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Function of Multimeric MADS Protein Complexes in Floral Organ Development of Plant

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Recent reports suggest that floral organs such as sepals, petals, stamens, and carpels are specified by quaternary MADS protein complexes with different combinations. The formation of quaternary complexes of ABCDE MADS proteins may be the molecular basis of ABCDE model for the floral organ development. The MADS complexes involved in each floral organ development seem to be conserved in at least dicot species although detailed molecular mechanism is slightly different depending on species. Even in monocot, at least rice, MADS complexes similar to those in dicot exist, suggesting that the floral organ specification by MADS protein complexes may be conserved in most of plants. The MADS protein complexes may have more specific recognition of target genes or more transcription activation ability than monomers or dimers, resulting in finely regulated floral organ development.

key words: MADS proteins, Multimeric complexes, ABCDE model, Floral organ development

INTRODUCTION

When plants receive environmental signals and genetic signals such as photoperiod, temperature, light, and activation of specific genes, they undergo the transition from vegetative to reproductive phase. During the reproductive phase, the flank of the shoot apical meristem converts into the floral meristem. In addition, the floral meristem gives rise to floral organ primordia, which develop into floral organs.

Most of the floral meristem identity genes and floral organ identity genes are members of the MADS transcription family. The MADS proteins, especially, MIKC type proteins that are mainly found in plants are composed of four regions; the MADS domain, I (intervening) domain, K (keratin-like) domain and C (C-terminal) domain. The MADS domain is conserved 56 amino acids that share a common DNA-binding function. The K domain is important for the dimerization and is predicted to form a coiled-coil structure because it encodes three amphipathic α-helices, K1, K2 and K3 [1-5]. Between the MADS domain and K domain is a variable region referred to as the I domain. The C domain has been postulated to mediate the formation of higher-order MADS protein complexes [6, 7]. The C domain has also been demonstrated to encode a transcriptional activation domain [7-9].

The floral organ development is explained as the ABCDE model. The development of each floral organ requires specific combinations of A, B, C, D, and E class genes, which encode MADS proteins [4, 10, 11]. For example, the A class genes specify sepals, the combination of A, B and E class genes specifies petals, the combination of B, C and E class genes specifies stamens, the combination of C and E class genes specifies carpels, and the combination of C, D and E class genes specifies ovules (Fig. 1).

As shown in Table 1, A, B, C, D, and E class genes have been reported from many species including *Arabidopsis*, *Antirrhinum*, and petunia on the basis of mutant phenotypes and sequence similarity. In *Arabidopsis*, the A class protein is APETALA1 (AP1), the B class proteins are APETALA3 (AP3) and PISTILLATA (PI), the C class protein is AGAMOUS (AG), the D class proteins are SEEDSTICK (STK), SHATTERPROOF1 (SHP1) and SHP2, the E class proteins are SEPALLATA (SEP) [12-17]. The PI and AP3 proteins, *Arabidopsis* B class proteins, interact with each other,

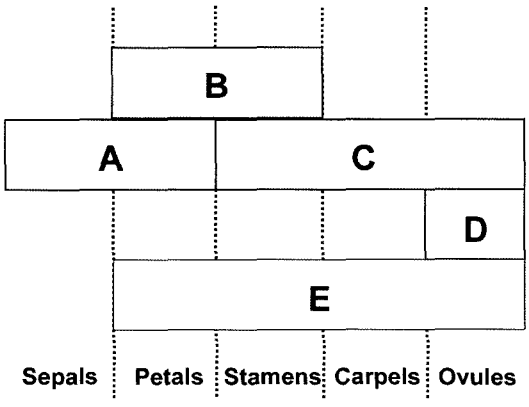

Figure 1. ABCDE model for floral organ specification in dicot.

