PERSPECTIVE

A terminator of floral stem cells

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Normal flower development requires the termination of stem cell activities in the floral meristem. The floral regulator *AGAMOUS* (*AG*) is necessary for this termination and represses the expression of the stem cell determinant *WUSCHEL* (*WUS*), but the repression mechanism was not clear. A recent study by Sun and colleagues (pp. 1791–1804) in this issue of *Genes & Development* has identified a direct target of *AG*, *KNUCKLES* (*KNU*), which encodes a transcriptional repressor of *WUS*, providing a key missing link in floral meristem determinacy.

Post-embryonic organ formation in flowering plants requires the activities of stem cells in the shoot, floral, and root meristems (Weigel and Jurgens 2002; Laux 2003). To produce multiple organs, the meristems need to maintain stem cells for sufficient periods of time. In *Arabidopsis*, this maintenance of stem cells requires the function of *WUSCHEL* (*WUS*), which was shown to be necessary and sufficient to promote stem cell activities in the meristems (Mayer et al. 1998). Another *Arabidopsis* regulator of meristem activity is *SHOOTMERISTEMLESS* (*STM*). *STM* and *WUS* function independently, though an increase in *WUS* function can partly compensate for the loss of *STM* function (Lenhard et al. 2002). *WUS* specifies a subset of cells in the center of the meristem as stem cells, whereas *STM* acts to suppress differentiation throughout the meristem, thus allowing the daughter cells of stem cells to proliferate before they are incorporated into organs (Lenhard et al. 2002).

The floral meristem differs from others in being determinate, generating a constant or limited number of floral organs before ceasing its activity. In addition, the termination of stem cell activities in the floral meristem is coupled to the initiation of reproductive floral organs and the timely determinacy of the floral meristem is important for proper formation of reproductive organs, particularly the female organ carpel. In *Arabidopsis*, the

floral homeotic gene *AGAMOUS* (*AG*) is required for both floral meristem determinacy and reproductive organ identities (Bowman et al. 1989, 1991; Yanofsky et al. 1990; Coen and Meyerowitz 1991; Mizukami and Ma 1995). *AG* is a key gene for the C function of the ABC model and a member of the MADS-box gene family (Yanofsky et al. 1990; Bowman et al. 1991; Coen and Meyerowitz 1991). In *ag* mutants, the floral meristem is active for a much longer period than normal, generating many more floral organs than the wild type (Bowman et al. 1989, 1991; Yanofsky et al. 1990). Similarly, reduction of *AG* function using sense or antisense transgenes causes extended floral meristem activities (Mizukami and Ma 1995; Mizukami et al. 1996).

Thus, proper stem cell activity in the floral meristem is controlled by the balance between the positive regulator *WUS* and the negative regulator *AG*. This is achieved through a positive–negative feedback loop (Fig. 1A). *WUS* is expressed in the early floral meristem and activates *AG* expression at floral stage 3 (as defined by Smyth et al. 1990). *AG* expression is reduced in the *wus* mutant, whereas ectopic *WUS* expression prolongs stem cell proliferation and also causes ectopic stamen and carpel development in an *AG*-dependent way (Lenhard et al. 2001; Lohmann et al. 2001). DNA-binding studies suggest that *WUS* cooperates with another positive regulator of floral meristem, *LEAFY* (*LFY*), to activate *AG* expression (Lohmann et al. 2001). On the other hand, *WUS* expression decreases when *AG* expression is activated and disappears by the time carpel primordia are initiated at stage 6 (Mayer et al. 1998). *WUS* is required for the floral meristem indeterminacy in the *ag-1* mutant because the flowers of the *wus-1 ag-1* double mutant appear indistinguishable from those of *wus-1* (Laux et al. 1996), supporting the hypothesis that *AG* negatively regulates *WUS* expression. Indeed, *WUS* remains active in the center of the indeterminate floral meristem of the *ag-1* mutant through the life of the flower (Lenhard et al. 2001; Lohmann et al. 2001), indicating that *AG* is required for the cessation of *WUS* expression at stage 6.

