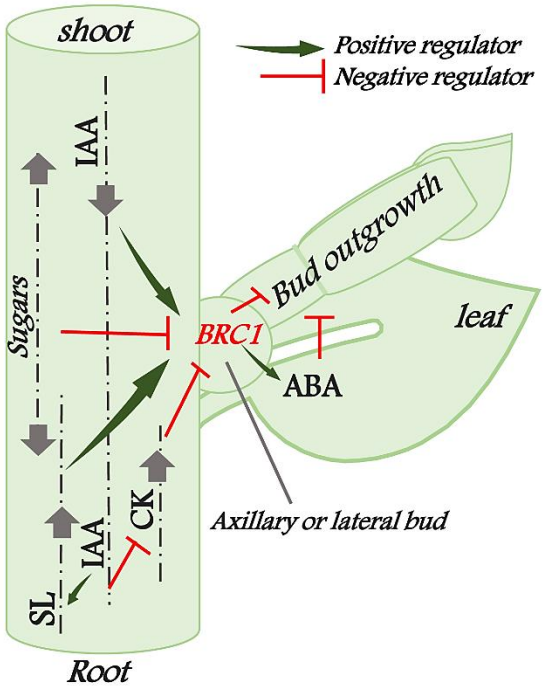

Figure 5. Hormonal signals control lateral bud outgrowth via BRC1. Hormones and nutrition control bud outgrowth. *BRC1* has a central role by interacting with different phytohormones. CK and sugars repress *BRC1*. Auxin represses CK either by repressing *IPT* genes or by promoting SL biosynthesis. In addition, BRC1 promotes ABA accumulation, which in turn suppresses bud development and outgrowth.

Axillary-meristem maturation and plant longevity

Plant longevity is defined as the period in which plants remain viable. Plant longevity has long been considered an essential trait for ecology, agronomy, and economy. However, the mechanisms regulating longevity in plants are mostly unknown. AMs, like the SAM, undergo phase changes. Upon flowering in Arabidopsis and many annual plants, when the SAM changes to an IM, vegetative development from AMs is highly suppressed (Amasino, 2009; Davies and Gan, 2012) and all energy is funneled toward reproductive activities (Thomas, 2013). The majority of AMs in axils of Arabidopsis leaves do not show vegetative development and immediately become IMs, and after producing a few cauline leaves are converted to flower meristems. However, in some Arabidopsis ecotypes such as Sy-0 this early maturation of the AMs is prevented, as some are maintained in the vegetative phase (Poduska et al., 2003). This heterochronic change of AM phase identity in Sy-0 leads to the formation of aerial rosettes in the axils of stem leaves, and significantly increases longevity and extends the period during which the plant is able to produce seed (Poduska et al., 2003). A dominant allele of the *FLC* gene has been suggested to underlie the increased life span of Sy-0 (Poduska et al., 2003). In addition, it has been shown that a double mutant of the Arabidopsis flowering-time genes *SOC1* and *FRUITFULL* (*FUL*) also shows enhanced vegetative development from AMs, leading to an increased life span (Melzer et al., 2008). FLC has previously been shown to negatively regulate *SOC1* and other flowering-time genes in the SAM (Deng et al., 2011; Matsoukas et al., 2012). However, our understanding of the mechanisms by which FLC keeps the AMs in the vegetative phase during floral transition is still limited.

Plants display a wide range of life cycles, varying from a few weeks for several annual species up to several thousands of years for some perennial species. In conjunction with their life span, plants have evolved two opposing growth habits. Many species are monocarpic, as they senesce and die after producing offspring once, even under appropriate growth conditions. By contrast, polycarpic plants do not die after flowering and typically produce multiple successful offspring over several consecutive years during their life history. All annual plants—but very few perennial plants—are monocarpic. Monocarpic perennials grow for many years, but die after producing offspring once (Munné-Bosch, 2008; Thomas, 2013).

