

Reproductive System RIKEN ECL Team (FY2024)
RIKEN ECL Team Leader: Reina Komiya (Ph.D.)



(0) Research fields

CPR Subcommittee: Biology

Keywords: Reproduction, Non-coding RNA, Chromatin Dynamics, Imaging, Crop Science

(1) Long-term goal of laboratory and research background

Our group major aim is to elucidate the reproductive non-coding RNA system in plants and the importance of non-coding genomic regions that are ubiquitously present in higher organisms.

We identified 771 reproductive long non-coding RNAs (lncRNAs), and numerous small RNAs derived from these lncRNAs, in rice. Furthermore, we proposed a model of spatial regulation via mobile Argonaute (mobile AGO), which function in migrating from soma to germ as carriers of these small RNAs derived from lncRNAs. However, non-cell-autonomous development through such migratory protein-RNA complexes remains unknown.

Our long-term goal is to elucidate mechanisms of information transfer within cells, between cells, and across generations through reproductive RNAs, as well as plant reproductive strategies generated from the diversity of environmentally adaptive non-coding RNAs. Ultimately, we intend to develop an RNA delivery system expanding this mobile project without the need for genome modification.

(2) Current research activities (FY2024) and plan

(A) Establishment of the Team

We established the Reproductive System RIKEN ECL Research Team at the RIKEN Yokohama Campus in October 2024.

(B-1) Inter-/Intracellular Dynamics of Reproductive Tissues Mediated by Mobile Factors

Argonaute protein (AGO) in association with small RNAs is the core machinery of RNA silencing, which causes target cleavage, translational repression and heterochromatin formation in many organisms. We identified two Argonaute proteins (mobile AGOs) as mobile carriers of small RNA from stamen somatic cells to germ cells (Tamotsu *et al.*, 2023, Figure 1). Additionally, mobile operators interacting with the mobile AGOs were also identified by our mass spectrometry, which we designated Mobile AGO Interactors I-IV, MAI-I, II, III, IV.

We focused on interactors of mobile AGOs, we generated rice mutants of MAI-I-IV using genome editing and evaluated their effects on reproduction, including fertility. Furthermore, through a collaborative study with Dr. Keiichiro Ishiguro (Kumamoto University; currently Chiba University), we generated antibodies against MAI-I, MAI-II, and MAI-III and analyzed their intracellular localization.

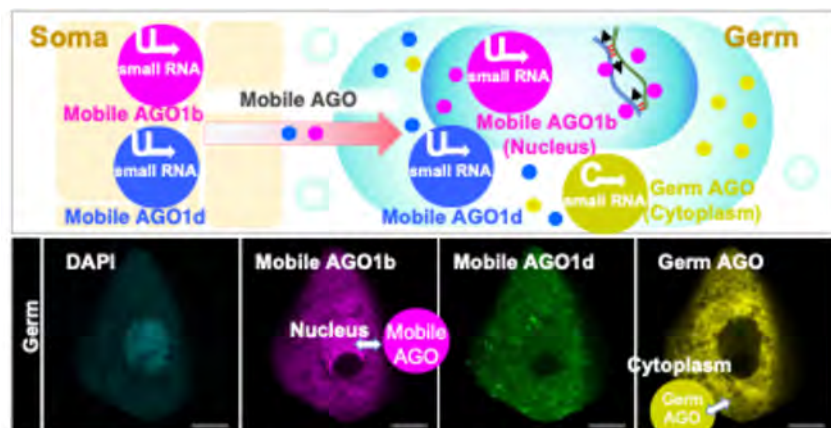


Figure 1. Spatial distribution of three ARGONAUTES mediated stamen-specific small RNA pathway.

(B-2) 4D Live Imaging of the Male Reproductive Organ in Rice

The stamen is the male reproductive organ in plants and contains the germ cells (Germ) and somatic cell layers (Soma). Since soma development affects germ cell development, their synchronization in stamen development is indicative of the importance of cell-to-cell interaction. However, the non-cell-autonomous mechanism remains unknown.

To establish 4D live imaging to visualize the intercellular movement of mobile AGOs and their effector factors, we attempted visualization of germ cells using gel-embedding methods in rice stamens expressing GFP specifically in reproductive tissues. In parallel, we are preparing plant materials utilizing photoconvertible proteins, thereby developing foundational technologies for manipulating soma-to-germ

communication.

(B-3) Transcriptional Regulation Mediated by Nuclear AGO

Mobile AGO formed nuclear foci, suggesting novel silencing functions in the nucleus. We conducted multi-omics analyses combining the following approaches:

1. Identification of genomic regions bound by mobile AGO using chromatin immunoprecipitation (ChIP)
2. DNA methylome
3. Transcriptome using mutant
4. Identification of small RNAs associated with mobile AGO

Future plan. We will advance the following studies: 1. Establishment of 4D live imaging of stamens, 2. Molecular function of effector factors that induce soma-to-germ communication, 3. Identification of mobile AGO targets, 4. Nuclear regulation and dynamics in reproduction. By integrating imaging technologies, intercellular movement, and intracellular functions, we aim to elucidate the core mechanisms of reproductive information transfer mediated by mobile signals.

(C) Sexual and Asexual Reproduction in Rice

Plant reproduction can be divided into asexual reproduction (vegetative propagation via mitosis) and sexual reproduction (seed propagation via meiosis and fertilization). The proper use of sexual or asexual reproduction is an effective reproductive strategy to benefit offspring in harsh environments.

I identified HMB (Histone Methylation Binding Protein) as a key regulator of the transition between asexual and sexual reproduction in rice. Focusing on lateral meristem that control reproductive mode switching, we conducted proteomic analyses in collaboration with Dr. Koshi Imami (RIKEN IMS). In parallel, we performed small RNA analyses to identify small RNAs and their target factors involved in reproductive mode transition. In addition, we are collaborating with Dr. Ayako Yokoi (NARO) to generate tag knock-in rice lines.

Future plan. As HMB encodes a bromodomain, we plan the ChIP using the tagged lines to identify of its target factors. Furthermore, comparative genomic analyses between vegetatively reproducing wild rice and seed-reproducing cultivated rice will be conducted to elucidate the molecular mechanisms regulating the environmental-dependent asexual and sexual reproductive patterns transition.

(3) Members

(Team Leader)

Reina Komiya

(Postdoctoral Researcher)

Zein Eddin Bader

(Technical Staff II)

Misato Ono, Hiroko Tsuchida

(3) Representative research achievements

I. Book in Japanese

1. **小宮怜奈** 誰でも再現できる NGS 「前」 サンプル調整プロトコール. 用土社. 389-398 (2024)

II. Presentations

2. **Komiya, R.** Nuclear Dynamics via RNA Silencing in Rice Reproduction. The 34th International Conference on Arabidopsis Research (July 15-19, 2024, Symposium invitation)
3. **Komiya, R.** Reproductive Non-coding RNA System in Rice. Tokyo RNA Club (November 6, 2024, Invitation)
4. **Komiya, R.** Reproductive Non-coding RNA System in Rice. Plant Science Symposium 2024 (December 9, 2024, Symposium invitation)
5. **Komiya, R.** Photoperiod-dependent Regulation of Sexual and Asexual Reproduction in Rice. Sexual Reproduction Symposium (March 6-8, 2025, Symposium invitation)