FLOWERING NEWSLETTER REVIEW

Regulation of flowering time and floral patterning by miR172

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Received 15 July 2010; Revised 2 September 2010; Accepted 2 September 2010

Abstract
Since the discovery of miRNAs in plants it has become clear that they are central to the regulation of many aspects of plant development and responses to the environment. miR172 regulates expression of a small group of AP2-like transcription factors in an evolutionarily ancient interaction. miR172 functions in regulating the transitions between developmental stages and in specifying floral organ identity. These two roles are conserved across monocotyledons and dicotyledons. Investigations into the roles of miR172 and its targets in phase changes in the model plant Arabidopsis have illustrated that this process is governed by complex regulatory systems. In addition to its conserved roles, miR172 has also acquired specialized species-specific functions in other aspects of plant development such as cleistogamy and tuberization.

Key words: Floral organ identity, flowering time, miR172, post-transcriptional regulation.

Introduction
Reproductive success in plants is dependent on the timing of the switch from vegetative to reproductive growth coinciding with optimal environmental and developmental conditions. Plants have evolved an elaborate regulatory network that integrates endogenous and environmental signals to ensure that flowering commences when conditions are most favourable. Flowering time is controlled by several endogenous and environmental pathways, including the autonomous, photoperiod, vernalization, gibberellic acid (GA), thermosensory, and ageing pathways, with about 180 genes that have been shown to be involved in flowering time control based on loss-of-function mutants or transgenic analysis using Arabidopsis (Fornara et al., 2010). The non-coding microRNA (miRNA) gene, MIR172, is one of these genes; it was one of the earliest plant MIRNA genes to be identified by small RNA cloning and sequencing in Arabidopsis (Park et al., 2002). The non-coding microRNA (miRNA) gene, MIR172, is one of these genes; it was one of the earliest plant MIRNA genes to be identified by small RNA cloning and sequencing in Arabidopsis (Park et al., 2002). It generates a 21-nucleotide (nt) mature miR172 sequence that regulates the mRNA abundance and/or translation of the plant-specific transcription factor gene APETALA2 (AP2) (Park et al., 2002; Chen, 2004) and a small group of AP2-like genes, including TARGET OF EAT1 (TOEI), TOE2, TOE3, SCHLAFMUTZE (SMZ), and SCHNARCHZAPFEN (SNZ) in Arabidopsis (Aukerman and Sakai, 2003; Schmid et al., 2003; Schwab et al., 2005), which contain sequences complementary to miR172. AP2 is a floral organ identity gene (Bowman et al., 1991; Drews et al., 1991) while the five AP2-like genes mainly act as flowering repressors (Aukerman and Sakai, 2003; Jung et al., 2007; Mathieu et al., 2009). The subsequent identification of miR172 and its targets in many species (see Supplementary Table S1 at JXB online) has shown that miR172 has an ancient origin. It has been found in ferns, gymnosperms, and flowering plants, although not in lycopods and moss (Axtell and Bartel, 2005). Recent studies in maize, rice, and barley have shown that miR172 is also important in regulating phase transition and in the determination of floral organ identity in monocotyledons (Lauter et al., 2005; Chuck et al., 2007b; Zhu et al., 2009; Nair et al., 2010). These investigations have provided new insights into the functions of miR172 and its targets in both these processes and demonstrated that the interaction between miR172 and its AP2 or AP2-like targets is deeply conserved and has an ancient role in developmental regulation in plants.