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Table 1. A, B, C, D and E class genes identified in *Arabidopsis*, *Antirrhinum*, and petunia

Function	Arabidopsis	Antirrhinum	Petunia
A	*API* [12]	?	?
B	*AP3* [13]	*DEF* [20]	*pMADS1* [22]
	PI [14]	*GLO* [18]	*FBP1* [23]
			pMADS2 [24]
C	*AG* [15]	*PLE* [21]	*pMADS3* [25]
			FBP6 [26]
D	*STK* [16]		*FBP7* [27]
	SHP1 [16]	?	*FBP11* [28]
	SHP2 [16]		
E	*SEP1* [17]	?	*FBP2* [29]
	SEP2 [17]		
	SEP3 [17]		
	SEP4 [19]		

forming a heterodimer [1]. Also, similar interactions between B class proteins as the DEFICIENS (DEF)-GLOBOSA (GLO) heterodimer is observed in *Antirrhinum* [18] and the OsMADS16-OsMADS4 heterodimer in rice [9].

Recently it has been suggested that MADS proteins function in the form of ternary or quaternary complexes containing more than two MADS proteins, generating "quartet model" for flower organ development [10]. In the quartet model, it is postulated that quaternary complexes of MADS proteins determine floral organ identity. Using yeast two-, three- and four-hybrid experiments and coimmunoprecipitation, several MADS protein complexes have been reported from mainly dicot. In this review, we highlight recent results about the MADS protein complexes involved in floral organ development and their molecular function.

Multimeric MADS protein complexes in dicot

As summarized in Table 2, the MADS protein complexes have been reported only in limited dicots such as *Arabidopsis*, *Antirrhinum*, petunia, and chrysanthemum [6, 7, 29, 30].

In *Arabidopsis*, the MADS protein complexes are very well characterized [10, 31]. Each complex, mostly quaternary complex, is proposed to consist of two MADS dimers. According to the quartet model, the composition of the quaternary complexes in the four whorls of the flower is predicted, suggesting that the formation of quaternary complexes of ABCDE proteins may be the molecular basis of the ABCDE model. For example, in whorl 2, a combination of AP3-PI-SEP3-AP1 is proposed to specify petals; in whorl 3, AP3-PI-SEP3-AG is proposed to specify stamens; and in whorl 4, AG-AG-SEP3-SEP3 is proposed to specify carpels. In the formation of the quaternary complexes of ABC proteins, SEP3 interacts with the AP3-PI heterodimer and also serves as a scaffold between AP3-PI and AG or between AP3-PI and AP1. These physical interaction in the MADS quaternary complex has been mainly confirmed using yeast two-, three-, or four-hybrid systems and coimmunoprecipitation [7].

The biological function of the MADS quaternary complex has been also identified in some cases. For example, ectopic expression of AP3-PI-SEP3 and AP3-PI-AP1 is sufficient to transform leaves into petaloid organs and that of AP3-PI-SEP3-AG is sufficient to convert cauline leaves into staminoid organs, suggesting that the MADS complexes function in the specification of floral organs. Also, a target gene of AP3-PI (*AP3::GUS*) is activated in non-floral organs when *SEP3* or *AP1* is ectopically expressed in addition to *AP3-PI*, indicating that the AP3-PI-SEP3 and AP3-PI-AP1 ternary complexes activate the expression of downstream genes [7].

Besides petal, stamen, and carpel identities, the MADS quaternary complex may function in the promotion of ovule identity. For instance, in the *shp1 shp2* double mutant, the complex composed of STK-SEP-AG is sufficient to promote normal ovule development [16, 32]. AG and SEP form a stable complex with STK or one of the SHP proteins. Each of these complexes is enough to promote ovule identity [16].

