Plant Cell Physiol. 41(6): 710–718 (2000)

Spatially and Temporally Regulated Expression of Rice MADS Box Genes with Similarity to *Arabidopsis* Class A, B and C Genes

Junko Kyozuka [1], Takeshi Kobayashi, Masakazu Morita and Ko Shimamoto

Laboratory of Plant Molecular Genetics, Nara Institute of Science and Technology, 8916-5, Takayama, Ikoma, 630-0101 Japan

The floral morphology of grass species is distinct from that of typical dicot plants. In order to achieve a better understanding of the molecular basis for this diversion, we isolated *RAP1A*, *RAP1B* and *RAG*, putative rice orthologs for the *Arabidopsis* class A gene *APETALA1 (AP1)* and class C gene *AGAMOUS (AG)*. The expression patterns of *RAP1A*, *RAG* and *OsMADS2*, a rice ortholog of the class B gene, were analyzed by in situ hybridization. *RAP1A* mRNA was expressed in the apical region of the floral meristem at an early stage of spikelet development, and then its expression was localized in developing lemma, palea and lodicules. The *OsMADS2* transcript was first observed in the region where stamen primordia are formed. Soon after, *OsMADS2* mRNA appeared in the lodicule primordia as well as the stamen primordia, and this RNA accumulation pattern persisted until late stages of floral development. The expression of *RAG* was observed in stamens and pistils of wild-type young spikelets. These RNA accumulation patterns are mostly similar to those of *Arabidopsis* class A, B, C genes, supporting the notion that the ABC model may be extended to rice.

Key words: ABC model — Floral organs — Rice.

Genetic analysis in *Arabidopsis* and *Antirrhinum* has demonstrated that floral organ identity genes act alone or in combination to specify the organ formed in each of the four whorls in a flower (for a review, see Weigel and Meyerowitz 1994, Yanofsky 1995, Riechmann and Meyerowitz 1997). Pattern formation of the floral organs is explained by the ABC model (Bowman et al. 1991). According to the model, the identity of the floral organs is defined by the action of genes in three classes (classes A, B and C). This model has been confirmed for many other plants and is accepted as a general concept, at least in dicot species. In *Arabidopsis*, class A genes *APETALA1 (AP1)* (Mandel et al. 1992a) and *APETALA2 (AP2)* (Jofuku et al. 1994), class B genes *APETALA3 (AP3)* (Jack et al. 1992) and *PISTILATA (PI)* (Goto and Meyerowitz 1994), and a class C gene *AGAMOUS (AG)* (Yanofsky et al. 1990) are known to be involved in the differentiation of each floral organ.

[1] Corresponding author: E-mail, junko@bs.aist-nara.ac.jp.

Many features of floral and inflorescence development of monocot species are distinct from those of dicot species. Among the monocot species, flowers in the grasses are the most highly diverged. Rice (*Oryza sativa* L.), which has several advantages for molecular biological studies (Izawa and Shimamoto 1996), produces flowers that are typical of a grass species (Hoshikawa 1989, Kyozuka 1999). An individual rice flower contains one gynoecium, six stamens and two lodicules, and is enclosed by two leaf-like structures, a lemma and a palea (Fig. 1). The whole structure, including the lemma, the palea and the flower, is referred to as a floret. A floret is enveloped by two extra bracts called upper and lower glumes. The whole unit, glumes plus floret, is termed a spikelet.

In spite of many comparative morphological studies, a satisfactory interpretation of the floral organs of a grass species is still not available. Among the floral organs, the lodicules have been the most puzzling ones (Clifford 1987, Schmidt and Ambrose 1998). According to Bossinger et al. (1992), lodicules represent the inner whorl of the perianth, where petals are formed in dicot species. The interpretation of the palea is also varied. Usually, the palea and the lemma are both considered bracts (Clifford 1987, Bell 1991); however, at the same time, the palea is often explained as the equivalent of dicot sepals (Schmidt and Ambrose 1998).

It is expected that the progress in the molecular and genetic understanding of floral development in dicot species can be utilized to interpret grass flower development. From rice, putative B- and C-function genes were isolated. *OsMADS2* and *OsMADS4* were reported as putative rice orthologs of *PI* (Chung et al. 1995), while *OsMADS16* was reported as an *AP3* counterpart (Moon et al. 1999). *OsMADS3* was proposed to be a rice ortholog of *AG* (Kang et al. 1995). Ectopic expression of *OsMADS3* in transgenic tobacco under the control of the 35S promoter caused homeotic alteration of sepals and petals to carpels and stamens, respectively, suggesting that *OsMADS3* acts as a class C gene (Kang et al. 1995). The function of these genes in rice flower development was analyzed by using an antisense approach. Transgenic plants expressing the antisense *OsMADS4* exhibited homeotic alterations of lodicules and stamens to palea/lemma-like structures and carpel-like organs, respectively (Kang et al. 1998). On the other hand, the transformation from stamens to lodicule-like structures and partial loss of floral determinacy was

observed in the plants expressing the antisense *OsMADS3* gene (Kang et al. 1998). The above mentioned results support the idea that the ABC model may be extended to rice.

