

Jack of Many Trades: The Multifaceted Role of miR528 in Monocots

MicroRNAs (miRNAs) are essential regulators, involved in almost all aspects of plant growth and development. In plants, miRNAs present in all angiosperms are regarded as conserved miRNAs; in contrast, miRNAs restricted to certain lineages (less-conserved) or a single species (species-specific) constitute the non-conserved miRNAs (Cuperus et al., 2011). Different members of a miRNA family usually target similar target genes from a gene family among different species. For instance, in most analyzed plants, the well-known miR156 family, usually consisting of a number of members in a given species, collectively target *SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE (SPL)* genes. Generally, conserved miRNAs target genes encoding transcript factors which function in diverse biological processes. This functional diversity of miRNAs is mainly achieved by the plasticity of their target genes from the same family, such as miR156-targeted *SPLs* and miR167-targeted *ARF (AUXIN RESPONSIVE FACTOR)* genes, on regulating distinct downstream genes.

miR528 was first identified in rice and later found to be restricted in monocots (Liu et al., 2005). In this issue, two articles have put a spotlight on miR528 in rice. Yao et al. (2019) uncovered the transcriptional regulation of miR528 by OsSPL9 in antiviral response in rice. In combination with previous studies from the same group, miR528, upon viral infection, prefers to associate with cleavage-defective Argonaute 18 (AGO18) protein instead of the canonical partner AGO1, thereby releasing its repression on its target gene encoding L-ascorbate oxidase (also referred to as L-ascorbic acid oxidase [AAO]); increased AAO activity leads to accumulation of higher basal reactive oxygen species and enhances antiviral defense (Wu et al., 2015, 2017). The other study (Yang et al., 2019) reported a novel role of miR528 in the regulation of flowering time in rice. miR528 was demonstrated to target a zinc-finger transcription factor gene, *RED AND FAR-RED INSENSITIVE 2 (OsRFI2)*, to promote heading under long-day conditions. The miR528 accumulation was fine-tuned by not only binding of OsSPL9 but also alternative splicing and alternative polyadenylation events of the primary *MIR528* transcript (Yang et al., 2019). Intriguingly, natural variation in the *MIR528* promoter, which is associated with a different number of OsSPL binding elements, likely contributes to the adaption of rice to growth at different latitudes, corresponding to different photoperiod rhythms (Yang et al., 2019).

In addition to these roles, miR528 also plays important roles in several other biological processes. It likely affects pollen development in rice (Personal communication with Prof. Yueqin Chen). In creeping bentgrass, overexpressing osa-miR528 alters plant development and enhances resistance to salt stress and N starvation by regulating both *AsAAO* and *COPPER ION BINDING PROTEIN1 (AsCBP1)* genes (Yuan et al., 2015). In contrast, miR528 affects lodging resistance of maize by targeting two copper-containing laccase genes, *ZmLACCASE3 (ZmLAC3)* and

ZmLACCASE5 (ZmLAC5), which are involved in lignin biosynthesis under nitrogen-luxury conditions (Sun et al., 2018). In banana, miR528 is an essential regulator in the response to cold stress of banana fruits (generally known as peel browning), via targeting of genes encoding polyphenol oxidases (PPO) (Zhu et al., 2019).

In summary (Figure 1), miR528, unlike most miRNAs, has evolved distinct target genes that are involved in various developmental processes or biotic and abiotic stress conditions. Indeed, besides these validated target genes, miR528 has many other potential target genes; for example, there are as many as 11 predicted target genes of miR528 belonging to nine different classes in rice (Zhou et al., 2010; Yuan et al., 2015). Our comprehensive target gene profiling of miR528 in more than 20 monocot genomes confirmed the great diversity of miR528 target genes (unpublished data). Interestingly, miR528 predominantly targets a large collection of genes encoding copper-containing proteins. In addition to those that are validated (AAO, CBP, LAC, PPO), targets include many other genes encoding monocopper proteins or multicopper oxidases. All these data uncover the multifaceted role of miR528, like a performer of the ancient Chinese dramatic art “Bian Lian”, who can change vividly colored marks instantly (Figure 1).

Due to its important roles in stress responses, miR528 is previously classified as a member of copper miRNAs, which are usually induced by copper deficiency and direct the post-transcriptional regulation of transcripts that encode copper-containing proteins (Pilon, 2017). Almost every monocot, except maize, has only one *MIR528* locus, although several whole-genome duplication events have happened after the split of monocots from their common ancestor with eudicots. Not only the miR528 sequence but also the hairpin structure of miR528 precursor are extremely conserved among different species, ensuring the greatly consistent processing of miR528. Therefore, the miR528 precursor sequence was commonly used as a backbone for the development of stable processing systems of artificial miRNAs (Warthmann et al., 2008).

