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Review

Defects in the biosynthesis of mitochondrial heme *c* and heme *a* in yeast and mammals

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Abstract

Defects in heme biosynthesis have been associated with a large number of diseases, but mostly recognized in porphyrias, which are neurovisceral or cutaneous disorders caused by the accumulation of biosynthetic intermediates. However, defects in the maturation of heme groups that are part of the oxidative phosphorylation system are now also recognized as important causes of disease. The electron transport chain contains heme groups of the types *a*, *b* and *c*, all of which are directly involved in electron transfer reactions. In this article, we review the effect of mutations in enzymes involved in the maturation of heme *a* (the prosthetic group of cytochrome *c* oxidase) and heme *c* (the prosthetic group of cytochrome *c*) both in yeast and in humans. *COX10* and *COX15* are two genes, initially identified in *Saccharomyces cerevisiae* that have been found to cause infantile cytochrome *c* oxidase deficiency in humans. They participate in the farnesylation and hydroxylation of heme *b*, steps that are necessary for the formation of heme *a*, the prosthetic group required for cytochrome oxidase assembly and activity. Deletion of the cytochrome *c* heme lyase gene in a single allele has also been associated with a human disease, known as Microphthalmia with Linear Skin defects (MLS) syndrome. The cytochrome *c* heme lyase is necessary to covalently attach the heme group to the apocytochrome *c* polypeptide. The production of mouse models recapitulating these diseases is providing novel information on the pathogenesis of clinical syndromes.

Keywords: Heme biosynthesis; Yeast; Mammal

1. Introduction

A heme is a metal-containing prosthetic group of several proteins, and consists of an iron atom embedded in a porphyrin ring system coordinated by four nitrogen atoms. The biosynthesis of heme is a multistep process that starts by the formation of a porphyrin ring from succinyl-CoA and glycine (Fig. 1).

Partial deficiencies in seven of the eight enzymes in the early biosynthetic pathway of heme result in a group of diseases known as porphyrias. Clinical manifestations in porphyrias are the result of accumulation of heme precursors. There are two types of porphyrias: neurovisceral and cutaneous. The molecular basis of porphyrias has been reviewed recently [1,2], and even though they may have a small effect on the levels of mitochondrial hemes [3], oxidative phosphorylation is not the primary target of these defects, and therefore they will not be discussed further in this article.

We will focus this review on maturation defects of two of the hemes that are intrinsic part of the oxidative phosphorylation complexes. There are three biologically important forms of heme (types *a*, *b*, and *c*), which differ by modifications in the porphyrin ring. The most common type of heme is called heme *b* (the prosthetic group of proteins like hemoglobin and myoglobin) and is also present in the mitochondrial bc_1 complex. Heme *b* is not covalently bound to its apoprotein partner. Heme *c* differs from heme *b* (or protoheme) in that the two vinyl side chains are covalently bound to the protein itself. Examples of proteins

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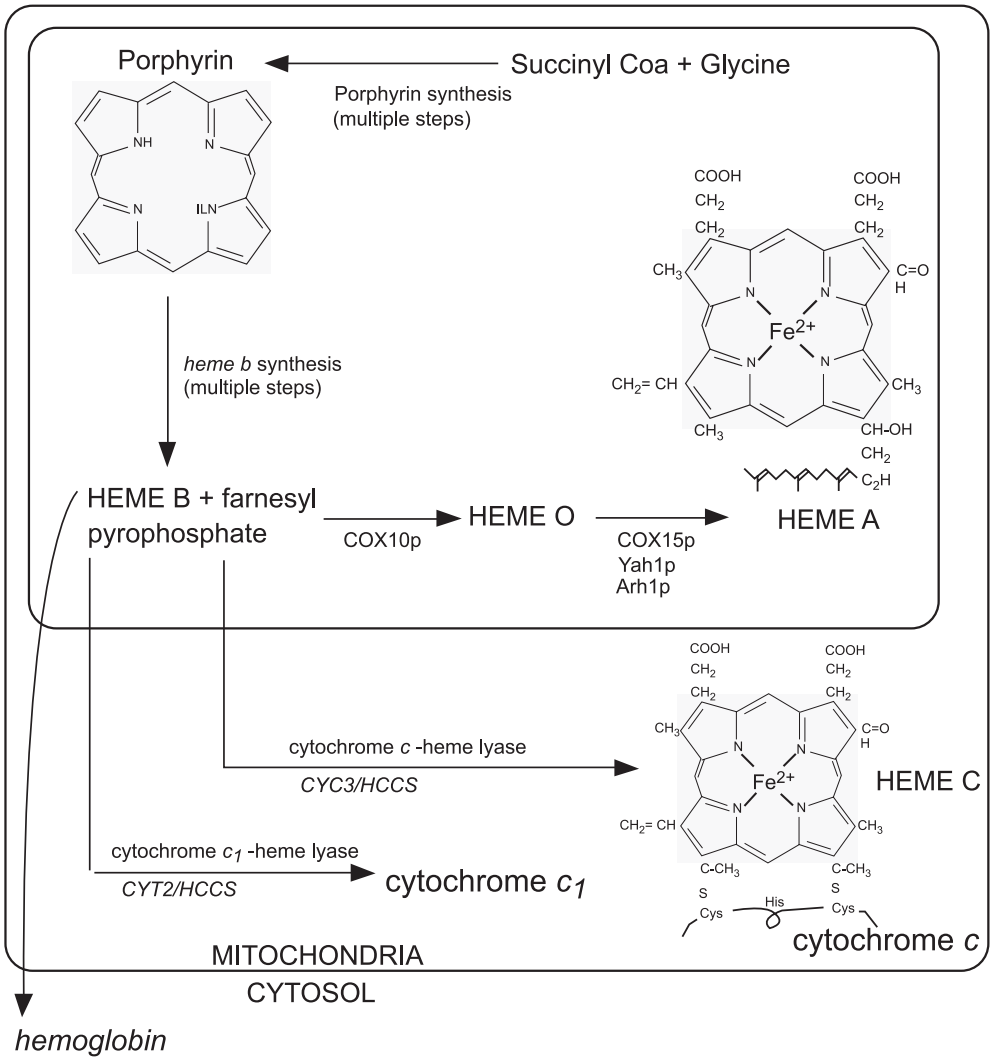

