LABORATORY OF CELL RESPONSES

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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, namely TORC1 and TORC2. TORC1 contains Tor2 as its catalytic subunit and is essential to suppress sexual differentiation in the presence of nitrogen. TORC2 contains Tor1 and, in contrast to TORC1, is required for onset of sexual differentiation under nitrogen starvation (Figure 1).

Temperature-sensitive *tor2* mutants initiate sexual differentiation on rich medium at the restrictive temperature. To gain insights into the TORC1 signaling pathway, we have isolated mutants that initiate sexual differentiation ectopically under nutrient-rich conditions. We are currently characterizing these mutants.

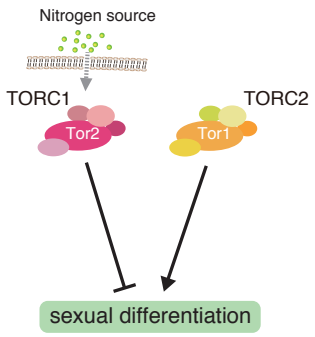

Figure 1. The two TOR complex pathways in *S. pombe*. TORC1, containing Tor2, and TORC2, containing Tor1, regulate sexual differentiation oppositely. TORC1 suppresses sexual differentiation in the presence of ample nitrogen.

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

Expression of hundreds of genes are 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 YTH-family RNA-binding protein Mmi1 plays a crucial role in the selective elimination system of meiosis-specific transcripts during the mitotic cell cycle. Mmi1 recognizes a region termed DSR (Determinant of Selective Removal) in meiotic transcripts, which is enriched with repeats of hexanucleotide motifs. Meiotic transcripts bound to Mmi1 are degraded by the RNA-degradation nuclear exosome machinery. Mmi1 also induces formation of facultative heterochromatin at a subset of its target genes.

During meiosis, a meiosis-specific nuclear body, called Mei2 dot, blocks the Mmi1-mediated elimination system. The Mei2 dot is composed of the RNA-binding protein Mei2 and a long non-coding RNA species termed meiRNA. Mei2 physically interacts with meiRNA and forms the dot structure at the chromosomal *sme2* locus, which encodes meiRNA. The Mei2 dot lures Mmi1 through numerous copies of the DSR motif on meiRNA and inhibits its function, so that meiotic transcripts harboring DSR are stably expressed (Figure 2).

We have demonstrated that Mmi1 prevents untimely translation of meiotic transcripts by regulating their localization. Multilayered suppression of meiotic genes by Mmi1 is vital for mitotic growth.

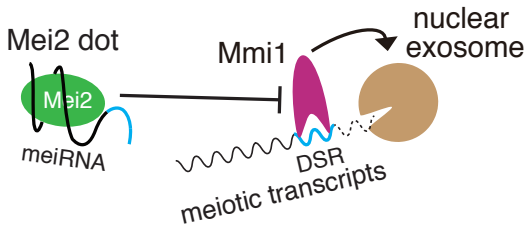

Figure 2. Selective elimination of meiosis-specific transcripts by the Mmi1/DSR system. Mmi1 binds to DSR in meiotic transcripts and induces their degradation by the nuclear exosome during the mitotic cell cycle. In meiotic cells, the Mei2 dot, composed of Mei2 and meiRNA, sequesters and inhibits Mmi1, so that DSR-harboring meiotic transcripts escape from Mmi1-mediated selective elimination.

III. Regulation of nuclear oscillation driven by cytoplasmic dynein during meiotic prophase

During meiotic prophase in fission yeast, the nucleus migrates back and forth between two poles of the cell. This oscillatory nuclear movement is called 'horse-tail' movement due to its characteristic shape and motion (Figure 3). Horse-tail nuclear movement enhances pairing of homologous chromosomes and facilitates meiotic recombination. Horse-tail movement is driven by cytoplasmic dynein, which forms a huge minus-end-directed microtubule motor complex, and dynactin, which is a protein complex that is required for most dynein-mediated cellular activities. Cytoplasmic dynein that is anchored to the cell cortex generates a pulling force on the microtubule emanating from the leading edge of the nucleus. This dynein-mediated pulling is the major contributor to horse-tail movement. Cortical anchoring of dynein is crucial for the generation of horse-tail movement.