Clearly, the precise duration of stem cell activity in the floral meristem is important for normal development. Terminating the stem cells too early would mean insufficient cells for floral organ formation, whereas overly extended

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Figure 1. Genes controlling stem cell activities in the floral meristem. (A) A regulatory loop between *WUS* and *AG*. *WUS* is expressed at floral stage 2 and then activates (arrow) *AG* expression at stage 3. By stage 6, *AG* has repressed (line with a bar) *WUS*. (B) Interactions between *AG*, *KNU*, and *WUS*. At stage 3, *AG* binds to the *KNU* promoter (dotted arrow), but H3K27 at *KNU* is trimethylated and *KNU* has no effect on *WUS*. At stage 6, the H3K27 trimethylation is removed and *KNU* is expressed, causing *WUS* repression. (C) Regulation of stem cell activity by *AG*, *KNU*, *WUS*, and other factors. At stage 3, *RBL*, *SQN*, and *ULT1* positively regulate *AG* and *SUP* expression, the latter of which might also repress *WUS* via an unknown mechanism. At stage 6, *AG* can activate the expression of *CRC*, which also represses *WUS*.

stem cell activity would result in abnormally large numbers of floral organs. Furthermore, because of the coupling of floral meristem termination with the formation of the carpels, the female reproductive organs, extended meristem activity is also linked to defects in carpel development. Thus, the above-mentioned positive-negative feedback loop must have a timing component: *WUS* activates *AG* expression first at stage 3, and *AG* represses *WUS* expression subsequently at stage 6. How is differential timing of the two aspects of the regulatory loop achieved? In particular, what is the mechanism for the delayed repression of *WUS* by *AG*?

Sun et al. (2009) have now obtained strong support from a series of genetic and molecular experiments for another gene, *KNUCKLES* (*KNU*), that serves to mediate the negative regulation of *WUS* by *AG*, and provide evidence for the timing mechanism of the termination of *WUS* expression. *KNU* was discovered as a positive regulator of floral meristem determinacy; unlike the normal flower, which produces carpels as the fourth and innermost whorl of organs, *knu* mutant flowers have extra stamens and carpels that form interior to the fourth-whorl carpels (Payne et al. 2004). In addition, the *KNU* protein contains a zinc finger and a putative transcriptional repression motif (Payne et al. 2004), suggesting that it might be a repressor of *WUS*. Therefore, Sun et al. (2009) hypothesized that *KNU* might act between *AG* and *WUS* to mediate the repression of *WUS* by *AG*.

To investigate how *AG* regulates floral meristem determinacy and the role of *KNU* in this process, Sun et al.

(2009) performed a series of genetic and molecular experiments. First, they ascertained the time at which *AG* is needed for normal determinacy, using an elegant experiment with inducible *AG* activity. It was shown previously that *AG* activity can be controlled by fusing it with a mammalian steroid hormone receptor (glucocorticoid receptor [GR]) that can be activated by an artificial hormone, dexamethosone (dex) (Ito et al. 2004; Gomez-Mena et al. 2005). By inducing *AG* activity at different floral stages in transgenic plants with inducible *AG* activity in an *ag* mutant background, Sun et al. (2009) showed that *AG* activity at stage 3, but not stage 4, was able to confer full determinacy, indicating that the normal onset of *AG* function at stage 3 is required for floral meristem determinacy.

