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Building Beauty: The Genetic Control of Floral Patterning

Review

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Floral organ identity is controlled by combinatorial action of homeotic genes expressed in different territories within the emerging flower. This review discusses recent progress in our understanding of floral homeotic genes, with an emphasis on how their region-specific expression is regulated.

Although flowers appear in a stunning diversity of forms, from the intricate and beautiful to the simple and inconspicuous, their basic plan is remarkably invariant across all species. The flowers of dicots, which represent one of the two major subdivisions of flowering plants and include the reference plant *Arabidopsis thaliana*, are organized into four concentric rings of organs, termed whorls (Figures 1A and 1B). The outer two whorls are occupied by sterile organs, with the normally green sepals that protect the emerging flower bud in the first whorl and the often showy and colorful petals that can serve to attract pollinators in the second whorl. The inner two whorls are devoted to reproduction, the central purpose of flower formation. Stamens, the male reproductive organs that produce pollen, are found in the third whorl, while the central fourth whorl is occupied by carpels, the female reproductive organs, which are normally fused to form the gynoecium (Figure 1A). After fertilization, the gynoecium develops into the fruit harboring the seeds. Organ number in the different whorls is typically fixed; in *Arabidopsis*, there are four sepals, four petals, six stamens, and two carpels (Figure 1B).

Flowers develop from primordia that arise on the flanks of the shoot apical meristem, a self-regulating population of undifferentiated cells that forms the growth point of the plant. Initially, the floral primordium is organized in a similar manner to the shoot apical meristem, with a central group of stem cells. For simplicity, the young floral primordium, before the emergence of floral organ primordia, is often called a floral meristem. After a few days, sepal primordia arise, followed by petal and stamen primordia. The floral meristem is consumed by the formation of the central carpels, which either arise fused or fuse shortly after they emerge.

During floral patterning, several processes need to occur coordinately, including the proper positioning of floral organs and specification of their identity in a position-dependent manner. Among these, most is known about the genetic and molecular control of floral organ identity, and here we summarize what has been learned about the mechanisms underlying this process. Because at this point there is a very large number of original publications in this field, we have cited reviews for most of the work published before the mid-1990s.

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The ABCs of Flower Development

Contemporary work on floral patterning began with the study of a series of mutants in which floral organs develop normally, but in the inappropriate whorl. Such mutants had been collected from garden snapdragon, *Antirrhinum majus*, by Hans Stubbe, and from the mustard relative *Arabidopsis thaliana* by Maarten Koornneef. In the late 1980s, three groups, headed by Enrico Coen in the United Kingdom, Elliot Meyerowitz in the United States, and Heinz Saedler in Germany, recognized the value of these mutants as homeotic mutants, and used them to initiate molecular and genetic studies of floral patterning. The initial genetic studies quickly led to proposal of the ABC model, now considered a milestone in plant developmental biology (Bowman et al., 1991; Coen and Meyerowitz, 1991). Based on phenotypic and genetic analyses, the model states that development of the four types of floral organs is governed by overlapping activities of three classes of regulatory genes. Termed A, B, and C, each class of genes is active in two adjacent whorls (Figure 1C). Activity of A class genes alone leads to formation of sepals in the first whorl, while combining their activity with that of B class genes promotes the formation of petals in the second whorl. Similarly, the combination of B and C class activity is required for stamen formation in the third whorl, while C class genes by themselves control formation of carpels in the fourth whorl.

To account for mutant phenotypes, the ABC model included another tenet, namely that A and C class activity are mutually exclusive and repress each other, since A and C class mutants are essentially mirror images of each other. In A class mutants, C class activity expands into all whorls, with sepals being replaced by carpels, and petals by stamens. Conversely, in C class mutants, A class activity expands into whorl three and four. In addition, the flower becomes indeterminate in C class mutants, that is, it no longer produces a limited number of organs, and new flowers form inside the original flower, giving rise to a flower consisting of (sepals, petals, petals)$_n$. Expression of B class genes is not affected by mutations in either A or C class genes. Therefore, inactivation of B class genes causes second whorl organs to adopt the same fate as first whorl organs, and third whorl organs the same as fourth whorl organs, giving rise to flowers consisting of sepals, sepals, carpels, carpels.

The original genes of the B and C classes turned out to be orthologs in *Antirrhinum* and *Arabidopsis* (Table 1). C class activity was initially represented by a single gene, *PLENA* (*PLE*) in *Antirrhinum* and its ortholog *AGAMOUS* (*AG*) in *Arabidopsis*. B class activity requires a pair of related genes in both species, *DEFICIENS* (*DEF*)/*GLOBOSA* (*GLO*) in *Antirrhinum* and *APETALA3* (*AP3*)/

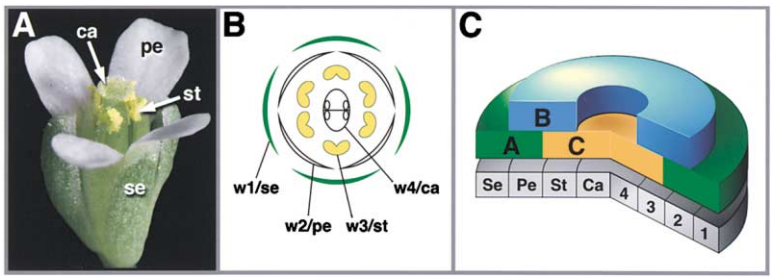

Figure 1. The Basics of Flower Development

(A) Mature *Arabidopsis* flower with sepals (se), petals (pe), stamens (st), and carpels (ca).

(B) Floral formula indicating whorls one to four (w1–4).

(C) Diagram of ABC model, indicating domains of ABC gene activities.

PISTILLATA (*PI*) in *Arabidopsis*. In contrast, the canonical A class gene *APETALA2* (*AP2*) from *Arabidopsis* has no direct counterpart in *Antirrhinum*, where A class activity was only represented by dominant mutations *ovulata* and *macho*, which later turned out to be gain-of-function alleles of the C class gene *PLE* (Weigel and Meyerowitz, 1994; Theissen et al., 2000; Zhao et al., 2001a).

