Floral stem cells: from dynamic balance towards termination

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Abstract

During early flower development in Arabidopsis, floral stem cells proliferate and produce a sufficient amount of cells that are recruited for organogenesis. However, after the central organ primordia initiate, stem cell activity in the floral meristem is terminated to ensure the differentiation of a fixed number of floral organs. Underlying this process, the genetic programme regulating the fate of floral meristems undergoes a shift from a spatially balanced signalling scheme for stem cell maintenance to a temporally controlled transcriptional scheme for stem cell termination. Precise timing of stem cell termination is a key issue for flower development, which is secured by the orchestration of multiple regulators in transcriptional and epigenetic regulation.

Introduction

In Arabidopsis reproductive development, SAMs (shoot apical meristems) produce inflorescences bearing flowers. Initiated from the flanks of the inflorescence meristem, floral meristems are originally spherically shaped group of cells, replicating the same cellular architecture with the SAM [1]. However, unlike the SAM, which produces a spiral vegetative phyllotaxy, the floral meristem contributes cells only to the formation of four concentric whorls of floral organs in a sequential manner from the outermost to the innermost: sepals, petals, stamens and carpels [2]. After the initiation of carpel primordia, floral stem cells are abolished and consumed in the differentiation of carpels, which enclose seeds for the next generation.

Maintenance of the floral meristem by the CLV (CLAVATA)–WUS (WUSCHEL) signalling pathway during early flower development

From floral bud initiation to the early stages of flower development [3], the floral meristem is maintained through the WUS–CLV signalling pathway (reviewed in [4]) (Figure 1A). The homeodomain transcription factor WUS is specifically expressed in a confined domain of the floral meristem and functions to specify and maintain the overlying two layers of epidermal cells with stem cell identity [5]. The stem cells specifically express CLV3, which encodes a small secreted peptide [6]. The CLV3 peptide is proposed to diffuse internally and becomes bound by the CLV1–CLV2 receptor complex in the cells of WUS expression domain [7]. Perceived by the receptor complex, CLV3 signals limit the expression of WUS as well as the size of WUS expression domain. As a dynamic balance, increase of CLV3 signal can rapidly repress WUS expression, which in turn leads to a fast reduction of CLV3 expression [8]. Through such a negative-feedback loop, the size of the WUS-dependent floral meristem is maintained at a proper level [7,9,10].

Termination of the floral meristem by AG (AGAMOUS)

Expression of the stem cell determinant WUS disappears by the time carpel primordia are initiated at stage 6 of flower development. This is caused by genetically programmed transcriptional events in which the MADS-box protein AG plays an essential role to terminate the floral meristem [11,12]. AG functions to specify reproductive organ identity [13]. Directly induced by WUS and the floral activator LFY (LEAFY) together, AG is activated from stage 3, a few days earlier than the repression of WUS at stage 6 in the centre of flower buds, where stamens and carpels would later arise [12] (Figure 1B). In turn, AG shuts off WUS activity by the end of stage 6 to allow further differentiation of floral organs [11,12]. Delayed AG activity leads to partially indeterminate flowers with an over-abundance of stamens, and loss-of-function mutations in AG gene produce indeterminate flower phenotypes [14,15]. In the ag-I null mutant and ag-4 weak allele, floral organs are homoeotypically converted and give rise to sepal–petal–petal and sepal–petal–stamen reiterations respectively [15,16]. This indeterminate growth in ag mutant flowers is largely due to the extended WUS activity in the floral meristem: WUS RNA is still detectable in the floral meristem of late-stage ag-I flowers [11,12], whereas, in ag-1 wus-I double mutant flowers, the floral meristem is precociously terminated into a central petal [17].

During early flower development, AG is expressed from an early stage (stage 3), whereas WUS is terminated by...
AG at a mid-stage (stage 6). From AG activation to WUS termination, there is an approx. 2-day interval under 24 h light growth conditions [3]. The 2-day timing is essential for flower development, because it allows enough cells to be produced and consumed to generate reproductive organs. In the null mutant wus-1, where stem cells are precociously terminated during flower development, only one or two stamens are produced and carpel development is fully abolished [17]. In contrast, prolonged ectopic WUS activity during flower development leads to the formation of an extra number of reproductive organs [11,12,18]. For example, in the null mutant cld-2, WUS is ectopically expressed and the overproduction of stem cells leads to supernumerary stamens and carpels [18]. Thus the precision of WUS expression domain and the timing for WUS termination are under strict regulation in flower development.