The fate of AMs plays a critical role in the opposing growth habits of monocarpic and polycarpic plants. In contrast to the conversion of all AMs to inflorescences and the eventual death of the plant body in monocarpic plants (Fig. 6), polycarpic plants maintain a number of AMs in the vegetative state after a successful round of offspring production. In this way polycarpic plants prolong their life span, with their maintained vegetative AMs producing new shoots during the next growing season (Fig. 6) (Amasino, 2009). In some polycarpic plants, however, all AMs do change to the reproductive phase. Still, vegetative development is maintained by reversing some IMs to the vegetative state under specific environmental conditions (Tooke et al., 2005). Many plants, such as petunia and tomato, can maintain some AMs in the vegetative state during flowering and, therefore, they can stay alive for a few years when grown as perennials in year-round tropical conditions (e.g. in greenhouses), but generally they are grown as annual plants because they cannot survive the cold temperature during the winter. However, these plants have a considerably longer life span compared to the typical annuals that only stay alive until the end of the reproductive phase.

The existence of both mono- and polycarpic species within many plant genera implies that the transition between polycarpic and monocarpic growth habit might be associated with relatively small genetic changes. In fact, the switch between mono- and polycarpy is considered as the most common growth form transition in angiosperms (Amasino, 2009). However, despite considerable interest in understanding the molecular mechanisms controlling the switch between mono- and polycarpy, only a few genes have yet been identified that are involved in the offspring-linked death in monocarpic plants and the survival of polycarpic plants even after multiple rounds of offspring production.

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 AMs during the reproductive phase. Indeed, the perennial life history in close relatives of Arabidopsis, such as *Arabis alpina*, relies on not all AMs converting to IMs in a single growing season. Some meristems undergo floral transition in spring, while several AMs are maintained in the vegetative state, allowing subsequent cycles of growth during the next growing season.

Studies in Brassicaceae species have recently started to reveal the genetic basis that differentiates between the monocarpic and polycarpic growth habit. The *FLC* MADS-box gene ortholog *PERPETUAL FLOWERING1* (*PEP1*) in the conditionally polycarpic perennial *A. alpina* controls the temperature-dependent switch from polycarpic to monocarpic growth (Wang et al., 2009). PEP1 blocks the vegetative-to-reproductive transition of meristems, resulting in continued vegetative development. Still, low-temperature-induced chromatin modifications (during winter) lead to repression of *PEP1* transcription and subsequently to flowering (in spring). However, the low temperature only transiently suppresses the expression of *PEP1*, as restoration of the chromatin modifications at the *PEP1* locus to their original levels after the cold period leads to reactivation of *PEP1*, causing the dormant meristems or the new meristems produced during the reproductive phase in *A. alpina* to maintain the vegetative fate. The outgrowth of these meristems during the next growth season supports a subsequent cycle growth (Wang et al., 2009). Lack of epigenetic memory at the *PEP1* locus after a cold period thus explains the temperature-dependent polycarpic life history of *A. alpina*. It has been shown that the Arabidopsis double mutant in the flowering genes *SOC1* and *FUL* shows a perennial-like lifestyle (Melzer et al., 2008). Interestingly, the PEP1-induced polycarpic behavior of *A. alpina* was also shown to be caused by suppression of *AaSOC1* expression (Bergonzi et al., 2013). This indicates that advances in the understanding of molecular mechanisms behind monocarpic life strategies can help to elucidate how polycarpic plants survive after multiple rounds of flowering.

The temperature-dependent polycarpic life history of *A. alpina* is also controlled by the age-dependent pathway. Unlike in Arabidopsis, the decrease in miR156 levels is uncoupled with miR172 in *A. alpina*, whereas the expression of *miR172* is up-regulated by cold temperatures independently of miR156 (Bergonzi et al., 2013). The induction of miR172 by low temperatures leads to the repression of the *APETALA2*-like transcription factor gene *PEP2*. PEP2 positively regulates PEP1 expression, thus providing negative feedback to the floral transition by promoting the maintenance of the vegetative identity in AMs. The decline in miR156 levels is essential for flowering in cold-treated *A. alpina* plants, as only AMs with low miR156 levels transit to the reproductive phase (Bergonzi et al., 2013), indicating that

polycarpic life history in *A. alpina* is also dependent on miR156 levels in AMs, this serving as a distant pathway independent of the vernalization pathway. Recently it was reported that cold exposure is not able to induce flowering in an *A. alpina spl15* loss-of-function mutant (Hyun et al., 2019), indicating that the different floral pathway integrator genes act in parallel to control polycarpic behavior in *A. alpina*.

