© 2015. Published by The Company of Biologists Ltd | Journal of Cell Science (2015) 128, 833–837 doi:10.1242/jcs.161729

CELL SCIENCE AT A GLANCE

Human mitochondrial COX1 assembly into cytochrome *c* oxidase at a glance

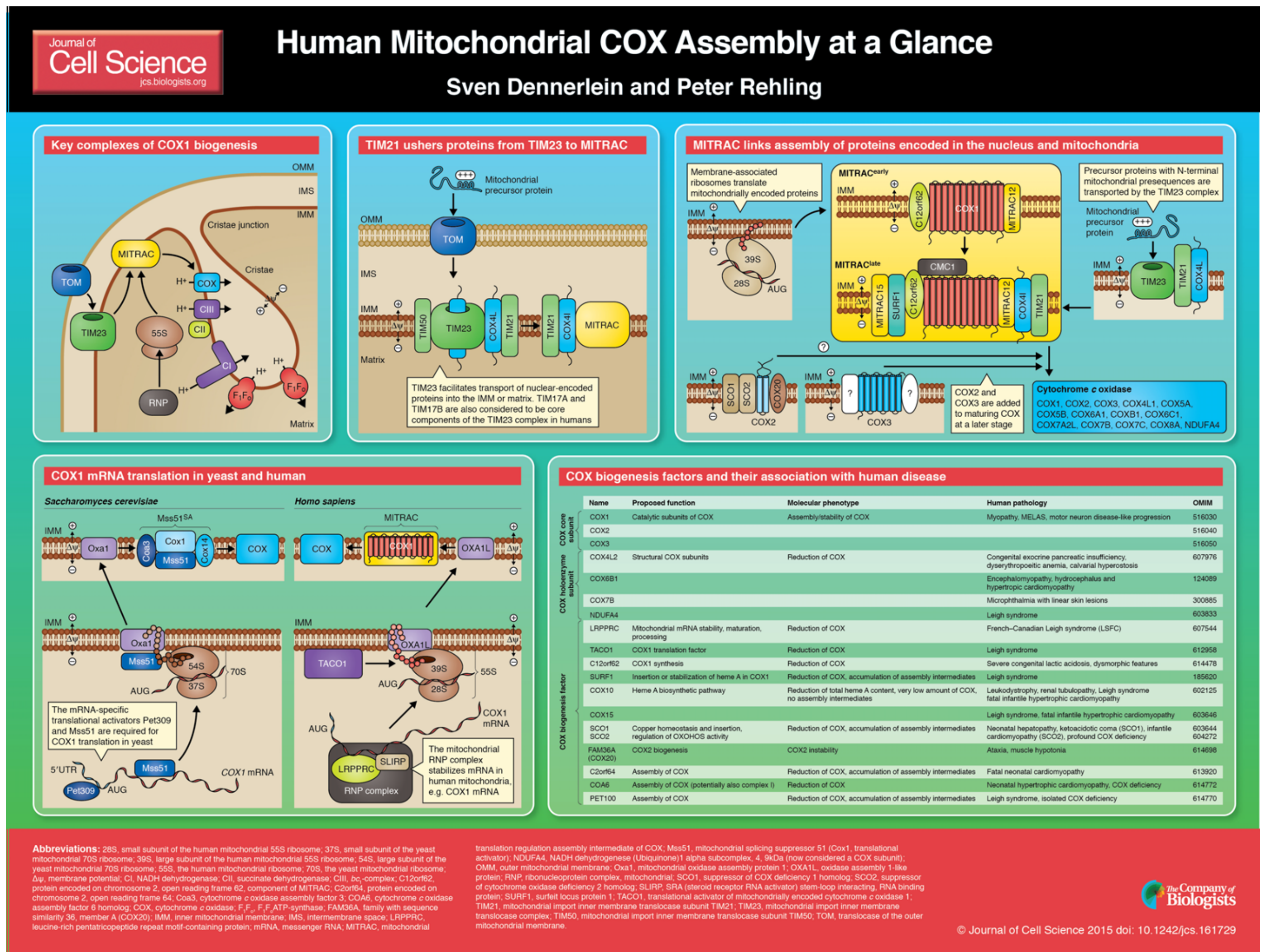
Sven Dennerlein[1] and Peter Rehling[1,2,*]

ABSTRACT

Mitochondria provide the main portion of cellular energy in form of ATP produced by the F_1F_o ATP synthase, which uses the electrochemical gradient, generated by the mitochondrial respiratory chain (MRC). In human mitochondria, the MRC is composed of four multisubunit enzyme complexes, with the cytochrome *c* oxidase (COX, also known as complex IV) as the terminal enzyme. COX comprises 14 structural subunits, of nuclear or mitochondrial origin. Hence, mitochondria are faced with the predicament of organizing and controlling COX assembly with subunits that are synthesized by different translation machineries and that reach the inner membrane by alternative transport routes. An increasing number of COX assembly factors have been identified in recent years. Interestingly, mutations in several of these factors have been associated with human disorders leading to COX deficiency. Recently, studies have provided mechanistic insights into crosstalk between assembly intermediates, import processes and the synthesis of COX subunits in mitochondria, thus linking conceptually separated functions. This Cell Science at a Glance article and the accompanying poster will focus on COX assembly and discuss recent discoveries in the field, the molecular

[1]Department of Cellular Biochemistry, University Medical Center Göttingen, 37073 Göttingen, Germany. [2]Max-Planck Institute for Biophysical Chemistry, 37077 Göttingen, Germany.

