Communicating across generations: The Bsister language

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Abstract

Bsister proteins form a clade of MADS-box transcription factors that originated 300 million years ago, after ferns diverged but before Angiosperms and Gymnosperms lineages did. Thus, Bsister proteins have been found in both Gymnosperm and Angiosperm species such as paddy oat (Gnetum gnemon), ginkgo, yew (Taxus baccata), rape seed, rice, maize, wheat, petunia, snapdragon, tomato and Arabidopsis. In all these species, they are expressed in female reproductive organs. In this review, we go over the evolution and pattern of expression of the Bsister proteins, and we have a glance at their interaction patterns in the form of high-order MADS-box complexes in different species. We describe the functions that have been assigned to them according to the analysis of mutants and RNA interference data. We finish this review discussing from a novel point of view the role that Bsister proteins might have, also in tetramer combinations with other MADS-box proteins, on the regulation of tissue communication occurring during reproduction. It is known that a cross-talk is essential for a proper ovule and seed development, and Bsister and their target genes might play key roles in these communication processes.

Keywords: Bsister, integument, MADS-box protein complexes, ovule development, reproductive cross-talk, seed development

Introduction

The evolutionary success of flowering plants likely depends on the novelties of their reproductive structures. On the female part, tissues with different genetic composition closely differentiate before and after fertilization. During ovule development, maternal sporophytic tissues, namely the ovule integument(s), coordinate together with the haploid generation, the embryo-sac. Next, the endosperm and embryo, which are the products of fertilization, grow in close proximity to the maternal seed coat which differentiates from the ovule integument. Mutant analysis supports the idea that a molecular cross-talk occurs in different directions before and after the fertilization: from the integument(s) to the internal tissues and vice versa. Disturbances in these communication mechanisms strongly decrease the fitness of the plant (for reviews see Nowack et al. 2010; Bencivenga et al. 2011). Successively, the description of female sporophytic sterile plants gave important clues on the signals from the maternal layers to the endosperm and embryo during seed formation (Nowack et al. 2010).

In the next paragraphs, we detail the role of the MADS-box genes belonging to the Bsister clade (Becker et al. 2002). Besides the observation that Bsister gene expression profile and protein interaction patterns are highly conserved in distantly related species, the reduced fertility observed in most of the Bsister mutant plants is often sporophytically controlled (de Folter et al. 2006; Deng et al. 2012; Mizzotti et al. 2012; Yang et al. 2012; Yin & Xue 2012; Chen et al. 2013; Lee et al. 2013). In our opinion, understanding the molecular pathways regulated by the Bsister transcription factors might strongly contribute to unveil the cell–cell communication mechanisms occurring between the maternal layers and the embryo-sac as well as between the seed coat and the products of fertilization.
The B\textsubscript{sister} clade: gene structure and phylogenetic analysis

Within the eukaryotes, MADS-box transcription factors play fundamental roles in developmental control and signal transduction. It is well documented that in higher plants, the MADS-box gene family underwent extensive duplications; following these events, neo- and sub-functionalization mechanisms strongly contributed to the evolution of plant form (Theißen 2000).

The plant MADS-box gene family can be subdivided into two major classes named type I and type II (Alvarez-Buylla et al. 2000). Plant type II MADS-box transcription factors are characterized by a conserved structure, indicated as MIKC-type, comprising a MADS (M), an intervening (I), a keratin-like (K) and a C-terminal (C) domain (Ma et al. 1991; Theißen et al. 1996; Munster et al. 1997). Phylogeny reconstruction of the MADS-box MIKC-type genes identified 12 clades known as AGAMOUS (AG)-, AGL2-, AGL6-, AGL12-, AGL15-, AGL17-, DEFIcIENS/GLOBOSA (DEF/GLO)-, FLOWERING LOCUS C (FLO)-, GGM13- (B\textsubscript{sister}), SQUAMOSA (SQUA)-, StMADS11- and TM3-like genes. Functional data showed that genes belonging to the same clade have similar expression profiles and highly related functions (Becker et al. 2000).