Cardamine flexuosa is another polycarpic close relative of Arabidopsis. Its polycarpic life history is mediated by cold temperatures, but unlike *A. alpina*, the age-decreased miR156 is associated with an increase in miR172 levels independent of cold temperature , which causes repression of its target *TOE1* and subsequently leads to repression of *CfPEP1* expression (Zhou et al., 2013a). This indicates that polycarpic behavior in Brassicaceae is controlled through distinct mechanisms.

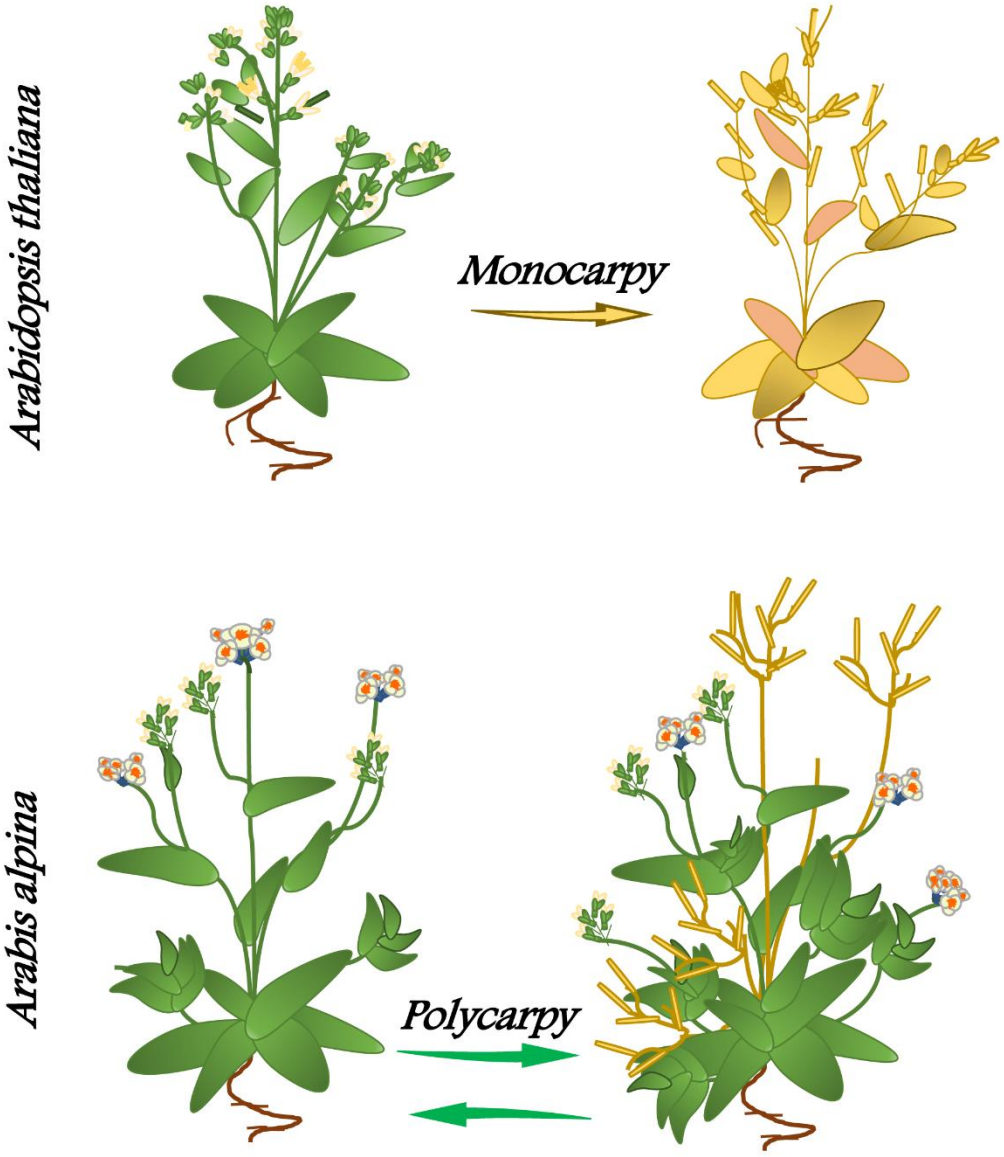

Figure 6. Monocarpic versus polycarpic life histories

Vascular meristems and primary and secondary growth

The evolution of vascular tissues composed of xylem and phloem for transport of various molecules such as water, nutrients, and other growth and defense factors between different tissue and organs has allowed higher plants to increase in size, and thus to outcompete smaller plants for light and nutrients. Vascular tissues also play an essential role in physical strength and resilience to wind and gravity. The vascular tissues connect all the plant organs by their conductive function, from the root tip to the various organs in the shoot. The xylem mediates the long-distance transport from root to shoot, such as water and solute minerals. In contrast, multiple signaling molecules and photosynthetic products are distributed throughout the entire plant by the phloem.

Vascular tissues in higher plants undergo two distinct growth phases during plant life history: primary and secondary growth. In young stems, the primary growth stage includes a number of vascular bundles. Each of these bundles contains procambium, primary phloem, and primary xylem (Fig. 7). After primary vascular growth, the procambium and its neighboring interfascicular parenchyma cells in stems are converted into the cambium, forming a ring of meristematic cells that in parallel to the production of phloem and xylem, respectively facing out and inside of the stem, promotes secondary growth of the stem. (Fig. 7) (Jouannet et al., 2015; Ragni and Greb, 2018). Apart from the mechanical support, this delivers through increased stem thickness, secondary growth plays a critical role in plant development as it also enhances the transport capacity of the stem by producing specialized cell types belonging to the phloem and xylem. However, in many flowering plants, especially monocots, all procambial cells eventually differentiate into non-meristematic vascular tissues during primary growth, and therefore cambium formation and secondary growth do not take place all.

