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QUARTERLY

Review

MADS-box genes are involved in floral development and evolution★

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MADS-box genes encode transcription factors in all eukaryotic organisms thus far studied. Plant MADS-box proteins contain a DNA-binding (M), an intervening (I), a Keratin-like (K) and a C-terminal C-domain, thus plant MADS-box proteins are of the MIKC type. In higher plants most of the well-characterized genes are involved in floral development. They control the transition from vegetative to generative growth and determine inflorescence meristem identity. They specify floral organ identity as outlined in the ABC model of floral development. Moreover, in *Antirrhinum majus* the MADS-box gene products DEF/GLO and PLE control cell proliferation in the developing flower bud. In this species the DEF/GLO and the SQUA proteins form a ternary complex which determines the overall "Bauplan" of the flower.

Phylogenetic reconstructions of MADS-box sequences obtained from ferns, gymnosperms and higher eudicots reveal that, although ferns possess already MIKC type genes, these are not orthologous to the well characterized MADS-box genes from gymnosperms or angiosperms. Putative orthologs of floral homeotic B- and C-function genes have been identified in different gymnosperms suggesting that these genes evolved some 300–400 million years ago. Both gymnosperms and angiosperms also contain a hitherto unknown sister clade of the B-genes, which we termed Bsister. A novel hypothesis will be described suggesting that B and Bsister might be involved in sex determination of male and female reproductive organs, respectively.

If the default program of plant growth is flowering, then a repressor is required to ensure vegetative growth of the plant. Indeed, recessive mutations are known in *Arabidopsis*

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Abbreviations: MYA, million years ago.

thaliana featuring immediate flowering of the plant giving rise to the above hypothesis (Chen *et al.,* 1997; Sung *et al.,* 1992). Two such repressor-encoding genes were identified to belong to the MADS-box gene family of transcription factors (Hartmann *et al.,* 2000; Michaels & Amasino, 1999; Sheldon *et al.,* 1999). However, in a certain developmental stage of plant growth and upon receiving the correct environmental signal phase transition occurs leading to the formation of an inflorescence thus initiating the generative mode of growth. At the base of floral development two genes both in *Arabidopsis thaliana* and in *Antirrhinum majus* are of importance, *Leafy/Apetala 1* and *Floricaula/Squamosa* respectively. Recessive mutants do not form floral primordia but instead produce secondary inflorescences. Hence the above genes control flower meristem identity. *Apetala 1* and *Squamosa* encode MADS-box proteins (Mandel *et al.,* 1992; Huijser *et al.,* 1992). Further floral development is guided by a series of interacting MADS-box proteins. How they act and how they specify the floral "Bauplan" will be described for *Antirrhinum majus.*

In addition the phylogeny of MADS-box genes will be described as well as possible scenarios for the origin of angiosperm flowers.

THE FLORAL "BAUPLAN"

Floral organs in most eudicots are arranged in whorls, as in *Antirrhinum majus,* a representative of this group of plants. Its floral "Bauplan" is shown in Fig. 1.

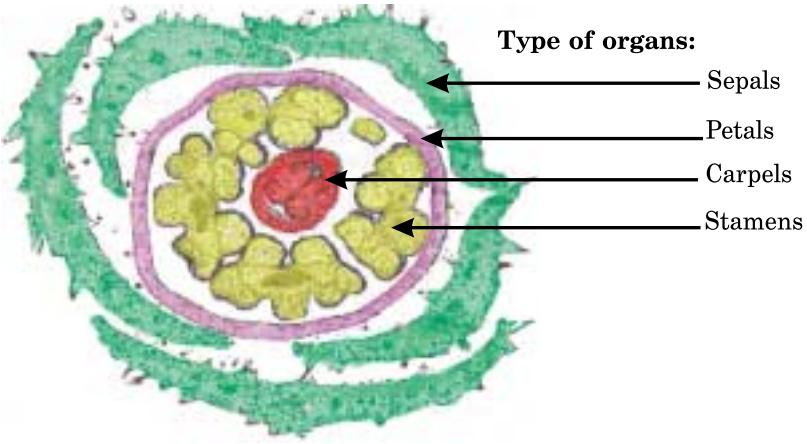

Figure 1. "Bauplan" of *Antirrhinum majus.*

Number of organs/whorl. In this case are 5 for w1, w2 (fused to a tube and seen here as a ring) and w3, while w4 has only 2 carpels. Arrangement of organs either is whorled as shown here, or decussate or spiral as in some other plants.