Expression profile of miR172 and its regulation
miR172 arises from MIR172 loci that are transcribed by RNA polymerase II. The primary transcript of MIR172 or
pri-miR172, has a 5' cap and a 3' poly(A) tail (Xie et al., 2005) and is predicted to form a characteristic imperfect stem-loop hairpin structure that is processed twice by the \( R\)Nase III-like enzyme DICER-LIKE1 (DCL1) acting in concert with the double-stranded RNA binding protein HYPERASTIC LEAVES1 and the zinc finger protein SERRATE (SE) to release a 21-nt miR172/miR172* duplex with 2-nt 3' overhangs. A 15-nt stem segment proximal from the miR172/miR172* duplex appears to be essential for accurate processing of pri-miR172 (Mateos et al., 2010; Werner et al., 2010). In the majority of plant species investigated so far, more than one MIR172 locus has been identified; for example, Arabidopsis, maize, and rice have 5, 5, and 4 MIR172 loci, respectively (see Supplementary Table S1 and Supplementary Fig. S1 at JXB online). In Arabidopsis, the expression of the MIR172a, b, and c genes is elevated as plants reach the reproductive growth stage, whereas the expression of the MIR172d and e genes is very low and does not alter as the plant develops (Jung et al., 2007). In rice, MIR172c is expressed in seedlings but not in developing grains (Sunkar et al., 2008; Zhu et al., 2008). Thus the expression of different members of the MIR172 family and, consequently, their corresponding mature miRNAs, depends on growth stages and tissue types. In addition, the expression level of miR172 is also affected by day length and temperature (Jung et al., 2007; Lee et al., 2010).

In both dicotyledons and monocotyledons, the expression level of miR172 increases as plants grow and after flowering it accumulates in leaves and floral buds (Aukerman and Sakai, 2003; Lauter et al., 2005; Chuck et al., 2007b; Jung et al., 2007; Zhu et al., 2009). The expression pattern of miR172 is inversely correlated with that of miR156 (Fig. 1; Chuck et al., 2007a; Wu et al., 2009). Studies in Arabidopsis have identified a regulatory link between miR156 and miR172. miR156 acts upstream of miR172 and regulates miR172 expression through the miR156 target SQUAMOSA PROMOTER BINDING PROTEIN-LIKE9 (SPL9), which promotes the expression of miR172 by directly activating its transcription (Wu et al., 2009). In transgenic Arabidopsis plants that express a miR156-resistant SPL9 transcript under the control of its native promoter, the pri-miR172b level was consistently higher throughout shoot development. This is in keeping with the presence of multiple copies of SPL binding elements in the miR172b promoter, at least one of which is strongly bound by SPL9 based on a ChIP assay (Wu et al., 2009). In addition to SPL9, SPL10 and several other SPL genes, such as SPL11 and SPL15, probably act redundantly in the transcriptional regulation of miR172 (Wu et al., 2009). This SPL-mediated regulatory module allows the sequential action of miR156 and miR172, which contributes to the regulatory network that governs developmental timing (Wang et al., 2009; Wu et al., 2009). Furthermore, the expression levels of miR172 and the miR172b precursor (pre-miR172b) reduced ~50% in the toei toel double mutants and the level of pre-miR172b was slightly elevated in plants over-expressing the miR172 target TOEI (Wu et al., 2009), suggesting the existence of a possible feedback loop in which a miRNA-regulated transcription factor regulates the transcription of its cognate miRNA.

The flowering time of plants is affected by ambient temperature. In Arabidopsis, this is achieved by regulating the thermosensory pathway genes FVE, FCA, and SHORT VEGETATIVE PHASE (SVP) (Blazquez et al., 2003). The expression level of miR172 was up-regulated at 23 °C compared with 16 °C based on a recent microarray experiment (Lee et al., 2010). In the syp-32 mutant, expression of miR172a was increased at both 16 °C and 23 °C, and a significant reduction of the expression levels of the miR172 targets AP2, SMZ, SNZ, TOEI, and TOE2 was observed at 23 °C; reduced levels of TOEI and TOE2 were also observed at 16 °C (Lee et al., 2010). This is consistent with the early flowering time phenotype of the syp-32 mutant, suggesting a role of the interaction between SVP and miR172 in the control of flowering time in Arabidopsis, although over-expressing miR172 leads to Arabidopsis plants insensitive to temperature for flowering (Lee et al., 2010). Meanwhile, miR172 abundance is also influenced by the autonomous pathway genes in Arabidopsis (Jung et al., 2007). These results suggest that the expression level of miR172 is transcriptionally regulated by a network with multiple layers of controls.

