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Plant development: Unveiling cytokinin's role in the end of flowering

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Plant reproductive duration is defined by the onset as well as the end of flowering. A new study characterizes the little-known process of flowering cessation by imaging cellular and molecular dynamics at the shoot apical meristem, revealing a critical role of cytokinin.

Monocarpy is a life-history strategy whereby an individual plant dies after a single reproductive period[1,2]. The majority of grain crops are monocarpic; therefore, understanding the mechanisms that regulate the length of the reproductive period is of significant importance for crop yield, given that the ability to prolong the single reproductive period could increase seed and fruit production. While extensive work has focused on the initiation of flowering and unraveled complex regulatory networks involving environmental and genetic factors[3], the end of flowering has received little attention. Such neglect could perhaps be due to the assumption that the end of flowering is a default process of meristem aging. However, classical studies[1,2] as well as a recent surge of research[4–8] have firmly established that the end of flowering, as characterized by proliferative arrest at the shoot apical meristem (SAM), is a regulated process. In fact, proliferative arrest represents an evolutionarily adaptative mechanism whereby the SAM undergoes growth cessation after producing a certain number of fruits and seeds, consequently directing resources toward fruit and seed production at the end of a plant's life[1,2,4]. In this issue of *Current Biology*, Merelo *et al.*[9] report detailed spatiotemporal analyses of cellular and molecular events leading to proliferative arrest at the SAM of *Arabidopsis thaliana* and reveal a critical role of the plant hormone cytokinin (CK) in regulating proliferative arrest.

Prior to the new study by Merelo *et al.*[9], two parallel pathways were identified that trigger proliferative arrest. One pathway involves the accumulation of a mobile signal produced by the fruit and/or seeds. The lack of fertilization in sterile plants or the removal of fruits from wild-type plants could delay proliferative arrest[1,2,10]. Recent reports suggest that auxin exported from the last-developing fruits triggers proliferative arrest through changes in auxin transport[5,7]. The other pathway is an age-dependent pathway involving the *FRUITFUL* (*FUL*) and *APETALA2* (*AP2*) transcription factor genes. *Arabidopsis* SAMs only become competent for proliferative arrest upon reaching an appropriate age. *FUL* promotes proliferative arrest through direct repression of *AP2* and *AP2-like* gene expression in the SAM[6]. As plants age, increased *FUL* and decreased *AP2* and *AP2-like* gene expression lead to decreased *WUSCHEL* (*WUS*), the specific expression of which at the organizing center is required to maintain the stem-cell pool at the SAM[11]. However, how the fruit/seed-signal pathway and the age-dependent pathway interact at the SAM and which cellular changes precede proliferative arrest remained unknown.

Merelo *et al.*[9] set out to provide a coordinated temporal and spatial framework that captures dynamic cellular and molecular events at the SAM leading up to proliferative arrest. In addition to measuring the number of open flowers and mature fruits produced by each inflorescence, the authors compared changes in cell number, cell size, and SAM area that occurred in bolting (rapid elongation of the main shoot) with the time of meristem arrest, using high-resolution imaging and a quantification algorithm. Since proliferative arrest is reversible, the authors then pruned the fruits after such arrest to reactivate the arrested meristem and continued to observe SAM development for two additional weeks. One important observation is that the reduction in flower and fruit number, meristem cell number, and meristem size starts three weeks after bolting (Figure 1A), considerably earlier than proliferative arrest, which takes place five weeks after bolting. This suggests that reduction of meristem function involves an early and gradual program. Second, a decrease in meristem area, as a result of reduced meristem cell number and cell size, is a key factor that limits meristem activity.

Further characterization of proliferative arrest involved observing spatiotemporal patterns of reporter gene expression at the SAM using high-resolution confocal imaging and MorphoGraphX software. CK is a plant hormone known to promote cell division by regulating cell-cycle components[12]. In addition, CK positively regulates *WUS* expression through B-type *Arabidopsis* response regulators (ARRs), which directly bind to the *WUS* promoter and promote *WUS* expression in the organizing center[13,14]. To examine how CK may regulate cell division and *WUS* expression in the context of proliferative arrest, Merelo *et al.*[9] examined the expression of a cell-cycle G2/M-phase reporter (*CYCB1;2–GFP*), a stem-cell-niche reporter (WUS_{pro}*:GFP–WUS*), and a CK-signaling reporter ($TCSn_{pro}$*:GFP–ER*) from the time of bolting to seven weeks after bolting (Figure 1A). Repression of *CYCB1;2–GFP* occurred as early as three weeks after bolting and matched closely in timing and extent with that of $TCSn_{pro}$*:GFP–ER*; repression of WUS_{pro}*:GFP–WUS* followed shortly afterwards. In reactivated meristems, the expression of all three reporters resumed with similar dynamics. The expression patterns of these reporters are consistent with the authors' hypothesis that a reduction of CK may be the early trigger of proliferative arrest, by both reducing CK-dependent cell