AG is required for WUS termination, but several results suggested that repression is indirect. First, when the second stem cell layer of the floral meristem is mutant for ag, normal AG function within the WUS expression domain is not sufficient to repress WUS, thus resulting in ag-mutant-like indeterminate flowers [19]. Secondly, 35S::AG does not lead to precocious termination of WUS and produces flowers with normal carpels [20]. This is unlike 35S::CLV3 flowers or flowers with induced over-expression of CLV3, both of which produced wus-mutant-like flowers [7,8].

**KNU (KNUCKLES) mediates WUS repression by AG**

A recent study shows that KNU functions to mediate the repression of WUS by AG [21] (Figure 1B). With a typical C-terminal EAR [ERF (ethylene-responsive factor)-associated amphiphilic repression]-like motif, KNU is a C2H2 zinc-finger protein which may function as a transcriptional repressor [22]. Mutation of KNU leads to male sterility and ectopic reiterations of stamens and carpels originated within primary carpels [21,22]. This indeterminate growth is essentially contributed by WUS activity, since flowers of the double mutant knu wus look the same as was flowers [21]. Consistently, in situ hybridization analysis has revealed persistent WUS expression in the ectopic floral meristem within knu carpels. Similarly, persistent WUS activity also leads to the indeterminacy of ag flowers [11,12]. Double mutant ag knu flowers are indistinguishable from those of ag, suggesting that AG functions as an upstream inducer of KNU [21].

Chromatin immunoprecipitation has shown that AG binds the non-typical binding consensus sequences on the KNU promoter directly to induce KNU expression [21]. Furthermore, mutagenesis of the consensus AG-binding sites in the KNU promoter leads to the abolishment of KNU-GUS reporter induction. These show the direct regulation of KNU by AG. Unlike another AG direct downstream target SPL (SPOROCYTELESS/NZZ (NOZZLE), which is activated immediately after AG induction [23], KNU induction requires an approx. 2-day lag after AG induction. The timed KNU induction is necessary for WUS to be expressed long enough for sufficient proliferation of floral stem cells before WUS is shut off. During the 2-day period, the repressive epigenetic mark H3K27me3 (histone H3 Lys27 trimethylation) on KNU chromatin is removed in an AG-dependent manner [21]. Improper maintenance of H3K27me3 in PcG (polycomb group) mutants or interruption of the integrity of H3K27me3 on KNU locus result in precocious and ectopic KNU expression in floral meristems, inflorescence meristems and even in vegetative tissues. This suggests a regulatory role of H3K27me3 in developmental timing control. This also indicates that there exists an AG-independent ubiquitous activator for KNU and that the function of AG in the regulation of KNU is to remove the repressive epigenetic mark at a specific time from the KNU locus.

KNU expression initiates in the floral meristem at the same stage as WUS expression terminates [21]. When KNU is overexpressed under the 35S promoter in wild-type flowers, floral meristems are precociously terminated, which results in flowers without carpels, resembling wus flowers. Overexpression of KNU also leads to the termination of floral meristems in ag-1 flowers, suggesting that KNU is the major player downstream of AG for WUS repression. The repression of WUS by KNU seems to be immediate, as WUS mRNA levels decrease significantly 24 h after KNU induction. However, whether KNU directly represses WUS or KNU represses an activator of WUS still remains to be studied.

In wild-type plants, WUS termination by AG is associated with the patterning of floral organs [12]. However, overexpression of KNU in an ag mutant background resulted in termination of floral meristems, indicating that floral meristem determinacy, i.e. the termination of WUS, can be induced without the induction of carpels, being fully uncoupled from the organ identity control [21].
Interactions between floral stem cell regulators in floral stem cell termination

Many regulators participate in the transcriptional repression of the stem cell determinant WUS. PAN is a direct activator of AG in the floral meristem. RBL, SQN, ULT positively regulate both AG and SUP, and SUP may function to repress WUS synergistically with AG. KNU is the major downstream mediator of AG to repress WUS expression. AG may also induce CRC, which is involved in WUS termination in parallel with KNU.