*Author for correspondence (peter.rehling@medizin.uni-goettingen.de)



	Name	Proposed function	Molecular phenotype	Human pathology	OMIM
COX core subunit	COX1	Catalytic subunits of COX	Assembly/stability of COX	Myopathy, MELAS, motor neuron disease-like progression	516030
	COX2				516040
	COX3				516050
COX holoenzyme subunit	COX4L2	Structural COX subunits	Reduction of COX	Congenital exocrine pancreatic insufficiency, dyserythropoeitic anemia, calvarial hyperostosis	607976
	COX6B1			Encephalomyopathy, hydrocephalus and hypertropic cardiomyopathy	124089
	COX7B			Microphthalmia with linear skin lesions	300885
	NDUFA4			Leigh syndrome	603833
COX biogenesis factor	LRPPRC	Mitochondrial mRNA stability, maturation, processing	Reduction of COX	French–Canadian Leigh syndrome (LSFC)	607544
	TACO1	COX1 translation factor	Reduction of COX	Leigh syndrome	612958
	C12orf62	COX1 synthesis	Reduction of COX	Severe congenital lactic acidosis, dysmorphic features	614478
	SURF1	Insertion or stabilization of heme A in COX1	Reduction of COX, accumulation of assembly intermediates	Leigh syndrome	185620
	COX10	Heme A biosynthetic pathway	Reduction of total heme A content, very low amount of COX, no assembly intermediates	Leukodystrophy, renal tubulopathy, Leigh syndrome fatal infantile hypertrophic cardiomyopathy	602125
	COX15			Leigh syndrome, fatal infantile hypertrophic cardiomyopathy	603646
	SCO1 SCO2	Copper homeostasis and insertion, regulation of OXOHOS activity	Reduction of COX, accumulation of assembly intermediates	Neonatal hepatopathy, ketoacidotic coma (SCO1), infantile cardiomyopathy (SCO2), profound COX deficiency	603644 604272
	FAM36A (COX20)	COX2 biogenesis	COX2 instability	Ataxia, muscle hypotonia	614698
	C2orf64	Assembly of COX	Reduction of COX, accumulation of assembly intermediates	Fatal neonatal cardiomyopathy	613920
	COA6	Assembly of COX (potentially also complex I)	Reduction of COX	Neonatal hypertrophic cardiomyopathy, COX deficiency	614772
	PET100	Assembly of COX	Reduction of COX, accumulation of assembly intermediates	Leigh syndrome, isolated COX deficiency	614770

functions of known factors, as well as new players and control mechanisms. Furthermore, these findings will be discussed in the context of human COX-related disorders.

KEY WORDS: COX1, MITRAC, Mitochondria, Cytochrome *c* oxidase, Complex IV

Introduction

Mitochondria are double-membrane-bounded organelles that originated from a bacterial ancestor through endosymbiosis. The outer and inner mitochondrial membranes (OMM and IMM) segregate mitochondria into two aqueous compartments, the mitochondrial matrix and the intermembrane space (IMS). The inner mitochondrial membrane forms cristae, which extend into the matrix and harbor the mitochondrial respiratory chain complexes and the F_1F_o ATP synthase at the cristae tips (Davies et al., 2013). In human mitochondria, complex I (NADH dehydrogenase) represents the largest respiratory chain complex with a size of 1 MDa; it consists of 44 structural subunits, seven of which are mitochondrially encoded (Hunte et al., 2010; Vinothkumar et al., 2014). In contrast to complex I, the smallest complex, complex II (succinate dehydrogenase) is composed of only four nuclear-encoded proteins. Complex III (*bc*-complex), which contains one mitochondrially (cytochrome *b*) and ten nuclear-encoded subunits, reduces cytochrome *c*, thereby providing electrons to the cytochrome *c* oxidase (COX, also known as complex IV). Cytochrome *c* oxidase is the terminal enzyme of the respiratory chain and electrons are transported by four redox-active centers (two copper centers and two heme *a* moieties) to molecular oxygen. In addition, COX contains several metal ions with yet unknown functions. Of the 14 structural subunits, the three core components, COX1, COX2 and COX3 (also known as MT-CO1, MT-CO2 and MT-CO3, respectively) are synthesized within the organelle, where they need to assemble with nuclear-encoded, peripherally localized subunits of the enzyme complex. An increasing number of factors required for cytochrome *c* oxidase assembly have been identified in recent years and mutations in the corresponding genes have been associated with various human diseases that are caused by deficiency of the enzyme complex. Interestingly, many of these proteins lack a clear yeast homolog.

