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Review

Floral organ identity: 20 years of ABCs

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ABSTRACT

One of the early successes of the application of molecular genetics to study plant development was the discovery of a series of genes that act together, in an apparently simple combinatorial model, to specify the identity of the different organs of a flower. Widely known as the ABC model, this framework for understanding has been investigated and modified over the course of the last two decades. The cast list of genes has been defined and, as other chapters in this volume will show, great progress has been made in understanding how they are regulated, how they act together, what they do and how they have contributed to the evolution of the flower in its varied forms. In this introductory review to the volume we will review the derivation and elaboration of the most current version of the ABC model, highlighting the modifications that have been necessary to ensure it fits the available experimental data. We will highlight the remaining difficulties in fitting the current model to the experimental data and propose a further modification to enable it to regain its applicability.

Contents

1. Introduction

Modern biology textbooks contain a simple and elegant model that explains how a few genes act together to specify the four organs types that make up a perfect flower. Known as the ABC model (Fig. 1), it was conceived in the early 1990s, based on a series of celebrated homeotic mutants in two model species, *Arabidopsis* and *Antirrhinum* [1,2]. Perfect flowers contain four types of floral organ arranged in four concentric rings, known as whorls. The four organ types are sepals (outermost or whorl 1), petals (whorl 2), male reproductive stamens (whorl 3) and female reproductive carpels (innermost or whorl 4). Deviations from this scheme, mainly in monocots and basal angiosperms, and their interpretation have been recently reviewed [3] and will not be considered here. The ABC model proposed that three functions, A, B and C, each defined by a class of homeotic mutant found in both *Arabidopsis* and *Antirrhinum*, specify the organs that form in the four whorls of the flower. The A-, B- and C-functions were each supposed to occupy two adjacent whorls, which overlap with each other so that each whorl is defined by the expression of a unique function or combination of functions (Fig. 1). The expression of the C-function alone in whorl four causes carpels to form. In whorl 3 both B- and C-functions are expressed, which specifies stamens. Petals form in whorl 2 due to the concomitant expression of A- and B-functions and the expression of A-function alone in whorl 1 results in sepals. Mutual repression between the A- and C-functions is integral to

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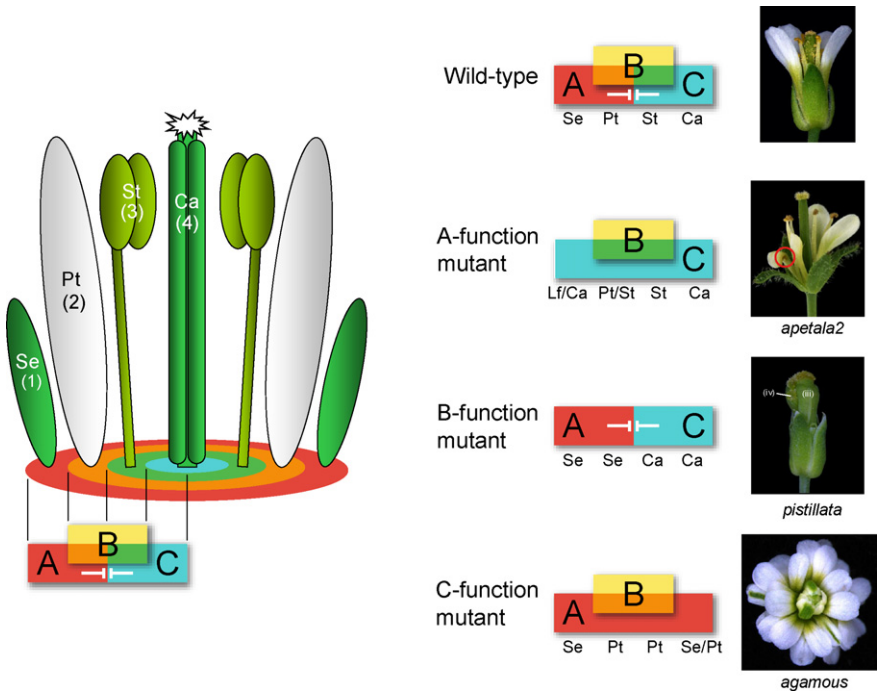

