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Flowering and determinacy in *Arabidopsis*

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Abstract

Meristems provide new cells to produce organs throughout the life of a plant, and their continuous activity depends on regulatory genes that balance the proliferation of meristem cells with their recruitment to organogenesis. During flower development, this balance is shifted towards organogenesis, causing the meristem to terminate after producing a genetically determined number of organs. In *Arabidopsis*, *WUSCHEL* (*WUS*) specifies the self-renewing cells at the core of the shoot meristems and is a key target in the control of meristem stability. The development of a determinate floral meristem is initiated by *APETALA1/CAULIFLOWER* (*AP1/CAL*) and *LEAFY* (*LFY*). The latter activates *AGAMOUS* (*AG*), partly in co-operation with *WUS*. *AG* then directs the development of the innermost floral organs and at the same time antagonizes *WUS* to terminate the meristem, although the mechanism of *WUS* repression remains unknown. All these genes participate in a series of regulatory feedback loops that maintain stable expression patterns or promote sharp developmental transitions. Although the regulators of meristem maintenance and determinacy in *Arabidopsis* are widely conserved, their interactions may vary in other species.

Key words: *AGAMOUS*, determinacy, flower development, shoot meristem, *WUSCHEL*.

Introduction

The apical meristems function as the main sources of new cells to sustain plant growth. The regular recruitment of meristem cells to form new organs and tissues is balanced by cell proliferation within the meristem to maintain its size relatively stable. This steady-state can persist throughout the life of the plant, but in many cases the meristem is genetically programmed to stop producing

new cells at a specific developmental stage. In these cases, the meristem is said to be determinate. A determinate meristem produces a part of the plant body with a predictable size and form, such as the flower, whereas indeterminate meristems produce parts of the plant whose size and shape depend on the local environment, such as branches and roots that grow to variable lengths. The positioning of determinate and indeterminate meristems varies between species and is a major determinant of plant architecture.

Here, the genetic control of meristem determinacy in *Arabidopsis* and the applicability of this knowledge to other species is reviewed. In the *Arabidopsis* shoot, determinacy is a property of the floral meristems, whereas the vegetative and inflorescence meristems are indeterminate. The indeterminate growth of the vegetative and inflorescence meristems requires a specific set of regulatory genes, whose activity is antagonized by flower-specific regulators. For this reason, it is useful to start with a description of how the activity of indeterminate meristems is maintained.

Maintenance of indeterminate meristems

The indeterminate growth of the vegetative and inflorescence meristems is sustained by small groups of self-renewing cells that are functionally similar to stem cells in animals (Sablowski, 2004). These cells are located in the central zone (CZ) of the meristem, while some of their descendants are displaced to the peripheral zone (PZ), where they are recruited to form new organ primordia (Fig. 1). Below the CZ, the rib meristem (RM) sustains stem growth. The CZ and PZ functions have been well studied in *Arabidopsis*, although the RM has received much less attention. Superimposed on the CZ/PZ organization, the typical tunica/corpus structure found in angiosperms can also be distinguished, with two external layers (L1 and L2) in which most cell divisions are oriented tangentially to the meristem surface, while the

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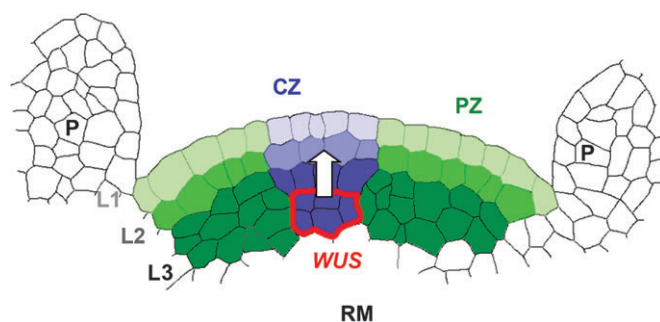