Although the ABC model proposed that the homeotic genes are only active in specific whorls, genetic analysis alone could not tell how their activity was regulated. Cloning of the ABC genes with subsequent expression and promoter studies revealed that regulation occurs mainly at the level of transcription, as the promoters of homeotic genes are predominantly active in those whorls where their function is required. An exception is the A class gene *AP2*, which is expressed uniformly in all whorls. *AP2* is also unusual in that it is the only floral homeotic gene that does not encode a MADS domain transcription factor. Subsequently, it was discovered that one MADS box gene, *APETALA1* (*AP1*), has dual roles: it acts during early stages of flower development redundantly with other factors to specify floral identity,

Table 1. Early Floral Patterning Genes

Arabidopsis	*Antirrhinum*	Gene Product
Meristem identity		
LEAFY (*LFY*)	*FLORICAULA* (*FLO*)	DNA binding, plant-specific
APETALA1 (*AP1*)	*SQUAMOSA* (*SQUA*)	MADS domain
B class regulators		
UNUSUAL FLORAL ORGANS (*UFO*)	*FIMBRIATA* (*FIM*)	F box
SUPERMAN (*SUP*)	*OCTANDRA* (*OCT*)?	Zinc finger
?	*CHORIPETALA*	?
?	*DESPENTEADO*	?
C class regulators		
WUSCHEL	?	Homeodomain
General ABC repressors		
CURLY LEAF (*CLF*)	?	Polycomb group (Enhancer of zeste)
INCURVATA2 (*ICU2*)	?	?
EARLY BOLTING IN SHORT DAYS (*EBS*)	?	?
EMBRYONIC FLOWER1 (*EMF1*)	?	Plant-specific, nuclear?
EMBRYONIC FLOWER2 (*EMF2*)	?	Polycomb group (Suppressor of zeste 12)
General ABC activators		
	POLYPETALA (*POLY*)	?
ABC genes		
A class		
APETALA1 (*AP1*)	—	MADS domain
APETALA2 (*AP2*)	?	AP2 domain
AINTEGUMENTA	?	AP2 domain
LEUNIG (*LUG*)	?	Tup1-like corepressor, WD40 repeats
STERILE APETALA (*SAP*)	?	Plant-specific, nuclear?
?	*STYLOSA* (*STY*)	?
?	*FISTULATA* (*FIS*)	?
B class		
APETALA3 (*AP3*)	*DEFICIENS* (*DEF*)	MADS domain
PISTILLATA (*PI*)	*GLOBOSA* (*GLO*)	MADS domain
C class		
AGAMOUS (*AG*)	*PLENA* (*PLE*) & *FARINELLI* (*FAR*)	MADS domain
CRABS CLAW (*CRC*)	?	YABBY domain
SPATULA (*SPT*)	?	bHLH domain
HUA1	?	Plant-specific, nuclear?
HUA2	?	RNA binding domain
ABC cofactors		
SEPELLATA1-3	?	MADS domain

Question marks indicate that an orthologous mutant has not been described; the dash indicates that the most closely related gene does not have the same function.

and it contributes during later stages to A function. Consistent with these two roles, *AP1* RNA is initially expressed throughout the flower, but becomes restricted to the A domain during later stages (Weigel and Meyerowitz, 1994; Theissen et al., 2000; Zhao et al., 2001a). However, *ap1* mutants, while defective in sepal and petal development, do not have as clear a homeotic phenotype as *ap2* mutants. Moreover, the homeotic function of *AP1* does not seem to be conserved in *Antirrhinum* (Theissen et al., 2000). Several other *Arabidopsis* and *Antirrhinum* genes that contribute to A function have now been described; they are discussed in more detail in the section on regulation of C function.

Cloning of the ABC genes also allowed for validation of the ABC model using gain-of-function experiments with transgenic plants. With the exception of *AP1*, ectopic expression of ABC genes leads to the formation of flowers that have phenotypes opposite to those observed in the respective loss-of-function mutants. For example, constitutive overexpression of both *AP3* and *PI* leads to the formation of flowers in which the first whorl is occupied by petals instead of sepals and the fourth whorl carpels are replaced by stamens (Krizek and Meyerowitz, 1996). Results from these experiments not only confirmed the predictions made by the ABC model concerning organ identity, but also corroborated the idea that regulation of ABC gene activity occurs mainly at the level of transcription.

The ABCs Begin with A…

A question that is central to our understanding of floral patterning is how the pattern of ABC gene expression is set up. Formally, the formation of individual flowers is downstream of floral induction, the process that underlies the transition from vegetative to reproductive development. One of the genes integrating the multiple endogenous and environmental signals that regulate the timing of floral induction is the meristem identity gene *LEAFY* (*LFY*), the *Arabidopsis* ortholog of *FLORICAULA* (*FLO*) from *Antirrhinum* (Blázquez and Weigel, 2000). Expression of ABC genes is much reduced or absent in *lfy* and *flo* mutants, in which flowers are replaced by shoot-like structures, but until recently it was unclear whether ABC genes were directly controlled by LFY and FLO (Weigel and Meyerowitz, 1994; Theissen et al., 2000; Zhao et al., 2001a).

Both FLO and LFY are expressed uniformly in young floral primordia as soon as these arise. The first hint that they might be direct regulators of floral homeotic genes came from the observation that constitutive ectopic expression of *LFY* not only causes plants to flower early, as expected from its role in floral induction, but also induces ectopic expression of the A class gene *AP1* (Parcy et al., 1998). Induction of *AP1* by LFY does not require protein synthesis, as shown with plants that constitutively express a hormone-regulated version of LFY (Wagner et al., 1999). Furthermore, fusion of LFY to a heterologous activation domain allows it to activate a reporter gene that is under the control of *AP1 cis*-regulatory sequences in yeast (Parcy et al., 1998), providing further evidence that the interaction is direct. In wild-type, *AP1* is activated shortly after *LFY* throughout the emerging floral primordium, in a pattern very similar to that of *LFY*. However, although *LFY* is an important regulator of *AP1*, *AP1* activation is merely delayed, not abolished, in *lfy* mutants, indicating that redundant factors contribute to *AP1* activation (Liljegren et al., 1999).

