CHAPTER V

GENERAL DISCUSSION

The present study elucidates the physiological role of copper chaperone Cox17p in mammalian system. In the CHAPTER II, the genomic structure of *COX17* gene was examined. It is known that deficiencies of the CCO enzymatic activity are associated with a wide range of encephalomyopathic disorders. Recently, mutations in several CCO assembly factors such as *SURF1*, *SCO2*, *SCO1* and *COX10* were identified in the patients with this disorders (Shoubridge *et al.*, 2001). It has been believed that yeast Cox17p is involved in copper recruitment to the mitochondria and functional assembly of the CCO (Glerum *et al.*, 1996). If mammalian *COX17* is also involved in the assembly of the CCO, its mutations may be identified as a responsible gene of the encephalomyopathies. Chromosomal localization analysis revealed that there were no genetic linkage between the *COX17* gene and other responsible genes described above. None has been reported about the mutations of the *COX17* gene in the encephalomyopathic disorders to date. Does the mammalian Cox17p really involved in the functional assembly of the CCO?

In the CHAPTER III, I obtained a hint for the answer of above question by identifying the promoter elements in this gene. Luciferase assays and EMSAs revealed that transcription of the *COX17* gene was regulated by both Sp1 and Nuclear Respiration Factors (NRFs, NRF-1, 2). Sp1 and NRFs are also involved in the transcriptional regulation of the several other respiratory genes such as cytochrome c, *COX 4*, *5b*, *6c* and *7a*. My preliminary experiment using cultured cells such as NIH3T3 or AtT-20 revealed that the addition or deprivation of copper in culture medium has not drastic effect on the expression of the *COX17* gene. These results indicate that the transcription of the *COX17* regulates by the common mechanism of the several respiratory genes rather than by the change of the environmental concentration of copper ions such as the regulation of metallothionein. And these supported the possibility that mammalian Cox17p is involved in the cellular respiration.

Targeted disruption of the *COX17* gene in mice resulted in embryonic lethal (CHAPTER IV). The embryonic development of the *COX17* (-/-) mice proceeded normally till E6.5 compared with their wild type littermates, although the CCO enzymatic activity of the *COX17* (-/-) embryos were severely reduced. From these results, I suggest a possibility that the CCO-dependent oxidative phosphorylation process may be not essential for the progress of embryogenesis at least before E6.5. Recently, a study that strongly supported the possibility mentioned above was reported. The study was generation and analysis of the targeted disruption of the COQ7/CLK-1 (Nakai *et al.*, 2001). The COQ7/CLK-1 is known to be required for biosynthesis of coenzyme Q, an essential cofactor that transfers electrons from complex I or complex II to complex III in the respiratory chain. The COQ7/CLK-1 (-/-) mice also exhibited abnormal embryonic development such as unformed neural tubes and failed to survive beyond E10.5. The enlarged mitochondria with vesicular cristae were observed in the mutant embryo and enlarged lysosomes filled with disrupted membranes (Nakai *et al.*, 2001). Because of mitochondrial inner membranes mature after neurulation at E10-E12 in rat (Shepard *et al.*, 1998; Shepard *et al.*, 2000), Nakai *et al.* suggested a possibility that the energy production switches from anaerobic glycolysis to aerobic glycolysis at E10-E12(Nakai *et al.*, 2001).

The reason of why the normal embryonic development of *COX17* (-/-) mice stopped was discussed in CHAPTER IV. Interestingly, many apoptotic cells were observed in the E8.5 *COX17* (-/-) embryo. In general, mitochondria play a key role not only in the production of ATP but also in the activation of apoptotic pathways. Considering that apoptotic pathways are energy consuming processes, apoptosis of the *COX17* (-/-) embryo cells which lack CCO activity seems to be contradictory to this dogma. It was reported that reduction of mitochondrial transmembrane potential that caused by an asymmetric distribution of protons on both sides of the inner membrane

constituted an early irreversible step of the apoptosis (Zamzami *et al.*, 1995). Because of the mitochondrial transmembrane potential is created by respiratory chain complex I, III and IV and is essential for ATP synthetase (complex V) activity, the severe reduction of CCO (complex IV) activity by the targeted disruption of the *COX17* gene may cause the reduction of this membrane potential. The reduction of this potential might induce the release of apoptogenic factors such as cytochrome c, Smac/Dialbo, Omi, AIF and endonuclease G and subsequent caspases activation. To date, little was known about the relationship between the mitochondrial physiology and the release of these factors. As the mitochondria of *COX17* (-/-) cells is absence of the CCO activity, the establishment of the *COX17* (-/-) cell line may be an important tool for elucidating this relationship.

As mentioned in the CHAPTER IV, the gene disruption of *CTR1*, *ATOX1* and *CCS* were reported by four independent groups. The *CTR1* (-/-) mice were also embryonic lethal, died between embryonic day 9.5 (E9.5) to E13.5. And the phenotype of the *CTR1* (-/-) embryo is very similar to the phenotype of the *COX17* (-/-) mice: Both of the *CTR1* (-/-) and *COX17* (-/-) embryos retarded after E6.5. On the other hand, the *ATOX1* (-/-) was died perinatally (Hamaza *et al.*, 2001) and the *CCS* (-/-) was born as a mature infant (Wong *et al.*, 2000). Taken together, the embryonic lethality of the *CTR1* (-/-) mice might be caused by the dysfunction of the CCO: The targeted disruption of *CTR1* gene caused deficiency of copper in the cytosol, and then, Cox17p was not able to catch and deliver it into mitochondria. And finally, the CCO was not able to express their enzymatic activity and the embryonic development was retarded. Therefore, I think that Cox17p may be a prominent candidate as the copper chaperone involved in the early embryogenesis downstream of *CTR1*. Most recently, characterization analysis of the *CTR1* -deficient embryonic cells was reported (Lee *et al.*, 2002). The *CTR1* (-/-) embryonic cells were rescued

by the adding of copper into the cultured medium (Lee *et al.*, 2002). If my hypothesis is not incorrect, over expression of the Cox17p might also rescue the lethality of *CTR1* (-/-) cells.