IMPRS CHEMICAL COMMUNICATION IN ECOLOGICAL SYSTEMS

Project 9

A miRNA taming floral homeotic genes

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Background:
The floral structure significantly influences the interaction of angiosperms with their environment, not least because it defines the set of species by which plants are pollinated. The genetic basis of how floral organ identities develop has, to a great extent, been elucidated: Mainly three clases of floral homeotic genes, termed A-, B- and C-class genes, determine in a combinatorial fashion which organs are formed where in a flower [1, 2]. According to the so called ABC-model of flower development, expression of only A-class genes leads to the development of sepals, expression of A- and B-class genes together leads to the formation of petals, B- and C-class genes expressed together determine stamens and the expression of C-class genes alone gives rise to carpels. All of the ABC genes encode transcription factors. However, also genes encoding for microRNAs (miRNAs) have been shown to be of major importance for development [for a review, see ref. 3]. ABC genes and miRNAs may even act together. One miRNA, miR5179, has been found to regulate members of one clade of B-class genes, the DEF-like genes in orchids [4]. This miRNA is quite remarkable. While genes encoding miRNAs (miR genes) have usually high birth and death rates and hence exist only for a short while on evolutionary time scales, very few acquired important developmental functions and hence were conserved in a broad range of taxa for hundreds of millions of years. However, miR5179 does not fit either pattern. Analyses of genome, transcriptome and miRNome data in our lab revealed that miR5179 likely originated in the stem group of flowering plants about 200 million years ago and was conserved in several plant lineages. It hence turns up in a number of extant species, like *Actinidia eriantha* (kiwifruit), *Citrus sinensis* (orange), *Musa accuminata* (banana) and *Oryza sativa* (rice), indicating an important function of miR5179. In contrast, however, miR5179 has been lost independently in many other lineages of flowering plants, like in the orders Vitales, Malvales and Pandanales showing that miR5179 was dispensable in these cases. So miR5179 provides a fascinating conundrum: it is ancient, but not universally conserved. Why is it functionally important in some plants, but dispensable in others?

By specifying stamen identity, DEF-like genes have an indispensable function in all flowering plants. Since miR5179 seems to control DEF-like genes, this raises the intriguing question: why is the evolutionary dynamic of the miR5179 gene so different from that of the

ultraconserved DEF-like target gene? In other words: what is the ultimate function of miR5179 in those species in which it still exists? Answering this question may require the study of a miR5179 knockout mutants. Such mutants have not been reported yet, however.

Project description:
We hypothesize that miR5179 functions in the restriction of the expression of members of one clade of B-class genes. This restriction may be necessary to prevent the development of aberrant floral structures, which would compromise the interaction of the respective flowering plants with their environment, especially pollinators. The species in which miR5179 was lost may have evolved other mechanisms to control the expression of B-class genes. To determine the function of miR5179 we will generate mutants of this miRNA. As many of the species in which miR5179 is conserved are not genetically tractable, we will focus our quest on the grass species *Brachypodium distachyon* and *Oryza sativa* (rice). We will use the CRISPR-Cas9 system according to the latest recommendations for the generation of miRNA mutants [5]. Towards this goal, we will cooperate with the group of Jochen Kumlehn at the IPK in Gatersleben, in which gene editing using CRISPR-Cas9 as well as transformation of grasses is well established [6]. The phenotypes of the produced mutants will be carefully characterized with special focus on floral structure and compared to the phenotypes of the wild-type plants. Moreover, we will closely monitor the expression of miR5179 and its target genes in floral buds and flowers of wild-type and mutant plants using qRT-PCR and *in-situ* hybridization, methods that have been well established in our lab [7]. The results of these experiments will help to elucidate as to whether miR5179 functions to tame B-class floral homeotic genes or may have another unexpected function.

Candidate profile:
We are looking for a candidate with proven skills in molecular biology and a strong interest in plant ecology, development, and evolution. The project involves cooperation with the IPK in Gatersleben, hence the ideal candidate has strong communication skills and the ability to cooperate with researchers with different backgrounds (bioinformatics, developmental biology, chemical ecology, evolution). Good time management and organizational skills as well as proficiency in written and spoken English are essential. The candidate should be keen on learning and applying several and diverse state-of-the-art techniques.

Reading: (*from our own lab)

1. Bowman JL, Smyth DR, Meyerowitz EM. (2012) The ABC model of flower development: then and now. *Development*. 139(22):4095-8.
2. Theißen G, Melzer, R, Rümpler F (2016) MADS-domain transcription factors and the floral quartet model of flower development: linking plant development and evolution. *Development.* 143:3259-3271.*
3. Gramzow L, Theißen G (2019) Plant miRNA Conservation and Evolution. In: de Folter S. (eds) Plant MicroRNAs. Methods in Molecular Biology, vol 1932. Humana Press, New York, NY, p. 41-50.*
4. Aceto S, Sica M, De Paolo S, D'Argenio V, Cantiello P, Salvatore F, Gaudio L. (2014) The analysis of the inflorescence miRNome of the orchid Orchis italica reveals a DEF-like MADS-box gene as a new miRNA target. *PLoS One*. 9(5):e97839.
5. Deng F, Zeng F, Shen Q, Abbas A, Cheng J, Jiang W, Chen G, Shah AN, Holford P, Tanveer M, Zhang D, Chen ZH (2022). Molecular evolution and functional modification of plant miRNAs with CRISPR. *Trends in plant science.* 27(9), 890-907.
6. Kumlehn J, Pietralla J, Hensel G, Pacher M, Puchta H. (2018) The CRISPR/Cas revolution continues: From efficient gene editing for crop breeding to plant synthetic biology. *J Integr Plant Biol*. 60(12):1127-1153.

7. Yang X, Wu F, Lin X, Du X, Chong K, Gramzow L, Schilling S, Becker A, Theißen G, Meng Z. (2012) Live and let die - the B(sister) MADS-box gene OsMADS29 controls the degeneration of cells in maternal tissues during seed development of rice (Oryza sativa). *PLoS One*. 7(12):e51435.