Fig. 1. Structure of the shoot apical meristem. The concentric L1, L2, and L3 cell layers are indicated by different shades of colour. The central zone (CZ) is marked in blue, the peripheral zone (PZ) in green, and the position of the rib meristem (RM) is indicated below the meristem dome. The region that expresses *WUS* is circled in red; the white arrow represents the intercellular signal produced by the *WUS*-expressing cells to maintain cell identity and proliferation in overlying CZ. P indicates organ primordia produced on the flanks of the meristem.

inner layer does not have clearly oriented divisions (Carles and Fletcher, 2003). Although the functional significance of the L1–L3 layering is not well understood, the existence of these clonally distinct layers has been essential to reveal the role of intercellular communication in meristem function.

SHOOT MERISTEMLESS (*STM*) and *WUSCHEL* (*WUS*) are two regulatory genes with central roles in shoot meristem development. *STM* and *WUS* genes function synergistically during meristem development and are required not only for the establishment of the shoot meristem during embryogenesis, but also for subsequent maintenance of the vegetative, inflorescence and floral meristems (Clark *et al.*, 1996; Laux *et al.*, 1996; Long *et al.*, 1996; Gallois *et al.*, 2002; Lenhard *et al.*, 2002). *STM* encodes a homeodomain protein expressed throughout the meristem (Long *et al.*, 1996) and has been proposed to delay differentiation to allow enough cells to bulk up before recruitment into organogenesis (Lenhard *et al.*, 2002).

WUS also encodes a homeodomain-containing protein and is essential to specify the stem cells present in the CZ: in *wus* mutants, the defective CZ cannot keep up with organ recruitment in the PZ, and the meristem is quickly consumed (Mayer *et al.*, 1998). Shoot meristem activity is eventually reinitiated in the axils of the leaves, only to terminate again; this intermittent meristem activity can carry on to the reproductive phase, when incomplete flowers are produced because of premature termination of the floral meristem (Fig. 3). Not only is *WUS* necessary to maintain the CZ cells, but ectopic expression of *WUS* is also sufficient to convert cells in organ primordia and even root meristems into cells with characteristics of the shoot meristem CZ (Schoof *et al.*, 2000; Gallois *et al.*, 2004). Although *WUS* is required to maintain stem cells in all layers of the CZ, it is expressed only in a few L3 cells

in the centre of the meristem (Mayer *et al.*, 1998) (Fig. 1). Because its effects are seen in cells that do not express *WUS*, an intercellular signal is believed to mediate these effects. The size and location of the *WUS*-expressing region of the meristem are maintained in spite of the continuous cell proliferation within the meristem, implying that *WUS* expression must be adjusted constantly according to the position of cells in the meristem.

Although both *STM* and *WUS* are essential for meristem maintenance, the evidence so far suggests that *WUS* has a more prominent role in the developmental control of meristem size and stability. One of the mechanisms that fine-tune *WUS* expression is the *CLAVATA* (*CLV*) signalling pathway, which represses *WUS* (reviewed by Carles and Fletcher, 2003). The signal in this case is the secreted polypeptide *CLV3*, whose biologically active form has recently been shown to be a dodecapeptide corresponding to the C-terminal region of the *CLV3* product (Ito *et al.*, 2006). *CLV3* is produced in the L1 and L2 layers of the CZ and moves into the inner layers, where it is perceived by a receptor containing the *CLV1* and *CLV2* polypeptides. Mutations in any of the *clv* genes have a similar effect: *WUS* expression increases and the CZ gradually enlarges. *WUS* activates expression of *CLV3* in the overlying region of the meristem and therefore limits its own expression, so it is believed that meristem size is stabilized by the *WUS/CLV* regulatory loop (Fletcher *et al.*, 1999; Brand *et al.*, 2000). Consistent with this idea, increases in *CLV3* rapidly repress *WUS* expression and shut down meristem activity (Reddy and Meyerowitz, 2005), but, surprisingly, it has been found that the meristem eventually adjusts to changes in *CLV3* expression and that plants can grow normally with *CLV3* levels ranging from 3-fold lower to 3-fold higher than the wild type (Muller *et al.*, 2006). The implication is that additional mechanisms, independent of the *CLV* loop, stabilize *WUS* expression and meristem size. It has also been revealed that *CLV3* itself has multiple functions, including the control of cell division rates in the meristem and repression of CZ identity in neighbouring PZ cells (Reddy and Meyerowitz, 2005).