Procambium and cambium formation

Procambium and cambium are a pool of stem cells formed in vascular plants that continuously generates the major plant vascular tissues, xylem and phloem, via asymmetric periclinal cell divisions. The basic characteristics and activity of procambium and cambium are very similar, and this is why they are considered the same set of vascular stem cells at different developmental stages. For instance, both in procambium and cambium, xylem is produced toward the inside and phloem toward the outside. However, cambium stem cells are usually more active than the ones in the procambium (Jouannet et al., 2015).

The SAM is the source of all primary vasculature system, containing several bundles. Each bundle includes procambium as a stem-cell provider between the phloem and the xylem (Fig. 7) (Jouannet et al., 2015). Cells located in the interfascicular region (the area between two bundles) gradually differentiate to parenchyma cells (Fig. 7). The cambium cylinder is generated from the procambium, by gradual fate change of interfascicular parenchyma cells to cambial cells, (Fig. 7). The cambium zone includes two parts: the division zone and the differentiation zone. The meristematic cells are located in vascular cambium named with vascular stem cells. These cells divide in two different directions, known as radial division toward xylem and phloem, which is responsible for stem diameter growth (Chiang and Greb, 2019). The extent of cambium activity can determine the number of xylem and phloem cells.

Once cells acquire a phloem or xylem identity, they elongate and expand their growth and eventually some cells undergo programmed cell death (Siedlecka et al., 2008). Environmental cues such as temperature, photoperiod, and water- and nutrient availability influence cambium activity. Many details about the factors and mechanisms behind cambium activity, regulation, and maintenance are still unknown.