Regulation of miR172 abundance by photoperiod is achieved through a clock-independent (or CONSTANS...
miR172 has been reported (Aukerman and Sakai, 2003; Kasschau et al., 2003; Schwab et al., 2005; Jung et al., 2007; Mathieu et al., 2009) but translational repression appears to be the major mode of action of miR172 (Aukerman and Sakai, 2003; Chen, 2004). miR172 was the first plant miRNA that was shown to regulate its targets mainly via translational repression. In the miR172b over-expressor, early activation tagged-Dominant (eat-D, an activation-tagged mutant), AP2 protein abundance was dramatically reduced, but the AP2 transcript accumulated to the normal level. TOE1 and TOE2 transcripts were also not reduced in eat-D (Aukerman and Sakai, 2003). Similarly, AP2 transcript abundance in hua enhancer1 (hen1) and dcl1 mutants

Dual modes of miR172-mediated gene regulation

miRNAs repress the expression of protein-coding genes through targeting their messenger RNAs (mRNAs) for cleavage or translational repression (Jones-Rhoades et al., 2006; Voinnet, 2009). The mode of regulation adopted by a particular miRNA generally depends on where its target site is located in the mRNA and the extent of sequence complementarity between the miRNA and the target site (Rhoades et al., 2002; Schwab et al., 2005). miRNAs with perfect or near perfect complementarity to their targets are predicted to cause RNA cleavage, whereas miRNAs that have a significant number of mismatches with their target sequence or that have target sites located in 3' UTR regions are predicted to cause translational repression. In plants, the majority of miRNAs have perfect or near perfect complementarity with their targets which are usually located in the coding region and thus their main mode of action is thought to be via mRNA cleavage. The high complementarity between miRNAs and their target sites allows relatively confident prediction of plant miRNA targets by bioinformatics approaches.

The miR172 target site is located in the coding region, but close to the 3' end, of a group of AP2-like transcription factors that contain two AP2 domains (Fig. 2; Kim et al., 2006; Shigyo et al., 2006). In Arabidopsis, cleavage of mRNAs of AP2, TOE1, TOE2, SMZ, and SNZ by
was similar to that in the wild type (WT), whereas AP2 protein amounts in these two mutants were ~2–3-fold higher (Chen, 2004). AP2 transcript accumulation in 35S:mIR172 plants was also similar to that in control plants, but AP2 protein was undetectable in 35S:mIR172 plants (Chen, 2004). mIR172 and TOE1 function through the photoperiod pathway to promote early flowering; mIR172 levels were changed in gi2 and photoreceptor mutants but the levels of TOE1 transcript were unaltered in these mutants (Jung et al., 2007). These results all indicate that mIR172 represses its targets at the level of translation. Recent evidence from Arabidopsis suggests that many plant miRNAs function at least partly by translational repression (Broderson et al., 2008).

In maize, using an antibody targeting the C-terminus of INDETERMINATE SPIKELET1 (IDS1), it was found that IDS1 was more abundant in tasselseed4 (ts4, a loss-of-function mutant of maize MIR172e, caused by transposon insertion) tassels than in WT during floral meristem initiation (Chuck et al., 2007b). However, unlike Arabidopsis where mIR172-mediated cleavage appears to be a minor mode of action, cleavage of mIR172 targets appears to be as important as mIR172-mediated translational repression. The expression of the maize GLOSSY15 (GL15) mRNA has an inverse relationship with that of mIR172 during the vegetative growth stage. GL15 transcripts rapidly decrease after the onset of mIR172 accumulation and mIR172-mediated cleavage was detected by 5’ RACE (Lauter et al., 2005). This mIR172-mediated degradation of GL15 seems to be sufficient for transition from the juvenile phase to the adult phase (Lauter et al., 2005). These results suggest that GL15 is targeted by mIR172 for mRNA degradation, although regulation of GL15 transcription cannot be ruled out, as changes in GL15 expression have not been analysed in mIR172 loss-of-function mutants. In barley, a synonymous nucleotide substitution at the mIR172 targeting site in Cleistogamy1 (Cly1) abolishes mIR172-mediated cleavage of Cly1 (Fig. 2) and results in the failure of flower (spikelet) opening when flowering, producing a cleistogamous phenotype (Nair et al., 2010).

