**IN BRIEF**

# CKI1-ARRs specify the central cell in Arabidopsis

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Like in animals, sexual reproduction in plants involves the fusion of male and female gametes to produce a diploid zygote that will develop into a new multicellular organism. However, a key innovation in flowering plants relies on the double fertilization process, in which a second sperm cell fuses with a homodiploid female gamete (i.e., the central cell) to generate a triploid tissue, called the endosperm, which will nourish the developing embryo within a small seed. In addition to the central cell, an egg cell is the other female haploid gamete that is fertilized by the other sperm cell to form the zygote. Prior to fertilization, the mature female gametophyte of several angiosperms also contains two synergid cells (responsible for pollen tube attraction) at the micropylar end, and three antipodal cells (important for the nutrition of the egg cell) at the chalazal end (**Li and Yang, 2020).** The establishment and maintenance of the correct identity of each of these four cell types in the female gametophyte ensure that double fertilization successfully proceeds to produce a fertile offspring. Nevertheless, the complete regulatory pathways that determine the identity of two female gametes are still to be defined, even in the model species Arabidopsis.

In this issue, **Mingsong Zhu and colleagues** (**Zhu et al., 2022**) deciphered the molecular mechanism acting downstream of CYTOKININ INSENSITIVE1 (CKI1), a histidine kinase previously implicated in the specification of the central cell through activation of the cytokinin signaling pathway (**Yuan et al., 2016**). The authors first analyzed a group of MYB transcription factors that generally mediate the cytokinin signaling pathway, the type-B ARABIDOPSIS RESPONSE REGULATORS (ARRs), and selected ARR10/ARR12/ARR18 based on their high expression in the ovary, and specific localization in the chalazal pole of the developing embryo sac.

The functional characterization of T-DNA insertion mutants indicated that the three ARRs redundantly regulate the development of the female gametophyte. While single and double mutants showed normal (double) fertilization and seed development, the triple mutant produced a degenerated embryo sac that caused fertility defects, phenocopying *cki1-9* plants. In both mutants, early stages of ovule development appeared normal, but the central cell and antipodal cells lost their identities. Similar results were observed in genome-edited plants in which *ARR18* and *CKI1* were knocked out using CRISPR-Cas9 technology in *arr10 arr12* and wild-type, respectively, suggesting that these factors might act together to specify cell fates in the female reproductive organs.

The authors elegantly visualized changes in cell identities by monitoring female gametophyte molecular markers in different genetic backgrounds. Remarkably, multiple *arr* mutants showed ectopic expression of egg cell-specific marker genes (**Figure 1**), pointing at a

function of ARR10/12/18 in the specification of the identities of the central cell and antipodal cells.

Zhu and coworkers also performed extensive genetic analyses to investigate the relationship between ARRs, CKI1, and three Arabidopsis histidine phosphotransfer proteins (AHP2/3/5), essential for proper formation of female reproductive structure (**Liu et al., 2017**). Higher order mutants showed very high seed abortion rate due to defective female gametophyte in 75% of ovules dissected from *arr10-5 arr12-1 arr18-2/+ cki1-9/+* and *arr10-5 arr12-1 arr18-2/+ ahp2-2 ahp3 ahp5-2/+* plants. These results implied that ARRs, together with CKI1 and AHP2/3/5, play key roles in female gametophyte development. Furthermore, the authors identified genes co-regulated by CKI1 and ARRs through transcriptomics profiling of wild-type and multiple mutant ovules. Differential gene expression analysis revealed an enrichment of categories related to signal transduction, and specifically, downregulation of gene families encoding signaling molecules such as the *LOW-MOLECULAR-WEIGHT CYSTEINE-RICH* (*LCR*) genes.

In summary, novel findings reported in this article shed new light on the critical components of the signaling pathway that specify cell fates during female gametophyte development, essential to guarantee the fertility of the offspring. Specifically, ARR10/12/18 act downstream of CKI1 to establish or maintain the polarity of the female gametophyte. How these proteins are polarly localized remains to be defined. Also, factors acting upstream and downstream of CKI1/ARRs are yet to be resolved. Nevertheless, small peptides represent promising candidates for future research as they may mediate communications among the different cell types of the female gametophyte.

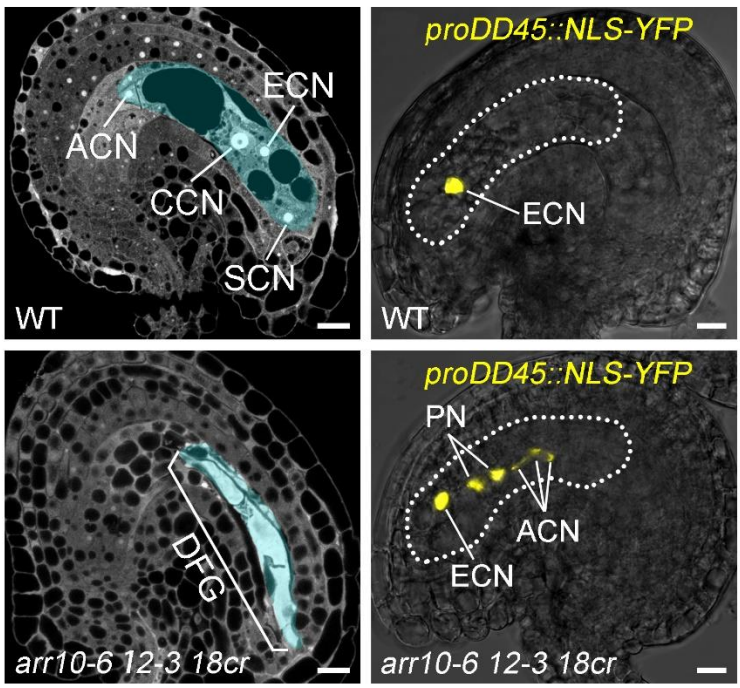

**Figure 1. *arr10-6 arr12-3 arr18-3* mutant lost its central cell identity**
Left, confocal laser scanning microscopy images showing defective female gametophyte at maturity of *arr10-6 12-3 18-3* mutant compared to wild-type. Right, ectopic expression of an important egg cell marker in the central cell and antipodal cells of *arr10-6 12-3 18-3* ovules. SCN, synergid cell nucleus; ECN, egg cell nucleus; CCN, central cell nucleus; ACN, antipodal cell nucleus. Adapted from Zhu et al. (2022), Figure 5.

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