Arabidopsis: Flower Development and Patterning

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Based in part on the previous version of this Encyclopedia of Life Sciences (ELS) article Arabidopsis: Flower Development and Patterning by John L Bowman

Introductory article

Article Contents

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The development of flowers and floral organs is directed by intricate genetic programmes, many aspects of which appear to be shared among all angiosperms. Early acting genes establish floral meristem identity in lateral organ primordia initiated at the periphery of the shoot apical meristem. Later, floral organ primordia arise at precise positions within these floral meristems and take on one of the four distinct identities (sepals, petals, stamens and carpels). A simple model (ABCE model), supported by both molecular and genetic experiments in *Arabidopsis*, explains how a small number of regulatory genes act in different combinations to specify these different organ types. These regulatory genes encode transcription factors that control the expression of many target genes responsible for organogenesis.

Introduction

Flowers are one of the defining features of angiosperms, the dominant group of land plants today. A universal theme underlies the enormous diversity in the size, shape and complexity among the flowers of the quarter of a million extant species of angiosperms. Most flowers are composed of four basic organ types (sepals, petals, stamens and carpels) arranged in concentric rings, called whorls. Sepals occupy the outermost whorl, with petals, stamens and carpels occupying successively more interior positions. Thus, a basic ground plan exists for organ type and position in all angiosperm flowers suggesting that a common genetic programme may be used to specify floral organ identity during the development of all flowers. **See also**: Flowers

Mutations affecting flowers and their organs provide a powerful means for studying the genetic interactions involved in their development. Differences in the development of mutant versus normal (wild-type) plants reveal the function of the mutated gene. Subsequent cloning of such genes then reveals the nature of their biochemical function. Initial studies on flower development and patterning have concentrated on a few genetically tractable model species. One such species, *Arabidopsis thaliana*, has a number of attributes that facilitate molecular genetic experiments. These attributes include a short generation time (approximately 6–8 weeks), ease of self- and cross-fertilization, ease of mutant isolation, ease of transformation to generate transgenic plants and a small genome that has been completely sequenced. **See also**: *Arabidopsis thaliana* as an Experimental Organism; Mutations and New Variation: Overview

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Establishment of the Floral Meristem

Arabidopsis is an annual plant. After seed germination, a small number of leaves are produced from a meristem at the tip of the shoot, referred to as the shoot apical meristem. This is the plant's vegetative stage of growth. Leaves exhibit a 'spiral phyllotaxy', arising one after another from the flanks of the meristem in a spiral pattern. After producing a rosette of leaves, the plant switches from vegetative to reproductive growth and the apical meristem becomes an inflorescence meristem that starts to produce floral meristems. This switch from vegetative to reproductive growth is sensitive to both environmental and endogenous signals and is timed to maximize seed set in the environment in which *Arabidopsis* evolved. Floral meristems are initiated in an indeterminate spiral from the flanks of the inflorescence meristem (**Figure 1a**). Floral meristems are determinate, each producing a single flower. Since the developmental fates of floral and inflorescence meristems differ, gene products must exist that distinguish newly formed floral meristem cells from the inflorescence

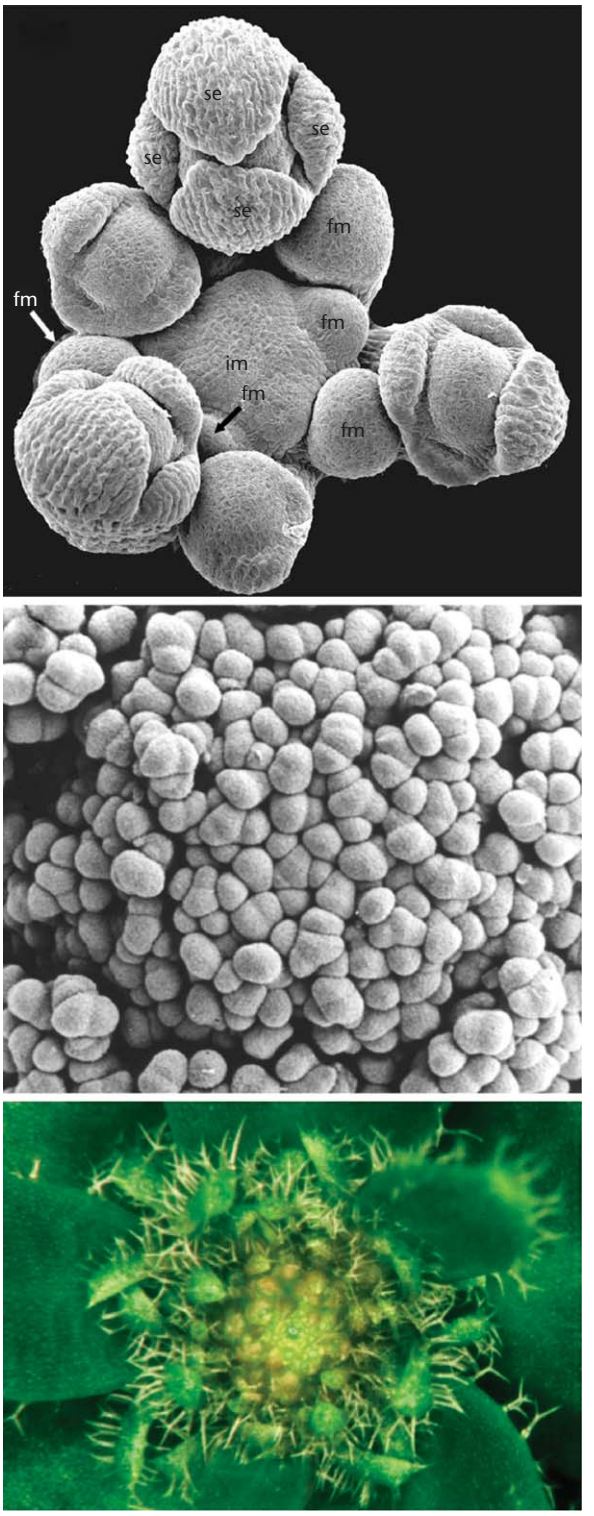

Figure 1 Establishment of the floral meristem. (a) Wild-type inflorescence meristem (im) and young floral meristems (fm). Four sepal primordia (se) have arised in the older flowers and are indicated on one flower. (b) *ap1 cal* inflorescence apex. (c) *ap1 lfy* inflorescence apex.