Fig. 1. The textbook ABC model. In the left hand panel is a diagram of a model flower with sepals (Se) in the outermost whorl (1), petals (Pt) in whorl 2, stamens (St) in whorl 3, and carpels (Ca) in whorl 4. Beneath the flower is the ABC model. In the first whorl the A-function genes are expressed (red). In whorl 2 A- and B-function (yellow) genes are co-expressed (orange). In the third whorl the B- and C-function (blue) act together (green), whilst in the fourth whorl the C-function acts alone. The ABC model predicts mutual repression between the A- and C-functions, as indicated by the barred lines. In the right hand panel are the *Arabidopsis* mutants from which the ABC model was proposed. At the top is the wild-type flower. Beneath that is an A-function mutant where loss of the A-gene results in expansion of the C-function into whorls 1 and 2. In the A-function mutant *apetala2* shown here, sepals are converted into carpel-like or leaf-like structures (depending on the allele), and petals into stamen-like organs (whorl 2 anther tissue circled). In *Arabidopsis* B-function mutants, such as *pistillata*, the first two whorls have sepal identity, and the third whorl is converted into a carpel. In the photograph, note how the third whorl carpels (iii) enclose the carpels in the fourth whorl (iv). At the bottom is shown the *Arabidopsis* C-function mutant, *agamous*. According to the ABC model loss of C-gene activity results in expansion of the A-function into all floral whorls. The result is conversion of reproductive organs (stamens and carpels) into perianth organs (sepals and petals) and loss of floral determinacy, giving the rose-like phenotype.

the ABC model to explain why the C-function expands into the outer whorls in A-function mutants, causing reproductive organs to develop in the first whorls. Since the model was based on homeotic mutants in both pioneering species, it was conceivable that the ABC model would provide a unified framework to explain flower development. Early ectopic expression experiments, in which the model was tested by different combinations of homeotic genes in inappropriate domains of the flower, provided broad support for the model [4,5]. Studies in a wide range of species have subsequently provided further general support for the model, with anomalies being largely attributed to idiosyncrasies of individual species (reviewed [6]). However, some experiments and observations were not immediately compatible with the ABC model and hinted at additional complexity.

2. Key players in the ABC model

The ABC model was formulated from the analysis of three classes of floral homeotic mutants with organ identity defects in two adjacent whorls of the flower. Importantly, similar classes of mutant were described in both *Arabidopsis* and *Antirrhinum*, suggesting that the regulation of organ identity was highly conserved in evolution [1]. Mutants with defects in the second and third whorls, which result in the homeotic conversion of petals to sepals and stamens to carpels, defined the B-function, and include the *Arabidopsis pistillata* (*pi*) and *apetala3* (*ap3*) mutants, and the *Antirrhinum globosa* (*glo*) and *deficiens* (*def*) alleles. Flowers in which perianth organs (sepals and petals) replace the reproductive organs in the third and fourth whorls are characteristic of the *Arabidopsis* and *Antirrhinum* C-function mutants *agamous* (*ag*) and *plena* (*ple*), respectively. However, as we will see later, true recessive A-function mutants, with defects in the first and second whorls, were only described for *Arabidopsis* (*apetala1* (*ap1*) and *apetala2* (*ap2*)).

During the time that the ABC model was being proposed, the identities of some of the genes were being revealed. Among the first ABC genes to be cloned were the *Antirrhinum* B-function gene *DEF* [7] and the *Arabidopsis* C-function gene *AG* [8], the products of which shared a high degree of homology with the DNA-binding domains of two known transcription factors identified in yeast (MCM1) and animals (SRF). These four proteins became the founding members of a new family of transcription factors known as the MADS-box proteins (MCM1, AG, DEF, SRF) [9]. By the mid-1990s many of the ABC genes, from several species, had been identified and, with the exception of the *Arabidopsis* A-function gene *APETALA2* (*AP2*) [10], were shown to encode MADS-box proteins.

MADS-box factors have subsequently been shown to be key regulators of plant developmental processes, and in *Arabidopsis* at least 107 MADS-box genes have been identified [11]. Plant MADS-box proteins belong to two large families: the type I class, which group with the human SRF protein, and the type II class that groups with yeast MEF2 [11,12]. The ABC MADS-box genes belong to the type II class and are characterized by four distinct domains (Fig. 2a). From the amino-terminal end these are the MADS-domain, the Intervening domain (I), the K-domain, and the C-domain. Together, the MADS-box and I-domain form the minimal DNA-binding domain. Plant MADS-box factors bind DNA as homo- or heterodimers, or in higher-order complexes. The I- and K-domains mediate the interactions between MADS-box proteins,

