REVIEW

New roles for MADS-box genes in higher plants


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Abstract

Putative transcription factors bearing a particular DNA-binding domain called "MADS-box", have been mainly involved in processes related to flower development. It is generally accepted that MADS-box genes may have played a central role in the evolution of plant reproductive structures. During the last years increasing evidence points to more general roles of these factors that spans to the control of the flowering time, but also to other non-reproductive processes. Moreover, sequencing of the Arabidopsis genome has led to the recognition of above hundred MADS-box genes in this model organism, most of them still uncharacterized. This opens the possibility of uncovering new roles for MADS-box genes in plant development and evolution.

Additional key words: Arabidopsis, flowering time, plant development, transcription factor, vegetative development.

Introduction

The "MADS" term gathers the initials from the Minichromosomal maintenance 1 (MCM1), Agamous (AG), Deficiens (DEF) and Serum response factor (SRF) genes, all of them sharing the so-called MADS-box (Schwartz-Sommer et al. 1990). MADS-box genes make up a multigene family encoding putative transcription factors which are characterized by a conserved DNA-binding/dimerization domain (Shore and Sharrocks 1995, Riechmann and Meyerowitz 1997). In addition to their wide distribution among animals, plants and fungi, some homology has also been found in bacteria, supporting the notion that the MADS-box could already be present in the common ancestor to prokaryotes and eukaryotes (Mushegian and Koonin 1996). During the last decade a huge amount of genetic and molecular information has accumulated, mainly from plant models like Arabidopsis, Antirrhinum and Petunia, leading to the notion that MADS-box genes could have played an important role in the evolution of the reproductive structures in plants. This has opened the way to investigate the evolutionary origin of the morphological diversity among plant species (for recent reviews see Irish 2000, Theissen et al. 2000, Ng and Yanofsky 2001). Although most of the available knowledge refers to the function of MADS-box genes in the differentiation of floral/inflorescence and floral organ meristems, growing evidence has pointed out to the involvement of this gene family also in the vegetative development of the plant. In this review we focus on the role of plant MADS-box genes in processes different to those of flower development.

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Abbreviations: AGL - agamous like; GA - gibberellins; LD - long day; MIKC - MADS-box intermediate keratine-like C-terminal domain structure; MADS - minichromosomal maintenance 1 agamous-deficiens serum response factor; NMKC - N-terminal MIKC domain structure; SD - short day.
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Structure and evolution of MADS factors

Most of plant MADS proteins initially described shared a similar structure designated as MIKC, according to the initials of their characteristic domains (Fig. 1A). The MADS-box domain, placed at the N-terminus, is composed by a sequence of about 60 amino acids pretty well conserved among different MADS-box genes and organisms. It folds to give a structural motif with an antiparallel coiled coil of two α-helices (one from each monomer) that is involved in the specific binding to DNA sequences (CARG boxes) conforming the consensus CC(A/T)XGG (Pellegrini et al. 1995). A second conserved domain, the K-box spans about 70 amino acids and is characterized by regularly spaced hydrophobic residues. The resulting structure is an amphipathic α-helix (keratin-like) that is responsible for protein dimerization. This particular domain seems to be an evolutionary acquirement exclusive of plant MADS factors, since it is absent from fungal or animal MADS proteins (Theissen et al. 2000, Alvarez-Buylla et al. 2000b). The intermediate (L) or linker (L) domain is a short variable segment of about 30 residues located between the K and MADS boxes. Early studies have shown that MADS proteins bind DNA as dimers, and that the molecular determinants controlling dimerization specificity are distributed along the MADS, I and K domains, varying among different MADS proteins (Riechmann et al. 1996). Recent interaction models propose that DNA binding could be achieved through the formation of multimeric complexes of MADS factors that would interact with a pair of CARG boxes brought into proximity through DNA bending (Theissen and Saedler 2001, Theissen 2001). Finally, the C-terminal domain is a sequence and length-variable amino-acid stretch that is though to mediate protein-protein interaction, acting as a transactivation domain. In addition, some MADS-box factors possess an additional N-terminal extension, the N-domain, and are accordingly designated as NMIKC.

