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Epigenetic remodeling by DNA glycosylases during rice reproduction

Cytosine methylation is a covalent modification of DNA that regulates important processes in eukaryotic genomes, including gene transcription, transposon silencing, and genomic imprinting (Law and Jacobsen, 2010). DNA methylation patterns are faithfully duplicated upon cell division to ensure genome integrity and to maintain lineage-specific cell fate. However, DNA methylation also needs to be dynamically reprogramed or reconfigured during development to allow establishment of new cellular identity and transcriptional state, which plays a prominent role in animal development and reproduction, and is increasingly being appreciated for reproductive success in flowering plants (Walker et al., 2018; Ono and Kinoshita, 2021). In mammals, germline cells and zygotes undergo genome-wide methylation resets to obtain cellular pluripotency. In flowering plants, multiple waves of localized smaller-scale epigenetic dynamics and remodeling have also been documented during reproduction (Gehring, 2019). For example, before fertilization localized demethylation in vegetative and central cells (VC and CC, companion cells of sperm and egg) was found to be essential for seed viability (Gehring, 2019). Upon fertilization, the genomes of endosperm and embryo undergo methylation reconfiguration in *Arabidopsis*, soybean, and rice (Park et al., 2016; Kim et al., 2019; Ono and Kinoshita, 2021). Although DNA glycosylases and the *de novo* methylation pathways are implicated in some of these processes, many of their biological functions remain to be fully elucidated.

EPIGENOME REMODELING IN GAMETE COMPANION CELLS OF *ARABIDOPSIS* AND RICE

In *Arabidopsis*, the genome of the CC undergoes extensive demethylation at thousands of loci directed by the DEMETER (DME) glycosylase to establish parent-of-origin-specific expression of many imprinted genes in endosperm. DME also demethylates the genomes of VCs and the lack of DME activity in VCs resulted in CHH hypomethylation in corresponding loci in sperm (Ibarra et al., 2012). Thus, the main functions of DME during *Arabidopsis* reproduction are to establish gene imprinting and to reinforce transgenerational TE silencing.

Although no DME ortholog was detected in monocots, the rice *ROS1a* (*DNG702*) was shown to be the functional counterpart of DME in rice cultivar Nipponbare (Ono et al., 2012). Similar to the VC of *Arabidopsis*, the rice VC genome is also extensively hypomethylated compared with the sperm genome in a *ROS1a*-dependent manner (Kim et al., 2019). In *ROS1a*/*ros1a* heterozygous plants, sperm CHH is hypomethylated at the loci where CG hypomethylation occurred in VCs, indicating that *ROS1a* activity in VCs is required for normal sperm CHH methylation. Furthermore, there is a large overlap between VC versus sperm and endosperm versus embryo hypomethylated DMRs, suggesting ROS1a also demethylates the rice CC genome (Kim et al., 2019). Thus, gamete companion cells epigenetic remodeling by DNA glycosylases is an evolutionarily conserved phenomenon in rice and *Arabidopsis*; species that diverged more than 150 million years ago.

Despite this conservation, many distinct features exist between rice and *Arabidopsis*. For example, *DME*'s expression is primarily restricted to the gamete companion cells of *Arabidopsis*, whereas *ROS1a* is broadly expressed throughout rice development. This suggests that the gamete companion cell function of *ROS1a* was delegated to *DME* in *Arabidopsis* and *ROS1a* might also play a role in rice gamete formation and seed development.

EPIGENOME REMODELING OF GAMETES AND ZYGOTE IN RICE

Rice has a persistent endosperm that serves as a staple food for humans and its genome contains more TEs than *Arabidopsis* (International Rice Genome Sequencing, 2005). Understanding its reproductive epigenetic mechanisms has direct relevance to food production and can complement the knowledge gained from research in *Arabidopsis* and other plant models. The Nipponbare reference genome encodes at least four DNA demethylases, DNG701–DNG704. Except for DNG702/ROS1a's roles in gamete companion cells, functions of these DNA glycosylases in germline cells and zygote were not known until now. A new report published in *Molecular Plants* attempted to address this question by conducting a comprehensive DNA methylome study in rice gamete, zygote, and developing embryos in wild-type and *dng* mutants (cultivar Dongjing) (Zhou et al., 2021). In addition, this new study also revealed methylation dynamics among these cell types/tissues. In wild-type plants, there is a moderate difference in bulk methylation level between egg and sperm, which is likely due to differential maintenance and *do novo* DNA methylase activities during male and female gametogenesis, a phenomenon consistent with what was observed during male sex lineage development in *Arabidopsis* (Walker et al., 2018; Long et al., 2021). Inclusion of the unicellular zygote methylome (at 6.5 h after pollination) allowed for a direct interrogation into changes in parental genomes after they fused to form the zygote. Importantly, significant differential methylations were detected between zygote versus egg or sperm that were not a simple summation of the methylomes of the gametes, suggesting that the zygote