In spite of these advances, basic information for determining to what extent the ABC model can be applied to rice is still lacking. First, class A genes have not been satisfactorily analyzed. Class A genes are required for specification of the non-reproductive portion of *Arabidopsis* flowers, which are highly modified in grass flowers. Therefore, isolation and characterization of class A genes is crucial for understanding the molecular basis of rice flower development. A rice cDNA clone with reasonably high sequence similarity to *AP1* has been identified through a large-scale cDNA analysis of the Rice Genome Project (Shinozuka et al. 1999). The putative class A gene of maize, *ZAP1* was isolated (Veit et al. 1993, Mena et al. 1996). However, there has been no report of a detailed analysis of either their expression or their function.

Furthermore, detailed analysis on the expression patterns of rice class B and C genes still remains to be reported. For the *OsMADS2, OsMADS4* and *OsMADS16*, class-B genes, and *OsMADS3*, RNA expression patterns were mainly examined by Northern blot analysis (Chung et al. 1995, Kang et al. 1995). In situ hybridization was only performed to examine the spatial distribution of RNA accumulation in mature florets, and the analysis did not include all organs in florets. In *Arabidopsis, AP1, AP3, PI* and *AG* are expressed in the regions of the flower that exhibit homeotic conversions when their function is disrupted by mutations. Thus, determination of their expression patterns in situ is an essential first step for unveiling the molecular mechanisms of floral development in grass species.

In this paper, we first describe the isolation of putative rice class A genes, *RAP1A* and *RAP1B*, and examine their expression patterns during rice panicle development. Furthermore, the patterns of RNA accumulation of the *OsMADS2* and *RAG* in rice flower development are determined by in situ hybridization.

Materials and Methods

Screening and cloning—The following pairs of primers were used to amplify the *AP1*- or *AG*-specific MADS box region by PCR: 5′-GTACTCGTAGAGCTTGCC-3′ and 5′-GGCAAGGTACAGCTGAAG-3′; 5′-GGGAAGATCGAGATCAAGAG-3′ and 5′-GTAGAGCCGGCCGCGGCT-3′. Approximately 10^6 plaques in a cDNA library made from young inflorescence RNA (Kyozuka et al. 1998) were screened. Washing was done under high-stringency conditions (65°C for hybridization and a wash with 2 × SSC at room temperature followed by two washes with 0.1 × SSC at 65°C). The plasmids containing positive cDNA were rescued in vivo from phages according to the manufacturer's protocol (Stratagene, La Jolla, CA, U.S.A.).

Phylogenetic analysis—Predicted amino acid sequences were used for phylogenetic analysis. A neighbor-joining tree was produced from the results of 1,000 bootstrap replicates using CLASTAW program of the DNA Data Bank of Japan (Mishima, Japan). The phylogenetic tree was displayed by TreeView software (Page 1996).

RNA analyses—RNA was isolated from various tissues by a published method (Chomczynski and Sacchi 1987). RNA blot analysis was carried out according to Kyozuka et al. (1998). Twenty μg of total RNA isolated from various tissues was separated by electrophoresis in 1.5% agarose gel and blotted onto a nylon membrane (Hybond N, Amersham, Little Chalfont, Buckinghamshire, U.K.). ^{32}P labeled RNA probes were produced and used for hybridization. To avoid cross-hybridization, the MADS-box region was deleted from the templates used for making the RNA probes.

In situ hybridization was done according to Kouchi and Hata (1993) with some modifications. Young panicles were fixed with FAA solution (3.7% [v/v] *p*-formaldehyde, 5% [v/v] acetic acid) at 4°C overnight, dehydrated, and then embedded in paraffin (Paraplast+, Oxford Labware, St. Louis, MO, U.S.A.). The tissues were sliced into 10-μm sections and dried overnight onto 3-amino-propyltriethoxy-silane-coated slides (ProbeOn Plus, Fisher Scientific, Pittsburgh, PA, U.S.A.). The C-terminus and 3′ untranslated regions of the *RAP1A, OsMADS2* and *RAG* were amplified by PCR and used as a template for making a digoxygenin-labeled RNA probe. Hybridization was performed overnight at 55°C. After the hybridization, sections were washed in 4 × SSPE followed by a washing with RNase solution (20 mg liter^{-1}) at 37°C for 30 min and 4 washings (30 min each) in 0.5 × SSPE solution at 65°C.

Results

Isolation of rice orthologs of AP1 and AG—The *AP1*-specific MADS region was amplified by PCR with a set of primers designed on the basis of the sequences conserved in *AP1* orthologs. The amplified fragment showed high sequence similarity with *AP1*; thus, it was used as a probe for screening a cDNA library prepared from young rice inflorescence mRNA. Screening yielded 2 MADS box cDNAs showing high sequence similarity to *AP1*. Thus, they were designated *RAP1A* and *RAP1B* (Rice homolog of *AP1*) (Fig. 2A). *RAP1A* was found to be a splicing derivative of an expressed sequence tag clone E31864, which was identified in the cDNA database of Rice Genome Project (Shinozuka et al. 1999). In this study, for convenience, we call this clone *RAP1A*. The gene phylogeny showed in Fig. 2C clearly indicated that *RAP1A* and *RAP1B* are members of the *AP1* sub-group and that *RAP1A* is more closely related to *ZAP1*, a maize *AP1* counterpart (Mena et al. 1995).