Next, Sun et al. (2009) tested the role of *KNU* by performing genetic experiments with various combinations of *ag*, *wus*, and *knu* mutations. They found that *ag knu* double mutants had similar phenotypes to those of *ag* mutants, consistent with the idea that *AG* and *KNU* act in the same genetic pathway and that *AG* is a positive regulator of *KNU*. In addition, the *knu wus* double mutant appeared the same as the *wus* mutant, indicating that *WUS* is downstream from *KNU*. Furthermore, when *KNU* was expressed using an *AG*-independent promoter (35S), *KNU* was able to rescue the indeterminate phenotype of the *ag-1* mutant, suggesting that *KNU* functions downstream from *AG*. On the other hand, *AG*-independent *KNU* expression caused *wus*-like floral phenotypes, supporting the hypothesis that *KNU* represses *WUS* expression. These experiments with mutants and transgenic plants provide key genetic evidence that *KNU* acts downstream from *AG* to repress *WUS* (Fig. 1B).

To examine the relationship between *AG*, *KNU*, and *WUS* further at the level of mRNA expression, Sun et al. (2009) used a transgenic plant system that allows flower development to be induced and found that *KNU* expression begins ~2 d after the start of *AG* expression, whereas *WUS* expression is repressed starting at ~2 d after *AG* expression. More directly, they showed that induction of *AG* activity using the *AG-GR* fusion system is followed by *KNU* expression ~2 d later. In contrast, *knu* mutants exhibited extended *WUS* expression, indicating that *KNU* is required for the termination of *WUS* expression in the floral meristem. Further support for the negative regulation of *WUS* by *KNU* came from transgenic plants with strong inducible *KNU* activity, which resulted in premature termination of meristem activity similar to that found in *wus* mutants. Sun et al. (2009) also determined that the repression motif at the C-terminal region of the *KNU* protein was required for termination of *WUS* expression, suggesting that *KNU* acts as a transcriptional repressor.

The delay in *KNU* expression in comparison with *AG* expression suggests that the activation of *KNU* expression might be a key to the timing of stem cell termination in the floral meristem, thereby allowing the needed amount of stem activity for floral organ generation. To examine this temporal relationship between *AG* and *KNU* further, Sun et al. (2009) combined the inducible *AG* (using the *AG-GR* fusion) with a *KNU-GUS* reporter gene and found that 1 d after *AG* induction, *GUS* expression was

not detected, but 2 d after AG induction, strong *GUS* expression was detected. As a positive control, another AG target gene, *SPL*, was activated immediately after AG induction. Although it is not known how *KNU* expression is delayed compared with AG induction, the delayed *KNU* expression is likely a key to allowing *WUS* to be expressed long enough for sufficient floral stem cell activity to generate floral organs before the termination of the floral meristem.

The next question Sun et al. (2009) addressed was whether *KNU* is a direct target of transcriptional regulation by AG. Sun et al. (2009) noted that the putative *KNU* promoter region does not contain a perfect AG target site, as defined by earlier in vitro studies (Huang et al. 1993; Shiraishi et al. 1993); nevertheless, there are three partial sites of AG-binding consensus sequences that were found previously to bind to AG weakly (Ito et al. 1997). To test whether AG binds to the *KNU* promoter region in vivo, Sun et al. (2009) performed chromatin immunoprecipitation (ChIP) experiments. They found that the *KNU* promoter region containing the partial AG-binding sites was enriched after 1 d of the induction of AG activity, and this enrichment was further strengthened 2 and 3 d after induction. Furthermore, when the partial AG-binding sites were mutated in a fusion of the *KNU* promoter to the *GUS* reporter gene, *GUS* expression was not detected in 88% of the transgenic plants, in contrast to *GUS* expression in 80% of the transgenic plants carrying a fusion with the wild-type *KNU* promoter. These results support the hypotheses that AG binds directly to the *KNU* promoter and that AG binding activates *KNU* transcription.