How does a single miRNA with almost identical sequence implement such a broad and diverse range of target capacity? One possible interpretation could be the rapid diversification rate of copper-containing proteins, which are significantly diverse due to the various compositions of different copper centers. Consistent with this is the fact that miR528 has evolved a distinct preference of target genes in different monocots. Another possibility might be the quick loss-and-gain of miR528 target sites during evolution, as demonstrated by the fact that miR528 target sites are always out of the functional domain region, and not at a

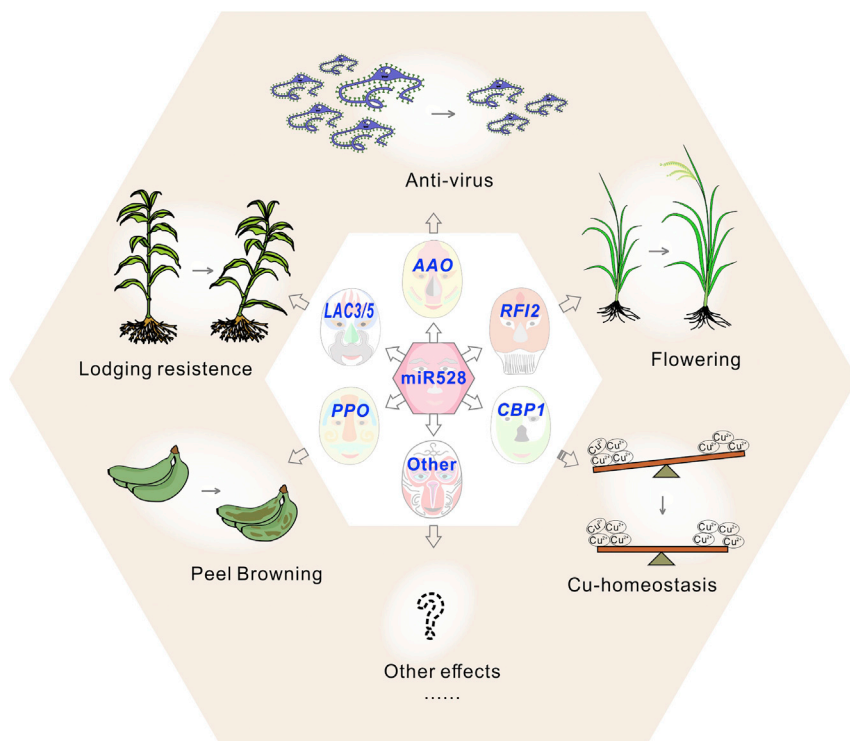


Figure 1. Multifaceted Roles of miR528 in Monocots.

Different cartoon masks, analogous to those used in the ancient Chinese dramatic art “Bian Lian”, denote the functional diversity of miR528. The function of CBP1 is inferred rather than experimentally demonstrated, as indicated with a different arrow.

consistent position, even for target genes from the same gene family. Therefore, there are some genes unrelated to copper-containing proteins that emerge as targets of miR528, such as *OsRFI2*. Another question is how the diverse functions of miR528 are orchestrated with the growth and development of a monocot plant. Perhaps, its proper function is accomplished by the coordinated expression of miR528 and its target genes via intertwined feedback loops (Pilon, 2017). In this regulatory network, miR528 is under the regulation of SPL proteins, which is responsive to copper availability, whereas the copper balance is modulated by copper-containing proteins, which are target genes of miR528. Noteworthy, despite miR156 consistently targets a series of SPLs in rice, *OsSPL9* is not its target gene (Xie et al., 2006; Yao et al., 2019).

Taken together, miR528 is an exceptional miRNA in plants, given its broad target capacity and distinct target preference among species. How this monocot-specific regulatory network of miR528 evolved and how each component of the network is coordinatively regulated remain open questions, worthy of further investigation.

FUNDING

This work was funded by the National Key Research and Developmental Program of China (no. 2018YFD1000104) and the National Natural Science Foundation of China (no. 31872063). This work was also supported by the Innovation Team Project of the Department of Education of Guangdong Province (no. 2016KCXTD 011), the Guangzhou Science and Technology Key Project (no. 201804020063).

ACKNOWLEDGMENTS

No conflict of interest declared. We are grateful to Prof. Yueqin Chen at Sun Yat-Sen University for sharing their data.

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<https://doi.org/10.1016/j.molp.2019.06.007>

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