Previously, *OsMADS3* was reported as a rice *AG* ortholog. However, the *OsMADS3* lacks an amino terminal extension which is present in all the class C MADS proteins of angiosperms. Therefore, the absence of this extension in the *OsMADS3* prompted us to re-screen for another *AG* ortholog of rice. Five independent clones were obtained by screening the cDNA library with a PCR fragment amplified with *AG* specific primers. Sequencing revealed that one

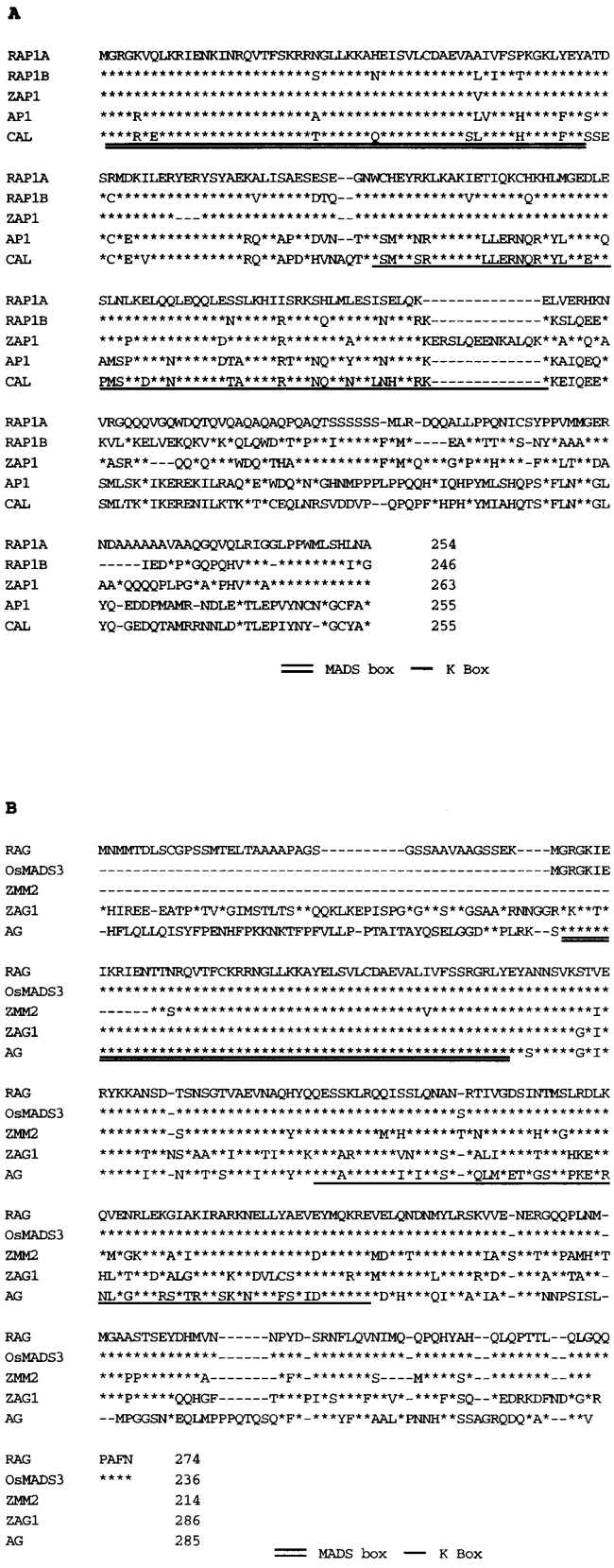

Fig. 2 Comparison of amino acid sequences of RAP1A, RAP1B and RAG with MADS box genes isolated from *Arabidopsis* and maize. Alignment of the amino acid sequences of RAP1A, RAP1B, ZAP1 and AP1 (A), and alignment of RAG, OsMAD3, ZMM2, ZAG1 and AG sequences (B). An asterisk indicates a residue identical to RAP1A or RAG, and a hyphen represents a gap introduced to optimize alignment. Numbers indicate the position of the amino acids in RAG from the putative translation start site. The conserved MADS-box and K-box regions are underlined. (C) Phylogenetic tree for rice (RAG, RAP1A, RAP1B, OsMADS1, OsMADS2, OsMADS3, OsMADS4, OsMADS6, OsMADS7, OsMADS8, OsMADS24, OsMADS45), *Arabidopsis* (AGL1, AGL2, AGL3, AGL4, AGL5, AGL6, AGL8/Ful, AGL9, AGL11, AGL12, AGL13, AGL14, AGL15, AGL17, AG, AP1, AP3, PI) and maize (ZAG, ZMM2, ZAP1) MADS box genes.

