Time to Stop: Flower Meristem Termination[1]

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Flowers are the reproductive structure of angiosperms. They are composed of four distinct types of organs: sepals, petals, stamens, and carpels, which typically develop on four concentric rings, or whorls (Fig. 1A). In Arabidopsis (*Arabidopsis thaliana*), floral organ identity relies on the combinatorial action of four classes of flower-specific transcription factors, expressed in partially overlapping domains, and inferred to form four distinct protein complexes (for review, see Ferrario et al., 2004; Krizek and Fletcher, 2005). Sepals are specified by complexes composed of class A (APETALA1 [AP1]) and E (SEPALLATA [SEP]) proteins, petals by a combination of class A, B (AP3 and PISTILLATA [PI]), and E proteins, stamens by a combination of class B, C (AGAMOUS [AG]), and E proteins, and carpels are specified by class C and E proteins. This model can be extrapolated to other taxa, except for the A function whose generalization remains ambiguous (for review, see Ferrario et al., 2004).

Floral organs are generated by a flower meristem (FM), a pool of pluripotent, dividing cells, which is itself produced by, or derives from the transformation of another meristem: the shoot apical meristem (SAM). SAM and FM share many regulators, which appear to be widely conserved among angiosperms, or at least among eudicots (for review, see Nardmann and Werr, 2007). KNOX I homeodomain transcription factors (SHOOT MERISTEMLESS [STM] and KNAT1, 2, and 6 in Arabidopsis) keep meristematic cells in an undifferentiated state, while the WUSCHEL/CLAVATA (WUS/CLV) negative feedback loop maintains a constant population of stem cells in the meristem center (for review, see Williams and Fletcher, 2005; Sablowski, 2007a). These genes are expressed in a similar way in the FM compared to the SAM, and their mutations cause similar defects in both SAM and FM: *stm* and *wus* meristems terminate prematurely, whereas *clv* meristems are enlarged and produce more organs than those of wild-type plants. Thus, during its first

developmental stages, FM homeostasis seems to be achieved by roughly the same molecular mechanisms as it is in the SAM.

However, the FM differs from the SAM in several ways: it produces floral organs instead of leaves, and neither generates axillary meristems nor elongating internodes. Also, FM growth pattern is determinate. Unlike the SAM, which maintains stem cells in its center and keeps producing new organs throughout the life of the plant, stem cells are only transiently maintained within the FM. In Arabidopsis, stem cell maintenance is disrupted at stage 6 of flower development (stages as described in Smyth et al., 1990), when carpel primordia are initiated, making the flower determinate, with a fixed number of whorls and organs per whorl. No more floral organs are produced after the carpels, which are congenitally fused, forming the placenta at their junction, where ovules develop (Figs. 1A and 2A). A similar disruption of stem cell maintenance is seen in most angiosperms, although some minor timing differences do exist between species with different placentation types (discussed by Colombo et al., 2008).
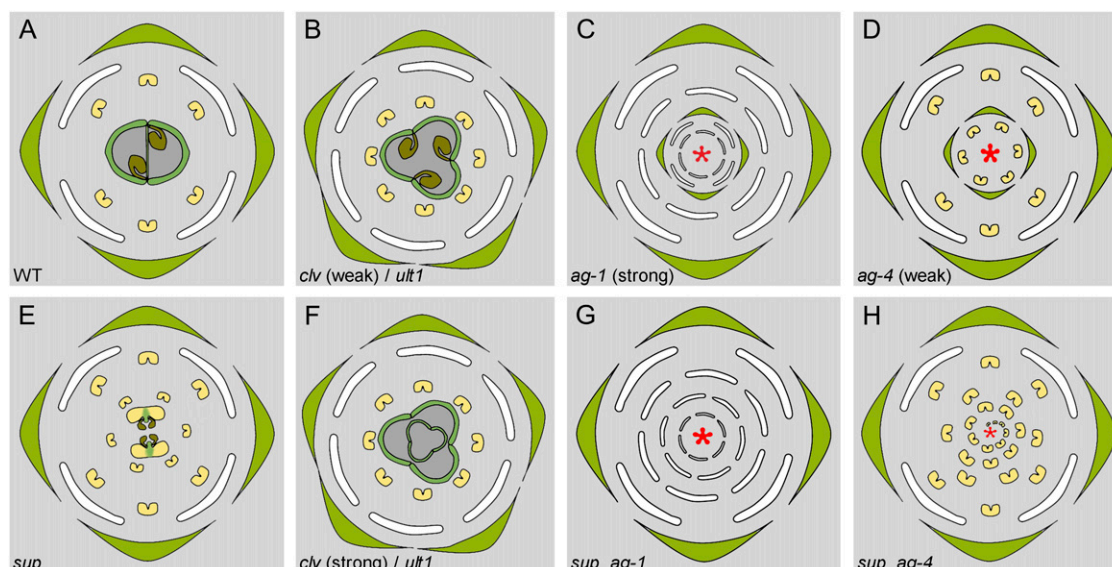
In several species, mutants with flowers displaying more organs than the wild type have been described. However, production of these extra organs may result from distinct processes generating different phenotypes. Mutants such as *clv* and *ultrapetala1* (*ult1*) in Arabidopsis have enlarged meristems, including FMs, which consequently produce more floral organs in each whorl (Fig. 1B; Clark et al., 1993, 1995; Kayes and Clark, 1998; Fletcher, 2001). This phenomenon is associated with an increase of the stem cell population (Schoof et al., 2000; Carles et al., 2005), and therefore results from spatial alterations of the FM. Conversely, mutants such as *ag* in Arabidopsis (Figs. 1, C and D, and 2B), *plena* (*ple*) in Antirrhinum (*Antirrhinum majus*), or *drooping leaves* in rice (*Oryza sativa*) exhibit a prolonged maintenance of stem cells within the FM, resulting in the production of extra organs borne on supernumerary whorls (Bowman et al., 1989, 1991; Yanofsky et al., 1990; Bradley et al., 1993; Sieburth et al., 1995; Nagasawa et al., 2003). This phenotype, referred to as flower indeterminacy or loss of FM termination, is thus due to a temporal, rather than spatial, alteration of flower development. Interestingly, supernumerary whorls are also seen in *clv* and sometimes *ult1* mutant flowers, though to a much lesser extent than in *ag* and *ple* flowers (Fig. 1F; Clark et al., 1993, 1995; Kayes and Clark, 1998; Fletcher, 2001;

