Cytochrome *c* oxidase biogenesis – from translation to early assembly of the core subunit COX1

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Mitochondria are the powerhouses of the cell as they produce the majority of ATP with their oxidative phosphorylation (OXPHOS) machinery. The OXPHOS system is composed of the F_1F_o ATP synthase and four mitochondrial respiratory chain complexes, the terminal enzyme of which is the cytochrome *c* oxidase (complex IV) that transfers electrons to oxygen, generating water. Complex IV comprises of 14 structural subunits of dual genetic origin: while the three core subunits are mitochondrial encoded, the remaining constituents are encoded by the nuclear genome. Hence, the assembly of complex IV requires the coordination of two spatially separated gene expression machinery. Recent efforts elucidated an increasing number of proteins involved in mitochondrial gene expression, which are linked to complex IV assembly. Additionally, several COX1 biogenesis factors have been intensively biochemically investigated and an increasing number of structural snapshots shed light on the organization of macromolecular complexes such as the mitoribosome or the cytochrome *c* oxidase. Here, we focus on COX1 translation regulation and highlight the advanced understanding of early steps during COX1 assembly and its link to mitochondrial translation regulation.

Keywords: complex IV; COX1; cytochrome *c* oxidase; mitochondria; OXPHOS

Mitochondria produce the majority of chemical energy in human cells by oxidative phosphorylation (OXPHOS). The OXPHOS system localizes to the cristae of the inner mitochondrial membrane (IMM). The F_1F_o ATP synthase and the electron transport chain (ETC) form the OXPHOS machinery. The latter one consists of the NADH: ubiquinone oxidoreductase (complex I), the succinate dehydrogenase (complex II), the ubiquinone cytochrome *c* oxidoreductase (cytochrome *bc*1 complex or complex III), and the cytochrome *c* oxidase (complex IV). Cytochrome *c*, which is reduced by the cytochrome *bc*1 complex is oxidized by the cytochrome *c* oxidase, complex IV, which finally converts molecular oxygen (O_2) to water (H_2O). With the transfer of electrons along the ETC, protons are pumped into the intermembrane space (IMS) by complexes I, III, and IV generating an electrochemical gradient, which is utilized by the F_1F_o ATP synthase to generate ATP.

At least 30 proteins are required to form the functional human complex IV. Among those are 14 structural subunits and a growing number of assembly factors, many are associated with human diseases [1]. Initially, 13 structural subunits of complex IV had been identified as components of the mature enzyme. However, NDUFA4, previously assigned as a constituent of complex I, is now allocated a complex IV subunit [2]. While 11 cytochrome *c* oxidase subunits are encoded in the nucleus, synthesized by cytosolic ribosomes, and imported into mitochondria, the three core proteins (COX1, COX2, and COX3) are encoded by the mitochondrial DNA and synthesized in mitochondria.

To assemble the 14 structural subunits into a functional complex IV enzyme with a size of ~ 440 kDa, several different assembly factors are required [1,3–5]. The fully assembled complex IV contains two heme molecules (heme a and a_3) and one mononuclear copper (CuB) center in the COX1 subunit and a binuclear copper (CuA) in COX2 that require the assistance of dedicated biogenesis factors, which facilitate coordinated cofactor embedding during the assembly process.

The proper insertion of these electron-transferring metal centers is crucial to prevent uncontrolled electron flux and to protect the cell from the generation of radicals, such as reactive oxygen species. However, as mentioned above, these metal ions are located within the central complex IV subunits COX1 and COX2, and hence, need to be inserted already during translation, but certainly prior to the final steps of enzyme maturation.

Recently, several publications shed light on the synthesis of mitochondrial-encoded proteins covering biological and biochemical aspects of the process, as well as structural details of ribosome function [6–10]. Here, we will focus on the synthesis of the central complex IV subunit COX1, as it represents the stepping stone of complex IV assembly, and emphasizes its translation initiation, termination, and regulation. We will discuss the factors required for COX1 expression and, furthermore, we will highlight our current knowledge of the early assembly steps of COX1.

