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Meristem Termination and Organ Number Control in Early Stage of Arabidopsis Flower Development

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Commentary

Unlike animals, plants are able to initiate new organs and tissues throughout their whole life after embryogenesis; the organogenesis relies on the continuous activity of pluripotent stem cells. Plant stem cells are embedded in specialized tissues called meristems. Two primary meristems, the shoot apical meristem (SAM) and the root apical meristem (RAM), which are located at the tips of shoot and root, are responsive for the plant continuous growth. After floral transition, floral meristems (FMs) develop from the franks of SAMs to form flowers. In angiosperms, flower development is well controlled by the precise coordination of stem cell proliferation and organ differentiation. Consequently, the number of organs in the flowers of a given species is defined mostly based on the size of FMs. For example, in a model plant Arabidopsis, each flower consists of four different types of floral organs, four sepals, four petals, six stamens and two fused carpels produced from outside (whorl 1) to the centre (whorl 4). While a mountain of evidence has shown that the FM activity is associated with the floral organ number, the precise developmental program behinds the species-specific numbers of floral organs and how 4 whorls are produced remains unknown.

In Arabidopsis, two known pathways control the FM activity at the spatial and temporal manners, respectively. Firstly, a negative feedback loop between the home domain transcription factor *WUSCHEL* (*WUS*) and the peptide *CLAVATA3* (*CLV3*) is conserved in FMs and shoots apical meristems (SAMs) [1]. *CLV3* is expressed at the stem cells in the central zone (CZ) within three clonally distinct layers (L1-3) and thus can be used as a stem cell marker. Directly underneath the stem cell population lies the organizing centre (OC), in which *WUS* is expressed and move to the stem cells to induce and maintain stem cell fates. Mutation in *WUS* leads to premature termination of stem cell activity in SAM and FM [2]. Stem cells express and secrete the *CLV3* peptide, which signal back to the OC to repress *WUS* expression via a signaling transduction pathway involving the LRR receptors CLV1/2/CORYNE. Once *CLV3* is mutated, stem cells over-accumulate due to unrestricted *WUS* expression, causing plants with more organs and flowers with increased floral organs in each whorl [3,4]. Secondly, another negative feedback between *WUS* and the class C floral homeotic gene AGAMOUS (AG) controls the FM activity termination [5-8]. Whilst SAM is indeterminate and able to initiate new organs continuously, FM is determinate and thus the proper timing to terminate the stem cell activity is important to define 4 whorls of floral organs [7]. In Arabidopsis, *WUS* activity is essential for stem cell maintenance, and its expression starts from the earliest floral stage during flower development. Together with the floral meristem regulator LEAFY, *WUS* induces AG expression at floral stage 3 in whorls 3 (stamen) and 4 (carpel) of floral primordia [7]. Approximately 2 days after AG induction at floral stage 6, AG deactivates the stem cell maintenance program both directly, by affecting the recruitment of Polycomb Group (PcG) proteins to the *WUS* locus, and indirectly repress *WUS* expression through the C2H2 zinc finger protein KNUCKLES (KNU) [6,9]. In *ag* and *knu* loss-of function mutants, *WUS* expression remains active beyond floral stage 6, which is sufficient to induce floral meristem indeterminacy with increased number of whorls [6,8].

WUS activity and phytohormone cytokinin signaling could reinforce each other in a positive feedback [9-12]. In many events of plant growth and development, auxin usually functions complementarily with cytokinin [13]. It has been known that auxin also controls the size of the root meristem non-cell autonomously, probably through it antagonistic function to cytokinin signaling [14]. Auxin and cytokinin show opposite functions in the regulation of shoot and root meristems, and further the function of auxin in FMs is not well understood [13,15].

We recently showed that the Arabidopsis boundary gene *SUPERMAN* (*SUP*) controls the FM activity by finely tuning of local auxin biosynthesis [16]. The increased organ number in *sup* mutants suggests that *SUP* is involved in both floral patterning and FM determinacy [17,18]. In a total loss-of-function mutant ag-1, flowers continue to grow repeated structures (sepals and petals) in the center of flower, as the consequence of homeotic transformations and prolonged expression of *WUS*. The *ag sup* double mutant flowers show the enlarged and fasciated floral meristems in the centers, indicating that *AG* and *SUP* have synergistic effects on the FM size [17]. Since the FM termination process is inactivated in *ag* mutant, the synergistic effect of *sup* mutation with *ag* mutant on the FM size suggests that *SUP* is involved in at least a spatial regulation of the FM activity. We firstly confirmed that the *SUP* function in FM regulation is dependent of *WUS* activity, as the *wus*-1 mutant is fully epistatic to *sup* in FM determinacy. We confirmed that there are more stem cells in *sup* at floral stages 4 to 6 compared to wild-type by counting the number of cells expressing p*CLV3*: GFP-ER. With the meristem marker pSTM: STM-VENUS, we also showed the increased FM size in *sup*. Notably, we also noticed the slightly prolonged *CLV3* and STM expression in the center of *sup* flowers. Altogether, our data showed that *SUP* control FM activity in both spatial and temporal manners.