Figure 1. Diagrams of wild-type and mutant Arabidopsis flowers displaying supernumerary organs. A, Wild-type flowers consist of four whorls bearing four sepals, four petals, six stamens, and two fused carpels, respectively. B, Weak *clv* and *ult1* mutant flowers exhibit supernumerary organs contained within the four primary whorls. C, Strong *ag-1* mutant flowers are indeterminate, with numerous supernumerary whorls of organs and homeotic transformations of reproductive organs into perianth ones. D, Weak *ag-4* mutant flowers are also indeterminate, with numerous supernumerary whorls of stamens and sepals. E, *sup* mutant flowers display a limited number of supernumerary whorls of stamens and sometimes staminoid carpels in the center. F, Strong *clv* and some *ult1* mutant flowers display supernumerary organs in each primary whorl but also exhibit a weak loss of FM termination, with a limited number of supernumerary whorls within the gynoecium. G, *sup ag-1* double mutant flowers display an indefinite number of petals inside the first whorl of sepals. H, *sup ag-4* double mutants have fully indeterminate male flowers, with the two inner whorls replaced by an indeterminate number of stamens borne on a spiral. Red asterisks indicate totally indeterminate FMs.

Carles et al., 2005), showing that spatial and temporal alterations of the stem cell population are not exclusive.

This review deals with the molecular mechanisms that control FM termination, which are closely linked to that of floral organ identity. Most of the discussion will be focused on Arabidopsis, but there will be reference to work with other plant species when available evidence exists that either extends or challenges models established in Arabidopsis.

## AG IS THE MAIN DEVELOPMENTAL SWITCH TOWARD FM TERMINATION

AG Triggers FM Termination by Turning WUS Off at Stage 6

AG does not only control floral organs identity. Strong *ag* alleles (*ag-1* to *-3*) cause homeotic transformations of stamens into petals, but also a total loss of FM termination (Fig. 1C; Bowman et al., 1989, 1991; Yanofsky et al., 1990). Carpels are not simply transformed into sepals: instead the whole fourth whorl is replaced by a new flower bud that in turn develops into a new abnormal flower. Thus new flowers are indefinitely produced within the initial flower as stem cells are maintained in the center of the FM. Weaker *ag*

alleles (*ag-4* [Fig. 1D] and *AG-Met-205*) have few or no defects in floral organ identity, but still exhibit a total loss of FM termination (Sieburth et al., 1995).

AG's role in flower determinacy appears widely conserved among angiosperms (for review, see Ferrario et al., 2004). However, two *AG* orthologs are found in several species, and they sometimes underwent subfunctionalization. For instance, *PLE* alone appears necessary for sexual organ identity and FM termination in Antirrhinum: *ple* mutants display an *ag*-like phenotype (except that nested flowers arise inside the fourth whorl instead of the third in strong *ag* mutants), while *farinelli* (*far*) mutants are unaffected (Bradley et al., 1993; Davies et al., 1999).