Undoubtedly, MADS-box gene activity is tightly correlated to the evolution of floral structures; the role of MADS-box genes in the determination of floral organ identity is described by the so-called ABCDE model (Coen & Meyerowitz 1991; Causier et al. 2010). Accordingly, different classes of gene activity are expressed in overlapping floral domains, thus determining organ identity. Class A (SQUA-like) genes determine sepal identity; petal formation depends on the activity of class A and B (DEF/GLO-like) genes; class B and C (AG-like) genes are required for stamen differentiation, while carpel identity is regulated by C class genes. In some species, members of the AG class have a specific role during ovule formation thus defining the D class (Angenent et al. 1995; Colombo et al. 1995; Favaro et al. 2003; Pinyopich et al. 2003). For almost 10 years, functional redundancy masked the existence of E (SEPI- or AGL6-like) class genes, which is indispensable for the differentiation of all floral organs (Pelaz et al. 2000; Ditta et al. 2004).

The founder of the B\textsubscript{sister} clade is the 	extit{Gnetum gnemon} GGM13 gene (Becker et al. 2002). Phylogenetic analysis of B\textsubscript{sister} genes identified in Angiosperms and Gymnosperms suggested that they represent a monophyletic group which is a sister clade of the B (DEF/GLO) genes. The DEF/GLO and GMM13 clades likely originated after the duplication of an ancestral gene in the lineage that led to extant seed plant before the Angiosperm and Gymnosperm lineages diverged 300 million years ago (MYA) (Becker et al. 2002). B\textsubscript{sister} proteins share with B proteins a shorter I domain compared with other MADS-domain factors, a subterminal PI motif-derived sequence and, in some cases, a PaleoAP3 motif in the C-terminal region (Becker et al. 2002).

A phylogenetic tree showing the common origin of the B and B\textsubscript{sister} clades as well as the relationship between selected Angiosperm and Gymnosperm B\textsubscript{sister} genes is represented in Figure 1. Eudicots B\textsubscript{sister} genes cluster together: a Brassica subclade composed of the four 	extit{Brassica napus} paralogous (BnTT16.1, BnTT16.2, BnTT16.3 and BnTT16.4) and Arabidopsis B\textsubscript{sister}/TRASPARENT TESTA 16 (ABS/TT16) (hereafter indicated as ABS) is clearly distinguishable from the other cluster composed of B\textsubscript{sister} genes identified in the three Asterid species 	extit{Antirrhinum majus}, 	extit{Petunia hybrida} and 	extit{Solanum lycopersicum}. The gene duplication event leading to ABS and GORDITA (GOA) occurred during diversification of the Brassicaceae (Erdmann et al. 2002).

Figure 1. Phylogenetic analysis of class-B and B\textsubscript{sister} genes. Nucleotide sequences were translated into proteins and aligned with the muscle algorithm, and a Maximum Likelihood tree was constructed. The number at the nodes are bootstrap values after 1000 replicates. Sequence accession numbers are the following: 	extit{Brassica napus} (BnTT16.1 (EU192029), BnTT16.2 (EE192029), BnTT16.3 (HM449990), BnTT16.4 (HM449989), Arabidopsis thaliana: AtABS (AJ318098), AtGOA (AY141243), AtAPI (M86357), AtPI (D30807); Antirrhinum majus: AntiDEFH21 (AJ307056), Petunia hybrida: PhFBP24 (AF335242), PhMADS2 (X69947), Solanum lycopersicum: SlFBP24 (XM_00249803), Oriza sativa: OsMADS29 (AK109552), OsMADS30 (AY174093), OsMADS31 (AY177698), Triticum aestivum: TaBsis (AM502893), Zea mays: ZmMADS16 (NM_001111166), ZmMADS17 (Q8VWM8), ZmMADS31 (GRMZM2G137387; maizesequence.org), ZmSILKY1 (AF181479), Gnetum gnemon: GGM13 (AJ132219), GGM2 (AJ132208) and Ginkgo biloba: GBM10 (AB029472).
The genetic distance between the Arabidopsis B\textsubscript{sister} genes, \textit{ABS} and \textit{GOA}, is supported by their functional characterization, which suggested that \textit{GOA} evolved under relaxed selection pressure and acquired novel roles compared to \textit{ABS} (Erdmann et al. 2010; Prasad & Ambrose 2010; Prasad et al. 2010). Among the monocots, two branches divide the putative canonical B\textsubscript{sister} genes orthologous to \textit{ABS} (\textit{ZMM17}, \textit{OsMADS29} and \textit{TaBsis}), and the others originated by duplication of the same ancestor (\textit{OsMADS30}, \textit{OsMADS31} and \textit{ZmMADS31}). Gymnosperm B\textsubscript{sister} genes, \textit{GGM13} and \textit{GBM10}, originated from the same ancestor of the Angiosperms.