Fig. 1. Heme *c* and heme *a* biosynthesis. Heme biosynthesis takes place inside the mitochondria and initiates with the formation of the porphyrin ring followed by the formation of heme *b*, which can be exported to the cytosol, where it can be used to mature hemoglobin and myoglobin. Heme *b* is transformed into heme *a* by the combined action of Cox10p, Cox15p, as well as other auxiliary enzymes. Heme *c* is formed by the covalent attachment of heme to cytochrome *c* or c_1. Specific heme lyases are required for these steps in yeast, but it seems that a single one is required in mammals.

that contain a *c*-type heme are cytochromes *c* and c_1. Although the heme group is similar in these proteins, the attachment site of heme *c* confers specific absorbance signatures, allowing the spectral discrimination of hemes *c* and c_1 [4]. Heme *a* differs from heme *b* in that a methyl side chain is oxidized into a formyl group, and one of the vinyl side chains (on C2) is replaced by an isoprenoid (farnesyl) chain [5] (Fig. 1). The only known protein to contain heme *a* is cytochrome *c* oxidase (COX) or complex IV of the oxidative phosphorylation system. COX contains two heme *a* groups, which are non-covalently bound to the mitochondrial DNA encoded subunit 1 or Cox1p. They contribute to the low-spin heme of cytochrome *a* and the high-spin heme of cytochrome a_3 [6].

COX is the terminal enzyme of the mitochondrial respiratory chain, which catalyzes the transfer of electrons from cytochrome *c* to molecular oxygen in a process that is coupled to transfer of protons through the mitochondrial inner membrane, which will contribute to the electrochemical gradient that drives the aerobic synthesis of ATP. In the electron transfer reaction, cytochrome *c* donates electrons to COX. The low spin heme *a* in subunit CoxIp receives electrons from the Cu_A center (located in subunit CoxIIp) and transfers them to a heterobimetallic active site formed by Cu_B and the high spin heme a_3 where molecular oxygen is bound and subsequently reduced to water. COX is a multimeric protein of dual genetic origin, mitochondrial and nuclear, and its assembly requires the assistance of a large number of nuclear gene products, including several involved in the biogenesis of the heme *a* groups and its insertion in the apoenzyme [7].

2. Yeast genes involved in heme *c* and *a* biosynthesis

The discovery of gene products involved in heme *c* and *a* biosynthesis has been greatly facilitated by research

performed in yeast models. The genes involved in these processes are functionally conserved, from yeast to human, as it has been demonstrated by heterologous complementation studies [8].