AG disrupts stem cell maintenance within the FM by turning *WUS* expression off at stage 6 of flower development (Fig. 3): *WUS* mRNA becomes undetectable at this stage in wild-type flowers (Mayer et al., 1998), but remains continuously expressed in the everlasting FM of *ag* mutant flowers, a phenomenon that is both necessary and sufficient to oppose FM termination (Lenhard et al., 2001; Lohmann et al., 2001). It is worth noting that this effect is specific to *WUS*, as prolonged expression of another promeristematic gene, *STM*, contrary to that of *WUS*, does not affect FM termination (Lenhard et al., 2001). *WUS* repression by AG occurs at least partly non-cell auton-
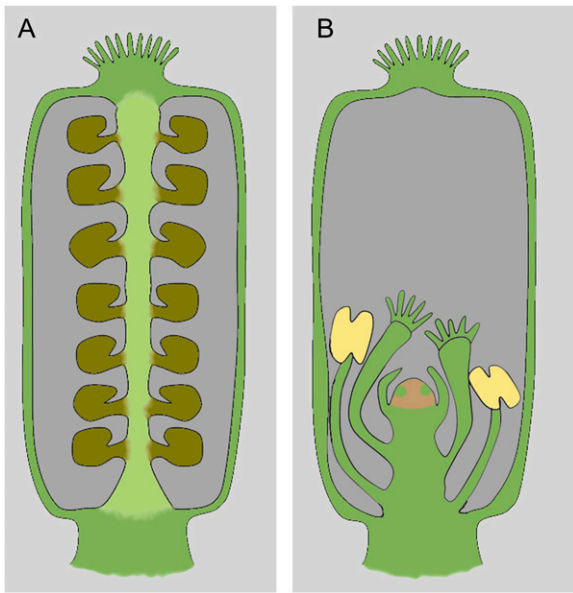
Figure 2. Siliques of wild-type and indeterminate flowers. A, Silique of a wild-type, determinate flower: Placenta develops between the fused carpels and bears ovules, and no floral organs are found within the gynoecium. B, Silique of an indeterminate flower, exhibiting supernumerary floral organs within the gynoecium, due to maintenance of the FM after stage 6; placenta usually develops (not shown), but at a much lesser extent than in wild type, and the seed set is greatly reduced due to the development of these supernumerary organs.

omously and is therefore probably indirect (Sieburth et al., 1998).

Interestingly, *AG* remains so far the only single gene strictly required for FM termination in Arabidopsis, making it the main switch toward stem cell arrest within the FM. However, a total loss of FM termination was more recently observed in several double mutants (Alvarez and Smyth, 1999; Prunet et al., 2008) and transgenic plants (Chen, 2004; Zhao et al., 2007), while some mutants, including *clv*, *ult1*, *perianthia* (*pan*), and *superman* (*sup*) only display a delay of FM termination, and produce a limited number of supernumerary whorls before stem cell maintenance eventually stops (Fig. 1, E and F; Schultz et al., 1991; Bowman et al., 1992; Clark et al., 1993, 1995; Kayes and Clark, 1998; Fletcher, 2001; Carles et al., 2004; Das et al., 2009; Maier et al., 2009). However, like in *ag* mutants, these phenotypes are always associated with a transient or persistent maintenance of *WUS* expression beyond stage 6, depending on the strength of the loss of FM termination (Schoof et al., 2000; Carles et al., 2004; Zhao et al., 2007; Prunet et al., 2008; Das et al., 2009; Maier et al., 2009).

FM Termination Requires AG Expression at High Levels and in the Very Center of the FM

A progressive decrease in AG levels triggers a wide range of phenotypes, from nearly normal flowers

without any homeotic conversion but with delayed FM termination—their gynoecium enclosing a secondary flower—to perfect phenocopies of strong *ag* mutant flowers (Mizukami and Ma, 1995; Chuang and Meyerowitz, 2000). Thus AG's three functions (promoting stamen and carpel identity and FM termination) are separable depending on the dose, and FM termination requires the highest amount of protein.