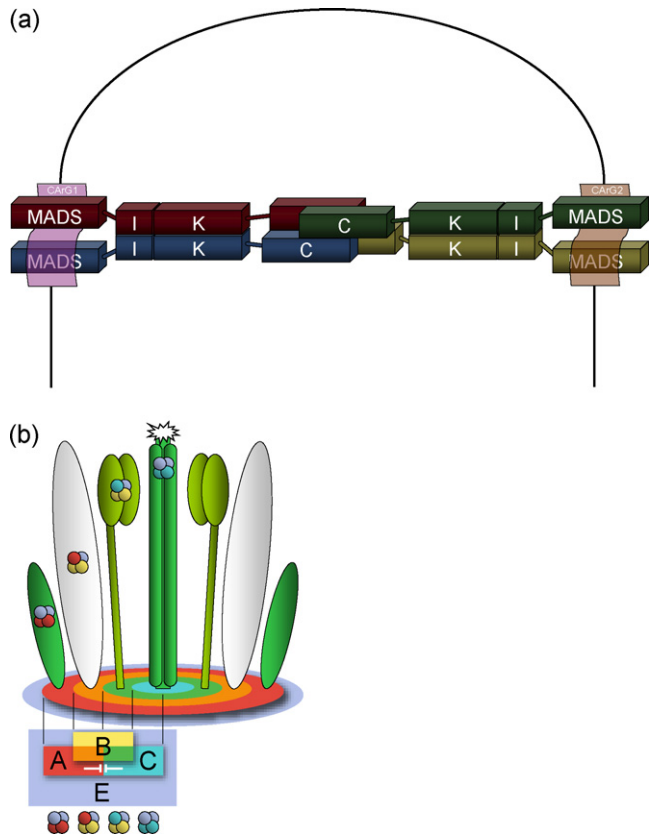

Fig. 2. The ABCE model and the protein interactions that specify organ identity. (a) The structure of the type II MADS-box factors and the floral quartet model. MADS-box factors that specify organ identity are composed of four adjacent domains, the DNA-binding MADS-box, and the I, K and C-domains. (b) The ABCE model. The E-function (purple) specifies the floral context in which the ABC genes operate. The E-function proteins form complexes with the appropriate ABC factors to direct floral organ identity. Each complex is composed of a quartet of MADS-box factors that interact through sequences within the C-termini of the proteins, as shown in (a), although sequences within the K-domain may also play a role [36]. The complex of MADS factors are thought to bind to multiple sites (known as the CArG box) within the target gene (a), causing bending of the DNA molecule (shown as a black line).

while the formation of higher-order complexes requires the C-domain.

3. Problems with the ABC model

In 1790 Johann Wolfgang Goethe proposed the theory that floral organs and leaves are variations of the same basic organ type [13]. For this theory to hold one would predict that expression of the floral organ identity genes in vegetative tissues would result in the formation of flower-like structures. However, constitutive co-expression of the B-function genes *AP3* and *PI* [4], or the C-function genes *AG* or *PLE* [5,14–16], does not alter the identity of vegetative organs. These findings suggested that the ABC-functions are necessary but not sufficient to define floral organ identity and revealed that the organ identity genes can only function within a pre-established floral context. The ABC model lacks the functions required to establish this floral context. The first clues to the identity of the genes that enable the organ identity functions to work came from mutant analyses in other species and from protein–protein interaction studies.

It was known that MIKC MADS-box factors bound DNA as dimers and heterodimers [17–19], which suggested that the combinatorial action of the ABC factors to promote floral organ identity was conferred by interactions between the proteins. For example, one might assume that stamens were specified by a physical interaction between B- and C-function proteins, and that the formation of specific MADS-box homo- and heterodimers provides a mechanism for differential DNA-binding. However, the experimental evidence did not support these hypotheses. Protein–protein and protein–DNA interaction studies revealed that the B-function proteins do not interact with any of the other organ identity factors, and are only able to pair with one another [19,20]. Other studies revealed that the biological function of MADS-proteins was independent of the DNA-binding specificity of the MADS-domain [4,21]. Consequently, the obvious one-on-one interactions between the appropriate MADS-box factors could not explain the combinatorial model for organ identity. Biological specificity of ABC proteins might instead rely on the formation of higher-order protein complexes with unrelated ternary factors, or other MADS-box factors. The first example of the formation of multimeric complexes between plant MADS-box proteins was the interaction between the *Antirrhinum* DEF/GLO dimer and the SQUAMOSA (SQUA) protein. The interaction was mediated by the C-domains of the proteins, and increased the level of DNA-binding in bandshift assays [22]. Importantly, it established a molecular basis for the combinatorial interactions of the floral organ identity genes.

