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SUMMARY OF DOCTORAL THESIS

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Title: Studies on a CoQ10 binding protein and CoQ10 synthesizing enzymes
（コエンザイムQ10結合タンパク質とコエンザイムQ10生合成系酵素の解析）

Summary

Coenzyme Q (CoQ or Q) plays an essential role in the electron transport chain, so its biosynthesis and regulation are very important for us to understand this basic metabolic route. The Q biosynthetic pathway has been elucidated in yeast and *E. coli*. To date, most of *COQ* genes (if additional ones exist) have been identified. The present focus of Q biosynthetic pathway is the functional characterization of *COQ* genes with unknown function and how the Coq polypeptides work in the organisms. On the other hand, its regulation is far from clearance relative to the Q biosynthesis. Especially, it is not known how the Q pool in the mitochondria is maintained and regulated. In the thesis, my works addressed some aspects of the above questions.

In chapter 1, I described a general introduction of the studies.

In chapter 2, I described an artificial polyprenyl diphosphate synthase (PDS). PDS is responsible for the synthesis of the side chain of CoQ and thus defines the CoQ species. PDS is classified as a homomer (such as IspB in *E. coli* and Coq1 in *S. cerevisiae*) or a heteromer (such as Dps1 and Dlp1 in *S. pombe* and human). Although Dps1 shares high sequence similarity with known homomeric PDS, Dps1 still needs Dlp1 as the partner to be functional in *S. pombe* and human. Surprisingly, when *dps1* or *dlp1* was expressed in an *E. coli* IspB (R321A) temperature-sensitive mutant, the strain showed a wild type-like growth at the restricted temperature, and IspB activity was restored with production of CoQ8. The further assays indicated that IspB interacted with Dlp1 (or Dps1) to form a high molecular weight complex that stabilized IspB, leading to full

functionality. These results indicate that a non-functional subunit of PDS retains the ability to regain the enzymatic function, thus providing important insights into the mechanism and evolution of PDS.

In the Chapter 3, I described a Q-binding protein required for the multiple functions of Q in *S. pombe*. It has been widely accepted that coenzyme Q (Q) exists freely in the mitochondrial membrane. However, the recent identification of a mitochondrial Q binding protein, termed Coq10, in budding yeast has the potential to change our current view of the Q status in membranes. Here, I studied the ortholog of budding yeast Coq10 (also termed Coq10) in fission yeast. Fission yeast *coq10*-null mutants exhibited a similar, albeit less severe, phenotype as Q-deficient fission yeast, including the requirement for antioxidants for proper growth on the minimal medium, increased sensitivity to H_2O_2, a high level of H_2S production, and a deficiency in respiration. The *coq10* null mutant produced nearly a normal level of Q10, suggesting that *coq10* does not belong to the group of Q biosynthetic genes. To elucidate the role of Coq10, I expressed recombinant *coq10* in *Escherichia coli*, and found that Q8 was present in purified recombinant Coq10. Mutational analysis of 13 conserved residues of Coq10 revealed that two hydrophobic amino acid residues, lysine 63 (L63) and tryptophan 104 (W104) play important roles in Coq10 binding to Q. An L63A/W104A double mutant of Coq10 exhibited lower Q-binding activity than either of the single mutants, and was unable to complement the *coq10* deleted fission yeast. In light of the observation that a human Coq10 ortholog was able to functionally compensate for the absence of *coq10* in fission yeast, the results suggest that the role of Coq10 is important for proper respiration in a variety of organisms.

From the above results, I drew the conclusions as following: (1) *E. coli* IspB is able to form a high-molecular weight complex with fission yeast Dlp1 or Dps1 to strengthen its stability, suggesting the conservation and potential evolutionary trend of PDS. (2) Coq10 is not required for the biosynthesis of Q, but is required for the functional roles of Q in every aspect. Coq10 is exclusive of the complexes of dehydrogenase in mitochondria and Coq10 binds to Q *in vivo*. Thus, the current data present the hypothesis that Q functions in a protein-mediated manner in mitochondrial membranes.