Recent phylogenetic analysis in Arabidopsis thaliana has led to the proposal of two evolutionary lineages represented by Type I and Type II MADS-box genes (Alvarez-Buylla et al. 2000b). They differ both in the amino acid sequence of the MADS-box as well as in the domain structure of the predicted protein. The MADS-box of Type I factors seems to have a higher homology to that of animal and fungi SRF-like factors, while for Type II proteins the MADS-box sequence is clearly more related to that of MEF2 (myocyte enhancer factor 2) like factors, also represented in animals and fungi. In addition,

![Fig. 1. Structure and domain function of plant MADS-box factors. Phylogenetic analysis and domain structure has lead to the proposal of two MADS-box classes or lineages in plants. A. Type I genes bear a MADS-box with a high homology to that of SRF-like factors and lack the K-box that is substituted by a C-terminal variable domain. B. Type II genes exhibit an MEF2-like MADS-box and the typical MIKC structure. Some of them also possess a variable N-terminal extension, not drawn in the scheme. Domain functions demonstrated or predicted for Type II factors are shown below the corresponding boxes.](image)

![Fig. 2. Phylogenetic analysis of the MADS-box family in Arabidopsis. A total of 104 protein sequences comprising the MADS-box plus some 20 - 50 additional residues were aligned using the Clustal X (v.1.8) program using the default settings. The alignment containing 120 positions was used to construct the unrooted neighbor-joining tree. Clusters for Type II factors were designated as in (Theissen et al. 1996), or arbitrarily, for the Type I class. Arrows indicate those Type I factors already cited in literature. Type II factors not previously reported are marked by an asterisk. Bootstrap values (over 1000 replicates) are indicated for relevant nodes of the Type I class. MADS-box proteins not previously reported were named according to their gene designation as stated in the Arabidopsis databank.](image)
Type I factors lack the K-box, conforming a structure with a MADS-box followed by a rather undefined and length-variable domain, while most of Type II factors exhibit the typical MIKC structure. In addition, an N-terminal variable extension is found in a number of both Type I and Type II factors. Nevertheless, monophyly of the Type I lineage was not well defined, as a number of genes lacking the K-box are also present in *Arabidopsis* whose MADS-box can be either clearly assigned to the MEF2 type, or difficult to classify in any of the SRF or MEF2 groups. The study was based on the available sequence information at that time (45 MADS-box genes). Upon complete sequencing of the *Arabidopsis* genome it is now possible to get a whole picture of the MADS-box family. In this work we present a whole phylogenetic analysis (Fig. 2), where MADS box proteins deduced from genes found in the *Arabidopsis* database, a total of 104, have been included. 38 of them correspond to MIKC/Type II genes, characterized by the classical MIKC structure and the presence of a MEF2-like MADS-box. These genes appear grouped in accordance to the clusters previously described, with the inclusion of three new members, not previously reported, placed in clusters SQUAMOSA (SQUA) and AGL15. The 66 remaining genes group together in a big cluster that include 12 agamous-like genes (AGLs) previously designated as Type 1 or unclassified genes (Alvarez-Buylla et al. 2000b). Although statistical support (bootstrap values) is, as in the previous report, not good for this clade, we will keep the Type-I designation of this group for simplicity. Within this group, an enormous heterogeneity exists related to both domain structure and MADS-box sequence, although they all lack the K-box. Thus, while the MADS-box of genes within the AGL35 cluster seem to have a higher homology to SRF-like MADS-box (based on the conservation of a number of characteristic residues), others, like those in the AGL23 or T13M22.2 clades, remain difficult to assign to any of the two groups. The question whether a SRF-like MADS-box is present in plant factors is relevant because it would indicate, as it has been proposed, that the evolutionary split between SRF and MEF2-like MADS-box genes took place before separation between animal/fungi and plant kingdoms (Alvarez-Buylla et al. 2000b). On the other hand, it is evident that the reduced number of the previously reported Type I genes just represented the iceberg tip of a quantitatively important group that contains even more members than the better known Type II class. This favours the notion that plant genes lacking the K-box may represent active genes and not merely evolutionary by-products (i.e. pseudogenes). Moreover, the almost absolute lack of information about these genes opens a wide investigation field that may uncover the participation of MADS-box genes in new cellular processes.