A number of additional regulatory genes control the position and number of cells expressing *WUS* and therefore also have a role in controlling meristem stability. One of them is *ULTRAPETALA* (*ULT*) (Carles *et al.*, 2004), which antagonizes *WUS*; accordingly, *ult* mutants have enlarged inflorescence meristems and supernumerary floral organs. The *HD-ZIP III* genes *CORONA* (*CNA*), *PHABULOSA* (*PHAB*), and *PHAVOLUTA* (*PHAV*) (the latter two better known for their role in controlling organ polarity) also restrict the size of the *WUS*-expressing domain and meristem size (Prigge *et al.*, 2005; Williams *et al.*, 2005). Chromatin remodelling factors participate in preventing *WUS* expression outside its normal domain (Kaya *et al.*, 2001; Bertrand *et al.*, 2003; Takeda *et al.*,

2004) and in directly activating it in its normal region (Kwon *et al.*, 2005). Another known positive regulator of *WUS* is *STIMPY* (*STIP*), which encodes a protein of the same family as *WUS* and is required to maintain *WUS* expression in the meristem; *STIP*, however, has a more general role in maintaining cell divisions in the meristem, a role that can be bypassed by exogenously added sucrose (Wu *et al.*, 2005). To integrate all these inputs, *WUS* could be expected to have complex *cis*-regulatory sequences. Surprisingly, however, much of the *WUS* expression pattern can be directed by a short (57 bp) sequence, suggesting that the integration of multiple inputs converges at a relatively simple regulatory element in the *WUS* gene (Baurle and Laux, 2005).

From the work reviewed above, *WUS* emerges as a central regulator of shoot meristem identity and stability. To balance the recruitment of cells away from the meristem with the supply of new meristem cells, a constant and precise pattern of *WUS* expression must be maintained within a population of cells that proliferates continuously. This is achieved by multiple regulatory inputs, many of which act to repress *WUS* outside its normal expression domain.

From vegetative to floral meristems

The dynamic balance between shoot meristem activity and organ initiation is maintained during most of the plant's growth, but it is eventually tipped in favour of organogenesis during floral development. The suppression of indeterminate growth in the floral meristem depends on floral-specific regulatory genes, whose expression is embedded within a programme of gene expression that is initiated at the transition to reproductive development. During this transition, the vegetative meristem is initially converted into the inflorescence meristem, which then produces floral meristems on its flanks. The transition from vegetative to reproductive development is controlled by multiple environmental and endogenous signals that ultimately converge on key regulators of floral identity: *APETALA1* (*API*)/*CAULIFLOWER* (*CAL*) and *LEAFY* (*LFY*) (reviewed by Komeda, 2004; Blazquez *et al.*, 2006) (Fig. 2).

API and *CAL* encode closely related MADS-domain transcription factors that are necessary and sufficient for the transition from inflorescence to floral meristem. In the double mutant *ap1-1 cal-1*, the primordia produced on the flanks of the inflorescence meristem fail to develop as flowers, and instead function as new inflorescence meristems, which go on to produce their own primordia, repeating the process until a large mass of meristems accumulates at the inflorescence apex, which resembles a cauliflower curd (Bowman *et al.*, 1993). Conversely, overexpression of *API* is sufficient to convert the