The translation process of COX1

Initiation of COX1 translation

The human mitochondrial translation machinery differs substantially from its cytosolic and bacterial counterparts. While the cytosolic ribosome represents an 80S complex, composed of a 60S large subunit (LSU) and a 40S small subunit (SSU), the human mitochondrial ribosome is a 55S particle, comprising a 39S mtLSU and a 28S mtSSU. As the mitochondrial translation apparatus is related to the bacterial one, a different subset of translation factors is present within mitochondria compared to the cytosol [11,12]. While at least 25 factors have been identified in the cytosol, 12 factors, which show not only similarities but also differences to bacterial translation factors, seem to suffice for mitochondrial translation. These are the initiation factors mtIF2 and mtIF3, the elongation factors mtEFTu, mtEFTs, and mtEFG1, and the mitochondrial release factors mtRF1 and mtRF1a [6,8–10,12–14]. Finally, the mitochondrial ribosome is getting dissociated into its subunits by mtRRF and mtEFG2 to be available for the next translation cycle [6,15,16]. Additionally, some key factors have been identified to be crucial to rescue stalled ribosome complexes, namely the two release factors ICT1 (mL62) [6,17] and C12ORF65 (mtRF-R) [18], and potentially, the alternative ribosome recycling factor GTPBP6 [12,19,20].

Although mitochondria have a bacterial ancestor, the mRNA architecture, the genetic code, and the mechanism of translation initiation differ substantially. Bacterial mRNAs carry the Shine Dalgarno sequence upstream of the start codon, which allows the correct positioning of the mRNA within the SSU due to base pairing with the anti-Shine–Dalgarno sequence of the 16S rRNA. In contrast, human mitochondrial mRNAs lack significant 5′ UTRs, and also the anti-Shine–Dalgarno sequence is missing in the 12S mt-rRNA. The transcripts encoding ND2, ND3, ND4L, ND5, ND6, CYTB, COX2, and COX3 do not contain any nucleotides upstream of the 5′-start codon, ATP8 contains one, ND1 two, and COX1 three nucleotides [21]. Nevertheless, there are two exceptions to this common trend: the two bicistronic mt-mRNA units, mtRNA7 (ND4L-ND4) and mtRNA14 (ATP8-ATP6). The 5′ region of ND4 represents the open reading frame of ND4L, while ATP8 contains the start codon of ATP6. In principle, the ND4 (296 nt) and the ATP6 (161 nt) transcript contain long 5′ UTR regions.

Initially, it has been assumed that mitochondrial translation initiation starts with mt-mRNA binding to the mtSSU. However, also for the prokaryotic system, the direct interaction of leaderless mRNAs with the 70S monosome has been reported, even when no translation initiation factor is present [22]. Recently, Remes *et al.* [23] reported that interaction of mt-mRNAs with the 55S particle seems to be more likely than their binding to the mtSSU prior to subunit joining.

To initiate translation, specific factors (initiation factors, IF) are required. A mitochondrial counterpart of bacterial IF1 is missing, however, homologs of IF2