…Then Comes B…

The picture of initial activation is more complex for B and C class genes, which require region-specific regulators for their expression. The investigation of B class genes *AP3* and *PI* as possible *LFY* targets seemed most promising, as their expression is much more reduced in strong *lfy* mutants than that of the A class gene *AP1* or the C class gene *AG*. However, despite this observation, it is still unclear whether LFY is a direct activator of B class genes. The first indication for interaction of *LFY* with region-specific coregulators in the activation of ABC genes came from an analysis of another gene required for B class gene expression, *UNUSUAL FLORAL ORGANS* (*UFO*). Unlike *LFY*, which is expressed throughout the young flower, *UFO* is expressed transiently in the flower in a domain similar to that of *AP3* and *PI* (Figure 2). In addition, *UFO* is expressed in the shoot apical meristem in a pattern that mimics that in the floral meristem, being excluded from the center and the periphery of the meristem (Lee et al., 1997). The interaction of *UFO* and *LFY* was most strikingly demonstrated by their ability to activate *AP3* and *PI* outside the flower, when both *UFO* and *LFY* are ectopically expressed (Parcy et al., 1998; Honma and Goto, 2000). Overall, based on these observations, it seems that region-specific expression of B class genes results from the interplay of LFY, which provides floral specificity, with *UFO*, which provides regional specificity within meristems.

Despite their strong gain-of-function effects, neither *LFY* nor *UFO* is absolutely required for B class gene expression. A candidate for another, possibly direct, activator of B class genes is *AP1*, which functions not only as a homeotic gene, but also as a floral identity gene. Ectopic *AP3* expression has been observed both in plants that express *AP1* ectopically and in plants that express an activated form of *AP1*, *AP1:VP16*, in the normal *AP1* domain (Sessions et al., 2000; Ng and Yanofsky, 2001). A direct role of AP1 in regulating *AP3* is further supported by the finding that AP1 binds to the *AP3* promoter and that the binding site is required for normal activity of this promoter (Hill et al., 1998; Tilly et al., 1998).

In contrast to B class activators LFY and AP1, UFO is not a DNA binding protein, but belongs to the family of F box proteins, many of which have been shown to provide substrate specificity to a class of E3 ubiquitin ligases known as SCFs (Samach et al., 1999). UFO interacts both in vitro and in vivo with another common SCF subunit, the SKP1 homolog ASK1, supporting the proposal that UFO acts by controlling the ubiquitination of *AP3* and *PI* regulators (Samach et al., 1999; Zhao et al., 2001b). The most common effect of ubiquitination is the targeting of proteins for proteasome-dependent degradation, and it is conceivable that *UFO* promotes degradation of an *AP3/PI* repressor, but ubiquitination can also regulate protein activity in other ways (e.g., Kaiser et al., 2000). An answer to the question of how

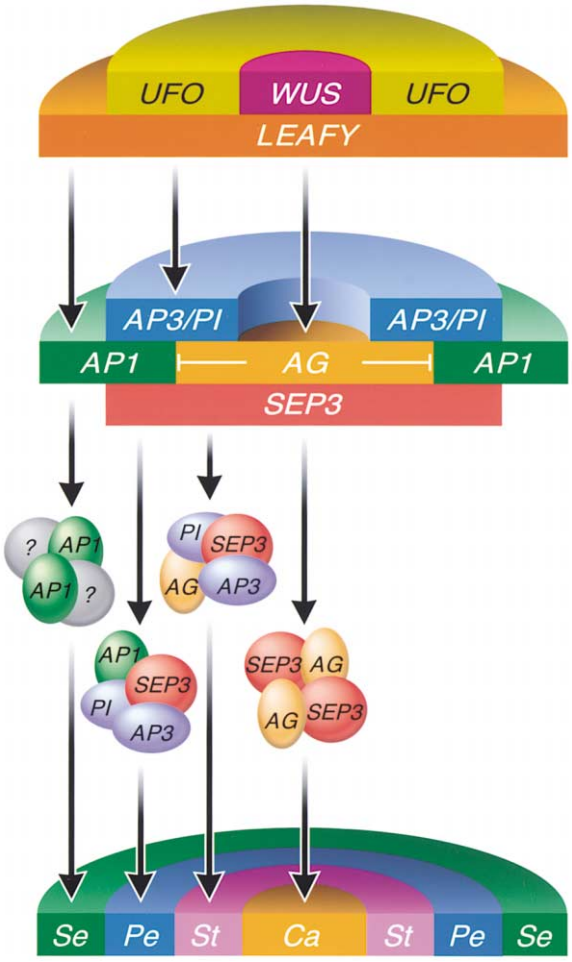

Figure 2. Flow Chart of Early Floral Patterning

Upstream regulators *LFY*, *WUS*, and *UFO* are expressed in specific domains, which, together with repression of *AP1* by *AG*, results in the ABC pattern. How the *SEP* pattern is regulated is not known. ABC gene products and SEP proteins, all of which are MADS domain proteins, assemble into higher order, most likely quaternary, complexes, which specify different organ identities. It is not known whether AP1 assembles into higher order complexes.

UFO acts may come from the investigation of two genes, *CHORIPETALA* and *DESPENTEADO*, which mediate the effects of the *UFO* homolog *FIMBRIATA* in *Antirrhinum* (Wilkinson et al., 2000).

