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Development of yeast strains that produce high levels of CoQ10

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Research aims

Coenzyme Q (CoQ, ubiquinone) is a component of the electron transport system in the mitochondria of eukaryotes and is essential for energy production. CoQ consists of a quinone moiety and an isoprenoid chain. Almost all living cells produce CoQ with different isoprenoid chain lengths. For example, CoQ10, which is a popular food supplement, possesses 10 isoprene units. CoQ10 is commercially produced by microorganisms, and as of yet, genetic engineering approaches to commercially synthesize this molecule have not been reported. Despite the importance of CoQ in living cells, the biosynthetic pathway of CoQ remains to be elucidated.[1)]

There were two major aims in the present study. The first was to unravel the biosynthetic pathway for Coenzyme Q in the fission yeast *Schizosaccharomyces pombe.* The second was to use the knowledge gained to produce CoQ10 in *S. pombe*. In contrast to *Saccharomyces cerevisiae,* which produces CoQ6, *S. pombe* naturally produces CoQ10, the same molecule found in humans, so it is beneficial to utilize *S. pombe* to produce CoQ10. As a first step, the genes for CoQ10 biosynthesis were investigated. The genes responsible for the synthesis of CoQ10 were then overexpressed and CoQ10 production was evaluated. Finally, CoQ10 was measured in protein kinase deletion strains.

Methods

Standard yeast culture media and genetic manipulations were used. *S. pombe* strains were grown in complete YES medium (0.5% yeast extract, 3% glucose, 225 mg/L each of adenine, leucine, uracil, histidine, and lysine hydrochloride) or in EMM medium (0.3% potassium hydrogen phthalate, 0.56% sodium phosphate, 0.5% ammonium chloride, 2% glucose, vitamins, minerals, and salts). Standard procedures were used for cloning genes in *Escherichia coli* and construction of gene deletion strains of *S. pombe*. PCR was done according to commonly used methods. Nucleotide sequences were determined by the dideoxynucleotide chain-termination method using an Applied Biosystems 3500 Genetic Analyzer. Crude lipid extracts were analyzed by normal phase thin layer chromatography (TLC) with authentic coenzyme Q10 as the standard. Normal-phase TLC was carried out on a Kieselgel 60 F_{254} plate with benzene/acetone (97 : 3, v/v). The band containing coenzyme CoQ10 was collected from the TLC plate following UV visualization and extracted with chloroform/methanol (1 : 1, v/v). The samples were dried and resolved in ethanol. Purified CoQ was further analyzed by HPLC with ethanol as the solvent. The production of H_2S was measured by the methylene blue method.[2)]

Results

The biosynthetic pathway that converts para-hydroxybenzoic acid (PHB) into CoQ10 consists of at least eight steps in *S. pombe*. Starting from the synthesis of PHB, seven enzymes are thought to be involved in CoQ10 synthesis. These steps include the condensation and transfer of the isoprenoid side chain to PHB, followed by methylations, decarboxylation and hydroxylations (Fig. 1). PHB-

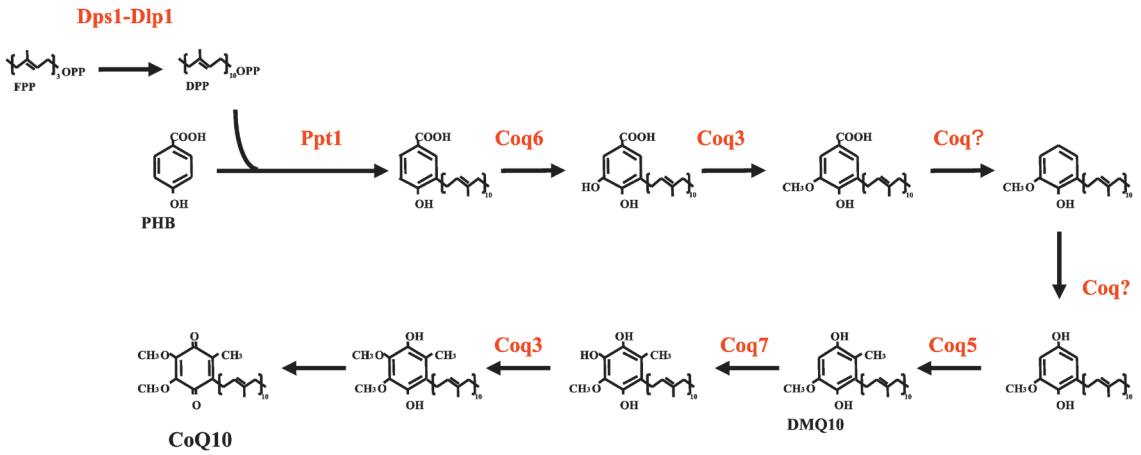

Fig. 1. Biosynthetic pathway for Coenzyme Q in fission yeast *Schizosaccharomyces pombe.* Name of gene products involved in CoQ synthesis are indicated. There are two undefined reactions in the pathway. Three genes (*coq4*, *coq8*, *and coq9*) are known to be essential for CoQ biosynthesis, but their roles in this pathway were not determined.