CYC3, in the yeast *Saccharomyces cerevisiae*, encodes cytochrome *c* heme lyase, the enzyme catalyzing the attachment of heme to apocytochrome *c* [9]. The cytochrome-c_1-heme lyase is encoded by the *CYT2* gene [10]. Both enzymes play a critical role in the localization of the respective cytochromes to mitochondrial compartments. It has been shown that no import of apocytochrome *c* occurred in mitochondria isolated from *cyc3* null strains. In addition, amino acid substitutions in apocytochrome *c* at either of the two cysteine residues that are the sites of heme covalent linkages (Fig. 1), or at an adjacent histidine that also participates as a ligand of the heme iron, resulted in a reduction in mitochondrial import. Therefore, covalent heme attachment seems to be a required step for import of cytochrome *c* into mitochondria [11], and the interaction of apocytochrome *c* with the cytochrome *c* heme lyase appears to drive the translocation process [12].

The biogenesis of cytochrome c_1 involves a number of steps including the synthesis as a precursor with a bipartite signal sequence. The first part of the targeting sequence is cleaved upon import into the matrix, whereas the second part appears to be cleaved upon re-export to the intermembrane space. Following translocation from the matrix side to the intermembrane-space side of the inner membrane, apocytochrome c_1 forms a complex with cytochrome c_1 heme lyase, and then holocytochrome c_1 formation occurs. However, heme attachment appears to take place on the intermembrane-space side of the inner mitochondrial membrane. The second proteolytic processing of cytochrome c_1 does not take place unless the covalent linkage of heme to apocytochrome c_1 precedes it. Therefore, cytochrome c_1 heme lyase is required for import of cytochrome c_1, but in contrast to cytochrome *c*, it is not involved in the transmembrane movement of the precursor polypeptide [13, 14].

Heme *a* is a unique heme compound present only in mitochondrial cytochrome *c* oxidase. Several genes products involved in the heme *a* biosynthetic pathway have been identified in yeast (reviewed in Ref. [7]). The first step in the heme *a* biosynthesis from protoheme is a farnesylation of the vinyl group at C2 of protoheme [15]. In yeast, this reaction is catalyzed by a farnesyl transferase encoded by the *COX10* gene to produce a new class of heme called heme O [16]. The yeast *COX10* was first reported as coding for a protein homologous to the *ORF1* product of *Paracoccus denitrificans* and required for the synthesis of cytochrome oxidase [17]. It was cloned by complementation of a particular COX mutant from a collection of respiratory deficient mutants [18] with a yeast genomic library. The COX deficiency in the original mutant was described as caused by recessive mutations in the nuclear gene *COX10*. Analyses of COX subunits in the mutant suggested that the product of *COX10* was providing an essential function at a posttranslational stage of enzyme assembly. Genes homologous to yeast *COX10* were found to be present in at least four different bacterial cytochrome oxidase operons. The discovery that the bacterial gene, termed *cyoE*, was coding for a farnesyl transferase that converts protoheme to heme O [19] was instrumental for the demonstration that Cox10p, like the product of cyoE was needed for heme *a* synthesis [16]. Interestingly, the human homolog of the *S. cerevisiae COX10* gene was subsequently cloned by functional complementation of a yeast *cox10* null mutant, even though the human *COX10* homolog does not complement the mutation as efficiently as the yeast *COX10*, likely due to the heterologous environment [20,21].

Heme O can function as a prosthetic group in some bacterial terminal oxidases [22]. For example, cytochrome O, one of the two terminal ubiquinol oxidases of *Escherichia coli*, is structurally and functionally related to cytochrome *c* oxidase of mitochondria and some bacteria. It has two heme groups, one of which binds CO and forms a binuclear oxygen reaction center with copper [22]. However, heme O cannot function as a prosthetic group of mitochondrial cytochrome oxidases and requires a second modification. The further conversion of heme O to heme *a* probably involves two steps. The first consists of a monooxygenase-catalyzed hydroxylation of the methyl group at carbon position 8, resulting in an alcohol that would then be further oxidized to the aldehyde by a dehydrogenase [23]. In yeast, the first reaction appears to be catalyzed by Cox15p, ferredoxin, and ferredoxin reductase [23, 24]. *COX15* is likely the yeast homolog of the *cta*A gene of *Bacillus subtilis* [23]. The product of *ctaA* is required for the conversion of heme O to heme *a* [25]. Interestingly, *E. coli*, which normally has only heme O, is able to synthesize heme *a* when transformed with *cta*A [26]. The fact that the purified ctaA protein was shown to have both protoheme and heme *a* associated with it suggested that it was likely to be a heme-dependent monooxygenase [27]. Several lines of evidence suggest that *COX15* is the homolog of bacterial *ctaA*. Cox15p exhibits some sequence similarity to the bacterial protein, but more significantly, *cox15* mutants, which are deficient in COX assembly and function, have no heme *a*, although they have low levels of heme O [23]. The phenotype of *cox15* mutants is clearly different from *cox10* mutants that lack both heme *a* and heme O [16]. Cox15p is an evolutionary conserved protein, distributed in eukaryotic organisms.