Studies in Arabidopsis found that cleavage of AP2 and TOE1 transcripts were substantially increased in the mIR172 over-expressor but the steady-state levels of these two mRNAs did not show a commensurate decrease (Schwab et al., 2005). The accumulation of the SUPERNUMERARY BRACT (SNB) mRNA in rice plants over-expressing mIR172 (Zhu et al., 2009) was also observed. A possible explanation for these findings is that these mIR172 targets are under direct or indirect feedback regulation by their own products to increase transcription of the target genes when mIR172 is acting to reduce their protein levels. This was first shown by an elegantly designed experiment, in which the wild-type AP2 transcript was distinguished from the mIR172-resistant AP2 (mAP2) transcript by digestion with restriction enzyme (Schwab et al., 2005). Wild-type AP2 mRNA abundance was increased in 35S::AP2 plants as expected, but strongly decreased in plants over-expressing mAP2, which presumably over-produce AP2 protein as they are no longer targeted by mIR172 (Schwab et al., 2005). It was also proposed that the efficiency of the feedback regulation differs for different target genes, which could explain the different responses observed for different mIR172 targets (Schwab et al., 2005). These results suggest that the amount of mIR172 relative to its target transcripts, the degree of complementarity between mIR172 and its targets, and feedback regulation of gene expression each contribute to whether transcript degradation or translational repression appears to predominate.

### miR172 and developmental phase transition

Flowering plants undergo three primary post-embryonic developmental stages, juvenile, adult, and reproductive. The juvenile phase is characterized by a variety of morphological traits, such as leaf shape, leaf size, and epidermal cell pattern and characteristics. The adult and reproductive phases are marked by an increase in reproductive competence and production of flowers (Poethig, 2009). The transition from the adult to the reproductive phase is quite obvious and has been studied intensively, but less is known about the juvenile-to-adult transition. It is now apparent that mIR172 and miR156 play a crucial role in these phase transitions in plants (Fig. 1).

In Arabidopsis, miR156 is required for the expression of all juvenile leaf characteristics, and regulates the timing of the juvenile-to-adult transition by co-ordinating the expression of several pathways that control different aspects of this process (Wu and Poethig, 2006; Wu et al., 2009). miR156 achieves its role by repressing the expression of a group of SPL transcription factors that possess distinct functions necessary for vegetative phase change. In turn, miR172 is activated by SPL9, and possibly by SPL10 as well, to promote adult phase identity via the repression of its targets TOE1 and TOE2 (Wu et al., 2009). Expression of TOE1 and TOE2 is high in young seedlings and gradually decreases throughout the life cycle, which is inversely correlated with the expression of mIR172 (Aukerman and Sakai, 2003; Jung et al., 2007). Plants expressing 35S::mIR172a or 35S::mIR172b produce leaves with abaxial trichomes (an adult leaf trait) earlier than controls; similarly toel1 or toel2 single mutants display accelerated generation of leaves with abaxial trichomes; this acceleration is more obvious in the toel toel2 double mutants (Wu et al., 2009). By contrast, both the MIR172a knock-out mutant and the 35S::TOE1 plants show delayed production of abaxial trichomes (Wu et al., 2009).

In maize, GL15 is required for the expression of juvenile leaf epidermal cell traits (such as epicuticular waxes and lack of leaf hairs). The onset of mIR172 expression between 6 d and 12 d after sowing coincides with the period when the identity of the first leaf expressing adult traits is specified; indicating that increased mIR172 expression is associated with the transition to the adult vegetative phase. mIR172-mediated down-regulation of GL15 mRNA may play a role during this transition (Lauter et al., 2005).
miR156 and miR172 also have complementary expression patterns in maize (Chuck et al., 2007a), but whether this is mediated by SPL genes has not been established.