The outermost whorl (w1) features five green sepals, while w2 holds five colored petals, which are fused to form a tubular structure with protruding lobes (Fig. 3a). These two whorls form the perianth, the protective organs. Further inside follow the sexual organs: w3 is male and features 4 stamens and 1 stamenoid, while the innermost female whorl (w4) contains a bilocular gynoecium.

The numbers of organs, the types of organs and their arrangement characterize the "Bauplan".

MADS-box genes seem to control these features. This is the subject of what follows.

THE ABC MODEL

The model is based on homeotic mutations in *Antirrhinum majus* and *Arabidopsis thaliana*, resulting in phenotypic replacements of organs in two adjacent whorls. Three classes of mutants are observed leading to the ABC model (Fig. 2):

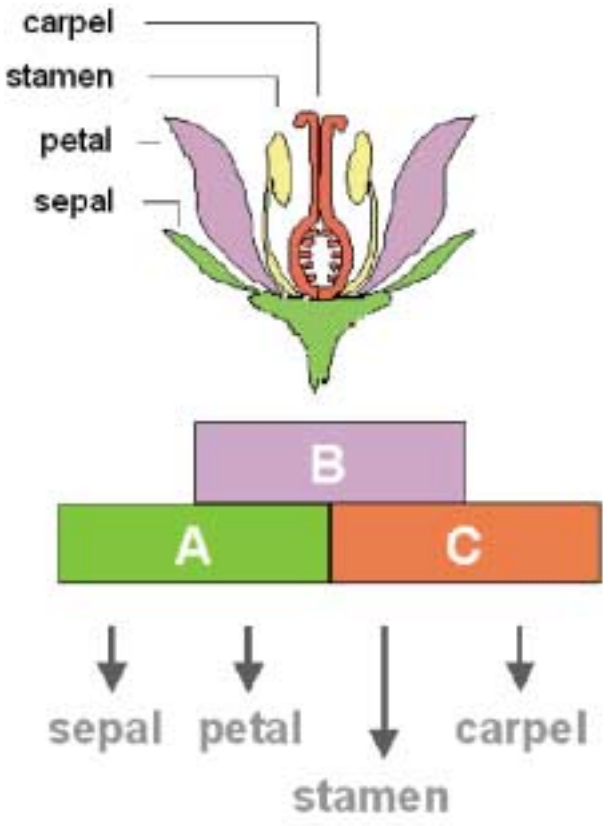

Figure 2. The ABC model.

- A-function mutants have organ replacements in w1 and w2. Instead of sepals and petals they feature carpeloid and stamenoid organs, respectively.
- B-mutants have altered organs in w2 and w3 where sepals and carpels are formed; these are thus male sterile.
- C-mutants feature no sexual organs at all; petals and sepals replace stamens and carpels in w3 and w4, respectively.

All said, the three functions seem to specify organ identity in a combinatorial way: the A-function specifies sepals and together with the B-function petals, while B- and C-functions are needed for stamens and C-function alone determines carpel formation.

However, there are also differences between species. In *Antirrhinum* for example, no recessive A-function mutations are found. Rather the A mutant phenotype is triggered in this species by a dominant mutation in a C-function gene indicating that the ABC model may be of mnemotechnic value, but certainly is not applicable to all species in its simplistic form.

Nonetheless, MADS-box genes encode ABC functions, which will be described below for *Antirrhinum majus*.

FUNCTION OF THE MADS-BOX GENES

Plant MADS-box genes encode transcription factors with MIKC domain structure. The M-domain represents the DNA-binding domain, while I is an intervening and K is a Keratin-like domain and is involved in protein–protein interactions as is the C-terminal domain in certain MADS-box proteins.

A-function genes

The *Squamosa* gene of *Antirrhinum majus* (Huijser *et al.,* 1992) encodes a protein orthologous to *Apetala 1* (Mandel *et al.,* 1992), one of the A-function genes of *Arabidopsis thaliana.* Nonetheless, *squamosa* mutants do not reveal an A-function deficient phenotype, but they rather produce secondary inflorescences instead of flowers. Therefore, *Squamosa* seems to be involved in floral meristem identity. On top of that, in conjunction with B-function genes, *Squamosa* is also involved in determining the floral "Bauplan".

B-function genes

Deficiens and *Globosa* are two genes located on different chromosomes in *A. majus* (Sommer *et al.,* 1990; Schwarz-Sommer *et al.,* 1990; Tröbner *et al.,* 1992). Mutants in each of them result in the almost same homeotic phenotype. Instead of petals a second whorl of sepals is formed in w2, and in w3 carpels replace stamens and form a tubular structure (Fig. 3b and d). w4 is missing altogether as can be seen in the cross section in Fig. 3d.