In *Schizosaccharomyces pombe COX15* is fused to *YAH1*, the structural gene for mitochondrial adrenodoxin [28]. Adrenodoxin is the second electron carrier that participates in a mitochondrial electron transfer chain that, in mammals, catalyses the conversion of cholesterol into pregnenolone, the first step in the synthesis of all steroid hormones. Yah1p is an essential protein in *S. cerevisiae* and it has been shown to play a central role in the

biosynthesis of iron–sulfur clusters [29]. Yah1p could serve as a link between heme and iron–sulfur cluster synthesis, the two major iron-consuming pathways. A fusion of the *S. cerevisiae COX15* and *YAH1* genes, when introduced in single copy into chromosomal DNA, is able to complement both the respiratory defect of a *cox15* null mutant and the lethality of a *yah1* mutant, demonstrating that the combined presence of the two proteins in a single polypeptide does not exert any effect on their respective activities [23]. These observations suggested that Cox15p in conjunction with Yah1p and its putative reductase, encoded by *ARH1* [30], functions as a three-component monooxygenase [23].

The identity of the putative gene product involved in the oxidation of the alcohol resulting from the Cox15p action to the corresponding aldehyde to yield heme *a* remains unknown at present.

3. Mammalian mutants

In humans as well as in yeast, mutations in genes involved in the heme *c* and *a* biosynthesis cause altered phenotypes and clinical syndromes. Here we summarize these findings and the diverse pathologies associated with such mutations.

3.1. Human holocytochrome c-type synthetase mutants

The human and murine holocytochrome *c*-type synthetase (*HCCS*) genes were the first mammalian HCCS to be described [31], and are the orthologues of the yeast *CYC3* gene [32]. In contrast to yeast, multicellular eukaryotes, including mammals, have a single heme lyase for both cytochromes *c* and c_1 [4]. The human *HCCS* gene was found to be in the critical region of Microphthalmia with Linear Skin defects syndrome (MLS). MLS is an X-linked male-lethal disorder associated with X chromosomal rearrangements resulting in monosomy of an Xp region. Features include microphthalmia, sclerocornea, linear skin defects, and agenesis of the corpus callosum in females [31].

HCCS is the only gene located entirely inside the MLS critical region in Xp22.31. To study the molecular basis of MLS, Prakash et al. [33] generated a deletion of the equivalent region in the mouse. This deletion inactivated the mouse *Hccs*. Ubiquitous deletions generated in vivo led to lethality of hemizygous, homozygous and heterozygous embryos early in development. This lethality was rescued by expression of a BAC transgenic human *HCCS* gene, resulting in viable homozygous, heterozygous and hemizygous deleted mice with no apparent phenotype. The association between defects in the corpus callosum, eyes and skin with gene dosage of *HCCS* is intriguing and still not understood. It is also not clear if the gene dosage defect of *HCCS* specifically affects the oxidative phosphorylation system.

3.2. Human COX10 mutants

Nuclear-driven defects in COX have been associated exclusively with mutations in assembly genes [34]. In 2002, Valnot et al. [21] reported for the first time mutations in the *COX10* gene associated with cytochrome oxidase deficiency in humans. They studied samples of a child from a consanguineous family. The pediatric patient studied presented clinical symptoms of ataxia at 18 months of age and its condition worsened into severe muscle weakness, hypotonia, ptosis, and pyramidal syndrome by 2 years of age. He had elevated lactate in blood and cerebrospinal fluid and proximal tubulopathy (suggested by elevated amino acids in urine) and died at 2 years of age. This patient had siblings that also had serious clinical symptoms. The oldest sister suffered of a mitochondrial encephalopathy associated with COX deficiency, and died at 5 years of age. The youngest sister also presented neurological symptoms with progressive deterioration and died at 3 years of age. Biochemical tests indicated that he was a case of isolated COX deficiency in muscle, lymphocytes and fibroblasts cultures [35]. DNA sequencing of the mitochondrial-encoded COX subunits or the tRNA genes did not reveal any mutations.

By genetic linkage studies, Valnot and colleagues were able to identify the locus of the disease on chromosome 17p13.1–q11.1. This region encodes two proteins involved in COX assembly: *SCO1* and *COX10*. The seven exons of *COX10* were sequenced and it was found a homozygous C to A transversion in exon 4. This transversion is located in nucleotide 612 of the cDNA and produces a change of an asparagine to a lysine (uncharged to a basic amino acid) at the protein level in the amino acid residue 204 (N204K). Sequencing analysis of the parents DNA showed that both were heterozygous for the transversion. Biochemical characterization of the mitochondrial cytochromes by difference spectrum showed the absence of the peak corresponding to cytochrome aa_3 at 600 nm in the patient cultured fibroblasts.