AG's functions can also be separated on a spatial basis. *pAP3::AG ag-3* transgenic plants, which lack functional AG in the whole fourth whorl, but still express it in the third, exhibit flowers with stamens but no carpel, and utterly lacking FM termination (Jack et al., 1997), showing the importance of *AG* expression in the fourth whorl to switch *WUS* expression. Moreover, the delay or loss of FM termination without alteration of floral organ identity observed in *clv*, *ult1*, and *pan* single mutants as well as in *crabs claw squint* (*crc sqn*) and *crc ult1* double mutants is associated with a transient or persistent defect in *AG* expression in an inner subdomain of whorl 4 (Clark et al., 1993; Fletcher, 2001; Prunet et al., 2008; Das et al., 2009;
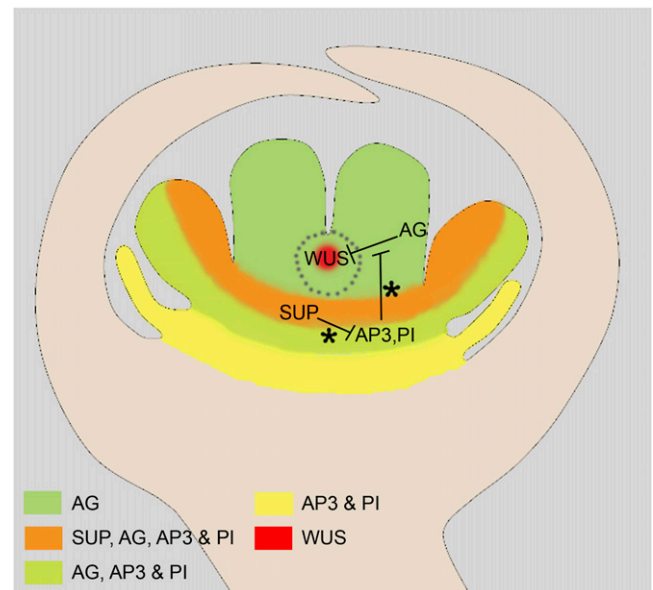
Figure 3. Main genetic pathways promoting FM termination. FM termination is due to the extinction of *WUS* expression at stage 6 of flower development, due to its repression by the MADS-box transcription factor AG. To perform this function, AG is required in a subdomain of whorl 4, marked off by gray dots. FM termination also requires the previous exclusion of AP3 and PI, which appear to oppose *WUS* repression by AG, from whorl 4. This exclusion is mediated by SUP. This may explain why AG, while expressed from stage 3 onwards, fails to shut *WUS* expression down before stage 6, when carpel primordia emerge. However, this delay was also proposed to be due to a progressive increase in *AG* expression level during flower development, a high dose of AG being specifically required for FM termination. This sketch represents a stage 6 flower bud, but it is worth noting that SUP's functions occur during earlier developmental stages, but condition the subsequent disruption of stem cell maintenance by AG. Asterisks indicate interactions that are still debated.

Maier et al., 2009). A similar, persistent defect of AG expression in the very center of the fourth whorl is also seen in the totally indeterminate flowers of transgenic plants constitutively expressing a miR172-resistant version of *AG*'s repressor AP2 (*35S::AP2m3*; Zhao et al., 2007). AG therefore appears to be required specifically in the very center of the FM (Fig. 3), where *WUS* is expressed, to switch it off and arrest stem cell maintenance.

FM Termination Relies on a Complex, Multilevel Activation of AG

Other mutations causing defects in FM termination were described, most of which affect genes encoding direct or indirect posttranscriptional activators of AG, thus unraveling the complex, multilevel regulation of this gene.

A modifier screen for enhancers of the weak *ag-4* allele led to the identification of HUA1 and 2 (Chen and Meyerowitz, 1999; Li et al., 2001). Both proteins, together with HUA ENHANCER (HEN) 2 and 4 (that were themselves identified in a screen for enhancers of *hua1 hua2*) are involved in the control of *AG* pre-mRNA correct splicing (Cheng et al., 2003).

Mutation of *HEN1*, which encodes a protein involved in microRNAs biosynthesis, causes pleiotropic phenotypes and sometimes triggers a strong loss of FM termination in a *hua1 hua2* background, while disruption of DICER LIKE1 (DCL1, also named CARPEL FACTORY), which also acts in the miRNAs processing pathway, has an even stronger effect on FM termination (Jacobsen et al., 1999; Chen et al., 2002; Park et al., 2002). Both proteins are necessary for the accumulation of miR172, which in turn enables *AG* activation by down-regulating AP2 (Chen et al., 2002; Aukerman and Sakai, 2003; Chen, 2004). Interestingly, the increase in AP2 protein level in *hen1* and *dcl1* plants is similar to that in miR172-resistent *AP2* over-expressing lines, but their flowers exhibit a much weaker loss of FM termination (Jacobsen et al., 1999; Chen et al., 2002; Chen, 2004; Zhao et al., 2007). This could be due to a defect in the accumulation of other miRNAs in *hen1* and *dcl1* plants, some of them having an antagonistic role to that of miR172 (Chen, 2004). Indeed, another family of miRNA, miR169, was shown to dampen the expression of *AG* orthologs in whorls 3 and 4 of Antirrhinum and petunia (*Petunia hybrida*) by repressing *AG* activators (Cartolano et al., 2007). Thus the weak loss of FM termination of *hen1* and *dcl1* flowers may be due to the lack of miR169 that would counterbalance that of miR172.