**Distantly related B\textsubscript{sister} genes share conserved expression profile**

The leitmotif of B\textsubscript{sister} gene is that they are mainly transcribed in female reproductive organs. This contraposition with the B-class genes, predominantly expressed in male organs, led to the intriguing hypothesis that the B\textsubscript{sister} genes played a pivotal role in the evolution of reproductive structures in Gymnosperms and Angiosperms (Becker et al. 2002).

Literature information, together with the availability of transcriptome datasets, allows a comprehensive scenario of B\textsubscript{sister} gene expression in distantly related species. Table I shows B\textsubscript{sister} gene expression profiles in selected Angiosperms and Gymnosperms. In some cases, detailed expression studies revealed a fine-tuned regulation of B\textsubscript{sister} gene transcription during ovule formation and early stages of seed development. The \textit{ABS} gene is expressed in the endothelium in mature ovules and young seeds (de Folter et al. 2006; Mizzotti et al. 2012). Dissection of \textit{B. napus} seeds into embryo, endosperm, inner integument and epidermis underlined a predominant transcription of the four \textit{B. napus} B\textsubscript{sister} genes in the inner integument (Chen et al. 2013). Among the Asterids B\textsubscript{sister} genes, \textit{FLORAL BINDING PROTEIN 24} (\textit{FBP24}) is transcribed in young ovules and, later in development, the expression is restricted to the endothelium (de Folter et al. 2006). Specific expression in the seed endothelium is reported also for the \textit{DEFICIENS HOMOLOG 21} (\textit{DEFH21}) gene (Becker et al. 2002).

Moving to monocots, the rice \textit{OsMADS29} gene is expressed during ovule differentiation and seed development. Within the seed, \textit{OsMADS29} can be detected in the cells originating from the inner epidermis that are involved in nutrient transfer from the mother plant to the next generation (Yang et al. 2012; Yin & Xue 2012; Lee et al. 2013). Ovule and seed integument expression is reported also for the \textit{ZMM17} and \textit{TaBsis} genes (Becker et al. 2002; Yamada et al. 2009).

B\textsubscript{sister} gene transcriptional regulation has been studied in \textit{G. gnemon}, \textit{G. biloba} and \textit{T. baccata}; these Gymnosperm species are extremely interesting from an evolutionary point of view because fertilized ovules form a fleshy fruit-like structure (Becker et al. 2002; Lovisetto et al. 2012, 2013). Conservation of the B\textsubscript{sister} gene expression pattern over at least 300 million years is demonstrated by the transcriptional regulation of the \textit{G. gnemon GGM13}, \textit{G. biloba GMB10} and \textit{T. baccata TbBS} genes which are all expressed in female reproductive organs and, more specifically, in the tissue layers surrounding the ovule.

Table I. Schematic representation of B\textsubscript{sister} gene expression levels during ovule and seed formation in selected species.

| Species | Young ovule | Ovule inner integuments | Ovule outer integuments | Nucellus | Embryo-Seed inner integument | Seed outer integument | Endosperm | Embryo Vegetative tissues |
|---------|-------------|------------------------|------------------------|---------|-----------------------------|----------------------|----------|--------------------------|
| \textit{AtABS} | | | | | | | | |
| \textit{AtGOA} | | | | | | | | |
| \textit{BnTT16.4} | | | | | | | | |
| \textit{PhFBP24} | | | | | | | | |
| \textit{AmDEFH21} | | | | | | | | |
| \textit{OsMADS29} | | | | | | | | |
| \textit{OsMADS30} | | | | | | | | |
| \textit{OsMADS31} | | | | | | | | |
| \textit{ZMM17} | | | | | | | | |
| \textit{TaBsis} | | | | | | | | |
| \textit{GGM13} | | | | | | | | |
| \textit{GBM10} | | | | | | | | |