…Followed by C

Arguably the most complete picture of ABC gene regulation has emerged for the C class gene *AG* and its *Antirrhinum* counterpart *PLE*. In line with the tenet of the ABC model that A and C function are mutually inhibitory, initial studies focused on repression of *AG* and *PLE* in the periphery of the flower. The importance of transcriptional repression was confirmed with the observation that *AG* RNA expands into the outer whorls of the A class mutants *ap2* and *leunig* (*lug*). However, *AG* is not only activated in a larger domain, but also earlier and more strongly in these mutants, suggesting that they are not merely region-specific repressors. Consistent with a broader role of the two genes, their expression is not restricted to the outer whorls of the developing flower (Jofuku et al., 1994; Conner and Liu, 2000). Both genes encode apparent transcription factors—*AP2* a member of a plant-specific class of DNA binding proteins and *LUG* a WD40 repeat protein with similarity to transcriptional repressors such as Tup1 from yeast or Groucho from *Drosophila*. Several regulatory elements that mediate repression of *AG* by *AP2* and *LUG* have been identified (Bomblies et al., 1999; Deyholos and Sieburth, 2000), but it is not known whether repression by *AP2* and *LUG* is direct. The same is true for *AINTEGUMENTA* (*ANT*) and *STERILE APETALA* (*SAP*; Table 1), both of which act redundantly with *AP2* in repressing *AG* and promoting organ identity in the outer whorls (Elliott et al., 1996; Klucher et al., 1996; Byzova et al., 1999; Krizek et al., 2000). Like *LUG*, *ANT* and *SAP* are expressed outside the flower and have other defects in addition to those resulting from *AG* misexpression. The most notable other role of *ANT* is in controlling organ initiation and organ size (Elliott et al., 1996; Klucher et al., 1996; Krizek, 1999; Mizukami and Fischer, 2000).

Another important negative regulator of *AG* is *CURLY LEAF* (*CLF*). *clf* mutant flowers have carpelloid sepals in the first whorl and staminoid petals in the second whorl, phenotypes reminiscent of *AG* derepression (Goodrich et al., 1997). In addition to ectopic expression in the flower, *AG* RNA is expressed widely in vegetative tissue of *clf* mutants. This vegetative expression of *AG* causes *clf* mutants to flower early, even though *AG* normally has no role in controlling flowering time. *CLF* itself is expressed throughout the plant and encodes a Polycomb group gene with closest similarity to *Enhancer of zeste* from *Drosophila*. Although Polycomb complexes have not yet been detected in plants, it is thought that the CLF product, like its animal counterparts, is involved in chromatin remodeling (Goodrich et al., 1997). Like Polycomb group proteins in animals, the primary role of CLF in the flower is maintenance, rather than establishment, of *AG* repression (Goodrich et al., 1997). Furthermore, there is weak ectopic *AP3* expression in *clf* mutants, pointing to a more general role of *CLF* in repressing homeotic genes (Goodrich et al., 1997; Serrano-Cartagena et al., 2000).

CLF acts redundantly with *INCURVATA2* (*ICU2*) in repressing *AG* in both flowers and vegetative tissue. *icu2* and *clf* single mutants have similar phenotypes, but double mutants show a much more severe phenotype, with carpelloid features on leaves along with *ap2*-like flowers (Serrano-Cartagena et al., 2000). It will therefore be interesting to learn whether *ICU2* also encodes a Polycomb group protein. Two other genes with more general roles in repressing a wide array of developmental regulators, including homeotic genes, are *EMBRYONIC FLOWER1* (*EMF1*) and *EMF2* (Aubert et al., 2001; Yoshida et al., 2001). *EMF2* is also a member of the Polycomb group genes and encodes a homolog of SU(Z)12 from *Drosophila* (Yoshida et al., 2001). Yet another repressor of *AG* and *AP3* is *EARLY BOLTING IN SHORT DAYS* (*EBS*), but, in contrast to the other genes discussed so far, expression of homeotic genes is only increased within their normal domains in *ebs* mutants (Gómez-Mena et al., 2001).

Given the large number of pleiotropic loci involved

in repression of *Arabidopsis* ABC genes, it is not too surprising that several *Antirrhinum* mutations, such as *stylosa* (*sty*) and *fistulata* (*fis*), cause ectopic expression of *PLE*, along with other complex phenotypes. It is not known whether these loci correspond to any of the *Arabidopsis* genes described above, but their unique phenotypes suggest that they define a different set of repressors (McSteen et al., 1998; Motte et al., 1998). Similarly, the *Antirrhinum* mutant *polypetala*, in which *PLE* as well as *DEF* expression are reduced, has no obvious counterpart in *Arabidopsis* (McSteen et al., 1998).

Because none of the cloned negative regulators of *AG* are expressed in a region-specific fashion, it appears that *AG* expression is globally repressed throughout the plant and that this repression is overcome by region-specific activators in the center of wild-type flowers. As with A and B class genes, the LFY transcription factor is an important upstream regulator of *AG*. The first indication that *AG* was directly regulated by LFY came from analysis of plants carrying an activated form of LFY, LFY:VP16. When expressed in the normal *LFY* domain, *LFY:VP16* causes phenotypes similar to those of transgenic or mutant plants with ectopic *AG* expression. More significantly, expression of *LFY:VP16* in vegetative tissue is sufficient for *AG* activation, similar to the activation of *AP3* and *PI* by the combination of *LFY* and *UFO* (Parcy et al., 1998). LFY binds to *AG* regulatory sequences in vitro, and the LFY binding sites are required for both the normal *AG* expression pattern and the response to *LFY:VP16* in vivo (Busch et al., 1999), providing strong evidence that LFY is indeed a direct regulator of *AG*.

