In plants, conserved microRNAs (miRNAs) tend to be encoded by gene families with multiple members. Two recent studies interrogated the functions of the 5-member MIR172 family in Arabidopsis and revealed complexities and intricacies of gene regulatory networks underlying floral transition.

MicroRNAs (miRNAs) are 20- to 24-nucleotide long, noncoding RNAs that regulate target genes at posttranscriptional levels [1]. Plants maximize their fitness by employing miRNAs to regulate various developmental processes, particularly in coordination with environmental cues [1]. Plant miRNAs are especially suited as developmental regulators as they, through a high degree of sequence complementarity, target a small number of genes (usually belonging to a gene family) at the nodes of gene regulatory networks. In addition, their own accumulation is subjected to spatial and temporal regulation through MIR gene promoters. MIR genes undergo dynamic birth and loss during evolution, such that only tens of miRNAs are conserved in seed plants [2,3]. Owing to whole genome, segmental, and gene duplication events in land plant evolution, conserved miRNAs tend to belong to gene families, in which identical or nearly identical mature miRNA sequences are encoded by different MIR genes [4]. Partial redundancy together with subfunctionalization and/or neofunctionalization among MIR family members potentially enhances regulatory dimensions and robustness.

Do MIR gene family members exhibit different spatiotemporal patterns in expression? Do they integrate different environmental signals to impact developmental processes? Do they act on the same or different sets of targets? These questions are still largely unknown for most MIR gene families in plants. Two recent studies [5,6] provided insights into some of these questions by interrogating the Arabidopsis 5-member MIR172 family, a miRNA family conserved in spermatophytes [3].

Several approaches have been routinely used to determine the biological functions of miRNAs, but none can readily reveal the functions of individual MIR gene family members. First, individual MIR genes can be over/ectopically expressed, and the phenotypic consequences are used to infer the biological functions of the miRNA. However, the approach itself alters the natural patterns of MIR gene expression and thus cannot confidently capture the endogenous functions of MIR genes. Second, expression of a miRNA-resistant target using its own promoter is a powerful approach to uncover the consequences when a particular target gene is released from miRNA regulation. However, this only reflects partial functions of a miRNA, as other targets are still being repressed by the miRNA. A third useful approach is target mimicry...
—the expression of an RNA with a non-cleavable miRNA target site [7]. The target mimic RNA leads to the degradation of the cognate miRNA or serves as a sponge to prevent the miRNA from accessing its natural targets. However, none of the approaches above can separate the regulatory contributions of individual MIR gene members. Finally, loss-of-function approaches can be used to study MIR genes as for protein-coding genes by taking advantage of the existing transfer DNA (T-DNA) mutants. Given the small size of precursor miRNAs (approximately 200 nucleotides), T-DNA insertion mutants in all MIR gene family members may not be readily available. Recently, gene editing technology based on clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 enables the construction of loss-of-function mutants for MIR genes and provides an opportunity to systematically interrogate the functions of individual members of MIR gene families.

Flowering at an appropriate time is critical for plants’ reproductive success and is thus regulated by sophisticated gene networks that monitor and respond to environmental changes and endogenous cues. In Arabidopsis, miR172 targets 6 members of the APETALA2 (AP2)-like family of transcription factor genes and plays critical roles in flowering time control [8,9]. However, little was known about how the presence of 5 MIR172 genes influences the gene regulatory networks underlying flowering. In the 2 studies published recently, the authors generated loss-of-function mutants in each of the 5 MIR172 genes and analyzed their developmental and particularly flowering time phenotypes. Moreover, they generated different combinations of higher-order mir172 mutants and further explored functional redundancy and/or specificity among the 5 genes. These studies, together with the use of reporter genes to monitor the expression patterns of each gene, demonstrate that the Arabidopsis MIR172 gene family members have divergent and common functions in integrating various endogenous and exogenous cues to execute flowering [5,6].

Upon floral induction, the shoot apical meristem (SAM) mainly produces flowers instead of leaves. Plants regulate flowering time via integration of environmental signals with endogenous cues. Genetic pathways that regulate flowering by responding to both environmental changes, such as seasonal changes in day length (photoperiod), and endogenous developmental information, such as plant age, have been elucidated [10]. These pathways converge on the floral integrators FLOWERING LOCUS T (FT) and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1), whose induction leads to flowering [10].

The photoperiod pathway (Fig 1) senses day length in leaves through a signaling cascade involving GIGANTEA (GI), FLAVIN-BINDING KELCH REPEAT F-BOX 1 (FKF1) and the transcription factor CONSTANS (CO) [10]. CO promotes flowering by activating the transcription of FT in the leaf vasculature [10]. AP2-like proteins harboring a transcriptional repression Ethylene-responsive element binding-factor-associated Amphiphilic Repression (EAR)-like motif interact with CO and repress FT expression [11], and this interaction was recently shown to be modulated by the blue light receptor CRYPTOCHROME2 (CRY2) [12]. The FT protein moves through the phloem to the SAM where it activates the floral developmental program. The 2 recent studies on the MIR172 family [5,6] demonstrated that MIR172A and MIR172B play major roles in promoting flowering under long days (LD) (Fig 1). Consistently, both genes are expressed in the leaf vasculature under LD.

Under short days (SD), the age pathway (Fig 1) plays a major role in flowering. Expression of the SQUAMOSA PROMOTER BINDING LIKE (SPL) family genes, which promote flowering, increases as plants age [10,13]. SPL genes are the targets of miR156, whose levels are high in young plants and progressively decrease as plants develop [10,13]. The levels of miR172 mirror those of miR156 as MIR172 genes are activated by SPL genes [13]. Under SD, the repression of AP2-like genes by miR172 induces the expression of SOC1, as well as FRUITFUL (FUL) and APETALA1, in the SAM to promote flowering. MIR172D functions as the major MIR172...
family member in the SAM to promote flowering under SD, with MIR172A and MIR172B playing a minor role (Fig 1). Consistently, MIR172D is highly expressed in the SAM.

The studies also provided insights into the intricacies of the gene regulatory networks underlying flowering (Fig 1). In leaf vasculature, the promoter activities of MIR172B and C, but not those of MIR172A, were reduced in an spl9 mutant. Thus, MIR172 members respond differently to this upstream activator. In the SAM, it was shown previously that miR172 levels are greatly reduced in an spl15 mutant [14]. Consistently, O’Maoiiléidigh and colleagues showed that the promoter activities of MIR172A-D were all increased in plants expressing miR156-resistant SPL15, suggesting that SPL15 activates these 4 genes [5]. Intriguingly, the late flowering phenotype of an spl15 mutant is strongly enhanced by mutations in either MIR172A or MIR172B, suggesting that another gene(s) activates the expression of these MIR172 genes in the absence of SPL15. In addition, the relationship between MIR172 and FUL, which repress the same AP2-like genes at the posttranscriptional and transcriptional levels, respectively, was also studied. Under both LD and SD conditions, MIR172 genes and FUL appear to act in parallel to promote the expression of the floral integrators. The work also begins the analysis of the effects of MIR172 genes on AP2 expression [5], but the regulatory relationships among the 5 MIR172 genes and the 6 AP2-like target genes remain largely unexplored. With mutants in each MIR172 family member, it is now possible to discern how MIR172 genes contribute individually or collectively to target gene repression. In summary, the 2 studies demonstrate that the redundancy and specificity of MIR172 family members increase the robustness and dimensions of the gene regulatory networks underlying flowering.

References


