Application of the methylotrophic yeast sexual mechanism in an artificial mating type switching system

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Research aims

A number of fungal species, including the two major model yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, have a homothallic sexual cycle (self-fertile) while heterothallic species (or strains) need to encounter the same species of the opposite mating type to reproduce [1]. Since the sexual cycle is generally advantageous for evolution, homothallic strains are better suited for breeding.

Yeast mating type is determined by the DNA sequence at the MAT gene locus. Mating type switching (MTS) is the mechanism of homothallism in *S. cerevisiae* and *S. pombe*. The molecular mechanism of MTS in *S. cerevisiae* has been intensively studied (Fig. 1). In contrast, the methylotrophic yeast *Ogataea polymorpha*, which is distantly related to *S. cerevisiae*, utilizes a different molecular mechanism to switch mating type [2, 3]. There are two MAT loci, of which only one is transcriptionally active. MTS occurs when the chromosomal positions of the two MAT loci are switched by chromosomal inversion through the inverted repeat (IR) sequences adjacent to the MAT loci [2]. The MTS mechanism in *O. polymorpha* appears to be simpler than that in *S. cerevisiae* and therefore is better suited as a model for an artificial MTS mechanism. This project aimed to identify factors and characteristics of the chromosome that are required for MTS in *O. polymorpha*.

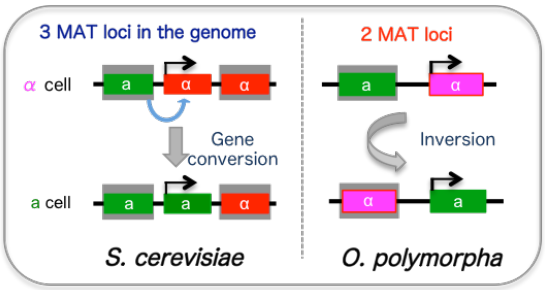

Figure 1 Mating type switching in yeast

Methods and Results

In *O. polymorpha*, MTS requires starvation conditions [2, 3]. This requirement can be overcome by constitutive expression of the transcription factor, Rme1 [4]. Since an inducible system of MTS is required for more detailed study, an attempt was made to develop an inducible expression system for Rme1 by combining it with the auxin-inducible degron.

Since that did not work well with Rme1, a tetracycline inducible expression system (Tet system) was established by transferring the *S. cerevisiae* system into *O. polymorpha*, with a few modifications. Next, we attempted to use the Tet system to express *RME1*.

A candidate approach was taken to identify factors that were necessary for MTS in *O. polymorpha*, with a particular focus on homologous recombination/repair factors and DNA replication factors. So far, several mutants have been identified as MTS-deficient mutants including *rad24*, *mec3*, *ddc1*, *mph1*, and *mus81*, in addition to the previously known *rad51*, *rad52*, and *rad17* mutants (Maekawa, unpublished) [5]. ChIP analysis indicated that Rad52 accumulates at IRs in response to nutritional starvation conditions.

Furthermore, chromosomal elements that play roles in MTS have been investigated. One of the IR sequences was inverted so that the repeat sequences make direct repeats (MAT-DR strain). In the MAT-DR strain, the intervening region would be looped-out if a recombination event occurred similar to that at MAT loci in the wild type (Fig. 2). The looped-out region was detected in the MAT-DR strain under starvation conditions, which was dependent on Mph1 helicase, but not the structure-specific endonuclease Mus81. These results suggest that the structure-specific endonuclease was not regulated to induce MTS.

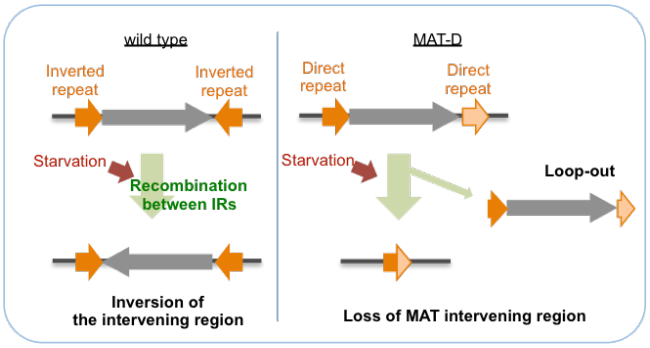

Figure 2 Schematics of chromosome structure surrounding MAT loci in cells with inverted repeats (wild type) and direct repeats (MAT-DR)

Conclusion

This study identified Ddc1, Mec3, Rad24, Mph1, and Mus81 as MTS factors in addition to the previously known Rad51, Rad52, and Rad17. Starvation-condition specific accumulation of Rad52 at IRs suggests that a double strand break was induced at or near IRs under starvation conditions. It may be possible that the MTS mechanism induced a crossover during repair of the DNA lesion at the IRs. However, MTS deficiency of the *mph1* mutant does not support this hypothesis because Mph1 in *S. cerevisiae* is known to inhibit crossovers [6]. Further investigation is required to elucidate the molecular mechanism of MTS in *O. polymorpha*.

Analysis of the MAT-DR strain suggests that the orientation of the IRs did not impact the recombination reaction and the regulation to induce MTS. MAT-DR cells loose essential gene(s) located within the intervening region when recombination occurs between repeat sequences, and become inviable. A large-scale screen can be set up to identify more MTS deficient mutants in the MAT-DR strain, which would enable a better understanding of

the molecular mechanism of MTS. Furthermore, in this study, the tetracycline inducible expression system was successfully established in *O. polymorpha*, which will benefit future research in this species.

References

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