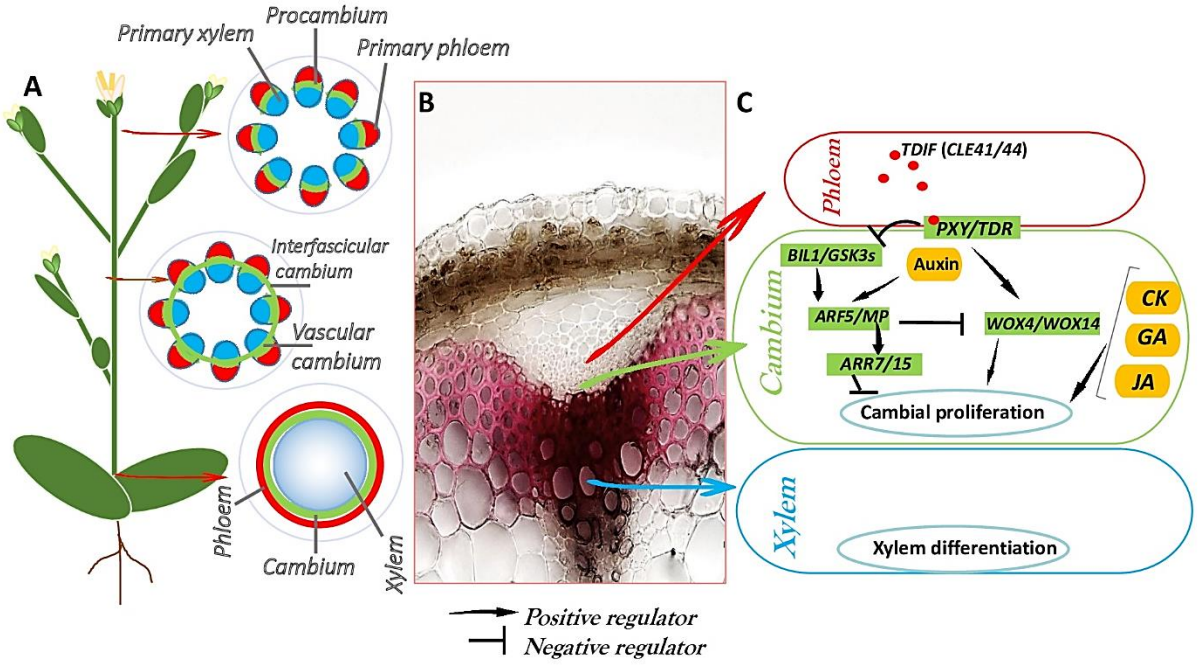

Figure 7. Dynamic nature of vascular cambium in the Arabidopsis inflorescence stem. (**A**) Schematic representation of an Arabidopsis plant with cross sections of the top-, middle- and bottom part of inflorescence stem showing different stages of vascular development. Very young stems from the top of the plant develop vascular bundles containing phloem, procambium, and xylem. In the middle part of the stem, cambial cell activity forms a ring of cambium extending into the interfascicular region. At the bottom, secondary phloem and xylem are formed as a consequence of cambium activity. (**B**) Bright field image of a lignin stained cross section of a middle part of an inflorescence stem showing the vascular bundle. (**C**) Hormonal and transcriptional control of vascular-cambium activity: the PXY receptor-like kinase, is activated by binding of the TDIF peptide ligand (red dots) synthesized in the phloem, and this induces *WOX4* transcription, which promotes cell proliferation in the cambium. PXY also promotes cambium activity by repressing *BIL1*expression and thus the auxin-dependent negative feed-back on cambial cell division. The plant hormones CK, jasmonate and GA all positively regulate cambium activity.

Hormonal and peptide signaling in secondary growth

Phytohormones are known to be involved in cambium regulation in parallel to environmental factors. The instrumental role of auxin in cambium regulation has been described in several reports. Auxin transport toward root from its source at the SAM affects cambium activity. In the stem of trees, auxin is highly accumulated in the cambium. Its levels are gradually decreased in cell types that are far from the cambium area (Tuominen et al., 1997; Aloni, 2013), suggesting the important role of auxin in cambium activity. However, no clear close correlation has been observed by now between auxin levels in the cambium and their effect

on cambium activity (Nilsson et al., 2008). A recent report showed that local auxin maximum in roots plays a role in promoting xylem identity (Smetana et al., 2019).