Since *AG* is activated only in a subset of *LFY*-expressing cells, region-specific coregulators must be required either to repress *AG* in whorls one and two or to enhance its activation in whorls three and four. Two recent publications support the latter idea by showing that the homeodomain protein WUSCHEL (WUS) contributes to activation of *AG* in the center of flowers (Lenhard et al., 2001; Lohmann et al., 2001; Figure 2). *WUS* was first identified because of its role in maintaining a stem cell population in the center of shoot apical and floral meristems. Because of their shoot meristem defects, *wus* mutants rarely make flowers, but the occasional flowers that are formed mostly lack stamens and carpels, the organs specified by *AG*. *WUS* is activated before *AG* in flowers and its RNA accumulates in a domain that is eventually included in the *AG* expression domain (Mayer et al., 1998). Although *wus* mutants can make a few stamens, *WUS* is required for normal *AG* activation, as plants with reduced *WUS* expression also have a reduced *AG* expression domain (Lohmann et al., 2001). Conversely, ectopic *WUS* expression leads to ectopic activation of *AG*, demonstrating that *WUS* is also sufficient to drive *AG* expression in flowers (Lenhard et al., 2001; Lohmann et al., 2001). WUS binds to sites adjacent to the LFY binding sites in the *AG* enhancer, and both act together to activate transcription from *AG* regulatory sequences in a yeast transactivation assay (Lohmann et al., 2001). Since LFY and WUS can bind DNA independently, activation is likely due to synergistic effects on the basal transcription machinery. Mutating the WUS binding sites strongly reduces the activity of the *AG* enhancer, confirming that WUS is a direct activator of *AG* (Lohmann et al., 2001). Thus, similar to the example of *LFY* interacting with *UFO* to activate *AP3* and *PI*, *LFY* interacts with WUS, which is expressed in a specific pattern in both shoot and floral meristems, to activate *AG*.

Refining the Floral ABCs

Like other cascades of transcriptional regulation during development, fine-tuning and maintenance are important aspects of ABC gene regulation. An interesting case is that of the B class genes *AP3* and *PI*, whose initial expression extends from whorls two and three, where both have a homeotic function, into adjacent whorls, with some expression of *AP3* in whorl one and of *PI* in whorl four. After initial activation, the products of both genes are required to maintain their own expression. At least for *AP3*, this autoregulation is likely to be direct, as the *AP3* promoter contains CArG boxes that are bound by AP3/PI heterodimers in vitro and that are required for promoter activity in vivo (Riechmann et al., 1996; Hill et al., 1998; Tilly et al., 1998). In the case of *PI*, the mechanism of autoregulation is less clear. Even though deletion studies have defined an *AP3*/*PI*-responsive element in the *PI* promoter, it does not contain a CArG box, nor is it bound by AP3/PI heterodimers (Honma and Goto, 2000). This contrasts with the situation in *Antirrhinum*, where both the *DEF* and *GLO* promoters contain CArG boxes bound by DEF/GLO heterodimers (Theissen et al., 2000; Zhao et al., 2001a).

Another level of B class gene regulation is provided by *SUPERMAN* (*SUP*), which is required to maintain the inner boundary of *AP3* expression. *SUP* itself is under control of the floral meristem identity gene *LFY*, which activates *SUP* through *AP3*/*PI*-dependent and -independent pathways (Sakai et al., 2000).

Finally, an important crossregulatory interaction occurs between *AP1* and *AG*. As mentioned before, *AP1* has dual functions—an early role as a floral identity gene and a later role as an A class homeotic gene. These dual functions are reflected in its expression pattern, with *AP1* initially being expressed throughout the floral primordium and later becoming restricted to presumptive whorls one and two. Repression of *AP1* in the center of the flower is *AG* dependent (Theissen et al., 2000; Zhao et al., 2001a), although it remains to be seen whether this is a direct effect of *AG*. Crossregulation of *AP1* by the C class gene *AG*, conforming to the third tenet of the ABC model that C class activity represses A class activity, provides an economical way of establishing the ABC pattern, as independent region-specific regulators are only required for *AG*.

Beyond the ABCs

One of the most satisfying findings of early experiments with floral homeotic mutants was that plants lacking all three classes of ABC gene activities formed flowers that had only leaf-like organs (Bowman et al., 1991), confirming Goethe's (1790) assertion made two centuries earlier that floral organs are modified leaves. It was disappointing, therefore, that overexpression of ABC genes, alone or in combination, failed to convert leaves into floral organs. Only recently has the missing piece of the puzzle been found. It turns out that at least B and C class genes cannot function without a trio of MADS box genes, the

SEPALLATA genes, whose combined knockout phenotype resembles that of plants without B and C function (Pelaz et al., 2000). Conversely, overexpressing *SEP* genes in combination with ABC genes leads to spectacular transformation of vegetative leaves into floral organs (Honma and Goto, 2001; Pelaz et al., 2001). The molecular basis of these effects is that that ABC gene products form higher order complexes with SEP proteins, which provide activation domains for those MADS domain proteins that cannot activate transcription on their own (Honma and Goto, 2001; Figure 2). A second way in which formation of higher order complexes may contribute to synergistic effects on the regulation of target genes is by increasing DNA binding affinity (Egea-Cortines et al., 1999).

Having found conditions in which ABC genes can induce floral organ fate throughout the plant should greatly facilitate the identification of their target genes. So far, little is known about such target genes, not very different from the situation for many developmental regulators in animals (Pradel and White, 1998). One of the most promising reports for *Arabidopsis* has been the one from Sablowski and Meyerowitz (1998), who used a hormone-dependent version of AP3 to search for direct target genes. Subsequent analysis of the *NAP* gene, which was identified with this method, revealed why the power of genetics is limited when it comes to a comprehensive picture of homeotic target genes: *NAP* expression is not confined to petals and stamens, where *AP3* is active, and modulating *NAP* activity in vivo has complex effects that do not obviously hint to a role of *NAP* in mediating *AP3* activity.

There are, however, some target genes that have organ-specific effects and that have been identified by genetic analyses. One example is that of the *SHATTERPROOF* (*SHP*) genes, which are regulated by *AG*, and which in turn control region-specific patterning within the carpel, an *AG*-dependent organ (Liljegren et al., 2000). The *SHP* genes are closely related to *AG*, and it will be interesting to learn whether the carpel-specific patterning function of the *SHP* genes originated only after the duplication event that gave rise to *AG* and *SHP* genes, or whether there was an ancestral version of *AG* that controlled all these functions.

