

Science and Technology Group Annual Report FY2018

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1 Introduction

non-coding RNAs (ncRNAs) are derived from intergenic regions that occupy more than 90% of genomes of higher organisms. The study of non-coding RNAs are currently an active research topic in biology. ncRNAs play important roles at various development stages in many organisms.

ncRNAs are divided into two groups, small RNAs and long ncRNAs. small RNAs are key matter of silencing. However, most of long ncRNA functions and molecules that interact with ncRNAs remain unknown in plants.

We have identified over 700 types of long intergenic non-coding RNAs (lincRNAs), specifically expressed during rice reproductive stages in which germ cell differentiation occurs. Furthermore, lincRNAs containing consensus sequences that are complementary to microRNA 2118 (miR2118), are cleaved within the miR2118 site. Cleaved lincRNAs are processed via endonuclease, DICER, resulting in the production of 21-nucleotide (nt) small RNAs, 21-nt phased small interfering RNAs (Figure 1, Komiya *et al.*, 2014).

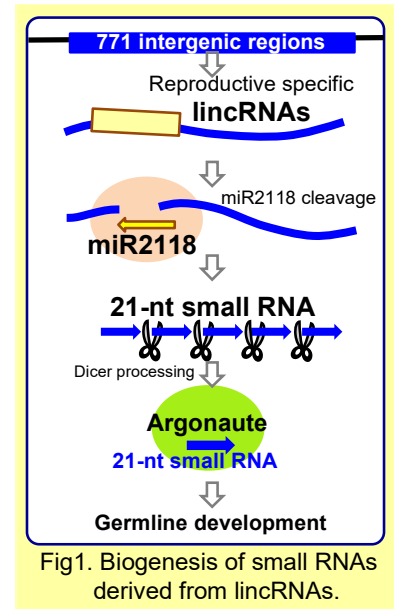


Fig1. Biogenesis of small RNAs derived from lincRNAs.

To reveal reproductive system through ncRNAs in plants, we are focusing on two main projects using rice:

1. Determining the roles of miR2118 and 21-nt small RNAs in early reproduction
2. Chromatin interaction through non-coding RNAs

By integrating 1 and 2, my goal is to construct an RNA/chromatin network model in germ cells and to reveal the reproductive system via numerous numbers of non-coding RNAs in rice.

2 Activities and Findings

2.1 Roles of microRNA2118 (miR2118) in rice reproduction

In the rice genome, there are 18 numbers of miR2118 which trigger 21-nt small RNA pathway. To reveal the function of miR2118 family members, we produced the 13 miR2118 deleted mutant by gene targeting. In these mutants, miR2118 decreased, although miR2118 which are expressed at premeiotic stages specifically. And most of lincRNAs are increased. small RNA transcriptome revealed that half of 21-nt small RNA production in over 1300 21-nt small RNAs clusters decreased of miR2118 mutants (with Dr. Tu; data not shown). Furthermore, the miR2118 mutants show low fertility and defects of anther development, suggesting that miR2118 is required for specifically anther development in early reproduction in rice. Currently, we are working on the publication of the role of miR2118 during plant reproduction.

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2.2 Production of gene targeting rice

By gene targeting using CAS9 system, we have produced over 20 mutant rice involved 21-nt small RNA production. We are screening the mutants which are essential for reproduction in plants.

2.3 Screening of reproductive specific Argonaute proteins

Argonaute proteins that are bound to small RNAs, cause silencing in animals and plants. We have performed proteomics to detect reproductive specific Argonaute. The proteomics revealed that 3 Argonaute candidates which may be interacting with 21-nt small RNAs during reproduction, have been detected (with Dr. Alejandro). To reveal the localization of the *Argonaute* transcripts in reproductive tissues, we performed *in situ* hybridization (with Dr. Koizumi; data not shown). We continue to elucidate the silencing mechanism regulated by reproductive specific Argonaute-small RNA machinery in FY2019.

3 Collaborations

- 3.1 **Dr. Tu N. Le**, OIST, Plant Epigenetics Unit. Informatics for small RNAs
- 3.2 **Dr. Koji Koizumi**, OIST, Imaging Section. *In situ* hybridization
- 3.3 **Dr. Alejandro Villar Briones**, OIST, Instrument Analysis Section. Proteomics support using mass spectrometry.
- 3.4 **Dr. Haruhiko Inoue**, National Agriculture and Food Research Organization (NAFRO). Planting of gene-targeting mutant lines in NAFRO green house.

4 Publications

- 4.1 **Kurokawa R, Komiya R, Oyoshi T, Matsuno Y, Tani H, et al. Multiplicity in Long Noncoding RNA in Living Cells. *Biomedical Sciences*. 4, 1-18 (2018).**
- 4.2 **小宮 怜奈. 多種のノンコーディング RNA が担う多様な機能. *生物工学*. 96, 349 (2018).**

5 External Funding

- 5.1 **JST PRESTO, PI: Komiya R.**
2017, September ~ 2021, March.
- 5.2 **JSPS Innovative area (RNA Taxonomy), PI: Komiya R.**
2017, April ~ 2019, March.