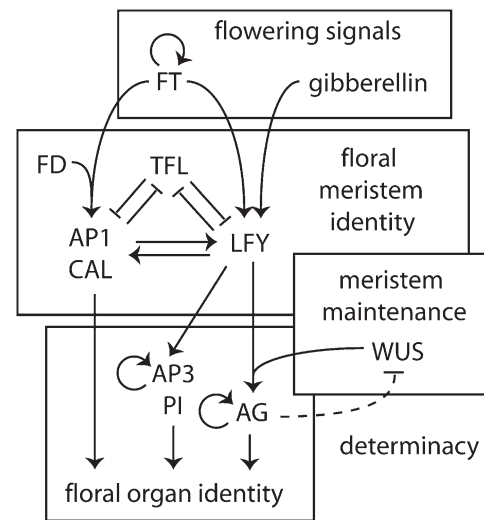


Fig. 2. Diagram of the interactions between regulators of floral meristem identity, floral organ development, and determinacy. Activation is indicated by an arrow and repression by a blunted line. The repression of *WUS* by *AG* is indicated by a dashed line to emphasize that it is likely to be indirect.

inflorescence meristem into a terminal flower (Mandel and Yanofsky, 1995). Consistent with their role in specifying floral meristems, *API* and *CAL* are expressed as soon as the floral primordium emerges from the inflorescence meristem (Kempin *et al.*, 1995).

LFY also encodes a transcriptional regulator that specifies floral identity and consequently promotes determinacy (Weigel *et al.*, 1992). This has been shown both by loss of *lfy* function, which converts floral meristems to inflorescence shoots (Schultz and Haughn, 1991), and by the effect of ectopic *LFY* expression, which converts the inflorescence meristem into a terminal floral meristem (Weigel and Nilsson, 1995). *LFY* promotes the transition from inflorescence to floral meristem largely by activating *API* (Mandel and Yanofsky, 1995; Wagner *et al.*, 1999), but subsequently has a central and *API*-independent role in controlling floral development.

In addition to being activated by *LFY*, *API/CAL* are redundantly activated by the *FT* gene (Ruiz-Garcia *et al.*, 1997) (Fig. 2). Recent evidence suggests that *FT* is expressed in leaves and that its RNA or protein is transported to the apex as part of a mobile flowering signal that is produced in response to long days (Abe *et al.*, 2005; Huang *et al.*, 2005). After reaching the apex, *FT* is believed to interact with the bZIP protein *FD* to activate *API/CAL* (Abe *et al.*, 2005; Wigge *et al.*, 2005). *LFY* itself is also activated by *FT* (Huang *et al.*, 2005), besides being activated by gibberellin, which also functions as a signal to promote the shift to reproductive development (Blazquez *et al.*, 1998).

As mentioned above, to maintain the indeterminate inflorescence meristem, *API/CAL* and *LFY* must be activated only in the floral primordia. The expression of *LFY*

and *API/CAL* in the inflorescence meristem is prevented by *TERMINAL FLOWER (TFL)*, which encodes a homologue of *FT* but has the opposite function, i.e. it antagonizes floral development (Bradley *et al.*, 1997; Kardailsky *et al.*, 1999; Kobayashi *et al.*, 1999) (Fig. 2). In the *tfl* mutant, ectopic expression of *LFY* and *API* transforms the whole inflorescence meristem into a floral meristem. *TFL* is expressed just below the inflorescence meristem, indicating that it functions non-cell-autonomously to prevent *LFY* and *API* expression and the consequent termination of the inflorescence meristem.

The interactions between *FT*, *API/CAL*, *LFY*, and *TFL* not only delimit where floral meristems develop, but also establish regulatory loops that ensure a sharp and stable transition to floral identity. After the initial activation by *FT/FD*, *LFY* and *API/CAL* reinforce each other's expression: *LFY* activates *API* directly (Wagner *et al.*, 1999) and *API* helps to maintain *LFY* expression in part by antagonizing *TFL* (Liljegren *et al.*, 1999). Together, *LFY* and *API/CAL* then activate the floral development programme.

Genetically programmed termination of the floral meristem

As described above, *API* and *LFY* promote floral meristem identity and consequently determinacy. Termination of the meristem, however, is not a direct effect of these regulatory genes, but part of the flower development programme set in motion by *API* and *LFY*.