Note: red: high expression; orange: medium expression; yellow: weak expression; white: no expression; grey: not known.
Furthermore, a derived “fruit” role can be postulated for the GBM10 gene that is expressed in the outermost seed integument from which the fleshly fruit-like structure originates after fertilization (Lovisetto et al. 2012, 2013). In some genomes, paralogous Bsister genes with not overlapping expression domains can be identified. As already described in the phylogenetic tree, a duplication event occurred within the Brassicaceae and led to the formation of GOA and ABS in the Arabidopsis genome. Similarly, a monocot-specific gene duplication gave rise to Bsister paralogous in the rice and maize genomes (OsMADS29, OsMADS30 and OsMADS31 in rice and ZMM17 and ZMM31 in maize). The not overlapping domains of ABS and GOA reflects the fact that GOA evolved new functions. Besides a wider expression in floral organs with respect to ABS, GOA is preferentially transcribed in the ovule and outer integument of the seed (Erdmann et al. 2010; Prasad et al. 2010). Concerning the monocots, OsMADS30 is expressed throughout all organs of the rice plant, while the expression of OsMADS31 is hardly detectable (Yang et al. 2012). It will be interesting to investigate which evolutionary mechanisms are acting on the function of the rice Bsister paralogous genes.

Comparing the Bsister expression profile in distantly related species confirms the first observation of extremely conserved female organ-specific genes (Becker et al. 2002). A closer look highlights that members of the GGM13-like genes clade are active in maternal tissues surrounding the haploid gametophytic generation before fertilization and the endosperm and embryo after the arrival of the sporadic nuclei.

Conserved Bsister protein interaction patterns

Protein interaction experiments in yeast and in planta (Egea-Cortines et al. 1999; Nougalli Tonaco et al. 2006) indicated that MADS-box floral identity proteins interact to form multimeric complexes also addressed as “floral quartets” (Theißen & Saedler 2001). These quaternary complexes are supposed to bind two cis-regulatory regions termed CArG boxes (for “CC-Arich-GG sequence) (Schwarz-Sommer et al. 1992; Riechmann et al. 1996). While in Angiosperms, the class E proteins play an indispensable role in the constitution of floral quartets, the formation of floral quartet-like complexes has been recently demonstrated through the interaction of Gymnosperm orthologs of class B and C MADS-box proteins (Wang et al. 2010). Diversification of MADS-box proteins function is therefore linked to the formation of different protein complexes; the interacting partners might mainly define the biological role of each complex.

The functional characterization of Bsister proteins includes the analysis of their capacity to assemble into MADS-box protein complexes. In Arabidopsis and Petunia, yeast three-hybrid assay demonstrated that ABS can interact with C and D class proteins in presence of the E class factors (Kaufmann et al. 2005; de Folter et al. 2006). Interestingly, in Arabidopsis, the complex between ABS and the class D protein SEEDSTICK (STK) has been recently proven to have a relevant role during fertilization and seed development (Mizzotti et al. 2012). Diverse interaction partners have been described for GOA, compared to its paralogous ABS (Erdmann et al. 2010; Prasad & Ambrose 2010).

In different conifers and Gnetum species, the AGL6-like genes represent the orthologs of the class E genes (Becker & Theißen 2003; Melzer et al. 2010). Y2H assays and pull-down experiments recently proved heterodimer formation between the GGM13 Bsister protein and orthologous of class B (GGM2), class C (GGM3) and AGL6-like (GGM11) proteins (Wang et al. 2010).

The identification of Bsister interacting partners represents an important tool to dissect the biological role of these MADS-box factors during plant development and evolution. Indeed, proteins involved in the formation of higher order complexes often share functional redundancy as the seed phenotype of the Arabidopsis abs-6 stk-2 double mutant recently demonstrated (Mizzotti et al. 2012).

B_sister gene activity contributes to reproductive cross-talk in the seed plant

Sequence analysis, expression profile data and protein interaction experiments strongly support the hypothesis that Bsister gene function has been conserved since their appearance 300 MYA before the separation of the Gymnosperm and Angiosperm lineages, but after the diversification of the fern lineage. Clues on the functional roles of Bsister genes are recently emerging; the comparison and integration of the results described in different species will contribute to highlight some aspects of the evolutionary mechanisms at the base of seed plant development and evolution.