In *Antirrhinum*, one type of MADS quaternary complex has been reported, so far [6]. SQUA, DEF and GLO interact at the molecular level, binding of DEF-GLO heterodimer and SQUA-SQUA homodimer to promoter sequences of common

Table 2. Ternary or quaternary MADS protein complexes in *Arabidopsis*, *Antirrhinum*, petunia, and chrysanthemum

Species	Complex	Class of ABCDE	Reference
Arabidopsis	AP3-PI-SEP3	B1-B2-E	[7]
	AP3-PI-AP1	B1-B2-A	[7]
	AP3-PI- SEP3-AG	B1-B2-E-C	[7]
	STK-SEP3-AG	D1-E-C	[36]
	SHP1/2-SEP3-AG	D2/3-E-C	[36]
Antirrhinum	SQUA-SQUA-GLO-DEF	?-B1-B2	[6]
Petunia	pMADS1-FBP1-FBP2	B1-B2-E	[29]
	pMADS1-pMADS2-FBP2	B1-B3-E	[29]
	pMADS1-FBP1-FBP2-pMADS3	B1-B2-E-C1	[29]
	pMADS1-pMADS2-FBP2-FBP6	B1-B3-E-C2	[29]
Chrysanthemum	CDM86-CDM115-CDM44	B1-B2-E	[30]
	CDM86-CDM19-CDM44	B1-B3-E	[30]

target genes [18, 33]. SQUA, DEF and GLO can also form ternary complexes in yeast, expanding the regulatory possibilities of MADS transcription factors [6]. However, neither DEF nor GLO can interact with SQUA in yeast two-hybrid assays [1].

Similarly, in petunia, yeast three- and four-hybrid experiments demonstrated the existence of complexes that consist of B-E and B-E-C MADS proteins [29, 34]. FLORAL BINDING PROTEIN2 (FBP2) showing high similarity to E class MADS protein in other species interacts with B class heterodimer, for example, pMADS1-FBP1 and the C class proteins, forming quaternary complexes. However, more specific interactions occur between B and C class proteins in petunia. Unlike in *Arabidopsis*, there are duplicated genes of the ancestral B and C class genes in petunia. FBP1 and pMADS2 are related closely to PI [23, 24], and FBP6 and pMADS3 are related to AG [25, 26]. Despite the high sequence similarity among the duplicated B and C proteins, there seems to be a high specificity in the pattern of interactions. The C class pMADS3 participates in the protein complex only if the B class FBP1 is present, whereas FBP6 requires the presence of pMADS2, leading to the formation of two different B-B-C-E quaternary complexes, FBP2-pMADS1-FBP1-pMADS3 and FBP2-pMADS1-pMADS2-FBP6. It was speculated that these two quaternary complexes have been recruited for slightly different functions within the flower identity program [29].

Recently, in chrysanthemum, which is also a member of the Compositae family, the ternary complexes between E class protein and B class heterodimer were demonstrated [30]. Two-hybrid analysis revealed that Chrysanthemum *Dendrathema grandiflorum* MADS (CDM) 86, a PI homolog, interacts with both CDM115 and CDM19, AP3 homologs, in a similar way as the B proteins PI-AP3, GLO-DEF, and FBP1-pMADS1 for *Arabidopsis*, *Antirrhinum*, and petunia, respectively [35]. Three-hybrid studies with the CDM proteins showed that CDM44, a SEP homolog, forms ternary complexes with the B class heterodimers CDM86-CDM115 and CDM86-CDM19 [30].

MADS protein complexes reported from *Arabidopsis*, *Antirrhinum*, petunia, and chrysanthemum suggest that conserved MADS protein complexes may function in the specification of each floral organ in dicots. Even though the detailed molecular mechanism may be a little bit different depending on species, similar combination of MADS proteins specify similar floral organs.

Molecular function of MADS protein complexes

On the basis of results about the MADS proteins, there might be two possible molecular mechanisms by which the MADS quaternary complexes function. One mechanism is that binding to two MADS binding sites of the MADS complexes might enhance the specificity of target gene recognition. Because the MADS quaternary complexes consist of two dimers, the quaternary complexes could bind to two CArG boxes, but the binding of the MADS dimers could be cooperative. Binding of one dimer in a quaternary complex would result in increased affinity for local binding of the second dimer in the quaternary complex. Indeed, SQUA-SQUA-DEF-GLO quaternary complex showed the enhanced affinity to target sequences [6], and some target genes such as *GLO* and *AP3* include multiple CArG sites in their promoters [18, 37, 38]. The ternary complex might result in a more specific regulation of downstream genes, thus promoting high reliability of the floral developmental program.