3.1. The ABCE model

The isolation of novel floral mutants in *Arabidopsis*, and other species, has led to an expansion of the ABC model to include the D- and E-functions. Discussion of the D-function, which specifies ovule identity in combination with members of the C-function group of genes [23–25], is beyond the scope of this chapter, but the E-function represents an important modification of the ABC model (Fig. 2b).

Factors affecting the activity of the organ identity genes were first identified in tomato (*TM5*) and petunia (*FBP2*). Silencing of these related MADS-box genes resulted in a phenotype that suggested a decreased influence of B- and C-functions on floral development [26–28]. Later, three genes belonging the *TM5*/*FBP2* group were identified in *Arabidopsis*, named *SEPALLATA 1* (*SEP1*), *SEP2* and *SEP3*. The *sep1 sep2 sep3* triple mutant has a similar phenotype to the silenced *tm5* and *fbp2* lines, with all floral organs being replaced by sepals [29]. The phenotypes of all these tomato, petunia and *Arabidopsis* mutants are reminiscent of a *bc* double mutant, suggesting that this group of genes is required for B- and C-function activity. Expression analysis of these genes, and related genes from *Antirrhinum* (*DEFH72* and *DEFH200* [20]), revealed that they are expressed after the floral meristem identity genes, but before the onset of the organ identity genes, and in most cases in all whorls of the flower. Together, these findings suggest that the role of these genes is to pre-establish the floral context in which the organ identity genes can function. Previously this group of genes was called the intermediate or identity mediating MADS-box genes [30], but they are now referred to as the E-function, in keeping with the nomenclature of the ABC model (Fig. 2b).

To determine whether the SEP proteins were sufficient to provide the floral context for organ identity gene activity, transgenic plants were generated in which SEP proteins in combination with ABC proteins were expressed constitutively. While ectopic expression of organ identity genes or *SEP* genes alone had no effect on vegetative tissues, co-expression of the A-function (*AP1*), the B-function (*AP3* and *PI*) and *SEP3* was sufficient to convert *Arabidopsis* rosette leaves into petaloid structures [31]. Interestingly, however, co-expression of the B-function and SEP3 in the absence of the A-function was also sufficient to drive petal identity in the vegetative phase [32], suggesting that the A-function is not required for petal identity. However, expression of AP1 together with PI and AP3 also resulted in conversion of leaves into petals [32], indicating that AP1

could replace SEP3 and that AP1 may also be involved in establishing the floral context. In keeping with the ABC model, expression of SEP3, PI, AP3 and AG converted vegetative tissues into stamen-like organs [32].

The conversion of leaves into floral organs established that the organ identity genes required the activity of SEP genes. Perhaps more remarkably though, it also experimentally demonstrated that the prediction made by Goethe over 200 years ago, held true. The question remained how do the *SEP* genes regulate the activity of the organ identity genes? Analysis of gene expression in the *sep1 sep2 sep3* triple mutant revealed that SEP is not required for activation of the B- and C-function genes [29]. Protein interaction studies revealed that the SEP class of proteins interacted directly with C-function proteins [20,33,34]. Later it emerged that the SEPs are also able to interact with the B-function heterodimer, suggesting that the SEPs form higher-order complexes with the organ identity proteins [30,32]. Furthermore, it was demonstrated that in the presence of SEP3 an interaction between the PI–AP3 heterodimer and the C-function protein AG could be detected [32]. These interactions provided the first evidence that the combinatorial ABC model requires the SEP proteins to mediate interactions between the organ identity proteins (Fig. 2b). Recently, a large-scale yeast three-hybrid study revealed a large number of complexes containing at least one SEP protein. It appears then that not only do the SEPs stabilize higher-order MADS-box complexes in floral organ development, but may also act as the 'glue' for MADS-box complexes that regulate many other developmental processes [35].

