Hum Genet (2000) 107 : 69–74
Digital Object Identifier (DOI) 10.1007/s004390000339

ORIGINAL INVESTIGATION

Fiona A. Punter · Denise L. Adams · D. Moira Glerum

Characterization and localization of human *COX17*, a gene involved in mitochondrial copper transport

Received: 13 April 2000 / Accepted: 31 May 2000 / Published online: 8 July 2000

Abstract Deficiencies in cytochrome oxidase (COX), the terminal enzyme of the mitochondrial respiratory chain, are relatively rare but most often lethal. The underlying causes are beginning to be elucidated, and most mutations are thought to affect the function of proteins involved in assembling the holoenzyme. COX17 is such an assembly protein and is thought to recruit copper to mitochondria for incorporation into the COX apoenzyme. Here we present the gene structure, the expression, and chromosomal localization for *COX17*, a candidate gene for COX deficiency. The *COX17* gene spans approximately 8 kb of human genomic DNA and encodes a transcript of approximately 450 bp that is expressed in all tissues tested. Although the *COX17* gene was previously mapped to chromosome 13q14-21, our results suggest that a *COX17* pseudogene maps to this region. The pseudogene contains several nucleotide changes, including one that would result in an altered amino acid in the putative copper binding domain. We have localized the gene encoding the COX17 protein to the long arm of chromosome 3 by radiation hybrid mapping. Deciphering of the *COX17* genomic structure will allow this gene to be assessed for mutations in COX deficient patients.

Introduction

Cytochrome oxidase (COX) is the terminal electron acceptor of the mitochondrial respiratory chain and contributes to the proton motive force that drives the synthesis of ATP (Capaldi 1990). This multi-subunit enzyme is remarkably conserved between lower and higher eukaryotes and consists of 13 polypeptide subunits (Saraste 1990; Taanman and Capaldi 1992). The three largest subunits are encoded in mitochondrial DNA and form the catalytic core of the enzyme. They contain the three copper atoms and two heme A molecules that serve as prosthetic groups in the holoenzyme and are directly involved in electron transfer. The remaining ten subunits of the COX holoenzyme are presumed to play a structural role and are encoded by nuclear DNA. In addition to the constituent subunits, there are a host of nuclear gene products that are required for assembly of the holoenzyme (McEwen et al. 1986; Tzagoloff and Dieckmann 1990). These assembly factors encompass proteins involved in expression of the individual subunits and in the provision of the requisite prosthetic groups to the apoenzyme. Studies in yeast have shown that these assembly proteins are essential components of the cytochrome oxidase biosynthetic pathway (Glerum et al. 1997). One such assembly factor is Cox17p (Cox17 protein, according to yeast nomenclature), which is a small protein presumed to shuttle copper from the cytosol into the mitochondrial intermembrane space (Glerum et al. 1996a). *COX17* was first identified and characterized in a yeast COX-deficient mutant in which the respiration deficient phenotype is characterized by an inability to complete cytochrome oxidase assembly. It appears that the failure to assemble a functional enzyme in *cox17* mutants is probably due to an inability to provide the apoenzyme with its required copper atoms (Beers et al. 1997; Srinivasan et al. 1998).

Specific deficiencies of cytochrome oxidase in humans have been described over the past two decades (DiMauro et al. 1987; Robinson et al. 1987), with most cases being associated with severe neonatal or infantile lactic acidosis and early death. Biochemical studies of COX deficient patients suggested that a failure to assemble the holoenzyme might underlie the majority of inherited COX deficiencies (Adams et al. 1997; Glerum et al. 1988; Lombes et al. 1991) and mutations in three assembly proteins have now been identified. Mutations in the *SURF1* gene have been shown to underlie the COX deficiency in patients

GenBank Accession numbers: AF269243, AF269244, AF269245, AF272832

F. A. Punter · D. L. Adams · D. M. Glerum (✉)
Department of Medical Genetics, University of Alberta,
8-33 Medical Sciences Building, Edmonton,
Alberta T6G 2H7 Canada
e-mail: moira.glerum@ualberta.ca,
Tel.: +1-780-492-4563, Fax: +1-780-492-1998

with Leigh syndrome (Tiranti et al. 1998; Zhu et al. 1998). Patients with a fatal cardioencephalomyopathy or hypertrophic cardiomyopathy, marked by a severe COX deficiency, have been shown to harbor mutations in the *SCO2* gene (Jaksch et al. 2000; Papadopoulou et al. 1999). Most recently mutations in *COX10* have also been shown to underlie an autosomal recessively inherited COX deficiency (Valnot et al. 2000). In all instances, previous work with the corresponding yeast homologs had demonstrated a role for these proteins in respiratory chain complex assembly (Glerum et al. 1996b; Mashkevich et al. 1997; Nobrega et al. 1990).