A second mechanism is that one or more monomers provide a transcription activation ability to the quaternary complex to allow for efficient transcriptional activation. For example, PI, AP3, and AG can not act as transcriptional activators, whereas SEP3 and AP1 can do so [7]. Thus, the SEP3 and AP1 might provide transcriptional activation ability to the quaternary complexes including them.

Recent results indicate that K domain is important for the multimeric MADS complex formation as well as homo- and heterodimer formation [7, 39, 40], and that the C domain may also contribute to complex formation in some cases [6, 40]. For example, mainly hydrophobic and some charged residues in K2 subdomain of PI, and a proline residue in the C terminal motif of PI play important roles in the formation of AP3-PI-SEP3-AG quaternary complex [40]. In *Antirrhinum*, the formation of homo- and heterodimers between plant MADS proteins occurs through mostly K domain [1, 2, 41], but DEF, GLO and SQUA can form ternary complexes via the C domain of SQUA, which suggests that the C domain of plant MADS proteins mediates quaternary complex formation [6]. These results suggest that the K domain, in association with the C domain, can mediate the MADS complex formation.

Role of E class MADS genes in the formation of MADS complex

In the MADS complexes, a central factor is the E class protein, represented by SEP1/SEP2/SEP3 in *Arabidopsis* [7], FBP2 in petunia [29], and CDM44 in chrysanthemum [30]. FBP2 is the only protein showing 83% identical amino acids to SEP3. Ectopic expression transgenic plants of *SEP3* and *FBP2* show similar phenotypes like early flowering, indicating that the two MADS proteins are functional homolog [29]. In *Antirrhinum*, DEFICIENS-Homolog72 (DEFH72) and DEFH200 show high similarity to FBP2 [1].

At least, SEP and FBP2 are always a component in multimeric MADS protein complex identified so far [29], suggesting that the E class protein is essential in the MADS complex formation.

The function of E class proteins seems to be redundant because *sep1*, *sep2* or *sep3* single mutants did not show any defect in floral organs as severe as in mutation of B, C class genes [17]. However, a striking phenotype occurs in *sep1 sep2 sep3* triple mutants, in which all flower organs resembled sepals [7], showing that the *SEP1*, *SEP2* and *SEP3* genes have overlapping functions required for petal, stamen

and carpel development. The *sep1 sep2 sep3* triple mutant phenotype is strikingly similar to BC (*ap3 ag, pi ag*) double mutants, indicating that the B and C class genes are inactive in the triple mutant, and that the B and C gene products require at least one of the SEP1, SEP2 and SEP3 proteins for activity [7, 17]. Recent results show that the ectopic expression of *SEP3* is sufficient to ectopically activate *AP3* and *AG*, suggesting that *SEP* genes are necessary for the activation of B and C class genes [42].

SEP4 gene, an *Arabidopsis* MADS gene shows extensive sequence similarity to and an overlapping expression pattern with the other *SEP* genes [19]. Floral organs are converted into leaf-like organs in *sep1 sep2 sep3 sep4* quadruple mutants that are more severe phenotype than in *sep1 sep2 sep3* triple mutants, indicating the involvement of all four *SEP* genes in the development of sepals. *SEP4* like other *SEP* genes also contributes to the development of petals, stamens, and carpels in addition to sepals, suggesting that the *SEP* genes play central roles in flower meristem identity and organ identity [19].

Multimeric MADS protein complexes in monocot

In monocot, MADS proteins are well elucidated in rice. On the basis of amino acid sequence similarity and phenotypes of mutants or ectopic expression transgenic plants, MADS genes belonging to A, B, and C classes have been reported (Table 3). Interaction between B class proteins, OsMADS4-OsMADS16 heterodimer is also demonstrated using yeast two-hybrid system [9]. However, MADS protein complexes have not been reported in rice, and even functional E class proteins are not clear, yet.

