Studies on Mammalian Copper Chaperone Cox17p

Division of Applied Biochemistry
Doctoral Degree Program in Agricultural Sciences
University of Tsukuba

Yoshinori Takahashi

TABLE OF CONTENTS

TABLE OF CONTENTS i

ABBREVIATIONS v

CHAPTER I GENERAL INTRODUCTION 1

CHAPTER II GENOMIC STRUCTURE OF MOUSE COX17 10

II-1. INTRODUCTION 11

II-2. MATERIALS AND METHODS 11

II-2-1. Materials 11

II-2-2. Genomic Southern hybridization analysis 12

II-2-3. Screening of mouse genomic DNA library 12

II-2-4. RNA preparation 12

II-2-5. 5'-Rapid amplification of cDNA ends 13

II-2-6. Radiation hybrid mapping 14

II-3. RESULTS 14

II-3-1. Genomic Southern hybridization analysis 15

II-3-2. Structure of the mouse *COX17* gene 15

II-3-3. Cloning of the 5'-noncoding region 16

II-3-4. Chromosomal assignment 16

II-4. DISCUSSION 17

II-5. FIGURES 20

CHAPTER III CHARACTERIZATION AND IDENTIFICATION OF PROMOTER ELEMENTS IN THE MOUSE *COX17* GENE 25

III-1. INTRODUCTION 26

III-2. MATERIALS AND METHODS 26
III-2-1. Materials 26
III-2-2. Cloning of the 5'-flanking region of the mouse *COX17* gene 27
III-2-3. RNA preparation 27
III-2-4. Primer extension analysis 27
III-2-5. Cell culture 28
III-2-6. Plasmid construction 28
III-2-7. Transient transfection 29
III-2-8. Luciferase and β-galactosidase assay 30
III-2-9. Preparation of nuclear extracts 30
III-2-10. Electrophoretic mobility shift assay (EMSA) 31
III-3. RESULTS 32
III-3-1. Cloning and characterization of the 5'-flanking region of the mouse *COX17* gene 32
III-3-2. Promoter activity of the 5'-flanking region of the mouse *COX17* gene 33
III-3-3. Functional analysis of the mouse *COX17* gene promoter 34
III-3-4. Contribution of the GC box and NRF-1 site to the promoter activity of the mouse *COX17* gene 34
III-3-5. EMSAs 36
III-4. DISCUSSION 37
III-5. TABLE AND FIGURES 39

CHAPTER IV GENERATION AND ANALYSES OF *COX17*-DEFICIENT MICE 50
IV-1. INTRODUCTION 51
IV-2. MATERIALS AND METHODS 52
IV-2-1. Materials 52

IV-2-2. Targeted disruption of *COX17* gene 52
IV-2-2-1. Plasmid construction 52
IV-2-2-2. Cell culture 53
IV-2-2-3. Transfection and analysis of ES cells 53
IV-2-2-4. Generation of chimeric and *COX17*-deficient mice 53
IV-2-3. RNA analysis 54
IV-2-4. Biochemical analysis of CCO 54
IV-2-5. Histochemical staining 54
IV-2-5-1. Hematoxylin and eosin staining 55
IV-2-5-2. Cytochrome c oxidase activity 55
IV-2-5-3. Succinate dehydrogenase activity 55
IV-2-5-4. Lactate dehydrogenase activity 55
IV-2-5. Immunohistochemistry 55
IV-3. RESULTS 55
IV-3-1. Targeted disruption of the*COX17* gene 56
IV-3-2. Analysis of *COX17* (+/-) mice 56
IV-3-3. Mice lacking *COX17* die during early embryogenesis 56
IV-3-4. Morphology and histochemistry of *COX17*-deficient embryos 57
IV-3-5. Oxidative phosphorylation and glycolysis of *COX17*-deficient embryos 57
IV-4. DISCUSSION 58
IV-5. TABLE AND FIGURES 62

CHAPTER V GENERAL DISCUSSION 72

CHAPTER VI CONCLUSION 77

ACKNOWLEDGMENTS 79

REFERENCES 80

ABBREVIATIONS

Abbreviations used are those recommended by the IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN): *Eur. J. Biochem.*, 138: 9 (1984).

AIF: apoptosis inducing factor

Atox1: antioxidant1

Ccc2: cross-complements the Ca^{2+}-sensitive phenotype of *csg1* mutants 2

CCO: cytochrome c oxidase

CCS: copper chaperone for SOD

COXI: CCO subunit I

COXII: CCO subunit II

COXIII: CCO subunit III

COXIV: CCO subunit IV

COX17: gene of Cox17p

Cox17p: CCO assembly protein 17

CRE: cAMP-responsive element

CREB: cAMP-responsive element binding protein

CTR1: gene of copper transporter 1

DIABLO: direct IAP-binding protein with low pI

HtrA: high temperature signaling complex A

MRE: metal regulatory element

NF-κB: nuclear factor κB

NGFI-B: nerve growth factor inducible clone B

NRF-1: nuclear respiratory factor 1

NRF-2: nuclear respiratory factor 2

LDH: lactate dehydrogenase

Lys7: involved in lysine biosynthesis, oxidative stress protection gene

Omi: a human homologue of the bacterial HtrA endoprotease

SCO1: gene of synthesis of CCO 1

SCO2: gene of synthesis of CCO 2

SDH: succinate dehydrogenase

Smac: second mitochondria-derived activator of caspase

SOD: super oxide disumutase

Sp1: specificity protein 1

SURF1: surfeit 1

AAP: abridged anchor primer

AUAP: abridged universal amplification primer

cDNA: complementary DNA

EMSA: electrophoretic mobility shift assay

dCTP: deoxy-cytidine 5'-triphosphate

DNA: deoxyribonucleic acid

EF cell: embryonic fibroblast cell

ES cell: embryonic stem cell

FITC: fluorescein isothiocyanate

GFP: green fluorescent protein

GSP: gene specific primer

LOD: logarithm of the odds

mRNA: messenger RNA

neo: neomycin phosphotransferase

neo^r: neomycin resistance gene

PCR: polymerase chain reaction

RACE: rapid amplification of cDNA ends

RH mapping: radiation hybrid mapping

RNA: ribonucleic acid

RSV: Rous sarcoma virus

SV40: simian virus 40

DAB: 3,3-diaminobenzidine

DMEM: Dulbecco's modified Eagle's medium

EDTA: ethylenediaminetetraacetic acid

EGTA: ethyleneglycol bis (2-aminoethylether) tetraacetic acid

FBS: fetal bovine serum

HEPES: N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid

LIF: leukemia inhibitory factor

β -NAD: β-nicotinamide adenine dinucleotide

NBT: nitro blue tetrazolium

PBS: phosphate buffered saline

PMSF: phenylmethanesulfonyl fluoride

SDS: sodium dodecyl sulfate

SSC: standard saline citrate

TPCK: N-tosyl-L-phenylalanyl chloromethyl ketone