meristem cells which gave rise to them. In *Arabidopsis* three genes that function to specify flower meristem identity are *LEAFY* (*LFY*), *APETALA1* (*AP1*) and *CAULIFLOWER* (*CAL*). Mutations in each of these genes lead to a partial loss of floral meristem identity, with the meristems produced from the flanks of mutant inflorescence meristems having both floral and inflorescence meristem characteristics. **See also**: Floral Meristems

In *ap1* mutants, the positions normally occupied by single flowers are instead occupied by branched structures composed of multiple flowers (**Figure 3d**). (Capitalized italics (*AP1*) denote the wild-type gene, whereas small italics (*ap1*) denote the mutant version of the gene.) The *ap1* phenotype has been interpreted as a partial loss of floral meristem identity. Mutations at a second locus, *CAL*, dramatically enhance the phenotype of *ap1* mutations. In *ap1 cal* double mutant plants, cells that would normally comprise a floral meristem instead behave as if they constituted an inflorescence meristem. In turn, these meristems produce additional meristems that also behave as inflorescence meristems. This pattern of development can be reiterated several times, resulting in structures composed of large numbers of inflorescence meristems (**Figure 1b**). This phenotype is strikingly similar to that of *Brassica oleracea* var. *botrytis*, the cultivated cauliflower. In *Arabidopsis*, *cal* mutations alone have no visible phenotypic effect, suggesting that the functions of *CAL* are fully redundant with those of *AP1*. The functional redundancy of these two genes is explained by the fact that *AP1* and *CAL* are closely related members of a gene family called the MADS (MADS is an acronym derived from the first four identified members of this protein family: *M*CM1 (from yeast), *A*GAMOUS (from Arabidopsis), *D*EFICIENS (from *Antirrhinum majus*), and SERUM RESPONSE FACTOR (from humans)) box family. MADS box genes encode transcription factors, proteins that regulate the expression of other genes, and members of this family are characterized by a deoxyribonucleic acid (DNA)-binding motif termed the MADS domain. Thus, *AP1* and *CAL* code for proteins with very similar biochemical activities. Furthermore, the expression patterns of these two genes are essentially indistinguishable. *AP1* and *CAL* are expressed at the messenger ribonucleic acid (mRNA) level throughout incipient floral meristems but not inflorescence meristems or leaves (**Figure 2b**). Because flowers are eventually formed in *ap1 cal* double mutants, additional genes capable of specifying floral meristem identity in *Arabidopsis* must exist.

Mutations in *LFY* also result in a partial conversion of floral meristems into inflorescence meristems. In *lfy* mutants, bract-like organs subtend each of these partially converted meristems. Bracts are organs that subtend flowers in many families of flowering plants, but are absent from those of the Brassicaceae. Thus, one function of the wild-type *LFY* gene is to suppress bract formation. The flowers that eventually develop in *lfy* mutants exhibit spiral phyllotaxis, like wild-type inflorescence meristems, rather than whorled phyllotaxis, like wild-type flowers. In

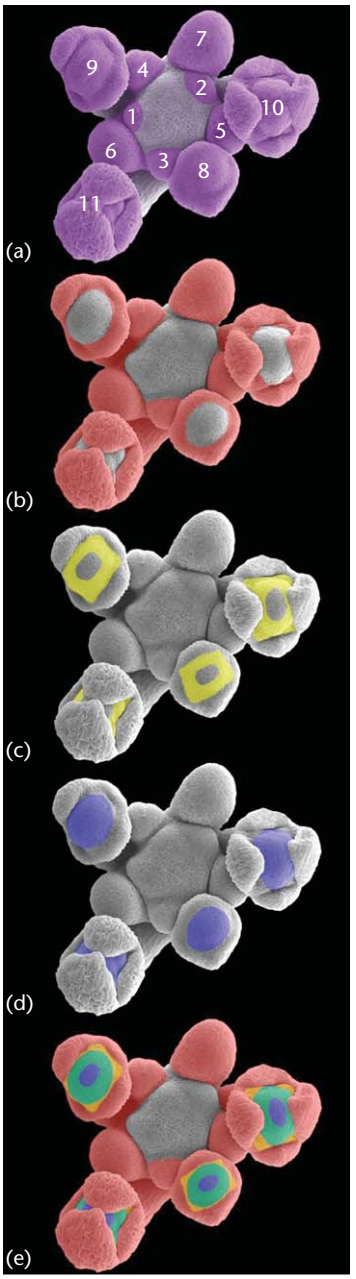

Figure 2 Expression patterns of the floral meristem identity and floral organ identity genes. (a) *LFY* (purple) is expressed in floral meristem anlagen (1, 2), flower meristems (3–7) and young developing flowers (8–11). The number 8 marks a stage 3 flower in which the 4 sepal primordia are first visible. (b) The A class gene *AP1* (red) is expressed in floral meristems, developing sepals and petals in whorls one and two of the flower, and the floral pedicel. (c) The B class genes *AP3* and *PI* (yellow) are expressed in whorls two and three, which develop into petals and stamens. (d) The C class gene *AG* (blue) is expressed in whorls three and four, which develop into stamens and carpels and (e) Composite of B, C and D; in whorl one A class genes are expressed (red), in whorl two both A and B class genes are expressed (orange), in whorl three both B and C class genes are expressed (green) and in whorl four C class genes are expressed (blue).

