Turning floral organs into leaves, leaves into floral organs
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The development of the floral organs is specified by the combinations of three classes of gene for organ identity in the ‘ABC’ model. Recently, molecular genetic studies have shown that this model is applicable to grass plants as well as most eudicots. Transcription factor complexes of ABC and homologous proteins form the molecular basis of the ABC model.

Introduction
The application of developmental genetics has led to progress in our understanding of how organ type is specified in the flower. The advances have been made through genetic analyses of mutants that specifically disrupt floral development, and through molecular cloning of the corresponding genes to reveal the nature of their biochemical function. A model (now known widely as the ‘ABC model’; Figure 1) based on genetic data was proposed to explain how a limited set of genes acting alone and in combination could specify the identity of the floral organs [1–4]. Subsequent molecular analyses of the relevant genes and ectopic expression studies in Arabidopsis have largely supported this genetic model and have led to numerous refinements and further insights (reviewed in [5–8]).

In this review we summarize recent progress made on three outstanding issues. First, how widely applicable is the ABC model, which was originally based on two evolutionarily divergent eudicot species — Arabidopsis thaliana and Antirrhinum majus? Second, how is the activity of the floral homeotic genes confined to flower meristems? Third, are there additional genes that act in parallel with the floral homeotic ABC genes to promote floral organ identity?

The floral homeotic mutants of Arabidopsis
The Arabidopsis flower consists of four concentric whorls of organs: sepals, petals, stamens and carpels. Mutations in a set of floral homeotic genes result in the misinterpretation of positional information in the developing flower, and subsequent homeotic transformation of floral organ types. These floral homeotic mutants fall into three classes, designated A, B and C, and each of the mutations results in organ identity defects in two adjacent whorls.

Class A mutants (apetala2 [ap2] and apetala1 [ap1]) have homeotic conversions in the first two whorls, with, in the case of ap2, the first whorl organs developing as carpels rather than sepals, and the second whorl organs developing as stamens rather than petals [1,4,9–15]. Class B mutants (pistillata [pi] and apetala3 [ap3]) have alterations in the middle two whorls, with sepals instead of petals developing in the second whorl and carpels instead of stamens in the third whorl [1,4,16–18]. The inner two whorls are affected in class C mutants (agamous [ag]), with petals developing in place of stamens and another flower replacing the carpels [1,4,19].

There are three basic tenets of the ABC model [4]: first, each of the classes of homeotic gene function acts in a field comprised of two adjacent whorls, the particular whorls being those that are altered when the corresponding genes are mutant; second, the combination of floral homeotic gene activities specifies the type of organ that develops; and third, 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.

Turning floral organs into leaves
The ABC model successfully predicts the phenotypes of the floral organs for most of the singly, doubly and triply mutant genotypes examined. But when all of the A, B and C classes of floral homeotic activities are removed, as in an ABC triple mutant, the model does not predict the type of organ that will develop. One might suppose that if all genes required for the specification of floral organ identity were removed, the identity of the resulting organs might represent a ground state, possibly a leaf-like organ. When the floral organs of the ABC triple mutant are examined, they exhibit features of both carpels (such as stigmatic tissue, fusion of organs along their margins and ovules) and leaves (stellate trichomes and stipules) [4,20**]. Thus, there must exist genes that promote carpel development in the absence of AG gene function.
The ABC model and the specification of floral organ identity. (a) A wild-type flower and its floral diagram. (b) The ABC model of the specification of floral organ identity depicts how three classes of floral homeotic genes can specify the identity of each of the four whorls of floral organs. Class A alone specifies sepals, classes A + B specify petals, classes B + C specify stamens, and class C alone specifies carpels. Abbreviations: ca, carpel; pe, petal; se, sepal; st, stamen.

Figure 1

In a series of elegant genetic experiments, Alvarez and Smyth [20••] have demonstrated that CRABS CLAW (CRC) and SPATULA (SPT) act in an AG-independent manner to promote several aspects of carpel differentiation. Both spt and crc mutations have a phenotypic effect in an ABC triple mutant background, reducing the amount and type of carpel tissues that develop, with mutations in spt having the more marked effect ([20••]; Figure 2). In the ap2 pi ag spt quadruple mutant, residual occasional ovule-like structures develop at the margins of the phyllody floral organs. Some of these marginal outgrowths also develop in ap2 pi ag spt crc pentuple mutants [20••], which suggests that additional genes also promote AG-independent development of marginal carpel tissues, candidates being LEUNIG and AINTEGUMENTA [21••]. Consistent with the presence of additional redundant genes is the observation that spt single mutants do not lack most marginal tissues, but instead primarily affect the differentiation of sepal tissues [20••]. Because ag mutations are epistatic to spt mutations, SPT, like AG, may also be negatively regulated by A class genes [20••].