Recent studies have provided unexpected insights into the biogenesis of mitochondrially encoded subunits and how this process is integrated into regulatory cycles. Here, we will first give a general overview of the complexes involved in COX assembly. Then we will illustrate the human mitochondrial TIM23 transport machinery and how it cooperates with COX assembly intermediates (see poster). As the assembly pathway of COX differs between yeast and human, we will compare these systems. We will then discuss the human COX1 assembly pathway in more detail and finally summarize known human diseases that are linked to COX deficiency.

The human presequence translocase – the TIM23 complex

Mitochondrial protein translocases that are responsible for the import of nuclear-encoded COX subunits into the mitochondria display high evolutionary conservation. Studies of the mechanisms underlying the transport processes have largely exploited yeast as a model organism (Chacinska et al., 2009; Dolezal et al., 2006; Dudek et al., 2013; Endo et al., 2011; Neupert and Herrmann, 2007; Sokol et al., 2014). However, the last few years have revealed several differences between the human and the yeast protein transport machineries. DNAJC19 and MCJ (also known as DNAJC15) initially have been considered to represent putative human orthologs of the yeast Pam18 protein, a component of the TIM23-complex-associated import motor that drives transport of precursors into mitochondria (D'Silva et al., 2008; Kozany et al., 2004; Li et al., 2004). Moreover, yeast Pam18 and its regulator Pam16 have been found to associate with respiratory chain complexes (Wiedemann et al., 2007). Defects in DNAJC19 have been linked to the syndrome dilated cardiomyopathy with ataxia (DCMA), a severe cardiomyopathy (Davey et al., 2006; Ojala et al., 2012). However, recent studies have shown that the majority of DNAJC19 associates with prohibitin complexes in human mitochondria and that DNAJC19 participates in cardiolipin metabolism (Richter-Dennerlein et al., 2014). Moreover, apart from its function in protein transport, another function of DNAJC19 that is not related to protein transport is further supported by the observation that only the second putative yeast Pam18 ortholog, MCJ, rescues *pam18Δ* cells (Schusdziarra et al., 2013). Strikingly, it has also been suggested that MCJ regulates the activity of complex I and promotes incorporation of complex I into respiratory chain supercomplexes (Hatle et al., 2013). These observations suggest that MCJ is present in two independent pools, because an association of the TIM23 complex with respiratory chain supercomplexes has not been described in humans. Nevertheless, it remains to be shown whether the observed interactions of MCJ with complex I and the TIM23 complex reflect a dynamic behavior.

Interestingly, TIM21 has been recently found to undergo dynamic associations between the TIM23 complex and COX assembly intermediates (Mick et al., 2012). TIM21 is a conserved subunit of the human TIM23 complex, but it also associates with the MITRAC (mitochondrial translation regulation assembly intermediate of COX) complex, an early assembly intermediate of the COX (see poster). TIM21 ushers imported, early COX subunits from the TIM23 complex to MITRAC (Mick et al., 2012) (see poster). The interaction between TIM21 and the TIM23 complex depends on active protein import, as a block of cytosolic translation inhibits the TIM23–TIM21 interaction, but does not affect the association of TIM21 with MITRAC. By contrast, a block in mitochondrial translation reduces the interaction of TIM21 with MITRAC, without affecting the interaction with TIM23.

Mitochondrial COX1 gene expression in yeast and human

The mitochondrial gene expression system differs substantially between lower and higher eukaryotes. The ribosome of mammalian mitochondria is a 55S particle with a 28S small subunit (mtSSU) and a 39S large subunit (mtLSU). In contrast, the yeast mitochondrial 70S ribosome comprises of a 37S SSU and a 54S LSU, respectively (for reviews, see Hällberg and Larsson, 2014; Lightowlers et al., 2014). Considering these differences, it is not surprising that fundamental changes in the synthesis and regulation of mitochondrial proteins have taken place during evolution. This section will focus on the translation of the COX1 protein by providing a short overview of Cox1 synthesis and regulation in yeast and summarizing the current view on the synthesis of human COX1.