In addition to a role in the juvenile-to-adult transition, the miR156-SPL9-miR172-TOEI/TOE2 cascade also contributes to the transition from the adult to the reproductive phase in Arabidopsis as gain- and loss-of-function mutations in TOEI and TOE2 have been shown to affect flowering time (Aukerman and Sakai, 2003; Jung et al., 2007). The toe1 toe2 double mutant flowers earlier than either individual mutant, suggesting that these two genes are functionally redundant (Aukerman and Sakai, 2003; Jung et al., 2007), but it is not as early flowering as the miR172 over-expressor, suggesting other AP2-like genes also act as flowering repressors. More recently, SMZ and SNZ have been shown to act redundantly with TOEI and TOE2 as floral repressors with over-expression of SNZ or SMZ causing late flowering (Mathieu et al., 2009). However, the toe1 toe2 smz snz quadruple mutant still flowers significantly later than plants that constitutively express miR172 (Mathieu et al., 2009), supporting a previous finding that AP2 acts as a flowering repressor (Chen, 2004). This has been confirmed by the detection of direct binding of AP2 to key flowering time promoting genes, such as APETALA1 (API), FRUITFULL (FUL), and SUPPRESSOR OF OVER-EXPRESSION OF CONSTANS1 (SOC1) (Yant et al., 2010). The function of TOE3 remains to be uncovered as its expression profile is different from that of other AP2-like genes and over-expression of TOE3 does not delay flowering time (Jung et al., 2007).

miR172 over-expressing plants exhibit early flowering under both LDs and SDs (Jung et al., 2007). In miR172 over-expressers and toe1 mutants, FLOWERING LOCUS T (FT) and floral homeotic genes, such as LEAFY (LFY) and API, are up-regulated, whereas expression of CO, FLOWERING LOCUS C (FLC) and other genes functioning in the autonomous and GA pathways is unaltered. Furthermore, miR172 abundance is substantially reduced in the cryptochrome mutants and gi-2, but unaffected in co-2 and increased significantly in phy-B-9 and hy1 (Jung et al., 2007). These results indicate that miR172 exerts its role by regulating expression of FT through a genetic pathway other than the autonomous and GA pathways (Jung et al., 2007). Based on these results, a working model in which miR172 mediates light signals from GI and promotes flowering by inducing FT through TOEI but independent of CO has been proposed (Jung et al., 2007). More recently, a direct interaction between SMZ and FT chromatin has been demonstrated using the ChiP-chip approach (Mathieu et al., 2009). The binding site of SMZ is located approximately 1.5 kb downstream of the FT protein coding sequence. This is consistent with the observation that no FT transcription was detected in smz-D (a dominant late-flowering mutant) or 3S::SMZ plants, and that FT expression reached a higher level in the toe1 toe2 smz snz quadruple mutant (Mathieu et al., 2009). In addition to FT, other flowering time regulators, such as SOC1 and API, were also repressed by direct binding of SMZ and AP2 upstream of their transcription start site (Mathieu et al., 2009; Yant et al., 2010). Interestingly, significant SMZ and AP2 binding signal was found less than 2 kb upstream of the transcription start site of TOE3 (Mathieu et al., 2009; Yant et al., 2010), which suggests that TOE3 could also be a flowering time regulator and achieve its role in an SMZ and AP2 dependent manner. This may explain why the role of TOE3 in flowering time regulation was not identified previously. Furthermore, SNZ and AP2 are SMZ targets (Mathieu et al., 2009), and SMZ, TOEI, and SNZ are targets of AP2 (Yant et al., 2010). TOEI is not a target of SMZ but plants over-expressing SMZ show reduced TOE1 levels (Mathieu et al., 2009), suggesting expression of TOE1 is also regulated by SMZ. Meanwhile, both AP2 and SMZ seem to be self-regulated (Mathieu et al., 2009; Yant et al., 2010). These results strongly suggest a complex negative feedback regulatory mechanism among the miR172 targets. The role of miR172 in regulating flowering time in other plant species is not yet clear, although over-expressing miR172 promotes flowering in potato and over-expression of miR172 targets (GL15 or ZmTOE1) delays flowering time in maize (Lauter et al., 2005; Salvi et al., 2007). This complex regulation by miR172 and its targets being uncovered in Arabidopsis may inform further investigations in other species.