At the same time, it was puzzling why AG binding was observed by ChIP 1 d after the induction of AG function, but *KNU* expression was not detectable until 2 d after the induction. To probe further into the mechanism of the activation of *KNU* expression, Sun et al. (2009) examined the status of transcription-repressive marker histone H3 Lys 27 trimethylation (H3K27me3). They found that the level of H3K27me3 was high near the *KNU* transcriptional start and in the coding region; moreover, the H3K27me3 level at *KNU* was reduced 2 d, but not 1 d, after flower development was induced, in an AG-dependent manner. So, the timing of the reduction of the H3K27me3 level is consistent with that of *KNU* expression. Also, Sun et al. (2009) found that a deletion of the *KNU* region with heavy H3K27me3 resulted in elevated expression of a *KNU-GUS* reporter gene.

Is the H3K27me3 level, or some other *cis* elements in the deleted *KNU* region, relevant to *KNU* regulation? Sun et al. (2009) addressed this question by testing for *KNU* expression in mutants that are defective in genes encoding polycomb group proteins, which maintain H3K27me3 levels (Katz et al. 2004; Schubert et al. 2006). Indeed, *KNU* was expressed ectopically in leaves of these mutants. Another test was to see whether another factor associated with H3K27me3, the epigenetic repressor TFL2/LHP1, was involved in repressing *KNU* expression. In the *tfl2* mutant, *KNU* was overexpressed in the flower and ectopically expressed in the inflorescence stem. Therefore, defects in maintaining H3K27me3 levels could de-

repress *KNU* expression, suggesting that relatively high levels of H3K27me3 at the *KNU* locus might be responsible for the repression of *KNU* expression, although the formal possibility that the epigenetic regulators polycomb group proteins and TFL2 might affect *KNU* expression indirectly could not be ruled out.

Whereas Sun et al. (2009) convincingly showed that AG likely activates *KNU* transcription by binding directly to the *KNU* promoter and that *KNU* expression is delayed relative to the onset of AG function, probably by repressive chromatin as marked by H3K27me3, several questions remain. First, it is not known whether and how AG acts to counter the repressive chromatin. Second, the difference for AG function between 1 d and 2 d after AG is induced is not known. In other words, why was AG able to counter the effect of repressive chromatin 2 d, but not 1 d, after AG induction? Sun et al. (2009) proposed that the repressive marker is removed in an AG-dependent manner and suggested that the period of 2 d might be required because of a need for active cell division. However, AG is not a histone demethylase. Could AG recruit a histone demethylase to the *KNU* promoter region? Is the removal of histone methylation achieved passively by preventing methylation following DNA replication during the cell cycle?

A second area that requires further investigation concerns the mechanism by which *KNU* represses *WUS* expression. Although Sun et al. (2009) showed that the *KNU* repression motif is required for the termination of *WUS* expression, it is possible that *KNU* represses an activator of *WUS* expression, thereby negatively regulating *WUS* expression in an indirect fashion. Sun et al. (2009) indicated that they performed a ChIP experiment for *KNU* binding to the *WUS* promoter, but the result was not conclusive. Perhaps more importantly, *KNU* has not been shown to be, or to associate with, a DNA-binding protein, although it has a C2H2-type zinc finger (Payne et al. 2004). Nevertheless, the observation by Sun et al. (2009) that the activity of a *KNU-GR* fusion protein can be induced by the artificial hormone dex suggests that *KNU* is a transcriptional regulator. Future experiments are needed to determine whether *KNU* is a DNA-binding protein and, if so, what the binding sequence for *KNU* is.

Yet another puzzle is the relationship between AG, *KNU*, and other genes that also play a role in regulating floral meristem determinacy (Fig. 1C). One such additional regulator is the C2H2 zinc finger protein SUPERMAN (SUP), which has been shown to repress stem cell proliferation at floral stages 3 and 4 (Bowman et al. 1992; Sakai et al. 1995). Genetic studies indicated that AG and SUP control floral meristem determinacy through independent pathways (Schultz et al. 1991; Bowman et al. 1992). SUP is also involved in the termination of *WUS* expression (Prunet et al. 2008). What is the relationship between the two C2H2 zinc finger proteins *KNU* and SUP? Another contributor to the floral determinacy is CRABS CLAW (CRC), which also negatively regulates stem cell proliferation (Bowman and Smyth 1999) and might act downstream from AG and two other floral organ identity genes—*APETALA3* and *PISTILLATA* (Lee et al. 2005).