After being initiated on the flanks of the inflorescence meristem, the floral meristem produces four whorls of organs, which in wild-type *Arabidopsis* typically contain four sepals, four petals, six stamens, and two fused carpels (Fig. 3). The identity of each type of organ is specified by a specific combination of MADS-domain proteins that are believed to form multiprotein complexes, each complex able to control the set of target genes required for the development of a particular organ type (reviewed by Krizek and Fletcher, 2005). One of these MADS-domain

proteins is *AP1* which, after its earlier role in specifying floral identity, participates in the development of the perianth organs (sepals and petals). *LFY* also remains active after floral initiation and activates genes encoding MADS-domain proteins required for stamen and carpel development: *APETALA3 (AP3)*, *PISTILLATA (PI)*, and *AGAMOUS (AG)* (Fig. 2). Among these, *AG* is especially relevant here because it has the additional role of controlling meristem determinacy. In strong *ag* mutants such as *ag-1* or *ag-3* (Fig. 3), stamens are replaced by petals (reflecting the organ identity function) and carpels are replaced by a reiteration of the sequence sepals–petals–petals, produced by an active meristem at the centre of the flower (revealing both the organ identity and the determinacy functions of *AG*).

To terminate the meristem, *AG* would be expected to antagonize the function of meristem maintenance genes such as *STM* or *WUS*. Given the prominent role of *WUS* in the control of meristem size and stability, an attractive idea would be that *AG* adds a further negative input to shut down *WUS* and terminate the floral meristem. Several lines of evidence have confirmed that this is the case. *WUS* is initially expressed in the floral meristem, but its expression decreases at the stage when *AG* is activated and disappears by the time carpel primordia are initiated (Mayer *et al.*, 1998). In contrast, *WUS* remains active in the centre of the indeterminate floral meristem of the *ag-1* mutant (Lenhard *et al.*, 2001; Lohmann *et al.*, 2001). As mentioned before, *WUS* is required for the maintenance of all shoot meristems, including the floral meristem; the premature termination of the floral meristem in the *wus-1* mutant (Fig. 3) is opposite to the extended meristem activity in *ag* mutants. *WUS* is essential for the indeterminacy seen in *ag* flowers, because the flowers of the double mutant *wus-1 ag-1* look indistinguishable from those of *wus-1* (Laux *et al.*, 1996). Conversely, forcing an increase in *WUS* expression in the floral meristem (using *LFY*, *AG*, or *AP3* promoters to express *WUS*) promotes indeterminacy in spite of *AG* activity (Lenhard *et al.*, 2001).

The experiments described above also revealed that *WUS* activated *AG*: ectopic *WUS* not only prolonged meristem activity, but also led to ectopic stamen and carpel development in an *AG*-dependent way (Lenhard *et al.*, 2001; Lohmann *et al.*, 2001). Supporting the suggestion that it can activate *AG*, the *WUS* protein bound *in vitro* to regulatory sequences present in *AG* and activated transcription of a reporter containing these sequences in yeast cells (Lohmann *et al.*, 2001). In the yeast experiments, however, transcription was only activated when *WUS* and *LFY* were combined, and not by either protein alone. The activation of *AG* by *WUS* and *LFY* combined would explain why *AG* is activated by *WUS* only during floral development. It must be noted, however, that *WUS* must be a redundant activator of *AG*,

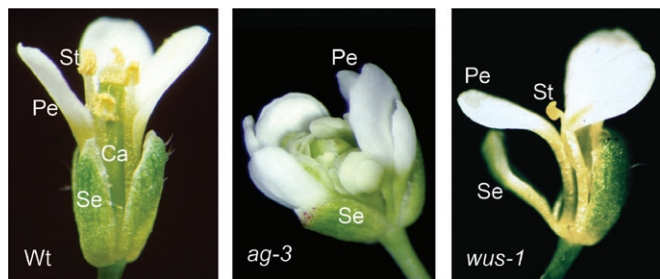


Fig. 3. Flowers of wild-type *Arabidopsis*, *ag-3* mutant, and *wus-1* mutant. Floral organs are indicated: sepals (Se), petals (Pe), stamens (St), and carpels (Ca). Note the indefinite production of sepals and petals in the *ag* mutant and the premature termination of flower development in the *wus* mutant.