**miR172 and floral organ identity**

Arabidopsis flowers have four whorls of floral organs. From outer to inner, they are sepals (whorl 1), petals (whorl 2), stamens (whorl 3), and carpels (whorl 4). The identities of these floral organs are specified by three classes of regulatory genes, the A, B, and C class genes (Causier et al., 2010). AP2 is an A class gene; it specifies sepal identity in whorl 1, and together with B class genes APETALA3 (AP3) and PISTILLATA (PI), petal identity in whorl 2. The stamen identity in whorl 3 is specified by the B class genes together with the C class gene AGAMOUS (AG). AG determines carpel identity in whorl 4. AP2 and AG act antagonistically to restrict each other’s activities to their proper domains of action within the flower meristem to specify the identities of the perianth and reproductive organs (Bowman et al., 1991; Drews et al., 1991; Zhao et al., 2007). Loss of AP2 function converts sepals and petals into carpels due to the expansion of AG activity into the outer two whorls of the flower (Bowman et al., 1991). The first indication of miR172 playing a role in the determination of floral identity in Arabidopsis was the identification of the miR172 activation-tagging mutant eat-D, which not only flowers very early but shows floral defects that are virtually identical to those observed for strong ap2 mutant alleles (Bowman et al., 1991), including the complete absence of petals and the transformation of sepals to carpels (Aukerman and Sakai, 2003). The same or similar floral defects were observed in plants over-expressing other members of the miR172 family (Chen, 2004).
In situ hybridization detected strong miR172 signals in stage 1 floral primordia; the signal persisted in all four floral whorls until stage 7, and after that stage the miR172 signal appears to be concentrated in the inner two floral whorls. This expression profile of miR172 may cause the AP2 protein to accumulate in the outer two floral whorls to specify perianth identities (Chen, 2004). Meanwhile, expression of a miR172-resistant version of the AP2 cDNA (35S::AP2m3), but not the WT AP2 cDNA (35S::AP2), leads to dramatic floral defects, indicating that miR172 acts in the flower to repress AP2 (Chen, 2004). The more precise roles of miR172-mediated repression of AP2 in floral patterning were revealed by over-expressing AP2 under the control of its own promoter (Zhao et al., 2007). It was discovered previously that AP2 promotes the expression domain of the B class genes, AP3 and PI, by antagonizing AG (Jack et al., 1992). It is now clear that the function of AP2 is restricted by miR172 to define the inner boundary of the expression domain of the B class genes to ensure proper floral patterning (Zhao et al., 2007). Furthermore, miR172-mediated repression of AP2 is crucial for maintaining floral meristem size and for floral determinacy, which is achieved through both AG-dependent and AG-independent pathways (Zhao et al., 2007).

Ectopic expression of 35S::AP2m3 in N. benthamiana induces floral patterning defects and the severity of the defects correlates with the AP2 mRNA and protein levels. Meanwhile, ectopic expression of Arabidopsis miR172 in N. benthamiana also causes early flowering as in Arabidopsis, indicating that miR172-mediated repression of AP2 in dicotyledons is conserved (Mlotshwa et al., 2006).