(mtIF2) and IF3 (mtIF3) have been reported [24,25] and a domain insertion within mtIF2 functionally replaces IF1 [7,26]. While mtIF3 has recently been linked to translation initiation of the bicistronic ATP8-ATP6 transcript [23], mtIF2 facilitates the binding of the initiator tRNA carrying a formylated methionine (fMet-tRNA^{Met}) to the mtSSU or the assembled ribosome. Loss of mtIF2 abolishes mitochondrial translation but does not influence the assembly of the mitochondrial ribosome [23]. Interestingly, defects in mtLSU assembly stabilize the initiation complex containing mtIF2, fMet-tRNA^{Met}, and a mRNA moiety with an AUG start codon in the P-site (Fig. 1, left side). As structural analyses reveal additional density for three nucleotides preceding the AUG codon, it has been suggested that the accumulated initiation complex contains exclusively the COX1 transcript [27]. The resolution of the cryo-EM data was not sufficient to determine the nature of these nucleotides, but it has been speculated that they interact with uS7m of the mtSSU. Nevertheless, the data do not rule out that the observed three nucleotides are rather part of a different transcript than COX1. Interestingly, in addition to the COX1 mRNA, Northern blot and qPCR analysis of gradient fractions clearly identified other mitochondrial-encoded transcripts in fractions where the mtSSU migrates, such as ND1, ND2, and COX2, indicating that also other mt-mRNAs could be loaded onto the mtSSU directly prior to monosome formation [28,29]. Further developments to reconstitute mitochondrial translation *in vitro* in combination with high-resolution cryo-EM structures are required to especially define the exceptional positioning of COX1 within the mtSSU.

Termination of COX1 translation

During the process of translation termination, dedicated factors (release factors, RF) sequence specifically recognize the STOP codon. These tRNA-like shaped proteins bind to the ribosomal A-site and provoke conformational changes within the ribosome to facilitate the hydrolysis of the ester bond between the nascent peptide chain and the peptidyl-tRNA. Bacteria contain two release factors (RF1 and RF2), which read the STOP codons UAA and UAG or UAA and UGA, respectively. However, mitochondria use a slightly different genetic code, for example, UGA encodes for tryptophan instead of being a STOP codon and AGA and AGG do not encode arginine, but have been reassigned as unconventional STOP signals to terminate COX1 and ND6 translation, respectively. How translation termination is achieved in

these two cases is controversially discussed. A −1 ribosomal frameshift by the human mitochondrial ribosome would generate the standard STOP codon UAG [30]. However, in some species, a U does not precede these unconventional STOP codons AGA or AGG, and in other species, canonical STOP codons are used for all mitochondrial-encoded transcripts. Thus, the factor responsible for terminating COX1 translation has been debated for a long time. Four proteins, related to the release factor family, have been identified in human mitochondria: mtRF1a, mtRF1, ICT1 (mL62), and C12ORF65 (mtRF-R) [14,17,31,32]. These factors share the highly conserved GGQ motive, which protrudes into the peptidyl transferase center once a release factor interacts with the ribosomal A-site [6]. In contrast to canonical release factors, ICT1 and C12ORF65 lack codon recognition motifs to sequence specifically read the STOP codon in the A-site. Both factors have been suggested to be required to rescue stalled ribosome complexes. ICT1 recognizes stalled mitochondrial ribosomes with truncated mRNA (non-stop scenario) and inserts its C-terminal extension into the mRNA channel [6,17]. C12ORF65 is involved in the mitochondrial ribosome-associated quality control system (mtRQC), which rescues ribosomes, for example, under aminoacyl-tRNA starvation or if translation factors are missing (no-go scenario) [18]. mtRF1a has been assigned as the major mitochondrial release factor as it shows the highest sequence similarity to bacterial RF1 and is capable of terminate translation at UAA and UAG codons *in vitro* [6,14]. In contrast, the function of mtRF1 was only determined recently. Three studies reported that mtRF1 is specifically responsible for translation termination of COX1, and thus for the biogenesis of complex IV (Fig. 1, left side) [8–10]. Loss of mtRF1 leads to a selective reduction in newly synthesized COX1 and to isolated complex IV deficiency. In agreement with a translation defect in COX1 are reduced levels of C12ORF62/ COX14, which is a constituent of the early complex IV assembly intermediate 'MITRAC' (Mitochondrial Translation Regulation Assembly intermediate of Cytochrome *c* oxidase) [9]. C12ORF62 interacts with ribosome-nascent chain complexes during COX1 synthesis (see the following section) (Fig. 1, left side). In contrast, mtRF1a ablation results in translation defects of other mitochondrial-encoded proteins including not only COX2 and COX3 but also ND6. Contrary to the effects observed in mtRF1-deficient cells, loss of mtRF1a results in an accumulation of COX1-containing MITRAC complexes as the assembly process is stalled downstream of this intermediate due to the reduction in COX2 and COX3 syntheses. Recently,