Interestingly, in addition to its early function in specifying carpel identity, *AG* itself is required for the patterning of specific carpel structures. Although *ag* single mutants lack carpels, because of expansion of A function into the center of the flower, removing A function in *ap2 ag* double mutants leads to the formation of carpelloid leaves in these flowers. The fact that these organs do not have the full inventory of pattern elements found in normal carpels indicates both that *AG* is required for patterning within the carpel and that other genes must act in parallel with *AG* in this process. Two genes that have such functions are *CRABS CLAW* (*CRC*) and *SPATULA* (*SPT*; Table 1), and, consistent with genetic studies, activation of *CRC* and *SPT* is at least partially independent of *AG* (Bowman and Smyth, 1999; Heisler et al., 2001). Carpel patterning also involves factors that do not have necessarily carpel-specific effects (e.g., Sessions et al., 1997). Other factors that act in parallel with *AG* and contribute to C function are the *HUA1* and *HUA2* (Chen and Meyerowitz, 1999; Li et al., 2001) as well as the *KNAT2* homeobox gene (Pautot et al., 2001; Table 1).

Summary

The regulatory system governing early floral patterning is well conserved in the two reference plants *Arabidopsis* and *Antirrhinum*, which represent the two major subdivisions of higher dicots. Consistent with the many similarities between *Arabidopsis* and *Antirrhinum*, the role of ABC genes is largely conserved in other dicots as well, and even in monocots such as grasses (e.g., Ambrose et al., 2000; Ma and dePamphilis, 2000). This observation not withstanding, there are variations in the manner in which B function genes contribute to the development of petals and stamens, as deduced from recent work on basal dicots (Kramer and Irish, 1999).

Other differences in the regulatory systems are due to gene duplication and loss, which has resulted in various degrees of redundancy and subfunctionalization. Examples are the multiple *AG* orthologs in *Antirrhinum*, petunia and cucumber, which differ in their ability to induce reproductive organ fate (Tsuchimoto et al., 1993; Kater et al., 1998; Davies et al., 1999), or the second whorl-specific phenotype of a mutation in the petunia B class gene *green petals* (*gp*; van der Krol et al., 1993). A more significant discrepancy is that there is no evidence for *AP2* orthologs controlling C class activity in other species (Maes et al., 2001). Thus, *AP2* may have acquired its role in *AG* regulation relatively recently during the evolution of *Arabidopsis*.

Although there has been significant progress in understanding the mechanisms of floral patterning, there are still many outstanding issues. The most significant is probably how the prepattern, which results in region-specific expression of homeotic activators such as *UFO* and *WUS*, is generated. The answer to this question will hopefully come from the rich body of work that deals with the origin, structure, and function of shoot meristems (Brand et al., 2001). Downstream of the homeotic genes, it seems likely that systematic global expression profiling will enable comprehensive identification of target genes. For both the upstream and downstream events, the major challenge remaining will be to decipher the logic of regulatory interactions that underlie the formation of flowers.

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References

Ambrose, B.A., Lerner, D.R., Ciceri, P., Padilla, C.M., Yanofsky, M.F., and Schmidt, R.J. (2000). Molecular and genetic analyses of the *silky1* gene reveal conservation in floral organ specification between eudicots and monocots. Mol. Cell *5*, 569–579.

Aubert, D., Chen, L., Moon, Y.H., Martin, D., Castle, L.A., Yang, C.H., and Sung, Z.R. (2001). EMF1, a novel protein involved in the control

of shoot architecture and flowering in *Arabidopsis*. Plant Cell *13*, 1865–1875.

Blázquez, M.A., and Weigel, D. (2000). Integration of floral inductive signals in *Arabidopsis*. Nature *404*, 889–892.

Bomblies, K., Dagenais, N., and Weigel, D. (1999). Redundant enhancers mediate transcriptional repression of *AGAMOUS* by *APETALA2*. Dev. Biol. *216*, 260–264.

Bowman, J.L., and Smyth, D.R. (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, J.L., Smyth, D.R., and Meyerowitz, E.M. (1991). Genetic interactions among floral homeotic genes of *Arabidopsis*. Development *112*, 1–20.

Brand, U., Hobe, M., and Simon, R. (2001). Functional domains in plant shoot meristems. Bioessays *23*, 134–141.

Busch, M.A., Bomblies, K., and Weigel, D. (1999). Activation of a floral homeotic gene in *Arabidopsis*. Science *285*, 585–587.

Byzova, M.V., Franken, J., Aarts, M.G., de Almeida-Engler, J., Engler, G., Mariani, C., Campagne, M.M., and Angenent, G.C. (1999). *Arabidopsis STERILE APETALA*, a multifunctional gene regulating inflorescence, flower, and ovule development. Genes Dev. *13*, 1002–1014.

Chen, X., and Meyerowitz, E.M. (1999). *HUA1* and *HUA2* are two members of the floral homeotic *AGAMOUS* pathway. Mol. Cell *3*, 349–360.

Coen, E.S., and Meyerowitz, E.M. (1991). The war of the whorls: genetic interactions controlling flower development. Nature *353*, 31–37.

Conner, J., and Liu, Z. (2000). LEUNIG, a putative transcriptional corepressor that regulates *AGAMOUS* expression during flower development. Proc. Natl. Acad. Sci. USA *97*, 12902–12907.

Davies, B., Motte, P., Keck, E., Saedler, H., Sommer, H., and Schwarz-Sommer, Z. (1999). *PLENA* and *FARINELLI*: redundancy and regulatory interactions between two *Antirrhinum* MADS-box factors controlling flower development. EMBO J. *18*, 4023–4034.

Deyholos, M.K., and Sieburth, L.E. (2000). Separable whorl-specific expression and negative regulation by enhancer elements within the *AGAMOUS* second intron. Plant Cell *12*, 1799–1810.

Egea-Cortines, M., Saedler, H., and Sommer, H. (1999). Ternary complex formation between the MADS-box proteins SQUAMOSA, DEFICIENS and GLOBOSA is involved in the control of floral architecture in *Antirrhinum majus*. EMBO J. *18*, 5370–5379.

