

## ACKNOWLEDGMENTS

I wish to express my sincere thanks to Professor Akiyoshi Fukamizu (Center for Tsukuba Advanced Research Alliance, Institute of Applied Biochemistry, University of Tsukuba) and Professor Eisuke Munekata (Institute of Applied Biochemistry, University of Tsukuba) for valuable advice, encouragement and support during the course of this study.

I would like to express my gratitude to Dr. Kako (Institute of Applied Biochemistry, University of Tsukuba) for helpful guidance and encouragement.

I am particularly grateful to Professor Tadashi Baba (Institute of Applied Biochemistry, University of Tsukuba) for his helpful discussion, advice and encouragement. I would like to express my thanks to Professor Jun-ichi Hayashi (Institute of Biological Sciences, University of Tsukuba), Dr. Hiroko Kodama (Teikyo University School of Medicine), Dr. Shin-ichi Kashiwabara (Institute of Applied Biochemistry, University of Tsukuba), Dr. Kazuto Nakada (Institute of Biological Sciences, University of Tsukuba) and Dr. Keiji Tanimoto (Institute of Applied Biochemistry, University of Tsukuba) for their helpful advice and encouragement for carrying out the generation and analysis of transgenic mice. I also wish to express my thanks to Mr. Ko Ohmura (Institute of Applied Biochemistry, University of Tsukuba) and Mr. Takayuki Ohishi (Institute of Applied Biochemistry, University of Tsukuba) for their help and helpful advise for the isolation of gene and determination of transcription start site.

I am deeply indebted to Dr. Hidehito Mukai (Peptide Biosignal Engineering Unit, Mitsubishi Kagaku Institute of Life Sciences) for his valuable discussions and encouragement. Finally, I wish to express my thanks to all the members of Laboratory of Peptide Biochemistry, University of Tsukuba, and my parents for continuous encouragement.

## REFERENCES

- Amaravadi, R., Glerum, D.M. and Tzagoloff, A. (1997). Isolation of a cDNA encoding the human homolog of *COX17*, a yeast gene essential for mitochondrial copper recruitment. *Hum. Genet.*, 99, 329-333.
- Bachman, N.J. (1995). Isolation and characterization of the functional gene encoding bovine cytochrome c oxidase subunit IV. *Gene*, 162, 313-318.
- Basu, A., Park, K., Atchison, M.L., Carter, R.S., Avadhani, N.G. (1993). Identification of a transcriptional initiator element in the cytochrome c oxidase subunit Vb promoter which binds to transcription factors NF-E1 (YY-1, delta) and Sp1. *J. Biol. Chem.*, 268, 4188-4196
- Beers J., Glerum, D. M. and Tzagoloff A. (1997) Purification, characterization, and localization of yeast Cox17p, a mitochondrial copper shuttle. *J. Biol. Chem.*, 272, 33191-33196.
- Benton, W. D. and Davis, R. W. (1977). Screening lambda<sub>gt</sub> recombinant clones by hybridization to single plaques in situ. *Science*, 196, 180-182.
- Bhat, B.M. and Wold, W.S. (1985). ATTAAA as well as downstream sequences are required for RNA 3'-end formation in the E3 complex transcription unit of adenovirus. *Mol. Cell Biol.*, 5, 3183-3193.
- Bird, A.P. (1986). CpG-rich islands and the function of DNA methylation. *Nature*, 321, 209-213.
- Capaldi, R. A., Marusich M. F., and Taanman J. W. (1995) Mammalian cytochrome c oxidase : characterization of enzyme and immunological detection of subunits in tissue extracts and whole cells. *Methods Enzymol.*, 260, 117-132.
- Carter, R.S. and Avadhani, N.G. (1991). Cloning and characterization of the mouse cytochrome c oxidase subunit IV gene. *Arch. Biochem. Biophys.*, 288, 97-106.
- Carter, R.S. and Avadhani, N.G. (1994). Cooperative binding of GA-binding