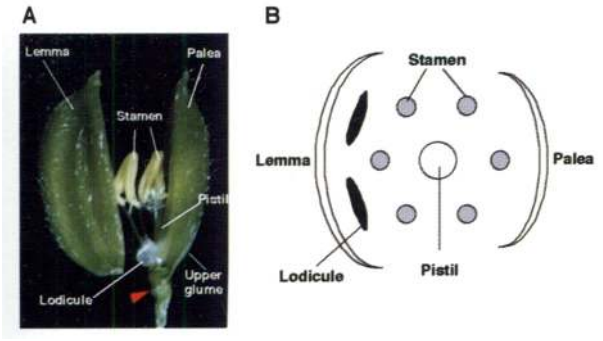

Fig. 1 A rice spikelet (A) and schematic diagram of a transverse section through a flower (B). A flower is composed of a pistil, six stamens and two lodicules. A rice flower is enclosed by a pair of bract-like structures called the lemma and palea. The structure that includes the lemma, the palea and the flower is referred to as a floret. A floret is enveloped by two extra bracts called upper and lower glumes. The lemma is detached from the original position (red arrow) to show lodicules.

of them exhibited high sequence similarity with *AG* and *OsMADS3* and was designated *RAG* (Rice ortholog of *AG*). The deduced amino acid sequence of *RAG* differs from that of *OsMADS3* only in 1 amino acid, except for the presence of 39 amino acids in the N terminal of RAG (Fig. 2B). The results that extensive sequence similarity was observed in the entire region of *RAG* and the *OsMADS3* cDNAs and Southern analysis revealed that both *RAG* and *OsMADS3* are single-copy genes in the rice genome (this study and Kang et al. 1995) indicated that *OsMADS3* and *RAG* represent the same gene. The differences in nucleotide sequences in the two cDNAs may reflect the polymorphism between cultivars since *OsMADS3* was isolated from rice cv. M201, whereas *RAG* was isolated from cv. Toride 1. The deduced amino acid sequence of *RAG/OsMADS3* is most closely related to that of *ZMM2* (Schmidt et al. 1993, Theissen et al. 1995) (Fig. 2C).

RAP1A, a putative rice class A gene, is expressed in lemma, palea and lodicules—The organ specificity of *RAP1A* and *RAP1B* mRNA expression was determined by RNA blot analysis (Fig. 3A). Loading of the same amount of RNA on each lane was confirmed by staining with ethidium bromide (data not shown). Both *RAP1A* and *RAP1B* mRNAs were most abundant in young panicles, with a lower level of expression also observed in mature spikelets but not in leaves or roots.

Next, we analyzed *RAP1A* mRNA expression during rice flower development by in situ hybridization (Fig. 3B through F). At the very early stage of rice spikelet development, floral meristems differentiate from the inflorescence meristem on each rachis branch. Then, lower and upper glumes, and subsequently lemma and palea primordia, differentiate in alternate phylotaxy. In the spikelet primordia before lemma and palea initiation, *RAP1A* mRNA was detected in the apical region of the floral meristem (Fig. 3B). After the lemma and palea initiated, *RAP1A* mRNA was excluded from the apical region of the floral meristem but accumulated in the developing lemma and palea (Fig. 3C). At a later stage of floral development, accumulation of *RAP1A* RNA was observed in lemma, palea and lodicules (Fig. 3D, E) but not in the stamen primordia (Fig. 3D) or the pistil (data not shown). Expression of *RAP1A* in the palea and lemma persisted to a later stage of spikelet development, although RNA accumulation was restricted in the inner and basal parts of the palea