which still functions in *wus* mutant flowers to direct the development of stamens (Fig. 3). Moreover, the *AG* expression domain is wider than that of *WUS*, so direct activation by *WUS* can only occur in a subset of the *AG*-expressing cells (so far, there is no evidence that the *WUS* protein moves between cells). Nevertheless, the overall conclusion is that *AG* functions in a negative feedback loop that terminates *WUS* expression and meristem activity in the floral bud.

While the activation of *AG* by *WUS* (at least in part of the floral meristem) appears to result from direct binding of *WUS* to the *AG* gene, the repression of *WUS* by *AG* is unlikely to be direct. Experiments using mosaic expression of *AG* have shown that determinacy is lost when *AG* expression is absent from the L2 layer of the floral meristem (Sieburth *et al.*, 1998), where *WUS* is not expressed (Mayer *et al.*, 1998). This implies that *AG* must function across cell boundaries to antagonize *WUS*, and that coincident expression of *AG* and *WUS* in the L3 layer is not sufficient to terminate the flower. Another reason why the repression of *WUS* by *AG* is probably indirect is that there is a delay between the activation of *AG* in the floral bud (stages 2–3) and down-regulation of *WUS* (stage 6, which occurs ~12 h later; Smyth *et al.*, 1990). Such a delay would be unexpected if *AG* functioned in a simple transcriptional cascade to down-regulate *WUS*.

What could be the signal that mediates the repression of *WUS* by *AG*? Non-cell-autonomous repression of *WUS* brings to mind the *CLV* pathway, so *AG* might stimulate the negative feedback loop involving *CLV3*. However, *clv* mutants have a much milder effect on floral determinacy than *ag* mutations; in other words, *AG* is still largely able to terminate the meristem in the absence of *CLV* function. In addition, the *ag-2 clv1-1* double mutant has a stronger increase in floral meristem activity than *ag-2* or *clv1-1* alone (Clark *et al.*, 1993), indicating that *CLV* and *AG* functions converge to limit meristem activity. Therefore, the *CLV* pathway appears unlikely to play a major role in mediating the determinacy effect of *AG*.

Other genes are known to promote floral determinacy, but are also unlikely to mediate *AG* functions. One of them is *SUPERMAN* (*SUP*), which limits stamen number and is believed to function non-cell-autonomously to control cell proliferation in the centre of the floral meristem (Schultz *et al.*, 1991; Bowman *et al.*, 1992; Sakai *et al.*, 1995). *WUS* is required for the decreased determinacy seen in the *sup* mutant, because the meristem termination in *wus-1* flowers is epistatic over the increase in organ number seen in the *sup-6* mutant (Laux *et al.*, 1996). The interaction between *sup-1* and *ag-1* mutations, however, is synergistic, indicating that they control meristem activity through parallel pathways (Bowman *et al.*, 1992). The *ULT* gene, which as described above antagonizes *WUS*, also limits the number of floral organs. In this case, strong *ag* mutations are epistatic over *ult*

(Carles *et al.*, 2004), suggesting that the role of *ULT* in floral determinacy is contained within the functions activated by *AG*. However, as in the case of *clv* mutants, the loss of determinacy in *ult* mutants is much weaker than in *ag* mutants, showing that *ult* is largely dispensable for the determinacy function of *AG*.