addition, *lfy* flowers lack petals and stamens and consist primarily of leaf-like and carpel-like organs. This is due to a second function of the wild-type *LFY* gene in activation of the floral organ identity genes (described later in the section on Transcriptional networks regulating flower development). The partial transformations of floral meristems into inflorescence meristems seen in the *ap1* and *lfy* single mutants become complete transformations in the *ap1 lfy* and *ap1 lfy cal* double and triple mutants, respectively (**Figure 1c**), suggesting that these genes work cooperatively and in a partially redundant manner to specify floral meristem identity. *LFY* encodes a transcription factor, but it is not a member of the MADS box family. Consistent with its proposed role, *LFY* is expressed in incipient floral meristems, but not in inflorescence meristems (**Figure 2a**). The initial timing of *LFY* expression precedes that of *AP1* and *CAL*, and *LFY* is in part responsible for the activation of *AP1* and *CAL* through direct binding of LFY protein to the *AP1* and *CAL* promoters.

Another approach in understanding gene function is to express cloned genes ectopically, i.e. at times and/or in places where the genes are not normally expressed. This can be done by putting a gene under the control of a constitutive promoter. When either *AP1* or *LFY* is expressed constitutively (in all tissues), meristems that would normally behave as inflorescence meristems now act as floral meristems. This phenotype is the opposite of that observed when the genes are mutated and corroborates the hypothesis that these genes specify floral meristem identity. In addition to showing a conversion of inflorescence meristems into floral meristems, these transgenic plants make the transition from vegetative to reproductive development earlier than wild-type plants. A shortened vegetative phase has also been demonstrated when *LFY* is constitutively expressed in other distantly related angiosperms, such as aspen and tobacco. Such reductions in flowering time are of great interest to plant breeders working with species in which flowering occurs only after several years.

Floral Homeotic Genes

After a group of cells on the flanks of the inflorescence meristem becomes specified as a flower by the action of the floral meristem identity genes, these floral meristems initiate the production of floral organs. The *Arabidopsis* flower consists of four concentric whorls of organs with four sepals in the first whorl, four petals in the second whorl, six stamens in the third whorl and two carpels in the fourth whorl (**Figure 3a** and **b**). Molecular genetics has been particularly useful in elucidating how the four types of floral organs acquire their distinct identities. These studies have focused on a set of homeotic mutants in which normal floral organs develop in inappropriate positions within the flower. For example, in one class of floral homeotic mutants, sepals develop in second whorl positions that are normally occupied by petals, and carpels develop in third whorl positions that are normally occupied by stamens.

