

**LABORATORY OF CELL RESPONSES**



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Cells sense the environment around them (e.g. the amount of nutrients and hormones present, 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 ambient conditions. Meiosis is essential for bringing forth genetically diverse progeny. In our laboratory, we use the fission yeast *Schizosaccharomyces pombe*, the simplest organism that performs meiosis (Figure 1), to research the mechanisms by which cells switch from mitosis (a kind of cell division that divides cells equally to create two identical cells) to meiosis.

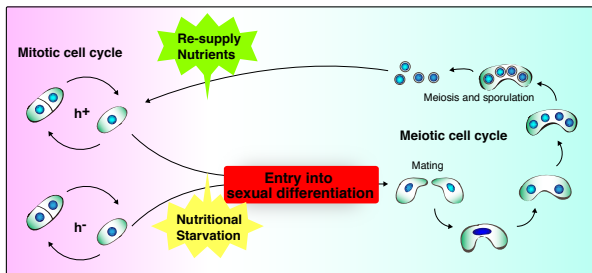


Figure 1. Life cycle of the fission yeast *S. pombe*. *S. pombe* cells proliferate by mitotic growth under nutrient-rich conditions. When starved of nutrients, especially nitrogen, *S. pombe* cells arrest the mitotic cell cycle and haploid cells conjugate with cells of the opposite mating type. Resulting diploid zygotes undergo meiosis and produce spores.

**I. Signaling pathways that regulate the onset of sexual differentiation**

We have been trying to elucidate how *S. pombe* cells switch their cell cycle mode from mitotic to meiotic. We focus on a highly conserved kinase, namely Target of rapamycin (TOR) kinase, which plays a key role in the recognition of nutrition and the onset of sexual differentiation in *S. pombe*. TOR kinase forms two types of complexes, TORC1 and TORC2. TORC1 contains Tor2 as its catalytic subunit and is essential in suppressing sexual differentiation in the presence of nitrogen. TORC2 contains Tor1 and, in contrast to TORC1, is required for the onset of sexual differentiation under nitrogen starvation.

Temperature-sensitive *tor2* mutants initiate sexual differentiation even on rich medium at restrictive temperatures. To gain an insight into the TORC1 signaling pathway, we have

isolated mutants that initiate sexual differentiation ectopically under nutrient-rich conditions, as *tor2* mutants do. We designated these mutants as *hmt*, standing for hypermating and temperature-sensitive growth. We cloned the responsible genes and found that five of the eight responsible gene encoded tRNA-related factors. The *hmt1* and *hmt2* genes encoded aminoacyl-tRNA synthetases for asparagine and proline, respectively. The *hmt3* gene encodes tRNA adenosine-34 deaminase. The *hmt4* is identical to *rpc34*, which encodes a subunit of RNA polymerase III. The *hmt5* is identical to *sfc4*, which encodes a subunit of the RNA polymerase III-specific general transcription factor IIIC. In the *hmt1-5* mutants, TORC1 activity is downregulated, suggesting that the products of these *hmt* genes may function upstream of TORC1. We also found that expression of tRNA precursors decreases upon nitrogen starvation. Furthermore, overexpression of tRNA precursors prevents TORC1 downregulation upon nitrogen starvation and represses the initiation of sexual differentiation. Based on these observations, we have proposed that tRNA precursors act as key signaling molecules in the TORC1 pathway in response to nitrogen availability (Figure 2).

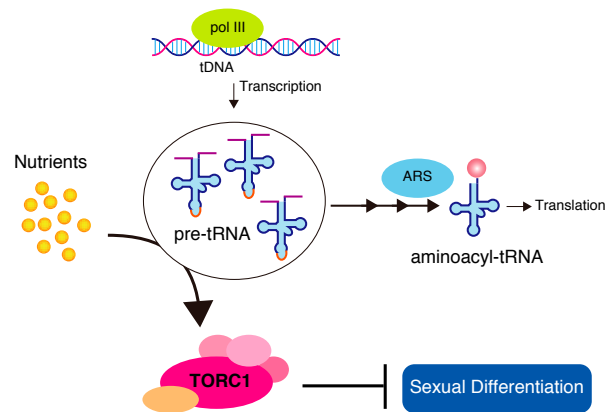


Figure 2. TORC1 regulation by tRNA precursors. TOR complex 1 (TORC1) promotes vegetative growth and suppresses sexual differentiation under nutrient-rich conditions. tRNA precursors (pre-tRNAs) positively regulate the TORC1 activity.

**II. The molecular mechanisms that establish the meiosis-specific gene expression profile**

The 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 the fission yeast *S. pombe*. 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 the formation of facultative hetero-

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2018. The former title is indicated by an asterisk (\*).

chromatin at a subset of its target genes. Furthermore, Mmi1 regulates the termination of transcription of its target genes. Mmi1-mediated termination of an upstream non-coding RNA ensures the expression of downstream genes, one of which encodes a mitogen-activated protein kinase kinase kinase (MAPKKK) essential for the initiation of sexual differentiation.

When *S. pombe* cells undergo 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.

We have shown that Mmi1 prevents untimely expression of meiotic proteins by tethering their mRNAs to nuclear foci. Mmi1 interacts with itself with the assistance of Erh1. Mmi1 self-interaction is crucial for nuclear foci formation, target transcripts elimination, their nuclear retention, and protein expression inhibition. Multi-layered suppression of meiotic genes by Mmi1 is vital for mitotic growth (Figure 3).

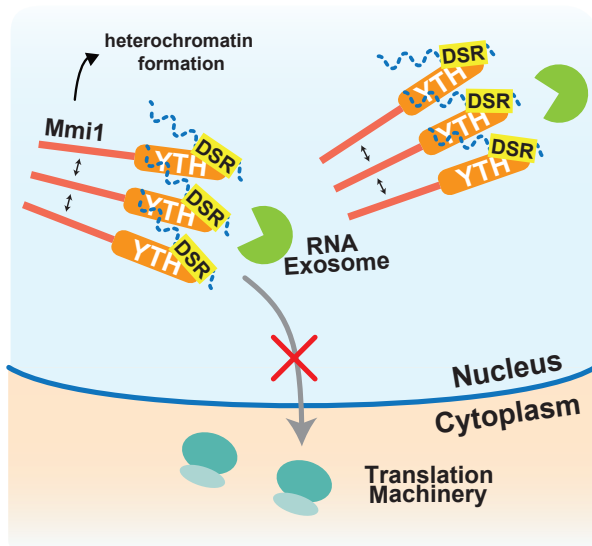


Figure 3. Mmi1-mediated multi-layered regulation to prevent mistimed expression of meiotic genes. In mitotically growing cells, meiotic transcripts carrying DSR are recognized by Mmi1, and are degraded by the RNA exosome. Mmi1 also induces heterochromatin formation. Furthermore, Mmi1 prevents nuclear export and ectopic protein expression of its targets.

### Publication List:

#### [Original papers]

- Otsubo, Y., Matsuo, T., Nishimura, A., Yamamoto, M., and Yamashita, A. (2018). tRNA production links nutrient conditions to the onset of sexual differentiation through the TORC1 pathway. *EMBO Rep.* 19, e44867.
- Shichino, Y., Otsubo, Y., Kimori, Y., Yamamoto, M., and Yamashita, A. (2018). YTH-RNA-binding protein prevents deleterious expression of meiotic proteins by tethering their mRNAs to nuclear foci. *eLife* 7, e32155.