Finally, to carry on its functions, AG also needs to interact with SEP proteins. Indeed, flowers from plants mutant simultaneously for three *SEP* genes still display a normal *AG* expression pattern, but entirely lack FM termination (Pelaz et al., 2000; Ditta et al., 2004). A similar commitment of class E proteins in FM termination was described in several other species, including petunia, gerbera (*Gerbera hybrida*), and tomato

(*Solanum lycopersicum*; Angenent et al., 1994; Pnueli et al., 1994; Vandenbussche et al., 2003; Uimari et al., 2004). It is worth noting that in both petunia and rice, ovule identity (also called class D) genes also appear to promote FM termination (Ferrario et al., 2006; Dreni et al., 2007). This raises the possibility of a similar but yet uncharacterized role of ovule identity genes in Arabidopsis, which could partly account for the delay between the onset of *AG* expression and *WUS* repression (Ferrario et al., 2006). Conversely, the requirement of class D proteins in FM termination could be specific to species with central placentation: In petunia and rice, the placenta and ovule, respectively, directly arise from the FM, which therefore terminates slightly later than in Arabidopsis flowers, at a stage corresponding to placenta/ovule rather than carpel initiation (discussed by Colombo et al., 2008).

FM TERMINATION IS CLOSELY LINKED TO THE FEMALE DEVELOPMENTAL PROGRAM

Control of FM Termination by SUP Is Independent of AG Transcription, But May Be Mediated by Class B Gene Repression

SUP, a C2H2 zinc-finger transcription repressor (Sakai et al., 1995; Hiratsu et al., 2002), also regulates floral organ number. Its mutation triggers the development of extra whorls of stamens inside the third whorl, usually at the expense of the gynoecium, which is often missing or replaced by staminoid carpels (Fig. 1E; Schultz et al., 1991; Bowman et al., 1992), although some alleles of *sup* also exhibit a mild increase in carpel number (Jacobsen and Meyerowitz, 1997; Rohde et al., 1999). Mutation of the ortholog of *SUP* in petunia triggers a similar phenotype, but also affects other aspects of flower development (Nakagawa et al., 2004), suggesting that SUP function in flower development is conserved to some extent. The *octandra* (*oct*) mutation (whose mapping has been elusive so far) also exhibits a *sup*-like phenotype in Antirrhinum, but interestingly, the *far ple* double mutant exhibits a phenotype similar to that of *ag sup* double mutant in Arabidopsis, showing that *AG* orthologs share OCT's role in this process (Davies et al., 1999).

Yet the origin of the *sup* phenotype remains a debated matter. It was first proposed that the *sup* phenotype is heterochronic, the switch from male program to female program being delayed. Thus, the FM seems transiently stuck in developmental time and keeps producing stamens instead of generating carpels and disrupting stem cell maintenance (compare Fig. 4, C and D; Schultz et al., 1991; Bowman et al., 1992). This first model suggests that SUP indirectly promotes FM termination. Interestingly, however, *AG* expression pattern remains unaltered in *sup* mutant flowers (Bowman et al., 1992), and no alteration of AG function has been shown so far. Indeed, the *sup* phenotype may be mediated by class B genes. The pro-
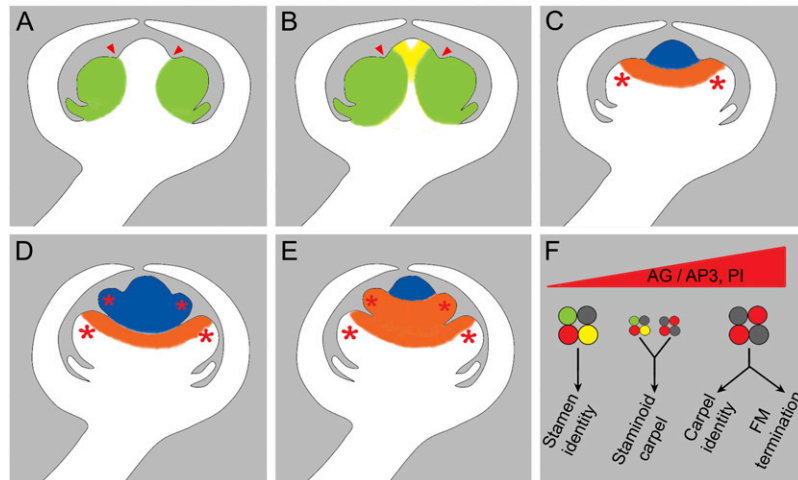
Figure 4. Control of flower determinacy by SUP and class B proteins. A and B, Effects of the *sup-1* mutation on class B genes expression pattern. Green staining marks simultaneous expression of *AP3* and *PI*; yellow staining marks that of *PI* alone. Red arrowheads mark the boundary between whorls 3 and 4. A, mRNA distribution pattern of class B genes *AP3* and *PI* in a wild-type flower at stage 5. At that stage, *AP3* and *PI* are coexpressed in the third whorl, but are excluded from the fourth whorl. B, mRNA distribution pattern of class B genes *AP3* and *PI* in a *sup-1* mutant flower at stage 5. Compared to wild type, expression of *AP3* and *PI* is expanded in the fourth whorl, toward the FM center. *PI* mRNA expansion appears wider than that of *AP3*. C to E, Two models of production of supernumerary stamens in *sup-1* mutant flowers. The sketches represent *sup-1* mutant flowers at stage 5 (C) and 7 (D and E). Different colors are used to follow the evolution of two cell populations, which correspond to two distinct domains of the flower at stage 5, during flower development. Red asterisks mark stamen primordia. C, Blue staining marks the center of the flower, which at this stage corresponds to the FM. Orange staining marks the inner part of whorl 3, which corresponds to *SUP* expression domain. D, First model: supernumerary stamens are produced from the FM whose termination is delayed. The identity of these supernumerary organs results from the ectopic expression of class B genes in the fourth whorl. E, Second model: supernumerary stamens are produced from the inner part of whorl 3 that overproliferates. Conversely, proliferation within the FM is strongly reduced. The broadening of *AP3* and *PI* mRNA distribution results from an overproliferation of class B-expressing cells rather than from an ectopic expression of these genes. F, Model of competition between B and C proteins to form MADS-box transcription factors complexes. The balance between class B and C proteins directs which MADS-box protein complexes are formed, and which developmental program subsequently takes place. MADS-box proteins are symbolized by colored circles; red, AG; green, AP3; yellow, PI; gray, SEP. For practical reasons, protein complexes are represented as heterotetramers, according to the quartet model (Theissen and Saedler, 2001), though their exact stoichiometry remains unknown. Quartet size indicates their relative amounts.