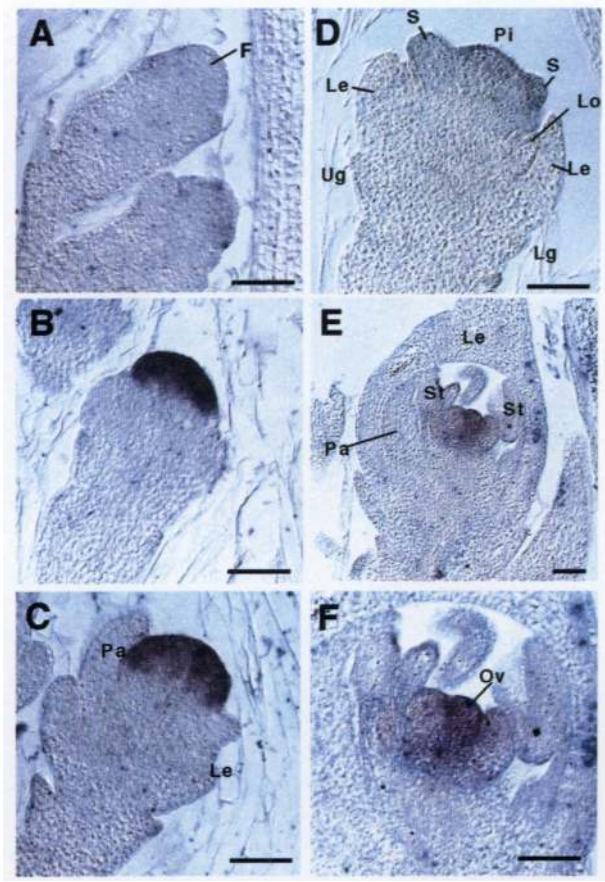

Fig. 5 Accumulation of *RAG* mRNA during rice flower development. (A) An early stage floral meristem. *RAG* mRNA is not detected. (B) A spikelet after the initiation of the lemma and palea. A strong signal of *RAG* expression is detected in the cells in the two inner whorls which will give rise to the stamens and the pistil. (C) A spikelet before stamen initiation. A signal of *RAG* expression is detected in the same region as in the floret shown in B; however, its expression level has started to decrease. (D) A spikelet with growing stamens. The *RAG* mRNA is observed in the stamens and the pistil primordium. (E) A floret at the later stage. All the floral organs have differentiated by this stage. A faint signal of *RAG* expression is observed in the stamens and the pistil. (F) A close-up of an ovule in the floret shown in E. Fm, floral meristem; Lo, lodicule; Pa, palea; Le, lemma; Pi, pistil; Ug, upper glume; Lg, lower glume; Ov, ovule. Bars, 100 μm.

and the lemma (Fig. 3E). Weak expression was also detected in the upper and lower glumes. In addition, a strong signal was observed in the vascular region in spikelets, rachis, and rachis branches (Fig. 3E, F).

Expression of OsMADS2, a putative rice class B gene, is restricted to lodicules and stamens—Two cDNAs have been reported as *PI* orthologs in rice (Chung et al. 1995). We chose *OsMADS2* as a probe to analyze the expression pattern of the rice class B gene because it has a higher sequence similarity to *PI* than *OsMADS4*. Figure 4 shows *OsMADS2* expression in longitudinal sections of a developing rice panicle indicating a highly localized accumulation of *OsMADS2* RNA in developing lodicules and stamens.

OsMADS2 mRNA was not detected in the early floral meristems before floral organ initiation (Fig. 4A)