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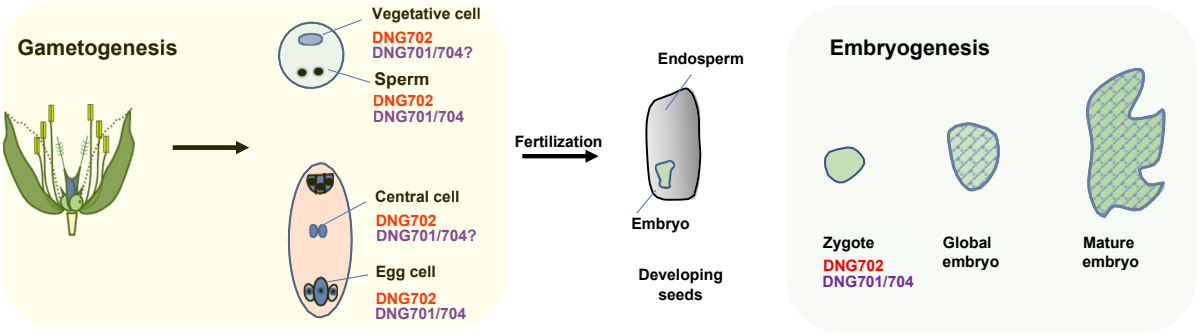

B

Cell	Glycosylase	Biological Function	Reference
Vegetative	DNG702	Ensure pollen germination, reinforce sperm methylation	Ono et al., 2012; Kim et al., 2019
Sperm	DNG702	Required for endosperm and embryo development*	Ono et al., 2012; Zhou et al., 2021
	DNG701/704	Required for endosperm and embryo development**	
Central	DNG702	Required for endosperm development and gene imprinting***	Ono et al., 2012; Park et al., 2016
Egg	DNG702	Primed for embryo development****	Zhou et al., 2021
	DNG701/704	Primed for embryo development****	
Zygote	DNG702	Required embryo development**	Zhou et al., 2021
	DNG701/704	Required embryo development**	

*Embryo development can proceed in heterozygous F1 zygotes. At least one copy of DNG702 is needed for embryo development.
** Homozygous *dng701/4* produced 50% aborted seeds, could be partially redundant with DNG702.
***Implied from ROS1a VC target sites and endosperm vs embryo DMRs overlap (see main text).
****Implied from DMRs between mutant and wild-type egg (see main text).

Figure 1. DNA glycosylases and their presumed functions during rice reproduction
(A) Diagram of rice reproduction (including gametes and zygotes where *DNGs* are known to be expressed).
(B) A brief summary of DNGs' functions in rice gametes and zygotes.

epigenome is quickly reconfigured after fertilization. As the zygote develops, the globular embryo (GE) exhibited a slightly higher CG methylation but a lower CHH methylation level compared with the zygote. In the mature embryo, CHH methylation remains low as in GE, but CHG methylation showed a more significant decrease from GE. Taken together, these observations show that there is a substantial DNA methylation remodeling and reconfiguration during rice embryo development that is distinct from what was observed in *Arabidopsis* and soybean (Ono and Kinoshita, 2021).

To understand the contributions of gamete- and zygote-expressed glycosylases (*DNG701*, *DNG702*, and *DNG704*) to the rice epigenetic dynamics during reproduction, the authors generated loss-of-function mutations in these genes and profiled methylomes from mutant eggs, sperm, and zygotes. Self-pollination of homozygous *dng702* plants can produce gametes but the zygotes failed to initiate embryogenesis. However, hybrid F1 seeds derived from reciprocal crosses between *dng702* and wild-type plants produced viable embryos with defective endosperm. These observations showed that, for zygotes to initiate embryogenesis, at least one functional copy of *DNG702* is needed; whereas for normal endosperm development, *DNG702* activity is required in both the CC and sperm (Figure 1). This is intriguing as most known gametophytic-related endosperm failures are caused by CC defects (Huh et al., 2008). If validated, this would indicate *DNG702* has a specific function in sperm that cannot be compensated by the wild-type CC genomes for endosperm development.

For *DNG701* and *DNG704*, reproductive defects were only observed in *dng701/4* double mutants that produced 50% aborted, 35% normal, and 15% retarded seeds. This suggests that *DNG701/4* also have a gamete formation function (see below) as well as a post-fertilization zygotic function that might be partially redundant with DNG702 (Figure 1). However, morphological phenotypes of F1 seeds derived from *dng701/4* and wild-type reciprocal crosses were not presented, making it difficult to assess their functions accurately.