duction of extra whorls of stamens in *sup* mutant flowers is associated with an expansion of *AP3* and *PI* expression toward the center of the FM (compare Fig. 4, A and B; Bowman et al., 1992; Goto and Meyerowitz, 1994). Additionally, the class B genes seem to be necessary for the establishment of the *sup* phenotype: *AP3* and *PI* were first described as epistatic to *SUP*, as *ap3 sup* and *pi sup* double mutant flowers are very similar to single *ap3* and *pi* flowers (Schultz et al., 1991; Bowman et al., 1992). Corroborating these data, flowers of plants overexpressing *AP3* alone (*p35S::AP3*) or together with *PI* (*p35S::AP3/PI*), as well as plants overexpressing *AP3*'s activator *UNUSUAL FLORAL ORGAN* (*p35S::UFO*) phenocopy the *sup* phenotype (Jack et al., 1994; Krizek and Meyerowitz, 1996; Lee et al., 1997). On the other hand, *ap3* or *pi* mutant flowers are often overdeterminate: Third whorl stamens are converted to carpels, but the overall number of organs in the two inner whorls is fewer than in wild-type flowers (2.7 carpels per flower on average in *pi-1*, instead of eight stamens and carpels; Bowman et al., 1989, 1991). Overdeterminacy is even more obvious in

flowers of Antirrhinum mutant for either *DEFICIENS* or *GLOBOSA*, the orthologs of *AP3* and *PI*, which entirely lack a fourth whorl (Sommer et al., 1990; Trobner et al., 1992). Thus, the class B genes *AP3* and *PI* appear to oppose FM termination by *AG* (Fig. 3), and their exclusion from the fourth whorl by *SUP* appears necessary for floral determinacy.

Alternatively, SUP was proposed to control the balance of cell proliferation between whorls 3 and 4 (Sakai et al., 1995; Sakai et al., 2000). This second model stipulates that SUP represses cell divisions in the inner third whorl where it is expressed, thus preventing the formation of extra whorls of stamens (compare Fig. 4, C and E). The reduction or disappearance of the gynoecium observed in the *sup* mutants would then be an indirect effect of an overproliferation in whorl 3, at the expense of whorl 4. There are several arguments in favor of this second model: *SUP* is not expressed in the fourth whorl, where it is supposed to repress the class B genes; instead, its expression pattern overlaps with that of *AP3* and *PI* in the inner third whorl (compare Fig. 4, A and C; Sakai et al., 1995). Also, *AP3*

and *PI* are still excluded from the very center of the FM in *sup* flowers (Fig. 4B), suggesting that the spatial extension of their expression pattern could be due to an excess of proliferation of *AP3*- and *PI*-expressing cells from inner whorl 3, rather than from an ectopic expression in whorl 4 (Sakai et al., 1995). Indeed, SUP's ability to repress cell divisions was confirmed by constitutive expression of *SUP* in Arabidopsis, tobacco (*Nicotiana tabacum*), and rice, which results in dwarfed plants with smaller flowers than wild type (Nandi et al., 2000; Bereterbide et al., 2001; Hiratsu et al., 2002), while local overproliferation was found in petunia flowers mutant for the ortholog of *SUP* (Nakagawa et al., 2004).