miR172-mediated regulation of AP2-like genes in the establishment of proper floral patterns is also important in monocotyledons. Maize has two distinct types of inflorescences. The male inflorescence, the tassel that has long branches, is apical, whereas the female inflorescence, the ear that does not have long branches, is formed at the axils of leaves on shortened lateral shoots. The inflorescence meristems (IMs) of the tassel and the ear each produce spikelet pair meristems (SPMs). Each SPM bears two spikelet meristems (SMs) that, in turn, produce a pair of floral meristems (FMs). Several tasselseed mutants that show partial to total sex reversal have been described in maize. ts4 mutants have a highly branched male inflorescence, in which pistils fail to abort and male floral organs do not develop, resulting in complete feminization of the male flowers. ts4 SPMs produce several SMs rather than two; the SM of ts4 is also indeterminate and produces multiple florets in a random pattern (Chuck et al., 2007b). Database searches and genetic analysis found that IDS1 is the major target of ts4 as the loss-of-function mutation of IDS1 suppresses the sex determination and branching defects in the ts4 mutants and the IDS1 protein expression domain is expanded in the ts4 mutants compared to that in WT (Chuck et al., 2007b). Furthermore, Ts6, a dominant tasselseed mutant caused by a G-to-A transition mutation within the miR172 targeting site, is an allele of IDS1 (Fig. 2) and phenocopies the mutant phenotypes of the ts4 mutants (Chuck et al., 2007b). Loss-of-function mutants of IDS1 delay the timely conversion of the spikelet meristem to the floral meristem and initiate extra florets instead of two (Chuck et al., 1998, 2007b). Based on analysis of spatial expression patterns of IDS1 transcript and protein in combination with the morphological changes in the ts4 and ids1 mutants, it appears that miR172 acts to define the determinacy of SPMs and SMs by restricting IDS1 protein activity at the base of the spikelet meristem. The formation of extra florets because of expansion of IDS1 expression in the ts4 mutant indicates that IDS1 functions in promoting the transition from SMs to FMs (Chuck et al., 2007b).

The ids1 mutant does not completely suppress the ts4 mutant phenotype (the ear of ids1 ts4 double mutants resembles that of ts4 single mutants), suggesting there is another miR172 target that contributes to the residual phenotypes observed in ids1 ts4 double mutants (Chuck et al., 2007b). This candidate miR172 target was later found to be SISTER OF INDETERMINATE SPIKELET1 (SID1) as the sid1 mutation enhances suppression of the ts4 mutant phenotype by the ids1 mutation (Chuck et al., 2008). The SID1 single mutant does not show an obvious phenotype and SID1 functions redundantly with IDS1 to specify the fate of several lateral meristems in the inflorescence (Chuck et al., 2008). This function of IDS1 and SID1 could be achieved by restricting expression of maize AG-like MADS-box transcription factors within the lateral organs of the spikelet, similar to the relationship between AP2 and AG in Arabidopsis (Bowman et al., 1991; Drews et al., 1991; Chuck et al., 2008).

Rice SNB is closely related to maize SID1 and IDS1 (Fig. 3). Loss-of-function mutants of SNB delayed the transition from spikelet to floral meristem and produced extra glumes before initiating florets (Lee et al., 2007), consistent with the loss-of-function mutant phenotypes of IDS1 (Chuck et al., 1998). In addition, in some spikelets of the snb mutants, the empty glumes and lodicules were transformed into lemma/palea-like organs (Lee et al., 2007). Transgenic rice plants over-expressing miR172b phenocopied the T-DNA insertion mutant of SNB: the severity of the mutant phenotypes observed in the miR172b over-expressers correlated with the expression level of miR172; furthermore, miR172-mediated cleavage of SNB mRNA was detected in panicles (Zhu et al., 2009). These results provide evidence that miR172-mediated regulation of SNB is important for the development of rice spikelets and florets. In addition to the mutant phenotypes of the snb mutant, the miR172b over-expressers displayed additional floral defects, suggesting that at least one other AP2-like target gene is required for correct floral development (Zhu et al., 2009). Based on phylogenetic analysis (Fig. 3) and expression profiles (Zhu et al., 2009), a possible candidate is Os03g60430. Based on in situ hybridization, SNB is initially expressed in the branch meristem and spikelet meristem, and is then primarily restricted to the boundary region of the spikelet and glume primordia. Once the spikelet meristem is converted into a floret meristem, a decreased expression of SNB is observed (Lee et al., 2007). Both miR172 and SNB were highly expressed in <1 cm long
amino acid substitutions per residue.

bootstrap probabilities are shown and the scale bar represents 0.1

MEGA version 4 using full-length protein sequences. Local

tree and molecular evolutionary analyses were performed by

rice (Os), maize (Zm), barley (Hv), and wheat (Ta). The phylogenetic

panicles (Zhu et al., 2009); therefore miR172 could act to

restrict the expression domain of SNB during floret de-

development in the same manner that maize miR172 restricts

the expression domain of IDS1 (Chuck et al., 2007b).