One way to reveal the downstream effectors that mediate the determinacy role of *AG* is to use expression arrays to screen for genes regulated by *AG*. Gomez-Mena *et al.* (2005) used inducible *AG* in an *ap1-1 cal* mutant background to screen for *AG* targets during the early stages of stamen and carpel development. Among the genes activated by *AG* in this study was *GA4*, whose product catalyses the final step in the biosynthesis of bioactive gibberellin, suggesting that one of the early functions of *AG* is to activate gibberellin biosynthesis. Because gibberellin is believed to antagonize meristem activity (reviewed by Shani *et al.*, 2006), a localized increase in gibberellin levels might mediate the meristem-antagonizing function of *AG*. To test this idea, it would be necessary to see whether floral meristems become indeterminate in the absence of gibberellin, but this has not been possible so far because even severe gibberellin-deficient mutants such as *gal-3* are believed still to produce low levels of gibberellin (Hedden and Phillips, 2000).

Activation of *GA4* in the early stages of floral development was confirmed by Wellmer *et al.* (2006), who used inducible *API* in the *ap1-1 cal-1* background to produce a time-course of changes in gene expression during early floral development. This study, however, also showed activation of genes that encode GA2-oxidases, which inactivate gibberellin. In the vegetative meristem, GA2-oxidases are expressed at the base of the meristem and organ primordia, and have been proposed to prevent diffusion of gibberellin from developing organs into the meristem (Jasinski *et al.*, 2005). Thus it is possible that GA2-oxidases are required to protect the floral meristem from gibberellin produced by the organ primordia before meristem termination is due. The exact location and timing of *GA4* and GA2-oxidase expression during early flower development, however, remain unknown.

Gibberellin appears unlikely to be the only phytohormone whose levels are relevant to meristem termination. It has been proposed that meristem activity requires at the same time low gibberellin levels and cytokinin biosynthesis, both of which are promoted by *STM* (Jasinski *et al.*, 2005; Yanai *et al.*, 2005). The positive role of cytokinin in meristem maintenance is also consistent with the finding that one of the functions of *WUS* is to repress genes that antagonize cytokinin responses (Leibfried *et al.*, 2005). Therefore, it might be expected that termination of the floral meristem would be associated not only with an increase in gibberellin activity, but also with a decrease in the levels or sensitivity to cytokinin. So far, however,

no connection has been noted between *AG* and genes involved in cytokinin production or responses.

In summary, during floral development, the repressive input that restricts the location and level of *WUS* expression is increased by genes such as *AG* and *SUP* to promote meristem determinacy. Precisely how these regulators antagonize *WUS* is still unknown, although phytohormones are plausible candidates to mediate the non-cell-autonomous effect of *AG* on meristem termination.

Relevance to other species

Of the regulators of meristem maintenance and determinacy described above, *AG* has been the most intensively studied from the evolutionary point of view (Theissen *et al.*, 2000; Irish, 2003). One of the interesting twists of *AG* function in other species is that the organ identity and determinacy functions are not always carried out by a single gene: in maize, these functions are performed by different *AG* paralogues (Mena *et al.*, 1996). The organ identity and determinacy functions of *AG* are also separable in *Arabidopsis*: loss of determinacy, but not of organ identity, occurs in plants with partial loss of *AG* function caused by antisense RNA or by a weak allele (*ag-4*, which produces a mutant *AG* protein with an internal deletion) (Mizukami and Ma, 1995; Sieburth *et al.*, 1995). The fact that the two functions of *AG* are genetically separable suggests that the organ identity function does not overlap significantly with the meristem termination function. This in turn is compatible with the idea that these functions of *AG* were acquired at different times during evolution (and were subsequently separated again in maize), although it is not clear what the ancestral function was. The association between *AG* homologues and reproductive shoot development in gymnosperms has been used to suggest that the role of *AG* in reproductive organ identity is ancient, but at the same time it has been noted that these reproductive shoots are determinate (Irish, 2003).