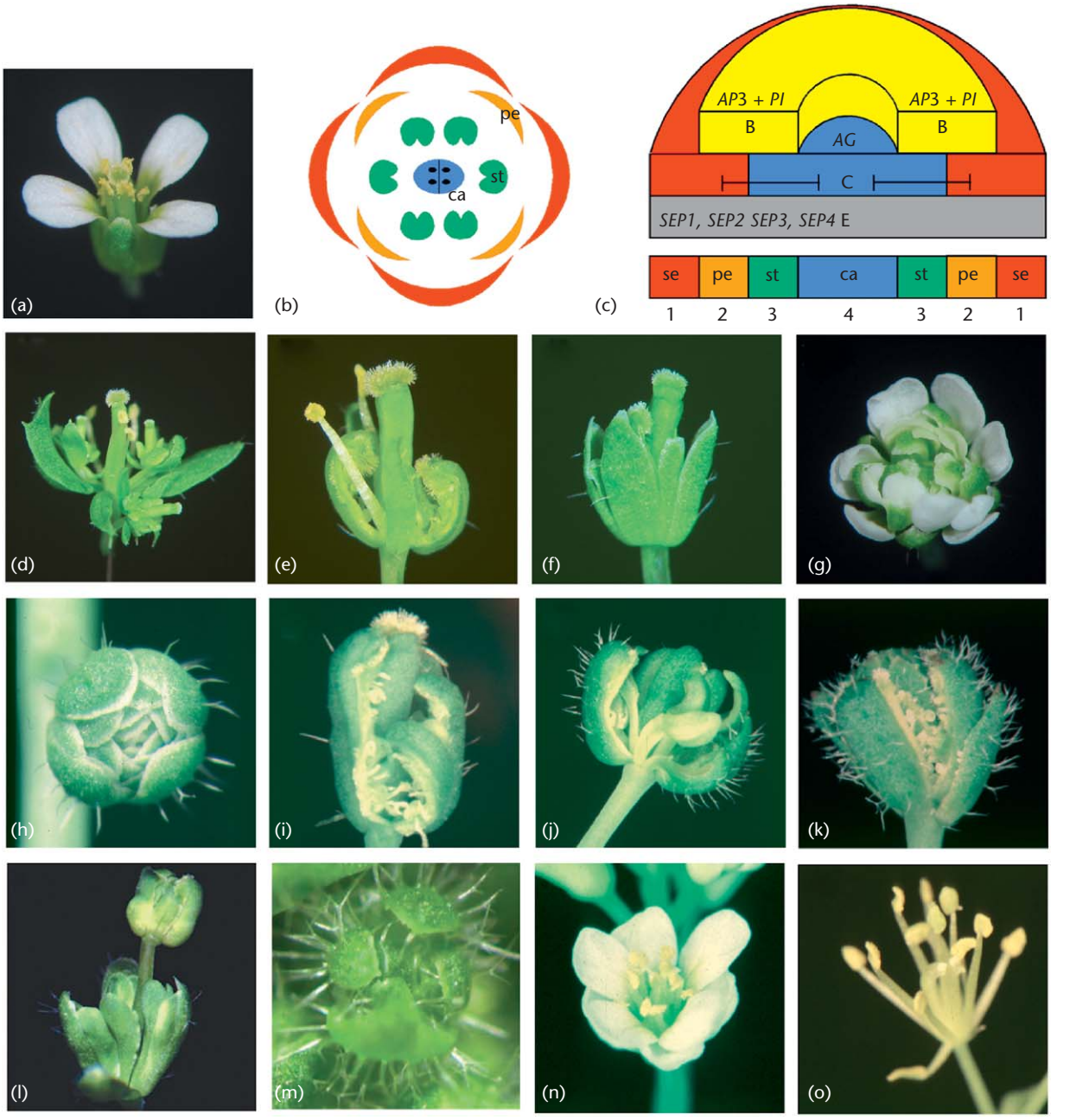

Figure 3 Specification of floral organ identity. (a) Wild-type flower. (b) Floral diagram of the wild-type flower. (c) The ABCE model of the specification of floral organ identity showing how four classes of gene activities act in different combinations to specify four distinct floral organ identities. A section through a floral primordium is represented as a set of boxes, with the regions representing each floral whorl shown at the bottom. In the top set of boxes, the A (red), B (yellow), C (blue) and E (grey) fields are shown and the floral homeotic gene products present in each field listed. The identity of the organs present in each whorl is shown in the lower set of boxes: sepal (red), petal (orange), stamen (green) and carpel (blue). (d) *ap1* flower (le, x, st, ca). (e) *ap2* flower (ca, st, st, ca). (f) *pi* flower (se, se, ca, ca). (g) *ag* flower ((se, pe, pe)$_n$). (h) *pi ag* flower ((se, se, se)$_n$). (i) *ap2 pi* flower (ca, ca, ca, ca). (j) *ap2 ag* flower ((le-ca, pe-st, pe-st)$_n$). (k) *ap2 pi ag* flower ((le-ca, le-ca, le-ca)$_n$). (l) *sep1 sep2 sep3* ((se, se, se)$_n$). (m) *sep1 sep2 sep3 sep4* ((le, le, le)$_n$). (n) Flower from a transgenic plant in which the B class genes are constitutively expressed (pe, pe, st, st). (o) *ap2* flower in which the B class genes are constitutively expressed (st, st, st, st). se, sepal; pe, petal; st, stamen; ca, carpel; le, leaf-like; le-ca, leaf-like carpel; pe-st, petaloid stamen and x, organs absent.

One interpretation of these phenotypes is that cells in the developing flower misinterpret their position, and consequently differentiate into the wrong cell types. In the specific case aforementioned, it is presumed that the function of the wild-type gene product is to specify the identities of the second and third whorl organs as petals and stamens, respectively. In the absence of this gene product, cells in the second and third whorls misinterpret their positions and

differentiate as if they were in the first and fourth whorls, respectively. **See also**: Carpels; Petals; Sepals; Stamens

The floral homeotic mutants of *Arabidopsis* make up three classes, designated A, B and C, with mutants in each class exhibiting organ identity defects in two adjacent whorls. The genes disrupted in these mutants are referred to as floral homeotic genes or floral organ identity genes. Mutations in the A class genes (*AP2* and *AP1*) display homeotic conversions in the outer two whorls. In the case of *ap2*, the first whorl organs develop as carpels, rather than sepals and the second whorl organs are either absent or develop as stamens, rather than petals (**Figure 3e**). In contrast, in *ap1* mutants the first whorl organs are bract-like, and the second whorls organs are most frequently absent (**Figure 3d**). Note that *AP1* is involved in both the specification of floral meristem identity and in the specification of floral organ identity. Mutations in either of the two B class genes (*PISTILLATA* (*PI*) and *APETALA3* (*AP3*)) lead to alterations in the middle two whorls, with sepals developing instead of petals in second whorl positions and carpels developing instead of stamens in third whorl positions (**Figure 3f**). Mutations in the C class gene *AGAMOUS* (*AG*) result in petals developing in place of stamens in the third whorl and the cells normally fated to become the fourth whorl carpels instead behave as if they constituted another floral meristem (**Figure 3g**). This new floral meristem reiterates the flower developmental process such that *ag* flowers are a series of nested flowers within flowers. Thus, the *ag* mutant phenotype can be summarized as (sepal, petal, petal)$_n$ with respect to organ identity. Mutants with similar phenotypes, that of indeterminate whorls of petals, are seen in many horticultural plants, such as roses and carnations, and these may be the result of alterations in C class function itself or its regulation.

A fourth class of floral organ identity genes affecting organ identity in all four whorls of the flower, has been designated the E class. There are four E class genes, *SEPALLATA1* (*SEP1*), *SEPALLATA2* (*SEP2*), *SEPALLATA3* (*SEP3*) and *SEPALLATA4* (*SEP4*), which act redundantly such that loss of any one *SEP* gene does not result in homeotic conversions within the flower. For this reason, the E class *SEP* genes were not originally identified in forward genetic screens. However, loss of multiple *SEP* genes results in homeotic changes in organ identity; *sep1 sep2 sep3* triple mutants produce indeterminate flowers consisting solely of sepals (**Figure 3l**), whereas *sep1 sep2 sep3 sep4* quadruple mutants produce indeterminate flowers consisting solely of leaf-like organs (**Figure 3m**). Thus, loss of E class gene activity results in a conversion of floral organs into vegetative organs, suggesting that the developmental ground state of a floral organ is a leaf.