**Functions performed by MADS-box genes**

A great diversity of functions have been described for MADS-box genes, covering both developmental and metabolic processes. Thus, the yeast ARG80 factor is involved in the control of arginine metabolism (Dubois et al. 1987), while MCM1 regulates transcription of different genes involved in cell cycle, growth and cell differentiation (Passmore et al. 1988). In insects, the MADS-box genes so far known have been involved in the development of the muscle and trachea (Affolter et al. 1994, Lilly et al. 1994). For vertebrates, described functions include muscle development (MEF-like factors) and mediation of the cellular response to growth factors (SRF). The first two MADS-box genes described in plants in early nineties were *deficient* from *Antirrhinum majus* and *agamous* from *Arabidopsis thaliana*, both involved in flower development. Since then, about 40 genes have been uncovered in *Arabidopsis*, a number that is probably less than half of the whole family size in this organism (Fig. 2, Riechmann et al. 2000), which is by now the main reference in higher plants. Most of the known functions are related to the flowering process either by controlling the appropriate development of floral meristem (meristem identity genes), or the different flower organs (organ identity genes). The analysis of mutants and the expression patterns of genes involved have led to the proposal of geneic models explaining gene interactions during flower development (reviewed in Theissen 2001). In addition to this, recent studies have demonstrated the involvement of MADS-box genes in the control of flowering time, fruit development, as well as in different aspects of the vegetative development.

**MADS-box genes and the control of flowering time**

The switch from vegetative to reproductive development is regulated by a complex genetic network that integrates both endogenous and environmental signals, mainly corresponding to the age of the plant, photoperiodic conditions and temperature. The analysis of mutants affected in flowering time, mainly in *Arabidopsis*, as well as molecular data from a number of the involved genes, have revealed the existence of several genetic pathways
leading to flowering (Fig. 3), for which a high redundancy exists (for review see Koornneef et al. 1998, Simpson et al. 1999, Reeves and Coupland 2000, Araki 2001). The following pathways have been described in Arabidopsis, although their characteristics may differ in other plant species depending on the particular photoperiodic/vernalization requirements.

The so-called autonomous pathway monitors somehow the developmental state (age) of the plant by sensing endogenous signals. The vernalization pathway allows the plant to flower early when exposed to low temperatures. Light quality and day-length signals are channelled through the so-called photoperiodic pathway which acts by accelerating flowering under long-day photoperiods (LD) relative to short days (SD). A fourth pathway has been proposed in which gibberellins phytohormones (GA) would promote flowering through a day-length independent pathway that is essential under short day conditions. A number of MADS-box genes have been shown to participate within this network (Table 1, Fig. 3).

Table 1. Arabidopsis MADS-box genes involved in the control of flowering time.

<table>
<thead>
<tr>
<th>Name</th>
<th>Effect on flowering transition</th>
<th>Flowering pathway</th>
<th>Other functions</th>
<th>Putative orthologues (organism)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLC/FLF (AGL25)</td>
<td>repressor</td>
<td>autonomous and vernalization</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>MAFI/FLM (AGL27)</td>
<td>repressor</td>
<td>autonomous and vernalization</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>SOC1 (AGL20)</td>
<td>promoter</td>
<td>autonomous, vernalization and photoperiodic</td>
<td>unknown</td>
<td>SAMADSA (Sinapis alba)</td>
</tr>
<tr>
<td>FUL (AGL8)</td>
<td>promoter</td>
<td>unknown</td>
<td>carpel and fruit development; floral identity</td>
<td>MADS1 (Nicotiana tabacum)</td>
</tr>
<tr>
<td>SVP (AGL22)</td>
<td>repressor (?)</td>
<td>autonomous (?)</td>
<td>unknown</td>
<td>SAMADSB (Sinapis alba)</td>
</tr>
<tr>
<td>AGL24</td>
<td>promoter (?)</td>
<td>unknown</td>
<td>unknown</td>
<td>STMA3516 (Solanum tuberosum)</td>
</tr>
</tbody>
</table>

