Science and Technology Group
Annual Report FY2019

Reina Komiya
Science and Technology Associate

1 Introduction

The genome of higher organisms is composed of more than 90% of non-coding genomic regions. In recent years, numerous non-coding RNAs transcribed from non-protein coded genomic regions have been identified in many organisms. Non-coding RNAs are divided into two groups: long non-coding RNAs (lncRNAs) and small RNAs. Small RNAs are a key gene-silencing system, which is conserved in plants and animals.

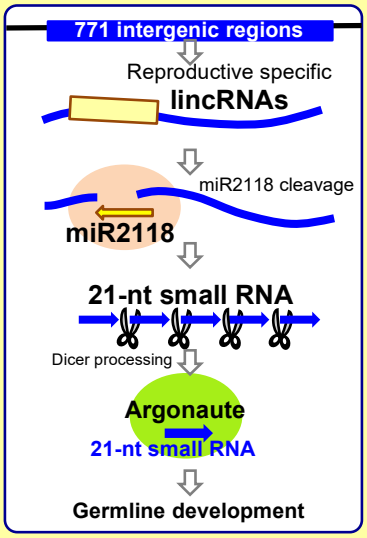

Fig1. Biogenesis of small RNAs derived from lincRNAs.

We have identified over 700 types of long non-coding RNAs, specifically expressed during rice reproductive stages. These long non-coding RNAs are processed via endonuclease, resulting in the production of 21-nucleotide (nt) secondary small RNAs, (Figure 1, Komiya *et al.*, 2014). However, the function of reproductive specific long non-coding RNAs and small RNAs, and their molecular mechanisms in plant reproduction remain unknown.

To elucidate the reproductive roles of long non-coding RNAs and small RNAs in plants, we have engaged in the following projects via small RNA profiling, imaging and proteome.

1. Generation of mutants of non-coding RNAs using genome editing
2. Biological functions of microRNA 2118 and secondary small RNAs in stamen
3. Identification of reproductive specific Argonaute proteins

2 Activities and Findings

2.1 Roles of microRNA2118 (miR2118) in rice reproduction

Reproductive specific small RNAs are key regulators in germline development in animals and flowering plants. microRNA2118 (miR2118) is conserved among plants, and is expressed at reproductive stages, producing secondary small interfering RNAs. 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 14 miR2118 deleted mutant by gene targeting.

mir2118 mutants showed low fertility, and is especially associated with morphological abnormalities in anther developments. Furthermore, small RNA profiling and proteomic analysis demonstrate the site-specific differences of small RNAs between soma (anther wall) and germ cells. **Our study highlights the significance of miR2118/secondary small RNAs functions in anther development and rice reproduction (Komiya *et al.*, under revision).**

2.2 Screening of reproductive specific Argonaute proteins

Argonaute proteins that are bound to small RNAs, cause silencing in animals and plants. We performed proteome analysis to detect reproductive specific Argonaute and identified three Argonaute candidates which may be interacting with 21-nt small RNAs during reproduction (with Dr. Alejandro). We continue to elucidate the silencing mechanism regulated by reproductive specific Argonaute-small RNA machinery in FY2020.

2.3 3D-imaging using immunostaining

To investigate the overall structure of anthers, anthers were visualized in 3D images using Lightsheet microscopy (with Dr. Koizumi; data not shown). Furthermore, we plan to detect the protein localization in reproductive tissues using immunostaining.

2.4 Production of gene targeting rice

By gene editing, we generated over 20 mutated variation lines, in which 21-nt small RNA production are affected (with Dr. Yokoi). We are screening the mutants which are essential for reproduction in plants.

3 Collaborations

3.1 **Dr. Tu N. Le,** OIST, Plant Epigenetics Unit.

3.2 **Dr. Koji Koizumi,** OIST, Imaging Section.

3.3 **Dr. Alejandro Villar Briones,** OIST, Instrument Analysis Section.

3.4 **Dr. Ayako Yokoi,** National Agriculture and Food Research Organization (NAFRO).

4 External Funding

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