Determination of the steady-state levels of COX subunits by immunoblot revealed that there was a 50% decrease of CoxIIIp as well as CoxVIcp subunits, whereas CoxIIp was barely detectable. In addition, Valnot et al. [21] performed complementation analysis of wild-type and mutated *COX10* (N204K mutation) in yeast *COX10* null mutants. They observed that both wild-type and mutant *COX10* were able to complement the yeast *COX10* defect when present in a high copy number. In contrast, at low copy number, only the wild-type *COX10* allowed yeast to grow in non-fermentable carbon source. These results suggest that the mutant Cox10p retains some residual activity that is able to complement the defect in yeast when present in high amounts. This is in concordance with the data observed in patient lymphocytes where there is a residual COX activity of 23–33% of control values.

Studies of COX-assembly intermediates in fibroblasts with this *COX10* missense mutation using blue-native gel

electrophoresis showed the presence of the same two CoxIp-containing subassemblies as the control but significantly reduced steady-state levels of the holoenzyme [36]. These studies suggested that heme *a* insertion into CoxIp occurs after formation of a subassembly with CoxIVp, but before the formation of CoxIp–CoxIVp–CoxVap subassembly.

Recently, other mutations in *COX10* gene were reported in patients with COX deficiency. Antonicka et al. [37] analyzed samples of two patients with different clinical phenotypes. One of the patients presented clinical symptoms at the first week of age leading to anemia, sensorineural deafness and hypertrophic cardiomyopathy and died at 5 months of age. Histochemistry of muscle samples showed a severe reduction or absence of COX activity in most of the muscle fiber even though it showed normal structures. Biochemical studies from mitochondria isolated from frozen muscle showed 5% COX activity of control samples whereas all the other respiratory complex activities were normal.

The second patient started with clinical problems at one and a half months of age. She had severe anemia, elevated lactate/pyruvate levels in blood and cerebrospinal fluid, hypotonia and brain lesions typical of Leigh syndrome. She died at 4 months of age. She also had a decreased COX activity in muscle biopsy while other respiratory enzyme complexes were normal.

Two missense mutations were detected in fibroblasts of the first patient: a transversion C791A in exon 4 and a C878T in exon 5. These mutations were heterozygous and were located in two evolutionary conserved sequence of the protein (T196K and P225L predicted amino acid, respectively). The second patient had two missense mutations in exon 7. One mutation was a transversion A1211T and the other one a transition A1211G that resulted in two amino acid substitutions at position D336V and D336G (also evolutionarily conserved positions).

Blue-native gel assays showed the absence of fully assembled complex IV in muscle mitochondria from the first patient and the authors were unable to detect COX subcomplexes as commonly observed in patients with *SURF1* mutations. These results also agreed with the findings of low enzyme activity of complex IV found in muscle mitochondria (less than 5% of control). In cultured fibroblasts, the deficiency of COX activity was not as severe (40% of control) as the one observed in muscle. In contrast, the second patient showed a stronger COX defect in fibroblast when compared to muscle (18% and 84% of controls, respectively). The assembly defect of COX was determined by two-dimensional blue native-SDS-PAGE in fibroblasts from the second patient. The authors showed a marked decrease in fully assembled COX using antibodies against subunits I and IV of the complex (about 15% of control) and subunit II was almost undetectable in this sample. None of the two patients had detectable levels of subassembled intermediates of complex IV, supporting the hypothesis that only heme *a*-containing CoxIp is stable and can promote holoenzyme assembly [37].

The levels of heme *a* were determined in muscle and fibroblast mitochondria from patients by HPLC. One patient had 7% of control levels of heme *a* and no heme O could be detected, suggesting that the step on the heme *a* biosynthesis catalyzed by Cox15p was not affected.

The *COX10* mutations reported in humans result in diverse clinical phenotypes, including tubulopathy, leukodystrophy, Leigh syndrome and infantile hypertrophic cardiomyopathy. The reasons for the different phenotypes and levels of COX deficiency in different tissues are still not clear, and remain under investigation.

3.3. Human COX15 mutants

Antonicka et al. [38] reported mutations in *COX15* gene that were responsible for isolated COX deficiency. The patient studied had midfacial hypoplasia, lactic acidosis, seizures and hypotonia shortly after birth and died at age 24 days from a massive biventricular hypertrophic cardiomyopathy [39]. Her heart had a severe loss of myofibers and increased number of mitochondria with abnormal shape and cristae and inclusions as determined by electron microscopy. In contrast, her skeletal muscle was normal. Biochemical analysis showed a severe deficiency of COX in heart (7%) and kidney and liver (<25%) when compared to controls. The levels of COX activity in muscle were within normal range. The other respiratory complex enzyme activities were normal. The cytochrome spectra showed very low levels of cytochrome aa_3 in heart, whereas the levels in muscle were 37% of control. Steady-state levels of CoxIIp, IIIp, VIap, VIbp, VIcp and VIIap were decreased whereas the levels of CoxIVp and Vap were less affected in heart.