Cytokinin has a central role in procambium/cambium initiation and development. Depending on side-chain structure, cytokinins are classified into four different groups (tZ-type, cis-zeatin-type, isopentenyladenine (iP)-type, and aromatic cytokinins). The most active cytokinins (iP-type and tZ-type) are synthesized by isopentenyltransferases (*IPTs*). Arabidopsis encodes eight *IPT* genes (*IPT1*, *3*, *4*, *5*, *6*, *7*, *8*) that mediate cytokinin biosynthesis (Miyawaki et al., 2004; Kakimoto, 2001). The Arabidopsis *ipt1*, *3*, *5*, *7* quadruple mutant shows no cambium activity and, subsequently, no secondary growth (Matsumoto-Kitano et al., 2008), whereas overexpressing *IPT7* in Populus strongly increases secondary growth and biomass in the stem (Immanen et al., 2016). The *LONELY GUY* (*LOG*) gene family is known to be involved in cytokinin homeostasis. LOG proteins can convert conjugated inactive forms of cytokinin to the active forms through different pathways. Interestingly, the expression of *LOG* genes in rice is required for meristem activity and maintenance. *Lonely guy (log)* mutant in rice shows defects in shoot meristem maintenance, resulting in abnormal branching patterns (Kurakawa et al., 2007). In hybrid aspen trees, high expression of *CYTOKININ DEHYDROGENASE 2* (*CKX2*) encoding a cytokinin-degrading enzyme reduces cytokinin levels and, concomitantly, secondary growth in the stem (Nieminen et al., 2008). Cytokinin's critical role in cambium dynamics and proliferation is less clear, and its key upstream and downstream partners remain elusive.

Other hormones and their effect on cambium dynamics, development, and secondary growth have often been discussed in different reports. Overexpression of gibberellin 20-oxidase (GA biosynthesis) increases the secondary growth in hybrid aspen (Mauriat and Moritz, 2009). In contrast, Arabidopsis GA biosynthesis mutants show an opposite effect on cambium activity in the hypocotyl (Ragni et al., 2011). Other hormones, such as jasmonic acid, brassinosteroids, strigolactones, and ethylene, positively regulate the cambium (Sehr et al., 2010; Ibañes et al., 2009; Hayward et al., 2009).

In addition to the hormonal pathways regulating secondary growth, peptide signaling constitutes another facet of the cambium regulatory system that plays an important role in secondary growth. The TDIF–PXY–WOX signaling pathway is the best-studied signaling pathway that controls cambium activity. The TRACHEARY ELEMENT DIFFERENTIATION INHIBITORY FACTOR (TDIF) peptide ligand encoded by the *CLAVATA3 (CLV3)/EMBRYO SURROUNDING REGIONRELATED 41 (CLE41)* and *CLE44* genes is synthesized in the phloem and diffuses through the apoplastic space to the cambium cells, where it binds to its cognate receptor PHLOEM INTERCALATED WITH XYLEM (PXY) (Fig. 7) (Etchells and Turner, 2010; Hirakawa et al., 2010). The PXY/TDIF complex regulates procambium/cambium cell division, inhibits xylem cell differentiation and is important for vascular-tissue patterning (Etchells and Turner, 2010; Fisher and Turner, 2007). The *WUSCHEL RELATED HOMEOBOX4 (WOX4)* and *WOX14* genes are downstream targets of PXY/TDIF, and they positively regulate the vascular cell division (Fig. 7) (Etchells et al., 2013; Hirakawa et al., 2010; Etchells et al., 2015; Kucukoglu et al., 2017). In Arabidopsis *wox4* or *wox14* single mutants cambium cell activity and -division rate is affected. In fact, a more substantial effect was observed in the *wox4 wox14* double mutant, surprisingly without any effect on vascular organization (Etchells et al., 2013). The WOX4