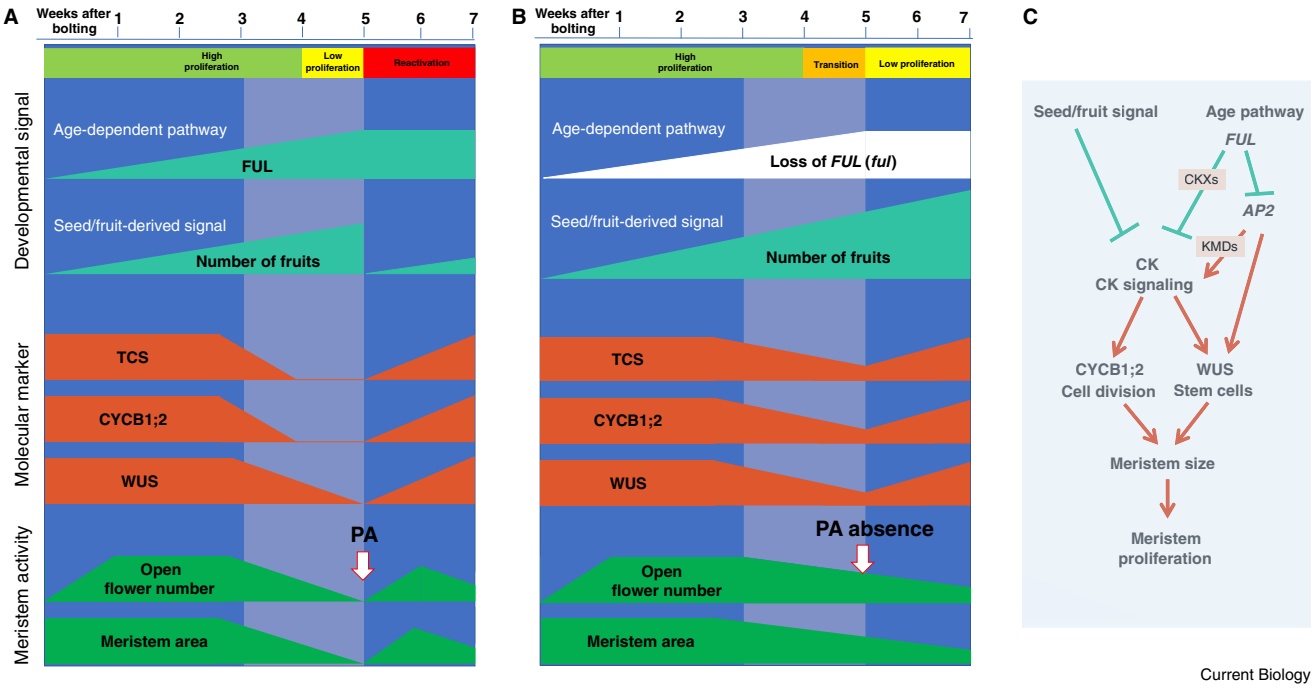

Figure 1. Temporal changes in cellular and molecular events leading to meristem arrest and reactivation in *Arabidopsis*.
(A) Summary of the timing of cellular and molecular changes at the SAM measured in weeks after bolting, where signal strength is indicated by the relative height of colored triangles and rectangles. Meristem reactivation is achieved by removing fruits. Significant reductions of molecular marker expression and meristem activities are evident three weeks after bolting and proliferative arrest ('PA'), depicted by a white arrow, occurs five weeks after bolting. (B) Summary of cellular and molecular changes at the SAM of *ful* mutants. Molecular marker expression and meristem activities are never reduced to zero (that is, an absence of proliferative arrest) five weeks after bolting, and the cellular and molecular changes at 5–7 weeks after bolting resemble those of the reactivated meristems, as shown in (A). (C) A model showing the integration of the fruit/seed-signal pathway and the age-dependent pathway with CK signaling in the SAM. The CK pathway promotes cell division and meristem maintenance, leading to meristem proliferation. Repression of the CK pathway results in decreased meristem size, leading to proliferative arrest. CK oxidases (CKXs) and KISS ME DEADLY (KMD) are intermediate regulatory targets through which *FUL* and *AP2*, respectively, regulate the CK pathway.

division as well as the stem-cell pool in the SAM.