DEF and GLO form the B-function and determine organ identity. In addition they also pro-

mote cell proliferation and as will be shown later they are involved in determining the floral "Bauplan" as well.

Molecularly DEF and GLO form a heterodimer which binds to the promoter of both genes, thus leading to an auto-regulated amplification of transcription as has been shown by *in vitro* studies as well as by *in situ* hybridization (Schwarz-Sommer *et al.,* 1992; Tröbner *et al.,* 1992; Zachgo *et al.,* 1995). *Def* and *Glo* are predominantly expressed in w2 and w3 in agreement with the mutant phenotype. However, some low-level expression is observed for *Def* in w1 and in w4. This raises the question of control of gene expression for *Def* and *Glo*. Numerous genes are involved in spatial and temporal control of expression of *Def* and *Glo* (Motte *et al.,* 1998; Wilkinson *et al.,* 2000), but these, except for *Ple* and *Far*, which will be described in the next paragraph, are not subject of this contribution.

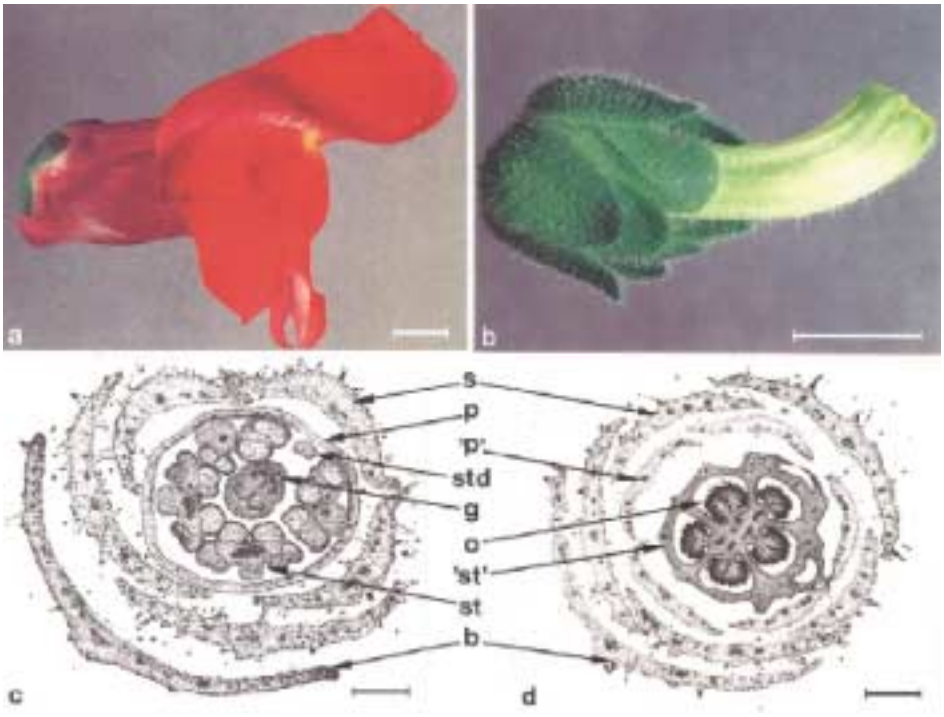

Figure 3. Mutant phenotype of B-function genes (b, d) compared to wild-type (a, c) in *Antirrhinum*.

C-function genes

In *A. majus* two very similar MADS-box genes are known, *Plena* (Bradley *et al.,* 1993) and *Farinelli* (Davies *et al.,* 1999). While *ple* knock-out mutants feature filled flowers, i.e. the sexual organs are replaced by perianth organs in a reiterated manner, in the dominant *plena*-macho allele, in which *Ple* is ectopically expressed, sepals and petals are replaced by carpels and stamens, and hence *macho* is fully sexualized (Fig. 4).

Since *far* mutants do not feature a homeotic phenotype, but rather are only male sterile, this gene seems not to classify as a C-function gene.

However, its structural relationship to *Ple* as well as the ectopic expression effects of *Far* in transgenic tobacco also reveals its functional relationship. Even though the first whorl organs are not affected in transgenic plants, the petals in w2 are replaced by stamens thus revealing the C-function. Why then are *far* mutants not homeotically transformed? The expression of *Far* in stamens for example is complementary to the expression of *Ple* and hence does not affect organ identity but rather the functionality of the organ resulting in male sterility upon mutation.