Elliott, R.C., Betzner, A.S., Huttner, E., Oakes, M.P., Tucker, W.Q.J., Gerentes, D., Perez, P., and Smyth, D.R. (1996). *AINTEGUMENTA*, an *APETALA2*-like gene of *Arabidopsis* with pleiotropic roles in ovule development and floral organ growth. Plant Cell *8*, 155–168.

Goethe, J.W.v. (1790). Versuch die Metamorphose der Pflanzen zu erklären (Gotha, Germany: C.W. Ettinger).

Gómez-Mena, C., Piñeiro, M., Franco-Zorrilla, J.M., Salinas, J., Coupland, G., and Martínez-Zapater, J.M. (2001). *early bolting in short days*: an *Arabidopsis* mutation that causes early flowering and partially suppresses the floral phenotype of *leafy*. Plant Cell *13*, 1011–1024.

Goodrich, J., Puangsomlee, P., Martin, M., Long, D., Meyerowitz, E.M., and Coupland, G. (1997). A Polycomb-group gene regulates homeotic gene expression in *Arabidopsis*. Nature *386*, 44–48.

Heisler, M.G., Atkinson, A., Bylstra, Y.H., Walsh, R., and Smyth, D.R. (2001). *SPATULA*, a gene that controls development of carpel margin tissues in *Arabidopsis*, encodes a bHLH protein. Development *128*, 1089–1098.

Hill, T.A., Day, C.D., Zondlo, S.C., Thackeray, A.G., and Irish, V.F. (1998). Discrete spatial and temporal cis-acting elements regulate transcription of the *Arabidopsis* floral homeotic gene *APETALA3*. Development *125*, 1711–1721.

Honma, T., and Goto, K. (2000). The *Arabidopsis* floral homeotic gene *PISTILLATA* is regulated by discrete cis-elements responsive to induction and maintenance signals. Development *127*, 2021–2030.

Honma, T., and Goto, K. (2001). Complexes of MADS-box proteins are sufficient to convert leaves into floral organs. Nature *409*, 525–529.

Jofuku, K.D., den Boer, B.G.W., Van Montagu, M., and Okamuro, J.K. (1994). Control of *Arabidopsis* flower and seed development by the homeotic gene *APETALA2*. Plant Cell *6*, 1211–1225.

Kaiser, P., Flick, K., Wittenberg, C., and Reed, S.I. (2000). Regulation of transcription by ubiquitination without proteolysis: Cdc34/SCF^{Met30}-mediated inactivation of the transcription factor Met4. Cell *102*, 303–314.

Kater, M.M., Colombo, L., Franken, J., Busscher, M., Masiero, S., Van Lookeren Campagne, M.M., and Angenent, G.C. (1998). Multiple *AGAMOUS* homologs from cucumber and petunia differ in their ability to induce reproductive organ fate. Plant Cell *10*, 171–182.

Klucher, K.M., Chow, H., Reiser, L., and Fischer, R.L. (1996). The *AINTEGUMENTA* gene of *Arabidopsis* required for ovule and female gametophyte development is related to the floral homeotic gene *AP2*. Plant Cell *8*, 137–153.

Kramer, E.M., and Irish, V.F. (1999). Evolution of genetic mechanisms controlling petal development. Nature *399*, 144–148.

Krizek, B.A. (1999). Ectopic expression of *AINTEGUMENTA* in *Arabidopsis* plants results in increased growth of floral organs. Dev. Genet. *25*, 224–236.

Krizek, B.A., and Meyerowitz, E.M. (1996). The *Arabidopsis* homeotic genes *APETALA3* and *PISTILLATA* are sufficient to provide the B class organ identity function. Development *122*, 11–22.

Krizek, B.A., Prost, V., and Macias, A. (2000). *AINTEGUMENTA* promotes petal identity and acts as a negative regulator of *AGAMOUS*. Plant Cell *12*, 1357–1366.

Lee, I., Wolfe, D.S., Nilsson, O., and Weigel, D. (1997). A *LEAFY* coregulator encoded by *UNUSUAL FLORAL ORGANS*. Curr. Biol. *7*, 95–104.

Lenhard, M., Bohnert, A., Jürgens, G., and Laux, T. (2001). Termination of stem cell maintenance in *Arabidopsis* floral meristems by interactions between *WUSCHEL* and *AGAMOUS*. Cell *105*, 805–814.

Li, J., Jia, D., and Chen, X. (2001). *HUA1*, a regulator of stamen and carpel identities in *Arabidopsis*, codes for a nuclear RNA binding protein. Plant Cell *13*, 2269–2281.

Liljegren, S.J., Gustafson-Brown, C., Pinyopich, A., Ditta, G.S., and Yanofsky, M.F. (1999). Interactions among *APETALA1*, *LEAFY*, and *TERMINAL FLOWER1* specify meristem fate. Plant Cell *11*, 1007–1018.

Liljegren, S.J., Ditta, G.S., Eshed, Y., Savidge, B., Bowman, J.L., and Yanofsky, M.F. (2000). *SHATTERPROOF* MADS-box genes control seed dispersal in *Arabidopsis*. Nature *404*, 766–770.

Lohmann, J.U., Hong, R., Hobe, M., Busch, M.A., Parcy, F., Simon, R., and Weigel, D. (2001). A molecular link between stem cell regulation and floral patterning in *Arabidopsis*. Cell *105*, 793–803.

Ma, H., and dePamphilis, C. (2000). The ABCs of floral evolution. Cell *101*, 5–8.

Maes, T., Van De Steene, N., Zethof, J., Karimi, M., D'Hauw, M., Mares, G., Van Montagu, M., and Gerats, T. (2001). Petunia *AP2*-like genes and their role in flower and seed development. Plant Cell *13*, 229–244.

Mayer, K.F.X., Schoof, H., Haecker, A., Lenhard, M., Jürgens, G., and Laux, T. (1998). Role of *WUSCHEL* in regulating stem cell fate in the *Arabidopsis* shoot meristem. Cell *95*, 805–815.

McSteen, P.C., Vincent, C.A., Doyle, S., Carpenter, R., and Coen, E.S. (1998). Control of floral homeotic gene expression and organ morphogenesis in *Antirrhinum*. Development *125*, 2359–2369.