Because *COX17* may also be involved in human COX deficiency, we have characterized and localized *COX17* in the human genome. The human *COX17* ortholog was originally identified by functional complementation of a yeast *cox17* null mutant (Amaravadi et al. 1997) but until recently was not mapped in the human genome. Here we present the genomic structure, expression analysis, and chromosomal localization of the *COX17* gene and a related processed pseudogene, *COX17P*.

Materials and methods

Clones

Bacterial artificial chromosomes (BACs) containing human *COX17* (BAC 274K10, BAC 26I10, and BAC 59M4) and *COX17P* (BAC 271E16, BAC 162M22, and BAC 271I16) were isolated by the Medical Research Council Genome Resource Facility at the Hospital for Sick Children, Toronto, Canada, using the human *COX17* cDNA as a probe. A P1 clone (P1 18110) harboring human *COX17* was similarly isolated by Genome Systems (St. Louis, USA).

Southern and northern blot analysis

Genomic DNA was isolated from human blood by standard methods, while BAC and P1 DNA were isolated according to the supplier's protocols. For Southern analysis 5 μg genomic DNA, 5 μg BAC DNA, and 2 μg P1 DNA, were digested with either *Eco*RI, or *Hin*dIII, or both (GibcoBRL, Burlington, Canada). Northern analysis was carried out on a human adult Multiple Tissue Northern blot (Clontech Laboratories, Palo Alto, USA), with human glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and S26 ribosomal protein (*S26*; Vincent et al. 1993) serving as control probes to normalize for loading differences in the RNA. All blotting was carried out according to standard protocols, using [α-^{32}P]dCTP-labeled (Random Primers DNA Labeling System, GibcoBRL) *COX17* cDNA as a probe.

Direct sequencing of *COX17* and *COX17P*

COX17 was sequenced from P1 18110 and BAC 274K10 with the Thermo-Sequenase radiolabeled terminator cycle sequencing kit (Amersham Pharmacia Biotech). *COX17P* was sequenced from BAC 271E16 and from a polymerase chain reaction (PCR) fragment amplified from human genomic DNA, using primers derived from the cDNA sequence.

Expression analysis of *COX17* and *COX17P* in lymphoblastoid RNA

Total RNA from a lymphoblastoid cell line was isolated using TRIzol reagent (GibcoBRL). First-strand cDNA synthesis was carried out with 2 μg RNA and an oligo(dT)$_{12–18}$ primer (GibcoBRL), followed by PCR of the reverse transcribed (RT) products. The forward primer, COX17H-1 (5′-ggaagtgactgcagacgaatcgg-3′), which is specific to the 5′ end of *COX17*, was used with the reverse primer COX17H-4 (5′-agttcgtcaaagaactccca-3′) at an annealing temperature of 65°C to detect expression of *COX17*. Similarly, primer COX17B-2 (5′-ttaaagaaatcggacgaattgg-3′), which is specific for the 5′ end of *COX17P*, was used with COX17H-4 at an annealing temperature of 50°C to test for the expression of *COX17P*.

Radiation hybrid mapping

Radiation hybrid mapping was carried out using the GeneBridge 4 Human/Hamster Radiation Hybrid Panel (Research Genetics, Huntsville, USA), which is composed of 93 radiation hybrids containing fragments of the whole human genome in a hamster background. Primers COX17H-3 (5′-tgagtctcaggagaagaagc-3′) and COX17H-4 were used to map *COX17P* with an annealing temperature of 58°C. *COX17* was localized using the intronic primers 18110-13 (5′-gccacttcctgtggaaag-3′) and 18110-18 (5′-gacttgaagagttagctac-3′) at an annealing temperature of 54°C.