In *Saccharomyces cerevisiae*, the mitochondrial mRNAs contain 5′UTRs that are recognized by specific translational activator proteins. In the case of *COX1* mRNA, these activators are Mss51 and Pet309 (Barrientos et al., 2004; Pérez-Martinez

Journal of Cell Science (2015) 128, 833–837 doi:10.1242/jcs.161729

et al., 2003; Tavares-Carreón et al., 2008; Zambrano et al., 2007; Zamudio-Ochoa et al., 2014). Pet309 belongs to the pentatricopeptide repeat (PPR) protein family, which is typically involved in mRNA metabolism. Pet309 has been shown to bind *COX1* mRNA and is required for its translation (Manthey and McEwen, 1995; Tavares-Carreón et al., 2008; Zamudio-Ochoa et al., 2014). Mss51 is able to bind to *COX1* mRNA, as well as to the newly synthesized Cox1 protein (Barrientos et al., 2004; Pérez-Martinez et al., 2003; Pérez-Martinez et al., 2009; Zambrano et al., 2007). Oxa1 facilitates insertion of Cox1 into the inner mitochondrial membrane. The first assembly step of newly synthesized Cox1 is its association with the two small inner membrane proteins, Cox14 and Coa3, as well as Mss51, forming a COX assembly intermediate (Mss51^{SA} complex) (Fontanesi et al., 2011; Mick et al., 2011) (see poster). Subsequently, several other proteins, among them Coa1, Shy1 and the nuclear-encoded Cox5 and Cox6 subunits, engage with the maturing COX complex. These steps also involve heme integration into Cox1, although it is not clear at which state precisely the incorporation occurs. A detailed overview of predicted COX assembly steps in yeast mitochondria can be found in recent reviews (e.g. Mick et al., 2011; Fontanesi, 2013).

The synthesis of COX1 in humans appears to differ significantly from the process in yeast (see poster). In contrast to yeast, mammalian mitochondrial mRNAs lack significant 5′UTR regions. Upstream of the translational start codon ATG, COX1 mRNA contains only three nucleotides in its 5′ region (Temperley et al., 2010). Thus, it appears unlikely that translational activators equivalent to yeast Pet309 or Mss51 could bind to the mRNA. Not surprisingly, no robust homologs of the translational activators have so far been identified in metazoa. Taking this into consideration, it is expected that alternative regulatory mechanisms for COX1 expression exist in human mitochondria.

The PPR-motive containing human protein LRPPRC, displays low sequence similarity to yeast Pet309 (Mootha et al., 2003; Sasarman et al., 2010). LRPPRC forms a mitochondrial ribonucleoprotein (RNP) complex with steroid receptor RNA activator (SRA) stem-loop interacting protein (SLIRP) in human mitochondria. This RNP complex interacts with polyadenylated mRNAs and is required for mitochondrial mRNA stability. In fibroblasts of patients with French–Canadian Leigh syndrome (LSFC), an early-onset progressive neurodegenerative disorder that is caused by mutations in LRPPRC, a specific reduction in the levels of COX has been observed (Sasarman et al., 2010). However, recent studies have shown that there is a tissue-specific involvement of LRPPRC in the assembly of respiratory chain complexes. Recently, in heart mitochondria of a conditional knockout mouse, a reduction of COX and impaired assembly of the F_1F_o ATP synthase has been observed (Mourier et al., 2014). When loss of LRPPRC function was assessed in different human tissues, the fibroblasts of LSFC patients displayed primarily a COX deficiency. However, upon a more severe reduction of LRPPRC levels, the entire respiratory chain was affected (Sasarman et al., 2015). Muscle cells from LSFC patients showed a combined NADH dehydrogenase and COX defect, whereas human heart-derived cells were only had mildly affected COX activity. Moreover, COX was almost undetectable in liver, underlining the importance of LRPPRC for COX maturation in specific organs. It is therefore tempting to speculate that tissue-specific pathways for the post-transcriptional handling of mitochondrial mRNAs exist.

The only known COX1-specific translation-regulating factor in higher eukaryotes is TACO1 (Weraarpachai et al., 2009) (see poster). Fibroblasts of patients with compromised TACO1 function present a slowly progressive Leigh syndrome and a reduction of COX1 synthesis that is concomitant with a faster turnover of COX2 and COX3. This finding clarifies the molecular basis of COX deficiency in these patients. However, the molecular function of TACO1 and how it affects COX1 synthesis on a biochemical level is still unknown.