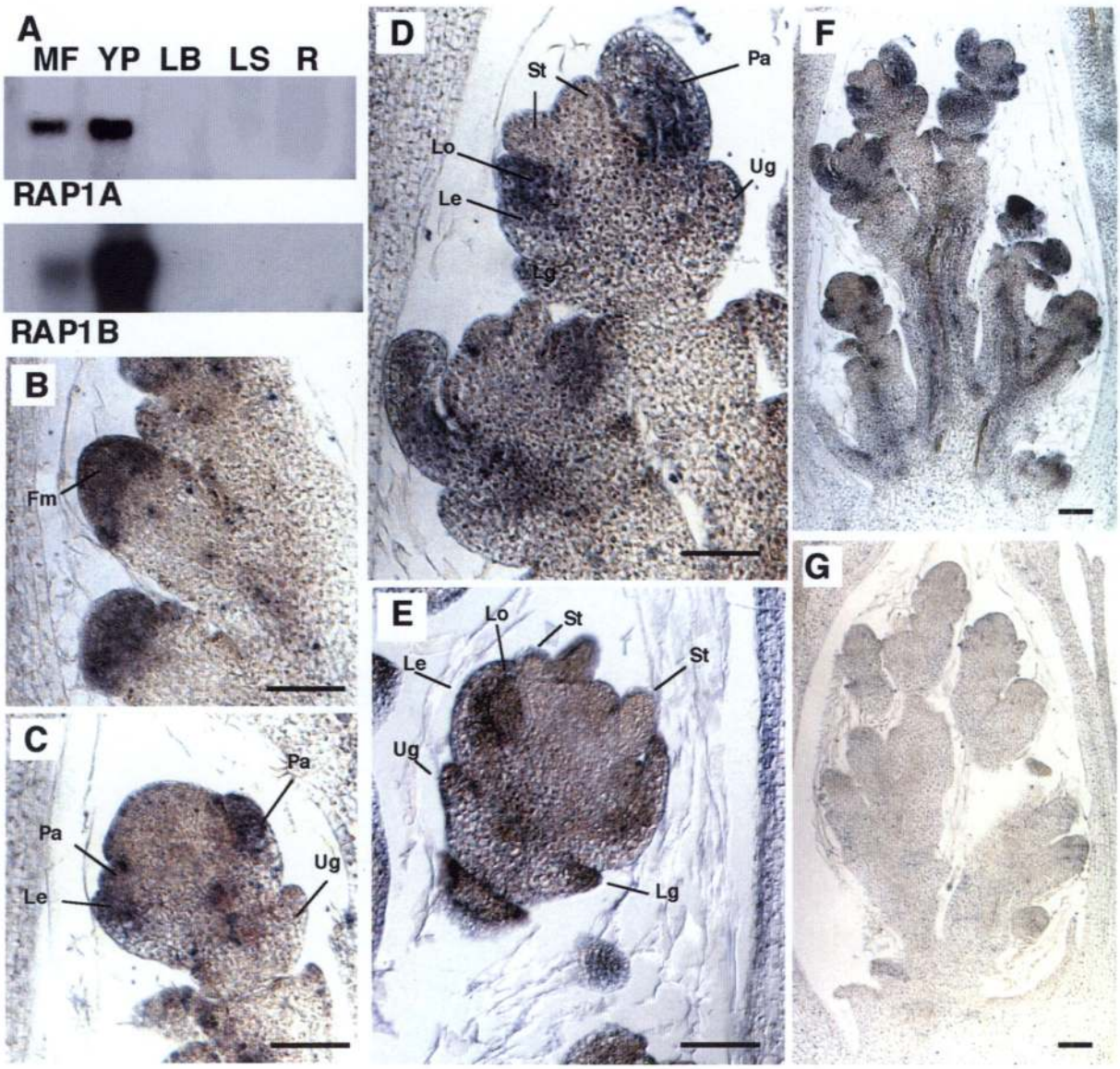

Fig. 3 *RAP1A* mRNA accumulation during rice flower development. (A) RNA blot analysis detecting *RAP1A* and *RAP1B* mRNA. Total RNA (20 μg) was loaded in each lane. MF, mature flower; YP, young panicle before floret initiation; LB, leaf blades; LS, leaf sheath; R, roots. (B) An early stage floral meristem before the initiation of glumes. *RAP1A* mRNA was detected at the apical region of the floral meristem. (C) A spikelet after the lemma and palea have initiated. A signal was detected in the developing palea and lemma. (D), (E), A spikelet at the stage of stamen development. *RAP1A* expression continues in the lemma, palea and lodicules but not in stamens. Weak *RAP1A* expression was also detected in the upper and lower glumes. (F) Whole view of a longitudinal section of a young panicle. (G) Sense control. No staining detected. Fm, floral meristem; Le, lemma; Pa, palea; Lo, lodicule; Ug, upper glume; Lg, lower glume; St, stamens. Bars, 100 μm.

through the stages of glumes, lemma and palea differentiation. Shortly after the palea primordia began to grow, *OsMADS2* mRNA was first detected in the cells that give rise to the stamens (Fig. 4B, C (upper floret), F). Since lodicules initiate after stamen primordia in rice (Clifford 1987), lodicule primordia are not visible at this stage. In the slightly later stage spikelet, when stamen and lodicule primordia became visible and the lemma and palea grew enough to cover the spikelet, *OsMADS2* mRNA was localized in both the lodicule and stamen primordia (Fig. 4C (lower floret), D, F). Interestingly, the intensity of the signal was reduced rapidly and extensively in the stamen primordia. Thus, a stronger expression was observed in the lodicules. This tendency of having a higher expres-