Genetic analysis of the plant response to vernalization allowed the identification of the locus flowering locus C. The corresponding gene (FLC) has been cloned independently by T-DNA tagging (Sheldon et al. 1999) and chromosome walking (Michaels and Amasino 1999), and shown to encode a MADS-box protein previously designated as AGL25. This factor acts as a dosage-dependent repressor of flowering as demonstrated by the fact that an increased gene activity causes a strong delay in flowering while an early-flowering phenotype is obtained by antisense reduction of the transcript content (Michaels and Amasino 1999, Sheldon et al. 1999, 2000). Those studies have shown that FLC is mainly regulated through inputs from the autonomous and the vernalization pathways (Fig. 3). Thus, FLC mRNA content is reduced by a cold treatment that lead to flowering acceleration. A negative effect is also achieved by the autonomous pathway as relatively high transcript amounts are detected in mutants of this pathway characterized by late-flowering phenotypes. FLC is generally expressed along the plant, even in vegetative tissues. However, no obvious alterations of the vegetative organs are observed in loss-of-function mutants. It is also remarkable that expression of FLC in the whole plant remains constant throughout development, something that is explained by considering that down-regulation of the repressor must take place in a limited number of cells within the apical meristem. Nevertheless, in situ expression analysis should give a clear answer to that question. Regarding FLC targets, it has been proposed that it negatively regulates two key flowering-time genes, FT and SOC1, (Fig. 3) that act as integrators of signals from different flowering pathways (Lee et al. 2000, Samach et al. 2000). Little is known about the molecular mechanism by which the FLC transcriptional activity is regulated. Interestingly, a reduction of the DNA methylation in the cell leads to a reduction of the FLC transcript content and, consequently, to early flowering, thus paralleling the vernalization effect (Finnegan et al. 1998, Sheldon et al. 1999). Although this correlation
implies that FLC is regulated by the methylation status, it remains unclear whether the methylation effect is achieved directly on the FLC gene or on other FLC regulators. On the other hand, a relationship between FLC and the gibberellin pathway has been proposed indicating that FLC may act counteracting the promotion of flowering by GA in the apical meristem (Sheldon et al. 1999).

Recently, a MADS-box gene, MADS affecting flowering 1 (MAFI), previously designated as AGL27, has been reported in Arabidopsis that behaves similarly to FLC (Ratcliffe et al. 2001). It has been suggested that MAFI acts either downstream or independently of FLC. MAFI exhibits a generalized expression pattern in the plant. The existence of multiple MAFI/AGL27 transcripts has been reported, possibly generated through alternative splicing (Sheldon et al. 1999, Alvarez-Buylla et al. 2000a). This is interesting as structurally different MADS proteins are predicted to be generated that could perform different functions in either the same or different tissues of the plant. Therefore, the study of the expression pattern for the individual transcripts is of paramount importance for an appropriate interpretation of results such as those obtained from "sense" and "antisense" analysis. In agreement with participating in the same process, MAFI lies in the FLC cluster (Fig. 2). This cluster also includes five still uncharacterized MADS-box genes whose functions could also be related to the control of the flowering time (Ratcliffe et al. 2001). The identification of the product of the flowering locus M has been achieved (Scortecchi et al. 2001), and shown to be identical to MAFI.