COX assembly – COX1 takes center stage

The crystal structure of the bovine COX complex originally revealed 13 structural subunits (Tsukihara et al., 1996). However, recently a 14th subunit, NDUFA4, has been described and needs to be considered as a COX component (Balsa et al., 2012; Pitceathly et al., 2013). The biogenesis of the COX, just as for other OXPHOS complexes, depends on the coordinated assembly of proteins encoded in the nucleus and mitochondria. This assembly process requires specific factors that protect cells from damage, stabilize complex intermediates and promote enzyme maturation. In *S. cerevisiae*, more than 20 proteins have been described to be involved in the maturation of COX (Barrientos et al., 2009; Kim et al., 2012; Mick et al., 2011). Only a few of these have been identified in humans. Our current knowledge of COX assembly factors is mainly based on the analysis of patients with complex IV deficiency or on homology to yeast proteins. A bioinformatic study exploiting yeast proteins identified C12orf62 as displaying sequence similarity to yeast Cox14 (Szklarczyk et al., 2012). At the same time, a patient with a defect in C12orf62, presenting with fatal neonatal lactic acidosis, was identified (Weraarpachai et al., 2012). However, although loss of Cox14 in yeast leads to increased synthesis of Cox1, which is subsequently degraded, loss of C12orf62 leads to a decrease in COX1 translation, suggesting that the function of the yeast Cox14 protein and the supposed human homolog C12orf62 are different and even provoke opposite effects upon protein loss (Clemente et al., 2013; Fontanesi et al., 2011; Mick et al., 2012; Weraarpachai et al., 2012).

The MITRAC complex has been identified as a COX assembly intermediate, integrating mitochondrially encoded and newly imported proteins (see poster). Because maturation of the enzyme complexes is a sequential process during which new components and cofactors are added, the composition of the MITRAC complex changes during the process. Current data indicate that early-MITRAC contains C12orf62 and MITRAC12, and assists COX1 in early assembly steps. TIM21 is likely to incorporate newly imported COX subunits into MITRAC at a later stage. However, precisely at which stage the interaction with TIM21 occurs and how the COX1 translational feedback mechanism is mediated remains unknown.

Several other proteins, many of which have been identified in the course of investigations of patients with a COX deficiency, have been described during the last decade. However, the molecular function for these proteins in COX biogenesis is often elusive. One of the most prominent examples is SURF1, for which a large number of mutations that lead to COX deficiency have been identified in patients (for a review, see Wedatilake et al., 2013). Patients with SURF1 mutations also display the phenotype of Leigh syndrome. In most cases, the disease is caused by instability of the SURF1 protein and a reduction in the amount of fully matured COX, although a concomitant accumulation of subcomplexes have been observed in these patients. Studies on SURF1 in bacteria and yeast indicate that the protein is involved in the insertion or stabilization of the heme-containing cofactors of COX1 (Bareth et al., 2013; Smith et al., 2005).

Journal of Cell Science (2015) 128, 833–837 doi:10.1242/jcs.161729

Two other proteins, C2orf64 and PET100 (also known as C19orf79), are potentially involved in the COX1 assembly process. A mutation in the C2orf64-encoding gene (*COA5*) leads to an accumulation of various COX assembly intermediates and reduced steady-state levels of COX1, thus explaining the complex IV deficiency (Huigsloot et al., 2011). A similar phenotype is observed in patients with mutations in the *PET100* gene (Lim et al., 2014; Oláhová et al., 2014). PET100 is present in a 300 kDa complex together with other subunits of the COX (Lim et al., 2014). However, the function of PET100 is still elusive.

Concluding remarks

Several factors involved in COX assembly have been identified recently and add new insights into the molecular details of this process. However, considering the complexity of the assembly process, very little is currently known about the sequences of events and the function of the assembly factors. The establishment of new techniques such as exome sequencing will help to identify new players. Although this is certainly essential for a comprehensive understanding, the challenge lies in the determination of the exact functions of the various proteins. To date we are still far from explaining how the COX1 protein is handled throughout the assembly process.

However, recent studies, as discussed above, provided a broader insight the COX1 assembly process and so bring us closer to a biochemical understanding of human diseases. These data could be the basis of further drug development and so might pave the way for COX therapy.

Acknowledgements

We are grateful to S. Callegarie and R. Richter-Dennerlein for critical comments on the article.

Competing interests

The authors declare no competing or financial interests.

Funding

Work in our laboratory is supported by the Deutsche Forschungsgemeinschaft; European Research Council [grant number 339580] to P.R.; and the Max-Planck-Society (to P.R.).

Cell science at a glance

A high-resolution version of the poster is available for downloading in the online version of this article at jcs.biologists.org. Individual poster panels are available as JPEG files at http://jcs.biologists.org/lookup/suppl/doi:10.1242/jcs.161729/-/DC1.

References

Balsa, E., Marco, R., Perales-Clemente, E., Szklarczyk, R., Calvo, E., Landázuri, M. O. and Enríquez, J. A. (2012). NDUFA4 is a subunit of complex IV of the mammalian electron transport chain. *Cell Metab.* **16**, 378-386.

Bareth, B., Dennerlein, S., Mick, D. U., Nikolov, M., Urlaub, H. and Rehling, P. (2013). The heme a synthase Cox15 associates with cytochrome c oxidase assembly intermediates during Cox1 maturation. *Mol. Cell. Biol.* **33**, 4128-4137.

Barrientos, A., Zambrano, A. and Tzagoloff, A. (2004). Mss51p and Cox14p jointly regulate mitochondrial Cox1p expression in Saccharomyces cerevisiae. *EMBO J.* **23**, 3472-3482.