- protein transcription factors to duplicated transcription initiation region repeats of the cytochrome c oxidase subunit IV gene. *J. Biol. Chem.*, 269, 4381-4387.
- Carter, R.S., Bhat, N.K., Basu, A., Avadhani, N.G. (1992). The basal promoter elements of murine cytochrome c oxidase subunit IV gene consist of tandemly duplicated ets motifs that bind to GABP-related transcription factors. *J. Biol. Chem.*, 267, 23418-23426.
- Chen, Z.W., Bergman, T., Östenson, C.G., Efendic, S., Mutt, V. and Jörnvall, H. (1997). Characterization of dopuin, a polypeptide with special residue distributions. *Eur. J. Biochem.*, 249, 518-522.
- Chomczynski, P. and Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.*, 162, 156-159.
- Clayton, D.A. (1991). Replication and transcription of vertebrate mitochondrial DNA. *Annu. Rev. Cell Biol.*, 7, 453-478.
- Culotta V.C., Klomp L.W., Strain J., Casareno R.L., Krems B., and Gitlin J.D. (1997). The copper chaperone for superoxide dismutase. *J. Biol. Chem.*, 272, 23469-23472.
- Darnell, J.E. Jr. (1982). Variety in the level of gene control in eukaryotic cells. *Nature*, 297, 365-371.
- DeBry, R.W. and Seldin, M.F. (1996). Human/mouse homology relationships. *Genomics*, 33, 337-351.
- Evans, M.J. and Scarpulla, R.C. (1989). Interaction of nuclear factors with multiple sites in the somatic cytochrome c promoter. Characterization of upstream NRF-1, ATF, and intron Sp1 recognition sequences. *J. Biol. Chem.*, 264, 14361-14368.
- Feinberg, A. P. and Vogelstein, B. (1983). A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal.*

*Biochem.*, 132, 6–13.

- Frohman, M.A., Dush, M.K. and Martin, G.R. (1988). Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. *Proc. Natl. Acad. Sci. USA*, 85, 8998-9002.
- Glerum, D.M., Shtanko, A. and Tzagoloff, A. (1996). Characterization of *COX17*, a yeast gene involved in copper metabolism and assembly of cytochrome oxidase. *J. Biol. Chem.*, 271, 14504-14509.
- Gopalakrishnan, L. and Scarpulla, R.C. (1994). Differential regulation of respiratory chain subunits by a CREB-dependent signal transduction pathway. Role of cyclic AMP in cytochrome c and COXIV gene expression. *J. Biol. Chem.*, 269, 105-113.
- Greene, J.M., Larin, Z., Taylor, I.C., Prentice, H., Gwinn, K.A. and Kingston, R.E. (1987). Multiple basal elements of a human hsp70 promoter function differently in human and rodent cell lines. *Mol. Cell Biol.*, 7, 3646-3655.
- Hamza, I., Faisst A., Prohaska J., Chen J., Gruss P. and Gitlin J. D. (2001). The metallochaperone Atox1 plays a critical role in perinatal copper homeostasis. *Proc. Natl. Acad. Sci. USA.*, 98, 6848-6852.
- Heaton, D., Nittis T., Srinivasan C., and Winge D. R. (2000). Mutational analysis of the mitochondrial copper metallochaperone Cox17. *J. Biol. Chem.*, 275, 37582-37587.
- Heinemeyer, T., Wingender, E., Reuter, I., Hermjakob, H., Kel, A.E., Kel, O.V., Ignatieva, E.V., Ananko, E.A., Podkolodnaya, O.A., Kolpakov, F.A., Podkolodny, N.L., Kolchanov, N.A. (1998). Databases on transcriptional regulation: TRANSFAC, TRRD and COMPEL. *Nucleic Acids Res.*, 26, 362-367.
- Herzig, R.P., Scacco, S., Scarpulla, R.C. (2000). Sequential serum-dependent

- activation of CREB and NRF-1 leads to enhanced mitochondrial respiration through the induction of cytochrome c. *J. Biol. Chem.*, 275, 13134-13141.
- Himmelblau, E., Amasino, R.M. (2000). Delivering copper within plant cells. *Curr. Opin. Plant Biol.*, 3, 205-210.
- Ho, S.N., Hunt, H.D., Horton, R.M., Pullen, J.K., Pease, L.R. (1989). Site-directed mutagenesis by overlap extension using the polymerase chain reaction. *Gene*, 77, 51-59.
- Inoue K, Nakada K, Ogura A, Isobe K, Goto Y, Nonaka I, Hayashi JI. (2000). Generation of mice with mitochondrial dysfunction by introducing mouse mtDNA carrying a deletion into zygotes. *Nat. Genet.*, 26, 176-181.
- Ishii, S., Kadonaga, J.T., Tjian, R., Brady, J.N., Merlino, G.T. and Pastan, I. (1986). Binding of the Sp1 transcription factor by the human Harvey ras1 proto-oncogene promoter. *Science*, 232, 1410-1413.
- Jaksch, M., Ogilvie, I., Yao, J., Kortenhaus, G., Bresser, H.G., Gerbitz, K.D. and Shoubridge, E.A. (2000). Mutations in SCO2 are associated with a distinct form of hypertrophic cardiomyopathy and cytochrome c oxidase deficiency. *Hum. Mol. Genet.*, 9, 795-801.
- Jaksch, M., Paret C., Stucka R., Horn N., Müller-Höcker J., Horvath R., Trepesch N., Stecker G., Freisinger P., Thirion C., Müller J., Lunkwitz R., Rödel G., Shoubridge E. A., and Lochmüller H. (2001). Cytochrome c oxidase deficiency due to mutations in SCO2, encoding a mitochondrial copper-binding protein, is rescued by copper in human myoblasts. *Hum. Mol. Genet.*, 10, 3025-3035.
- Kako K, Wakamatsu H, Hamada T, Banasik M, Ohata K, Niki-Kuroiwa T, Suzuki S, Takeuchi J, Ishida N. (1998). Examination of DNA-binding activity of neuronal transcription factors by electrophoretical mobility