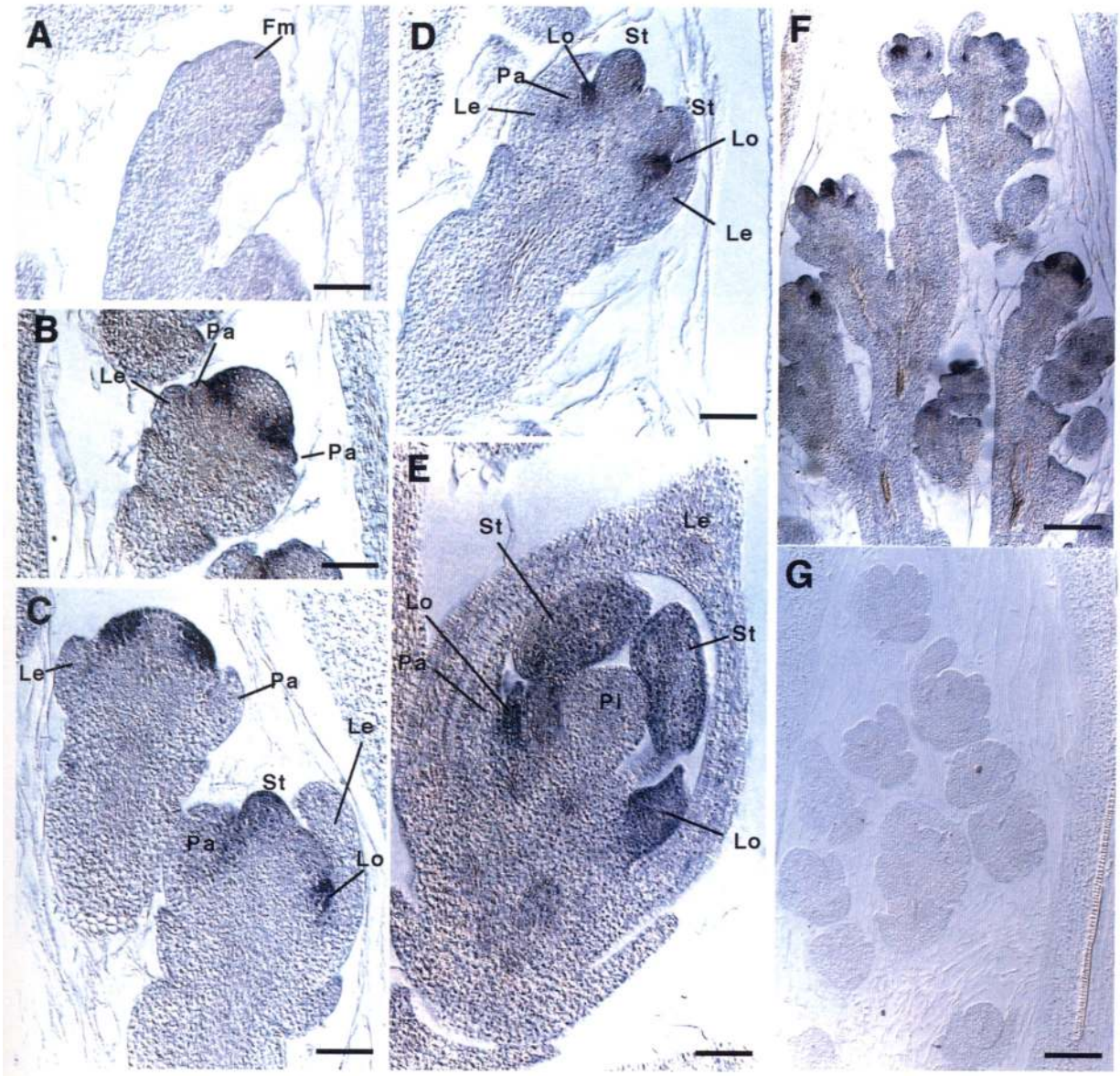

Fig. 4 Accumulation of *OsMADS2* mRNA during rice flower development. (A) An early stage floral meristem. *OsMADS2* mRNA is not detected at this stage. (B) A spikelet after initiation of the lemma, palea and lodicules. A strong signal of the *OsMADS2* expression is detected in the cells which will later give rise to stamens. (C) A spikelet before stamen initiation (upper floret) and a spikelet with stamen primordia (lower floret). A strong signal of the *OsMADS2* expression is detected in the cells that will give rise to stamens in the upper floret. *OsMADS2* RNA is expressed in both lodicules and stamen primordia in the lower floret. (D) A spikelet with growing stamens. *OsMADS2* mRNA is observed in lodicules and stamens. (E) A floret at the later stage. All the floral organs have differentiated. A signal of *OsMADS2* expression is observed in lodicules and stamens. (F) A transverse section of the whole panicle. (G) Sense control. No staining was detected. Fm, floral meristem; Lo, lodicule; Pa, palea; Le, lemma; Pi, pistil; R, rachis. Bars, 100 μm.

sion in lodicules than in stamens was kept to later developmental stages in which all the floral organs are well differentiated (Fig. 4E). Accumulation of the *OsMADS2* RNA was not observed in pistil primordia or developing pistils. In our analysis, *OsMADS2* mRNA was not detected in the glumes, the lemma, or the palea.

RAG, a putative class C gene, is expressed in the two inner whorls—The localization of *RAG* RNA accumulation was also determined by in situ hybridization (Fig. 5). *RAG* expression was not detected in the early flower meristems and young flowers at the palea and lemma primordia initiation stage (Fig. 5A). Intense expression of *RAG* mRNA was first observed in cells in the two inner whorls where the stamens and the pistil are formed (Fig. 5B, C). After the stamen primordia had initiated, a low but significant level of *RAG* RNA expression was observed in the stamens and the pistil primordia (Fig. 5D). This expression pattern was kept until the later stage of flower development, and a very low level of *RAG* mRNA was present during the development of the stamens and the pistil (Fig. 5E). A relatively strong signal was detected in the ovule at this stage (Fig. 5E, F). *RAG* expression was kept under a detectable level in other spikelet organs, such as lodicules, the palea and the lemma throughout the stages of floral development.

Discussion

In this report, we described the isolation of rice *AP1* and *AG* orthologs. Sequence similarity and expression pattern suggested that *RAP1A* is a cognate ortholog of *AP1*. Furthermore, we determined the spatial and temporal expression patterns of *OsMADS2* and *RAG*, putative rice class B and C genes, respectively, in rice floral development. The accumulation of *RAG* RNA in the two inner whorls and *OsMADS2* expression in the next two whorls fit the ABC model well. Although differences in floral morphology make it difficult to compare rice and *Arabidopsis* flower development accurately, our results support the notion that the ABC model can be extended to grasses (Schmidt and Ambrose 1998, Kan et al. 1998). In the near future, analysis of *RAG* and *RAP1A* expressions in their loss of function mutants and transgenic rice plants in which expression of the rice class A, B, C genes are altered will provide a more comprehensive view concerning the model explaining floral organ formation in rice.