Saurer *et al.* [8] solved the structure of the mtRF1 within the mitochondrial ribosome by cryo-electron microscopy and thereby provided insight into the mode of action of mtRF1 compared to mtRF1a. Both proteins differ substantially in their N-terminal portions, in which mtRF1 contains an extension of positively charged residues. This amino acid stretch seems to extend into a pocket below the L7/L12 stalk of the mtLSU. Both proteins contain a PTH domain with the conserved GGQ motif but differ in the codon recognition domain, in which mtRF1 displays insertions in the α5 helix and the codon recognition loop. This rather atypical codon recognition domain enables mtRF1 to recognize non-canonical stop codons with A and G at the first two positions of the codon by distorting the mRNA backbone, reorienting the first two bases, and by utilizing a network of interactions including the 12S mt-rRNA and mt-mRNA [8]. Although these data mechanistically explain how mtRF1 terminates COX1 translation, it remains to be addressed why mtRF1-deficient cells display an isolated COX deficiency but not complex I defect, if mtRF1 is also responsible for recognizing the non-canonical STOP codon AGG in ND6 transcripts.

Nevertheless, mtRF1-deficient cells are still able to synthesize a fraction of COX1, which is able to assemble into respiratory chain supercomplexes and enables cells to respire. Interestingly, C12ORF65 as part of the mtRQC, showed increased levels in mtRF1-deficient cells, while the levels of mtRF1a and mL62 remained unaffected [9]. Additionally, siRNA-mediated depletion of C12ORF65 led to a further decrease in COX1 synthesis compared to sole ablation of mtRF1 without affecting the synthesis of other mitochondrial-encoded proteins. Hence, the interruption of translation termination of COX1 induces the mtRQC system involving C12ORF65. This also underlines the central importance of the mtRQC system, since a growing number of patients with mutated *C12orf65* have been described, developing Leigh syndrome [32–34]. However, as these patients with usually undetectable levels of C12ORF65 display a general translation defect, mtRQC mediated by C12ORF65 seems to be generally involved in ribosome rescue and not selectively responsible for the release of stalled COX1-translating ribosomes. Interestingly, the reduction in COX1 translation termination due to mtRF1 ablation specifically reduces the level of COX1 mRNA while all other mitochondrial transcripts remained unaffected [9,10]. The specific reduction in COX1 mRNA might reflect a feedback mechanism that prevents an overload of the mtRQC if mtRF1 is missing. However, this observation requires further investigation.