and WOX14 transcription factors promote stem cell proliferation by interacting with the GRAS-domain transcription factor HAIRY MERISTEM 4 (HAM4) (Zhou et al., 2015). In addition, PXY promotes the proliferation of cambium cells by repression of *BRASSINOSTEROID-INSENSITIVE 2-LIKE 1* (*BIL1*) expression (Fig. 7) independent of *WOX4/14.* The BIL1 protein kinase was shown to phosphorylate MP/ARF5 and thereby enhance its inhibitory effect on cambium activity in the Arabidopsis stem and hypocotyl. The Arabidopsis *bil1* mutant showed increased cambial activity and subsequently vascular-tissue production. Interestingly, BIL1-MP/ARF5 signaling acts by activation of the A-type *ARABIDOPSIS RESPONSE REGULATOR (ARR)* genes *ARR7* and *ARR15* (Fig. 7), encoding known negative regulators of cytokinin signaling (Han et al., 2018).

The role of AT-hook-motif nuclear proteins in plant development

All plant species, from the moss *Physcomitrella patens* to monocot and dicot plants whose genomes have been sequenced, contain highly conserved *AT-HOOK MOTIF NUCLEAR LOCALIZED (AHL)* genes (Rensing et al., 2008; Kaul et al., 2000; Paterson et al., 2009; Goff et al., 2002; Tuskan et al., 2006; Zhao et al., 2014). The high conservation of this family among land plants suggests the important role of AHL in plant growth and development. However, our knowledge about the exact role of this family in terms of biological function is limited.

AHL proteins contain two different conserved units: the AT-hook motif and plant- and a prokaryote-conserved (PPC) domain. They bind to the minor groove of DNA in AT-rich areas via the AT-hook motif, which contains an Arg-Gly-Arg conserved palindromic core. This binding occurs when the DNA backbone interacts with the AT-hook core via the side chain of an arginine residue inserted into the minor groove of the DNA (Huth et al., 1997). The second important functional unit of AHL proteins is the PPC domain—120 amino acid in length—which exists in bacteria and archaea as a single protein that forms a trimer (Fujimoto et al., 2004; Lin et al., 2007). Among land plants, the PPC domain has been identified in AHL proteins, where it is located in the carboxyl end. The PPC domain is responsible for AHL's nuclear localization and physical protein–protein interaction with other AHLs or common transcription factors (Fujimoto et al., 2004; Street et al., 2008; Zhao et al., 2013a).

The Arabidopsis genome encodes 29 AHL proteins with either one or two AT-hook DNA-binding motifs (Fujimoto et al., 2004; Matsushita et al., 2007; Zhao et al., 2014). *AHL* family members are involved in different aspects of growth and development in Arabidopsis, including flowering time, flower development, vascular-tissue differentiation, hypocotyl growth, and hormonal regulation (Weigel et al., 2000; Jin et al., 2011; Gallavotti et al., 2011; Street et al., 2008; Zhao et al., 2014; Zhou et al., 2013b; Matsushita et al., 2007). Overexpression of *AHL18* and *AHL22* delay flowering time (Street et al., 2008; Xiao et al., 2009; Yun et al., 2012; Xu et al., 2013), *SUPPRESSOR OF PHYTOCHROMEB-4 (SOB3/AHL29), ESCAROLA (ESC/AHL27),* and *AHL22* control the hypocotyl elongation of light-grown seedlings (Street et al., 2008; Xiao et al., 2009), overexpression of *SOB3/AHL27* and *ESC/AHL29* results in bigger organs such as leaves, flowers, and fruit (Street et al., 2008), overexpression of *AHL25* and *AHL15* maintains the negative feedback of *GA 3-oxidase* involve in GA biosynthesis (Matsushita et al., 2007), *AHL18* modulates primary root