The large increase in the number of perianth organs seen in *ag* mutants is reminiscent of double flowers, such as roses and carnations, that have been selected in many species by horticulturalists. The resemblance raises the question of whether similar genes are involved, and in some examples this appears to be the case. In Japanese morning glory, a double mutant flower phenotype first described in the 18th century is caused by mutation of an *AG* homologue (Nitasaka, 2003). In rose, two *AG* homologues have been identified, one of which has the same type of internal deletion as the *ag-4* allele, which as described above causes loss of determinacy (Kitahara and Matsumoto, 2000). Whether this allele has played a role in the selection of modern double roses, however, remains to be seen. It must be noted that the much increased

number of petals and stamens and eventual termination of the floral meristem in roses could also be caused by a localized enlargement of the floral meristem, as seen in the *sup* or *ult* mutants.

Other key players in meristem identity and determinacy, such as *WUS*, *LFY*, and *TFL*, are also clearly conserved and carry out comparable functions in other species (Stuurman *et al.*, 2002; Schwarz-Sommer *et al.*, 2003; Angenent *et al.*, 2005; Kieffer *et al.*, 2006). Some of the regulatory connections between these genes, however, are variable. In tomato, for example, the *TFL* orthologue *SELF-PRUNING* maintains indeterminacy in the inflorescence meristem, but does not do so by antagonizing expression of the *LFY* orthologue, *FALSIFLORA* (Pnueli *et al.*, 1998; Molinero-Rosales *et al.*, 1999). The floral-specific repression of *WUS* may also differ in plants in which the central region of the floral meristem gives rise to the placenta instead of carpel primordia. This is the case in *Impatiens*, where *AG* does not appear to be sufficient to terminate the floral meristem (Ordidge *et al.*, 2005; Chiurugwi, 2007). Similarly, it has been noted in petunia that repression of *WUS* does not coincide with the activation of *AG* orthologues, but occurs later, when other MADS proteins are expressed in the centre of the flower to specify ovule identity. When expressed during the vegetative phase, these ovule identity genes terminate the meristem, suggesting that they could mediate *WUS* repression (Ferrario *et al.*, 2006).

Other aspects of meristem determinacy are even more clearly divergent, particularly when determinacy is controlled during developmental steps that have no obvious equivalent in *Arabidopsis*. In maize, the inflorescence meristem does not give rise to floral meristems directly, but instead gives rise to two intermediate types of meristems, the spikelet pair and the spikelet meristem (see review by Bortiri and Hake, 2007). The regulatory genes *ramosa1* (Vollbrecht *et al.*, 2005), *ramosa2* (Bortiri *et al.*, 2006), and *branched silkless1* (Chuck *et al.*, 2002) control the determinacy of the spikelet pair and spikelet meristems, and do not appear to have counterparts that control meristem determinacy in dicotyledonous plants.

Another way in which the control of determinacy differs across plants is in its reversibility. In an annual plant such as *Arabidopsis*, it is clear why commitment to flowering and floral development should be irreversible and followed by death of the plant. In perennial plants, reversion to vegetative growth after the flowering season occurs from meristems that have not been converted to reproductive development (i.e. there is no reversion), but in some cases true reversion occurs, exemplified by plants showing pseudovivipary and by *Impatiens* shifted to long days after flowering (reviewed by Tooke *et al.*, 2005). Stable developmental transitions are often caused by autoregulatory loops that translate a transient stimulus into a stable regulatory change (Davidson *et al.*, 2002). In

Arabidopsis, such autoregulatory loops occur in multiple stages in the control of flowering and determinacy, including autoactivation by *FT* (Huang *et al.*, 2005), the reciprocal activation of *LFY* and *API/CAL* mentioned above, and positive autoregulation of *AG* (Gomez-Mena *et al.*, 2005). In *Impatiens*, reversion to vegetative development in long days correlates with the interrupted production of a leaf-derived flowering signal (Tooke *et al.*, 2005) and could be due to a failure to establish autoregulatory loops, such as the *FT* autoactivation loop.

In conclusion, to understand evolutionary variation in meristem determinacy and in plant development in general, a future challenge will be to reveal not only the conserved and divergent regulators of meristem activity, but also how diversity is created by changes in the regulatory connections between those genes.

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