COX1 translation regulation in human mitochondria

In addition to the described regulation of translational processes within mitochondria, the synthesis of COX1 underlies further, specific control mechanisms that involve several essential factors. TACO1 is a translational regulator of COX1 [35]. Patient-derived cell lines with TACO1 dysfunction displayed reduced COX1 protein synthesis and concomitant complex IV deficiency. TACO1 is a mRNA-binding protein that possesses a DUF28 domain (Fig. 1, left side). So far, it is the only translation activator in human mitochondria that appears to regulate the synthesis of one specific mitochondrial-encoded protein [35,36]. In addition to TACO1, C12ORF62 and MITRAC12 affect COX1 protein biogenesis. Both proteins have been linked to mitochondrial disorders. Mutations in C12ORF62 and MITRAC12 resulted in isolated complex IV deficiency. A homozygous missense mutation in C12ORF62 (c.88G>A) leads to fatal neonatal lactic acidosis [37], while a heterozygous mutation in MITRAC12 (c.199dupC, c.215A>G) caused neuropathy, exercise intolerance, obesity, and short stature [38]. C12ORF62 and MITRAC12 form an early complex IV intermediate, termed MITRAC (Mitochondrial Translation Regulation Assembly intermediate of Cytochrome *c* oxidase) (Fig. 1, left side) [38–41]. The MITRAC complex facilitates the coordinated assembly of the nuclear-encoded complex IV subunits, which have been synthesized in the cytosol and imported into the organelle, with the mitochondrial-encoded COX1 protein. The newly imported complex IV subunits are transferred by the TIM21 protein from the presequence translocase to the MITRAC complex [39]. Hence, TIM21 acts as a shuttling protein between the mitochondrial import machinery and the assembly intermediate. C12ORF62 and MITRAC12 interact with the nascent COX1 polypeptide chain and thus associate with actively translating mitochondrial ribosomes. Hence, it is tempting to speculate that mitochondrial ribosomes are dedicated to the translation of a certain mRNA, such as COX1, and that specific assembly factors interact with them. Pharmacological inhibition of mitochondrial translation affects the interaction of C12ORF62 and MITRAC12 with the mitochondrial ribosome but not with the OXA1L protein insertase that facilitates membrane insertion of the polypeptide chains in a cotranslational manner [40,42,43]. This observation implies that C12ORF62, MITRAC12, and OXA1L form a stable complex in the membrane independent of a translating ribosome and that a population of OXA1L is dedicated to the

insertion of COX1 into the IMM (Fig. 1, left side). However, OXA1L is considered to be the general insertase for mitochondrial-encoded proteins. OXA1L patient-derived cell lines showed a general reduction in the mitochondrial OXPHOS system [44]. In OXA1L-depleted cells, only the NADH:ubiquinone oxidoreductase and the F_1F_o-ATP synthase were found to be affected [4,45]. Hence, cells subjected to siRNA-mediated depletion of OXA1L behave differently from the patient-derived cells upon loss of OXA1L. This obvious difference requires further analysis (Fig. 1).

Another key aspect of the interaction between MITRAC/OXA1L and COX1 is the acquirement of translational plasticity of COX1 synthesis. In this process, the synthesis of COX1 adapts to the influx of cytosolic synthesized precursors of the complex IV into mitochondria [40,46], potentially by regulation of the function of C12ORF62 and MITRAC12.

Interestingly, the relative abundance of these early MITRAC constituents is coupled to the synthesis and subsequent assembly of COX1 as demonstrated by a reduction in C12ORF62 in mtRF1-deficient cells and the elevated levels of the MITRAC complex due to the stalling in COX1 assembly in mtRF1a-ablated cells. This highlights the dynamic nature of the MITRAC complex and its ability to react to changes in COX1 biogenesis [9].

Early assembly steps of cytochrome *c* oxidase – linear or modular?