Analysis of COX by Blue Native-PAGE from the patient heart mitochondria reveals low levels of fully assembled enzyme and small amounts of non-assembled COXIV subunit. In patient fibroblasts the same pattern was obtained although the protein level and enzyme activity of COX was less affected. Overexpression of *COX15* complemented the COX deficiency to 60% of controls. Blue Native-PAGE showed that the levels of the fully assembled COX increased to 60% of controls in the patient fibroblast [38].

DNA sequencing of the *COX15* gene showed two different mutations: a C700T transition in exon 5 that resulted in an amino acid substitution of conserved arginine 217 to tryptophan (R217W) in one of the alleles. The second allele had a mutation in the splice-acceptor site of intron 3 (C447-3G). The patient was also homozygous for a T1171C in variant 2 of exon 9 that resulted in an amino acid change F374L. The mutation in the splice site produced a transcript lacking exon 4, resulting in a frame shift mutation producing a premature stop codon.

Analysis of heme *a* levels in the patient's heart mitochondria showed a marked decrease (6% of control) while heme O levels were increased. The heme *a* levels in patient fibroblasts were also low, but heme O was

undetectable. Overexpressing *COX15* in patient fibroblasts increased the levels of heme *a* to 65% of control. As mentioned earlier, *COX15* has two splice variants, COX15.1 and COX15.2, the role of which still unknown. Antonicka and colleagues used COX15.1 for their complementation experiments and they did not obtain full rescue of COX deficiency. It was suggested that the two variants are necessary for complete activity [38].

4. Mouse *COX10* conditional knockout

To get a better insight into the pathophysiology of cytochrome oxidase deficiencies as well as the role of heme *a* in assembly and activity of this enzyme, we are in the process of creating knockout mice for *COX10*. Due to the severity of the pathology and the fatal outcome associated with mutations in assembly factors, we took the conditional knockout approach using the loxP–Cre system [40]. We created a mouse with the exon 6 flanked by loxP sites (floxed). Even though none of the patients described above presented any mutation in this region, exon 6 forms part of the second cytoplasmic domains (domain 2b) that has been shown to be part of the active site of the enzyme in *E. coli* [15]. By using a transgenic mouse expressing the P1-Cre recombinase under a tissue-specific promoter, we were able to created several conditional knockout animals in muscle, neurons and liver. The exon 6 skipping in both the human and mouse genes predicts an out-of-frame mRNA, which would also eliminate the domain 2b and the last three membrane spanning regions.

Our work with the *COX10* conditional KO remains in progress and incomplete. Therefore, we will describe only a brief summary of our approach. We are creating a muscle-specific knockout mouse, by crossing the floxed *COX10* mouse with a mouse transgenic for a Cre recombinase driven by a myosin light chain promoter, which should lead to a myopathy resembling the ones observed in mitochondrial diseases.

We are also in the process of creating a COX deficiency in the CNS through the deletion of the *COX10* gene by the synapsin-Cre and by the CamKIIα-Cre. The former is expressed early in fetal development whereas the latter is expressed at higher levels in the adult brain. Therefore, we expect to have both an infantile and an adult model of COX deficiency in the CNS.

5. Concluding remarks

Defects in heme *c* and *a* biosynthesis and their insertion in the corresponding apoenzymes are associated with human diseases. The use of yeast models has allowed for the identification of the genes involved in this process and will continue to be useful in the identifications of novel genes and the characterization of their products. In addition, the creation of mouse models will constitute valuable tools to better understand the pathophysiology of mitochondrial heme defects in mammals.