The ABC and updated ABCE model

The study of double mutants has revealed that B class activity is independent of both A- and C class activities, but that the A- and C class activities are mutually antagonistic. Combining these observations with the activity domains deduced from the single mutant phenotypes led to the formulation of the ABC model for the specification of floral organ identity (**Figure 3c**). The basic tenets of the ABC model are as follows: (1) each of the A, B and C classes of homeotic gene function acts in a field composed of two adjacent whorls: A class activity in whorls 1 and 2, B class activity in whorls 2 and 3 and C class activity in whorls 3 and 4; (2) the combination of floral organ identity gene activities present in any particular whorl specifies the type of organ that develops in that whorl, e.g. class A alone specifies sepals, classes A + B specify petals, classes B + C specify stamens and class C alone specifies carpels and (3) the class A and class C activities are mutually antagonistic such that loss of A results in C activity in all four whorls and vice versa. This original ABC model has been updated to include the class E genes which act in combination with classes A, B and C genes to specify sepal, petal, stamen and carpel identities in floral whorls one to four, respectively. Thus in the ABCE model, classes A + E specify sepal identity in the first whorl, classes A + B + E specify petal identity in the second whorl, classes B + C + E specify stamen identity in the third whorl and classes C + E specify carpel identity in the fourth whorl.

The model successfully predicts the phenotypes of the floral organs in double mutant combinations. For example, in *pi ag* (or *ap3 ag*) double mutants, in which both B and C class activities have been lost, A class activity is found in all floral whorls due to the absence of the antagonistic C class activity. Since A and E class activities specify sepals, the prediction is that *pi ag* flowers should consist entirely of sepals, and indeed, this is the phenotype observed (**Figure 3h**). Conversely, *ap2 pi* double mutant flowers lack both A and B class activities, leaving only C and E class activities in all floral whorls. Thus, *ap2 pi* flowers consist entirely of carpels (**Figure 3i**). In *ap2 ag* double mutants, only B and E class activities remains in the second and third whorls. B class activity does not normally occur alone, but rather acts in combination with A- and C class activities to specify petal and stamen identity, respectively. The second and third whorl organs of *ap2 ag* flowers are neither wild-type petals nor wild-type stamens, but instead have characteristics of both petals and stamens at both the organ and individual cell levels (**Figure 3j**). Thus, these organs, petal–stamen blends, represent a type that is not normally found in wild-type flowers. In the first and fourth whorls of *ap2 ag* double mutants, where none of the A, B or C classes of floral homeotic activities are present, leaf-like organs are produced (**Figure 3j**). These leaf-like organs display some features of carpels, suggesting that additional genes specify carpel identity in the absence of class C gene function. In *ap2 pi ag* triple mutants, where none of the A, B and C classes of floral homeotic activities are present, carpelloid leaf-like organs are formed in all four whorls (**Figure 3k**). This phenotype is very similar to that resulting from the loss of class E function (**Figure 3m**), demonstrating that A-, B- and C class genes require E activity for their floral organ identity functions. The development of leaf-like structures throughout the flower in the absence of either E function or

the combined absence of A, B and C functions provides support for the idea that floral organs are evolutionarily derived from leaves.

The floral homeotic genes encode transcription factors that are active in distinct regions of the floral meristem

All of the known floral homeotic genes encoding the A, B, C and E functions have been cloned in *Arabidopsis*, and encode transcription factors. Intriguingly, one of the A class genes (*AP1*) and all of the B, C and E class genes (*AP3*, *PI*, *AG*, *SEP1*, *SEP2*, *SEP3* and *SEP4*) encode transcription factors belonging to the MADS box gene family, suggesting that diversification within this gene family may have been instrumental in the evolution of flowers. *AP2* also encodes a transcription factor but of a different family.

Most of the class A, B and C genes are expressed at the mRNA level in spatially restricted regions of a developing flower consistent with where these genes have activity in the ABCE model (**Figure 2b**, **c** and **d**). The expression of *AP1* is initially throughout the flower meristem, but by the time sepal primordia arise (a stage 3 flower), *AP1* expression becomes restricted to the first and second whorls (**Figure 2b**). Simultaneous with the restriction of *AP1* expression to the outer two whorls is the initiation of *AG* expression in whorls three and four (**Figure 2d**). Unlike *AP1*, expression of the other class A gene, *AP2*, is observed in all four floral whorls. Expression of the class B genes, *AP3* and *PI*, is restricted to the second and third whorls (**Figure 2c**). The four class E genes, *SEP1*–*SEP4*, show somewhat different patterns of expression. *SEP1* and *SEP2* are expressed in all four whorls whereas *SEP3* is expressed in whorls two, three and four and *SEP4* is expressed in whorls one and four.

The molecular mechanisms mediating the antagonism between the A and C class gene activities are beginning to be revealed. The C class gene *AG* negatively regulates the transcription of the A class gene *AP1* in the third and fourth whorls. This regulation is likely to involve direct binding of AG to *AP1* regulatory sequences. The class A gene *AP2* negatively regulates the transcription of the C class gene *AG* in the first and second whorls. However, not all of the mutual antagonism occurs at the transcriptional level since *AP2* is transcribed in all four whorls. The restriction of its organ identity activity to the outer whorls involves post-transcriptional regulation of *AP2*. AP2 protein does not accumulate in the inner two floral whorls because of translational repression mediated by a microRNA (miRNA). miRNAs are endogenous small RNAs of 21–24 nucleotides that regulate gene expression through either translational repression and/or cleavage of mRNA sequences containing a partially complementary miRNA-binding site. The coding region of *AP2* contains a binding site for miRNA172. miR172 is initially expressed throughout a floral meristem but later becomes predominately restricted to whorls three and four where it is thought to inhibit the translation of *AP2* mRNA. **See also**: MicroRNAs (miRNAs) and Plant Development