Although the formation of tetrameric complexes between the SEP proteins and the ABC proteins (also known as floral quartets; see Fig. 2) explained the specification of floral organ identity, experimental proof for the stoichiometry of these complexes was only recently reported. In bandshift assays using a DNA probe containing two MADS-box binding sequences, it was shown that AP3 and PI do not bind the probe in a co-operative manner, suggesting that they do not easily form tetramers. However, co-operative binding of AP3–PI to the probe was observed in the presence of SEP3 [36]. That the DNA-binding complex contained four component MADS-box proteins was confirmed by measuring the stereospecificity of binding, thus providing compelling support for the floral quartet model.

4. Problems with the ABCE model

The ABCE model addresses some of the inconsistencies between the original model and the experimental data. By defining a further class of MADS-box transcription factors that are required to establish the floral context it explains the inability of the ABC genes alone to confer floral organ identity onto leaves. It also provides a model to explain the combinatorial nature of the ABC model, by facilitating interactions between the ABCE factors as part of higher-order quartets (Fig. 2). Despite this progress, however, there are still some outstanding issues to deal with. There are inconsistencies even in the apparently comparable homeotic mutants from which the original model was derived. Some of these, such as the fact that *Arabidopsis* B-function mutants produce a different number of whorls before termination compared to B-function mutants of *Antirrhinum*, point to important species-specific refinements of the underlying mechanism [37]. We are not concerned with these subtle differences in the regulatory circuitry here, since the B- and C-functions remain fundamentally similar. It is, however, impossible to ignore the deviations from the predictions of the model when one considers the A-function. Although the model was derived from homeotic mutants in *Arabidopsis* and *Antirrhinum*, no true recessive *Antirrhinum* A-function mutant has ever been identified. Instead, dominant mutants that perfectly mimic the idealised A-function mutant phenotype have been reported [14,38]. These mutants, which have an A-function-like carpel, stamen, stamen carpel phenotype, are gain-of-function mutants of the C-function gene *PLE*, not A-function mutants. In fact, not only does one of the two founding species lack A-function mutants, but no recessive mutant conforming to the expected A-function phenotype (Fig. 1) has subsequently been reported in any other species [39]. One cannot help but ask then if the A-function is unique to *Arabidopsis* or if it is ill-defined in this species as well.

Part of the difficulty in understanding the deficiencies of the ABC model with respect to the A-function comes from the fact that the A-function was proposed to play two distinct roles in flower development (Fig. 1); the control of organ identity in whorls 1 and 2 and the spatial restriction of C-function activity. We will deal with each of these functions separately.

4.1. Control of perianth organ identity

In *Arabidopsis* two genes are usually thought to comprise the A-function; *APETALA2* (*AP2*) and *APETALA1* (*AP1*) (reviewed [39–41]). Although mutants in these genes affect organ identity in the outer whorls, neither makes a wholly convincing case for the A-function as it is usually understood and taught in a classroom. *ap2* mutants affect a range of tissues including the outer floral whorls (the perianth), which are altered in an allele- and environment-specific manner. Strong *ap2* alleles at elevated temperatures produce first whorl organs with carpeloid characteristics and second whorl organs with stamenoid features, in accordance with the ABC model. However, the first and/or second whorl organs of *ap2* mutants can also assume a variety of other fates, including leaf or bract-like structures (see Fig. 1) or be missing or replaced by secondary flowers (see [39]). Similarly, *ap1* mutants mainly form bract-like first whorl organs and have missing organs in whorl 2, where secondary flowers sometimes develop (see [39]). The development of vegetative/inflorescence lateral organs and the formation of new flowers in the outer whorls of mutant flowers cannot be explained by the ABC model. These aspects of the phenotype suggest an incomplete establishment of the floral state, rather than a defect in floral organ identity [42]. Indeed, the requirement for floral meristem identity to be specified is not restricted to *Arabidopsis*; mutations affecting sepal identity also affect meristem identity in all species tested [39,43]. The link between sepal production and floral meristem identity is most obvious in *Antirrhinum* where mutants of the orthologue of *AP1*, *SQUA* rarely flower and instead replace flowers with new inflorescences bearing no floral organs [44].