- shift assay. *Brain Res. Brain Res. Protoc.*, 2, 243-249.
- Kako, K., Tsumori, K., Ohmasa, Y., Takahashi, Y. and Munekata, E. (2000). The expression of Cox17p in rodent tissues and cells. *Eur. J. Biochem.*, 267, 6699-6707.
- Kodama, H. and Murata, Y. (1999). Molecular genetics and pathophysiology of Menkes disease. *Pediatr. Int.*, 41, 430-435.
- Kuo, Y. M., Zhou B., Cosco D., and Gitschier J. (2001). The copper transporter CTR1 provides an essential function in mammalian embryonic development. *Proc. Natl. Acad. Sci. USA.*, 98, 6836-6841.
- Lee, J., Prohaska J. R., and Thiele D. J. (2001). Essential role for mammalian copper transporter Ctr1 in copper homeostasis and embryonic development. *Proc. Natl. Acad. Sci. USA.*, 98, 6842-6847.
- Lenka, N., Vijayasarathy, C., Mullick, J., Avadhani, N.G. (1998). Structural organization and transcription regulation of nuclear genes encoding the mammalian cytochrome c oxidase complex. *Prog. Nucleic Acid Res. Mol. Biol.*, 61, 309-44.
- Lin S.J. and Culotta V.C. (1995). The ATX1 gene of *Saccharomyces cerevisiae* encodes a small metal homeostasis factor that protects cells against reactive oxygen toxicity. *Proc. Natl. Acad. Sci. USA.*, 92, 3784-3788.
- Mansour, S. L., Thomas K. R., and Capecchi M. R. (1988). Disruption of the proto-oncogene int-2 in mouse embryo-derived stem cells: a general strategy for targeting mutations to non-selectable genes. *Nature*, 336, 348-352.
- McCarthy, L.C., Terrett, J., Davis, M.E., Knights, C.J., Smith, A.L., Critcher, R., Schmitt, K., Hudson, J., Spurr, N.K. and Goodfellow, P.N. (1997). A first-generation whole genome-radiation hybrid map spanning the mouse genome. *Genome Res.*, 7, 1153-1161.
- Mitchell, P.J. and Tjian, R. (1989). Transcriptional regulation in mammalian cells by sequence-specific DNA binding proteins. *Science*, 245, 371-

378.

- Mount, S.M. (1982). A catalogue of splice junction sequences. *Nucleic Acids Res.*, 10, 459-472.
- Nishihara, E., Furuyama, T., Yamashita, S. and Mori, N. (1998). Expression of copper trafficking genes in the mouse brain. *Neuroreport*, 9, 3259-3263.
- O'Halloran, T. V. and Culotta V. C. (2000). Metallochaperones, an intracellular shuttle service for metal ions. *J. Biol. Chem.*, 275, 25057-25060.
- Punter F.A., Adams D.L., and Glerum D.M. (2000) Characterization and localization of human COX17, a gene involved in mitochondrial copper transport. *Hum. Genet.*, 107, 69-74.
- Papadopoulou, L.C., Sue, C.M., Davidson, M.M., Tanji, K., Nishino, I., Sadlock, J.E., Krishna, S., Walker, W., Selby, J., Glerum, D.M., Coster, R.V., Lyon, G., Scalais, E., Lebel, R., Kaplan, P., Shanske, S., De Vivo, D.C., Bonilla, E., Hirano, M., DiMauro, S. and Schon, E.A. (1999). Fatal infantile cardioencephalomyopathy with COX deficiency and mutations in *SCO2*, a COX assembly gene. *Nat. Genet.*, 23, 333-337.
- Paret, C., Ostermann, K., Krause-Buchholz, U. Rentzsch, A. and Rodel, G. (1999). Human members of the *SCO1* gene family: complementation analysis in yeast and intracellular localization. *FEBS Lett.*, 447, 65-70.
- Ricardo U., Manuel O., and Mauricio G. (1998) Essentiality of copper in humans. *Am. J. Clin. Nutr.*, 67, 952S-959S
- Roise, D. and Schatz, G. (1988). Mitochondrial presequences. *J. Biol. Chem.*, 263, 4509-4511.
- Sanger, F., Nicklen, S., Coulson, A.R. (1977) DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. U S A*, 74, 5463-5467.
- Sanger, F., Coulson, A.R., Barrell, B.G., Smith, A.J., Roe, B.A. (1980). Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. *J. Mol. Biol.*, 143, 161-178.
- Scarpulla, R. C. (2002). Transcriptional activators and coactivators in the