Lodicules correspond to dicot petals—Lodicules are the organs in grass spikelets whose identity has not been clearly defined (Schmidt and Ambrose 1998). The most accepted interpretation is that lodicules represent petals, although there have been many previous discussions on this point (for example, see Clifford 1987). In wild-type dicot flowers, class B genes determine the identities of petals and stamens, which are the organs generated in whorls 2 and 3. Since class B genes are expressed in whorls 2 and 3 in later stages of flower development (Jack et al. 1992, Goto and Meyerowitz 1994), it has been anticipated that the RNA accumulation pattern of grass class B genes will aid in interpreting the structures within grass spikelets. Kang et al. (1995) examined the expression of *OsMADS2* and *OsMADS4* by RNA blot analysis and in situ hybridization analysis. However, lodicules were not included in the organs they analyzed (Chung et al. 1995). Here, we showed that *OsMADS2* mRNA accumulated in lodicules and stamens. In addition, *RAP1A* expression was also observed in lodicules. Thus, our results strongly suggest that lodicules are organs formed in whorl 2, which means that they correspond to petals in dicot flowers.

Lodicules were transformed to palea/lemma-like structures by expressing antisense *OsMADS4* in transgenic rice plants (Kang et al. 1998). In the maize *silky1 (si1)* mutant, the function of the maize ortholog of *AP3*, another class B homeotic gene of *Arabidopsis*, was disrupted (Schmidt and Ambrose 1998). In *si1* spikelets, lodicules are replaced by palea-like structures, and the homeotic transformation from stamens to the carpel-like structures occurs. Although details of the *si1* gene have yet to be reported, their results also imply that lodicules are floral organs corresponding to petals in dicot species.

RAP1A, a rice AP1 ortholog—In this study, we revealed that the mRNA of the rice class A gene, *RAP1A*, was mainly localized in the developing palea, lemma and lodicules in the later stage florets. In *Arabidopsis, AP1* is expressed in the sepals and petals (Mandel et al. 1992a). Thus, our current observation further supports the view that lodicules are homologous organs of dicot petals. However, the expression of *RAP1A* in the palea and lemma should be interpreted carefully. From the expression pattern of *RAP1A*, the simplest interpretation is that both the palea and lemma are organs corresponding to sepals. However, the observation that the palea and lemma differentiate in alternate phylotaxy and are not formed in a single whorl makes this interpretation unlikely. The phenotype of maize *si1* mutant and *OsMADS4* antisense plants, in which a palea/lemma-like organ is formed instead of lodicules, might suggest a homology between the palea, lemma and dicot sepals. However, it is not possible to precisely determine whether the identity of the transformed organ is a palea or a lemma, especially when the homeotic conversion is incomplete. *SQUAMOSA (SQUA)*, an *Antirrhinum* counterpart of *AP1*, is expressed in the bract at a low level as well as in sepal and petal primordia, although no phenotypic alteration is found in the bracts of *squa* mutants (Huijser et al. 1992). Therefore, an alternative explanation is that the expression of *RAP1A* in palea reflects the homology between the palea and sepals; on the other hand, *RAP1A* expression in the lemma represents the expression of class A genes in the bract.

In dicot species, overexpression of *AG* homologs under the control of the 35S promoter causes homeotic transformation from petals and sepals to stamens and carpels, respectively. This was demonstrated in many plant species including *Arabidopsis* (Mandel et al. 1992b, Mizukami and Ma 1992, Riechmann and Meyerowitz 1997), *Brassica napus* (Mandel et al. 1992), tobacco (Kempin et al. 1993), petunia (Tsuchimoto et al. 1993) and tomato (Pnueli et al. 1994). A palea and/or a lemma might be transformed to a carpel if they are homologous organs of dicot sepals in transgenic rice plants ectopically expressing the class C gene. Ectopic expression of *RAG* in rice as well as loss of function analysis of *RAP1A* should provide more hints for elucidating the identities of the palea and lemma.

The uniform expression of *RAP1A* in early floral meristems is analogous with the expression of *AP1* in floral meristems (Mandel et al. 1992a). The *AP1* has dual functions as a floral meristem identity gene as well as a floral organ identity gene to specify organs in whorls 1 and 2. From the resemblance between the overall expression patterns of *RAP1A* and *AP1*, it is likely that *RAP1A* is a rice ortholog of *AP1* that plays a major role in floral meristem initiation in rice. In *Arabidopsis, AP1* and *LEAFY (LFY)* (Weigel et al. 1992) are considered to play primary roles in initiating floral meristems. Previously, we showed that *RFL*, a rice *LFY* ortholog, is not expressed in floral meristems but is expressed in very young panicles much earlier than floral meristem initiation, implying that the function of *RFL* has diverged from that of *LFY* (Kyozuka et al. 1998). Thus, the difference found in the RNA accumulation patterns of *RAP1A* and *RFL* raised the interesting possibility of the functions of *LFY* and *AP1* orthologs being differentiated in rice. The loss of the function phenotypes of *RFL* and *RAP1A* should be examined to test this notion.

We thank Emi Kurokawa for providing excellent technical assistance. This work was supported in part by the Japan Society for the Promotion of Science, "Research for the Future" Program, JSPS-RFTF97-R16001.

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(Received February 7, 2000; Accepted March 21, 2000)