The absence of a role for the A-function in determining petal identity is illustrated by the fact that petals are still produced in *ap1* mutants in combination with certain other mutants. These include combinations with the C-function mutant *agamous* (*ap1 ag* [45]), with an inflorescence meristem identity mutant *agl24* (*ap1 agl24* [46]), and with plants constitutively expressing a gene required for the floral context, *SEPALLATA3* (*ap1 35S:SEP3* [47]). The lack of influence of *AP1* on petal development in *Arabidopsis* is also in agreement with mutant studies in several other species [39]. For example, on the rare occasions when *squa* mutants flower, the flowers are misshapen, but do not show altered organ identity [44]. On the other hand, *AP2* in *Arabidopsis* and the orthologous *LIP* genes in *Antirrhinum* seem to play a role in the growth and terminal differentiation of petals [48,49], although in *Arabidopsis* this is complicated by the influence of *AP2* on the spatial control of the C-function (discussed below).

In conclusion, there is little evidence to support a central role for the A-function in determining petal identity in any species. The influence of the A-function on sepal identity is inseparably linked to its role in the establishment of floral meristem identity, although species can differ in their abilities to produce floral organs from incompletely specified floral meristems.

4.2. The spatial control of C-function genes expression

The expansion of the C-function into the outer whorls of *Arabidopsis* A-function mutants revealed the second aspect of the A-function—the spatial regulation of the C-domain. According to the ABC model, mutual antagonism between the A- and C-functions defines the expression domains of these genes. That C antagonises A-gene expression is proven in other species, at least for *AP1*-like genes, suggesting that this conserved function of C-genes is important for termination of organ initiation in the centre of the flower (floral determinacy). However, with the exception of *Arabidopsis*, none of the described *ap1* or *ap2*-like mutants from any species show ectopic C-function in the perianth (reviewed [39]). This may therefore be an independently acquired function, unique to *Arabidopsis*. Thus, spatial control of the C-function, which seemed a straightforward integral role of the A-function in the ABC model, cannot form part of a generally applicable flowering model.

According to the ABC model, each organ identity gene is specifically expressed in two adjacent whorls of the flower. In contrast, the *Arabidopsis* A-function gene *AP2* is involved in several floral and non-floral developmental events with a correspondingly broad expression pattern. These features of *AP2* are shared by several other negative regulators of the C-function, which are typified by the production of reproductive organs in the outer whorls of their mutants (reviewed [6,41,50–52]). Originally, these regulators of the C-function were not considered part of the ABC model.

The ubiquitous expression of these negative regulators of the C-function makes it difficult to explain how they can specifically exclude C-function expression from the outer whorls. Two recently published models propose viable mechanisms to solve this problem for *Arabidopsis* [53] and *Antirrhinum* [54]. While there are differences between these models, they both describe a system in which the C-function is repressed in all whorls of the flower and is only induced in cells in which activators antagonise the repressive state. In *Antirrhinum* the balanced activities of activators (e.g. FLORICAULA [55]) and ubiquitously expressed repressors (e.g. STYLOSA (STY) and FISTULATA (FIS) [50,51,54]) of *PLE* expression controls the outer boundaries of the C-function expression domain. Consistent with this, loss of *FIS* activity results in increased *PLE* expression at the centre of the flower and an outward expansion of the C-domain, and production of ectopic reproductive structures in the perianth. In *Arabidopsis* SEP3 and AP1 (and likely other related proteins [56]) repress the C-function by direct interaction with the repressor proteins LEUNIG (the orthologue of STY) and its partner SEUSS in all whorls, but this repression is antagonised by activators such as LEAFY and WUSCHEL in the inner whorls and by converting SEP3 in combination with AG into an activator [57]. As in *Antirrhinum*, enhanced levels of the C-function gene at the centre of the developing *Arabidopsis* flower was shown to result in expansion of the C-domain into the perianth and conversion of those organs into reproductive structures [58]. In the centre of a wild-type flower a self-tuning control system reinforces the C-domain once a particular threshold of C-function gene product is reached [54,57,58]. Perianth specific repressors, including RABBIT EARS [59], ensure that the C-domain is restricted to the centre of the flower as other repression mechanisms begin to breakdown during floral maturation [58].

