Science and Technology Group
Annual Report FY2022

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1 Introduction

The genome of higher organisms is composed of more than 90% of non-coding genomic regions. A recently large number of non-coding RNAs transcribed from these intergenic regions have been identified in many organisms. However, most biological functions remain unknown in plants and animals.

I identified more than **770 types of reproductive long non-coding RNAs and numerous small RNAs derived from these long non-coding RNAs in rice** (Komiya *et al.*, 2014; Fig.2A). **The major aim of this research is to elucidate the comprehensive reproductive system via NON-coding RNAs in plants and the significance of non-coding genomic regions presented in higher organisms.**

2 Activities and Findings

2-1 3D imaging of the male organ in rice

The stamen is the male reproductive organ in plants that contains the germ cells (Germ) and somatic wall (Soma). We successfully developed a method to visualize the three-dimensional (3D) structure of the whole rice stamens, which can also be used for distinguishing the internal structure of the stamens (Koizumi and Komiya 2022; Figure 1).

Furthermore, we successfully developed a **3D organ-immunoimaging technique with single-cell and intracellular resolution** (Araki *et al.*, 2022).

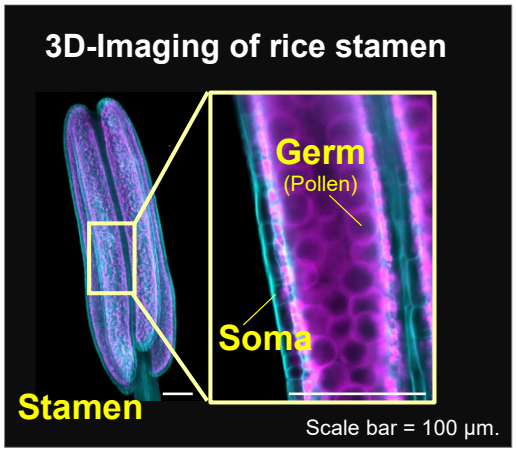

Figure 1. 3D-Imaging of rice stamen. Soma (light blue) and germ, pollen (magenta) of stamen were detected.

2-2 Spatial control of ARGONAUTEs-mediated silencing in stamen

Argonaute protein (AGO) in association with small RNAs is the core machinery of RNA silencing, an essential mechanism for precise development and defense against pathogens in many organisms.

We identified two AGOs, **AGO1b and AGO1d, that cell type-specifically regulate stamen development by acting as mobile carriers of small RNAs from the soma to the germ cells.** Our study also highlights a new mode of reproductive RNA silencing via the specific nuclear and cytoplasmic localization of three AGOs, AGO1b, AGO1d, and MEL1, in rice germ cells (Figure 2B; Tamotsu *et al.*, 2022).

Moreover, I reviewed the biogenesis, diversification, spatiotemporal expression pattern and function of these small RNAs in plants (Komiya 2022; Komiya 2023).

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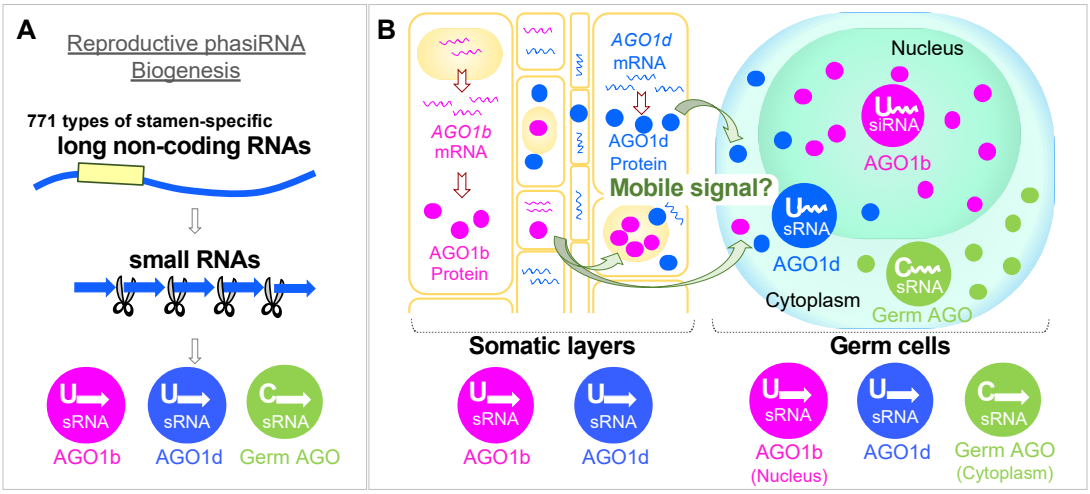

Figure 2. Spatial RNA silencing via small RNA delivery in stamen developments.
Reproductive Non-coding RNA pathways in rice. Reproductive long non-coding RNAs processing results in the production of numerous small RNAs. During stamen development, 1st Uracil (U) small are loaded into Soma Argonautes, which are distinct from Germ Argonautes association with 1st Cytosine small RNAs.
(A) The spatial RNA silencing via small RNA delivery in stamen developments.

3 My group members and Collaborators

1. **Ms. Hinako Tamotsu,** Technician
2. **Ms. Holly Morris,** Technician
3. **Dr. Koji Koizumi,** OIST Science imaging section
4. **Dr. Ayako Yokoi,** National Agriculture and Food Research Organization

4 Publications (†:Corresponding author)

1. Tamotsu, H., Koizumi, K., Briones, A. and **Komiya, R**†. Spatial distribution of three ARGONAUTEs regulates the anther phasiRNA pathway. ***bioRxiv***, DOI: https://doi.org/10.1101/2022.09.27.509800 (2022).

2. Araki, S., Tamotsu, H. and **Komiya, R**†. 3D-multiple immunoimaging using whole male organs in rice. ***Scientific Reports*** 12:15426 (2022).

3. Koizumi, K. and **Komiya, R**†. 3D imaging and in situ hybridization for uncovering the functions of microRNA in rice anther. ***Methods in Molecular Biology*** 2509, 93-104 (2022).

4. **Komiya, R**†. Spatial distribution of three ARGONAUTEs regulates the anther phasiRNA pathway. ***The Journal of Biochemistry***, Japanese (2023, *in press*).

5 External Funding

1. **Japan Science and Technology Agency, FORESTO research**
   PI: Komiya R. FY2021 ~ FY2023 (~Max FY2026).

2. **The Naito Foundation**
   PI: Komiya R. December 2020 ~ March 2024.