We identified novel subunits of dynactin, and showed that dynein-related cortical factor, Num1, cooperates with dynactin to establish dynein anchoring at the cell cortex. We are now studying how the oscillatory movement of the nucleus is accomplished by combining computational simulations with live cell imaging (Figure 4).

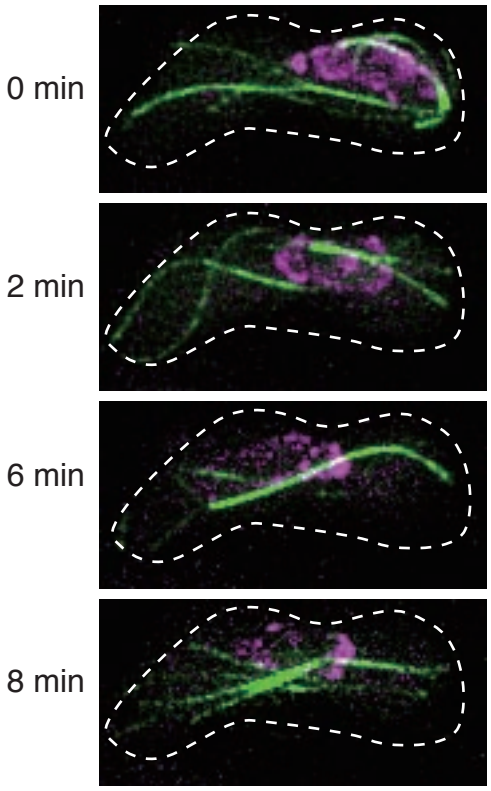

Figure 3. Horse-tail nuclear movement during meiotic prophase in *S. pombe*. Time-lapse images of nuclear membrane (Cut11, magenta) and microtubules (Atb2, green) in the wild-type strain. The cellular contour is shown by the dotted line.

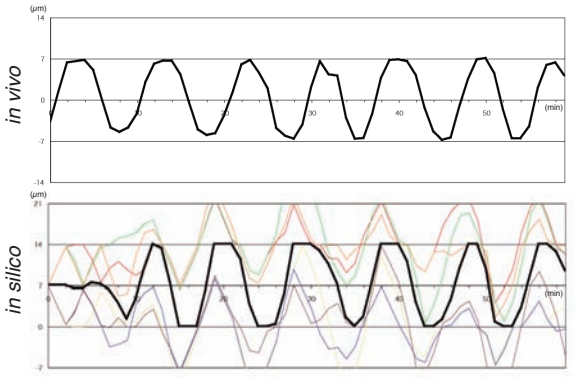

Figure 4. Tracks of the leading edge of the nucleus during horse-tail nuclear movement in a living cell and in a simulation model. Colored lines in the model indicate tracks of microtubule plus ends.

Publication List:

〔Review articles〕

- Yamashita, A., Shichino, Y., and Yamamoto, M. (2016). The long non-coding RNA world in yeasts. Biochim. Biophys. Acta Gene Regul. Mech. *1859*, 147-154.
- Yamashita, A., Sakuno, T., Watanabe, Y., and Yamamoto, M. (2016). Analysis of *Schizosaccharomyces pombe* Meiosis. Fission Yeast, A Laboratory Manual, 251-259.
- Yamashita, A., Sakuno, T., Watanabe, Y., and Yamamoto, M. (2016). Live imaging of chromosome segregation during meiosis in the fission yeast *Schizosaccharomyces pombe*. Fission Yeast, A Laboratory Manual, 260-263.
- Yamashita, A., Sakuno, T., Watanabe, Y., and Yamamoto, M. (2016). Synchronous induction of meiosis in the fission yeast *Schizosaccharomyces pombe*. Fission Yeast, A Laboratory Manual, 264-267.
- Yamashita, A., Sakuno, T., Watanabe, Y., and Yamamoto, M. (2016). A simple method to induce meiosis and sporulation semisynchronously in the fission yeast *Schizosaccharomyces pombe*. Fission Yeast, A Laboratory Manual, 268-270.