

Science and Technology Group Annual Report FY2020

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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 key matters of the silencing system, which is conserved in plants and animals.

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.

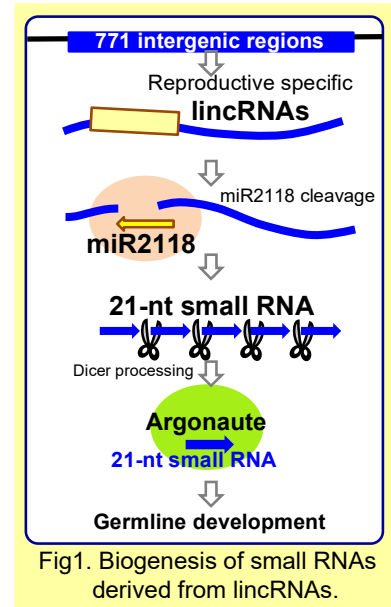


Fig1. Biogenesis of small RNAs derived from lincRNAs.

To elucidate the reproductive roles of long non-coding RNAs and small RNAs in plants, I 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 Mutant resources for non-coding RNAs

To elucidate the biological functions of these reproductive long non-coding RNAs and small RNAs, I generated the over 50 mutant lines via genome editing. These over 50 mutant lines that affect the multiple non-coding RNAs, show the various reproductive phenomena.

2.2 miR2118-dependent U-rich small RNA production in somatic stamen development

miR2118 that is conserved among plants, produces secondary small RNAs (Figure 2). *mir2118* mutants showed severe male and female sterility associated with somatic abnormalities in stamen developments (Figure 2a, b). Furthermore, small RNA profiling and proteome demonstrate the site-specific differences of small RNAs between soma and germ cells. Our study highlights the significance of miR2118 and secondary small RNAs functions in somatic development during rice reproduction (Figure 2). We reported the roles of miR2118 in *Nature Communications*.

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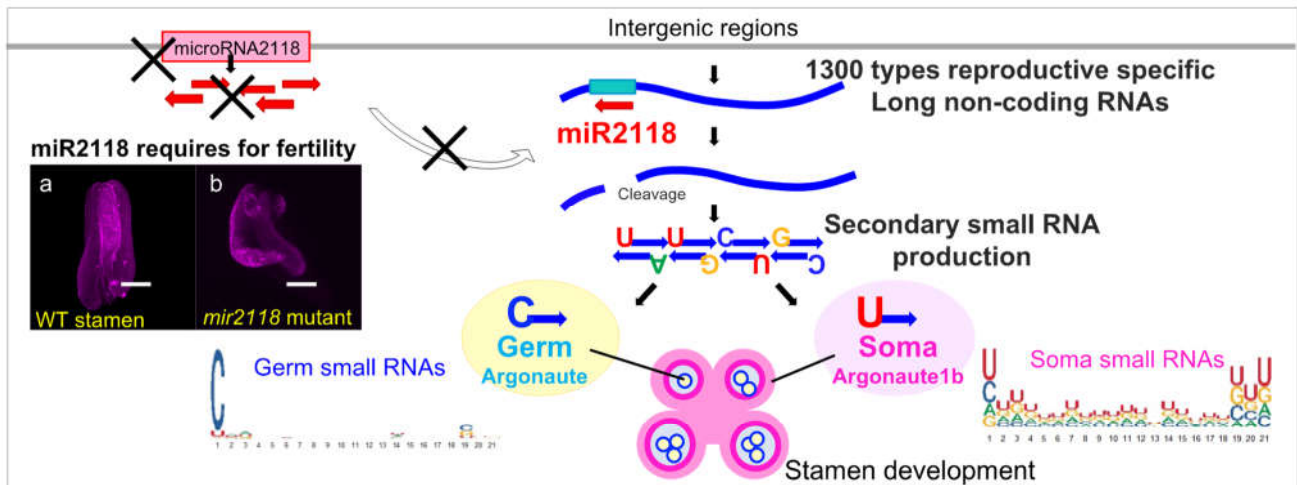


Figure 2. The model of cell-lineage-specific small RNA production during stamen development. microRNA2118 (miR2118) targets and cleaves over 1300 long non-coding RNAs, resulting in the production of the secondary small RNAs. *mir2118* mutants showed sterility with abnormalities in the stamen development (a and b), indicating that miR2118 is crucial for reproduction. During stamen development, the Uracil (U)-rich nucleotides are critical to the Soma small RNAs, which are distinct from 1st Cytosine small RNAs in Germ.

2.3 3D imaging of anthers structure (a part of stamen)

To investigate the overall structure of anthers, anthers were visualized in 3D images using Lightsheet microscopy (with Dr. Koizumi; data not shown). We succeeded in the 3D-imaging to detect the stamen structure, and this protocol is under reviewed in *Methods in Molecular Biology*.

3 Publications

Araki, S., Le, TN., Koizumi, K., Briones, A., Nonomura, K., Endo, M., Inoue, H., Saze, H. and **Komiya, R.**[†] miR2118-dependent U-rich phasiRNA production in rice anther wall development. *Nature Communications* 11, 3115 (2020).

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4 Invited Presentations

Komiya, R. Diverse non-coding RNAs involved in plant reproductive system. Young research meeting of the Japanese Biochemical Society (2020).

Komiya, R. miR2118-dependent U-rich phasiRNA production in rice anther wall development. 43rd Annual Meeting of the Molecular Biology Society of Japan (2020).

Komiya, R. Diverse non-coding RNAs involved in plant reproductive system. Kyusyu and Okinawa meeting of the Japanese Society of Breeding (2020).

Komiya, R. Diverse non-coding RNAs involved in plant reproductive system. The 62nd Annual Meeting of the Japanese Society of Plant Physiologists (2021).

5 External Funding

JST PRESTO, PI: **Komiya R.** September 2017 ~ March 2021.

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

Kato Memorial Bioscience Found of the meeting, PI: **Komiya R.** November 2020.