Barrientos, A., Gouget, K., Horn, D., Soto, I. C. and Fontanesi, F. (2009). Suppression mechanisms of COX assembly defects in yeast and human: insights into the COX assembly process. *Biochim. Biophys. Acta* **1793**, 97-107.

Chacinska, A., Koehler, C. M., Milenkovic, D., Lithgow, T. and Pfanner, N. (2009). Importing mitochondrial proteins: machineries and mechanisms. *Cell* **138**, 628-644.

Clemente, P., Peralta, S., Cruz-Bermudez, A., Echevarría, L., Fontanesi, F., Barrientos, A., Fernandez-Moreno, M. A. and Garesse, R. (2013). hCOA3 stabilizes cytochrome c oxidase 1 (COX1) and promotes cytochrome c oxidase assembly in human mitochondria. *J. Biol. Chem.* **288**, 8321-8331.

D'Silva, P. R., Schilke, B., Hayashi, M. and Craig, E. A. (2008). Interaction of the J-protein heterodimer Pam18/Pam16 of the mitochondrial import motor with the translocon of the inner membrane. *Mol. Biol. Cell* **19**, 424-432.

Davey, K. M., Parboosingh, J. S., McLeod, D. R., Chan, A., Casey, R., Ferreira, P., Snyder, F. F., Bridge, P. J. and Bernier, F. P. (2006). Mutation of DNAJC19, a human homologue of yeast inner mitochondrial membrane co-chaperones, causes DCMA syndrome, a novel autosomal recessive Barth syndrome-like condition. *J. Med. Genet.* **43**, 385-393.

Davies, K. M., Daum, B., Gold, V. A. M., Mühleip, A. W., Brandt, T., Blum, T. B., Mills, D. J. and Kühlbrandt, W. (2013). Visualization of ATP synthase dimers in mitochondria by electron cryo-tomography. *J. Vis. Exp.* **2014**, 51228.

Dolezal, P., Likic, V., Tachezy, J. and Lithgow, T. (2006). Evolution of the molecular machines for protein import into mitochondria. *Science* **313**, 314-318.

Dudek, J., Rehling, P. and van der Laan, M. (2013). Mitochondrial protein import: common principles and physiological networks. *Biochim. Biophys. Acta* **1833**, 274-285.

Endo, T., Yamano, K. and Kawano, S. (2011). Structural insight into the mitochondrial protein import system. *Biochim. Biophys. Acta* **1808**, 955-970.

Fontanesi, F. (2013). Mechanisms of mitochondrial translational regulation. *IUBMB Life* **65**, 397-408.

Fontanesi, F., Clemente, P. and Barrientos, A. (2011). Cox25 teams up with Mss51, Ssc1, and Cox14 to regulate mitochondrial cytochrome c oxidase subunit 1 expression and assembly in Saccharomyces cerevisiae. *J. Biol. Chem.* **286**, 555-566.

Hällberg, B. M. and Larsson, N.-G. (2014). Making proteins in the powerhouse. *Cell Metab.* **20**, 226-240.

Hatle, K. M., Gummadidala, P., Navasa, N., Bernardo, E., Dodge, J., Silverstrim, B., Fortner, K., Burg, E., Suratt, B. T., Hammer, J. et al. (2013). MCJ/DnaJC15, an endogenous mitochondrial repressor of the respiratory chain that controls metabolic alterations. *Mol. Cell. Biol.* **33**, 2302-2314.

Huigsloot, M., Nijtmans, L. G., Szklarczyk, R., Baars, M. J. H., van den Brand, M. A. M., Hendriksfranssen, M. G. M., van den Heuvel, L. P., Smeitink, J. A. M., Huynen, M. A. and Rodenburg, R. J. T. (2011). A mutation in C2orf64 causes impaired cytochrome c oxidase assembly and mitochondrial cardiomyopathy. *Am. J. Hum. Genet.* **88**, 488-493.

Hunte, C., Zickermann, V. and Brandt, U. (2010). Functional modules and structural basis of conformational coupling in mitochondrial complex I. *Science* **329**, 448-451.

Kim, H. J., Khalimonchuk, O., Smith, P. M. and Winge, D. R. (2012). Structure, function, and assembly of heme centers in mitochondrial respiratory complexes. *Biochim. Biophys. Acta* **1823**, 1604-1616.

Kozany, C., Mokranjac, D., Sichting, M., Neupert, W. and Hell, K. (2004). The J domain-related cochaperone Tim16 is a constituent of the mitochondrial TIM23 preprotein translocase. *Nat. Struct. Mol. Biol.* **11**, 234-241.

Li, Y., Dudek, J., Guiard, B., Pfanner, N., Rehling, P. and Voos, W. (2004). The presequence translocase-associated protein import motor of mitochondria. Pam16 functions in an antagonistic manner to Pam18. *J. Biol. Chem.* **279**, 38047-38054.