COX1 contains 12 transmembrane spans in the inner membrane, which are connected by small loops in the IMS or the mitochondrial matrix. Initially, it was believed that the complex IV assembly pathway is a sequential order of events, during which nuclear-encoded subunits,
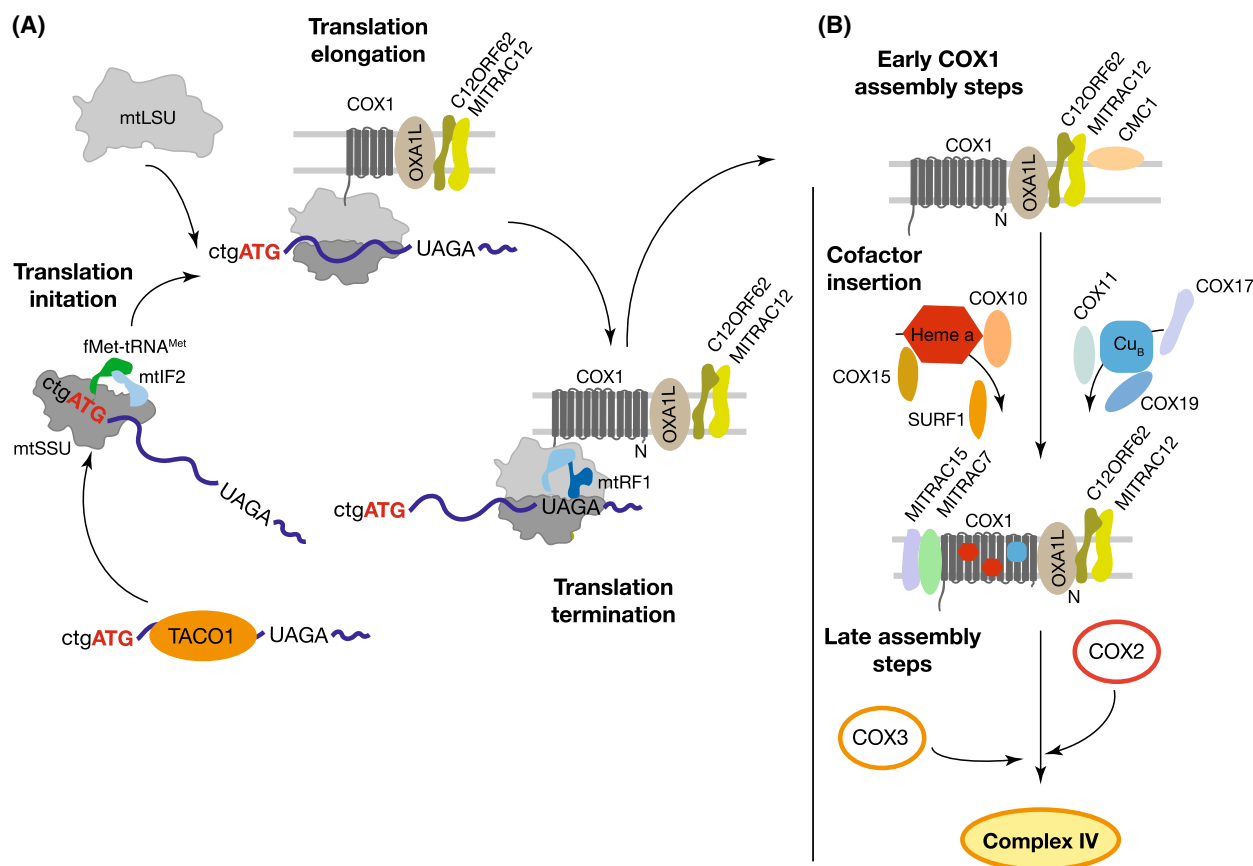
Fig. 1. Translation regulation and the early assembly steps of COX1. (A) Translation is initiated by mtIF2 binding together with a fMet-tRNA[Met]. After translation by the large ribosomal subunits (mtLSU) starts, and while the nascent COX1 protein is inserted into the inner membrane (IM) by OXA1L, the first COX1-specific proteins bind to the nascent chain. COX1 translation is terminated by binding of mtRF1. (B) After ribosome release, further COX1-specific assembly factors, such as CMC1, bind to the complex. Furthermore, the heme and copper moieties have to be incorporated in the COX1 protein. Within the final steps of COX1 assembly, MITRAC15 and MITRAC7 interact with COX1, prior to the addition of COX2 and COX3 to finalize the complex IV enzyme. (Only interaction assembly factors are illustrated).

with the help of assembly factors, are added one after another until the holoenzyme is established. However, this model of a linear assembly pipeline of the complex IV is controversially discussed in the field as it has also been proposed that the complex IV biogenesis occurs in a modular manner [47–49]. The modular model divides the assembly pathway into four distinct stages (S1–S4), in which the mitochondrial-encoded subunits COX1, COX2, and COX3 form the core components of each stage [47,48].

