LABORATORY OF CELL RESPONSES

Director General
YAMAMOTO, Masayuki

Specially Appointed Associate Professor
YAMASHITA, Akira

Postdoctoral Fellow: *SHICHINO, Yuichi*
Visiting Scientist: *OTSUBO, Yoko*
Secretary: *SAKAGAMI, Mari*

Cells sense the environment around them, for example the amount of nutrients and hormones, as well as the temperature and pressure, and decide what kind of activities to undertake using this information. Germ cells, which produce sperm and eggs, begin halving their number of chromosomes during a special kind of cell division called meiosis, in response to the ambient conditions. In our laboratory we use the fission yeast *Schizosaccharomyces pombe*, the simplest organism that performs meiosis, to research the mechanism by which cells switch from mitosis, the kind of cell division that divides cells equally to create two identical cells, to meiosis, which is essential for bringing forth genetically diverse progeny.

I. Signaling pathways that regulate the onset of sexual differentiation

We have been trying to elucidate how fission yeast cells switch their mode of cell cycle from mitotic to meiotic. We focus on a highly conserved kinase, namely Target of rapamycin (TOR) kinase, which plays key roles in the recognition of nutrition and the onset of sexual differentiation in fission yeast. TOR kinase forms two types of complexes, TORC1 and TORC2. TORC1 contains Tor2 as its catalytic subunit and is essential to suppress sexual differentiation in the presence of nitrogen. However, the critical effectors of TORC1 that function in the suppression of sexual differentiation have remained largely unknown.

We demonstrated that TORC1 could phosphorylate an RNA-binding protein, Mei2, *in vitro*. Mei2 is the master regulator that switches the cell cycle from mitotic to meiotic. Mei2 is also known to be involved in earlier steps of sexual differentiation. We found that non-phosphorylatable Mei2, in which the nine phosphorylation sites were changed to alanine, became more stable than the wild-type in the presence of nitrogen. We further showed that Mei2 was polyubiquitylated *in vivo* in a TORC1-dependent manner.

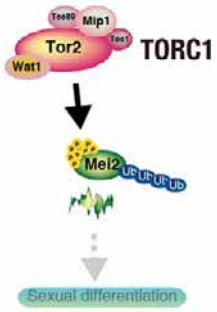

Figure 1. A schematic of the suppression of sexual differentiation by TORC1 through phosphorylation of Mei2.

From these observations we propose that TORC1 contributes to the suppression of sexual differentiation in the presence of rich nutrition through phosphorylation and destabilization of Mei2 (Figure 1) (Otsubo et al., 2014).

II. The molecular mechanisms that establish the meiosis-specific transcription profile

Expression of hundreds of genes is upregulated during meiosis. We have shown that specific control of the stability of meiotic transcripts, which is orchestrated by the interplay between RNA-binding proteins and a long non-coding RNA, contributes to the meiosis-specific gene expression in fission yeast. Understanding precise mechanisms of this control will shed light on the regulation of timely gene expression during meiosis.

A meiosis-specific nuclear body in fission yeast, called Mei2 dot, promotes the progression of meiosis by sequestering and inhibiting Mmi1 protein, a crucial factor involved in the selective elimination of meiosis-specific transcripts (Figure 2). The Mei2 dot is composed of the RNA-binding protein Mei2 and a long non-coding RNA species termed meiRNA. We have shown previously that Mei2 physically interacts with meiRNA and forms the dot structure at the chromosomal *sme2* locus, which encodes meiRNA, depending on the transcription of meiRNA (Figure 2). We have further shown that meiRNA carries numerous copies of the DSR motif, which is recognized specifically by Mmi1, and that meiRNA is indeed a target eliminated by Mmi1. Thus, the molecular mechanisms underlying the specific localization of meiRNA to its genetic locus and the importance of this localization in the inactivation of Mmi1 provide interesting questions.

We found that Mmi1 is essential to anchor meiRNA to its coding locus *sme2*. Meanwhile, overexpression of meiRNA promoted accumulation of Mmi1 to the *sme2* locus and reduced active Mmi1 in the cell. These findings indicate that Mmi1 facilitates the retention of meiRNA at its genetic locus as it is transcribed, and that the anchored meiRNA then attracts Mmi1 and inhibits the function of this protein (Shichino et al., 2014).

III. Meiosis-specific cell cycle regulation

In both mitosis and meiosis, the accumulation and activation of M-phase promoting factor (MPF), composed of Cdk and B-type cyclin (Cdc2 and Cdc13 in *S. pombe*, respectively) is required before nuclear division. When cells exit from M phase to enter the next phase of the cell cycle, the MPF level must be reduced. In meiosis, how is the MPF

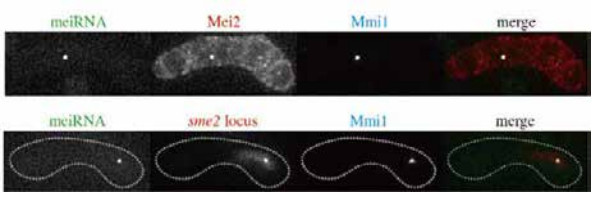

Figure 2. Co-localization of the Mei2 dot components. The upper panels display localization of meiRNA, Mei2 and Mmi1, and the lower panels display localization of meiRNA, the *sme2* locus and Mmi1. The cellular contour is shown in a dotted line.

level regulated between the first meiotic division (meiosis I) and the second meiotic division (meiosis II), during which two sequential nuclear divisions take place? We are interested in the molecular mechanisms enabling the meiotic cell cycle. We have shown previously that a meiosis-specific inhibitor of the anaphase-promoting complex/cyclosome (APC/C), namely Mes1, protects Cdc13 from complete destruction at anaphase of meiosis I. This secures the retention of a sufficient level of MPF activity to conduct meiosis II.