Lightowlers, R. N., Rozanska, A. and Chrzanowska-Lightowlers, Z. M. (2014). Mitochondrial protein synthesis: figuring the fundamentals, complexities and complications, of mammalian mitochondrial translation. *FEBS Lett.* **588**, 2496-2503.

Lim, S. C., Smith, K. R., Stroud, D. A., Compton, A. G., Tucker, E. J., Dasvarma, A., Gandolfo, L. C., Marum, J. E., McKenzie, M., Peters, H. L. et al. (2014). A founder mutation in PET100 causes isolated complex IV deficiency in Lebanese individuals with Leigh syndrome. *Am. J. Hum. Genet.* **94**, 209-222.

Manthey, G. M. and McEwen, J. E. (1995). The product of the nuclear gene PET309 is required for translation of mature mRNA and stability or production of intron-containing RNAs derived from the mitochondrial COX1 locus of Saccharomyces cerevisiae. *EMBO J.* **14**, 4031-4043.

Mick, D. U., Fox, T. D. and Rehling, P. (2011). Inventory control: cytochrome c oxidase assembly regulates mitochondrial translation. *Nat. Rev. Mol. Cell Biol.* **12**, 14-20.

Mick, D. U., Dennerlein, S., Wiese, H., Reinhold, R., Pacheu-Grau, D., Lorenzi, I., Sasarman, F., Weraarpachai, W., Shoubridge, E. A., Warscheid, B. et al. (2012). MITRAC links mitochondrial protein translocation to respiratory-chain assembly and translational regulation. *Cell* **151**, 1528-1541.

Mootha, V. K., Lepage, P., Miller, K., Bunkenborg, J., Reich, M., Hjerrild, M., Delmonte, T., Villeneuve, A., Sladek, R., Xu, F. et al. (2003). Identification of a gene causing human cytochrome c oxidase deficiency by integrative genomics. *Proc. Natl. Acad. Sci. USA* **100**, 605-610.

Mourier, A., Ruzzenente, B., Brandt, T., Kühlbrandt, W. and Larsson, N.-G. (2014). Loss of LRPPRC causes ATP synthase deficiency. *Hum. Mol. Genet.* **23**, 2580-2592.

Neupert, W. and Herrmann, J. M. (2007). Translocation of proteins into mitochondria. *Annu. Rev. Biochem.* **76**, 723-749.

Ojala, T., Polinati, P., Manninen, T., Hiippala, A., Rajantie, J., Karikoski, R., Suomalainen, A. and Tyni, T. (2012). New mutation of mitochondrial DNAJC19 causing dilated and noncompaction cardiomyopathy, anemia, ataxia, and male genital anomalies. *Pediatr. Res.* **72**, 432-437.

Oláhová, M., Haack, T. B., Alston, C. L., Houghton, J. A., He, L., Morris, A. A., Brown, G. K., McFarland, R., Chrzanowska-Lightowlers, Z. M., Lightowlers, R. N. et al. (2014). A truncating PET100 variant causing fatal infantile lactic acidosis and isolated cytochrome c oxidase deficiency. *Eur. J. Hum. Genet.*

Pérez-Martinez, X., Broadley, S. A. and Fox, T. D. (2003). Mss51p promotes mitochondrial Cox1p synthesis and interacts with newly synthesized Cox1p. *EMBO J.* **22**, 5951-5961.

Pérez-Martinez, X., Butler, C. A., Shingú-Vazquez, M. and Fox, T. D. (2009). Dual functions of Mss51 couple synthesis of Cox1 to assembly of cytochrome c oxidase in Saccharomyces cerevisiae mitochondria. *Mol. Biol. Cell* **20**, 4371-4380.

Pitceathly, R. D. S., Rahman, S., Wedatilake, Y., Polke, J. M., Cirak, S., Foley, A. R., Sailer, A., Hurles, M. E., Stalker, J., Hargreaves, I. et al.; UK10K Consortium (2013). NDUFA4 mutations underlie dysfunction of a cytochrome c oxidase subunit linked to human neurological disease. *Cell Reports* **3**, 1795-1805.

Richter-Dennerlein, R., Korwitz, A., Haag, M., Tatsuta, T., Dargazanli, S., Baker, M., Decker, T., Lamkemeyer, T., Rugarli, E. I. and Langer, T. (2014). DNAJC19, a mitochondrial cochaperone associated with cardiomyopathy, forms a complex with prohibitins to regulate cardiolipin remodeling. *Cell Metab.* **20**, 158-171.

Sasarman, F., Brunel-Guitton, C., Antonicka, H., Wai, T., Shoubridge, E. A.; LSFC Consortium (2010). LRPPRC and SLIRP interact in a ribonucleoprotein complex that regulates posttranscriptional gene expression in mitochondria. *Mol. Biol. Cell* **21**, 1315-1323.

