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.

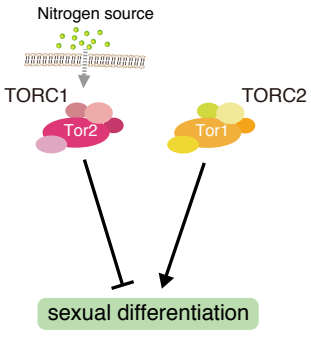

Figure 1. The two TOR complex pathways in *S. pombe*. TORC1, containing Tor2, and TORC2, containing Tor1, regulate sexual differentiation oppositely. TORC1 suppresses sexual differentiation in the presence of ample nitrogen.

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 shown, in collaboration with a group at the University of Cambridge, that a conserved multifunctional protein complex Ccr4/Not is recruited by Mmi1 to its target transcripts and plays an essential role for heterochromatin formation in the Mmi1-dependent pathway (Cotobal *et al.*, 2015).

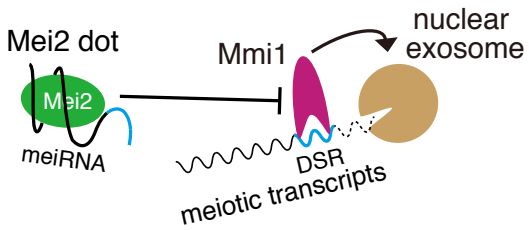

Figure 2. Selective elimination of meiosis-specific transcripts by the Mmi1/DSR system. Mmi1 binds to DSR in meiotic transcripts and induces their degradation by the nuclear exosome during the mitotic cell cycle. In meiotic cells, the Mei2 dot, composed of Mei2 and meiRNA, sequesters and inhibits Mmi1,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

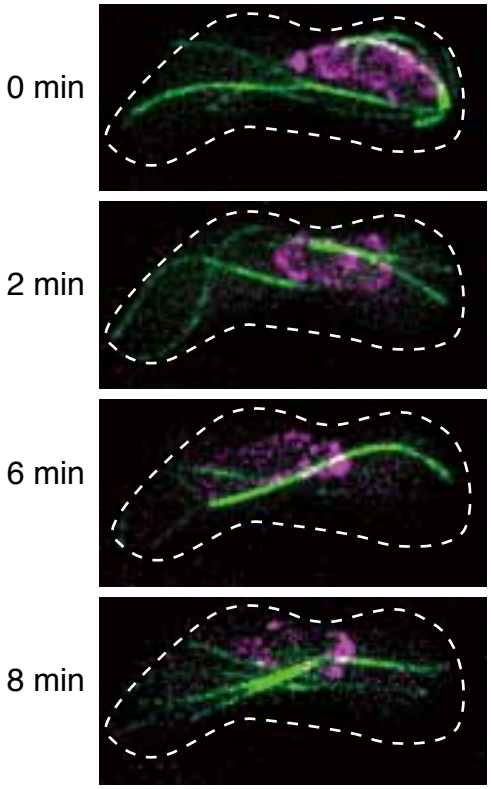

Figure 3. Horse-tail nuclear movement during meiotic prophase in *S. pombe*. Time-lapse images of nuclear membrane (Cut11, magenta) and microtubules (Atb2, green) in the wild-type strain. The cellular contour is shown by the dotted line.

We identified three subunits of dynactin, a protein complex that is required for most dynein-mediated cellular activities (Fujita *et al*., 2015). The three subunits, namely Arp1, Mug5 and Jnm1, transiently colocalized with dynein at the cell cortex and were essential for the cortical anchoring of dynein. We also found that another dynein-related cortical factor, Num1, cooperates with dynactin to establish dynein anchoring at the cell cortex (Figure 4).

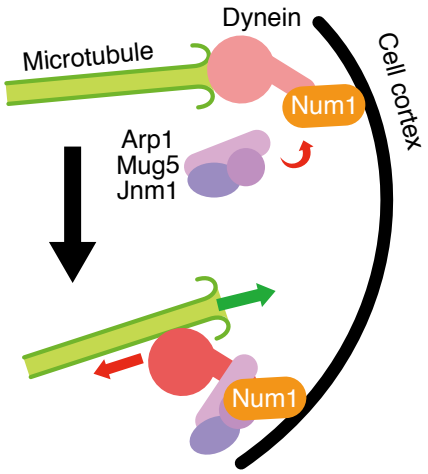

Figure 4. Cortical anchoring of cytoplasmic dynein in *S. pombe*. Dynein is captured by Num1 at the cell cortex. Dynactin subunits, Arp1, Mug5 and Jnm1 assemble with dynein at the cell cortex and activate dynein. Dynactin and Num1 cooperate to establish dynein anchoring and enable dynein to generate microtubule-pulling force.

Publication List:

〔Original papers〕

- Cotobal, C., Rodríguez-López, M., Duncan, C., Hasan, A., Yamashita, A., Yamamoto, M., Bähler, J., and Mata, J. (2015). Role of Ccr4-Not complex in heterochromatin formation at meiotic genes and subtelomeres in fission yeast. Epigenet. Chromatin *8*, 28.
- Fujita, I., Yamashita, A., and Yamamoto, M. (2015). Dynactin and Num1 cooperate to establish the cortical anchoring of cytoplasmic dynein in *S. pombe*. J. Cell Sci. *128*, 1555-1567.