5. A new (A)BC model with widespread applicability

Several points illustrate the fact that there is no A-function comparable to the B- and C-functions in any species. Only the B- and C-functions act subsequent to the establishment of meristem identity to determine organ identity. Only the expression domains of the B- and C-functions align with an organ identity function in two adjacent whorls. Only the B- and C-functions have both individual and combined roles in establishing floral organ identity and only these functions are exclusively composed of MADS-box transcription factors. Finally, unlike the A-function genes that have additional non-floral roles, expression of the B- and C-function genes outside the flower has no consequence, because they lack the floral meristem environment they need to exert their effects. In strict terms of organ identity therefore we have a "BC" model, analogous to that proposed by Schwarz-Sommer et al. [9]. However, we would like to incorporate a newly defined "(A)" function into the model to provide a more complete representation of the current results from many species. In this model (Fig. 3) the (A)-function fulfils several roles. It is expressed before the B- and C-functions and acts to establish the floral meristem identity, thus facilitating the production of the ground state of floral organs, the sepals. The (A)-function is also required for the later activation and regulation of the B- and C-functions, which results in the establishment of B- and C-function boundaries. Its role in setting the floral context means that the (A)-function includes the *SEPALLATA* (E-function or Im) genes. These genes, unlike B- and C-function genes, contribute to both the B- and C-functions and establish a floral context by their involvement in the control of floral meristem identity. In short, the (A)-function comprises those requirements necessary to enable the B- and C-functions to exert their control over floral organ identity.

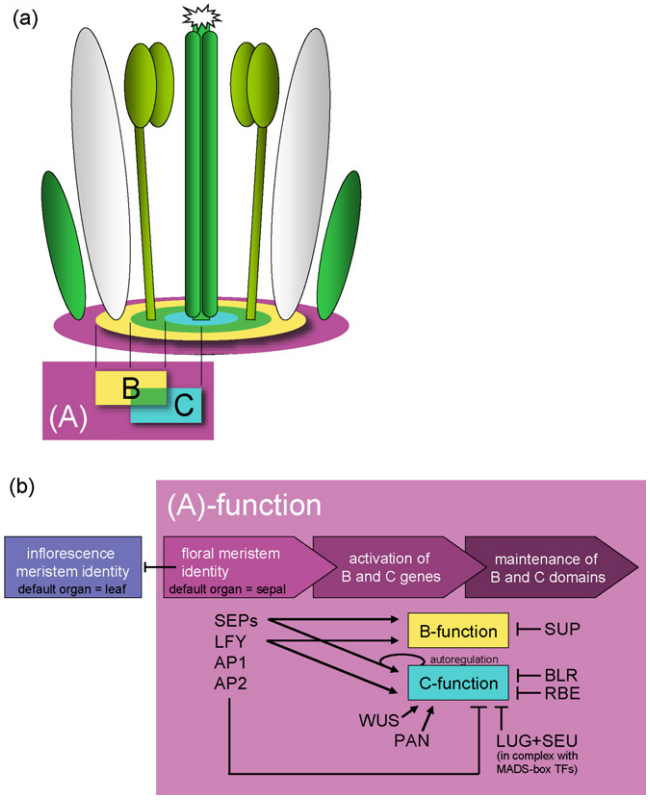

Fig. 3. The (A)BC model. (a) The newly applicable (A)BC model. As in the textbook ABC model, the B- and C-function genes are expressed in two adjacent whorls of the flower to promote petal, stamen and carpel identities (scheme as used in Fig. 1). The newly defined (A)-function, which replaces the textbook A-function in this model, provides the context in which the B- and C-functions are active, and regulates their spatial and temporal expression domains. (b) The multifaceted roles of the (A)-function, using *Arabidopsis* as an example. The (A)-function has roles in floral mersitem identity, activation of the B- and C-function genes, and regulation of the B- and C-gene expression domains. The transition from inflorescence identity to floral identity relies on the expression of (A)-function genes that repress inflorescence identity and promote the floral context. Once the floral context is established, the (A)-function induces expression of the floral organ identity genes and subsequently restricts activity of the B- and C-function genes to specific domains. Arrows represent activation, bars represent repression.

This concept of the (A)-function enables the (A)BC model to regain its widespread applicability and provides a framework with which the existing mutants can be interpreted. The complexity and diversity of the individual components of the (A)-function are accommodated, whilst it is not necessary to understand them in detail to make a basic use of the model. As new genes are identified, such as those controlling the outer boundaries of B-function expression, they can be added to the (A)-function without affecting the model. Finally, the (A)BC model represents a move away from the solid blocks of mutually exclusive gene expression that characterised the previous model and that failed to fit the observable data. The (A)BC model permits a more flexible dynamic control of the domains of expression of the organ identity genes, providing an easy way to explain variations in expression patterns seen in different species. As more regulatory genes are discovered, it will be interesting to see how the different components of (A) vary between species, and the extent to which (A) is composed of conserved genes and convergent functions.

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