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学 位 論 文 要 旨

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題目: Comparison and features of prenyl diphosphate synthases from budding yeast and fission yeast

出芽酵母と分裂酵母の長鎖プレニル二リン酸合成酵素の比較特性

Ubiquinone (UQ), which is composed of a benzoquinone moiety and an isoprenoid side chain with various lengths, is present in almost all living organisms. UQ localizes mainly in the plasma membrane of prokaryotes and in the inner mitochondrial membrane of eukaryotic cells. UQ is an essential factor for the electron transfer system and is also a known lipid antioxidant. The biosynthetic pathway of UQ has been deciphered almost entirely in *Escherichia coli* and *Saccharomyces cerevisiae*, and 8 enzymes and nine steps are thought to be involved in the pathway. The length of the UQ isoprenoid side chain is determined by polyprenyl diphosphate synthase (poly-PDS) and differs among living organisms. For examples, *S. cerevisiae* produces UQ-6, *E. coli* produces UQ-8, and *Schizosaccharomyces pombe* and humans produce UQ-10. Our group proved that the UQ side chain lengths could be altered by genetic engineering. It was proved that poly-PDS can be classified into homodimer-, heterodimer-, and heterotetramer- types based on the pattern of components.

In chapter 1, the author described the general introduction of the study.

In chapter 2, the author described the characterization of budding yeast hexaprenyl diphosph-
ate synthase Coq1 in *S. pombe* or in *E. coli*. In budding yeast, Coq1 catalyzed the synthesis of UQ isoprenoid side chain. When the author expressed the *COQ1* gene in an *E. coli ispB* disrup-
tant and detected only Hex-PDS activity and UQ-6, indicating that expression of Coq1 alone results in bacterial enzyme-like functionality. In fission yeast the isoprenoid side chain of UQ is catalyzed by a heterotetramer formed from decaprenyl diphosphate synthase Dps1 and Dlp1. Deletion of which of these two genes will cause phenotypes of UQ deficiency and growth delay on minimal medium. These phenotypes can be rescued by introducing a complementary gene on a plasmid. When the author expressed the *COQ1* gene in fission yeast Δ*dps1* or Δ*dlp1* strains, the *COQ1* gene restored growth on minimal medium in the Δ*dlp1* but not in the Δ*dps1* strain. Intriguingly, UQ-9 and UQ-10, but not

UQ-6, were identified and deca-PDS activity was detected in the *COQ1*-expressing Δ*dlp1* strain. No enzymatic activity or UQ was detected in the *COQ1*-expressing Δ*dps1* strain. The author then demonstrated the binding of Coq1 and Dps1 by co-immunoprecipitation, and the formation of tetramer consisting of Coq1 and Dps1 was detected in *S. pombe*. Thus, Coq1 is functional when expressed alone in *E. coli* and in budding yeast, but is only functional as a partner with Dps1 in fission yeast. These results provide important insights into the process of how PDSs evolved from homo- to hetero-types.

In chapter 3, the author analyzed the relationship between sulfide metabolism and UQ in fission yeast. To know the mechanism how sulfide is highly accumulated in UQ deficient fission yeast cells, the author compared the sulfide production level in mutants involved in sulfide metabolism. The result indicated that sulfide is generated only by sulfite reductase. It also indicated that sulfide is consumed mostly by the synthesis of cysteine. Moreover, addition of cysteine lowered the amount of sulfide in sulfide production strains. To better understand the relation between sulfide metabolism and UQ, the author next expressed *cys1a*, *met17* and *hmt2*, which encoding the cysteine synthase, homocysteine synthase and sulfide-quinone oxidoreductase, respectively, in UQ deficient fission yeast mutants and other mutants. Lowered productions of sulfide were detected from the transformants only except for *hmt2* in UQ deficient mutants. This result indicated that amount of sulfide is controlled by both UQ dependent and independent mechanisms.

In chapter 4, the author described the modification of the isoprenoid side chain length of MK in *E. coli* by genetic engineering. The isoprenoid side chain of UQ and MK are determined by octaprenyl diphosphate synthase IspB. Expression of the *ispB* homologs from other organisms in an *ispB* disruptant KO229 would result in production of UQ-6, UQ-7, UQ-9 and UQ-10 as main products, respecitvely. To know if the MK species in KO229 were converted, the author tested the species of MK, but only little amount of MK was detected. The author next expressed plasmids pBSSK-COQ1, pMN18, pRC10 and pLD5, which habor the hexaprenyldiphosphate synthase gene (*COQ1*) from *S. cerevisiae*, the heptaprenyl diphosphate synthase gene from *Haemophilus influenzae*, the solanesyl diphosphate synthase gene (*sdsA*) from *Rhodobacter capsulatus*, and the decaprenyl-diphosphate synthase gene (*ddsA*) from *Gluconobacter suboxydans*, respectively, in an *ubiA* disruptant MU1227. MK-9 and MK-10 were detected as the additional products besides MK-8 from the transformants of pRC10 and pLD5, respectively. These suggested that MenA showed low substrate specificity to poly-PDS and the side chain length of MK could be converted by expressing poly-PDS genes from other organisms.

From the results above, the author concluded three things: (1) *S. cerevisiae* Coq1 functions as homo- type PDS when expressed alone in *E. coli* or in budding yeast, while in *S. pombe* Coq1 only works as a partner with Dps1 in *S. pombe*. The author proved that a heterotetramer was formed by Coq1 and Dps1 in *S. pombe*. (2) The author suggested that sulfide is generated by lack of metabolic absorption of sulfide into amino acids or by attenuating the function of the electron transfer system. UQ plays an important role in sulfide oxidation in fission yeast. (3) The author established that MK species could be altered by expression of poly-PDS genes from other organisms in *E. coli*.