Lodicules are grass family specific floral organs, which
develop at the base of the carpel and stamens, and are

considered to be homologous to petals in eudicots (Bommert

et al., 2005). They swell when flowering to open the floret.

A recent study in barley has shown that a single nucleotide

substitution in the miR172 binding site in Cly1 results in

smaller lodicules and cleistogamous (or closed) flowering

(Nair et al., 2010). In transgenic rice plants over-expressing

miR172b, an enlarged and/or increased number of lodicules

was frequently observed and, in most cases, lodicules were

elongated and transformed into lemma/palea-like organs;

consequently, these spikelets were unable to close after

flowering and resulted in naked grains (Zhu et al., 2009). It

is hypothesized that this might be a result of increased

suppression of expression of Os04g55560, the most closely

related of the five rice miR172 targets to Cly1 (Fig. 3). These

results suggest that miR172-mediated cleavage of Cly1 and

Os04g55560 (the most frequently cleaved

among the five miR172 targets in WT rice plants; Zhu

et al., 2009) is essential for normal flower opening in barley

and rice. Both non-cleavage and over-cleavage results in

defects in development of the lodicules; therefore, normal

non-cleistogamy, an ancestral and dominant character in

barley (perhaps in all grass species), seems to be main-
tained by a balance of miR172-mediated repression. How

this balance is achieved warrants further investigation. In

addition, the wheat domestication gene Q, which confers

reduced plant height, a free-threshing character and spike

compactness to cultivated wheat, is also a miR172 target

although it is not clear whether miR172-mediated regulation

had a role in the domestication course of the gene

(Simons et al., 2006).

**Does miR172 act as a long-distance signal?**

There is increasing evidence that small interfering RNAs

can act as mobile silencing signals (Dunoyer et al., 2010;

Molnar et al., 2010). In most cases miRNAs are thought to

act in a cell autonomous manner; however, miR399 has

been shown to be a long-distance signal involved in the

regulation of plant phosphate homeostasis (Pant et al.,

2008) which suggests there may be circumstances in which

miRNAs act as mobile signals. In potatoes, miR172

expression increases under tuber-inducing short days and is

present in vascular bundles and phloem (Martin et al.,

2009). Over-expression of miR172 promotes tuberization,

an effect which is graft transmissible from scion to stock.

This raises the possibility that miR172 can act as a mobile

signal in this system, although to date this has not been

directly demonstrated.

**Conclusion**

miR172 is one of the more ancient miRNAs in plants and

regulates a small group of AP2 domain-containing plant-
specific transcription factors whose functions are broadly

conserved although with differing morphological outcomes

in different species (Fig. 1). The detection of miR172 in the

majority of plants investigated and the demonstrated regula-
tion of predicted target miRNAs indicates that the miR172-

AP2 family interaction has been retained throughout plant

evolution. This suggests that the regulation of the AP2-
domain genes by miR172 has long been a critical component

of plant development. The functions of miR172 targets in

*Arabidopsis* have been characterized in detail using knockout

mutants or transgenic plants over-expressing miR172, pro-

viding clues for the functional analysis of miR172 targets in

other species, in particular rice, maize, barley, and wheat,

where *AP2*-like targets of miR172 are likely to play an

important role in reproductive development and the forma-
tion of floral organs. Understanding the role of miR172 and

its targets in these species could provide new ways for

manipulating reproductive development to improve crop

productivity. This is exemplified by the recent demonstration

that alteration of the miR156 regulation of *SPL14* expression

can be used to change plant architecture and improve grain

yield in rice (Jiao et al., 2010; Miura et al., 2010).

**Supplementary data**

Supplementary data are available at *JXB* online.

**Supplementary Table S1.** miR172 family and its AP2-
domain containing targets in plants.
Supplementary Fig. S1. Alignment of published miR172 sequences in plants.

References