Sasarman, F., Nishimura, T., Antonicka, H., Weraarpachai, W., Shoubridge, E. A. (2015). Tissue-specific responses to the LRPPRC founder mutation in French Canadian Leigh Syndrome. *Hum. Mol. Genet.* **24**, 480-491.

Schusdziarra, C., Blamowska, M., Azem, A. and Hell, K. (2013). Methylation-controlled J-protein MCJ acts in the import of proteins into human mitochondria. *Hum. Mol. Genet.* **22**, 1348-1357.

Smith, D., Gray, J., Mitchell, L., Antholine, W. E. and Hosler, J. P. (2005). Assembly of cytochrome-c oxidase in the absence of assembly protein Surf1p leads to loss of the active site heme. *J. Biol. Chem.* **280**, 17652-17656.

Sokol, A. M., Sztolsztener, M. E., Wasilewski, M., Heinz, E. and Chacinska, A. (2014). Mitochondrial protein translocases for survival and wellbeing. *FEBS Lett.* **588**, 2484-2495.

Szklarczyk, R., Wanschers, B. F., Cuypers, T. D., Esseling, J. J., Riemersma, M., van den Brand, M. A., Gloerich, J., Lasonder, E., van den Heuvel, L. P., Nijtmans, L. G. et al. (2012). Iterative orthology prediction uncovers new mitochondrial proteins and identifies C12orf62 as the human ortholog of COX14, a protein involved in the assembly of cytochrome c oxidase. *Genome Biol.* **13**, R12.

Tavares-Carreón, F., Camacho-Villasana, Y., Zamudio-Ochoa, A., Shingú-Vázquez, M., Torres-Larios, A. and Pérez-Martínez, X. (2008). The pentatricopeptide repeats present in Pet309 are necessary for translation but not for stability of the mitochondrial COX1 mRNA in yeast. *J. Biol. Chem.* **283**, 1472-1479.

Temperley, R. J., Wydro, M., Lightowlers, R. N. and Chrzanowska-Lightowlers, Z. M. (2010). Human mitochondrial mRNAs – like members of all families, similar but different. *Biochim. Biophys. Acta* **1797**, 1081-1085.

Tsukihara, T., Aoyama, H., Yamashita, E., Tomizaki, T., Yamaguchi, H., Shinzawa-Itoh, K., Nakashima, R., Yaono, R. and Yoshikawa, S. (1996). The whole structure of the 13-subunit oxidized cytochrome c oxidase at 2.8 A. *Science* **272**, 1136-1144.

Vinothkumar, K. R., Zhu, J. and Hirst, J. (2014). Architecture of mammalian respiratory complex I. *Nature* **515**, 80-84.

Wedatilake, Y., Brown, R. M., McFarland, R., Yaplito-Lee, J., Morris, A. A. M., Champion, M., Jardine, P. E., Clarke, A., Thorburn, D. R., Taylor, R. W. et al. (2013). SURF1 deficiency: a multi-centre natural history study. *Orphanet J. Rare Dis.* **8**, 96.

Weraarpachai, W., Antonicka, H., Sasarman, F., Seeger, J., Schrank, B., Kolesar, J. E., Lochmüller, H., Chevrette, M., Kaufman, B. A., Horvath, R. et al. (2009). Mutation in TACO1, encoding a translational activator of COX I, results in cytochrome c oxidase deficiency and late-onset Leigh syndrome. *Nat. Genet.* **41**, 833-837.

Weraarpachai, W., Sasarman, F., Nishimura, T., Antonicka, H., Auré, K., Rötig, A., Lombès, A. and Shoubridge, E. A. (2012). Mutations in C12orf62, a factor that couples COX I synthesis with cytochrome c oxidase assembly, cause fatal neonatal lactic acidosis. *Am. J. Hum. Genet.* **90**, 142-151.

Wiedemann, N., van der Laan, M., Hutu, D. P., Rehling, P. and Pfanner, N. (2007). Sorting switch of mitochondrial presequence translocase involves coupling of motor module to respiratory chain. *J. Cell Biol.* **179**, 1115-1122.

Zambrano, A., Fontanesi, F., Solans, A., de Oliveira, R. L., Fox, T. D., Tzagoloff, A. and Barrientos, A. (2007). Aberrant translation of cytochrome c oxidase subunit 1 mRNA species in the absence of Mss51p in the yeast Saccharomyces cerevisiae. *Mol. Biol. Cell* **18**, 523-535.

Zamudio-Ochoa, A., Camacho-Villasana, Y., García-Guerrero, A. E. and Pérez-Martínez, X. (2014). The Pet309 pentatricopeptide repeat motifs mediate efficient binding to the mitochondrial COX1 transcript in yeast. *RNA Biol.* **11**, 953-967.