However, the relationship between *CRC* and *KNU* is unclear, and the interactions between *AG*, *SUP*, and *CRC* at the molecular level are yet to be understood. Recent studies have uncovered other positive regulators of floral meristem determinacy—*REBELOTE* (*RBL*), *SQUINT* (*SQN*), and *ULTRAPETALA1* (*ULT*)—that function upstream of *AG* and *SUP* (Fig. 1C; Carles et al. 2004; Prunet et al. 2008). The proteins encoded by these genes have various predicted activities, suggesting that they act with different mechanisms. With rapid progress fueled by new technologies, future investigations will likely yield new insights regarding the control of stem cell activities in the floral meristem, ever enriching us in this fertile ground of plant development.

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References

- Bowman JL, Smyth DR. 1999. *CRABS CLAW*, a gene that regulates carpel and nectary development in *Arabidopsis*, encodes a novel protein with zinc finger and helix-loop-helix domains. *Development* **126**: 2387–2396.
- Bowman JL, Smyth DR, Meyerowitz EM. 1989. Genes directing flower development in *Arabidopsis*. *Plant Cell* **1**: 37–52.
- Bowman JL, Smyth DR, Meyerowitz EM. 1991. Genetic interactions among floral homeotic genes of *Arabidopsis*. *Development* **112**: 1–20.
- Bowman JL, Sakai H, Jack T, Weigel D, Mayer U, Meyerowitz EM. 1992. *SUPERMAN*, a regulator of floral homeotic genes in *Arabidopsis*. *Development* **114**: 599–615.
- Carles CC, Lertpiriyapong K, Reville K, Fletcher JC. 2004. The *ULTRAPETALA1* gene functions early in *Arabidopsis* development to restrict shoot apical meristem activity and acts through *WUSCHEL* to regulate floral meristem determinacy. *Genetics* **167**: 1893–1903.
- Coen ES, Meyerowitz EM. 1991. The war of the whorls: Genetic interactions controlling flower development. *Nature* **353**: 31–37.
- Gomez-Mena C, de Folter S, Costa MMR, Angenent GC, Sablowski R. 2005. Transcriptional program controlled by the floral homeotic gene *AGAMOUS* during early organogenesis. *Development* **132**: 429–438.
- Huang H, Mizukami Y, Hu Y, Ma H. 1993. Isolation and characterization of the binding sequences for the product of the *Arabidopsis* floral homeotic gene *AGAMOUS*. *Nucleic Acids Res* **21**: 4769–4776.
- Ito T, Takahashi N, Shimura Y, Okada K. 1997. A serine/threonine protein kinase gene isolated by an in vivo binding procedure using the *Arabidopsis* floral homeotic gene product, *AGAMOUS*. *Plant Cell Physiol* **38**: 248–258.
- Ito T, Wellmer F, Yu H, Das P, Ito N, Alves-Ferreira M, Riechmann JL, Meyerowitz EM. 2004. The homeotic protein *AGAMOUS* controls microsporogenesis by regulation of *SPOROCTELESS*. *Nature* **430**: 356–360.
- Katz A, Oliva M, Mosquana A, Hakim O, Ohad N. 2004. *FIE* and *CURLY LEAF* polycomb proteins interact in the regulation of homeobox gene expression during sporophyte development. *Plant J* **37**: 707–719.
- Laux T. 2003. The stem cell concept in plants: A matter of debate. *Cell* **113**: 281–283.
- Laux T, Mayer KFX, Berger J, Jurgens G. 1996. The *WUSCHEL* gene is required for shoot and floral meristem integrity in *Arabidopsis*. *Development* **122**: 87–96.