Results

Southern blot analysis of *COX17*

To determine the genomic structure of *COX17*, a number of clones harboring the gene were obtained. A single P1 and six BAC clones were obtained by screening genomic libraries with the *COX17* cDNA. DNA from each of the clones was purified, and 2–5 μg was digested with *Eco*RI, *Hin*dIII, and both enzymes together. Subsequent Southern blot analysis using the *COX17* cDNA as a probe suggested the presence of two copies of *COX17* in the human

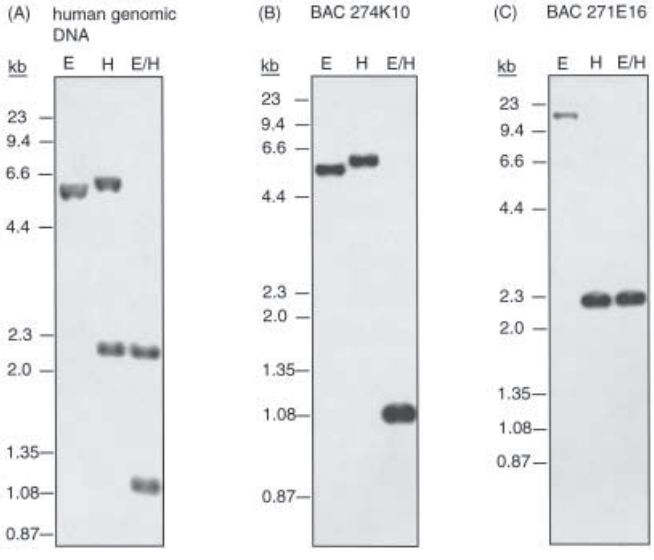

Fig. 1A–C Southern blot analysis of *COX17*. Hybridization of the *COX17* cDNA to human genomic DNA (**A**), BAC 274K10 DNA (**B**), and BAC 271E16 DNA (**C**) was carried out as described in the text. The migration of molecular weight markers is indicated *on the left side of each blot*. *E* DNA digested with *Eco*RI; *H* DNA digested with *Hin*dIII; *E/H* DNA digested with *Eco*RI and *Hin*dIII

genome. As seen in Fig. 1A, digestion of genomic DNA with *Eco*R1 and *Hin*dIII together yields two fragments. The smaller fragment, which is approximately 1.2 kb in size, is also present in the double digest lane of BAC 274K10 (Fig. 1B). The larger fragment in the double digest lane of the genomic DNA Southern blot, which is approximately 2.3 kb in size, is present in digested BAC 271E16 DNA (Fig. 1C). These two distinct banding patterns were corroborated in the other BAC clones examined.

Genomic structure of *COX17* and *COX17P*

Results from the Southern analysis suggested that several of the BAC clones contained similar or identical inserts and one representative clone for each of the two banding patterns was selected for further study. DNA sequencing subsequently confirmed that there are two distinct copies of *COX17* in the human genome, which we have denoted *COX17* and *COX17P*.

COX17 contains coding sequence that is identical to the published cDNA sequence, in addition to previously undescribed intronic sequence. The *COX17* gene consists of three exons and two introns and is depicted in schematic form in Fig. 2A. Partial sequencing of the intronic DNA verified that the intron-exon boundaries conform to the canonical GT/AG rule. The approximate size of the first intron was determined by PCR, using primers that anneal to the 3′ end of exon 1 and the 5′ end of exon 2. A PCR product of approximately 2 kb was generated (data not shown). Interestingly, the 5′ end of the first intron revealed sequence that is rich in a CTGCCCT repeat (data not shown), which is not recognized by RepeatMasker (from http://www.dot.imgen.bcm.tmc.edu:9331/). The significance of this motif is currently unknown. PCR with primers that anneal to the 3′ end of exon 2 and the 3′ end of intron 2 (primer 18110-13; 5′-gccacttcctgtggaaag-3′) demonstrated that intron 2 spans approximately 5.5 kb of DNA (data not shown). Taken together, these data indicate that the *COX17* gene is contained in approximately 8 kb of genomic DNA.