The linear assembly pathway favors COX1 as the stepping stone of complex IV assembly, which after dissociation of the mitochondrial ribosome is present in an assembly intermediate of a size between 140 and 230 kDa. This assembly model is based on the observations that the core proteins of complex IV, COX1, COX2, and COX3 are assembled one after another, while a deficiency of one of them influences the synthesis of the others. For example, the loss of COX1 also leads to a reduction in COX2 and COX3 [39]. Additionally, subunit-specific assembly factors have been identified in association with all mitochondrial-encoded complex IV subunits, for example, the COX1-specific assembly factors MITRAC12 and C12ORF62 are also in contact with newly synthesized COX2 and COX3 [39,40], or COX2-specific biogenesis factor COA6 co-purifies in addition to the newly synthesized COX2 also COX1 [50].

In contrast, the modular way proposes that the COX1 module gets added onto a so-called 'seed' which contains HIGD1A, COX4-1, and COX5A. Although HIGD1A has been described to bind COX1 and influence its activity during hypoxia, BN-PAGE analyses identified HIGD1A exclusively in complexes higher than 242 kDa, which would correspond to the expected size of COX1 in its first assembly intermediate MITRAC [3,39,51]. Yet, another study that postulated the modular model of COX biogenesis defined the 'seed' as a much faster-migrating complex of ~ 50 kDa [48]. This study also used BN-PAGE analysis in combination with mass spectrometry of cybrid cell lines that carried a mutated COX3 version [48]. In summary, further experiments will be required to define the 'seed' as a physiological unit within cells. Recently, Timón-Gómez *et al.* [3] investigated the role of HIGD1A and additional HIGD2A using HEK293 knockout models. Strikingly, they reported that HIGD1A is involved in complex IV as well as in the assembly pathway of the cytochrome *c* reductase (complex III), while HIGD2A appeared to be preferentially involved in complex IV assembly [3]. Accordingly, the precise functions of HIGD1A and HIGD2A remain to be defined.

Factors assisting early COX assembly steps

As described above, ribosome-associated C12ORF62 and MITRAC12 are the first proteins that interact with the newly synthesized COX1 protein and associate with the nascent chain [40]. Regardless of whether the linear or modular assembly model is favored, the first assembly factor that interacts with the new fully translated COX1 and the associated C12ORF62 and MITRAC12 appears to be CMC1 (Fig. 1, right side). CMC1 is a twin CX_9C motive-containing protein [52,53]. Although COX1 synthesis is not abolished in CMC1-deficient cells, later assembly steps are affected and newly synthesized COX1 is rapidly degraded.

Critical steps during COX1 assembly are the incorporations of the heme a/heme a_3 and the Cu_B moiety (Fig. 1, right side). COX10 and COX15 participate in heme asynthesis in mitochondria [4,54] and both proteins have been linked to human mitochondrial diseases. Mutations in COX10 resulted in Leigh syndrome [55,56], while COX15 mutations provoked Leigh-like syndrome and cardioencephalomyopathy [57,58]. In addition, SURF1 participates in heme insertion, specifically heme a_3 (Fig. 1, right side) [59]. Hence, it is not surprising that mutations in SURF1 have been identified in several patients also presenting Leigh syndrome [1]. In bacteria, Surf1 directly interacts with heme [60], while in higher eukaryotes, it could either be that SURF1 directly interacts with the heme or by facilitating its incorporation, acting rather as a chaperone-like factor. Although SURF1 seems to be critical for COX biogenesis, low levels of the matured oxidase are still formed in the absence of SURF1 [4].

For the insertion of copper to form the Cu_B site in human complex IV, COX11 is required. The insertion of the Cu_B is facilitated by COX11 assisted by COX19 (Fig. 1, right side). In yeast, the soluble copper-binding protein COX17 carrying a twin CX_9C motive and located within the IMS delivers copper to the IMS-exposed domains of COX11 [61]. To address the mechanism of Cu_B incorporation in human cells, Nývltová *et al.* [62] have generated knockout cell lines of COX11 and COX19. The authors proposed that first heme a gets incorporated into COX1, while heme a_3 and the Cu_B moiety are incorporated into COX1 after its association with COX2 [62].