Another MADS-box gene recently identified as a flowering promoter, integrating signals from the different pathways is the supressor of overexpression of constans (SOC1) or AGL20. This has been independently shown through different experimental approaches. Late-flowering mutants affected in AGL20 have been identified by transposon tagging (Borner et al. 2000), thus indicating an involvement of this gene in the control of the flowering time. On the other hand, AGL20, later called SOC1, has been identified as a direct target of constans (CO), a gene involved in the photoperiodic pathway (Samach et al. 2000). AGL20 is also regulated by genes belonging to the autonomous pathway, as demonstrated by a down-regulation of this gene in mutants affected in both the photoperiodic and the autonomous pathways. In addition, regulation of AGL20 by gibberellins has been shown (Borner et al. 2000). According to this, SOC1/AGL20 has been proposed to play a central role as an integrator of the signals from the different flowering pathways. Expression of AGL20 in Arabidopsis shows again a generalized pattern that is affected by the photoperiod. A low content of the AGL20 transcript is found in the apical meristem when plants are grown under SD, while a considerable and fast increase is obtained when they are induced to flowering by shifting to LD. Up-regulation is also obtained during the transition to flowering under SD, something that is consistent with the proposed regulation by signals from the different pathways. AGL20 is also expressed at later stages in the inflorescence meristem, and later on, in the centre of the flower meristem. This would indicate a possible role in flower development, although AGL20/SOCI mutations apparently do not affect flower morphology. An AGL20 orthologous gene has been identified in the related species Sinapis alba (Bonhomme et al. 2000, Borner et al. 2000). Interestingly, overexpression of this gene in a SD cultivar of Nicotiana tabacum was able to overcome the absolute requirement of SD for flowering, thus circumventing the photoperiodic block imposed under LD (Borner et al. 2000). Moreover, activation of this gene by gibberellins and cytokinins is observed, according to the notion that AGL20 activation is one of the steps of the gibberellin/cytokinrin pathway leading to flowering (Bonhomme et al. 2000).

Fruitful (FUL, former AGL8) is another MADS-box gene that has been involved in the control of the floral transition. Early studies on this gene revealed that it plays a clear role during carpel and fruit development (Gu et al. 1998), as it will be discussed in a next section. In addition, it has been shown that FUL also participates in the acquisition of the floral meristem identity together and redundantly with apetala 1 (API) and cauliflower (CAL). Although the ful mutant is only slightly affected in flowering time, triple mutants for FUL, CAL and API causes a non-flowering phenotype, thus indicating its role in promoting floral transition (Ferrándiz et al. 2000a). According to this function, FUL is expressed weakly in rosette leaves during vegetative development and strongly upregulated in the shoot apex upon transition to flowering (Gu et al. 1998). In addition, FUL (together with API and CAL) acts, in turn, as a regulator of leafy (LFY) and terminal flower 1 (TFL1), two other meristem identity genes (Ferrándiz et al. 2000a). Consistent with the similarity of functions, FUL lies within the same cluster than API and CAL. It is remarkable the finding during our search of two new genes, T6J22.1 and F28B23.25, belonging to this cluster and not previously reported in Arabidopsis (Fig. 2). It is likely that their function could also be related to the control of the flowering time and/or the specification of meristem identity.

The short vegetative phase (SVP or AGL22) is another MADS-box gene identified through transposon tagging as a dose-dependent repressor of flowering (Hartmann et al. 2000). Loss-of-function mutants for SVP are characterised by early-flowering phenotypes under any photoperiodic condition. Expression analysis indicates the existence of two different transcripts with a predominantly vegetative pattern. As one would predict for a flowering repressor, expression in the apical meristem is strongly reduced after floral transition. Transcript levels are not affected by the photoperiodic
conditions or vernalization, thus indicating a possible location within the autonomous pathway. SVP shares a high homology with AGL24, making up a together a separate genetic cluster (Fig. 2). It has been suggested that AGL24 acts as a promoter of the flowering process (Ferrándiz et al. 2000a), although this question still awaits further investigation. In this context, a putative AGL24 orthologue has been identified in potato (STMADS16) with a similar expression pattern (Garcia-Maroto et al. 2000). Over-expression of STMADS16 in a tobacco heterologous system accelerates flowering under SD but not in LD thus suggesting a possible role in promoting flowering and a limiting function under SD.