Manipulation of floral organ identity in *Arabidopsis*

Ectopic expression of the B- and C class genes has shown that they are sufficient to specify organ identity within the flower. For example, ectopic expression of both B class genes in an otherwise wild-type background (**Figure 3n**) results in a flower in which petals are present in the first and second whorls (A + B + E) and stamens are present in the third and fourth whorls (B + C + E). By utilizing combinations of mutant and transgenic lines in which the A, B or C genes have been ectopically expressed, it is possible to manipulate organ identity in each whorl of the flower. Flowers consisting entirely of sepals or carpels are formed in the *pi ag* (**Figure 3h**) and *ap2 pi* (**Figure 3i**) double mutants, respectively. Flowers consisting of all petals or all stamens can also be generated. Expression of the B class genes in all four whorls of a C class mutant (*ag*) results in a flower consisting of petals in all whorls. Conversely, expression of the B class genes in all four whorls of an A class mutant (*ap2*) results in a flower consisting of stamens in every whorl (**Figure 3o**). Although ectopic expression of the A-, B- and C class genes is sufficient to convert one floral organ type into another, it is not sufficient to convert a vegetative organ into a floral organ. However, this can be achieved by misexpressing the E class genes in combination with the A-, B- and C class genes. Ectopic expression of *AP1*, *AP3*, *PI* and *SEP3* results in the conversion of rosette leaves into petals and ectopic expression of *AP3*, *PI*, *AG* and *SEP3* converts cauline leaves into stamens. These results indicate that A, B, C and E class genes are sufficient to confer a floral organ fate in all lateral organs produced by the shoot apical meristem.

The quartet model

Although, genetic evidence from both loss-of-function (mutants) and gain-of-function (ectopic expression) studies strongly support the ABCE model of floral organ specification, a complete understanding of the biochemical basis for this combinatorial model is still lacking. MADS domain proteins are present in plants, animals and fungi where they bind to CArG ($CC[A/T]_6GG$) box DNA sequences as either homodimers or heterodimers. In *Arabidopsis*, the class A MADS domain protein AP1 and the C class MADS domain protein AG bind to DNA as homodimers whereas the two class B proteins AP3 and PI form a DNA-binding heterodimer. The DNA-binding specificities of these three dimers *in vitro* are quite similar and the regulation of distinct target genes is likely to be a consequence of interactions with additional proteins that lead to the formation of higher order protein complexes. These higher order complexes appear to involve additional MADS domain proteins. For example, it has been shown in yeast that the AP3-PI heterodimer can interact with AP1

and SEP3 and it can interact with AG when SEP3 is also present. Thus the organ identity MADS domain proteins (AP1, AP3, PI, AG, SEP1, SEP2, SEP3 and SEP4) may function in ternary and/or quaternary complexes *in vivo*. **See also**: Regulatory Genes in Plant Development: MADS

These and other observations have led to the 'quartet model' of floral organ specification which proposes that a unique tetrameric complex of MADS domain protein is formed in cells of each floral whorl, leading to the regulation of distinct set of target genes. Thus, in the first whorl, a tetramer of two AP1-SEP heterodimers might regulate genes required for sepal development (**Figure 4**). In the second whorl, a tetramer formed from the association of an AP1-SEP heterodimer and an AP3-PI heterodimer would regulate genes required for petal development (**Figure 4**). In the third whorl, a tetrameric MADS domain complex consisting of an AP3-PI heterodimer and an AG-SEP heterodimer would regulate genes involved in stamen development, and in the fourth whorl, a tetrameric complex composed of two AG-SEP dimers would regulate genes required for carpel development (**Figure 4**). As the binding of MADS domain dimers bends DNA, individual CArG boxes located at a distance may be brought in proximity such that each dimer within the tetrameric complex interacts with a single CArG box. The exact composition of the multimeric MADS domains complexes that specify different floral organ identities await biochemical characterization.

Transcriptional Networks Regulating Flower Development

Several recent technological advances including DNA microarrays and chromatin immunoprecipitation (ChIP) are helping to unravel the transcriptional networks that regulate flower development and patterning. DNA microarrays are small chips containing many short DNA segments representing the entire genome of an organism. Hybridization of these microarrays with labelled complementary (cDNA) synthesized from RNA isolated from a particular tissue or stage of development provides gene expression information on a global level. Microarrays are useful in identifying potential regulatory targets of a transcription factor when used in combination with transgenic plants containing an inducible form of that transcription factor. For example, fusion of the ligand-binding domain of the glucocorticoid receptor to a transcription factor makes the activity of the transcription factor dependent on a steroid. Monitoring gene expression changes after treatment of transgenic plants carrying such constructs with a steroid can identify genes regulated by that transcription factor. Confirmation that a particular gene is a direct target of a transcription factor can be achieved using ChIP. In this technique, nuclei are isolated from plant tissues and exposed to a fixative that crosslinks transcription factors to DNA sites where they are physically bound. The chromatin with the attached transcription factors is then extracted,