The incorporations of the metal centers into COX1 appear to occur in a coupled manner since the metallo-chaperones COX15, COX10, and COX11 have been identified in the interactome of SURF1. SURF1, on the other hand, is associated with C12ORF62- and MITRAC12-containing complexes [39,40] as well as

with the first nuclear-encoded complex IV constituents COX4-1 and COX5A [4,48]. Mutations in the respective genes have been linked to mitochondrial diseases. While COX4-1 patients [63,64] present, for example, short stature and Leigh-like syndrome, COX5A mutations have been associated with psychomotoric delay, cortical atrophy, or lactic acidemia [65,66]. Although PET117 has been reported as a COX15 interactor [67], it has been also defined as an interactor of the late assembly factor PET100 [48,68]. Hence, its potential function during heme insertion and COX1 assembly requires clarification. Nevertheless, mutations in PET117, as well as PET100, are also associated with human diseases. PET117 patients present neurodevelopmental regression, reduced exercise availability, and lactic acidosis [68], and mutations in PET100 lead to Leigh syndrome and lactic acidosis [69,70].

During subsequent steps of COX1 assembly, MITRAC15 (COA1) [39,71] followed by MITRAC7 (SMIM20) (Fig. 1, right side) [72] are recruited to the maturing COX1 protein. MITRAC15 not only was identified as a component of the MITRAC complex [39] but also interacts with ACAD9 in the ND2 module during NADH:ubiquinone oxidoreductase (complex I) maturation [71,73]. Akin to the function of C12ORF62 and MITRAC12 during COX1 biogenesis, ACAD9 and MITRAC15 bind to the nascent chain of ND2 during translation. The function and potential regulatory role of this dual interaction of MITRAC15 with MITRAC and the ND2 module require further investigations. However, it is tempting to speculate that MITRAC15 acts as a mediator between complexes I and IV coordinating the assembly of COX1 and ND2 potentially in response to cellular demands. This hypothesis would predict a dynamic shuttling of MITRAC15 from MITRAC to the ND2 module and vice versa. Alternatively, dedicated MITRAC15 pools are exclusively involved in COX1 or in ND2 biogenesis, which would probably require MITRAC15 regulation.

Probably the last key regulator during the early steps of COX1 assembly is MITRAC7 [72]. MITRAC7 acts as a COX1-specific chaperone, the relative abundance of which needs to be tightly controlled as loss of MITRAC7 provokes a fast turnover of newly synthesized COX1, while elevated levels of MITRAC7 lead to stalling of COX1 within MITRAC [72]. Taken together, the early assembly steps of the cytochrome *c* oxidase are highly regulated and involve several crucial assembly factors.

Concluding remarks

Studies during the last decade have substantially advanced our understanding of complex IV biogenesis and especially of COX1 synthesis as well as assembly. Structural analysis of the mitochondrial ribosome and associated factors provide important insights into the molecular mechanisms of translation initiation, elongation, and termination. Additionally, the availability of knockout cell lines in combination with biochemical approaches stimulated the progress of our understanding of complex IV biogenesis. However, considering the complexity of factors involved in the translation process on one hand, and the assembly of the multi-membrane spanning protein COX1 on the other, a comprehensive mechanistic understanding of the biogenesis process and especially of regulatory fine-tuning is still not achieved. Therefore, it will be essential to obtain structural insights into the interaction of the maturing COX1 with its assembly and translation regulators, such as C12ORF62 and MITRAC12. Using defined knockout cell lines will certainly help to accumulate translationally arrested COX1 assembly intermediates, increasing the options for structural analysis.

Acknowledgements

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