MADS-box genes in fruit development

Fruit development is a poorly known process, even though one can envision that its understanding may have a considerable interest, as a way to improve certain fruit qualities. In Arabidopsis, three MADS-box genes have been involved up to now. FUL, a gene also regulating floral transition and meristem identity, participates in the development of the fruit valves, while shutterproof 1 (SHP1) and shutterproof 2 (SHP2) are essential for the differentiation of the valve dehiscence zone. Fruit dehiscence and, consequently, seed dispersal in Arabidopsis and other plants bearing siliques is achieved through differentiation of a few cell layers at the valve margin. Upon fruit maturation a dehiscence zone develops allowing detachment of the valves and releasing of the seeds. FUL function in fruit development was uncovered through the analysis of the transposon-generated mutant fruitful (ful-1). In ful-1 plants elongation of the siliqua after fertilization is abolished due to the failure of valve cells to elongate and differentiate, leading to crowded seeds and dehiscence failure (Gu et al. 1998). FUL exhibits a complex expression pattern (see previous section) that includes the carpel walls, consistently with its role in valve development. Loss-of-function mutants of SHP1 and SHP2 have been generated by T-DNA insertion that allowed functional studies (Liljegren et al. 2000). Even though single mutations do not show an apparent effect on fruit development, in the double mutant the dehiscence zone fails to develop thus indicating that both genes act redundantly to specify cell-fate within the valve margin. Expression of SHP1 and SHP2 takes place, in addition to the valve margin, in other tissues as in developing ovules, septum, nectaries and style. Nevertheless, development of these organs is not apparently affected in the double mutant perhaps due to functional redundancy with other genes in those cells. FUL and SHP genes play different specialized roles during fruit development and their expression is regulated in an antagonistic way. Thus, FUL acts as a negative regulator of SHP1/2 ensuring that their expression and, consequently, fruit abscission takes place exclusively at the valve margin (Ferrándiz et al. 2000b). On the other hand, upstream positive regulation of SHP1/2 is achieved by the product of AG (Savidge et al. 1995), a MADS-box gene involved in flower development.

Another interesting aspect of these group of genes comes from their phylogenetic relationships. SHP1 and SHP2 (previously designated as AGL1 and AGL5) belong to a cluster of genes that include to AG among other genes (Fig. 2). Members of this group share similar expression patterns and functions, mainly related to stamen and carpel development. On the other hand, FUL belongs to a cluster integrated by genes like AP1 and CAL whose functions are related with the specification of flower/inflorescence meristem identity. Although FUL still conserve this kind of function, its participation in fruit development may be interpreted as another example of functional recruitment in the course of evolution (Theissen et al. 2000).

Investigation in plants other than Arabidopsis still remains very scarce. Recently, a novel gene, DEFH28, from the snapdragon Antirrhinum majus has been described that is likely to represent the ortholog of FUL (Muller et al. 2001). A dual role both in the control of floral meristem identity and fruit development was also shown for DEFH28. Another gene called jointless has been characterized in Lycopersicon esculentum that seems to be essential in the process leading to pedicel abscission. Jointless was isolated by chromosome walking, and further shown to be responsible for the jointless mutation by co-segregation analysis (Mao et al. 2000). Jointless mutants are characterized among other features by the failure to develop the abscission zone that normally allows shedding of the fruit. In this sense jointless function resembles that of SHP genes although at a different spatial location in the fruit. However, assignation of JOINTLESS to phylogenetic clusters puts JOINTLESS in a different clade, together with AGL24 and SVP, two flowering time genes. In agreement to this, jointless mutants are also affected in the regulation of the
meristem fate, with inflorescence meristems reverting to vegetative growth after forming few flowers (Szymkowiak and Irish 1999). As in the case of their clade partners, JOINTLESS also shows a generalized expression pattern, with the exception of the green tissue of the fruit calix.

Initial studies of B-function genes like deficiens and globosa from Antirrhinum majus, which are involved in the determination of flower organ identity, revealed that expression of these genes was maintained throughout the development of the flower and that it also included carpel tissues. Consequently, it was postulated that they could play additional roles during fruit development (Tröbner et al. 1992, García-Maroto et al. 1993). This turned out to be true as it has been recently reported the involvement of the globosa orthologous gene from Malus domestica (MdPI) in the parthenocarpic development of the fruit (Yao et al. 2001), thus uncovering a new function for these MADS-box sub-family. This is of biotechnological importance as it opens the possibility for the generation of high-quality seedless cultivars through down-regulation of this gene.