On the other hand *Far* also controls, in conjunction with *Ple* the expression level of

B-function genes resulting in a rather complex regulatory network for cell proliferation. Based on this and the facts that:

- B-mutants do not form 4. whorl organs, and
- C-mutants show excessive number of whorls,

a model for cell proliferation in floral development has been suggested (Davies *et al.,* 1999) according to which PLE/FAR block cell proliferation in w3, but this block is counteracted by DEF/GLO directly or through OCTANDRA. On the other hand PLE/FAR also block the expression of *Def/Glo* such that in w4 only PLE/FAR are present thus terminating development of further whorls.

Having thus developed a flower, how is its "Bauplan" laid down?

Squa AND *Def/Glo* AND THE FLORAL "BAUPLAN"

While the wildtype and *squa Antirrhinum* flowers are made up of 4 distinctive whorls, the first three of that hold 5 and w4 has only 2 organs. The B-function mutants *def* and *glo* feature basically the same structure, except that w4 is missing. Recently we have shown that the "Bauplan" is different in the double mutant *squa, def* (Egea-Cortines *et al.,* 1999). As is shown in Fig. 5 only one whorl of organs is seen, all other inner organs, though they have been homeotically transformed, are in a spiral arrangement and their numbers have increased also. More than a dozen additional sepals followed by a multitude of spirally arranged carpels is seen.

Hence, SQUA and DEF together seem to secure the architecture of the typical *Antirrhinum* flower.

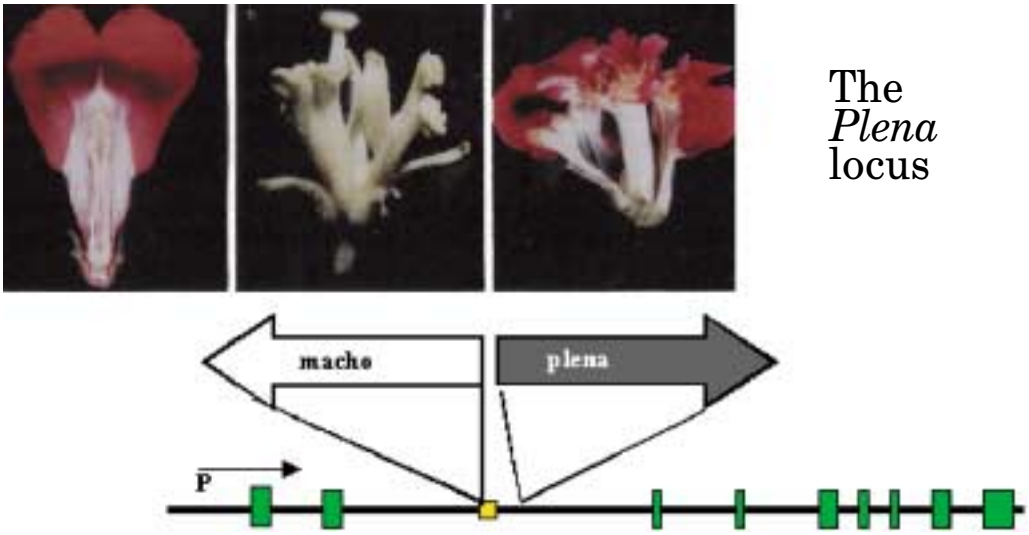

Figure 4. Mutant phenotypes of a C-function gene in *Antirrhinum*. Left: wild-type flower; middle: *plena*-macho flower; right: *plena* loss-of-function flower.

How can that be achieved molecularly?

Yeast three hybrid studies using a DEF/GLO heterodimer as bait fished out SQUA thus indicating that "A-" and the B-functions indeed seem to interact. Subsequently this could be corroborated through *in vitro* studies. It turned out that the interaction

Figure 5. Schemes of mutant phenotypes of Squa and *Def/Glo* and the floral "Bauplan".

is mediated through the C-terminal domains of these MADS-box proteins. The ternary complex thus formed seems to recognize two CArG boxes within target genes needed to establish the floral "Bauplan".

In summary. MADS-box proteins are needed for floral meristem identity (SQUA), for floral organ identity (DEF/GLO, PLE), for promoting cell proliferation (DEF), blocking cell proliferation (PLE), male fertility (FAR) and maintaining floral architecture (SQUA, DEF/GLO).

