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SUMMARY OF DOCTORAL THESIS

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Title: Molecular and genetic analysis of Moc interacting proteins that regulate sexual differentiation of *Schizosaccharomyces pombe*

(分裂酵母の有性生殖を制御する Moc に相互作用するタンパク質の分子遺伝学的解析)

The fission yeast *Schizosaccharomyces pombe,* is an excellent model organism to study the mitosis-meiosis decision mechanism. Meiotic differentiation and cell growth are mutually exclusive programs, and in the yeast the switch between cell proliferation and sexual differentiation is triggered by nutrient starvation. The sexual differentiation of *S. pombe* is down regulated by the major nutrient sensing pathway. In *S. pombe,* a series of molecular event are responsible for regulation of the cAMP pathway. The cellular cAMP level is dependent upon relative activities of the biosynthetic enzyme (adenylyl cyclase) and the degradative enzyme (phosphodiesterases). Adenylyl cyclase, encoded by the *cyr1* gene, generates cAMP from ATP and trimeric G proteins (Gpa2, Git5, Git11) control the activity of adenylyl cyclase through a nutrient-sensing mechanism of the Git3 receptor. Adenylyl cyclase associated protein (Cap1) is involved in Cyr1 regulation. A cAMP degradative enzyme Pde1 (phosphodiesterase) down regulates cAMP by converting it to AMP. Thus, concentration of intracellular cAMP plays a key role for cells to switch mitosis to meiosis through induction of Ste11.

In this thesis the author focused on a new Moc protein complex and Moc interacting proteins that regulate sexual differentiation of fission yeast. The *moc* (multicopy suppressor of over-expressed *cyr1*) genes (*moc1-4*) have been isolated to by-pass the sterile phenotype of *S. pombe* caused by an elevation of cAMP. Moc1 isolated as a potential regulator of M-phase progression and is involved in meiosis and its two orthologs, budding yeasts SDS23 and SDS24 are functionally complementary. Sds23 forms the stable complex with type 2A protein phosphatase (PP2A) and as phosphatase inhibitor. This protein contains two CBS (cystathionine-ß-synthase) domains, which are predicted to have a multiple trafficking function for protein-protein interaction and metabolic regulations. Moc2 (Ded1), which is a general translational regulatory factor, and works as an RNA helicase, is involved in both sexual differentiation and cell growth. Moc3, a novel Zn finger type protein localized in nucleus, is involved in sexual differentiation, ascus formation, and stress response. Moc4 (Zfs1), an mRNA binding and destabilizing protein, is involved in sexual differentiation and septum formation. Moc3 binds with Moc4/Zfs1. All Moc proteins positively induced sexual differentiation in *S. pombe* to different degrees.

In chapter II, to investigate possible interactions among Moc1, Moc2, Moc3 and Moc4 proteins, the author first screened for individual Moc-interacting proteins using the yeast two-hybrid system and verified the interactions with other Moc proteins. Using this screening process, Cpc2 and Rpl32-2 were highlighted as factors involved in interactions with multiple Moc proteins. Cpc2 interacted with Moc1, Moc2 and Moc3, whereas the ribosomal protein Rpl32-2 interacted with all Moc proteins in the two-hybrid system. The physical interactions of Cpc2 with Moc1, Moc2 and Rpl32-2, and of Rpl32-2 with Moc2 were confirmed by co-immunoprecipitation. In addition, results revealed that each Moc protein exists as a large complex, using Blue Native PAGE. Over-expression of Moc1, Moc2, Moc3, Moc4 and Rpl32-2 resulted in the efficient induction of a key transcription factor Ste11, suggesting that all proteins tested are positive regulators of Ste11. Considering that Moc2/Ded1 is a general translation factor and that Cpc2 associates with many ribosomal proteins including Rpl32-2, it is possible that a large Moc-mediated complex, detected in this study, may act as a translational regulator involved in the control of sexual differentiation in *S. pombe* through the induction of Ste11.

In chapter III, The author isolated a novel gene, named *ers2*, encoding mitochondrial glutamyl tRNA synthetase (mGluRS) as a Moc3 interacting element by the yeast two-hybrid system. Cytoplasmic glutamyl tRNA synthetase (cGluRS) also interacted with Moc3 in a yeast two-hybrid system. Disruption of *ers1* (cGluRS) and of *ers2* (mGluRS) indicated that these genes are both essential for the cell growth of *S. pombe*. Interestingly, *ers2* severely affected cell growth and decreased viability, but induced sexual differentiation of *S. pombe* when it was over-expressed. Over-expression of *ers1* also stimulated sexual differentiation in *S. pombe*. These observations led to test the effects of various amino acids on sexual differentiation. The author found that glutamic acid, as well as other specific amino acids, such as tryptophan, methionine, and threonine, efficiently induced sexual differentiation in *S. pombe*. These findings suggest a new regulatory mechanism where GluRS and glutamic acid are involved in sexual differentiation in fission yeast.

In this thesis, the author identified a large complex mediated by Moc proteins, ribosome-associated protein Cpc2 and the ribosomal protein Rpl32-2 that involved in translational regulation for controlling the sexual differentiation of fission yeast through activation of the key transcription factor Ste11. The author also proposes the existence of a new regulatory mechanism where Moc3 interacting proteins, GluRSs and glutamic acid are involved in sexual differentiation in fission yeast.