COX17P does not contain any intronic DNA and several nucleotide changes exist between the gene and the cDNA. Within the 5′ untranslated region the first 13 nucleotides of *COX17P* and the *COX17* cDNA do not share any identity. A nucleotide difference also exists at position –64, where a C→T transition is present in *COX17P* (nucleotides are numbered as in the published cDNA sequence; Amaravadi et al. 1997). In addition to the differences found in the 5′ untranslated region, four nucleotide changes, consisting of 32 C→T (Pro→Leu), 53 A→G (Lys→Arg), 73 G→A (Ala→Thr), and 96 G→A (silent), are found within the coding region (Fig. 2B). As shown in Fig. 3, the nucleotide difference at position 73 occurs within the putative copper-binding domain of COX17 at an alanine residue that is conserved in all mammalian

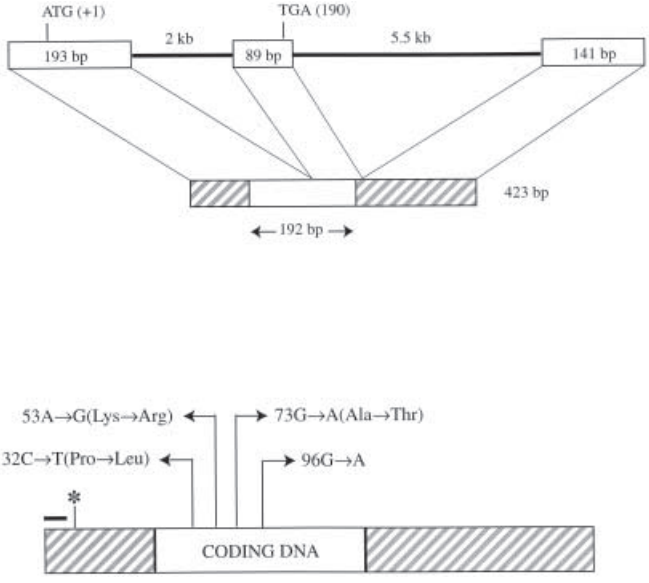

Fig. 2A,B Organization of *COX17* and *COX17P*. **A** A schematic representation of the *COX17* gene and the corresponding cDNA transcript is shown. In the *COX17* gene exons are depicted as *white boxes* (exon size is shown *within the box*), while introns are represented by *solid black lines*. The first methionine codon at nucleotide position +1 is shown, as is the stop codon at nucleotide position 190. Within the *COX17* transcript *hatched lines* represent the untranslated region; *white box* coding region. The length of the *COX17* transcript given does not include the polyA tail. The lines joining the gene and cDNA indicate how the three exons contribute to the transcript. The diagram is not to scale. **B** *COX17P* is similar, but not identical to the *COX17* cDNA. The four nucleotide differences occurring within the coding region are shown. *Hatched lines* represent noncoding DNA; *bar* above the gene represents the nucleotide differences at the 5′ end of *COX17P*; *asterisk* nucleotide difference at position 23

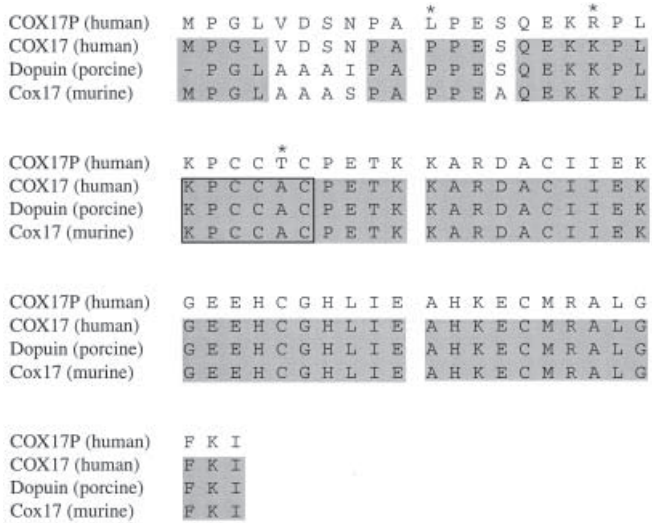

Fig. 3 Alignment of the predicted amino acid sequences of homologous mammalian COX17 proteins ("dopuin" is the original name given to porcine Cox17). Identical residues in the three expressed proteins are highlighted by gray shading; *asterisks* amino acid differences in human COX17P; *black box* putative copper-binding domain. Sequence references are noted in text

COX17 proteins identified to date. None of the *COX17* expressed sequence tags (ESTs) in the database (GenBank; http://www.ncbi.nlm.nih.gov/) contain the nucleotide changes seen in *COX17P*, which also has the polyadenylation signal seen in the cDNA. These results suggest that *COX17P* is a processed pseudogene.