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References

[1] M. Lecha, C. Herrero, D. Ozalla, Diagnosis and treatment of the hepatic porphyrias, Dermatol. Ther. 16 (2003) 65–72.
[2] S.E. Foran, G. Abel, Guide to porphyrias. A historical and clinical perspective, Am. J. Clin. Pathol. 119 Suppl. (2003) S86–S93.
[3] J. Krijt, P. Maruna, P. Petrovicky, V. Janousek, The effect of protoporphyrinogen oxidase inhibitors on microsomal and mitochondrial cytochromes, Obes. Res. 3 (Suppl. 5) (1995) 785S–788S.
[4] D.G. Bernard, S.T. Gabilly, G. Dujardin, S. Merchant, P.P. Hamel, Overlapping specificities of the mitochondrial cytochrome *c* and c_1 heme lyases, J. Biol. Chem. 278 (2003) 49732–49742.
[5] W.S. Caughey, G.A. Smythe, D.H. O'Keeffe, J.E. Maskasky, M.I. Smith, Heme A of cytochrome *c* oxicase. Structure and properties: comparisons with hemes B, C, and S and derivatives, J. Biol. Chem. 250 (1975) 7602–7622.
[6] M. Saraste, Structural features of cytochrome oxidase, Q. Rev. Biophys. 23 (1990) 331–366.
[7] A. Barrientos, M.H. Barros, I. Valnot, A. Rotig, P. Rustin, A. Tzagoloff, Cytochrome oxidase in health and disease, Gene 286 (2002) 53–63.
[8] A. Barrientos, Yeast models of human mitochondrial diseases, IUBMB Life 55 (2003) 83–95.
[9] M.E. Dumont, J.F. Ernst, D.M. Hampsey, F. Sherman, Identification and sequence of the gene encoding cytochrome *c* heme lyase in the yeast *Saccharomyces cerevisiae*, EMBO J. 6 (1987) 235–241.
[10] A. Zollner, G. Rodel, A. Haid, Molecular cloning and characterization of the *Saccharomyces cerevisiae* CYT2 gene encoding cytochrome-c_1-heme lyase, Eur. J. Biochem. 207 (1992) 1093–1100.
[11] M.E. Dumont, J.F. Ernst, F. Sherman, Coupling of heme attachment to import of cytochrome *c* into yeast mitochondria. Studies with heme lyase-deficient mitochondria and altered apocytochromes *c*, J. Biol. Chem. 263 (1988) 15928–15937.
[12] M.E. Dumont, T.S. Cardillo, M.K. Hayes, F. Sherman, Role of cytochrome *c* heme lyase in mitochondrial import and accumulation of cytochrome *c* in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 11 (1991) 5487–5496.
[13] D.W. Nicholson, W. Neupert, Import of cytochrome *c* into mitochondria: reduction of heme, mediated by NADH and flavin nucleotides, is obligatory for its covalent linkage to apocytochrome *c*, Proc. Natl. Acad. Sci. U. S. A. 86 (1989) 4340–4344.
[14] D.W. Nicholson, R.A. Stuart, W. Neupert, Biogenesis of cytochrome c_1. Role of cytochrome c_1 heme lyase and of the two proteolytic processing steps during import into mitochondria, J. Biol. Chem. 264 (1989) 10156–10168.
[15] K. Saiki, T. Mogi, K. Ogura, Y. Anraku, In vitro heme O synthesis by the cyoE gene product from *Escherichia coli*, J. Biol. Chem. 268 (1993) 26041–26044.
[16] A. Tzagoloff, M. Nobrega, N. Gorman, P. Sinclair, On the functions of the yeast COX10 and COX11 gene products, Biochem. Mol. Biol. Int. 31 (1993) 593–598.
[17] M.P. Nobrega, F.G. Nobrega, A. Tzagoloff, 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 (1990) 14220–14226.
[18] A. Tzagoloff, C.L. Dieckmann, PET genes of *Saccharomyces cerevisiae*, Microbiol. Rev. 54 (1990) 211–225.
[19] K. Saiki, T. Mogi, Y. Anraku, Heme O biosynthesis in *Escherichia coli*: the cyoE gene in the cytochrome *bo* operon encodes a protoheme IX farnesyltransferase, Biochem. Biophys. Res. Commun. 189 (1992) 1491–1497.
[20] D.M. Glerum, A. Tzagoloff, Isolation of a human cDNA for heme A:farnesyltransferase by functional complementation of a yeast cox10 mutant, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 8452–8456.
[21] I. Valnot, J.C. von Kleist-Retzow, A. Barrientos, M. Gorbatyuk, J.W. Taanman, B. Mehaye, P. Rustin, A. Tzagoloff, A. Munnich, A. Rotig, A mutation in the human heme A: farnesyltransferase gene (COX10) causes cytochrome *c* oxidase deficiency, Hum. Mol. Genet. 9 (2000) 1245–1249.