SPT encodes a basic helix–loop–helix transcription factor and its expression in the developing carpel is consistent with the proposed role of promoting the growth of marginal tissues [22••]. SPT is also expressed in many different tissues outside the carpel, suggesting that it may act redundantly in diverse developmental processes. The negative regulation of SPT by the A class genes is at the transcriptional level and, furthermore, the restriction of SPT expression to the marginal tissues of the carpel is mediated by ETTIN activity [22••]. In ettin mutants, marginal tissue (e.g. septum) develops ectopically in non-marginal positions [23]. The ectopic development of septum in ettin mutants is mediated by ectopic SPT activity, as confirmed by the epistasis of SPT mutations over ettin mutations with respect to this phenotype [22••,24].

Is ABC model applicable to the grass flower?

Grass species have flowers with highly derived structures, and the identity of the lodicules, palea and lemma, which surround the reproductive organs has been unclear (Figure 3; [25–27]). Issues regarding the evolution of floral organs in grass species are now being addressed using the ABC model as a baseline.

Grass flower homologs of ABC genes

The isolation of putative class A, B and C genes from rice and maize suggests that the basic mechanisms of flower development are probably conserved between grasses and eudicots [28–33,34••,35••]. In both maize and rice, class C genes are expressed in the inner two whorls, class B gene transcripts accumulate in stamens and lodicules, and a putative rice class A gene, RAP1A, is expressed in lodicules, the palea and lemma [34••]. Although expression patterns can be correlative, a functional assay is needed to further assess any potential homology.

Recently, Ambrose et al. [35••] have identified silky1 (si1) as the AP3 ortholog in maize. Remarkably, in si1 mutants, stamens and lodicules are homeotically transformed into carpels and palea/lemma-like structures, respectively [35••]. Likewise, reduced function of the rice PI ortholog (OsMADS4) by antisense methodology results in a similar phenotype [36]. These data imply that there is a homologous relationship between lodicules and petals, and that B function is conserved in grass flowers.
ap2 pi ag (ABC) triple mutants develop placenta bearing ovules, septum containing transmitting tract tissue, style tissue, and stigmatic papillae along the margins of their floral organs [4,20**]. The only major carpel tissue not found in ap2 pi ag triple mutants is the specialized histology of the ovary wall [20**]. In contrast, floral organs of ap2 pi ag spt quadruple mutants lack most of the carpelloid marginal tissues found in the ABC triple mutant, indicating that SPT promotes the differentiation of all these tissue types — a role not obvious from the spt single mutant phenotype in which only the septum is lacking [20**].

**Identity of lemma and palea**

In contrast to the progress in understanding the identity of lodicules, the nature of lemma and palea is still uncertain. Palea/lemma-like structures develop in place of the lodicules in si1 mutants, which suggests that there is a possible homology between palea/lemma and sepals [35••]. In addition, the expression pattern of the RAP1A is consistent with the palea/lemma representing the calyx [34••].

In contrast, an absence of any morphological changes in the palea or lemma in Act1::RAG plants (J Kyoizuka, unpublished data) argues against this view and raises the possibility that the palea and lemma may not be floral organs whose identities are controlled by class A, B and C genes. However, it is also possible that ectopic expression of the class C gene is not sufficient to specify carpel identity in grasses.

Recently, Jeon et al. [37] demonstrated that a mutation in OsMADS1, an AGL9 subfamily rice MADS-box gene, resulted in the elongation of palea and lemma and a reduction in floral meristem determinacy. Analysis of loss-of-function phenotypes of the grass class A genes, as well as genes with related sequences, such as OsMADS1, will be critical in elucidating the nature of the palea/lemma.

**Homology between lodicules and eudicot petals**

In eudicots, the ABC model has enabled the prediction of modified flower structures through manipulating the expression of the floral organ identity genes. For example, ectopic expression of AG by a constitutive promoter results in homoeotic transformations of sepals to carpels and petals to stamens in eudicot flowers [38,39]. In rice, the ectopic expression of RAG/OsMADS3, a rice AG ortholog driven by the strong Actin1 promoter, caused homoeotic conversions of lodicules to stamens (J Kyokuka, unpublished data).

This increases the evidence in favour of lodicules being genetically homologous to petals. Together, homology between lodicules and eudicot petals is supported by three different lines of evidence: first, analyses of RNA expression patterns of class A and B genes; second, loss-of-function mutant phenotypes of class B genes; and third, gain-of-function alleles of class C genes. Together, these results suggest that the ABC genes control floral organ development in a similar manner in the grasses and the eudicots, which span the majority of angiosperm phylogenetic diversity [40].