Expression of *COX17*

A single transcript was detected when the *COX17* cDNA was hybridized to a human multiple tissue northern blot. As seen in Fig. 4, *COX17* appears to be most highly expressed in tissues with a high aerobic demand. Although not clear from the figure, longer exposure revealed the presence of *COX17* mRNA in all lanes, suggesting that it is ubiquitously expressed, as expected for a gene that is involved in an essential process such as oxidative phosphorylation. Because the *COX17* transcript was smaller than the lowest marker on the commercial northern blot, the blot was also probed with the *S26* cDNA. The *COX17* cDNA hybridizes to a transcript that migrates below the 700 bp *S26* transcript, consistent with an expected transcript size of approximately 450 bp.

To determine whether both *COX17* and *COX17P* are expressed RT-PCR was performed with RNA isolated from a lymphoblastoid cell line. Using primers COX17H-1 (specific to the 5′ end of *COX17* and the *COX17* cDNA) and COX17H-4, a 350-bp product was amplified from the lymphoblastoid cDNA pool (*COX17*, +RT, Fig. 5). When the same experiment was carried out using primers COX17B-2 (specific for the 5′ end of *COX17P*) and COX17H-4, no product was amplified from the lymphoblastoid cDNA pool (*COX17P*, +RT, Fig. 5). These primers, however, did amplify a product from BAC 271E16 and human genomic DNA (*COX17P*, Fig. 5). These results indicate that *COX17P* is not expressed in the tissue tested and further suggest that *COX17*, and not *COX17P*, is the gene producing the single transcript seen on northern blots. In addition to the nucleotide differences present in *COX17P*, the absence of *COX17P* expression further suggests that *COX17P* represents a processed *COX17* pseudogene.

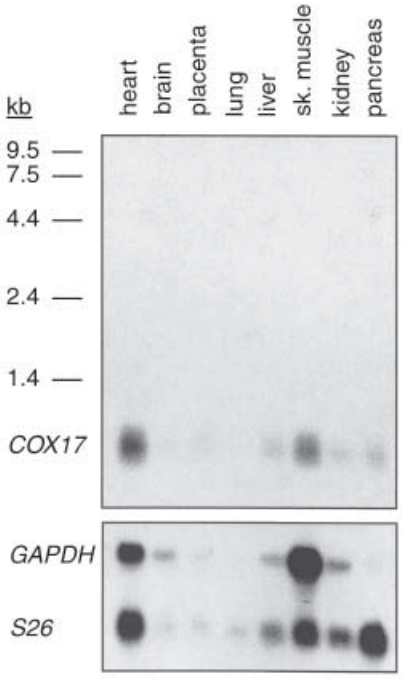

Fig. 4 Northern blot analysis of *COX17*. The *COX17* cDNA was hybridized to a human multiple tissue northern blot (Clontech) as described in the text. The blot was also probed with the *GAPDH* (1.3 kb) and *S26* (0.7 kb) cDNA control probes. *Left side of the blot* migration of molecular weight markers. *Sk muscle* skeletal muscle

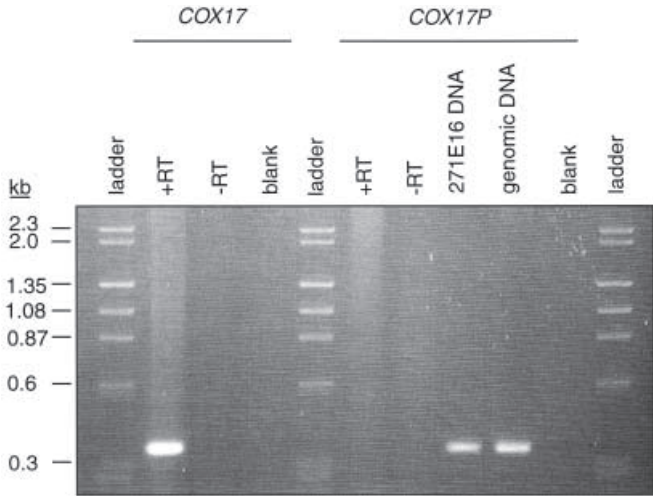

Fig. 5 Expression studies of *COX17* and *COX17P*. RT-PCR was performed on RNA isolated from a lymphoblastoid cell line. Expression of both genes was detected by PCR using gene-specific primers as described in the text. Positive controls for *COX17P* included BAC 271E16 DNA and human genomic DNA. *+RT* RT-PCR carried out in the presence of reverse transcriptase; *–RT* RT-PCR carried out in the absence of reverse transcriptase; *blank* PCR carried out in the absence of template DNA