Analysis of DMRs between mutant and wild-type gametes and zygotes enabled identification of loci targeted by each glycosylase. Interestingly, the DMRs of *dng702* and *dng701/704* versus wild-type egg and sperm are largely non-overlapping, indicating that DNG702 and DNG701/DNG704 have distinct targets in egg and sperm. Comparing targets of DNG701/4 in unicellular zygotes with those in eggs or sperm revealed that DNG701/4 have almost entirely new targets in zygotes, indicating that they have distinct functions before and after fertilization. Furthermore, loci demethylated by DNG702 in sperm or eggs are hypermethylated in wild-type zygotes, indicating that those gametic targets of DNG702 are quickly remethylated upon gamete fusion,

presumably by the *de novo* methylation pathways. Likewise, DNG701/4 target sites in gametes were also remethylated in the unicellular zygotes.

In conclusion, Zhou and colleagues provided a large amount of exciting new data that greatly enrich and extend our knowledge on the epigenetic dynamics during rice reproduction. Particularly, the generation of *dng mutant plants and methylomes of gametes and zygotes provide the research community a rich epigenetic resource that will spark new discoveries and inspire motivations for deeper understanding of how these glycosylases function.* This study strongly suggests that localized demethylation by these three DNG glycosylases in the sex cells likely serves to "prime" the gametes for successful fertilization and prepare the zygotes for rapid cellular division and differentiation during early embryogenesis. Once the gametes successfully fuse, parental imprints are quickly removed by *de novo* methylation and the glycosylases resume their zygotic functions by marking new target loci to ensure robust progression of embryogenesis. This notion is further supported by the transcriptomic analysis of zygote versus egg which revealed that the number of differentially expressed genes between zygote and egg is reduced by ~50% in *dng701/4*, indicating that the zygotic function of *DNG701/4* contributes substantially to the transcriptional state of the unicellular zygotes.

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REFERENCES

Gehring, M. (2019). Epigenetic dynamics during flowering plant reproduction: evidence for reprogramming? New Phytol. **224**:91–96. https://doi.org/10.1111/nph.15856.

Huh, J.H., Bauer, M.J., Hsieh, T.-F., and Fischer, R.L. (2008). Cellular programming of plant gene imprinting. Cell **132**:735–744.

Ibarra, C.A., Feng, X., Schoft, V.K., Hsieh, T.F., Uzawa, R., Rodrigues, J.A., Zemach, A., Chumak, N., Machlicova, A., Nishimura, T., et al. (2012). Active DNA demethylation in plant companion cells reinforces transposon methylation in gametes. Science **337**:1360–1364. https://doi.org/10.1126/science.1224839.

International Rice Genome Sequencing, P. (2005). The map-based sequence of the rice genome. Nature **436**:793–800. https://doi.org/10.1038/nature03895.

Kim, M.Y., Ono, A., Scholten, S., Kinoshita, T., Zilberman, D., Okamoto, T., and Fischer, R.L. (2019). DNA demethylation by ROS1a in rice vegetative cells promotes methylation in sperm. Proc. Natl. Acad. Sci. U S A **116**:9652–9657. https://doi.org/10.1073/pnas.1821435116.

Law, J.A., and Jacobsen, S.E. (2010). Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nat. Rev. Genet. **11**:204–220.

Long, J., Walker, J., She, W., Aldridge, B., Gao, H., Deans, S., Vickers, M., and Feng, X. (2021). Nurse cell-derived small RNAs define paternal epigenetic inheritance in *Arabidopsis*. Science **373**. https://doi.org/10.1126/science.abh0556.

Ono, A., and Kinoshita, T. (2021). Epigenetics and plant reproduction: multiple steps for responsibly handling succession. Curr. Opin. Plant Biol. **61**:102032. https://doi.org/10.1016/j.pbi.2021.102032.

Ono, A., Yamaguchi, K., Fukada-Tanaka, S., Terada, R., Mitsui, T., and Iida, S. (2012). A null mutation of ROS1a for DNA demethylation in rice is not transmittable to progeny. Plant J. **71**:564–574. https://doi.org/10.1111/j.1365-313X.2012.05009.x.

Park, K., Kim, M.Y., Vickers, M., Park, J.S., Hyun, Y., Okamoto, T., Zilberman, D., Fischer, R.L., Feng, X., Choi, Y., et al. (2016). DNA demethylation is initiated in the central cells of *Arabidopsis* and rice. Proc. Natl. Acad. Sci. U S A **113**:15138–15143. https://doi.org/10.1073/pnas.1619047114.

Walker, J., Gao, H., Zhang, J., Aldridge, B., Vickers, M., Higgins, J.D., and Feng, X. (2018). Sexual-lineage-specific DNA methylation regulates meiosis in *Arabidopsis*. Nat. Genet. **50**:130–137. https://doi.org/10.1038/s41588-017-0008-5.

Zhou, S., Li, X., Liu, Q., Zhao, Y., Jiang, W., Wu, A., and Zhou, D.X. (2021). DNA demethylases remodel DNA methylation in rice gametes and zygote and are required for reproduction. Mol. Plant https://doi.org/10.1016/j.molp.2021.06.006.