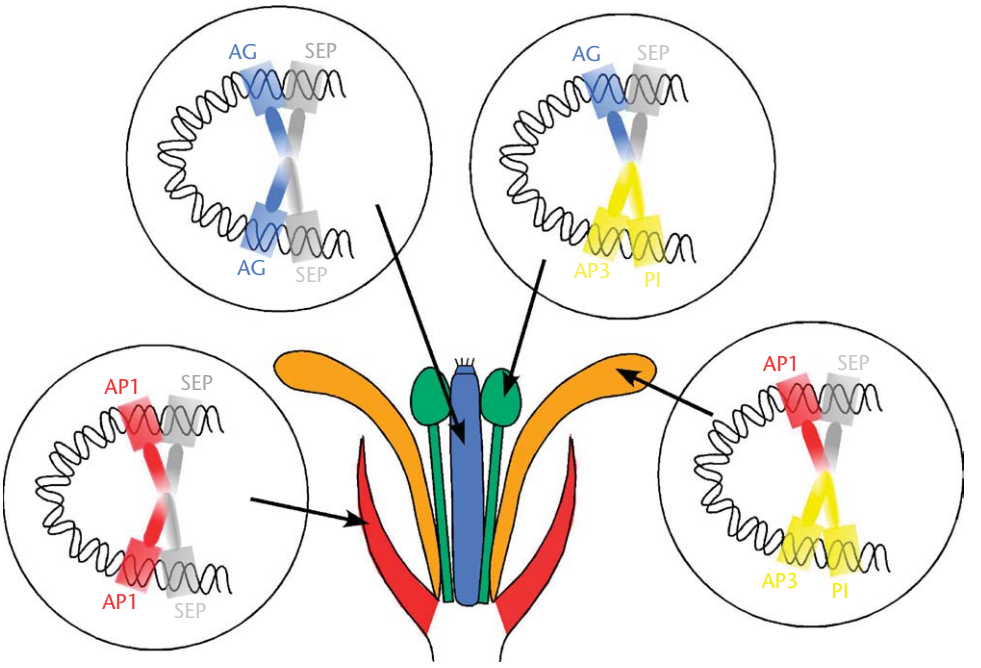

Figure 4 Quartet model for the specification of floral organ identity. A unique tetrameric MADS domain protein regulatory complex is proposed to form in cells of each floral whorl. In first whorl cells, a tetrameric complex composed of two AP1-SEP heterodimers is proposed to regulate the expression of genes involved in sepal (red) development. In second whorl cells, a tetrameric complex composed of one AP1-SEP heterodimer and one AP3-PI heterodimer regulates genes required to make a petal (orange). In third whorl cells, a tetrameric complex composed of one AP3-PI heterodimer and one AG-SEP heterodimer regulates genes needed for stamen (green) development. In fourth whorl cells, a tetrameric complex composed of two AG-SEP heterodimers regulates genes required for carpel (blue) development.

sheared and immunoprecipitated using an antibody specific for the transcription factor. After reversal of the crosslinks, the immunoprecipitated DNA is subjected to the polymerase chain reaction (PCR) using primers within the regulatory region of the candidate target gene. If the gene is a direct target of the transcription factor, the PCR product from the immunoprecipitated DNA template should be enriched compared to that from the input (non-immunoprecipitated) DNA. **See also**: DNA Chips and Microarrays; Gene Expression in Plants; Transcriptional Profiling in Plants

Transcriptional activation of *LFY* within groups of cells in the periphery of the inflorescence meristem confers their identity as floral meristems. Within these cells, LFY specifies floral meristem identity and promotes expression of the floral homeotic genes. LFY binds directly to *AP1* regulatory sequence to promote *AP1* expression initially throughout a floral meristem. *AP1* expression later becomes restricted to the first and second whorls due to repression by AG (**Figure 5**). Although LFY is present throughout young floral meristems, it confers region-specific activation of the B- and C class genes because of its requirement for co-activators that are present in spatially restricted regions of the flower. LFY acts with WUSCHEL (WUS) to activate *AG* expression in the central region of a stage 3 flower (**Figure 5**). WUS, a homeodomain protein, is expressed in a small number of cells in the centre of shoot and floral meristems where it acts to promote stem cell identity. LFY and WUS bind independently to adjacent sites within *AG* regulatory sequence and both transcription factors appear to be required for high-level *AG* expression. Later in flower development, AG feeds back to repress *WUS* expression in the centre of the flower, thus terminating the floral meristem (**Figure 5**). In *ag* mutants, continued expression of *WUS* in the central region of the flower is responsible for the indeterminacy of these flowers. In addition to the positive role of LFY and WUS in activation of *AG* expression in whorls three and four, other proteins, including AP2 and two transcriptional co-repressors, LEUNIG (LUG) and SEUSS (SEU), prevent ectopic expression of *AG* in the outer two whorls.

LFY acts in combination with UNUSUAL FLORAL ORGANS (UFO) to activate expression of the class B gene *AP3* in the second and third whorls. UFO is expressed dynamically during flower development; in stage 2 and 3 flowers *UFO* mRNA is detected in a ring-like domain that corresponds to presumptive second and third whorl cells. UFO does not bind directly to the *AP3* promoter but is recruited to the promoter through its interaction with LFY. UFO encodes an F-box protein that has been shown to be a component of a SCF (Skp1-Cullin-F-box protein) E3 ubiquitin ligase. E3 ubiquitin ligases are enzymes that catalyse the final step in the covalent attachment of the small protein ubiquitin to target cellular proteins. Typically, a polyubiquitin tag marks these proteins for degradation by the 26S proteasome, a large protein complex that removes unnecessary or damaged cellular proteins by breaking them into smaller peptides and eventually single amino acids. The LFY protein is ubiquitylated *in vivo*, a modification partly dependent on UFO activity. In