Next, we carried out a series of experiments to answer how *SUP* regulates FM activity in Arabidopsis. Previous study showed that the ectopic expression of *SUP* in Arabidopsis and tobacco leads to dwarf plants with organs of reduced size, and these phenotypes were associated with both auxin and cytokinin signaling defects [19,20].

However, the transgenic plants with altered morphology in these reports made it difficult to distinguish the causal factors. Another study had shown that CUP-SHAPED COTYLEDON2 (CUC2) mRNA is ectopically expressed at the center of FMs in *sup*-1 [21]. CUC1-3 genes encode three closely related members of the NAC transcription factors, which participate in meristem and organ boundary formation [22,23]. CUC2 can be induced by low levels of auxin but repressed by high levels of auxin [24]. With a reporter pCUC2: CUC2-3xVENUS-N7, we detected ectopic CUC2 expression in the FM region in *sup* [24], implying that the auxin signaling or accumulation is disturbed in *sup* mutants. To test this hypothesis, we compared auxin distribution pattern between *sup* and WT flowers with the auxin reporters pDR5rev:: GFP-ER and pDR5rev::2xGFP-N [25,26]. We detected the increased auxin signal around the whorl 3/4 boundary regions at stage 4 floral buds in *sup*. We next utilized another more sensitive auxin marker DII-VENUS, which expression under the control of the RPS5A promoter, and marks auxin accumulation with the absence of fluorescence as the auxin-dependent degradation of fusion domain II of an Aux/IAA protein [27]. With the marker, we confirmed that auxin accumulated at the whorl 3/4 boundary regions of the *sup* mutant flower.

Next, we treated *sup* mutant floral buds with an auxin signaling inhibitor, p-chlorophenoxyisobutyric acid (PCIB), to check whether increased auxin accumulation in *sup* mutants is responsible for the floral indeterminacy [28]. We demonstrated that PCIB treatment could almost fully rescue both the stamen number and carpel defects in *sup*-5. At the same time, we generated the transgenic line p*SUP*: aaH with the bacterial auxin biosynthetic gene iaaH under the control of the *SUP* promoter, in which iaaH can convert indoleacetamide (IAM) to IAA in Arabidopsis [29]. With IAM treatment, the flowers of the transgenic plants showed *sup* mutant phenotypes, suggesting that locally increased auxin at *SUP* expression region is sufficient to trigger FM indeterminacy phenotype.

To identify the *SUP* direct targets that link with auxin signaling pathways, we utilized the inducible transgenic line p35S: *SUP*-GR, in which the steroid-binding domain of the rat glucocorticoid receptor (GR) was translationally fused with the *SUP* protein. Microarray analyses with p35S: *SUP*-GR inflorescences have identified YUC flavin monooxygenases YUC1/4 and a TRP-a-transferase TRYPTOPHAN AMINOTRANSFERASE RELATED 2 (TAR2) as the potential *SUP* downstream genes. We next checked their expression levels between WT and *sup* at stage 4 floral buds with the help of the floral induction system ap1 cal p35S: AP1-GR [30]. As *SUP* is a strong active repressor [20], the transcriptionally up-regulated YUC1/4 was selected as the potential *SUP* targets. By using GUS reporter lines, we verified that YUC1/4 is ectopically expressed in *sup* mutant flowers. Consistent with the depression of YUC1/4 genes, the auxin increase in the *sup* mutant floral buds was confirmed by measure of the major form of nature auxin indole-3-acetic acid (IAA).

Next we confirmed that YUC1/4 is *SUP* direct binding targets with a chromatin immunoprecipitation (ChIP) binding assay. We further showed that *SUP* could recruit PcG complex to YUC1/4 loci to repress their expression. In addition, we have shown that either yuc1 or yuc4 loss-of-function mutant can partially rescue *sup* at the stamen and carpel numbers, or the floral defect of yuc1 yuc4 is epistatic to *sup*-5 in phenotypes, implying that YUC1 and YUC4 are two major targets responsible for *sup* mutant phenotype in the floral morphology.

Although we have shown that *SUP* participates in FM regulation through finely tuning of auxin amount, we still have no idea how auxin affects the FM activity. There are two alternative but not exclusive hypotheses for auxin regulation of FM in *sup* mutant. First, Auxin affects cell division [13], and thus increased cell division rates may be responsible for the increased number of stem cells in *sup* mutants. While we still have no data of division rates for stem cells or OC in *sup* mutant flowers, BrdU incorporation assays showed cell division rates at the whorl 3/4 boundary were increased [21,31]. Auxin, together with cytokinin, also contributes to cell differentiation [13]. Indeed, we observed that ectopic expression of *SUP* promotes differentiation of FMs in Arabidopsis. The expanded and prolonged *CLV3* and STM expression in sup flowers suggest that *SUP* promotes the FM differentiation. Future studies will reveal how auxin regulates the FM activity.

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