Interestingly, these two models are not exclusive. Indeed, constitutive expression of *SUP* in rice does not only reduce cell proliferation, it sometimes causes replacement of third whorl stamens by stamen-carpel mosaic organs, a phenotype associated with a reduced expression of *OsMADS2*, a rice *PI* ortholog (Nandi et al., 2000). Similarly, in Arabidopsis, ectopic expression of *SUP* under the control of the *AP1* promoter (*pAP1::SUP*) causes both a decrease in overall floral organ number and transformations of petals into sepals and stamens into carpels (Yun et al., 2002). The decrease in floral organ number is likely to be due to repression of cell proliferation in the *pAP1::SUP* lines, but the changes in petal and stamen identity is symptomatic of a defect in B function. Indeed, it was shown that both *AP3* and its activator *UFO* are down-regulated in *pAP1::SUP* flowers compared to wild type (Yun et al., 2002). SUP is thus apparently able to repress both cell proliferation and *AP3* and *PI* expression.

However, according to the second, proliferation-based model, the balance of cell divisions between whorls 3 and 4 is affected in *sup* mutant flowers, inferring that the extra stamens result from an overproliferation of the inner part of whorl 3 at the expense of the FM center, which stops proliferating. Nonetheless, in both *sup* and wild-type flowers, cells incorporate BrdU on both sides of the boundary between whorl 3 and FM center, indicating that the latter is still proliferating (Breuil-Broyer et al., 2004). Additionally, the delay in FM termination in *sup* mutant flowers has recently been associated with transient maintenance of *WUS* expression beyond stage 6 (Prunet et al., 2008), suggesting that the FM keeps functioning normally while the extra whorls of stamens are produced. Also, the occasional fasciation of *ag-1 sup-1* double mutant flowers (Bowman et al., 1992) indicates that *SUP* represses cell divisions in the FM center, and not only in whorl 3. It is therefore unlikely that the extra whorls of stamens in the *sup* mutant are produced only from an excess of cell proliferation within whorl 3.

Given that *SUP* does repress the class B genes, and the strong similarities between the *sup* and *p35S::AP3/PI* flowers, it is likely that their phenotype results at least partly from the same process, that is, an ectopic expression of *AP3* and *PI* in the center of the flower, which somehow opposes FM termination (Fig. 3).

AP3 and PI May Antagonize AG's Fourth Whorl Functions by Competing with AG and SEP to Form MADS-Box Protein Complexes

With the exception of the occasional fasciation, which may result from SUP's role (independent of B and C genes) in controlling proliferation within the FM, FM termination is not more affected in *ag-1 sup-1* than in *ag-1* flowers (Bowman et al., 1992). Similarly, ectopic expression of both *AP3* and *PI* delays FM termination in a wild-type *AG* context, but does not enhance the indeterminacy phenotype of *ag* mutant flowers (Krizek and Meyerowitz, 1996). This epistasy of *ag* to *sup* and *p35S::AP3/PI* regarding FM termination suggests that the effects of mutations in *SUP* or the ectopic expression of class B genes on FM termination depends on *AG*. Indeed, ectopic AP3 and PI in whorl 4 of *sup* and *p35S::AP3/PI* flowers may repress AG function in this region. As *AG* mRNA expression pattern remains unaltered in *sup* mutant flowers (Bowman et al., 1992), this putative repression is likely to occur at a posttranscriptional level.

Floral organ identity within the third and fourth whorls is thought to rely on two different complexes of MADS-box transcription factors. The former includes AG and SEP together with AP3 and PI, while the latter, which likely promotes FM termination also, is composed of AG and SEP without AP3 and PI (Goto et al., 2001; Theissen, 2001; Theissen and Saedler, 2001; Krizek and Fletcher, 2005). It is therefore tempting to propose that class B genes antagonize AG's fourth whorl functions by competing in the formation of the aforementioned complexes (Fig. 4F). The balance between AP3/PI and AG would then determine whether the FM shifts toward the female program and terminates.

Interestingly, this hypothesis is consistent with the dose-dependent effects of ectopic expression of class B genes, with an increase in stamen number observed between *p35S::AP3*, *sup1*, and *p35S::AP3 sup1* flowers, respectively (Jack et al., 1994), and between *p35S::AP3*, *sup1*, *p35S::AP3/PI*, and *p35S::AP3/PI sup1* flowers (Krizek and Meyerowitz, 1996). Conversely, *ag-1/AG sup-2* and *ag-4 sup-1* flowers are totally indeterminate male flowers, consisting in an indefinite number of stamens produced inner to whorl 2 by an everlasting FM (Fig. 1H; Schultz et al., 1991; Prunet et al., 2008), suggesting that the defect in FM termination of *sup* mutant flowers is enhanced by a reduced dose of functional AG. Another strong evidence in favor of a competition for the formation of complexes of MADS-box transcription factors is provided by the phenotype of *pAP3::WUS* transgenic flowers. As a result of *AG* activation by WUS, *AG* expression level is increased in the third whorl, causing stamens to become carpelloid (Lohmann et al., 2001). Thus, increasing the dose of AG within a domain where it is normally already present together with both AP3 and PI is sufficient to promote the female program, at the expense of the male one.