In the last 10 years, mutant plants affected in Bsister activity have been described in Arabidopsis, Petunia, Brassica and rice (Nesi et al. 2002; Kaufmann et al. 2005; de Folter et al. 2006; Erdmann et al. 2010; Prasad & Ambrose 2010; Prasad et al. 2010; Deng et al. 2012; Yang et al. 2012; Yin & Xue 2012; Chen et al. 2013). Intriguingly, the defects due to mutations in Bsister genes are
sporophytically controlled. The first Bsister mutant described is the Arabidopsis abs mutant (Nesi et al. 2002). The morphological analysis of the pale abs mutant seeds demonstrated that ABS activity correlated with endothelium differentiation where it controls cell structure and pigment accumulation. Endothelial cells in abs mutant seeds appeared flatter and irregular in shape compared to wild-type; furthermore, these cells lack proanthocyanidins accumulation. In the abs mutant background, seed coat defects are visible immediately after fertilization (Nesi et al. 2002). ABS function in Arabidopsis is partially masked by redundancy with the MADS-box gene STK (Mizzotti et al. 2012). The phenotype of the abs-6 stk-2 double mutant suggested that the MADS-box protein complex composed of ABS and STK is required for ovule and seed formation. Simultaneous lack of ABS and STK activity caused complete absence of endothelium with repercussions on embryo-sac formation, fertilization and seed development. Interestingly, the abs-6 stk-2 double mutant is female sporophytic sterile as ABS/abs-6 STK/stk-2 heterozygous plants are fully fertile (Mizzotti et al. 2012). Seeds with a reduced amount of endosperm have been described in petunia plants where the two STK orthologous (FLORAL BINDING PROTEIN 7 – FBP7 – and FBP11) were down-regulated (Colombo et al. 1997). Genetic analysis showed that this phenotype was sporophytically controlled as the abs-6 stk-2 phenotype. Taken together, these results suggest that some overlapping functions between the Bsister and D-class genes might be conserved in different species.

Target gene identification of the Arabidopsis protein complex composed of ABS and STK will represent a starting point to dissect the molecular cross-talk occurring between the ovule integuments and the developing gametophyte as well as between the seed coat and the fertilization products.

Different phenotypes between goa and abs mutant plants definitely demonstrated the fact that GOA evolved new regulatory roles not overlapping with ABS (Erdmann et al. 2010; Prasad & Ambrose 2010; Prasad et al. 2010). Developmental defects in goa mutant plants suggest that GOA contributes to the control of fruit cell expansion and to the differentiation of the outer integument of the seed (Erdmann et al. 2010; Prasad & Ambrose 2010; Prasad et al. 2010). The goa-1 abs-1 double mutant phenotype showed that ABS and GOA have additive roles during seed coat development (Prasad et al. 2010).

Four paralogous ABS genes (BnTT16.1, BnTT16.2, BnTT16.3 and BnTT16.4) have been identified in the allotetraploid species B. napus (Deng et al. 2012; Chen et al. 2013). Simultaneous down-regulation of the four BnTT16 genes using an RNA interference (RNAi) approach influences plant size, flowering time, floral morphology, embryo formation, seed development and seed set (Deng et al. 2012). Interestingly, shorter siliques and reduced seed set are visible when the female plants carry the RNAi construct, independent from the male parent genetic background (Deng et al. 2012).

Among the Asterids, functional characterization of a member of the GGM13 clade, the FBP24 gene, is reported in Petunia (de Folter et al. 2006). FBP24 loss of function resulted in a maternally-controlled phenotype. Silencing the FBP24 gene affects endothelium cell identity with repercussions on plant fertility; reciprocal crosses showed a reduced seed set compared to wild-type plants only when the FBP24 gene is down-regulated in the mother plant. Occasionally, morphological defects are described before fertilization because a few ovules lacking the embryo-sac have been identified (de Folter et al. 2006). This phenotype is visible only when the FBP24 endogenous gene is down-regulated as a consequence of co-suppression; on the contrary, transposon insertion within the FBP24 locus did not cause defects compared to wild-type plants. Taken together, these results suggest functional redundancy with unknown factors which might be silenced in the fbp24 co-suppressed lines (de Folter et al. 2006).