Table 2. MADS-box genes involved in fruit development.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Organism</th>
<th>Function in fruit development</th>
<th>Other functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>FUL (AGL8)</td>
<td>Arabidopsis</td>
<td>valve development</td>
<td>flowering time/ floral meristem identity/ leaf development</td>
</tr>
<tr>
<td>DEFH128</td>
<td>Antirrhinum</td>
<td>carpel wall development</td>
<td>floral meristem identity</td>
</tr>
<tr>
<td>SHP1(AGL1)</td>
<td>Arabidopsis</td>
<td>valve margin abscission</td>
<td>unknown</td>
</tr>
<tr>
<td>SHP2(AGL5)</td>
<td>Arabidopsis</td>
<td>valve margin abscission</td>
<td>unknown</td>
</tr>
<tr>
<td>JOINTLESS</td>
<td>Lycopersicum esculentum</td>
<td>pedicel abscission</td>
<td>inflorescence/ floral meristem identity</td>
</tr>
<tr>
<td>MDPI</td>
<td>Malus domestica</td>
<td>parthenocarpic development</td>
<td>flower organ identity</td>
</tr>
</tbody>
</table>

MADS-box genes in non-reproductive processes

Some of these genes were likely to participate in the development of other plant structures, as they are expressed in vegetative tissues such as leaves, stem and roots (Ma et al. 1991). Although there is a general correlation between expression pattern and place of function, one should keep in mind that the presence of transcript in a given tissue would not necessarily mean that gene activity is required for its proper development. We will first described those cases in which direct evidence exists for the involvement of MADS-box genes in vegetative processes, and finally some genes for which such a role might be inferred from their expression patterns.

Root development: The MADS-box gene ANRI was identified in Arabidopsis as a result of a screening for nitrate inducible genes (Zhang and Forde 1998). Expression of ANRI is restricted to the roots of plants and is positively regulated by nitrate. This behaviour seems to be part of a response of the plant to the presence of nitrate in the soil, a situation in which lateral root growth is stimulated. Knocking-out of ANRI by antisense technology eliminates this response thus indicating that ANRI is required for lateral root development. It has been proposed that ANRI could act as a transcriptional regulator of genes modulating the rate of lateral root elongation. Phylogenetic analysis shows that ANRI is located in a cluster that includes to AGL17, a gene that is also specifically expressed in roots, among other genes like AG/L6 and AG/L7, for which little information is still available (Fig. 2).

Root nodulation: Another MADS gene, NMH7, has been reported in Medicago sativa whose expression is specifically induced in root nodules upon infection by Rhizobium (Heard and Dunn 1995). Interestingly, homology search for a putative orthologue of NMH7 in the Arabidopsis genome does not give any clear

Table 3. MADS-box genes involved in vegetative development.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Organism</th>
<th>Arabidopsis orthologue</th>
<th>Expression pattern</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANRI</td>
<td>Arabidopsis</td>
<td>---</td>
<td>Root</td>
<td>Lateral root development</td>
</tr>
<tr>
<td>NMH7</td>
<td>Medicago sativa</td>
<td>Unknown</td>
<td>Nodule</td>
<td>Nodule differentiation (?)</td>
</tr>
<tr>
<td>NMH5</td>
<td>Medicago sativa</td>
<td>AGL17</td>
<td>Root/Nodule</td>
<td>Unknown</td>
</tr>
<tr>
<td>AGL17</td>
<td>Arabidopsis</td>
<td>---</td>
<td>Root</td>
<td>Unknown</td>
</tr>
<tr>
<td>FUL</td>
<td>Arabidopsis</td>
<td>---</td>
<td>Generalized</td>
<td>Leaf development and others</td>
</tr>
</tbody>
</table>
candidate. The most similar is *apetala 3* (90% identity at the MADS-box, 54% overall identity), a B-function gene classically involved in the development of flower sepals and stamens. Thus, *NMH7* could represent another case of MADS-box gene recruitment, in this case from the cluster of B-function genes, to perform some specialized function at least in legume plants. Moreover, an *AGL17* orthologous gene (*NMH5*) has been described in alfalfa whose transcripts are also found in nodules (Heard et al. 1997), although its function in these symbiotic organs still remains obscure. In this context, it is interesting to notice that the development of root nodules is a response of the legume plant to a low soil nitrogen content. As *ANR1* is up-regulated by the nitrate, it is possible that the activation of the closely related gene, *NMH5*, was induced by the nitrate accumulation within the root-derived nodule cells.