architecture (Širl et al., 2020), *SOB3/AHL27* and *AHL20* regulate plant innate immune responses (Lim et al., 2007; Lu et al., 2010), and *AHL15*, *AHL19* and *AHL20* are involved in embryogenesis (Karami et al., 2020).
The absence of clear phenotypes in single and double Arabidopsis *ahl* loss-of-function mutants suggests a high functional redundancy among *AHL* genes. Therefore, most reports about this family and its effects come from overexpression of these genes in Arabidopsis (Street et al., 2008; Xiao et al., 2009; Zhao et al., 2013). High levels of functional redundancy across the AHL genes can be overcome by deleting six conserved amino acids at the core of the PPC domain. Removal of this region in AHL29 confers a dominant-negative effect when expressed in wild-type Arabidopsis, suggesting a possible interaction among AHLs with themselves and also other transcription factors by forming different protein complexes (Zhao et al., 2013b).
In animals, most AT-hook proteins are localized to MARs (Fusco and Fedele, 2007), where they are generally associated with proteins that modulate chromatin architecture (Fusco and Fedele, 2007). Matrix attachment regions (MARs) are DNA sequences rich in AT nucleotides that interact with the nuclear matrix or protein network inside the nucleus. MARs regulate gene expression by organizing chromatin at a higher order (Heng et al., 2004; Girod et al., 2007; Wilson and Coverley, 2013). Also plant AHL proteins were shown to preferentially bind to the AT-rich sequences in MARs (Morisawa et al., 2000; Fujimoto et al., 2004; Xu et al., 2013). Some evidence exists showing that AHL proteins function by altering the organization of the chromatin structure (Lim et al., 2007; Ng et al., 2009; Yun et al., 2012; Xu et al., 2013). It was recently shown that *AHL15* overexpression extensively re-organizes the chromatin configuration (Karami et al., 2020). In addition, it has been demonstrated that AHL proteins repress the transcription of several essential developmental regulatory genes, possibly through modulation of the epigenetic code in the vicinity of their DNA-binding regions (Lim et al., 2007; Ng et al., 2009; Yun et al., 2012). Despite the progress mentioned above with regard to AHL function in Arabidopsis, the exact molecular mechanism behind their role in plant development remains elusive.

Outline of this thesis

In **chapter 2** of this thesis we confirmed that *AHL15* acts as an AM maturation suppressor, resulting in plant longevity extension. *ahl15* loss-of-function accelerates AM maturation. Conversely, high expression of *AHL15* suppresses AM maturation and induces polycarpic features in monocarpic Arabidopsis and tobacco. Interestingly, in short-day grown Arabidopsis with promoted longevity, the expression of *AHL15* was quite high in AMs. Our genetic studies indicate the crucial role of *AHL15* in suppressing axillary-meristem maturation and promoting plant longevity, directly downstream of *SOC1*, *FUL*, and upstream of GA.
More-detailed analyses revealed that *AHL15* is not only and specifically involved in suppressing AM maturation, but that interestingly, the protein rather has a crucial role in an earlier development phase transitions. In **chapter 3**, we verified that *ahl15* loss-of-function leads to early VPC and flowering. A dramatic delay in the VPC and flowering was observed

upon *AHL15* overexpression in Arabidopsis and tobacco. Tissue-specific expression of *AHL15* in the SAM and in young leaves confirmed the direct and indirect role of AHL15 in phase change and flowering in Arabidopsis. Furthermore, we found that AHL15 represses *SPLs* independent of miR156 (master regulator of VPC). AHLs and SPLs suppress each other in a negative feedback manner, since the effect of SPLs on VPC turned out to be mediated by repression of *AHL* expression. Our findings indicate genetic interaction between AHLs and SPLs in controlling the VPC.

We also noticed that *AHL15* overexpression surprisingly affects secondary growth in Arabidopsis. In **chapter 4**, we showed that *AHL15* plays a central role in inducing vascular-cambium activity downstream of *SOC1* and *FUL* and upstream of the *IPT* cytokinin biosynthesis genes. Overexpression of *AHL15* lead to extensive secondary xylem production in Arabidopsis. In contrast, *ahl15* loss-of-function plants showed significant reduction in cambium activity and xylem production. By tracking the expression pattern of *AHL15*, we confirmed that *AHL15* is specifically expressed in the intra- and interfascicular parts of the vascular cambium. By expressing *AHL15* under a cambium-specific promoter (*pPXY:AHL15*), we verified that cambium-specific enhancement of *AHL15* expression is sufficient to promote cambium activity and enhance xylem formation.

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