In rice, OsMADS1, OsMADS7, and OsMADS8 are considered to be E class proteins. Based on amino acid sequence homology and expression pattern, *OsMADS7* and *OsMADS8* are the closest relatives to *SEP* genes [43, 50]. *OsMADS7* and *OsMADS8* show high levels of similarity with the *SEP* genes and almost identical expression pattern, suggesting that these genes have a similar function in rice. *OsMADS7* and *OsMADS8* are probably redundant genes since their amino acid sequences are very similar and also their expression patterns [51, 52]. Yeast three-hybrid experiments show that both OsMADS7 and OsMADS8 can form ternary complex with rice B class heterodimer, OsMADS4-OsMADS16, resulting in OsMADS4-OsMADS16-OsMADS7 and OsMADS4-OsMADS16-OsMADS8 ternary complexes (Park and Moon, unpublished data). These data suggest that B-E MADS complex similar to that in dicot also exist in rice, suggesting that MADS complexes involved in floral organ development may be conserved in plants.

Table 3. A, B, C, D, and E class genes in rice

Function	Gene	Evidence	Reference
A	*OsMADS14*	H[a]	[3]
	OsMADS15	H, E[b]	[44]
	OsMADS18	H	[3]
B	*OsMADS4*	H, E, M[c], P[d]	[45]
	OsMADS16	H, E, M, P	[9]
C	*OsMADS3*	H, E, M	[46]
D	*OsMADS13*	H	[47]
E	*OsMADS1*	H, M	[48]
	OsMADS5	H	[49]
	OsMADS7	H, E	[43]
	OsMADS8	H, E	[43]

[a]amino acid sequence homology
[b]expression patterns
[c]mutant phenotypes
[d]physical interaction

Mutant phenotype demonstrates that OsMADS1 possesses E class function [53]. Severe loss-of-function mutations of *OsMADS1* cause complete homeotic conversion of lodicules, stamens, and carpels into lemma- and palea-like structures. These phenotypes resemble the phenotypes caused by mutations of *Arabidopsis SEP1/SEP2/SEP3* and petunia *FBP2*, suggesting that *OsMADS1* play a very similar role in rice to that of E class genes in dicot plants. However, physical contact between OsMADS1 and OsMADS4-OsMADS16 heterodimer has not been confirmed, yet.

Several evidences such as amino acid sequence similarity, expression pattern, phenotypes of mutants and ectopic expression plants, and physical interaction among MADS proteins suggest that rice MADS proteins have conserved functions with those of MADS proteins in dicot [3, 9, 53-57]. Thus it is possible that both monocot and dicot have multimeric MADS protein complexes with similar molecular mechanism. However, further studies are necessary to support the similarities between dicot and monocot MADS complexes.

Conclusion and prospective

At present, ABCDE model for floral organ development can be explained with specification of each floral organ by multimeric MADS protein complexes even though the nature of MADS protein complexes is completely not characterized. Different combination of MADS proteins in the complexes can specify different floral organs such as sepals, petals, stamens, and carpels. The MADS complexes seems to be conserved in at least dicot species although detailed molecular mechanism is a little bit different depending on species. Even in monocot, at least rice, the similar MADS complexes to those in dicot seem to exist, suggesting that floral organ specification by MADS protein complexes may be conserved in most of plants.

Considering that MADS proteins have high similarity of amino acid sequences and conserved domains, most of MADS proteins in plants including MADS proteins involved in floral organ development may function in the form of complexes, having more specific recognition of target genes or more transcriptional activation than monomers or dimers. However, components of MADS protein complexes have

been isolated in very limited species, and are not fully characterized yet. Future work will focus on the isolation and biochemical characterization of MADS protein complexes in plant cells.

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