- nuclear control of mitochondrial function in mammalian cells. *Gene.*, 286, 81-89.
- Sciacco, M. and Bonilla E. (1996). Cytochemistry and immunocyto-chemistry of mitochondria in tissue sections. *Methods Enzymol.*, 264, 509–521.
- Seelan, R.S., Gopalakrishnan, L., Scarpulla, R.C. and Grossman, L.I. (1996). Cytochrome c oxidase subunit VIIa liver isoform. Characterization and identification of promoter elements in the bovine gene. *J. Biol. Chem.*, 271, 2112-2120.
- Seelan, R.S. and Grossman, L.I. (1992). Structure and organization of the heart isoform gene for bovine cytochrome c oxidase subunit VIIa. *Biochemistry*, 31, 4696-4704.
- Seelan, R.S. and Grossman, L.I. (1997). Structural organization and promoter analysis of the bovine cytochrome c oxidase subunit VIIc gene. A functional role for YY1. *J. Biol. Chem.*, 272, 10175-10181.
- Shoubridge, E.A. (2001). Cytochrome c oxidase deficiency. *Am. J. Med. Genet.*, 106, 46-52.
- Srinivasan, C., Posewitz, M.C., George, G.N., and Winge, D.R. (1998). Characterization of the copper chaperone Cox17 of *Saccharomyces cerevisiae*. *Biochemistry*, 37, 7572-7577.
- Sucharov, C., Basu, A., Carter, R.S., Avadhani, N.G. (1995). A novel transcriptional initiator activity of the GABP factor binding ets sequence repeat from the murine cytochrome c oxidase Vb gene. *Gene Expr.*, 5, 93-111.
- Takahashi, Y., Kako K., Arai H., Ohishi T., Takehara A., Fukamizu A., and Munekata E. (2002). Characterization and identification of promoter elements in mouse *COX17* gene. *Biochimica. Biophysica. Acta.*, 1574, 359-364.
- Takahashi, Y., Kako, K., Ohmura, K., Tsumori, K., Ohmasa, Y., Kashiwabara, S.,



- Baba, T., Munekata, E. (2001). Genomic Structure of mouse copper chaperone, COX17. *DNA Seq.*, 12, 305-318.
- Takahashi, Y., Kako K., Kashiwabara S., Takehara A., Inada Y., Arai H., Nakada K., Kodama H., Hayashi J., Baba T. and Munekata E. (2002). Mammalian copper chaperone Cox17p has an essential role in activation of cytochrome c oxidase and embryonic development. *Mol. Cell. Biol.*, 22, 7614-7621.
- Takenouchi, T., Fujimoto, M., Shimamoto, A. and Munekata, E. (1999). Isolation and characterization of Cox17p from porcine heart by determining its survival-promoting activity in NIH3T3 cells. *Biochim. Biophys. Acta*, 1472, 498-508.
- Tazi, J. and Bird, A. (1990). Alternative chromatin structure at CpG islands. *Cell*, 60, 909-920.
- Yoshikawa, S., Shinzawa-Itoh, K., Nakashima, R., Yaono, R., Yamashita, E., Inoue, N., Yao, M., Fei, M.J., Libeu, C.P., Mizushima, T., Yamaguchi, H., Tomizaki, T. and Tsukihara T. (1995). Redox-coupled crystal structural changes in bovine heart cytochrome c oxidase., *Science*, 280, 1723-1729.
- Valentine, J.S. and Gralla, E.B. (1997). Delivering copper inside yeast and human cells. *Science*, 278, 817-818.
- Virbasius, C.A., Virbasius, J.V. and Scarpulla, R.C. (1993). NRF-1, an activator involved in nuclear-mitochondrial interactions, utilizes a new DNA-binding domain conserved in a family of developmental regulators. *Genes Dev.*, 7, 2431-2445.
- Virbasius, J.V. and Scarpulla, R.C. (1991). Transcriptional activation through ETS domain binding sites in the cytochrome c oxidase subunit IV gene. *Mol. Cell Biol.*, 11, 5631-5638.
- Wong, P. C., Waggoner, D., Subramaniam, J. R., Tessarollo, L., Bartnikas, T. B.,

Culotta, V. C., Price, D. L., Rothstein, J., and Gitlin, J. D. (2000). Copper chaperone for superoxide dismutase is essential to activate mammalian Cu/Zn superoxide dismutase. *Proc. Natl. Acad. Sci. USA.*, 97, 2886-2891.

Zamzami, N., Marchetti, P., Castedo, M., Zanin, C., Vayssiere, J.L., Petit, P.X., Kroemer, G. (1995). Reduction in mitochondrial potential constitutes an early irreversible step of programmed lymphocyte death in vivo. *J. Exp. Med.*, 181, 1661-1672.