While these data reveal a correlation between CK signaling and meristem activities, further experimentation by exogenous application of CK to the shoot apex showed that CK application alone was sufficient to prevent proliferative arrest and even reverse it. This result supports a causal role of CK in inhibiting proliferative arrest and further suggests that CK may act to integrate signals from the fruit/seed-signal pathway and the age-dependent pathway at the SAM (Figure 1C), given that CK application alone could block the effects of both pathways. Finally, the data strongly support the mechanism proposed by the authors that decreasing CK at the SAM leads to a smaller meristem size, which limits the meristem's proliferative ability.

Merelo *et al.*[9] went on to characterize *fruitful* (*ful*) mutants, which flower indefinitely without entering proliferative arrest. Detailed cellular and molecular analyses of *ful* mutant SAMs defined several sequential phases: a high proliferation phase (0–4 weeks after bolting), a transition phase (4–5 weeks after bolting), and a low proliferation phase (5–7 weeks after bolting) (Figure 1B). From 0–4 weeks after bolting, the cell size and number, cell-division activities, CK signaling, and *WUS* expression in *ful* mutants exhibited similar dynamics as those in wild-type SAMs, which reflected a high proliferative ability initially, followed by a decline three weeks after bolting. However, cell division, CK signaling, and *WUS* expression in *ful* mutants decreased more slowly and never reached proliferative arrest five weeks after bolting (Figure 1B). The decline of these parameters, albeit at a slower rate, suggests that *FUL* is not the only inhibitory factor in proliferative arrest. From 5–7 weeks after bolting, SAMs remained active in *ful* mutants and even started to slowly increase in activity, resembling reactivated meristems (Figure 1A,B), suggesting that *FUL* may be required to completely shut down meristem proliferation at a later phase. Merelo *et al.*[9] went on to suggest two ways by which *FUL* may negatively regulate the CK pathway (Figure 1C). First, *FUL* promotes the expression of cytokinin oxidases that degrade CK. Second, FUL directly represses *AP2*, and AP2 positively regulates CK signaling by repressing the expression of several *KISS ME DEADLY* (*KMD*) genes, encoding Kelch-repeat F-box proteins involved in the degradation of B-type ARRs.

The elegant molecular and cellular framework presented here should attract increased interest in this important yet understudied area of flowering cessation. One key unanswered question is how the CK-related pathway is integrated with the fruit/seed-derived signal to regulate proliferative arrest. Prior studies revealed similarities in the transcriptomes of arrested meristems and dormant axillary buds[4,8], prompting the question of

whether the induction of proliferative arrest in SAMs by fruit/seed-produced auxin might involve similar mechanisms as arrest in the dormant axillary buds induced by apically produced auxin in apical dominance. The dormant axillary buds were proposed to result partly from auxin-mediated inhibition of CK biosynthesis in the stem or from reduced auxin export from the axillary bud[5,15]. These potential mechanisms could be tested in arrested meristems by analyzing CK signaling and biosynthesis pathway mutants and their genetic interactions with fruit/seed-pathway mutants, including those with defects in auxin biosynthesis and transport. Additionally, since fruit/seed-derived auxin only causes proliferative arrest in older SAMs[5], how the age-dependent pathway confers competence to arrest is another important question. Finally, as monocarpy has evolved several times from polycarpy, there may be species-specific regulatory mechanisms[2]. Comparative analyses of the end of flowering in different monocarpic species will illuminate strategies that balance growth with reproduction.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Infectious disease: Dog diets may drive transmission cycles in human Guinea worm disease

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Domestic dogs have an important role in the ecology of transmission of the Guinea worm, a debilitating human parasite. A new study documents how fish content in dogs' diets can predict Guinea worm infection status, suggesting additional avenues for control.

In the early 2000s, Guinea worm (*Dracunculus medinensis*) seemed poised to become the second human infectious disease ever eradicated, after smallpox (Variola sp.). Prior to eradication initiatives, Guinea worm caused painful and debilitating infections in millions of people annually and, like smallpox, had no known animal reservoir, making it a prime candidate for the next eradication[1,2]. Guinea worm eradication initiatives found much success, resulting in the number of endemic countries shrinking to a handful by the early 2010s[1]. It was at this point,