Until now, the only mutant phenotype correlating with lack of Bsister function within the monocots is reported in rice (Yang et al. 2012; Yin & Xue 2012; Lee et al. 2013). The role of OsMADS29 was recently elucidated using an RNAi construct which specifically silences the endogenous OsMADS29 gene (Yang et al. 2012; Yin & Xue 2012) and through the characterization of the spontaneous female sterile (fst) mutation which resulted to map in the OsMADS29 locus (Lee et al. 2013). In the plant analyzed, lack of activity of OsMADS29 is responsible for sporophytic female sterility. In fst homozygous plants, defects are visible before fertilization mainly during ovule integument development (Lee et al. 2013). Following fertilization, both the fst mutant and OsMADS29 RNAi plants produced aborted or shrivel seeds mostly lacking endosperm (Yang et al. 2012; Yin & Xue 2012). Detailed morphological analysis showed that the down-regulation of the OsMADS29 gene correlates with reduced or delayed cell degradation of the maternal tissue named nucellar projection (NP). In wild-type plants, fertilization is followed by programmed cell death of the NP tissue; this event is required for the efficient nutrient transfer to both embryo and endosperm. Because OsMADS29 is not expressed in the endosperm, aborted seeds in mutant plants are likely the consequence of defects during nutrient transportation from the maternal tissues to the next generation (Yang et al. 2012; Yin & Xue 2012). Interestingly, a possible role for the wheat Bsister gene
(TaBis) during seed vascular bundle formation has been postulated (Yamada et al. 2009).

Ectopic expression of Bsister genes has been performed in different species (Kaufmann et al. 2005; de Folter et al. 2006; Erdmann et al. 2010; Prasad et al. 2010; Lovisetto et al. 2013). While in some cases, the phenotype of transgenic plants confirmed the data observed in knock-out plants (35S::GOA plants are smaller than wild-type), in other situations, ectopic expression of Bsister genes is responsible for pleiotropic defects (Kaufmann et al. 2005; Erdmann et al. 2010; Prasad et al. 2010).

Widespread developmental defects as a consequence of ectopic expression of MADS-box transcription factors might likely depend on the unbalanced changes in protein complexes that can be formed when a MADS-box protein is over-produced. Because Bsister proteins participate in the formation of quaternary MADS-box protein complexes, the effects of their ectopic expression might not completely reflect the Bsister physiological function.

Overall, both monocots and dicots Bsister mutant plants indicated that Bsister factors play key roles in the differentiation of the ovule sporophytic tissues and maternal-derived seed compartments with consequences on plant fertility. In some cases, this function is redundantly shared with other MADS-box genes as recently described in Arabidopsis (Mizzotti et al. 2012) while in other species, it seems restricted to Bsister genes as it can be deduced by the defects observed in the rice osmads29 mutant plants (Yang et al. 2012; Yin & Xue 2012; Lee et al. 2013).

Concluding remarks and future perspective

In the aim to dissect molecular aspects of reproduction in higher plants, the increasing information regarding Bsister gene function in distantly related species represent an important achievement.

The fine-tuned regulation that MADS-box transcription factors play during plant development is strongly dependent on the formation of interchangeable protein complexes which act as functional units. In this perspective, the identification of protein complexes comprising Bsister factors might therefore represent an important step to highlight the biological roles of GGM13-like genes. Furthermore, the possibility to identify target genes of MADS-box protein complexes comprising the Bsister proteins will represent the chance to dissect part of the molecular components involved in tissue communication during ovule and seed formation in higher plants. Promisingly, the Gene Ontology classification of genes differentially expressed in fst rice mutant compared to wild type plants comprises auxin efflux and polarity, hormone regulation, signal transduction, sugar metabolism and apoptosis related genes (Lee et al. 2013). Target genes of the Bsister factors likely belong to these functional classes.

Excitingly, we are now at the beginning of understanding the general principles of tissue communication during ovule and seed formation. These aspects are important not only for basic research but also in the perspective to manipulate seed quality for food source.

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