Localization of *COX17* and *COX17P*

Radiation hybrid mapping was used to determine the chromosomal locations of both the *COX17* and *COX17P* genes. Primers COX17H-3 and COX17H-4 were used to amplify a 235-bp fragment corresponding to *COX17P*, which localizes to chromosome 13q14-q21. The *COX17* mRNA was also assigned to this chromosome 13 locus by two different consortia (RHdb RH67876 and RHdb RH 75245). However, these primers cannot amplify a 235-bp product from *COX17* since they are specific for exons 1 and 3, which are separated by approximately 6 kb of DNA. To obtain the correct chromosomal location for *COX17*, primers 18110-13 and 18110-18 were used to generate a 220-bp fragment corresponding to a portion of

intron 2, which localized to the long arm of chromosome 3 (3q13.1-q21), between the markers D3S9340 and D3S1765.

Discussion

COX17 is a small copper chaperone that is responsible for recruiting copper to mitochondria (Beers et al. 1997; Glerum et al. 1996a). Studies in yeast have shown that the presence of Cox17p is essential for assembly of a functional cytochrome oxidase. Our current study, which investigates the human orthologue of *COX17*, has determined that there are two distinct copies of *COX17* in the human genome. The *COX17* gene, which is ubiquitously expressed, is composed of three exons and two introns and spans approximately 8 kb of genomic DNA. *COX17P*, in contrast, does not appear to be expressed. While similar to the *COX17* cDNA, *COX17P* harbors a number of nucleotide differences, including one that would alter a conserved amino acid residue in the putative copper-binding domain of COX17. Of the over 65 human *COX17* ESTs that are available in the current database, none of the ESTs contains the same nucleotide differences as *COX17P*. Although the possibility that *COX17P* is expressed in a tissue that has not been examined cannot be excluded, our results support the notion that *COX17P* is a processed pseudogene.

Two different consortia, Genethon and the Wellcome Trust Centre for Human Genetics (WTCHG), have previously localized *COX17* to chromosome 13 by radiation hybrid mapping (RHdb RH67876 and RHdb RH75245, respectively). However, the primers designed by Genethon from the *COX17* cDNA sequence would anneal at the exon 1/intron1 and intron 2/exon 3 boundaries of *COX17*. Primers at these positions would not amplify a product from the *COX17* gene. WTCHG used primers that anneal to exons 1 and 3, which could only amplify a 235-bp product from *COX17P*, given that *COX17* contains several kilobases of intervening DNA. In both cases it appears that the processed pseudogene, *COX17P*, was mapped. The results presented here indicate that the expressed copy of *COX17* lies on the long arm of chromosome 3, most likely in the pericentromeric region. This portion of chromosome 3 is very poorly characterized, with relatively few markers and a dearth of sequence information. At present none of the human diseases mapped to this region seem likely to be associated with a defect in COX17.

Recently, several mammalian COX17 homologs have been identified. Alignment of the predicted protein sequences of murine (GenPept accession number P56394), porcine (Chen et al. 1997), and human (Amaravadi et al. 1997) COX17 show that mouse and pig have 92% and 93% identity, respectively, with human COX17 (Fig. 3). Along with yeast Cox17p, all three mammalian COX17 proteins share the KXCCXC motif, which is believed to bind copper and is also present in all mouse and human metallothioneins (Amaravadi et al. 1997). Currently the precise physiological role of COX17 within mammalian cells has not been established. Functional studies must now be undertaken to delineate whether mammalian COX17 proteins act in the same fashion as their yeast counterpart. In this regard it is interesting to note that COX17 is a member of a conserved family of cellular copper chaperones (Harrison et al. 1999; Valentine and Gralla 1997) that all appear to traffic copper to a specific target protein within the cell. While the cellular role of COX17 is similar to that of the copper chaperones ATOX1 and CCS, it does not share any sequence similarity with these two proteins. ATOX1 chaperones copper to ATP7B, the Wilson disease protein (Hamza et al. 1999) and may be involved in copper storage disorders. CCS provides copper to SOD1, the cytosolic superoxide dismutase, and may therefore play a role in the etiology of amyotrophic lateral sclerosis (Schmidt et al. 1999). In light of the very specialized function of these copper chaperones, further functional studies may help to determine whether COX17 is involved in the phenotypes of copper storage disorders, in addition to its potential role in inherited COX deficiencies.