Whatever the exact molecular basis of the inhibition of FM termination by AP3 and PI, it is worth noting that this process is closely linked to the female developmental program: In wild-type flowers, FM termination coincides with female organs initiation. In *sup* mutant flowers also, FM termination is usually associated with the production of carpelloid organs (Schultz et al., 1991; Bowman et al., 1992). *AG* is the master gene promoting FM termination, yet it controls both male and female programs within the flower, and appears unable to disrupt stem cell maintenance while the FM is still producing male organs. Exclusion of class B genes from the fourth whorl is responsible for the shift from male to female program, and allows FM termination to occur.

CRC, a Gene Involved in the Female Program, also Controls FM Termination

Other mutants confirm the close link between female program and FM termination. Mutations in *CRC* and *SPATULA* (*SPT*) affect both growth and congenital fusion of carpel primordia (Alvarez and Smyth, 1999). *CRC* and to a lesser extent *SPT* also have a role in FM termination, as *crc spt* double mutant flowers sometimes produce supernumerary whorls of stamens and carpels within the gynoecium. This loss of determinacy is even stronger in *crc AG/ag-1* flowers (Alvarez and Smyth, 1999). Thus, CRC, which specifically controls the female developmental program and is expressed from stage 6 on (Bowman and Smyth, 1999), also shares AG's function in FM termination, hence confirming the close link between these two processes. In the case of *CRC*, both functions seem to be widely conserved in angiosperms, as orthologs of *CRC* in various species display similar expression patterns in carpel primordia and loss-of-function phenotypes (Nagasawa et al., 2003; Yamaguchi et al., 2004; Fourquin et al., 2005; Lee et al., 2005; Orashakova et al., 2009). In rice, tobacco, petunia, and California poppy (*Eschscholzia californica*), this loss of function alone is sufficient to cause a strong loss of FM termination, suggesting that CRC orthologs play a more important role in this process in most species compared to Arabidopsis (Yamaguchi et al., 2004; Lee et al., 2005; Orashakova et al., 2009).

CRC is a YABBY transcription factor expressed in developing carpels, but neither in stamens nor in the FM center, suggesting that it modulates stem cell maintenance in a non-cell-autonomous fashion (Bowman and Smyth, 1999). *CRC* expression is directly activated by AG (Bowman and Smyth, 1999; Gomez-Mena et al., 2005). Therefore, part of AG function in promoting carpel development and FM termination could be mediated by the downstream action of CRC. The possibility that *CRC* could in turn activate *AG* in a positive feedback was suggested (Gomez-Mena et al., 2005), but no direct evidence supports it so far. Indeed, *CRC* is expressed in floral organs in the absence of *AG* when *AP2* is also mutated, and confers on them some carpelloid features, showing that both CRC activation and function are at least partially independent of AG (Alvarez and Smyth, 1999; Bowman and Smyth, 1999).

How could CRC promote FM termination independently of *AG*? Several members of the YABBY gene family exhibit complex interactions with meristematic genes including KNOX I genes, *WUS* and *CLV3* (Sawa et al., 1999; Siegfried et al., 1999; Kumaran et al., 2002; Goldshmidt et al., 2008). However, whether similar interactions are responsible for CRC's function in FM termination remains unclear, as *YAB2*, which is not well characterized so far, is the only YABBY gene able to complement *crc-1* (Meister et al., 2005; Fourquin et al., 2007).

CONCLUSION

Most angiosperm flowers consist of a determinate number of organs organized in a precise, conserved architecture. In Arabidopsis, the genetic bases of flower determinacy have been progressively unraveled using mutants showing an increase in floral organ number, due to either spatial or temporal defects in the FM. FM termination—the arrest of stem cell maintenance at the appropriate moment in the course of flower development—is of major importance for the generation of determinacy, and relies on a regulatory network centered on the single transcription factor, AG. Consistent with this central role, AG's expression and function are tightly controlled by numerous factors, including activators within the two inner whorls, and inhibitors in the outer whorls. This complex regulation results in a close association between FM termination and the female developmental program: AG is only able to turn off stem cell maintenance after the shift from the male to the female program, when class B genes have been excluded from the center of the flower. This exclusion is likely due to the action of SUP. Despite recent advances in our understanding of the spatial and temporal control of FM termination, one major question remains unanswered. AG promotes FM termination by indirectly repressing *WUS* (Sieburth et al., 1998; Lenhard et al., 2001; Lohmann et al., 2001) but no intermediate between AG and *WUS* has been found so far. However, *GA4*, a gene involved in the biosynthesis of gibberellins, has recently been identified among the targets of AG (Gomez-Mena et al., 2005). Gibberellins oppose meristem activity (for review, see Shani et al., 2006), raising the possibility that AG promotes FM termination by modifying phytohormone levels within the FM (discussed by Sablowski, 2007b). Clarifying the roles of SUP, CRC, and phytohormones appears as the next frontier in a better understanding of this developmental process.

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