We isolated mutants defective in *spo5*, which encodes a meiosis-specific RNA-binding protein, in a new screening to identify mutants deficient in the progression of meiosis II. We found that *spo5* mutant cells lost Cdc13 prematurely prior to meiosis II (Figure 3). The defect in meiosis II in the *spo5* mutants was recovered by increasing the CDK activity. Furthermore, accumulation of *cdc13* transcripts during meiosis II was lower in *spo5* mutant cells than in wild-type cells. These findings indicate that Spo5 is a novel factor that contributes to the regulation of the expression of Cdc13 during meiosis (Arata *et al*. 2014).

Publication List

〔Original papers〕

- Aoi, Y., Kawashima, S.A., Simanis, V., Yamamoto, M., and Sato, M. (2014). Optimization of the analogue-sensitive Cdc2/Cdk1 mutant by in vivo selection eliminates physiological limitations to its use in cell cycle analysis. Open Biol. 4, 140063.
- Arata, M., Sato, M., Yamashita, A., and Yamamoto, M. (2014). The RNA-binding protein Spo5 promotes meiosis II by regulating cyclin Cdc13 in fission yeast. Genes Cells 19, 225-238.
- Hirai, H., Arai, K., Kariyazono, R., Yamamoto, M., and Sato, M. (2014). The kinetochore protein Kis1/Eic1/Mis19 ensures the integrity of mitotic spindles through maintenance of kinetochore factors Mis6/CENP-I and CENP-A. PLoS One 9, e111905.
- Okada, N., Toda, T., Yamamoto, M., and Sato, M. (2014). CDK-dependent phosphorylation of Alp7-Alp14 (TACC-TOG) promotes its nuclear accumulation and spindle microtubule assembly. Mol. Biol. Cell 25, 1969-1982.
- Otsubo, Y., Yamashita, A., Ohno, H., and Yamamoto, M. (2014). S. pombe TORC1 activates the ubiquitin-proteasomal degradation of the meiotic regulator Mei2 in cooperation with Pat1 kinase. J Cell Sci. 127, 2639-2646.
- Shichino, Y., Yamashita, A., and Yamamoto, M. (2014). Meiotic long non-coding meiRNA accumulates as a dot at its genetic locus facilitated by Mmi1 and plays as a decoy to lure Mmi1. Open Biol. 4, 140022.
- Togashi, N., Yamashita, A., Sato, M., and Yamamoto, M. (2014). Functional significance of nuclear export and mRNA binding of meiotic regulator Spo5 in fission yeast. BMC Microbiol. 14, 188.

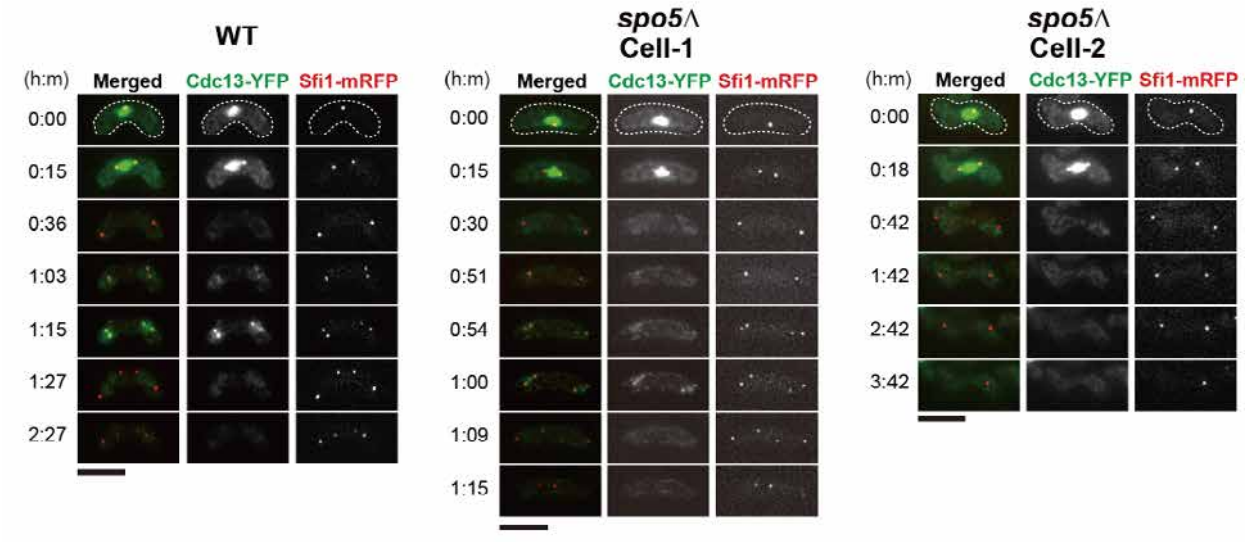

Figure 3. Cdc13 disappears prematurely in the *spo5* mutants. Cdc13 and a spindle pole body marker Sfi1 were monitored in wild-type and *spo5* mutant cells in the course of meiosis. In wild-type cells, Cdc13 signal diminished at anaphase of meiosis I but reappeared at the beginning of meiosis II. In *spo5* mutant cells, Cdc13 signal reappeared only faintly (Cell-1) or did not reappear (Cell-2).