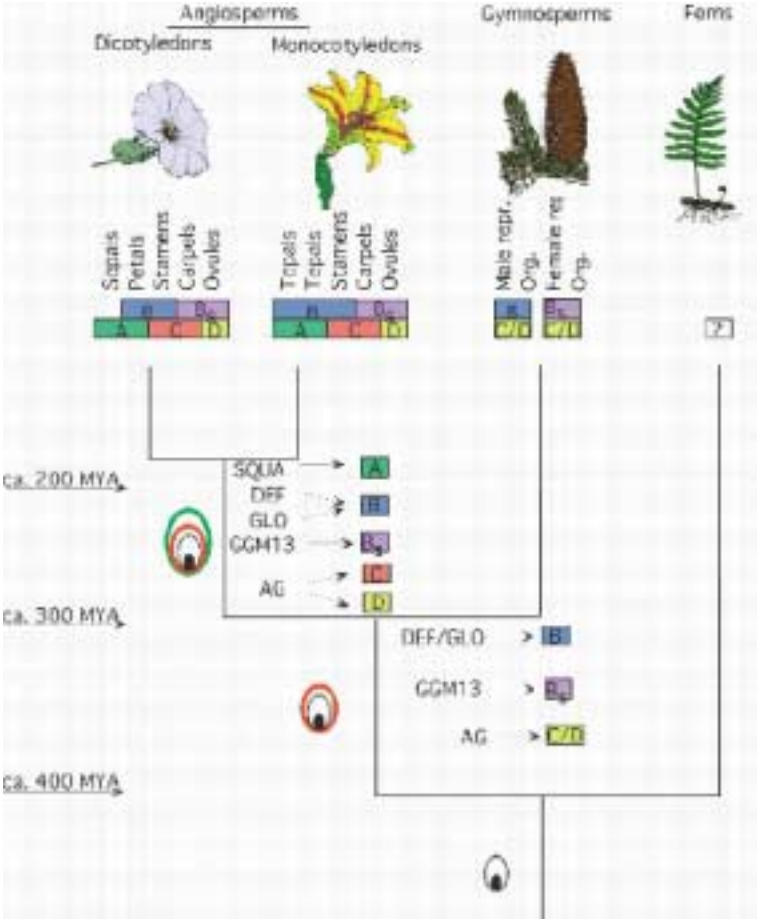

Figure 6. Phylogeny of major land plant taxa and the evolution of floral homeotic functions.

Therefore, understanding the phylogeny of MADS-box genes may provide insights into flower origin and evolution.

PHYLOGENY OF MADS-BOX GENES

MADS-box genes from informative taxa such as the moss *Physcomitrella*, the ferns *Ceratopteris* and *Ophioglossum*, the gymnosperm *Gnetum* and the monocotyledonous flowering plants *Zea*, *Tulipa* and *Lilium* were characterized. For review see: "A short history of MADS-box genes in plants" (Theißen *et al.*, 2000). The evidence obtained through DNA-sequencing and phylogeny reconstructions by computer suggests that the last common ancestor to extant ferns and seed plants about 400 million years ago (MYA) had homologs, but not orthologs, of floral homeotic genes of the MIKC type (Fig. 6).

However, putative orthologs of floral homeotic B- and C-function genes have been identified in different gymnosperms, suggesting that these genes originated 300–400 MYA (Winter *et al.*, 1999).

In addition, both angiosperms and gymnosperms also contain members of a hitherto unknown sister clade of the B genes, termed Bsister (Bs) genes.

EXPRESSION OF B AND Bs

In contrast to the B genes, which are predominantly expressed in male reproductive organs in both gymnosperms and angiosperms, expression of the Bs genes was found to be predominantly in female reproductive organs in *Gnetum gnemon* (Fig. 7). Moreover, we could show that a Bs gene isolated from *Zea mays* was also predominantly expressed in female organs.

Figure 7. Expression of B- and Bs-genes in *Gnetum gnemom*.

A NOVEL HYPOTHESIS

The above mentioned data suggest an ancestral system for the specification of reproductive organ identity which was established at the base of extant seed plants, about 300 MYA. We assume that it is the ancestral function of C genes to distinguish between reproductive (C expression on) and non-reproductive organs (C expression off). Superimposed is the differential expression of B (and probably also Bs) genes to discriminate between male and female reproductive organs. This possibly represents the ancestral sex determination system of extant seed plants.

We hypothesize that a superclade of B and Bs genes played an important role during two key innovative events in the evolution of seed plant reproductive structures.

First, the establishment of distinct male B and female Bs gene lineages after duplication of an ancestral gene may have been a crucial event during the origin of male microsporophylls and female megasporophylls (400–300 MYA).

Second, during flower origin (300–200 MYA), expression of the Bs genes expanded into one of the key structures of angiosperms, the carpel, while B expression and function expanded into the petal, another evolutionary novelty of flowering plants.

These hypotheses provide novel starting points for scenarios, which describe how flowers may have evolved out of gymnosperm cones.

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