**Carpel development in grass flowers**

Grass species may have acquired unique mechanisms for carpel development. It has been proposed that in maize the C function is shared by two AG orthologs, ZAG1 and ZMM2 [29,30]. The expression patterns of ZAG1 and ZMM2 suggest that these genes may be involved in determining the carpel and stamen identities, respectively.

In the zag1 mutant, however, in spite of a biased expression of ZAG1 in the carpel, there were no clear morphological alterations in this organ and only floral meristem determinacy was affected [29]. This raises the possibility that carpel identity may not depend solely on the class C homeotic genes in grass species, and that the function of another unknown gene(s) may also be required for the specification of carpels.

**Turning leaves into floral organs**

Molecular cloning of the ABC genes in Arabidopsis has revealed that all except AP2 share a conserved DNA-binding domain, called the MADS domain or ‘MADS box’ [3,14,15,17–19]. By manipulating the expression of the ABC genes, one can construct flowers with of any floral organs in any whorl [4,39,41]. Ectopic expression of the ABC genes in leaves fails, however, to convert them...
into floral organs [39,41]. Thus, the ABC genes are necessary for the formation of floral organs, but they are not sufficient for the conversion from vegetative leaves into floral organs.

**Another factor is needed**

More than 200 years ago, Goethe proposed that floral organs are the result of a transformation (‘metamorphosis’) of the basic leaves. Thus, there should exist as yet unidentified factors required for this transformation or, alternatively, it might be that vegetative leaves are not the ‘basic organ’ from which the floral organs were derived. Recently, Honma and Goto [42**] found the missing factor by searching for proteins that interact with the ABC MADS-box proteins.

The B gene proteins, PI and AP3, act as a heterodimer and autoregulate their own transcription ([41,43–45]; reviewed in [8]). The transcription of an AP3:GUS reporter gene is activated by PI–AP3 without de novo protein synthesis in the flower [43], but is not activated outside the flower even when PI and AP3 are expressed constitutively ([41,43]; Figure 4). However, AP3::GUS expression is observed in the leaves when a viral transactivation domain, VP16, is fused with PI (35S::PI:VP16; Figure 4). This suggested that a flower-specific cofactor might supply a transactivation domain to the PI–AP3 complex.

Thus, the putative missing factor was isolated by fishing for proteins that interact with the PI–AP3 complex, and was identified as another MADS-box gene, AGL9 (agamous-like 9) [42**,46]. Other kinds of proteins were not isolated in the yeast two-hybrid screening, suggesting that the entire complex may be composed solely of MADS-box proteins, and that AGL9 may supply a transactivation activator domain. Notably, Davies et al. [47] and Egea-Cortines et al. [48**] have demonstrated that Antirrhinum ABC MADS-box proteins exhibit a higher affinity and specificity with target DNA sequences when part of a ternary complex than when part of a dimer.

**AGL9 (SEP3) is the missing factor**

Recently, AGL9 was renamed SEPALLATA3 (SEP3) based on its mutant phenotype [49**]. The sep3 mutant itself shows only a subtle phenotype, but in combination with sep1 and sep2 mutants (knockouts of the closely related genes AGL2 and AGL4, respectively), all floral organs develop as sepal-like organs. Thus, the triply mutant sep123 flowers resemble those of bc double mutants [49**].

The most striking evidence demonstrating that SEP3 is the missing factor is the phenotypes of doubly, triply or quadruply transgenic plants combining ectopic expression of SEP3 with ectopic expression of the ABC genes. Remarkably, leaves are converted into petals by ectopic expression of PI–AP3–SEP3, AP1–PI–AP3 [42**] or AP1–PI–AP3–SEP3 [50**], into stamens by PI–AP3–AG–SEP3 [42**], and into carpels by AG–SEP3 (K Goto, unpublished data; Figure 5). These observations indicate that when SEP3 gene function is added in combination with ABC genes, vegetative leaves can be converted into floral organs, supporting Goethe’s theory.

**Complexes of the MADS-box proteins are the molecular basis of the ABC model**

Further analyses of protein–protein interactions suggest that SEP3 not only interacts with PI and AP3, but also serves a scaffold between PI–AP3 and AG [42**]. Although PI–AP3 and AG (the B and C proteins) cannot interact directly, if SEP3 is added then the quartet complex, PI–AP3–SEP3–AG (third whorl complex), can form. The AG–SEP3 interaction suggests that the fourth whorl complex should be AG–SEP3. Likewise, PI–AP3 can interact with both AP1 and SEP3, and AP1 and SEP3 interact with each other [42**]; therefore, the second whorl complex should be AP1–PI–AP3–SEP3.