- Lee JY, Baum SF, Alvarez J, Patel A, Chitwood DH, Bowman JL. 2005. Activation of *CRABS CLAW* in the nectaries and carpels of *Arabidopsis*. *Plant Cell* **17**: 25–36.
- Lenhard M, Bohnert A, Jurgens G, Laux T. 2001. Termination of stem cell maintenance in *Arabidopsis* floral meristems by interactions between *WUSCHEL* and *AGAMOUS*. *Cell* **105**: 805–814.
- Lenhard M, Jurgens G, Laux T. 2002. The *WUSCHEL* and *SHOOTMERISTEMLESS* genes fulfill complementary roles in *Arabidopsis* shoot meristem regulation. *Development* **129**: 3195–3206.
- Lohmann J, Huang R, Hobe M, Busch M, Parcy F, Simon R, Weigel D. 2001. A molecular link between stem cell regulation and floral patterning in *Arabidopsis*. *Cell* **105**: 793–803.
- Mayer KFX, Schoof H, Haecker A, Lenhard M, Jurgens G, Laux T. 1998. Role of *WUSCHEL* in regulating stem cell fate in the *Arabidopsis* shoot meristem. *Cell* **95**: 805–815.
- Mizukami Y, Ma H. 1995. Separation of *AG* function in floral meristem determinacy from that in reproductive organ identity by expressing antisense *AG* RNA. *Plant Mol Biol* **28**: 767–784.
- Mizukami Y, Huang H, Tudor M, Hu Y, Ma H. 1996. Functional domains of the floral regulator *AGAMOUS*: Characterization of the DNA-binding domain and analysis of dominant negative mutations. *Plant Cell* **8**: 831–845.
- Payne T, Johnson SD, Koltunow AM. 2004. *KNUCKLES* (*KNU*) encodes a C2H2 zinc-finger protein that regulates development of basal pattern elements of the *Arabidopsis* gynoecium. *Development* **131**: 3737–3749.
- Prunet N, Morel P, Thierry A, Eshed Y, Bowman J, Negrutiu I, Trehin C. 2008. *REBELOTE*, *SQUINT*, and *ULTRAPETALA1* function redundantly in the temporal regulation of floral meristem termination in *Arabidopsis thaliana*. *Plant Cell* **20**: 901–919.
- Sakai H, Medrano LJ, Meyerowitz EM. 1995. Role of *SUPERMAN* in maintaining *Arabidopsis* floral whorl boundaries. *Nature* **378**: 199–203.
- Schubert D, Primavesi L, Bishopp A, Roberts G, Doonan J, Jenuwein T, Goodrich J. 2006. Silencing by plant Polycomb-group genes requires dispersed trimethylation of histone H3 at lysine 27. *EMBO J* **25**: 4638–4649.
- Schultz EA, Pickett FB, Haughn GW. 1991. The *FLO10* gene product regulates the expression domain of homeotic genes *AP3* and *PI* in *Arabidopsis* flowers. *Plant Cell* **3**: 1221–1237.
- Shiraishi H, Okada K, Shimura Y. 1993. Nucleotide sequences recognized by the *AGAMOUS* MADS domain of *Arabidopsis thaliana* in vitro. *Plant J* **4**: 385–398.
- Smyth D, Bowman JL, Meyerowitz EM. 1990. Early flower development in *Arabidopsis*. *Plant Cell* **2**: 755–767.
- Sun B, Xu Y, Ng K-H, Ito T. 2009. A timing mechanism for stem cell maintenance and differentiation in the *Arabidopsis* floral meristem. *Genes & Dev* (this issue). doi: 10.1101/gad.1800409.
- Weigel D, Jurgens G. 2002. Stem cells that make stems. *Nature* **415**: 751–754.
- Yanofsky MF, Ma H, Bowman JL, Drews GN, Feldmann K, Meyerowitz EM. 1990. The protein encoded by the *Arabidopsis* homeotic gene *agamous* resembles transcription factors. *Nature* **346**: 35–39.

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