Given that mutations in yeast *COX17* result in a respiration deficient phenotype, it is entirely possible that errors in mitochondrial copper recruitment also play a role in human COX deficiency. The elucidation of the genomic structure and the correct chromosomal location of the *COX17* gene provide new information on a candidate gene for inherited COX deficiencies. This information is an important supplement to the standard RT-PCR approaches currently possible, which do not allow examination of promoters or intron/exon boundaries and are prone to false positives from contamination with genomic DNA. With the information presented here we are now in a position to assess whether mutations in *COX17* are an underlying cause of some cases of human mitochondrial disease.

Acknowledgements We thank the Molecular Diagnostics Laboratory, University of Alberta Hospital, for the human genomic DNA, Dr. John Forbes for the GAPDH and S26 cDNA probes, Mr. James Friedman for the lymphoblastoid RNA, and Dr. Michael Walter for the radiation hybrid panel. We also thank Mr. James Friedman and Dr. Michael Walter for many helpful discussions. This work was supported by grants from the Medical Research Council (MRC) of Canada and the Alberta Heritage Foundation for Medical Research (AHFMR). F.A.P. is the recipient of an AHFMR Studentship, and D.M.G. is an MRC and AHFMR Scholar.

References

Adams PL, Lightowlers RN, Turnbull DM (1997) Molecular analysis of cytochrome *c* oxidase deficiency in Leigh's syndrome. Ann Neurol 41:268–270

Amaravadi R, Glerum DM, 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

Beers J, Glerum DM, Tzagoloff A (1997) Purification, characterization and localization of yeast Cox17p, a mitochondrial copper shuttle. J Biol Chem 272:33191–33196

Capaldi RA (1990) Structure and function of cytochrome *c* oxidase. Annu Rev Biochem 59:569–596

Chen Z, Bergman T, Ostenson C, Efendic S, Mutt V, Jornvall H (1997) Characterization of dopuin, a polypeptide with special residue distributions. Eur J Biochem 249:518–522

DiMauro S, Servidei S, Zeviani M, DiRocco M, DeVivo DC, DiDonato S, Uziel G, Berry K, Hoganson G, Johnsen SD, Johnson PC (1987) Cytochrome *c* oxidase deficiency in Leigh syndrome. Ann Neurol 22:498–506

Glerum DM, Yanamura W, Capaldi RA, Robinson BH (1988) Characterization of cytochrome-*c* oxidase mutants in human fibroblasts. FEBS Lett 236:100–104

Glerum DM, Shtanko A, Tzagoloff A (1996a) Characterization of *COX17*, a yeast gene involved in copper metabolism and assembly of cytochrome oxidase. J Biol Chem 271:14504–14509

Glerum DM, Shtanko A, Tzagoloff A (1996b) *SCO1* and *SCO2* act as high copy suppressors of a mitochondrial copper recruitment defect in Saccharomyces cerevisiae. J Biol Chem 271:20531–20535

Glerum DM, Muroff I, Jin C, Tzagoloff A (1997) *COX15* codes for a mitochondrial protein essential for the assembly of yeast cytochrome oxidase. J Biol Chem 272:19088–19094

Hamza I, Schaefer M, Klomp LWJ, Gitlin JD (1999) Interaction of the copper chaperone HAH1 with the Wilson disease protein is essential for copper homeostasis. Proc Natl Acad Sci USA 96:13363–13368

Harrison MD, Jones CE, Dameron CT (1999) Copper chaperones: function, structure and copper-binding properties. J Biol Inorg Chem 4:145–153

Jaksch M, Ogilvie I, Yao J, Kortenhaus G, Bresser H-G, Gerbitz K-D, Shoubridge EA (2000) Mutations in *SCO2* are associated with a distinct form of hypertrophic cardiomyopathy and cytochrome *c* oxidase deficiency. Hum Mol Genet 9:795–801