[22] A. Puustinen, M. Wikstrom, The heme groups of cytochrome o from *Escherichia coli*, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 6122–6126.
[23] M.H. Barros, C.G. Carlson, D.M. Glerum, A. Tzagoloff, Involvement of mitochondrial ferredoxin and Cox15p in hydroxylation of heme O, FEBS Lett. 492 (2001) 133–138.
[24] D.M. Glerum, I. Muroff, C. Jin, A. Tzagoloff, COX15 codes for a mitochondrial protein essential for the assembly of yeast cytochrome oxidase, J. Biol. Chem. 272 (1997) 19088–19094.
[25] B. Svensson, K.K. Andersson, L. Hederstedt, Low-spin heme A in the heme A biosynthetic protein CtaA from *Bacillus subtilis*, Eur. J. Biochem. 238 (1996) 287–295.
[26] B. Svensson, M. Lubben, L. Hederstedt, *Bacillus subtilis* CtaA and CtaB function in haem A biosynthesis, Mol. Microbiol. 10 (1993) 193–201.
[27] B. Svensson, L. Hederstedt, *Bacillus subtilis* CtaA is a heme-containing membrane protein involved in heme A biosynthesis, J. Bacteriol. 176 (1994) 6663–6671.
[28] M.H. Barros, F.G. Nobrega, YAH1 of *Saccharomyces cerevisiae*: a new essential gene that codes for a protein homologous to human adrenodoxin, Gene 233 (1999) 197–203.
[29] H. Lange, A. Kaut, G. Kispal, R. Lill, A mitochondrial ferredoxin is essential for biogenesis of cellular iron–sulfur proteins, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 1050–1055.
[30] L. Manzella, M.H. Barros, F.G. Nobrega, ARH1 of *Saccharomyces cerevisiae*: a new essential gene that codes for a protein homologous to the human adrenodoxin reductase, Yeast 14 (1998) 839–846.
[31] L. Schaefer, A. Ballabio, H.Y. Zoghbi, Cloning and characterization of a putative human holocytochrome *c*-type synthetase gene (HCCS) isolated from the critical region for microphthalmia with linear skin defects (MLS), Genomics 34 (1996) 166–172.
[32] Q.P. Schwarz, T.C. Cox, Complementation of a yeast CYC3 deficiency identifies an X-linked mammalian activator of apocytochrome *c*, Genomics 79 (2002) 51–57.
[33] S.K. Prakash, T.A. Cormier, A.E. McCall, J.J. Garcia, R. Sierra, B. Haupt, H.Y. Zoghbi, I.B. Van Den Veyver, Loss of holocytochrome *c*-type synthetase causes the male lethality of X-linked dominant microphthalmia with linear skin defects (MLS) syndrome, Hum. Mol. Genet. 11 (2002) 3237–3248.
[34] N. Darin, A.R. Moslemi, S. Lebon, P. Rustin, E. Holme, A. Oldfors, M. Tulinius, Genotypes and clinical phenotypes in children with cytochrome-c oxidase deficiency, Neuropediatrics 34 (2003) 311–317.
[35] H. Ogier, A. Lombes, H.R. Scholte, B.T. Poll-The, M. Fardeau, J. Alcardi, B. Vignes, P. Niaudet, J.M. Saudubray, de Toni–Fanconi–Debre syndrome with Leigh syndrome revealing severe muscle cytochrome *c* oxidase deficiency, J. Pediatr. 112 (1988) 734–739.
[36] S.L. Williams, I. Valnot, P. Rustin, J.W. Taanman, Cytochrome *c* oxidase subassemblies in fibroblast cultures from patients carrying mutations in COX10, SCO1, or SURF1, J. Biol. Chem. 279 (2004) 7462–7469.
[37] H. Antonicka, S.C. Leary, G.H. Guercin, J.N. Agar, R. Horvath, N.G. Kennaway, C.O. Harding, M. Jaksch, E.A. Shoubridge, Mutations in COX10 result in a defect in mitochondrial heme A biosynthesis and account for multiple, early-onset clinical phenotypes associated with isolated COX deficiency, Hum. Mol. Genet. 12 (2003) 2693–2702.
[38] H. Antonicka, A. Mattman, C.G. Carlson, D.M. Glerum, K.C. Hoffbuhr, S.C. Leary, N.G. Kennaway, E.A. Shoubridge, Mutations in COX15 produce a defect in the mitochondrial heme biosynthetic pathway, causing early-onset fatal hypertrophic cardiomyopathy, Am. J. Hum. Genet. 72 (2003) 101–114.
[39] N.G. Kennaway, R.D. Carrero-Valenzuela, G. Ewart, V.K. Balan, R. Lightowlers, Y.Z. Zhang, B.R. Powell, R.A. Capaldi, N.R. Buist, Isoforms of mammalian cytochrome *c* oxidase: correlation with human cytochrome *c* oxidase deficiency, Pediatr. Res. 28 (1990) 529–535.
[40] A.S. Perkins, Functional genomics in the mouse, Funct. Integr. Genomics 2 (2002) 81–91.