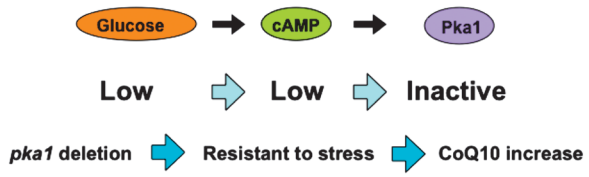

Fig. 2. cAMP-PKA signaling and the production of CoQ10. When glucose levels are decreased, the synthesis of cAMP becomes lower due to lack of activation of adenylyl cyclase. Under low levels of cAMP, PKA is inactive due to formation of a complex between the catalytic subunit (Pka1) and regulatory subunit (Cgs1). Under low glucose conditions, CoQ10 is highly produced.

polyprenyl diphosphate transferase (Ppt1/Coq2) catalyzes the condensation reaction between PHB and the isoprenoid side chain. Coq3 (O-methyltransferase) catalyzes the two O-methylation steps in the CoQ biosynthetic pathway. Coq4 is absolutely required for biosynthesis, but its enzymatic function remains to be elucidated. Coq5 (C-methyltransferase) catalyzes the single C-methylation step in the CoQ10 biosynthetic pathway. Coq6 has been identified as C5 oxygenase. Coq7 (mono-oxygenase) is involved in the penultimate step of CoQ biosynthesis. Coq8 functions as a protein kinase that phosphorylates some of the Coq proteins and stabilizes the protein complex for CoQ synthesis. Coq9 is required for CoQ biosynthesis, but its enzymatic function is not yet known. Recently it has been shown that para-aminobenzoic acid also serves as a precursor for CoQ biosynthesis in budding yeast, but it is not clear if this is true in other organisms.

Ten deletion strains for CoQ10 synthesis were constructed in *S. pombe*. These included three genes that have not yet been assigned to the CoQ biosynthetic pathway. We then investigated the phenotypes of these deletion strains and observed that they did not grow well on the EMM minimum plate. Because of the lack of CoQ10 synthesis, these strains were unable to survive in stationary phase, produced a high amount of H_2S[3] and lack of de novo uridine monophosphate synthesis.[4] We then determined the product of the precursor(s) of CoQ10 and identified DMQ10 in an *S. pombe coq7* deletion mutant. We also tried to characterize CoQ10 precursors in the *coq5* deletion strain but were unable to determine the structures of these products.

The effect of CoQ10 encapsulated with cyclodextrin was also investigated. Cyclodextrin allowed solubilization of CoQ10 so that it could be dissolved in water. We found that CoQ10 encapsulated with cyclodextrin restored the growth of *S. pombe coq* deletion strains by supporting the antioxidant property of CoQ10.[5]

Each gene that was involved in CoQ biosynthesis was then expressed in *S. pombe*. Ten genes were individually expressed and CoQ10 production was measured. As a result, *coq2,* which encodes PHB-prenyltransferase, increased the production of CoQ10 effectively, although only by 30%. We attempted to express all 10 *coq* genes in the same cells, but this resulted in growth inhibition in many cases. We then tested the deletion strains of protein kinases to measure the amount of CoQ. We found that loss of Pka1 increased the production of CoQ10, and loss of its regulatory subunit Cgs1 lowered the production of CoQ10 (Fig. 2). Pka1 is the catalytic subunit of cAMP-dependent protein kinase A (PKA), which is activated by glucose in yeast to regulate cellular proliferation. Consistently, CoQ10 was highly produced under low glucose conditions and was decreased in the presence of high glucose. Therefore, we found that PKA contributes to the production of CoQ10 in fission yeast.

Conclusion

There are at least ten genes involved in CoQ10 synthesis. Individual *coq* gene deletion strains displayed phenotypes related to respiration and oxidation-reduction of enzymes. Some strains accumulated putative precursors. DMQ10 was found in *S. pombe coq7* deletion mutant. Expression of CoQ biosynthetic genes in *S. pombe* led to the increase of CoQ10 synthesis by up to 30%. By deletion of *pka1,* a significant increase in CoQ10 was observed. The PKA pathway regulates the synthesis of CoQ10 in fission yeast *S. pombe.*

References

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