Lombes A, Nakase H, Tritschler H-J, Kadenbach B, Bonilla E, DeVivo DC, Schon EA, DiMauro S (1991) Biochemical and molecular analysis of cytochrome *c* oxidase deficiency in Leigh syndrome. Neurology 41:491–498

Mashkevich G, Repetto B, Glerum DM, Tzagoloff A (1997) *SHY1*, the yeast homolog of the mammalian *SURF-1* gene, encodes a mitochondrial protein required for respiration. J Biol Chem 272:14356–14364

McEwen JE, Ko C, Kloeckener-Gruissem B, Poyton RO (1986) Nuclear functions required for cytochrome *c* oxidase biogenesis in *Saccharomyces cerevisiae*. J Biol Chem 261:11872–11879

Nobrega MP, Nobrega FG, Tzagoloff A (1990) *COX10* codes for a protein homologous to the ORF1 product of *Paracoccus denitrificans* and is required for the synthesis of yeast cytochrome oxidase. J Biol Chem 265:14220–14226

Papadopoulou LC, Sue CM, Davidson MM, Tanji K, Nishino I, Sadlock JE, Krishna S, Walker W, Selby J, Glerum DM, VanCoster R, Lyon G, Scalais E, Lebel R, Kaplan P, Shanske S, DeVivo DC, Bonilla E, Hirano M, DiMauro S, Schon EA (1999) Fatal infantile cardioencephalomyopathy with COX deficiency and mutations in *SCO2*, a COX assembly gene. Nat Genet 23:333–337

Robinson BH, DeMeirleir L, Glerum M, Sherwood G, Becker L (1987) Clinical presentation of mitochondrial respiratory chain defects in NADH-coenzyme Q reductase and cytochrome oxidase: clues to the pathogenesis of Leigh disease. J Pediatr 110:216–222

Saraste M (1990) Structural features of cytochrome oxidase. Q Rev Biophys 23:331–366

Schmidt PJ, Ramos-Gomez M, Culotta VC (1999) A gain of superoxide dismutase (SOD) activity obtained with CCS, the copper metallochaperone for SOD1. J Biol Chem 274:36952–36956

Srinivasan C, Posewitz MC, George GN, Winge DR (1998) Characterization of the copper chaperone Cox17 of *Saccharomyces cerevisiae*. Biochemistry 37:7572–7577

Taanman J-W, Capaldi RA (1992) Purification of yeast cytochrome c oxidase with a subunit composition resembling the mammalian enzyme. J Biol Chem 267:22481–22485

Tiranti V, Hoertnagel K, Carrozzo R, Galimberti C, Munaro M, Granatiero M, Zelante L, Gasparini P, Marzella R, Rocchi M, Bayona-Bafaluy MP, Enriquez J, Uziel G, Bertini E, Dionisi-Vici C, Franco B, Meitinger T, Zeviani M (1998) Mutations of *SURF-1* in Leigh disease associated with cytochrome *c* oxidase deficiency. Am J Hum Genet 63:1609–1621

Tzagoloff A, Dieckmann CL (1990) *PET* genes of *Saccharomyces cerevisiae*. Microbiol Rev 54:211–225

Valentine JS, Gralla EB (1997) Delivering copper inside yeast and human cells. Science 278:817–818

Valnot I, von Kleist-Retzow J-C, Barrientos A, Gorbatyuk M, Taanman J-W, Mehaye B, Rustin P, Tzagoloff A, Munnich A, Rotig A (2000) A mutation in the human heme A: farnesyltransferase gene (*COX10*) causes cytochrome *c* oxidase deficiency. Hum Mol Genet 9:1245–1249

Vincent SV, Marty L, Fort F (1993) S26 ribosomal protein RNA: an invariant control for gene regulation experiments in eucaryotic cells and tissues. Nucleic Acids Res 21:1498

Zhu Z, Yao J, Johns T, Fu K, DeBie I, Macmillan C, Cuthbert AP, Newbold RF, Wang J, Chevrett M, Brown GK, Brown RM, Shoubridge EA (1998) *SURF1*, encoding a factor involved in the biogenesis of cytochrome *c* oxidase is mutated in Leigh syndrome. Nat Genet 20:337–343