Science and Technology Group
Annual Report FY2016

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1 Introduction

Sexual reproduction transmits genetic information to the next generation and increases genetic diversity in offspring. Successful sexual reproduction in flowering plants depends on accurate germ cell differentiation. Molecular mechanisms of germ cell development during pre-meiotic stages 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 that contain consensus sequences complementary to microRNA 2118 (miR2118) are cleaved within the miR2118 site. Cleaved lincRNAs are processed via DICER-LIKE4 (DCL4) protein, resulting in the production of 21-nucleotide phased small interfering RNAs, phasiRNAs (Figure 1, Komiya *et al.*, 2014).

The study of non-coding RNAs (ncRNAs) are currently an active research topic in biology. More than 90% of the genomes of higher organisms comprise intergenic regions. Endogenous ncRNAs play important roles at various developmental stages in many organisms. However, most ncRNA functions and molecules that interact with ncRNAs remain unknown in plants.

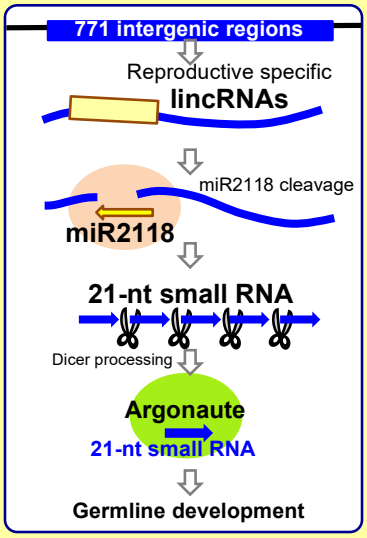

Fig1. Biogenesis of small RNAs derived from lincRNAs.

To reveal reproductive mechanisms specific to pre-meiotic stages involving lincRNAs, I am engaged in two main projects using rice:

1. Determining the roles of over 700 lincRNAs, miR2118 and 21-nt small RNAs in early reproduction

2. Chromatin regulation between germ cells and somatic cells

By integrating **1** and **2,** my goal is to construct an RNA/chromatin network model in pre-meiotic germ cells, including the topological organization of ncRNA, multichromosomal regions and chromatin regulators.

2 Activities and Findings

2.1 Evolution and diversification of phasiRNAs triggered by miR2118

Biogenesis of phasiRNAs derived from lincRNAs are conserved in monocots including rice and maize. Dicots, including *Arabidopsis thaliana* and *Medicago*, also have 21-nt phasiRNAs triggered by miR2118 family members. Unlike in monocots, most phasiRNA precursors in dicots are derived from leucine-rich repeat (NB-LRR) family genes, coding-genes that are pathogen-defense genes, not lincRNAs (Zhai et al., 2011).

Interestingly, in gymnosperms, such as Norway spruce, miR2118 targets both NB-LRR RNAs and reproductive non-coding RNAs (Xia et al., 2015). This indicates that dual miR2118 phasiRNA biogenesis in gymnosperms emerged more than 300 million years ago, and the defensive phasiRNA pathway in dicots and the reproduction-specific phasiRNA pathway in monocots may have developed following the divergence of angiosperm lineage (Fig 2). This fiscal year, I wrote a review about phasiRNA pathways and adaptation to environmental changes in land plants (Komiya 2017).

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2.2 Roles of miR2118 families in rice reproduction

To reveal the function of lincRNAs and miR2118, we identified the following four types of mutants by the gene-targeting system that causes deletion at specific targeting loci :

1. single lincRNA mutants
2. multiple lincRNAs mutants
3. miR2118 mutants
4. super-cluster mutants

In FY2016, we focused on miR2118 mutants. 18 miR2118 families exist in the rice genome. Within miR2118 mutants, 13 miR2118 families are deleted, we found low fertility in phenotypes, and lincRNA expression is increased (Komiya unpublished data). These results suggest that miR2118 families regulate reproduction in rice.

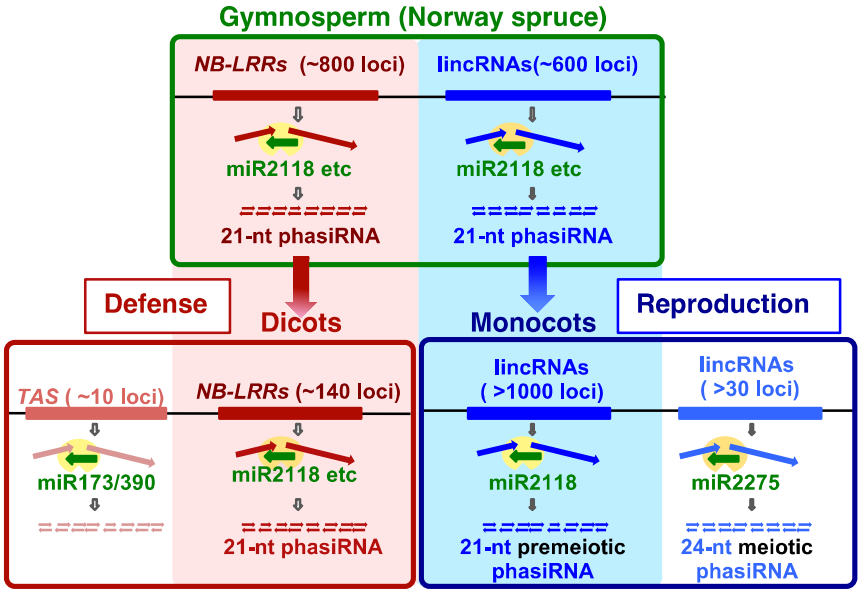

Fig2. miR2118 are conserved in gymnosperm, dicots and monocots

2.3 Biological role of Chromatin Regulation Factor (CRF) in rice

CRF is a transcriptional repressor that directly binds to modified histones for transcriptionally silenced heterochromatin in animals (Bannister and Kouzarides, 2005). We created *CRF* promoter:CRF:GFP transgenic rice, and also performed CRF protein expression and mass spectrometric confirmation for CRF antibody creation. In FY2017, I hope to reveal the biological roles of CRF1 by chromatin immuno-purification and transcriptome using the CRF:GFP rice and the antibody.

3 Collaborations

3.1 Dr. Alejandro Villar Briones, OIST. Rice CRF proteins were sequenced by Dr. Alejandro Villar-Briones using mass spectrometry.

3.2 Dr. Masaki Endo and Ms. Masahiro Mikami, National Institute of Agrobiological Sciences (NIAS). A CRISPER CAS9 vectors were provided by Dr. Endo.

4 Publications

4.1 *Komiya, R. Biogenesis of diverse plant phasiRNAs involves an miRNA-trigger and Dicer-processing. *Journal of Plant Research*. 130, 17-23 (2017).
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5 External Funding

5.1 JSPS Young Scientist (B) PI: **Komiya, R.** 2014, April ~ 2017, March.
5.2 JSPS Innovative area (RNA Taxonomy), PI: **Komiya, R.** 2015, April ~ 2017, March.

April~July, 2016 Maternity leave & Child care leave