Mizukami, Y., and Fischer, R.L. (2000). Plant organ size control: *AINTEGUMENTA* regulates growth and cell numbers during organogenesis. Proc. Natl. Acad. Sci. USA *97*, 942–947.

Motte, P., Saedler, H., and Schwarz-Sommer, Z. (1998). *STYLOSA* and *FISTULATA*: regulatory components of the homeotic control of *Antirrhinum* floral organogenesis. Development *125*, 71–84.

Ng, M., and Yanofsky, M.F. (2001). Activation of the *Arabidopsis* B class homeotic genes by *APETALA1*. Plant Cell *13*, 739–754.

Parcy, F., Nilsson, O., Busch, M.A., Lee, I., and Weigel, D. (1998). A genetic framework for floral patterning. Nature *395*, 561–566.

Pautot, V., Dockx, J., Hamant, O., Kronenberger, J., Grandjean, O., Jublot, D., and Traas, J. (2001). *KNAT2*: evidence for a link between Knotted-like genes and carpel development. Plant Cell *13*, 1719–1734.

Pelaz, S., Ditta, G.S., Baumann, E., Wisman, E., and Yanofsky, M.F. (2000). B and C floral organ identity functions require *SEPALLATA* MADS-box genes. Nature *405*, 200–203.

Pelaz, S., Tapia-Lopez, R., Alvarez-Buylla, E.R., and Yanofsky, M.F. (2001). Conversion of leaves into petals in *Arabidopsis*. Curr. Biol. *11*, 182–184.

Pradel, J., and White, R.A. (1998). From selectors to realizators. Int. J. Dev. Biol. *42*, 417–421.

Riechmann, J.L., Krizek, B.A., and Meyerowitz, E.M. (1996). Dimerization specificity of *Arabidopsis* MADS domain homeotic proteins APETALA1, APETALA3, PISTILLATA, and AGAMOUS. Proc. Natl. Acad. Sci. USA *93*, 4793–4798.

Sablowski, R.W., and Meyerowitz, E.M. (1998). A homolog of *NO APICAL MERISTEM* is an immediate target of the floral homeotic genes *APETALA3*/*PISTILLATA*. Cell *92*, 93–103.

Sakai, H., Krizek, B.A., Jacobsen, S.E., and Meyerowitz, E.M. (2000). Regulation of *SUP* expression identifies multiple regulators involved in *Arabidopsis* floral meristem development. Plant Cell *12*, 1607–1618.

Samach, A., Klenz, J.E., Kohalmi, S.E., Risseeuw, E., Haughn, G.W., and Crosby, W.L. (1999). The *UNUSUAL FLORAL ORGANS* gene of *Arabidopsis thaliana* is an F-box protein required for normal patterning and growth in the floral meristem. Plant J. *20*, 433–445.

Serrano-Cartagena, J., Candela, H., Robles, P., Ponce, M.R., Pérez-Pérez, J.M., Piqueras, P., and Micol, J.L. (2000). Genetic analysis of *incurvata* mutants reveals three independent genetic operations at work in *Arabidopsis* leaf morphogenesis. Genetics *156*, 1363–1377.

Sessions, A., Nemhauser, J., McColl, A., Roe, J., Feldmann, K., and Zambryski, P. (1997). *ETTIN* patterns the *Arabidopsis* floral meristem and reproductive organs. Development *124*, 4481–4491.

Sessions, A., Yanofsky, M.F., and Weigel, D. (2000). Cell-cell signaling and movement by the floral transcription factors LEAFY and APETALA1. Science *289*, 779–782.

Theissen, G., Becker, A., Di Rosa, A., Kanno, A., Kim, J.T., Münster, T., Winter, K.U., and Saedler, H. (2000). A short history of MADS-box genes in plants. Plant Mol. Biol. *42*, 115–149.

Tilly, J.J., Allen, D.W., and Jack, T. (1998). The CArG boxes in the promoter of the *Arabidopsis* floral organ identity gene *APETALA3* mediate diverse regulatory effects. Development *125*, 1647–1657.

Tsuchimoto, S., van der Krol, A.R., and Chua, N.-H. (1993). Ectopic expression of *pMADS3* in transgenic petunia phenocopies the petunia *blind* mutant. Plant Cell *5*, 843–853.

van der Krol, A.R., Brunelle, A., Tsuchimoto, S., and Chua, N.-H. (1993). Functional analysis of petunia floral homeotic MADS box gene *pMADS1*. Genes Dev. *7*, 1214–1228.

Wagner, D., Sablowski, R.W.M., and Meyerowitz, E.M. (1999). Transcriptional activation of APETALA1 by LEAFY. Science *285*, 582–584.

Weigel, D., and Meyerowitz, E.M. (1994). The ABCs of floral homeotic genes. Cell *78*, 203–209.

Wilkinson, M., de Andrade Silva, E., Zachgo, S., Saedler, H., and Schwarz-Sommer, Z. (2000). *CHORIPETALA* and *DESPENTEADO*: general regulators during plant development and potential floral targets of FIMBRIATA-mediated degradation. Development *127*, 3725–3734.

Yoshida, N., Yanai, Y., Chen, L., Kato, Y., Hiratsuka, J., Miwa, T., Sung, Z.R., and Takahashi, S. (2001). EMBRYONIC FLOWER2, a novel Polycomb group protein homolog, mediates shoot development and flowering in *Arabidopsis*. Plant Cell *13*, 2471–2481.

Zhao, D., Yu, Q., Chen, C., and Ma, H. (2001a). Genetic control of reproductive meristems. In Meristematic Tissues in Plant Growth and Development, M.T. McManus and B. Veit, eds. (Sheffield: Sheffield Academic Press), pp. 89–142.

Zhao, D., Yu, Q., Chen, M., and Ma, H. (2001b). The *ASK1* gene regulates B function gene expression in cooperation with *UFO* and *LEAFY* in *Arabidopsis*. Development *128*, 2735–2746.