**Leaf development:** Although a MADS-box gene specifically involved in leaf development has not been described yet there is evidence that *FUL* activity is required for leaf cellular differentiation. This is inferred from the fact that cauline leaves from the *ful-1* mutant are broader than those in the wild type, together with a reduction in the number of internal cell layers (Gu et al. 1998). Association of *FUL* expression to the vascular system of vegetative tissues argues in favour of an effect on vein differentiation as the cause of changes in the leaf shape, although this interpretation still awaits further studies.

**Inferred functions from expression patterns:** For a number of MADS-box genes the only available information is the expression pattern, although some inferences have been done about their roles. Among them, other genes from *Arabidopsis* whose transcripts are exclusively found in root cells, and that could be related to some aspect of root development are *AGL14* and *AGL19*, both included in the *TM3* clade, and the solitary *AGL12* (Rounsley et al. 1995, Alvarez-Buylla et al. 2000a). Other MADS-box genes have been proposed to be involved in seed development. Detailed expression studies have been performed with the seed specific gene *AGL15* (Heck et al. 1995, Rounsley et al. 1995, Perry et al. 1996). *AGL15* protein is found very early in embryo development and maintained at a high level until initiation of seed desiccation. *AGL15* seems to be also present in the endosperm tissues of dicot and monocot species (Perry et al. 1996), although some discrepancy exists since in some studies the corresponding transcript was not detected in the endosperm (Rounsley et al. 1995). In addition, nuclear localization of AGL15 and binding of this protein to a CArG motif has been shown, indicating a role of AGL15 as a transcriptional regulator. Search in the *Arabidopsis* genome database reveals the existence of an *AGL15* related gene designated MJM18.2, not previously reported. In the cluster analysis (Fig. 2) both genes appear grouped, and it is therefore conceivable that they are involved in the same developmental process.

*AGL18* is another MADS-box gene whose transcripts are found in the seed (Alvarez-Buylla et al. 2000a). Nevertheless, this gene has a complex expression pattern that includes both vegetative and reproductive tissues, being remarkable its expression during pollen development. Within the seed, *AGL18* mRNA is specifically found in the endosperm but not in embryonic cells, a characteristic that makes it a useful seed marker for this kind of tissue.

*AGL16* is another MADS-box gene belonging to the *AGL17* cluster (Fig. 2). However, its expression is not restricted to the roots, exhibiting a generalized expression in the plant (Alvarez-Buylla et al. 2000a). Interestingly, expression in the leaf, where the mRNA is found at a higher level, is mainly associated to stomatal guard cells and trichomes, while in the roots it is again found in the epidermal cells. Consequently, a possible a role in epidermal differentiation processes giving raise to those structures has been suggested. It is likely that future studies will reveal the participation of MADS-box factors like *AGL16* in cell-type specification networks that are being uncovered in leaves and roots (Larkin et al. 1997, Schiefelbein et al. 1997).

**Conclusions**

The MADS-box gene family encode transcriptions factors that perform different functions during plant development. Among their roles are not only the development of flower organs, but also the control of the flowering time and the development of organs such as fruits, roots, leaves, and other specialized tissues. Investigation in this field is still very scarce for most of plants. Even in the plant model *Arabidopsis* the number of MADS-box factors characterized so far is much below the whole number uncovered by the sequencing of its genome. This makes likely that new functional aspects of MADS-box genes are still revealed as knowledge increase in the next years.
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