What about the first whorl? The SEP proteins may not be required for a first whorl complex as the sep123 triple mutant develops normal sepals. Because MADS-box proteins form a dimer to bind their target DNA sequence (called the ‘CArG-box’; reviewed in [8]), AP1 might form

Figure 4

AP3::GUS reporter gene expression in plants constitutively expressing (a) PI–AP3 and (b) PI:VP16–AP3. AP3::GUS is activated by PI–AP3 in the floral organs, but not in the vegetative organs (i.e. leaves). When the viral transactivation domain VP16 is fused to PI (PI:VP16), AP3::GUS becomes activated in the leaves. This implies that a flower-specific cofactor with a transactivation domain is required for complete B gene activity. 

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homodimers or heterodimers with CAULIFLOWER (CAL). AP1 and CAL can interact with each other (K Goto, unpublished results), but cal single mutants do not exhibit any aberrant phenotype although they enhance ap1 phenotypes [13,51]. Ectopic expression of AP1 or CAL does not result in a transformation of leaves into sepals [51,52], however, which suggests the existence of an as yet unidentified factor required for the promotion of sepal identity in conjunction with AP1. On these molecular bases, the classical ABC model should be modified by adding SEP as a limiting factor that restricts the activity of the ABC genes to the floral organs (Figure 6).

The functional redundancy of the SEP genes has been shown recently by both loss-of-function alleles [49**] and gain-of-function alleles [42**,50**]. Ectopic expression of AP1–PI–AP3–SEP3, as well as of AP1–PI–AP3–SEP1–SEP2, can convert leaves into petals [50**]. The three SEP genes form a monophyletic clade within the MADS-box genes of Arabidopsis (Figure 7, SEP clade).

The observation that both AP1–PI–AP3 and PI–AP3–SEP3 can convert leaves into petals when constitutively expressed [42**] argues that AP1 and SEP3 are also redundant in some of their functions. This is supported by the fact that the SEP genes and AP1 belong to the SEP/AP1 superclade (Figure 7). One possible redundant function may be to act as a transactivation component in the complexes. B and C genes do not show any transactivation activity, but genes in the SEP/AP1 superclade shows such activity, although their relative activities vary (Figure 7).

**Conclusions and perspectives**

The ABC model has been surprisingly successful in explaining how a small number of regulatory genes, acting alone and in combination, specify the identity of the floral organs. If all the ABC gene activities were compromised, one might expect a ground state of the floral organ to be revealed — what Goethe referred to as the ‘ideal basic organ’. abc triple mutant flowers comprise not leaves, but organs that have characteristics of both leaves and carpels.

It is tempting to speculate that the pathways mediated by SPT and other marginal-tissue promoting genes might represent evolutionarily ancient genetic networks directing the development of sporophylls that predate the evolution...
of flowers. Subsequently, the ABC genetic network would have been recruited and integrated with these pre-existing genetic programs, leading to the evolution of the flower in angiosperms. In this scheme, the marginal tissues and non-marginal tissues of the Arabidopsis carpel would have separate evolutionary origins, and a ground state of the angiosperm floral organ might be a sporophyll. Studies of orthologous genes in other angiosperms and in non-flowering plants are needed to address this issue.

The ABC model is widely applicable to the angiosperms, including grasses. Grass flowers are morphologically quite different from those of other angiosperms, especially in the peripheral two whorls. Molecular genetic studies of maize and rice suggest that the palea and lemma may be similar to the sepals of eudicots, and that lodicules are probably homologous to petals of eudicots.

Protein complexes are the molecular basis of the genetic ABC model: A + B + SEP3 specifies petals; B + C + SEP3 specifies stamens; C + SEP3 specifies carpels. Because leaves can be converted into floral organs, these complexes must be sufficient for floral organ identity. It is noteworthy that SEP3 as well as the ABC genes are homologous MADS-box transcription factors, and that different complexes of homologous proteins result in specific floral organ activity. Biochemical studies are now required to reveal how DNA-binding affinities are modulated by these complexes in vivo, because all MADS-box proteins bind to CArG boxes in vitro.

It is also intriguing that some MADS-box proteins belonging to the AP1/SEP superclade have transactivation activity, whereas others belonging to the PI/AP3 or AG clades do not. When and how they obtained or lost such activity during their evolution might have influenced the evolution of floral organs.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
** of outstanding interest

The genetic interactions between Arabidopsis thaliana have been studied extensively. The authors propose that restricting 3P activity to the marginal regions of the carpel is mediated in part by the activity of ETTIN.

Additionally, the differentiation of the ovary wall, whereas genes that control carpel development in parallel with tissue formation by activity of leunigauclei has been studied. Specifically, APETALA2 gene in Arabidopsis thaliana encodes a MADS-box and is expressed in floral development.


PI–AP3–SEP3–AG are possible candidates for the second and third whorl complexes, respectively. They also prove by transgenic analyses that these protein complexes are sufficient for the formation of petals (second whorl organ) and stamens (third whorl organ). Thus, these protein complexes are the molecular basis of the ABC model.