Termination of the floral meristem by other factors

Besides the AG–KNU–WUS pathway, a number of genes are also known for their functions in floral meristem regulation (Figure 2).

ULT1 (ULTRAPETALAI), which encodes a SAND (Sp100/aire-1/nucP41/75/DEAF-1) domain protein, is a negative regulator of stem cell accumulation in the floral meristem [24]. ult1 mutant flowers retain prolonged WUS activity, which results in larger floral meristems with more floral organs than wild-type flowers. Genetic and molecular studies revealed that ULT1 negatively regulates the size of the WUS-expressing domain in the floral meristem. This repression may act upstream of AG and establish the proper floral meristem determinacy through the WUS–AG temporal feedback loop [24,25].

RBL (REBELOTE), a protein with unknown functional domain, and SQN (SQUINT), a cyclophilin, were also found to redundantly affect floral stem cell termination [26]. The mutations of RBL, SQN and ULT1, in combination with each other, lead to indeterminate flower phenotypes with reiterating reproductive organs in the centre of the flower. This indeterminacy may be attributed to the compromised AG expression in the centre of the floral meristem, which in turn leads to extended WUS activity. Thus RBL, SQN and ULT1 are all required for floral meristem termination and subsequent development of flowers, presumably by reinforcing AG expression.

PAN (PERIANTHIA) encodes a bZIP (basic helix-loop-helix zipper) transcription factor and plays a role in regulating floral stem cell fate [27,28]. Genetic and biochemical data have shown that PAN is a direct regulator of AG [28]. PAN binds the AG regulatory sequence directly and functions to control AG activity in the centremost region of the AG expression domain [27]. Under short-day conditions, PAN is essential for AG activation in early flowers and AG RNA is reduced in young flowers produced in pan mutants, indicating that PAN is an upstream activator of AG in the floral meristem [28].

SUP (SUPERMAN) encodes a C2H2 zinc finger protein with a C-terminal conserved EAR-like motif. Mutants of SUP have supernumerary stamens at the expense of carpels, whereas sup wus double mutants resemble wus, suggesting that SUP functions to restrict the WUS expression domain in a non-cell-autonomous manner (reviewed in [26]). In ag-1 sup double mutants, the floral meristem is greatly enlarged with reiterating whorls of petals [29]. In ag-1, SUP still remains its expression pattern as in wild-type at the boundary between the third and fourth whorl organs [30]. These suggest that SUP acts in parallel with AG to control floral meristem determinacy.

CRC (CRABS CLAW) encodes a YABBY family zinc-finger protein [31]. CRC expression initiates from stage 6 on the abaxial sides of carpel primordia, and is mostly constrained in carpels and nectaries. Thus CRC is not expressed in the floral meristem. The null mutant crc-1 produces determinate flowers with stunted carpels, which are unfused at the tip [32]. However, the crc mutation enhances floral meristem indeterminacy: ectopic growth of reproductive organs within the primary carpels was observed in crc ag-1/+; crc spathula-2; crc ult1-4; crc rbl-1 and crc sqn-4 double mutants, indicating that CRC may also control floral meristem determinacy non-cell-autonomously [26,32]. Because it is reported that CRC RNA is significantly reduced in the null allele ag-1 and CRC may be activated by AG [33,34], CRC could be another downstream mediator of WUS repression by AG in the floral meristem. In knu crc double mutant flowers, reiterated whorls of reproductive organs are observed within the primary carpels (B. Sun, unpublished work), suggesting that CRC may function synergistically with KNU to terminate floral stem cell activity.

Prospects

Precisely timed termination of floral stem cell activities involves a complex genetic program. The CLV–WUS signalling pathway first maintains the floral meristem, whereas AG functions to shut off stem cell activity at a certain floral developmental stage by inducing KNU, encoding a C2H2 zinc-finger repressor-like protein. In addition, many other genes also participate in this process, but the detailed mechanisms of their functions remain to be determined. Even for the termination of WUS by AG, how AG functions to reduce the repressive epigenetic marks on the KNU chromatin and how KNU exerts its repression on WUS are still unknown. The answers to these questions will deepen our comprehension of flower development.

Acknowledgements

We thank Dr Yu Hao and Dr Jose Dinneny for a critical reading of the manuscript.
References


Received 7 September 2009
doi:10.1042/BST0380613