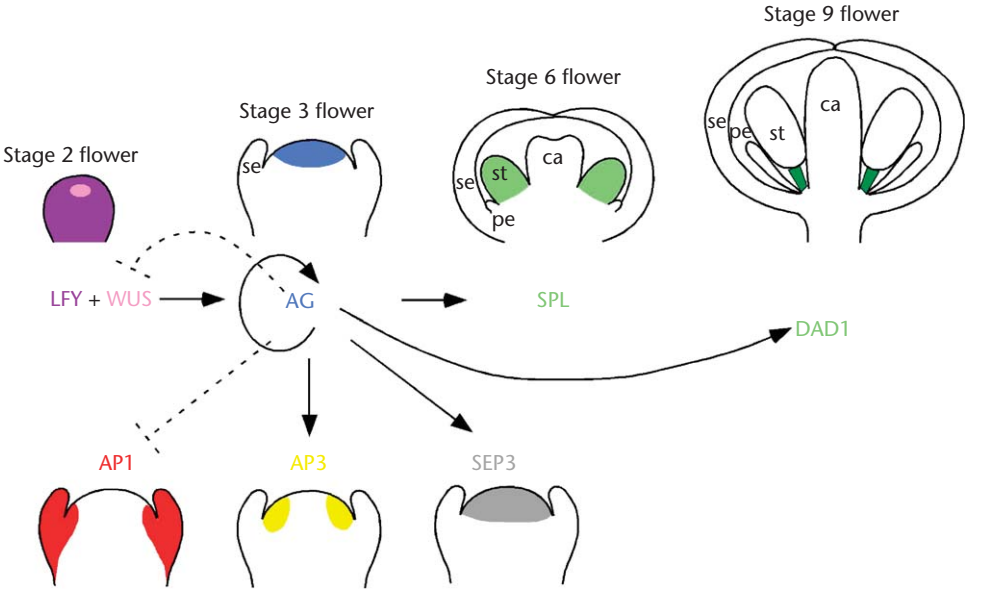

Figure 5 Transcriptional network showing regulatory interactions involving AG during different stages of flower development. LFY (purple) acts with WUS (pink) in stage 1 and 2 flowers to activate expression of *AG* (blue) in the two inner whorls of a stage 3 flower. AG later acts to repress *WUS* expression in the centre of developing flowers. LFY activates *AP1* (red) expression throughout a young floral meristem but *AP1* expression becomes restricted to the outer two floral whorls in a stage 3 flower due to repression of *AP1* expression by AG. AG binds directly to its own promoter and that of *AP3* (yellow) and *SEP3* (grey) to maintain their expression in developing flowers. AG promotes stamen and carpel identity through early and late activation of target genes. An early target of AG regulation in stamen primordia is *SPL* (light green). A later target of AG regulation in developing stamens is *DAD1* (dark green). Solid arrows indicate direct regulation. Bars shown in dashes indicate interactions that have not yet been shown to be direct. se, sepal; pe, petal; st, stamen and ca, carpel.

addition, it has been shown that inhibition of proteasome activity interferes with the ability of LFY to activate *AP3* expression. This suggests that ubiquitylation and subsequent degradation of LFY may somehow stimulate its transcriptional activation activity, although the mechanism by which this is accomplished is unknown. An alternative hypothesis is that UFO ubiquitylates another unidentified protein present at the *AP3* promoter, perhaps a repressor of *AP3* expression. Degradation of this repressor would then lead to *AP3* activation. **See also**: Ubiquitin Pathway

Because only a few downstream regulatory targets of the floral organ identity proteins have been identified and functionally characterized, the mechanisms by which these homeotic proteins regulate organogenesis remain elusive. Several regulatory targets of AG, identified using a steroid-inducible form of the class C protein in combination with gene expression microarrays, have been characterized in some detail. One interesting conclusion from these experiments is that AG regulates distinct target genes at different stages of stamen and carpel development. Thus rather than acting at the top of a transcriptional cascade, AG likely acts to initially turn on a set of genes specifying stamen and carpel identity and later activates other genes that contribute to stamen and carpel morphogenesis and differentiation. This conclusion is consistent with the expression pattern of *AG*; *AG* mRNA is found throughout third- and fourth whorl organ primordia during early floral stages but becomes restricted to particular cell types later in stamen and carpel development. An early target of AG activity is *SPOROCYTELESS* (*SPL*), a gene required for microsporogenesis, the developmental process by which mature pollen grains are generated (**Figure 5**). A later target of AG regulation is *DEFECTIVE IN ANTHER DEHISCENCE1* (*DAD1*), which encodes a biosynthetic enzyme involved in production of the plant hormone jasmonic acid (JA) (**Figure 5**). Activation of JA production by AG coordinates different events in stamen maturation including filament elongation, anther dehiscence and pollen maturation. In addition, AG acts in a positive feedback loop to maintain its own expression and to maintain the expression of other MADS box genes (*AP3* and *SEP3*) encoding AG-interacting partners required for stamen and carpel development (**Figure 5**).

Summary

Genes that specify floral meristem identity have been found in evolutionarily divergent plants, suggesting that a common genetic programme governs this process in angiosperms. The ABCE model, based on molecular genetic experiments, explains how a small number of regulatory genes act in different combinations to specify floral organ identity. Mutations in genes similar to those described here have been found in a number of different angiosperm species, suggesting that many aspects of the ABCE model are likely to be true for all flowering plants. However, class A genes that specify both sepal and petal identities and repress C function have not been discovered in other plants, casting doubt on the universality of the class A function. Furthermore in basal angiosperms, which represent plants most closely related to the earliest lineages of angiosperms, class B gene homologues are often expressed in broader domains within the flower that sometimes lack sharp whorl boundaries. For example, in monocots such as tulips and lilies that form two outer whorls of showy coloured floral organs (tepals) in place of distinct sepals and petals, class B genes are expressed in whorls one, two and three rather than being restricted to whorls two and three.

Although the ABCE model successfully explains the specification of organ identity, it does not address other aspects of floral patterning, such as how the number, position, size and shape of the floral organs are established. These features vary widely between flowers of different species and are likely to involve genes that act downstream or independently of the floral homeotic genes. **See also**: Carpels; Meristems; Petals; Phyllotaxy; Sepals; Stamens; Transgenic Plants

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