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SUMMARY OF DOCTORAL THESIS

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Title: Multistep regulation of protein kinase A upon glucose starvation in fission yeast

分裂酵母でのグルコース飢餓におけるプロテインキナーゼAの多段階調節機構

Several signal transduction pathways regulate mitosis to meiosis transition of fission yeast *Schizosaccharomyces pombe* depending on environmental conditions. Nutritional signal constitues one of the most important signaling cascades that decide the fate of fission yeast whether it continues to grow by mitosis or enter into meiosis. The other signaling pathways that also contribute in mitosis to meiosis switching are pheromone signaling and stress responsive pathways. Nutrient signaling consists of two signaling cascade: first is the glucose sensing cAMP-PKA pathway and the second is the nitrogen sensing TOR pathway. Under nutrient- rich conditions (high glucose or carbon source) fission yeast, proliferates by mitosis, but they arrest their cell cycle at the G1 phase under nutrient-starved conditions and proceeds to meiosis ending up to form four haploid spores. The cAMP-PKA pathway negatively regulates the meiosis in fisson yeast. The catalytic subunit of cAMP dependent protein kinase A (Pka1) phosphorylates the transcription factor Rst2 which otherwise induces the expression of Ste11, a key transcription factor of the onset of meiosis. On the other hand, disruption of *cgs1*, the regulatory subunit of PKA negatively regulates the sexual differentiation of fission yeast,

Katayama et al. isolated nine *sam* (skip the requirement of starvation for mating) mutants that are characterized to sporulate on nutrient rich medium. But these mutants were not completely characterized. To identify the *sam* mutant alleles, the author sequenced the genes that are possibly involved in sexual differentiation.

In chapter II the author described the identification of *sam6* mutant. All *sam* mutants were found to be sensitive to 1M KCl and showed high mating frequency on rich medium like a *pka1* mutant. This characteristics of the *pka1* mutant led to investigate whether one or more of the *sam* mutations carried the *pka1* allele. Sequencing of the *pka1* locus of recessive *sam* mutants revealed that *pka1* locus of *sam6* mutant contain a nonsense mutation on the kinase domain of Pka1 and over expression of *pka1* could suppress the *sam* phenotype. Moreover, when *pka1* gene locus from *sam6* mutant was introduced into wild type genome the resulting strain was indistinguishable from *sam6* mutant. So the author concluded that *sam6* mutant carried a *pka1* mutation. The author found that Pka1 protein interacted with Cgs1 (a regulatory subunit of PKA) under low glucose condition (low cAMP in cells) by forming a Pka1-Cgs1

complex. The author also observed that Pka1 protein was in a phsphorylated form under glucose starved conditions and the phosphorylation of Pka1 was dependent on the regulatory subunit Cgs1. Pka1 protein moved from the nucleus to the vacuoles after 6-8 hours of glucose starvation and is distributed in the cytosol after 12 hours. Thus, glucose starvation reduces the activity of Pka1 by forming the Pka1-Cgs1 complex, which resides in cytosol and positively regulates sexual differentiation in fission yeast.

In chapter III, the author described the identification of other two *sam* mutants i.e., *sam5* and *sam7*. Sequencing of *pka1* locus of *sam5* and *sam7* mutants revealed that these mutants contain a point mutation (G441E or G441R) on the kinase domain of Pka1. In these mutants the Pka1 proteins were normally synthesized but remained in an inactive stage, and that could be confirmed by the observation of that the Rst2 and Ste11 were localized in the nucleus in *sam5* and *sam7* mutant under glucose-rich conditions. Localization of Ste11 in the nucleus is required for execution of sexual differentiation of fission yeast. The inactive Pka1 protein remained in the cytosol under glucose-rich conditions whereas the wild type Pka1 protein remained in the nucleus. Furthermore those inactive Pka1 proteins did not interact with Cgs1 and starvation induced hyperphosphorylation was inhibited, suggesting that the residue may serve as a surface for the regulatory subunit (Cgs1) and required for active conformation of Pka1 protein for nuclear import. It has been shown that phosphorylation at Thr-356 residue of the activation loop region of Pka1 is important for optimum activity and replacement of this residue by alanine inactivates the kinase. The author re-examined the functions of the Thr-356 residue in terms of phosphorylation, localization and binding with regulatory subunit (Cgs1). The author found that phosphorylation of this residue is important for both nuclear localization and interaction with Cgs1. The author also detected the interaction of Pka1 with Cgs1 occurred before hyperphosphorylation. And the mutant Pka1 proteins that failed to interact with Cgs1 were not hyperphosphorylated under glucose starvation.

In this thesis the author identified three recessive *sam* mutants, which contain the *pka1* mutations. An analysis of these *sam* mutant revealed that the activity of Pka1 is important for the nuclear localization of Pka1 and Cgs1 is required for hyperphosphorylation of Pka1. Pka1 is controlled spatially and physically by glucose signaling and phosphorylation in fission yeast.